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T-1

Development of Genotoxicity Assays with Transgenic Rodents

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Human genome is continuously exposed to endogenous and exogenous genotoxic agents. These agents interact with DNA, thereby inducing mutations and chromosome aberrations. If mutagenic events occur in functionally important regions of the chromosome, e.g., oncogenes or tumor suppressor genes, the events promote cellular carcinogenesis. Therefore, identification and risk assessment of genotoxic compounds are critically important for primary prevention of human cancer. Of various genotoxicity assays, *in vivo* assays play crucial roles in evaluation of cancer risk of chemicals. To this end, we have developed *gpt* delta mouse by introduction of lambda EG10 DNA into fertilized eggs of C57BL/6J mice. The mice carry about 160 copies of lambda EG10 DNA in both chromosomes 17. *gpt* delta transgenic mice enable us to detect mutations in any organs of mice, and identify mutational changes by DNA sequencing. In addition, two distinct types of mutations, i.e., point mutations and deletions, can be analyzed by two separate selections, i.e., *gpt* selection and Spi⁻ selection, respectively, in the same organ and the same animal. We have characterized mutations induced by various chemical and physical agents such as environmental hazardous compounds, cancer therapeutics, radiation and ultraviolet light. We also revealed antigenotoxicity of nobiletin, a major component of citrus polymethoxyflavones, against mutations in the lung of mice induced by NNK, a tobacco-specific nitrosamine. Because rats are more frequently used for cancer bioassays than mice, we developed *gpt* delta rats by introduction of lambda EG10 DNA into fertilized eggs of Sprague Dawley rats. Recently, F344 *gpt* delta rats were established by backcrosses of S.D. *gpt* delta rats to wild-type F344 rats more than 15 generations. F344 *gpt* delta rats allow integration of *in vivo* genotoxicity assays and short-term carcinogenicity assays. The integrated assay may be useful for early identification of genotoxic and non-genotoxic carcinogens in a reduced number of experimental animals.

T-2

Role of Epigenetic Modifications in Multistage Tumorigenesis of The Colon

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Genome-wide DNA hypomethylation and concomitant promoter specific tumor suppressor gene hypermethylation are among the most common molecular alterations in human neoplasia. Previous our study using *Dnmt1* hypomorphic alleles to reduce genomic methylation revealed that global DNA hypomethylation results in suppression of tumorigenesis in the colon, stomach, esophagus and tongue. We further investigated the mechanistic insight, in which forced reduction of DNA methylation levels inhibits tumorigenesis in the colon. We herein show that colonic tumors consist of heterogeneous cell populations and that DNA methylation prevents tumor cells from differentiation. These findings suggest that epigenetic modifications may play a role in the fixation of tumor cells at an undifferentiated state, and that the expansion of stem-like tumor cells may be a driving force for the tumorigenesis. Finally, we would like to introduce our recent attempt to apply the technologies of inducing iPS cells for better understanding of the role of epigenetic modifications in multistage tumorigenesis of the colon.

S1-1

Chemoprevention Research for Prostate Cancer using TRAP Rats

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Chemoprevention is one of attractive approaches for prostate cancer because of the high incidence and long latent period, and several dietary factors as well as genetic background have been linked to risk and progression of prostate cancer. Prostate cancer is known to be associated with aging, that is, about three-quarters of cases worldwide occur in men aged 65 years or more. Therefore, the main strategy with chemoprevention for prostate cancer is to delay the development of clinically evident disease due to suppression of progression from precancerous lesions to invasive cancer.

We have established an animal model whereby 3,2'-dimethyl-4-aminobiphenyl (DMAB) administration induces ventral prostate carcinomas which are microscopic in size, non-invasive and androgen-dependent, while additional long-term treatment with testosterone propionate causes development of invasive and metastatic androgen-independent adenocarcinomas, arising from dorsolateral and anterior prostate and seminal vesicles. However, a long period of about 60 weeks is required to induce prostate cancers and the frequency of lesion development is relatively low. Therefore, we established transgenic rat for adenocarcinoma of prostate (TRAP) model using rat probasin promoter/SV40 T antigen gene construct. TRAP rats develop high-grade prostatic intraepithelial neoplasia from 4 weeks of age and well-moderately differentiated adenocarcinomas with high incidences by 15 weeks of age. These adenocarcinomas were androgen dependent and almost all of them were noninvasive phenotype. The characteristics of the prostatic lesions developed in TRAP rats are suitable for evaluation of strategy for chemoprevention and treatment, and the data will be summarized.

S1-2

Colon Carcinogenesis Study with A Novel *Apc*-mutant Rat

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Tumor suppressor APC protein has multiple functional domains. *In vitro* studies have revealed that C terminus domains of APC, such as basic domain, EB1-binding domain, and DLG-binding domain, are involved in cell migration, cell adhesion, and chromosome segregation. However, *in vivo* functions of these domains remain unknown, because no animal model lacking individual domain is available. Here, to elucidate *in vivo* functions of the C terminus domains in colon carcinogenesis, we developed a novel *Apc*-mutant rat and carried out chemically induced colon carcinogenesis. A nonsense mutation (S2523X) in the *Apc* gene induced by ENU mutagenesis on the F344 genetic background was isolated. We backcrossed mutation-carriers 5 times to F344 rats to remove other mutations induced by ENU and established Kyoto *Apc* Delta (KAD) strains. KAD is homozygous for the mutation and expresses APC lacking a part of basic domain, EB1-binding, and DLG-binding domains. Although KAD rats showed no difference in susceptibility to AOM-induced colon carcinogenesis, they showed significantly higher susceptibility to AOM and DSS-induced colon carcinogenesis, compared with its control F344 rats. Interestingly, KAD rats showed severe diarrhea even after secession of DSS treatment. In addition, KAD rats showed severe inflammation of colonic mucosa after treatment of DSS. These results suggest the C terminus of APC would be involved in DSS-induced colonic inflammation.

S1-3

Inflammation and Cancer: Lessons from Pathway Specific Model Mice

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Epidemiological studies have shown that infection-associated chronic inflammation promotes cancer development. For example, *H. pylori* infection can cause gastric carcinogenesis. To examine the role of inflammatory responses in tumorigenesis, we have constructed “pathway specific” mouse models. *K19-Wnt1* mice, in which canonical Wnt signaling is activated in gastric mucosa, developed small preneoplastic lesions consisting of dysplastic epithelial cells. However, *K19-Wnt1* mice did not develop gastric tumors. In contrast, *K19-C2mE* mice, in which prostaglandin E₂ (PGE₂) level is increased in the stomach, developed metaplastic hyperplasia with severe inflammatory responses. Epidemiological studies indicate that both Wnt signaling and PGE₂ pathway play an important role in gastric cancer. We thus crossed these mouse models to generate *K19-Wnt1/C2mE* mice, in which both Wnt and PGE₂ pathways are activated simultaneously. Importantly, *K19-Wnt1/C2mE* double transgenic mice developed large tumors in the glandular stomach, and their histology was similar to that of human glandular type gastric cancer. Consistently, gene expression profile of *K19-Wnt1/C2mE* mouse gastric tumors was similar to that of human intestinal-type gastric cancer. These results suggest that activation of Wnt signaling is required for dysplastic changes of epithelial cells, and induction of PGE₂ pathway or PGE₂-dependent inflammation causes proliferation of such dysplastic epithelial cells, resulting in development of gastric tumors. Thus, PGE₂ or PGE₂-related inflammation will be an effective target for chemoprevention against gastric cancer.

S1-4

Promising Applicability of Reporter Gene Transgenic Rodents in Cancer Research

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Several types of transgenic rodents carrying reporter genes such as MutaTM Mouse and Big Blue^R have been developed to investigate *in vivo* genotoxicity of environmental chemicals. Among of them, we have used the *gpt* delta mouse/rat established by Dr. Nohmi and their colleagues. In this model, both point mutations in the *E. coli gpt* gene (*gpt* assay) and deletions with the sizes of more than 1 K bps in the *red/gam* genes of λ phage (*Spi* assay) can be detected. It is well known that there are discrepancies between *in vivo* long-term carcinogenicity and genotoxicity using several standard batteries of genotoxicity test, which means we must focus our attention on the mode of action in terms of the risk assessment for environmental agents. In this respect, reporter gene transgenic rodents may be useful tools to predict genotoxicity as well as carcinogenicity because studies can be performed with similar protocols as for the long-term bioassay. In addition, we have noted that various proposed mechanisms underlying the actions of genotoxic or non-genotoxic carcinogens are able to be investigated concurrently with transgenic mutation assays. In the present symposium, I will introduce our accumulating data on *in vivo* mutation assays using *gpt* delta rats or mice given various environmental carcinogens together with concurrent examinations of DNA modifications, oxidative stress, glutathione *S*-transferase placental form (GST-P) immunohistochemistry, and/or mRNA levels of drug metabolizing enzymes. To expand the application of the transgenic rodents, we explore a possibility of the transgenic rodents being available in the comprehensive or integrated toxicological studies. The outlines of those on-going studies will also be presented.

S1-5

Cytogenesis of Pancreatic Ductal Adenocarcinoma in the Rat

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Pancreatic ductal adenocarcinoma (PDA) is one of the most debilitating malignancies in humans. A thorough understanding of the cytogenesis of this disease will aid in establishing successful treatments. We have developed an animal model which uses adult Hras^{G12V} and Kras^{G12V} transgenic rats in which oncogene expression is regulated by the Cre/loxP system and neoplastic lesions are induced by injection of adenovirus expressing Cre recombinase. Adenoviral infection of injected animals was exclusive to the pancreas; infected cells could be identified in duct, intercalated duct, centroacinar and acinar cells. After injection, proliferative lesions in the duct epithelium, intercalated ducts and centroacinar cells, but not acinar cells, were widespread. Preneoplastic lesions derived from ductal and centroacinar cells that developed into adenocarcinomas were induced. Furthermore, adult transgenic rats were injected with adenovirus with Cre recombinase under the control of acinar cell specific promoters. Notably, injected animals did not develop any observable proliferative or neoplastic lesions. These results indicate that pancreatic ductal adenocarcinomas arise from centroacinar cells, intercalated duct or pancreatic duct epithelium, but not from acinar cells.

S2-1

Pathogenesis of renal interstitial fibrosis, based on the relationship between macrophages and myofibroblasts

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Fibrosis is a suitable aspect after tissue injury. Chronic renal disease is a lesion characterized by renal fibrosis. We have investigated the pathogenesis using cisplatin-induced rat renal models. As a result, it has been found that macrophages and myofibroblasts induced by factors which are produced by macrophages play important roles in renal fibrosis. Immunohistochemically, exudative and tissue macrophages appear in early stages, and then antigen-presenting cells are developed in advanced stages. Lymphoid vessels and lymphocyte aggregations are characteristically formed in advanced stages, suggesting the complicated pathogenesis. Myofibroblasts are formed under TGF-beta1; the cells are derived from renal fibroblasts, undifferentiated mesenchymal cells and renal epithelial cells undergoing epithelial-mesenchyma transition (EMT). PDGF-BB has synergistic effects for TGF-beta1 influence in myofibroblast development. Osteopontin and BMP-6 expressions increase in renal fibrotic lesions; osteopontin stimulates renal epithelial regeneration and BMP-6 has inhibitory effects for myofibroblast development, indicating that these factors may improve renal fibrosis. On the other hand, these factors have roles to develop myofibroblasts from undifferentiated mesenchymal cells. Endogenous PGE₂, produced through COX-1 rather than COX2, may be related to renal epithelial regeneration via EP4 receptor. The clarification of pathogenesis of renal fibrosis would lead to therapeutic strategies for chronic renal diseases.

S2-2

Pathological features of kidney and peripheral nerve lesions in spontaneous and drug-induced diabetic rats.

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Appropriate animal models are essential tools for understanding the pathogenesis and developing therapeutic agents of diabetes. Spontaneous diabetic WBN/Kob rats develop diabetic nephropathy and peripheral neuropathy, however they were still mild even in animals at end of the life span. This animal develops late onset diabetes beginning from about 40 weeks of age and diabetic conditions persist for about 50 weeks. The objective of this study is to evaluate the modification of renal and nerve lesions by prolonged diabetic condition, unilateral nephrectomy and the therapeutic insulin treatment.

Prolonged diabetic condition and unilateral nephrectomy deteriorated urinary albumin secretion, glomerular hypertrophy, diffuse glomerular sclerosis and Armanni-Ebstein lesions, but advanced glomerular lesions such as mesangiolysis, capillary aneurysm and nodular sclerosis were not induced in these animals. Insulin treatment waned enlargement of glomerular mesangial volume and Armanni-Ebstein lesions, but an inhibitory effect on fibrin cap and podocyte injury was not clear. Therefore, these lesions may be attributable to chronic progressive nephropathy rather than diabetic nephropathy.

Nerve lesions with delayed motor nerve conduction velocity included segmental demyelination, remyelination, axonal atrophy, endoneurial fibrosis and thickening of endoneurial capillary wall. Treatment of insulin has apparent inhibitory effects of progression of nerve lesions. However, accelerated diabetic condition and longer affected period by alloxan treatment did not enhance the severity of diabetic peripheral neuropathy in WBN/Kob rat.

S2-3

Histopathological Features of Diabetic Ocular Complications in the Spontaneously Diabetic Torii (SDT) Rat

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The Spontaneously Diabetic Torii (SDT) rat is a novel rat model recently established for type 2 diabetes mellitus. The onset of diabetes is as early as 15 weeks of age in males and 40 weeks in females. The cumulative incidence of diabetes reaches 100% by 40 weeks of age in males and 33% by 65 weeks of age in females. SDT rats develop hyperglycemia but can survive for long period of time without insulin therapy. As the result, SDT rats develop various diabetic ocular complications in all of the animals of both sexes at ages over 60 weeks.

In the lens, swelling, vacuolation, liquefaction and disintegration of the lens fibers, and Morgani's globules were observed in the cortex showing hypermature cortical cataracts. The nucleus of the lens was often extruded into the vitreous due to rupture of the posterior lens capsule. The vitreous body was shrunk and the vitreous cortex was detached from the retina (posterior vitreous detachment), resulting in tractional retinal detachment around the optic disc. Proliferative fibrovascular membrane containing fine capillaries was formed and connected the vitreous cortex with the retina. The capillary vessels in the fibrovascular membrane were very fine with thin walls and vitreous hemorrhage and slight inflammatory cell infiltration were sometimes observed. At the site where the fibrovascular membrane was anchored, the retina was locally thickened and formed folds. At the bottom of the folds, retina was detached from the retinal pigmented epithelium (tractional retinal detachment). Dilated capillaries and a newly formed capillary network were another characteristic feature of the thickened retina. Hemosiderin deposition indicating previous hemorrhage was sometimes observed in the retina. In the iris, neovascularization was evident. The iris was often continuous with the fibrovascular tissue that covered the surface of the anterior lens capsule. In the ciliary body, neovascularization and hemosiderin deposition were observed in the trabecular meshwork and severe hemorrhage filling with erythrocytes in the anterior chamber was sometimes observed. The anterior chamber was often dilated and filled with proteinaceous fluid strongly suggesting development of neovascular glaucoma.

There has been no adequate animal model for diabetic ocular complications morphologically resembling those in diabetic patients and the SDT rats represent the first animal model for diabetic ocular complications. In conclusion, SDT rats of both sexes develop ocular complications morphologically consistent with those reported in diabetic patients, and they provide a useful animal model for investigating therapeutic agents.

S2-4

Development of Hepatitis, Hepatic Fibrosis and Hepatic Tumor in Diet-induced Non-alcoholic Steatohepatitis

Model Rats

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Non-alcoholic steatohepatitis (NASH) is a clinicopathological entity characterized by the development of histopathological changes such as hepatocyte fatty change, inflammation, hepatocyte ballooning and fibrosis, similar to alcoholic hepatitis, and occur in the absence of viral infection, autoimmunity and excess alcoholic consumption. NASH is thought to be a kind of metabolic syndrome because of a close association with obesity, diabetes and hyperlipidemia. Because NASH is an asymptomatic ill and insidiously progresses to hepatocellular carcinoma, developing diagnostic procedures and medical treatments are urgent. For the sake of clarifying the pathogenesis and developing new drugs, a number of animal models have been developed. Above all, diet-induced NASH models are widely used for the simplicity of producing and the high reproducibility. I have reported a comparative study between three diet-induced models, methionine-choline deficient model (MCD), choline deficient L-amino acid defined model (CDAA), and high-fat model (HF). MCD rats showed moderate fibrosis with severe inflammatory foci. CDAA rats developed severe fibrosis while inflammatory response was minimal. HF rats showed moderate inflammation with minimal fibrosis. Altered hepatocellular foci were found in all models.

In this symposium, I present detailed histopathological and clinical data of above three models and discuss the prospects of diet-induced NASH models.

S2-5

Pathogenesis of Pulmonary Fibrosis Following Intratracheal Instillation of DDAC in Mice

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Didecyldimethylammonium chloride (DDAC) is used worldwide as a germicide and a wood preservative. We report that DDAC induces pulmonary fibrosis in mice. Male C57BL/6L mice were intratracheally instilled with DDAC to collect bronchoalveolar lavage fluid (BALF) and lung tissue samples. Pulmonary cytotoxicity was in association with inflammation, which was confirmed by expression of MCP-1, MIP-1 α , MIP-1 β , and RANTES in BALF; these changes were accompanied by altered lung gene expression of their chemokine (C-C motif) receptor (Ccr) 1, Ccr2, Ccr3, and Ccr5. *in vitro* cytotoxicity was more sensitive in macrophage (J774.1) than in epithelial cell (A549) and isolated mouse lung fibroblast-like cell (MLF). The cytotoxic and inflammatory phases were accompanied or followed by pulmonary remodeling, i.e., fibrosis, which was evident in increases of interstitial connective tissues, fibroblasts/myofibroblasts, and macrophages, as demonstrated by Masson trichrome stain, immunohistochemistry for α -SMA, vimentin, and Mac3, and the mRNA expression of type I procollagen. Developing fibrotic foci were likely associated with increased expression of TGF- β 1 mRNA and decreased expression of BMP-7 mRNA. In consistent with these, phosphorylated smad2/3 was expressed higher than phosphorylated smad1/5 in fibrotic lung samples. To further explore the contribution of TGF- β /smad signaling, we treated with DDAC in MLF. Pretreatment with SD208, a TGF- β RI (ALK5) kinase inhibitor attenuated DDAC-induced TGF- β mRNA and α -SMA expression through suppressing phosphorylation of smad2/3. These results suggest that administering DDAC by intratracheal instillation causes a sequence of pulmonary changes, i.e. cytotoxicity, inflammation, and fibrosis, in mice, and the pulmonary fibrosis might be mediated by TGF- β 1/smad2/3 signaling.

O-1

Mechanism of Rat Lung Carcinogenesis Promotion by Inhalation Exposure of C60 and MWCNT

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Nanotechnology has considerable global socioeconomic value, and the benefits afforded by engineered nano materials are expected to have significant beneficial impacts in fields such as plastics, energy, electronics, aerospace and medicine. However, there is an urgent need to determine potential human health hazards before wide spread introduction of nano materials into the market. Previously, by a novel intra-pulmonary spraying (IPS)-initiation-promotion protocol, we demonstrated that nano-scale titanium dioxide (nTiO₂), which is evaluated by WHO/IARC as a Group 2B carcinogen, exerts carcinogenic activity in the lung. We also demonstrated that promotion of lung carcinogenesis by nTiO₂ was mediated by MIP1 α expressed by TiO₂ laden alveolar macrophages, acting locally in the alveoli. In the present study, we investigated whether nano particles other than nTiO₂ could act via a similar mechanism. Three types of nano particles, nTiO₂, fullerene(C60) and multi walled carbon nanotube (MWCNT), were administered to SD rats by IPS 5 times over 9 days. Slight inflammatory changes and induction of alveolar macrophage were observed in the lung, and aggregates of nanoparticle were phagocytosed by macrophages regardless of particle type. Significant increase in 8-OHdG formation in the lung tissue was observed in the nTiO₂ and C60 groups but not in the MWCNT group. Protein array analysis revealed that MIP1 α was significantly increased in the nTiO₂ group, but no significant increase in 12 inflammatory cytokines was observed in the C60 or MWCNT groups. These results suggest that the mechanisms of lung carcinogenesis by inhalation exposure of C60 and MWCNT are not similar to that of TiO₂

O-2

Pulmonary effect of MWCNT by intratracheal instillation to rats.

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Nanoparticles have specific optical and electric properties. The production of highly functional materials by utilizing such properties has already begun; however, the effects of nanoparticles on humans have not been elucidated. Concerns about the biological effects of these nanoparticles being produced for use in industrial products have arisen since epidemiologic data have shown a correlation between airborne nanoparticles, as typified by PM2.5 derived from the combustion of fossil fuel, and cardiovascular diseases.

We evaluated the pulmonary effects of multiwall carbon nanotube by instillation to rats. Dispersion of the sample MWCNT was confirmed before experiment. MWCNT (The mean diameter based on volume and mass by dynamic light scattering technique was 392 nm.) was instilled to male Wistar rats at the concentration of 0.2 mg or 1mg with the 0.05 % tritonX distilled water. In BALF samples, the number of total cells and PMNs were increased in 1mg instilled group at the day 3 and 1 month. In lung section, the increased alveolar macrophage, infiltrated inflammatory cells including neutrophils and eosinophils, hyperplastic formation of alveolar epithelial cells and slight fibrogenic changes were observed at 3 days after instillation. Pointcounting evaluation of lung tissue showed that significant inflammatory changes were seen especially in 1month after instillation. No specific pathological changes were seen in other organs (brain, liver, kidney, spleen and testis).

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O-3

Serum N-ERC/mesothelin Level of Untreated Rats with Different Strains, Sexes and Ages.

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N-ERC/mesothelin is a product of the *ERC* gene expressed in the Eker rat, a hereditary renal carcinoma strain, and is identified as the human mesothelin /megakaryocyte potentiating factor. N-ERC/mesothelin is applied to diagnose and monitor on efficacy of treatment of mesothelioma. On the other hand, we are studying on the usefulness of N-ERC/mesothelin as a biomarker to detect early stage mesotheliomas in rats. In this study, we examined serum N-ERC/mesothelin levels in untreated rats with different strains, sexes and ages.

F344/Jcl (F344), BrlHan:WIST@Jcl (WIST) and Jcl:SD (SD) rats of both sexes with the age of 3, 10 and 48 week-old (15 rats for each group) were used. Serum N-ERC/mesothelin were detected using a sandwich ELISA assay kit for rat N-ERC/mesothelin (IBL, No.27765).

When serum levels (ng/mL) of rats with a particular age were compared, that of F344 was higher than those of SD and Wistar, the levels being in the similar range in the latter 2 strains. Sex difference was not observed in any rats with a particular age. In all strains, serum N-ERC/mesothelin levels showed a tendency to decrease with aging. From the results of this study, it is indicated that serum N-ERC/mesothelin levels were measurable in different strains, sexes and ages of untreated rats. N-ERC/mesothelin could be applied for the observation of induction and progression of mesothelial proliferating lesions in rats.

O-4

Effects of diacylglycerol on PKC expression in Hras128 rat mammary carcinogenesis

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Diacylglycerol (DAG) is involved in cell proliferation through activation of protein kinase C (PKC). We examined the effects of DAG on the expression levels of PKC in DAG/triacylglycerol (TAG)-induced Hras128 mammary carcinogenesis. The rats were treated (oral administration) as follows: G1, 0.5 mL TAG x2/wk; G2, 0.5 mL DAG x2/wk; G3, 0.5 mL DAG x1/wk + 0.5 mL TAG x1/wk; G4, 0.5 mL DAG x1/2wks + 0.5 mL TAG x3/2wks. Experiment was terminated at 15 weeks after the start. Mammary tumors were histologically adenocarcinoma. Tumor incidence of G2 (77%) was higher than G1 (22%). Tumor multiplicity of G2 (1.3) was also higher than the other 3 groups (0.4-0.9) ($P < 0.05$). In tumor tissues, the mRNA expression levels of 6 PKC isoforms increased compared to the adjacent normal tissues. Treatment of Hras128 rats with DAG may enhance mammary carcinogenesis by inducing expression of several PKC isoforms.

O-5

Analysis of the Mechanisms of Chemical-inducible Liver Hypertrophy in CAR Deficient Mice.

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[Introduction] Constitutive Androstane Receptor (CAR), an orphan nuclear receptor playing an essential role for induction of CYP2B, is supposed to be related to hepatocyte proliferation. To clarify the role of CAR in liver hypertrophy-mediated hepatocarcinogenesis process, liver hypertrophy-inducible chemicals were administered to CAR deficient mice, and histopathological changes in the liver and the alterations of xenobiotic metabolism-related gene expressions were examined for the first step.

[Materials and Methods] 6-week-old, male, CAR+/+ (wild, C3H strain) and CAR-/- (KO) mice were administered piperonyl butoxide (PBO, pesticide synergist), decabromodiphenyl ether (DBDE, brominated flame retardant), phenobarbital (PB) or compound X (X) in diet for 4 weeks at doses of 5000, 50000, 500 or 500 ppm, respectively.

[Results] Liver weights were significantly increased in all treated groups of wild mice. In KO mice, increases of liver weight were seen only in PBO and X groups. Histopathologically, severe centrilobular or diffuse hepatocellular hypertrophy was detected in the animals showing increased liver weight. The morphological patterns of hypertrophied hepatocytes were dependent on the chemicals. In immunohistochemistry, hypertrophied hepatocytes of wild mice were strongly positive for Cyp2b10, although the signal was faint in KO mice except PBO group showing weak but diffuse stainability. The expression of Cyp2b10 mRNA was significantly increased in all treated groups of wild mice and slightly in PBO and X groups of KO mice. The expression of Cyp3a11 mRNA was increased in PBO and X groups of both genotypes. The expressions of Cyp1a1, 1a2 and 1b1 mRNA were increased in DBDE group of both genotypes.

[Discussion] Our results indicate that PBO and X induce liver hypertrophy through CAR-independent pathway. On the other hand, CAR is essential for liver hypertrophy induced by PB. CAR is also involved in DBDE-induced liver hypertrophy, however, its mechanism might be different from that of PB.

O-6

Estrogen and androgen signaling in hepatocellular hypertrophy in rats

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It is not always clear whether hepatocellular hypertrophy is an adverse effect or an adaptive response, although it is a common aspect in toxicity tests. Sex steroid hormones have been implicated in development of hepatocellular carcinogenesis. In the present study, we investigated changes in estrogen and androgen signaling status during the development of hepatocellular hypertrophy induced by phenobarbital (PB), chlofibrate (CF), piperonyl butoxide (PBO) and acetaminophen (AA). 6 week-old F344 rats were fed with chemicals and killed at day 3, weeks 4 and 13, and at week 17 after the 4 week recovery period on standard diet. Liver tissues were dissected and subjected to the total RNA extraction. The mRNAs including estrogen and androgen receptors (ER, AR) as well as their responsive genes such as LIFR, MAP and α 2M were quantified by the real-time RT-PCR method. During the treatment, expression of ER α and its responsive gene, LIFR, was decreased in CF, PBO and AA groups. Androgen responsive α 2M gene expression increased in CF and PBO groups. Interestingly, LIFR was recently identified as a suppressor gene of HCC. α 2M has reported to be a HCA maker. Taken together, our results suggested that altered estrogen and androgen signaling along with hepatocellular hypertrophy may indicate adverse toxic effects.

O-7

Microangiopathy Associated with MNU-induced Retinal Degeneration in Mice

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Introduction: Although acellular capillary or pericyte loss are known to be pathognomonic changes of early diabetic retinal microangiopathy, we have reported the appearance of similar vascular changes in non-diabetic WBN/Kob rats with inherited progressive retinal atrophy. To clarify the association between intraretinal vascular change and retinal degeneration, morphologic and morphometric changes of vessels in trypsin-digested retinal tissue were examined in mice with drug-induced retinal degeneration.

Materials & Methods: N-methyl-N-nitrosourea (MNU) was intraperitoneally injected (60 mg/kg) to male ICR mice once at 5-week of age to induce retinal degeneration. The animals were sacrificed 2 and 4 weeks after MNU treatment. Age-matched and intact ICR mice were used as control animals. Eyeballs from each group were fixed in 4 % paraformaldehyde. The retinal tissues of right eyeballs were digested in trypsin solution at 4°C according to Ashton's method, then stained by PAS and hematoxylin for microscopic examination of vascular tissue. The left eyes were prepared for histological examination.

Results: MNU-induced retinopathy was characterized by progressive loss of nuclei of outer granular layer and rod and cone layer from 2 to 4 weeks after the MNU treatment. The numbers of acellular capillaries and ghost cells in trypsin-digested retinal vessels were gradually increased with time after treatment of MNU, and they were significantly higher at 2 and 4 weeks after treatment compared to age-matched and intact control mice.

Conclusion: Vascular changes similar to early diabetic microangiopathy are consistently induced in retinal tissues of mice with drug-induced retinal degeneration like those of hereditary retinopathy in WBN/Kob rats.

O-8

Modifying Effects of Type 2 Diabetes Mellitus in Rat Carcinogenesis

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Type 2 diabetes mellitus (DM2) has been reported to affect the risk of carcinogenesis in colon, liver, pancreas, breast and uterus, but the relationship between DM2 and carcinogenesis is still unclear. Recently, we have reported that DM2 promotes the incidence of bladder and colon cancer using DMBDD model, a well-known rat multi-organ carcinogenesis test.

To investigate the mechanisms of carcinogenic effects of DM2, 6 week-old male Zucker Diabetic Fatty (ZDF) type 2 diabetes rats and control Lean rats were given 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine in the drinking water from week 1 to 4. At week 4, the experiment was terminated and analysis of rat serum levels of several biologically active materials and gene expression in the mucosa of the urinary bladder were performed.

The serum level of insulin and leptin were high in the non-treated ZDF rats as compared to the non-treated Lean rats. Furthermore, in ZDF rat bladder mucosa, up-regulation of PI3K and overexpression of PCNA, a marker of cell proliferation, were found in concordance with inhibition of p53 expression.

Our results indicated that the mechanism of high sensitivity of type 2 DM rat to bladder carcinogenesis is related to the abnormalities in PI3K pathway resulting in elevation of PI3K and decrease of p53 gene expression in consequence of increase of serum insulin and leptin levels.

O-9

Disruption of Smad-dependent Signaling for Growth of GST-P-positive Lesions from the Early Stage in a Rat Two-stage Hepatocarcinogenesis Model

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The immunohistochemical distribution of signaling molecules of transforming growth factor (TGF)- β was analyzed in relation with liver cell lesions expressing glutathione *S*-transferase placental form (GST-P) during liver tumor promotion by fenbendazole, phenobarbital, piperonyl butoxide, or thioacetamide, using rats. Our study focused on early-stage promotion (6 weeks after starting promotion) and late-stage promotion (57 weeks after starting promotion). With regard to Smad-dependent signaling, cytoplasmic accumulation of phosphorylated Smad (phospho-Smad)-2/3 — identified as Smad3 by later immunoblot analysis — increased in the subpopulation of GST-P⁺ foci, while Smad4, a nuclear transporter of Smad2/3, decreased during early-stage promotion. By late-stage promotion, GST-P⁺ lesions lacking phospho-Smad2/3 had increased in accordance with lesion development from foci to carcinomas, while Smad4 largely disappeared in most proliferative lesions. With regard to Smad-independent mitogen-activated protein kinases, GST-P⁺ foci that co-expressed phospho-p38 mitogen-activated protein kinase increased during early-stage promotion; however, p38-downstream phospho-activating transcriptional factor (ATF)-2, ATF3, and phospho-c-Myc, were inversely downregulated without relation to promotion. By late-stage promotion, proliferative lesions that downregulated phospho-ATF2 and phospho-c-Myc had increased along with lesion development, although they lacked p38. These results suggest that from the early stages, carcinogenic processes were facilitated by disruption of tumor suppressor functions of Smad-dependent signaling, while Smad-independent activation of p38 was an early-stage phenomenon. GST-P⁺ foci induced by promotion with agonists of peroxisome proliferator-activated receptor- α did not change Smad expression, suggesting an aberration in the Smad-dependent signaling prerequisites for induction of GST-P⁺ proliferative lesions.

O-10

Carcinogenic Potential and Formation of Specific DNA Adducts of Estragole in the Rat Liver

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【PURPOSE】Estragole (ES), a natural constituent of several herbs such as tarragon, basil, fennel and anise, has been used as a food additive for flavoring agent. It has been reported that ES has the potential for inducing tumors along with DNA adduct formation in the mouse liver. Various genotoxicity tests for ES showing negative, there have been no reports regarding hepatocarcinogenicity in rats. In the present study, to evaluate hepatocarcinogenicity of ES in the rat liver, quantitative analysis of GST-P positive foci and the specific DNA adducts were performed in the livers of rats treated with ES. 【METHOD】Male F344 rats (6-week-old) were given ES (600 mg/kg b.w.) by gavage 5 days per week, or 10 ppm diethylnitrosamine (DEN, as a positive control) in the drinking water for 16 weeks. Rats were sacrificed at weeks 4, 8 and 16, and the livers were sampled for GST-P immunohistochemical examination. Additionally, ES specific DNA adducts in the livers at week 4 were measured by LC-MS/MS. 【RESULT】Body weight gain was significantly reduced from week 2 in the ES-treated group, the relative liver weights being significantly increased in the ES and DEN-treated groups throughout the experimental period. In addition to detection of GST-P foci in the ES-treated group from week 4, number and area of GST-P positive foci at week 16 were significantly increased as in the DEN-treated group. ES-3'-N²-dG, ES-3'-8-dG and ES-3'-N⁶-dA as ES-specific DNA adducts were detected in the livers at week 4. 【DISCUSSION】Quantitative analysis of GST-P positive foci strongly suggests that ES could be a hepatocarcinogen even in the rat. Furthermore, detection of ES-specific DNA adducts at early stage might indicate the involvement of genotoxicity in ES hepatocarcinogenesis. Hereafter, quantitative analysis of the DNA adducts will be performed.

O-11

Toxicological evaluation of furan using a newly developed short-term comprehensive method with *gpt* delta rats

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A newly short-term comprehensive method with *gpt* delta rodents is considered to be a powerful tool for evaluating systemic toxicity, genotoxicity and carcinogenicity under an identical experimental protocol. Furan, a basic structure of various furan derivatives used as flavoring agents, has been known as a carcinogen in the livers of rodents in spite of its genotoxicity being equivocal. In the present study, in addition to validating the new method, the toxicity profile of furan was investigated. Male and female 7-week-old F344 *gpt* delta rats were given furan in corn oil at a dose of 0, 2 or 8 mg/kg of body weight by gavage 5 days per week for 13 weeks. At necropsy, significant decrease of body weight was observed in the highest dose males. Significant increases of the spleen and liver weights were also observed in both sexes of the highest dose groups. Significant increase of serum ALP levels was observed at doses of 2 mg/kg or higher in males. In micronucleus tests for bone marrow cells, the micronucleus frequency in the highest dose males (0.32 ± 0.07) was significantly higher than that in the control group (0.13 ± 0.07 , $P < 0.05$). In conclusion, the toxic effects of furan observed in this new method were in line with those in the previous studies. However, the positive results in micronucleus test disaccorded with the previous data, which might be attributed to the difference of administration methods (repeat vs. single doses). For the future, histopathologic examination, *gpt in vivo* mutation assay and quantitative analysis of GST-P positive foci in the livers will be performed.

O-12

Mechanism of Promotion Effect of Diphenylarsenic Acid on Rat Liver Carcinogenesis

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Diphenylarsinic acid (DPAA) is an environmental degradation product of diphenylarsine chloride or diphenylarsine cyanide, which were chemical warfare agents produced by Japan during the World War II. DPAA is suspected of inducing health effects that include articulation disorders, involuntary movements, and sleep disorders in kamisu, Irbaraki (Japan). We previously showed that DPAA had promotion activities and increased liver weights at 20 ppm but not at below on rat liver carcinogenesis in medium-term bioassay (Ito test). The purpose of present study is to clarify the mechanism of DPAA-induced toxicity and promotion activity in rat liver. We found that DPAA at 20ppm increased significantly in oxidative DNA damage, mRNA expression of CYP1B1, cyclin D1, and c-Myc, and protein level and transcriptional activity of AhR. Proteome analysis showed that 69 proteins were differentially up or down-regulated in 20ppm DPAA group compared with DEN alone including 18 proteins of AhR pathway. These results suggested that activity of AhR pathway and oxidative DNA damage on rat liver carcinogenesis involved in promotion activity of DPAA.

O-13

Suppressive Effect of Global DNA Hypomethylation on Gastric Carcinogenesis: Analysis by *Dnmt1* Hypomorphic Mouse

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Global DNA hypomethylation and concomitant site-specific gene hypermethylation are among the most common molecular alterations in human neoplasia. Although site-specific DNA hypermethylation has been shown to be associated with the development of various tumors accompanied by transcriptional silencing of target genes, the functional significance of global DNA hypomethylation in tumorigenesis remains unclear. Previous studies have revealed that a genetic reduction of the DNA methylation levels leads to opposing effects on tumor development, depending on the tumor cell type and the stage of tumorigenesis. In the present study, we investigated the effect of DNA hypomethylation on gastric carcinogenesis in mice. The genetic reduction of DNA methylation levels suppressed the incidence, number and size of gastric tumors in two different mouse models for gastric tumorigenesis: the *N*-methyl-*N*-nitrosourea (MNU)-induced model and the *Apc*^{Min/+} mouse model that spontaneously develops gastric tumors with aging. Histological analyses revealed DNA hypomethylation to completely inhibit the development of invasive gastric tumors. These findings indicate, for the first time, that the reduction of DNA methylation levels suppresses gastric carcinogenesis and suggest that the pharmacological modification of the epigenetic status might therefore be a potentially useful strategy for the prevention and treatment of gastric cancers.

O-14

A Review for Histopathological Changes Induced by an Oncolytic Adenovirus (Telomelysin, OBP-301) in Preclinical and Clinical Studies

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Telomelysin (OBP-301) is an oncolytic adenovirus (serotype 5) which was constructed to selectively replicate in tumor cells and induce tumor-cell lysis. It is now under clinical development as a novel approach for the treatment of malignant tumors. Telomerase activity is increased in many tumor cells closely with human telomerase reverse transcriptase (hTERT) expression. In Telomelysin, hTERT gene promoter drives expression of E1 genes which are indispensable for adenovirus replication so that the propagation of the virus is restricted in tumor cells expressing hTERT. Telomelysin replicated efficiently and induced cell death in a panel of human cancer cell lines in vitro. In tumor-bearing nude mice, Telomelysin showed growth inhibition of the tumors by intratumoral (IT) injection.

When Telomelysin was injected IT in hepatitis B virus X protein (HBx) transgenic mice bearing hepatic tumors, foci of liquid necrosis were seen in the cancerous areas, not in the non-cancerous areas in the surviving mice. The tumor cells showed swelling and cell lysis suggesting of intracellular viral propagation. Single cell necrosis was also observed. In toxicity studies, Telomelysin was injected intramuscularly (IM) or intravenously (IV) to cotton rats which is known to be susceptible to adenoviruses. When injected once, Telomelysin induced local irritation at the injection sites including degenerative changes of the muscle fibers and interstitial mononuclear cell infiltration. In the liver, increased reticuloendothelial cells in the sinusoid and single cell necrosis of the hepatocytes were seen in a high-dose IV treated animals, which were considered due to hepatotropic nature of adenoviruses.

A phase-I dose-escalation clinical study was conducted to assess safety, pharmacokinetics and also tumor response in a total of 22 patients with advanced solid tumors. Telomelysin was once or 5-times injected IT and well tolerated. Histopathological evaluation of biopsy samples (taken before injection, 28 and/or 56 days after injection) was performed in 12 cases. One patient with melanoma, who had injection at left thigh mass, showed obvious tumor regression. No apparent tumor cells were seen in the tissues on Days 28 and 56, whereas typical infiltrative melanoma was seen before injection. In addition, three other patients showed pathological changes which may be indicative of possible antitumor effects of Telomelysin.

These pathological changes from preclinical (HBx transgenic mice and cotton rats) and clinical studies will be reviewed in the presentation.

O-15

Increase of GABAergic Interneurons in the Hippocampal Dentate Gyrus by Developmental Exposure to Acrylamide

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Although concern has long been raised on the developmental neurotoxicity of acrylamide (ACR), apparent offspring toxicity except for decreased body weight has not been detected by maternal oral exposure with doses causing apparent neurotoxicity to dams, experimentally. In the present study, developmental neuronal toxicity of ACR was examined using rats in terms of aberrant neuronal migration as well as well-known axon terminal injury. Pregnant SD rats were administered ACR at 0, 25, 50, or 100 ppm in the drinking water from the day 6 of pregnancy until day 20 after delivery. As a positive control for direct exposure, ACR was intraperitoneally injected three times/week to offspring at 50 mg/kg BW during lactation period. Dams exposed to 100 ppm ACR showed apparent increase of neurotoxic gait scores after delivery as well as axonal degeneration of sciatic nerves and aberrant synaptic aggregation immunoreactive for synaptophysin in the cerebellar molecular layer, both of them reflecting axon terminal injury by ACR. With regard to offspring, direct exposure animals alone showed gait abnormality and histopathological changes suggestive of axon terminal injury. On the other hand, maternally exposed offspring showed decreased body weight at 100 ppm; however, they lacked axon terminal injury. As well as direct exposure animals, dose-dependent increases of Reelin- or GAD67-immunoreactive cells suggestive of GABAergic interneurons were observed in the hippocampal dentate hilus of maternally exposed offspring with statistical significance from 25 and 50 ppm, respectively. Although dose-dependent, the level of ACR-Hb adducts in offspring was one tenth or less than that in dams at postnatal day 14. In summary, although preweaning rats have susceptibility to ACR-induced axon terminal injury, the internal level of ACR in offspring exposed through maternal oral administration is insufficient to induce this type of neurotoxicity due to limited lactational transfer. However, increase of Reelin-expressing interneurons suggestive of neuronal mismigration was induced by ACR from low doses that did not cause growth retardation.

O-16

Establishment of a new invasive urinary bladder cancer model using human c-Ha-ras proto-oncogene transgenic rats

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To establish animal models for identifying mechanisms of urinary bladder cancer (UBC) invasion, male human c-Ha-ras proto-oncogene transgenic rats (Hras 128) were divided into 5 groups and treated with 0.05% N-butyl-N-(hydroxybutyl) nitrosamine (BBN) in drinking water and/or 0.1% phrnylethyl isothiocyanate (PEITC) in diet, respectively, as follows: BBN (8 wks) → PEITC (8 wks); PEITC (8 wks) → BBN (8 wks); PEITC alone (16 wks); BBN alone (16 wks); non-treatment. At the end of week 16, incidences of invasive UBC in BBN → PEITC and BBN alone groups were 77% (10/13 rats), respectively. The average number of invasive UBC in BBN → PEITC and BBN alone groups were 1.3 ± 0.9 and 0.7 ± 0.7 /rat, respectively. Both incidence and numbers of invasive UBC tended to be higher in BBN → PEITC group compared to the BBN alone group. Furthermore, non-invasive UBC were also found in other all rats of above 2 groups. No tumors were found in other groups. These findings indicated that treatment of Hras 128 rats with BBN and PEITC simultaneously induced high incidences of both non-invasive and invasive UBC, and therefore provide a useful comparison model for explore the mechanisms of UBC invasion.

O-17

Establishment of an animal model by allogeneic and orthotopic transplantation of a rat prostate cancer cell line

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【Background】 Previously, we established a cell line (PLS10) from a prostate cancer induced by treatment with 3,2'-dimethyl-4aminobiphenyl and testosterone propionate in a male F344 rat. We have now establishment of an animal model of prostate cancer by allogeneic and orthotopic transplantation of using this cell line.

【Experiments】 5×10^6 PLS10 cells in a 50% matrix gel were injected into the ventral prostate of 6-week-old F344 rats. Rats were sacrificed at week 4 and week 8. Prostate, lung, liver, kidney, and lymph node were fixed in formalin and examined histologically for tumor and metastasis.

【Results】 Tumor incidence was 100% in injected rats. The average tumor volume was $343 \pm 246 \text{ mm}^3$ at week 4 and $2,114 \pm 390 \text{ mm}^3$ at week 8. At week 4, perineural invasion was observed in 6 of 6 rats examined; lymphatic invasion was observed in 4 of 6 rats examined; vascular invasion was observed in 5 of 6 rats examined; and lymph node metastasis was observed in 2 of 6 rats examined. At week 8, perineural invasion, lymphatic invasion, vascular invasion, and lymph node metastasis was observed in all animals examined.

【Summary】 This model uses animals with an intact immune system and the prostate cancer resembles human prostate cancer both biologically and histologically. Therefore, this model can be used to study prostate carcinogenesis and investigate therapies.

P-1

Isolated glomerular proteome analysis of rats with the non-insulin-dependent diabetes mellitus

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[Background]

Proteins of the transforming growth factor beta (TGF- β) superfamily mediate the increase of the mesangial matrix in diabetic nephropathy. However, many key molecules and pathways that mediate diabetic nephropathy have not been elucidated. To this end, we performed isolated glomerular proteome analysis of rats with non-insulin-dependent diabetes mellitus.

[Materials and Methods]

We performed preliminary experiments on Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are representative models for obesity and diabetes; Long-Evans Tokushima Otsuka (LETO) rats were used as controls. At week 27 (early-stage of diabetic nephropathy) and 38 (proteinuria stage), we isolated glomeruli from the OLETF and LETO rats using sieving method. We performed proteome analysis to investigate the intergroup differences in protein expression in the glomeruli. Further, we investigated the changes in the mesangial matrix in both groups.

[Result]

We identified and quantified 165 proteins with 95% confidence or higher by using QSTAR Elite LC-MS/MS system and ProteinPilot 2.0 software, respectively and found that the expression of 13 proteins was upregulated in OLETF rats, while that of 14 proteins was downregulated. We could not detect proteins of the TGF- β superfamily. However, the expression of α -actinin-4 and vimentin was downregulated at week 27 in the OLETF rats.

[Summary]

We could not detect TGF- β superfamily proteins at week 27, since there was negligible change in the mesangial matrix. However, we confirmed the downregulation of α -actinin-4 and vimentin expression. In diabetic nephropathy, podocyte injury leads to proteinuria. We intend to perform further investigations once data of 38 weeks is compiled.

P-2

Histopathological Analysis of Diabetic Nephropathy in Spontaneously Diabetic Torii (SDT) Rats, Type 2 Diabetes

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Diabetic nephropathy in type 2 diabetes has been increasing in recent years. Diabetic nephropathy leads to renal failure. This disease requires further elucidation of the mechanisms and for development of new pharmaceutical drugs. In this study, we investigated the histopathological feature of diabetic nephropathy in SDT rats as a type 2 diabetic model.

[Materials and Methods] Twelve 9-week-old male SDT rats were obtained from CLEA Japan. Seven untreated SDT rats surviving to 69 weeks old were used in this study. Five of them were sacrificed by exsanguination under anesthesia. The other two were perfused under anesthesia, and the kidneys were collected. The kidneys were fixed in 10% neutral buffered formalin, and routinely processed for the histopathological examination. Diffuse diabetic glomerulosclerosis were evaluated according to the human histopathological classification of diabetic nephropathy (Gellman classification). In addition, the kidneys were examined by electron microscopy.

[Results] In glomerular tufts, diffuse glomerulosclerosis (Gellman classification II) and exudative lesions were observed. Glomerulosclerotic lesions were characterized by diffuse mesangial expansion (increase in extracellular matrix) and basement membrane thickening. Moreover, macrophages (positive for ED-1) tended to appear in the part of vascular pole. Increased extracellular matrix and infiltrating macrophages were also noted in the Goormaghtigh cell area. Furthermore, glycogen accumulated in cytoplasm of renal distal tubular cells.

[Conclusion] Diffuse glomerulosclerosis, exudative lesions, and macrophages occurred in SDT rat glomerulus, which were similar to the feature of human diabetic nephropathy. As for humans, inflammation may be related to progressing of glomerulosclerosis in SDT rats. On the other hand, glycogen accumulation was noted in the distal tubule, which was different from that in humans (proximal tubule).

P-3

Carcinogenic mechanism of squamous cell carcinoma in the forestomach of Alloxan-induced Diabetic Rats.

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Alloxan-induced diabetic rats frequently exhibited proliferative lesions of squamous epithelium accompanied by chronic inflammation and microbial infection in the forestomach, and some lesions progressed to squamous cell carcinoma (SCC). In the present study, we tried to clarify the mechanism of forestomach carcinogenesis in this model.

We examined the immunohistochemical expression of p53, cyclin D1, Ki67 and β -catenin protein in 12 alloxan-induced diabetic rats with SCC. In addition, genetic alterations of *H-ras*, *p53* and *β -catenin* were examined in 5 cases.

Histologically, varying degree of hyperplasia of mucosal squamous epithelium was detected in the forestomach of alloxan-treated rats apart from the area of SCC. All SCC were well-differentiated type. Immunohistochemical analyses revealed occasional p53-positive nuclei in neoplastic cells of the forestomach. Moreover, nuclear overexpression of cyclin D1 and Ki67 was detected in hyperplastic cells of basal layer and neoplastic cells. The nuclear/cytoplasmic expression of β -catenin was also exhibited in hyperplastic and neoplastic cells. The nuclear expression of β -catenin was more frequent in the proliferated squamous cells in the basal layer.

Five cases of forestomach SCC had no mutation of *H-ras*, *p53* and *β -catenin* genes.

Enhanced p53 expression in the neoplastic cells of forestomach SCC and higher nuclear expression of β -catenin, cyclin D1 and Ki67 in proliferative lesions in present study suggested that Wnt pathway plays important role in this carcinogenic mechanism. Additionally, genetic alteration of *H-ras*, *p53* and *β -catenin* were not detected in 5 cases. These results might be an evidence of epigenetic theory of the carcinogenesis of this model. However, further study is needed to clarify the mechanism of carcinogenesis.

P-4

High susceptibility to azoxymethane-induced ear duct and intestinal carcinogenesis in OLETF rats

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Diabetes and obesity are thought to be risk factors for colorectal cancer in humans. In our recent study, whole body X-irradiated Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model animal of type 2 diabetes, developed low incidence of small intestine adenocarcinoma, but not in nondiabetic Long-Evans Tokushima Otsuka (LETO) rats. In the present study, six-week-old male OLETF and LETO rats (n=24) were given weekly s.c. injections of 15 mg/kg azoxymethane (AOM) for three weeks and killed after 30 weeks from the initial treatment. The incidences of ear duct tumors in LETO and OLETF rats were 0% and 67% ($P<0.001$), respectively. Those of small intestine tumors in these strains were 0% and 43% ($P<0.001$), respectively, and those of colon tumors were 46% and 79% ($P<0.05$), respectively. Serum triglyceride and free fatty acid levels in OLETF rats were significantly higher than those in LETO rats at sacrifice, but serum insulin was lower than that in LETO rats. These data suggest that hyperlipidemia plays an important role in high susceptibility to AOM-induced ear duct, small intestine and colon carcinogenesis in OLETF rats.

P-5

N-Methyl-*N*-Nitrosourea-Induced Retinal Degeneration in Mouse Is Independent of p53 Gene

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A single systemic administration of *N*-methyl-*N*-nitrosourea (MNU) causes retinal degeneration involving photoreceptor cell loss within 7 days. MNU-induced photoreceptor cell loss is due to apoptosis and is a reliable animal model for human retinitis pigmentosa. The purpose of this study was to determine if p53 contributes to the development of MNU-induced retinal degeneration in mice.

Methods: Eight-week-old p53 $-/-$, p53 $+/-$ and p53 $+/+$ mice received an intraperitoneal injection of 60 mg/kg body weight of MNU. Age-matched p53 $+/+$ mice received vehicle only (physiologic saline containing 0.05% acetic acid). Mice were sacrificed and necropsied 7 days after the treatment. Both eyes were examined histologically and morphometrically to determine retinal thickness, photoreceptor cell ratio, and retinal damage ratio.

Results: No mice died during the experiment, but the p53-null mice treated with MNU had a statistically significant weight loss as compared to the other groups. Histologically, all MNU-treated mice, regardless of p53 gene status, experienced retinal degeneration characterized by photoreceptor cell loss (the disappearance of the outer nuclear layer and photoreceptor layer) in both central and peripheral retina. All MNU-treated mice had significantly decreased retinal thickness and photoreceptor cell ratios at the central and peripheral retina and an increased retinal damage ratio as compared to the vehicle-treated control. The retinal changes caused by MNU in p53 $+/+$, $+/-$ and $-/-$ mice were not significantly different.

Conclusion: Because the absence of p53 did not prevent photoreceptor cell loss, we conclude that p53 is not essential for MNU-mediated photoreceptor cell degeneration.

P-6

Triparanol-induced Cataract in the Rat—Morphological and Molecular Analyses—

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Triparanol (TPN) was once used therapeutically as a cholesterol-reducing drug but withdrawn from the market because of adverse effect cataract. The mechanism of TPN-induced cataract is not well known but may be due to lipids or electrolytes imbalance. To explore the pathophysiological mechanism of cataract, we examined the histological changes of rat lens, analysis of lipid and gene expression.

TPN was administered orally at doses of 0, 10, 30 and 45 mg/kg/day for 13 weeks to male Crl:CD (SD) rats at 8-week old. The ophthalmologic examination was repeated during study. At the end of the experiment period, all animals were necropsied and eyes were fixed in glutaraldehyde-formalin and routinely processed for histopathological assessment. In the molecular biological study, TPN was administered orally at 60mg/kg/day for 4 weeks to male SD rats at 8-week old. Right lens and plasma cholesterol/desmosterol were extracted using methanol/chloroform and analyzed by LC-MS. Total RNA from left lens were analyzed for GeneChip (Affymetrix®).

Ophthalmologically, the opacity development in 45mg/kg group was perceptible from 8-week, the lens in the 30mg/kg group was also similar lesion by 13-week. Histologically, there was cortical cataract which was characterized by swollen/disruption fiber in the posterior and anterior subcapsular cortex and nuclear fragmentation of epithelial cells at the equator. There was increase in desmosterol content of rat lens but no change in the amount of lens cholesterol. Microarray analysis of cataract lenses showed changes in gene expression of ion channels and component.

The TPN cataract may be caused by disruption of lens fiber followed by the disturbance of lipid metabolism and modulation of ion transport in the lens.

P-7

Microvascular Architecture of the Eye of Aging Royal College of Surgeons (RCS) Rat: A Scanning Electron Microscopic Study of Vascular Corrosion Casts

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Royal College of Surgeons (RCS) rat has been considered an animal model of human inherited retinal dystrophy. In their eyes, photoreceptor cells have degenerated and disappeared with aging, and small blood vessels are recognized at the outer granular zone of the retina. To investigate the microvascular architecture, we prepared methylmethacrylate (MMC) corrosion castings and examined with scanning electron microscopy.

Eighteen RCS rats, 5-8 months old, were injected Mercox CL (DIC, Tokyo, Japan) or Batson's 17 (Polyscience, Inc., Warrington, PA, USA) MMC media into the orbital blood vessels. After polymerization, the eyes were macerated in a 6M KOH solution. The specimens were investigated using a scanning electron microscope (JSM-6360LV, JEOL, Tokyo).

The retinal capillaries were not uniform in diameter and were narrow and distended in parts. They were folded or tangled. These changes were often recognized at capillaries connected to the central retinal vein. The globular capillaries were located outside of the original position. We could not find any choroidal neovascularization. Small blood vessels which were recognized at the retinal outer granular layer possibly correspond to the abnormally distributed retinal capillaries.

Further examinations are needed to clarify the behavior of the retinal capillaries and to discuss application as other animal model from the inherited retinal dystrophy.

P-8

Retinal Lesion in the *hhy* Hydrocephalus Mutant Mouse
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Mutant animals are useful models to study the pathogenesis of intractable hydrocephalus. Hemorrhagic hydrocephalus (*hhy*) mouse is a spontaneous mutant with dilatation of lateral ventricles, intracerebral hemorrhage, which start from 2 weeks of age. The *hhy* mouse also has a heterotopic gray matter in the cerebral cortex. Detailed pathogenesis of the *hhy* mouse remains to be elucidated. Causative gene of *hhy* mouse is located in the mouse Chr12 and recently Mori *et al* have identified the gene (unpublished data). In this study, we examine the eye lesion in the *hhy* mouse to disclose the roles of *hhy* gene for the eye development.

We examined *hhy/hhy* and control (*hhy/+* or *+/+*) mice from embryonic day 15 to 6 months of age. Frozen or paraffin sections of eyes were made and evaluated histopathologically. A polyclonal rabbit antibody was obtained using a synthetic peptide of causative gene and used for immunohistochemistry and immunoelectron microscopy.

Retinal dysplasia was found in the adult *hhy/hhy* mouse with varied severity and frequency of occurrence. Mice at postnatal day 0 have disarranged neural layer of the retina and rosette-like structure consisting of immature neural cells was observed. Immunoreactivity for antibody against *hhy* peptide was located the outermost layer of the developing retina with liner pattern at embryonic day 15.

Immunoelectron microscopy revealed positive signals for *hhy* peptide seemed to be located in the cell junction of the outermost layer. From these findings, retinal dysplasia develops in the *hhy* mouse as well as hydrocephalus and subcortical heterotopia, and it is suggested that *hhy* gene plays important roles for the retinal development.

P-9

Inhibitory Effects of Bitter Melon Leaf Extract on Prostate Cancer Cell Migration and Invasion

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Extracts of bitter melon have been reported to possess anti-tumor activity. Our previous study revealed that bitter melon leaf extract (BMLE) inhibited P-glycoprotein activity and reverse multidrug-resistant in cervical carcinoma cell line. In this study, we examined the effects of BMLE against invasiveness of prostate cancer in vitro using rat prostate cancer cell line (PLS10). Using migration and invasion chamber, treatment of BMLE with non-toxic dose significantly reduced PLS10 migration and invasion. Gelatin and plasminogen-casein zymography demonstrated that matrix metalloproteinase (MMP)-2, MMP-9 and urokinase-type plasminogen activator (uPA) secretions were significantly decreased by BMLE. For determination of MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-2 gene expression, using real time PCR, BMLE significantly decreased gene expression of MMP-2 and MMP-9 whereas markedly increased mRNA level of TIMP-2 which is known to have inhibitory effect on the activity of MMP-2. The collagenase type IV activity was inhibited by BMLE detected with an EnzChek Gelatinase/Collagenase Assay kit. These results suggest that BMLE exerts anti-invasion effect on PLS10 cells through the inhibition of cancer cell motility, decreasing the secretion, expression and activity of extracellular matrix degradation enzymes, and up-regulation of the expression of MMP inhibitor. **Acknowledgement:** This work was supported by grants from the Royal Golden Jubilee Ph.D. Program of Thailand and the Society for Promotion of Pathology of Nagoya, Japan.

P-10

Relationship of heat shock protein 25 with reactive macrophages in thioacetamide-induced rat liver injury

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Heat shock protein 25 (Hsp25) has anti-inflammatory activity. Relationship of Hsp25 expression with macrophage appearance was investigated in thioacetamide (TAA)-induced rat acute hepatic lesions. TAA was administered at a dose of 300 mg/kg in male Crl:CD(SD) rats aged 6 weeks. Rats were sacrificed on 1, 2, 3, 5, 7 and 10 days after a single administration. Liver was removed, and examined by real time RT-PCR used a primer of Hsp25, Tumor necrosis factor- α (TNF- α), Monocyte chemoattractant protein-1 (MCP-1), Osteopontin (OPN), histopathology and immunohistochemistry for Hsp 25, ED1, ED2 and OPN antibody.

TAA-induced lesions were developed in the centrilobular areas, consisting of hepatocyte coagulation necrosis and reactive macrophages. Macrophages immuno-reacting to ED1 (CD68; exudative macrophages) were mainly seen within the lesions, whereas macrophages reacting to ED2 (CD163; resident macrophages and Kupffer cells) appeared mainly in the periphery of the lesions, having abundant cytoplasm. Hsp25-immunopositivity was seen in hepatocytes around the lesions; the distribution of Hsp25 expression related to ED1- and ED2-positive macrophages in and around the centrilobular lesions, respectively. Because macrophages appearing in early stages of hepatic lesions produce various pro-inflammatory factors, mRNA expressions of TNF- α , MCP-1 and OPN were examined in correlation with Hsp25 mRNA expression. Hsp25 mRNA expression generally correlated to TNF- α , MCP-1 and OPN expressions, suggesting that these factors might be associated directly or indirectly with Hsp25 expression. Taken together, it was considered that Hsp25 might have a function as cytoprotection against macrophages appearing in hepatic lesions, and that factors produced probably by macrophages in very early stages of hepatic lesions might have influenced Hsp25 expression. Hsp25 expression would be used as an index of anti-inflammatory action in the evaluation of hepatotoxicants *in vivo*.

P-11

Decrease of Liver Tumor Formation in CYP2E1-null Mice Treated With Diethylnitrosamine

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CYP2E1 metabolizes many low-molecular-weight toxins and carcinogens. Some *in vitro* experiments suggest that CYP2E1 may be involved in the metabolic activation of diethylnitrosamine (DEN). However, there has been no direct evidence demonstrating a role for CYP2E1 in DEN-mediated carcinogenesis *in vivo*. To clarify this, we carried out a DEN-induced hepatocarcinogenesis experiment using CYP2E1-null mice. Male 14-day-old wild-type and CYP2E1-null mice were treated with DEN (10 mg/kg of body weight) and killed at weeks 24 and 36 after DEN treatment for investigation of tumors and at 6, 24 and 48 h for examination of apoptosis and gene expression. Liver weights of CYP2E1-null mice were significantly different at weeks 24 and 36 compared to wild-type mice ($p < 0.01$). Liver tumor incidences of CYP2E1-null mice were significantly decreased at weeks 24 and 36 compared to wild-type mice ($p < 0.01$). CYP2E1-null mice showed significant decrease in the multiplicities of hepatocellular adenoma at weeks 24 and 36 ($p < 0.05$, $p < 0.01$, respectively), and of hepatocellular carcinoma at week 36 ($p < 0.01$) compared to wild-type mice. Apoptotic index and caspase-3 and/or Bax mRNA expression of CYP2E1-null mice were significantly different at 6, 24 and 48 h after DEN treatment compared to wild-type mice ($p < 0.05$). We conclude that CYP2E1-null mice show lower tumor incidence and multiplicity compared to wild-type mice in DEN-induced hepatocarcinogenesis, and it is suggested that CYP2E1 completely participates in DEN-induced hepatocarcinogenesis, and high frequency of tumors in wild-type mice could be associated with the increased apoptosis.

Key words: Diethylnitrosamine (DEN), CYP2E1-null mice, CYP2E1, Hepatocarcinogenesis

P-12

The Metabolic Relationship of CYP1A2 Expression to MeIQx-induced Rat Hepatocarcinogenesis

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Interaction of more than 2 chemicals is a very important factor for carcinogenic risk assessment and management. 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), one of the most abundant carcinogenic heterocyclic amines in cooked foods, is speculated to be a human liver carcinogen. MeIQx is metabolically activated by CYP1A2 and then N-acetyltransferase (NAT), findings which suggest that its carcinogenic potential might be enhanced by simultaneous exposure to chemical(s) inducing CYP1A2. Therefore, we investigated the effects of alpha- and beta-naphthoflavone as CYP1A2 inducers on MeIQx-induced rat hepatocarcinogenesis in a medium-term rat liver bioassay. Unexpectedly, no modifying influence of naphthoflavones on MeIQx-induced hepatocarcinogenesis was demonstrated with reference to glutathione S-transferase placental form (GST-P) positive foci in the liver, although up-regulation of CYP1A2 was detected on Western blot analysis. Activity of NAT was not affected. In MeIQx-treated rats, CYP1A expression was mainly detected in Zone 3 of the liver where GST-P positive foci were preferentially located, while naphthoflavones alone or combinations of naphthoflavones and MeIQx induced CYP1A expression in Zone 1. This difference in intralobular distribution of CYP1A might be related to the fact that MeIQx hepatocarcinogenesis was not modified by the two CYP1A inducers.

P-13

Aging effects on susceptibility to chemical hepatocarcinogenesis in Cx32 dominant negative transgenic rats

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Gap junctions have important roles in the maintenance of tissue homeostasis and the control of cell growth and differentiation. In the liver, connexin 32 (Cx32) is a major gap junction protein. We previously established transgenic rats carrying a dominant negative mutant of Cx32 under control of albumin promoter (Tg), which have much decreased capacity for gap junctional intercellular communication (GJIC), and they were found to be susceptible to diethylnitrosamine (DEN)-induced hepatocarcinogenesis compared to littermate wild-type rats (wild).

In this study, we examined whether aging affects GJIC function and susceptibility to DEN-induced hepatocarcinogenesis with the transgenic rat model.

Male Tg and wild rats of 10, 30 or 85 weeks-old were given a single intraperitoneal administration of 40 mg/kg DEN and sacrificed 12 weeks later. The number and area of GST-P foci were significantly increased in the liver of Tg compare to wild rats at 10 and 30 weeks-old. On the other hand, in the 85 weeks-old rats, both Tg and wild rats had large number and area of GST-P foci and the difference in these values between Tg and wild was not significant.

Next, GJIC capacity in the liver of male 10 and 100 weeks-old wild rats were measured by dye-loading assay and protein expression of Cx32. At 100 weeks-old, abilities for GJIC and Cx32 expressions located at hepatocyte membrane were much decreased compared to those of 10 weeks-old.

These results suggest that ability of GJIC is decrease with aging, resulting in increasing susceptibility for DEN-induced hepatocarcinogenesis in wild-type rats.

P-14

Oxidative DNA Damage and *In Vivo* Mutagenicity in the Livers of *gpt* delta Rats Given Non-genotoxic Hepatocarcinogens with CYP-inducible Potency

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Much attention has been paid to participation of oxidative DNA damages resulting from CYPs induction in non-genotoxic carcinogenesis. In the present study, oxidative DNA damage and *in vivo* mutation were examined in the livers of *gpt* delta rats given piperonyl butoxide (PBO) or phenobarbital (PhB), non-genotoxic hepatocarcinogens. Six-week-old male F344 *gpt* delta rats (5 rats/group) were fed diet containing PBO at a dose of 2% or PhB at a dose of 0.1% for 4 and 13 weeks. At necropsy, the livers were removed for measurements of mRNA levels of CYP1A1, 1A2 and 2B1, 8-hydroxydeoxyguanosine (8-OHdG) levels and *gpt* mutation. Quantitative analysis for PCNA or GST-P in the liver slices was also performed. While PhB induced mRNA levels of CYP 2B1 with statistical significance, PBO caused significant elevations of CYP 1A1 and 1A2 as well as CYP 2B1. Four weeks exposure to PBO resulted in significant increase of 8-OHdG levels, further elevation being found at 13 weeks, although there were no statistically significant changes in the PhB-treated groups. No significant changes in *gpt* mutant frequencies along with no alterations of specific mutation frequencies were observed in all treated groups throughout the experimental period. Labeling index of PCNA was significantly elevated only in the PBO-treated group at 4 weeks. There were no changes in the numbers and areas of GST-P positive liver cell foci in all treated groups. The present data for CYP mRNA levels were in concord with the previous reports. The fact that only PBO caused oxidative DNA damage might be attributed to the effective generation of oxidative stress due to CYP 1A1/2. However, together with no increase of GST-P positive foci, administration of PBO or PhB failed to affect *in vivo* mutagenicity. Thus, it is unlikely that oxidative stress resulting from CYP induction takes part in PBO or PhB hepatocarcinogenesis.

P-15

Analysis of Metabolic Pathway of Dimethylarsinic Acid in Rats

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【Background】 We have previously demonstrated the bladder carcinogenicity of dimethylarsinic acid (DMA^V) in rats. However, little is known about the mechanism underlying its carcinogenicity. Dimethylmonothioarsinic acid (DMMTA^V) is a metabolite of DMA^V and is found in the urine of both humans and rats exposed to arsenic. We hypothesized that DMMTA^V involve in the DMA^V-induced bladder carcinogenesis. The purpose of the present study was to evaluate the metabolic pathway of DMA^V in rats.

【Method】 Ten week-old male SD rats were divided into 4 groups and treated with DMA^V (50mg/kg) in different routes as follows: group 1, intravenous [i.v.] administration and bile collection from 2 hours after treatment; group 2, i.v. administration and bile collection immediately; group 3, intragastric [i.g.] administration and bile collection from 2 hours after treatment; and group 4, i.v. administration and bile collection immediately. Biliary arsenic concentrations were determined by LC-ICP-MS.

【Result】 DMA^V and DMMTA^V were found in all samples, and the proportion of DMMTA^V was 2.9%, 0.2%, 6.7% and 2.0% in groups 1- 4, respectively. The proportion of DMMTA^V were higher in i.g. treatment groups than i.v. treatment groups at all time points. Furthermore, the proportion of DMMTA^V were higher in groups collecting bile from hour 2 than groups collecting bile right after treatment in both routes.

【Conclusion】 Metabolism of DMA^V in intestines is important for the formation of DMMTA^V

P-16

Effects of CCl₄-induced Hepatic Injury on *in vivo* Mutagenicity of MeIQx in the Livers of *p53*-proficient or -deficient *gpt* delta Mice

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Chronic inflammation is regarded as a risk factor of cancer development. To investigate whether chronic inflammation affects mutagenicity induced by 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), a mutagen derived from cooked foods, we examined *in vivo* mutation assay in the livers of *p53*-proficient or *p53*-deficient *gpt* delta transgenic mice. Female B6C3F₁ *p53*-proficient or -deficient *gpt* delta mice were given a diet containing 300 ppm of MeIQx for 13 weeks, and were intraperitoneally injected with 0.2 mL/kg body weight carbon tetrachloride (CCl₄) for once a week during the experimental period. At necropsy, the livers were removed for *in vivo* mutation assay and measurement of mRNA expression levels of *CYP1A2* and *GSTα4*. In the *gpt* mutation assay for detection of point mutations on the *gpt* locus, the mutant frequency (MF) in *p53*-proficient mice fed MeIQx was significantly increased as compared with the basal diet control. In the co-treatment group with MeIQx and CCl₄, the MF was significantly elevated as compared with the MeIQx alone group. Likewise, in the Spi⁺ mutation assay for detection of deletion mutations on the *red/gam* locus, the MF in mice fed MeIQx was significantly elevated as compared to the control value, the combined treatment elevating the MF. Although the enhancing effects of CCl₄ treatment were observed in *p53*-deficient mice, there were no differences in *gpt* and Spi⁺ MFs between *p53* genotypes in terms of the effects of CCl₄. In the *p53*-proficient mice, *gpt* mutation spectra analysis revealed that GC:TA transversion mutation was predominant spectra independent of CCl₄ treatment, and this type of mutation was increased by co-treatment with CCl₄. There were no significant changes in *CYP1A2* and *GSTα4* mRNA levels between MeIQx-treated groups with and without CCl₄. In the present study, *in vivo* mutagenicity of MeIQx was enhanced due to CCl₄ injection. Although the increased clonal expansion of *gpt* mutant colony in the combined treatment group suggests that induction of cell proliferation might play an important role in increment of mutagenicity of MeIQx. Moreover, the present data showing no significant changes in *CYP1A2* and *GSTα4* mRNA levels between MeIQx-treated groups with and without CCl₄ suggest that the increments by CCl₄ co-treatment might result from the modifications of some inflammation-related factors. On the other hand, the enhancing effects of CCl₄ co-treatment on mutagenicity of MeIQx may not involve *p53* functions.

P-17

Identification of prostate cancer stem cells in the bone microenvironment.

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[INTRODUCTION] Bone metastasized prostate cancer is therapy resistant and threat of QOL, and new therapeutic approach is needed. Cancer stem cell is known to initiate and maintain malignancy, and also to be associated with the formation of metastasis. We hypothesized that prostate cancer stem cell may play a vital role in tumor growth in the bone microenvironment. We examined expression of prostate cancer stem cell surface molecules in prostate cancer tissue and prostate cancer cell line. We also attempted to obtain candidates of rat prostate cancer stem cells using limiting dilution assay. [Materials & Methods] Rat prostate cancer cell line (PLS10) and human prostate cancer cell lines (LNCaP, PC3 and DU145) were used. PLS10 cells were cultured in limiting dilution assays. Colonies derived from single cells were cultured again in limiting dilution assays. Expression of cancer stem cell surface molecules (CD44 and CD133) was determined by fluorescence associated cell sorting (FACS) analysis. Expression of CD44 and CD133 in prostate cancer bone infiltration / metastasis was determined by immunohistochemistry of anti-CD44 and anti-CD133 antibodies. Prostate cancer tissues obtained from human bone as well as in rat bone were also used. Cell proliferation was assessed by WST-1 assay. [Results] FACS analysis showed that CD44 was highly expressed but CD133 was weakly expressed in PLS10. FACS analysis also showed that CD133 was weakly expressed in all three human prostate cancer cell lines. CD44 positive cancer cells were immunohistochemically readily observed in prostate cancer tissues obtained from human bone as well as in rat bone. We generated additional two sublines of PLS10 (SCC2, SCC3) for the limiting dilution assay. WST-1 assay showed that SCC2 and SCC3 had higher growth potential than the original one. [Discussion] We could find CD44 positive prostate cancer cells in the bone microenvironment. This finding might be associated with the existence of cancer stem cell in the bone microenvironment. Further study is needed to determine prostate cancer stem cells in the bone microenvironment and to obtain cell lines including prostate cancer stem cells.

P-18

Uterine masses of the rabbit uterus-abdominal wall adhesion model

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【Introduction】 Uterine masses were induced in the rabbit uterus-abdominal wall adhesion model to cause the adhesion of the uterus and the abdominal wall surgically.

【Methods】 Female New Zealand White rabbits were given a single intravenous injection of 30U/body of chorionic gonadotropin to synchronize the estrous cycle and uniformize the uterus shape before operation. Under anesthesia, rabbits underwent a laparotomy, and their uterus and the abdominal wall were cauterized with electric scalpel. Thereafter, the cauterized area of the uterus and the abdominal wall were ligated. At 14 and 60 days after operation, rabbits were sacrificed under the pentobarbital sodium anesthetizing and pathological examination was performed. In the histopathological examination, paraffine-embedded sections of uterus were routinely prepared and stained with hematoxyline and eosin for the light microscopic observation.

【Results】 <At 14 days after operation> In 17 of all 37 sites, polypoid masses were observed in the intrauterine cavity. In the center of the masses, there were many clear cells. And eosinophilic large cells were seen in the marginal area of the masses and blood vessel surroundings. The hemorrhage and degeneration/necrosis were observed in the vascularized area of the masses. However, there were not masses in the uterus which were not cauterized.<At 60 days after operation>The masses were not observed in the treated uterus of all 18 sites.

【Conclusion】 We diagnosed the masses of the uterus as the deciduoma which has been reported in dogs, rats and rabbits. It has already been reported that the deciduoma is induced by the promotion of the ovarian function and the mechanical stimulation to the uterus. In the present study, animals were given chorionic gonadotropin before operation. Chorionic gonadotropin has the action to promote the production of progesterone and the luteinization to cause the ovulation. Therefore, it was thought that the cause of the deciduoma might be the pseudopregnant which was induced by chorionic gonadotropin and mechanical stimulation of cauterization.

P-19

Morphological Image Analyses to Assess the Effect of Telmisartan on Bleomycin-induced Lung Fibrosis in Mice.

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Several types of morphological image analysis are used to predict the effect of drugs on the bleomycin (BLM) induced lung fibrosis in animals. The purpose of this study is to assess the effect of telmisartan, angiotensin II receptor antagonist, on bleomycin-induced lung fibrosis with the morphological image analysis by histopathological approach.

Adult male 7-week-old C57BL/N6 Crl:Crj mice were used. These were divided into three groups: control group (n=4), BLM treated group (n=6, BLM group), BLM and telmisartan treated group (n=8, BLM+TS group). BLM was treated intratracheally to the BLM and BLM+TS group mice at a dose of about 1 mg/kg body weight. Telmisartan was orally administrated to the BLM+TS group mice at a dose of 10 mg/kg/day for 28 days. The lungs were removed and instilled intratracheally with 10% formaldehyde at a constant pressure of 15 cm H₂O for 15 minutes. The paraffin sections were stained with hematoxylin-eosin, trichrome, and lysozyme immunohistochemical stains. Modified Ashcroft scale, fibrosis fraction, and macrophage density were measured with the captured lung images.

Multifocal fibrosis formed under the visceral pleura in both the BLM and BLM+TS group mice. Focal and alveolus fibrosis were slight in the BLM+TS group mice relative to the BLM group mice. Alveolar macrophages increased and were active in the BLM group mice relative to the BLM+TS group mice. Modified Ashcroft scale was not significantly different between the BLM and BLM+TS group mice. Fibrosis fraction decreased significantly in the BLM+TS group mice relative to the BLM group mice. Macrophage density increased significantly in the BLM group mice relative to the control and BLM+TS group mice. The approximate line of the fibrosis area plotted against macrophage density showed a negative slope in the BLM group mice only.

The morphological image analysis by histopathological approach clearly explained the effects of telmisartan. We conclude that telmisartan reduces the fibrosis and macrophage activity. Besides, measurement of cytokines and stability of the model animals may be needed to validate our results.

P-20

The proteome analysis of mice lung tumors by QSTAR Elite LC-MS/MS

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We have previously shown that lung squamous cell carcinoma (SCC) is induced by 20 weeks treatment with N-nitroso-tris-chloroethylurea (NTCU). And histopathologically, it was appreciated that NTCU-induced Squamous metaplasia-Dysplasia-SCC arise in terminal bronchiole. The aim of the present study was to identify the origin of cancer cell in lung adenocarcinoma (AdCa) and SCC, and proteins specific or common in two cancers.

Seven mice were treated with single i.p. injection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, 2 mg/0.1 ml saline/mouse) on the first experimental day and sacrificed after 55 weeks (AdCa-induced model). Twenty six mice were treated topically with NTCU (0.01 M 75 µl/mouse) twice a week for eighteen weeks and sacrificed after 22 weeks (SCC-induced model). SCC and AdCa samples were collected from each group and proteome analysis was carried out by using QSTAR Elite LC-MS/MS.

187 proteins were identified with 95% confidence or higher and quantified with ProteinPilot 2.0 Software. Significant overexpression of 52 proteins (e.g. SP-C, TIM) were detected in lung AdCa compared to normal-appearing tissues and 46 proteins (e.g. cytokeratins 6A, 5, 19) were detected in SCC.

Expression patterns of proteins largely differed between AdCa and SCC. Furthermore AdCa and SCC are suggested to be derived from type II pneumocytes and clara cell, respectively.

P-21

Chemopreventive Effects of PJJ-34 on Mice Lung Squamous Cell Carcinoma

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We have established the novel carcinogenesis model for lung squamous cell carcinoma (SCC) by administration of N-nitroso-tris-chloroethylurea (NTCU). PJJ-34 is a newly-identified serratane type triterpenoid, extracted from cuticles of *Picea jezoensis*. In rat multi-organ carcinogenesis bioassay (DMBDD model), the multiplicity of lung tumors (adenomas + adenocarcinomas) were significantly reduced by PJJ-34. Therefore, we investigated the chemopreventive effects of PJJ-34 on lung SCC. Female A/J mice, 6-week-old, were administered NTCU by topical application to the skin twice a week for 4 weeks. After a week finishing NTCU treatment, mice were administered 0, 2.5 or 5.0 mg/kg PJJ-34 by intragastric administration for 15 weeks. NTCU treatment induced lung squamous metaplasia almost in all mice. Incidences of lung SCCs were 41, 47 and 30% in NTCU□Vehicle, NTCU□2.5 and 5.0 mg/kg PJJ-34 groups, respectively. No evidence was obtained the inhibitory effects of PJJ-34 on mice lung SCC. These result indicate that developmental mechanisms for lung SCC and adenocarcinoma might be different in mice.

P-22

Morphological characteristics of the carcinogen-induced thyroid proliferative lesions in BrIHan:WIST@Jcl (GALAS) rats with vacuolar change/hydropic degeneration of thyroid follicular cells

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Vacuolar change/hydropic degeneration of thyroid follicular cells was recognized in BrIHan:WIST@Jcl (GALAS) rats, however there have been few reports about the thyroid proliferative lesions of the rats with vacuolar change. We found them in a medium-term multi-organ carcinogenicity bioassay using male GALAS rats. The rats were given DEN, MNU, DMH, BBN and DHPN for a total multiple initiation period of 4 weeks (DMBDD treatment), and all survivors were sacrificed at the end of week 29 (35 weeks old). Vacuolar change of thyroid was seen in 9 out of 114 rats, and focal hyperplasia and multifocal adenoma in 5 of them. These proliferative lesions consisted of high columnar epithelium with huge vacuole contained slightly eosinophilic substance, low columnar epithelium with small vacuole, or basophilic epithelium without vacuole. Staining properties of follicular colloid in the proliferative lesions for PAS, thyroglobulin and throxine were poorly-matched with the morphological characteristics. Meanwhile, PCNA-positive cells were increased in the basophilic epithelium compared to vacuolar-type. From these findings, it was presumed that there is a morphological variety of the carcinogen-induced thyroid proliferative lesions in GALAS rats with vacuolar change of thyroid, and some relationship between cell proliferative activity and disappearance of vacuole.

P-23

Development of a Short Term Two-stage Skin Carcinogenicity Model Using rasH2 Mice

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[Purpose] We investigated skin tumor promoting effects of benzoyl peroxide (BPO), a skin tumor promoter and oleic acid diethanolamine condensate (OADC), a non-carcinogen, using 7,12-dimethylbenz [*a*] anthracene (DMBA) as an initiator to establish an ultra-short-term skin carcinogenicity model using rasH2 mice. And we also examined the dose and frequency of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as a positive control.

[Methods] DMBA (50 µg/100 µL acetone) was applied to shaved dorsal skin of female rasH2 mice (7 weeks old, 10 mice/group). BPO (20 or 1 mg/200 µL) was applied 5 times a week, and OADC (30 mg/kg b.w.) was applied everyday in accordance with the two-year carcinogenicity studies for 8 weeks from 1 week after DMBA initiation. TPA was applied at a dose of 8 µg/200 µL once a week, 4 µg/200 µL twice a week, and 4 µg/200 µL once a week. Moreover, non-initiated mice (5 mice/group) were also similarly treated with BPO (20 mg/200 µL), OADC, or TPA (8 µg/200 µL).

[Results] At 4 weeks after DMBA treatment, skin nodules were observed in all DMBA-treated groups. Increase in the number of nodules in the OADC group was not observed during the treatment period, and the incidence and average number at sacrifice was 30% (0.4). In the BPO group, the incidence with the high dose was reached 100% at 6 weeks, and the incidence and average number of skin nodules at sacrifice were 100% (39.7) and 70% (0.9) in the 20 mg or 1 mg/200 µL groups, respectively. In all TPA-treated groups, the incidence reached 100% at 6 weeks, and average numbers of skin nodules at sacrifice were 47.1, 63.3, and 32.8 with 8 µg/100 µL (1 time/week), 4 µg/100 µL (twice/week), and 4 µg/100 µL (1 time/week), respectively.

[Conclusion] It was confirmed that BPO induced skin nodules in female rasH2 mice after DMBA initiation with dose-dependence. OADC was without significant influence. In the TPA groups, a sufficient quantity of skin nodules was already observed with treatment at 4 µg/100 µL (1 time/week).

P-24

A 3-Month Oral Dose Toxicity Study of Acrylamide in Syrian Hamsters

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Acrylamide (AA) induces tumors in various organs/tissues in rats and mice. Epidemiological studies for the oral exposure to AA have revealed controversial results, and mortality studies of AA workers showed increased rates of pancreatic cancer among the workers. In the present study, for the dose selection of long term carcinogenicity studies, subchronic toxicities of AA was evaluated in Syrian hamsters, which is sensitive to the pancreatic ductal carcinogenesis, at concentrations of 0 (control), 20, 30 and 50 mg/kg body weight in drinking water. Treatment with AA caused abnormal gait and ataxia in all males and females at 50 mg/kg, and the animals were sacrificed at weeks 5-13 based on the ethics of animal experimentation. Body weights in 30 and 50 mg/kg males and water consumption in all treated groups were lower than the control. At terminal, elevation of ALP in 30 mg/kg males and γ -GTP in 20 and 30 mg/kg females were noted. Microscopically, axonal/myelin degeneration of sciatic nerves was observed in 4/9, 9/9 and 9/9 males and 5/9, 9/9 and 9/9 females at 20, 30 and 50 mg/kg, respectively. The results indicated that the maximum tolerated dose for the carcinogenicity studies of AA to be 20 mg/kg or less in both male and female Syrian hamsters.

P-25

Promoting Effects of Sugar-rich Diet on N-nitrosobis (2-oxopropyl) amine-induced Pancreatic Carcinogenesis in Hamsters

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We reported that a sugar-rich diet promoted BOP-induced pancreatic carcinogenesis in hamsters at the 24rd annual meeting of JSTP. In the present study, we examined effects of carbohydrate metabolism on pancreatic carcinogenesis by using a sucrose, glucose and fructose-rich diet. Seven-week-old male Syrian golden hamsters were used. The diets in which starch was replaced at rates of 20% by sucrose, glucose and fructose, respectively, were used in order to administer the same levels of calories and the other nutrients. BOP at a dose of 10 mg/kg body weight administered subcutaneously at 0, 3 and 6 day. At 2 week, animals were divided into 4 groups and administered following diets, Group 1 (n=19); basal diet, Group 2 (n=19); sucrose diet, Group 3 (n=18); glucose diet, Group 4 (n=18); fructose (n=18) diet. All animals were sacrificed at 35 week. The serum and pancreas were supplied to biochemical test and histopathological examination, respectively. There were no significant differences in the serum glucose, triglyceride, total and LDL-cholesterol, AST, and ALT in each groups. In Group 2, the incidence and number of proliferative ductal lesions including atypical hyperplasia and adenocarcinoma increased significantly compared to Group 1. In Group 3, the number of proliferative ductal lesions increased significantly compared to Group 1. These results show that sucrose and glucose-rich diet promoted pancreatic carcinogenesis, but fructose did not. It is suggested that increasing level of the postprandial insulin affects pancreatic carcinogenesis.

P-26

A Novel Immunohistochemical Marker, Integrin $\alpha_v\beta_3$ for BOP-induced Early Lesions of Pancreatic Ductal Carcinoma in Hamsters

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N-Nitrosobis(2-oxopropyl)amine (BOP)-induced pancreatic ductal carcinomas and early lesions in hamsters have been reported to show histopathological resemblance to those in human. As for human carcinoma cases, some specific protein expression profiles have been demonstrated by proteome/immunohistochemical analyses, but a detailed molecular approach to the dissection of BOP-induced pancreatic carcinogenesis have not yet been performed. In this study, we conducted immunohistochemical analyses of hamster lesions with a focus on 5 proteins reported to be altered in human, to clarify the similarity of the phenotypes between hamsters and humans. Integrin $\alpha_v\beta_3$ was expressed in 6 of 6 adenocarcinomas (ACs) and more importantly 13 of 14 atypical hyperplasias (AHs). Immunoreactivities for galectin-1/3 or α -enolase in epithelial cells and kallikrein 7 in stromal cells were also detected in both ACs and AHs, but their expression frequencies were lower than those for Integrin $\alpha_v\beta_3$. Thus some similarities of tumor-associated protein expression were confirmed between in human ACs and hamster ACs/AHs, and particularly integrin $\alpha_v\beta_3$ appeared as a useful immunohistochemical marker for the early lesions in hamsters.

P-27

Effects of Propolis on Inflammation-related Rat Colon Carcinogenesis Induced by DMH and DSS

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Propolis has been reported to exert anti-inflammatory, anti-oxidative and anti-tumor effects. The purpose of the present study was to evaluate effects of ethanol-extracted propolis (EEP) and water-extracted propolis (WEP) on inflammation-related rat colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) and dextran sodium sulfate (DSS). Six week-old F344 male rats were divided into 3 groups of 20 rats each. All rats were given subcutaneous injections of DMH at a dose of 40 mg/kg b.w. twice a week, followed by 1% DSS in drinking water for 1 week. After 1 week on DSS, rats were fed diet containing 1% EEP, 1% WEP or basal diet for 29 weeks. The multiplicities of tubular adenocarcinoma were 2.2 ± 3.5 , 0.4 ± 0.6 and 1.2 ± 1.7 per rat in basal diet control, EEP and WEP groups, respectively. The number of tubular adenocarcinoma was significantly decreased in EEP group compared to the control. Furthermore, protein expression of iNOS in the colon tubular adenomas and tubular adenocarcinomas showed tendency to decrease in EEP but not in WEP-treated rats compared to the control animals. The results indicated that EEP exerts suppression effect on inflammation-related rat colon carcinogenesis induced by DMH and DSS, due to its anti-inflammatory activity.

P-28

Suppression of inflammation-associated colonic tumorigenesis in Min mice with a cyclooxygenase-2 inhibitor

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Individuals with long-standing ulcerative colitis have increased risk of colorectal adenocarcinomas. Dextran sulfate sodium (DSS) has been known to cause severe inflammation in rodents resembling ulcerative colitis and to accelerate colon carcinogenesis. We have previously demonstrated that DSS treatment induced repeated division of crypts as well as proliferation of colonic epithelial cells surrounding the mesenchyme lacking covering epithelial cells, where COX-2 was exclusively expressed. To assess a role of COX-2 in repairing process, Min mice and wild type C57BL/6J mice, 7 weeks old, were administered 2% DSS in drinking water for 7 days, fed diet with or without 300 ppm Etodolac from day 7, and sacrificed sequentially at experimental day 10 (10D), 14D, 21D, week 5 (5W), and 8W. The number of colonic polyps in Min mice was decreased from 19.6 ± 1.4 /animal (AVE \pm SE) in a basal diet group to 9.0 ± 3.3 in Etodolac group ($P < 0.05$) at 5W. Later at 8W, however, the number of tumors did not show significant difference. Reduction of tumor volume was revealed to be 7.5 ± 1.2 mm³ in Etodolac-treated Min mice from 17.9 ± 2.4 in basal diet group ($P < 0.0001$). These results indicated that Etodolac could be effective in the suppression of tumor progression.

P-29

Effects of adriamycin on the development of hereditary glomerular lesions in infant ICGN mice.

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ICR-derived glomerulonephritis (ICGN) mouse is known as a hereditary nephritic model. We have reported the usefulness of infant ICGN mice, a sensitive animal model to evaluate renal toxicity targeting human infants. To confirm the usefulness of this murine model, infant ICGN mice were treated with a renal toxicant adriamycin (ADR) to evaluate the effects on the development of glomerular lesions. Male 3-week-old ICGN mice were treated intravenously with 0 (control), 1.0, 3.5 and 10.5 mg/kg BW of ADR for 1 week. As results, final body and renal weights, and levels of proteinuria did not differ between the non-treatment control and treated groups. Histopathologically, the number of glomeruli showing focal or diffuse mesangial expansion, which is a glomerular lesion of ICGN mice, tended to increase with the treatment of 10.5 mg/kg BW ADR, although glomeruli with α -smooth muscle actin (a marker for mesangial cell activation)-positive mesangial cells were not increased. Although WT-1-positive podocytes were detected in both the control and ADR 10.5 groups, the number of WT-1 positive cells per glomerulus tended to decrease by ADR, indicating possible podocyte damage. ADR did not affect other renal lesions such as microaneurysm in the glomeruli, urinary cast and tubule regeneration. In conclusion, ADR affected the hereditary glomerular lesions in infant ICGN mice.

P-30

Cancer cell death by capsaicin treatments

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Capsaicin is the major pungent ingredient in red peppers. Many papers suggested capsaicin has chemopreventive and chemotherapeutic effects. However, the mechanisms are not well known. In this presentation, we investigated the capsaicin effects in various cancer cell lines, following Human prostate cancers; LNCaP, DU145, PC-3. Human mammary cancers; MCF-7, T47D, MBA-MB-231. Human colon cancer; HT-29. Human hepatic cancer; HepG2, Human renal cancer; Caki-1. Human bladder cancer; T24. Several rat cancer cell lines were also applied. These cell lines were treated with capsaicin and observed the morphological alterations, and then investigated protein expression changes by western blotting analysis. And then, we have tried to define the cell death mechanisms according the "The Nomenclature of Cell Death: Recommendation of an ad hoc Committee of the Society of Toxicologic Pathologists, and "Classification of cell death: recommendation of the Nomenclature Committee Cell Death 2009" Cell Death and Differentiation (2009) 16,3-11.

P-31

Hypothermia and Related-Lesions in Rats Treated With a Single Dose of Chlorpromazine

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Chlorpromazine was reported to induce hypothermia in rats. We investigated whether hypothermia would cause organ toxicity in chlorpromazine-treated rats. Two groups of 10 male rats were administered with a single dose of 750 mg/kg of chlorpromazine, and each dosed group was housed under different conditions for 4 days; one consisted of housing the rats individually in stainless-steel bracket cages, and the other consisted of housing the rats together in a group of five in polycarbonate cages with chips. In the rats housed individually, hypothermia below 30°C was observed at 6 hours post dosing and thereafter. In contrast, the rats housed together in a group maintained the rectal temperature of 34°C or higher. Thus, alleviation of hypothermia was demonstrated by housing the rats together in a group. Alleviation of hypothermia in the rats housed together in a group prevented the development of the lesions observed in the rats housed individually such as microvesicular change of the hepatocytes in the liver, microvesicular change of the proximal tubular epithelium in the kidney, necrosis of the cortical cells, vacuolation of the medullary cells and fibrin deposition in the adrenal, and nuclear vacuolation of the round spermatids and retention of step 19 spermatids in the testis.

These results indicated that hypothermia-related lesions were observed in the liver, kidney, adrenal and testis in the rats treated with a single dose of chlorpromazine.

P-32

Effects of fasting and refeeding on hematopoietic cells in bone marrow

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In toxicity study, failure of administration or toxicity of test article occasionally leads to transient fasting or feed intake reduction, due to loss of condition. Then we studied at the effects of fasting and recovery on toxicological parameters

Twenty Crl:CD (SD) male rats were divided into 5 groups (G1-5). We fasted G1-4 for 0, 1, 2, 3 days, respectively. G5 was fasted for 3 days, and fed for 10 days (recovery group). After sacrifice under isoflurane anesthesia, we performed to study hematology, blood biochemistry (Triglyceride: TG, Glucose: Glu, Total-cholesterol: T-Chol, HDL-cholesterol: HDL-C) necropsy, and histopathological evaluation.

In hematology, reticulocyte counts decreased in a fasting duration-dependent manner. In blood biochemistry, though Glu and TG decreased after 1 day fasting, further decrease was not admitted following extension of fasted period. There were no changes in T-Chol and HDL-C. At G5, most of parameters in hematology and blood biochemistry were similar levels at G1. In histopathological finding, hematopoietic cells in femur and sternum decreased slightly in 1 of 4 examples at G4. Moreover, hematopoietic cells in femur decreased slightly 3 of 4 examples in G5, but not in sternum. In thymus, apoptosis increased after 2 days fasting, in particular, G3, G4, and G5 were recognized 2, 4, and 1 of 4 examples, respectively. In mesenteric lymph nodes, lymphocyte decreased only in G5, and the degrees of changes were slight and moderate for 2 and 1 of 4 examples, respectively. In liver, mitosis and cell proliferation decreased in a fasting duration-dependent manner. There was no change with other organs.

It was found that hematopoietic organ, liver, reticulocyte, Glu and TG were affected by fasting. When food consumption changes, it is necessary to evaluate carefully the association between the changes of these parameters and test article.

P-33

Chronic Toxicity and Carcinogenicity of Dietary Administered Magnesium Chloride in F344 Rats

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Magnesium chloride ($MgCl_2$) has been widely utilized as a food additive and is therefore present in our environment. It has found particular application as a main constituent of coagulating agents for tofu. In the present study, chronic toxicity and carcinogenicity of dietary administered $MgCl_2$ was investigated in male and female F344 rats. Chronic toxicity and carcinogenicity studies of $MgCl_2$ were performed in 6-week-old male and female F344 rats at dietary concentrations of 0, 0.25, 0.8, 2.5 or 5.0% in a 52-week toxicity study and 0, 1.25 or 2.5% in a 104-week carcinogenicity study. As results, in the chronic toxicity study, loose stool and increased water intake were observed in the 2.5 and 5.0% groups of both sexes and decreased food intake and reduction of body weight gain were observed in 5.0% males. In hematology, decrease in white blood cell count in 2.5 and 5.0% males, and increase in red blood cell count, hemoglobin concentration and hematocrit in 5.0% females were observed. In serum biochemistry, total cholesterol and triglyceride levels were decreased and blood urea nitrogen levels were increased in the 5.0% groups of both sexes. Increase in creatinine levels in 5.0% females, magnesium levels in 5.0% males and 2.5 and 5.0% females and inorganic phosphorus in 2.5 and 5.0% males and 5.0% females were detected. The relative kidney weights were increased in 5.0% males. Histopathological observation revealed no significant treatment-related findings. Regarding carcinogenicity study, water intakes were increased in treatment groups of both sexes, but no effects were found on survival rate and body weights. Treatment with $MgCl_2$ did not exert any significant influence on the incidences of tumors in any of the organs and tissues examined. It was concluded that the no observed adverse effect level of $MgCl_2$ was 0.8% in diet, which is equivalent to 368 and 439 mg/kg bw/day in males and females, respectively, and the compound is non-carcinogenic under the conditions of the present study.

P-34

A 90-day Feeding Toxicity Study of L-Proline in Fischer 344 Rats

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L-Proline (L-Pro) is a non-essential amino acid that can be formed from and converted to glutamic acid. L-Pro is approved in Japan as an existing food additive for seasoning of the diet and has recently become widely used as an ingredient of supplements, health foods and cosmetics. However, there is minimal data on adverse effects of L-Pro in either experimental animals or humans.

In the present study, a subchronic oral toxicity of L-Pro was investigated with groups of 10 male and 10 female Fischer 344 rats fed a purified 20% casein diet containing 0, 0.625, 1.25, 2.5 or 5.0% of L-Pro for 90 days. There were no toxicologically significant, treatment-related changes with regards to body weight, food intake or urinalysis data. Regarding several hematology, serum biochemistry and organ weight parameters, significant changes were observed between some of the treated groups and the controls. All these changes, however, were slight and lacked dose-dependence, and have no corresponding pathological findings. The histopathological assessment revealed only sporadic and/or spontaneous lesions.

In conclusion, the no-observed-adverse-effect-level (NOAEL) for L-Pro was, therefore, determined to be a dietary dose of 5.0% (2772.9 mg/kg body weight/day for males and 3009.3 mg/kg body weight/day for females) under the present experimental conditions.

P-35

A Subchronic Feeding Toxicity Study of Nisin A, An Antimicrobial Peptide Produced by *Lactococcus Lactis* Subsp. *Lactis*, in Rats

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Nisin A, an antibacterial peptide produced by *Lactococcus lactis* subsp. *lactis*, which belongs to the Class I bactericins called lantibiotics, is a small (3.5 kDa), 34-amino acid, cationic, hydrophobic peptide with five characteristic (beta-methyl) lanthionine rings. The objective of the present investigation was to evaluate the subchronic toxicological potential of nisin A given 90 days in the diet to F344 rats.

Groups of 10 male and 10 female F344 rats (6-week old) were administered nisin A at dietary levels of 0%, 0.2%, 1.0% and 5.0% for 90 days. Animals given NaCl at a dietary level of 3.712% (equivalent to the NaCl content in 5.0% nisin A group) served as a reference material treated group. The animals were observed daily for clinical signs, and individual body weights were recorded weekly. Food and water consumption was measured over a 2-day period once a week. Urinalysis was performed at weeks 4, 8 and 13, and ophthalmologic examination was performed at week 13. At the end of week 13, all rats were fasted overnight and blood samples were collected via the abdominal aorta from all rats. Hematological estimations and blood biochemistry were carried out. Gross observations and determinations weighing of selected organs were made at necropsy, and then full histopathological examination was performed.

There were no deaths, and the treatment had no adverse effects on clinical signs, body weights, food consumption, ophthalmology, hematology, or gross pathology.

Statistically significant increases of water consumption, urine volume, and urinary sodium and chlorine, and decreases of urinary potassium and serum sodium, along with increases of absolute and relative kidney weight, and incidences of minimal squamous cell hyperplasia of limiting ridge in the forestomach, were found in nisin A treated groups. It was considered that these changes were related to NaCl, since they were also noted in rats given diet containing the reference substance.

Thus, no toxicologically significant changes were apparent in both sexes of F344 rats fed diet containing 0, 0.2%, 1.0% and 5.0% nisin A for 90 days. Therefore, the no-observed-adverse-effect level (NOAEL) for nisin A was concluded to be a dietary level of 5.0% (2996 mg/kg/day for males and 3187 mg/kg/day for females).

P-36

Effects of Magnetite Nanoparticles on Lungs of Fischer 344 Rats after a Single Intratracheal Instillation.

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Ferric oxide nanoparticles are of considerable interest for the application in nanotechnology related fields. However, as iron being a highly redox-active transition metal, the safety of iron nanomaterials need to be further studied. In this study, ferric oxide nanoparticles (magnetite) were used to test the pulmonary responses in rats by an intratracheal instillation.

Ten-week-old male and female Fischer 344 rats (n=5/group) were exposed a single intratracheal instillation of 0 (vehicle), 5, 15 or 45 mg/kg body weight (BW) magnetite. After 14 days, the rats were sacrificed and biological consequences were investigated.

The lung weights of treated rats were increased, and the weights of the 15 and 45 mg/kg BW male and female groups were significantly higher than those of the controls. The lungs of treated rats showed enlargement and black patches originating from the color of magnetite, macroscopically. The histopathological main changes in lungs of treated rats were infiltration of macrophages with magnetite, inflammatory cell infiltration in the walls and spaces of the alveoli and alveolar type II cell proliferation. The hypertrophy and proliferative changes of bronchial epithelia, increase of goblet cells in bronchi and perivascular edema were observed in treated rats. There were no significant changes with regards to body weight, hematology and serum biochemistry.

In the acute toxicity test, magnetite caused a foreign body inflammation with the magnetite accumulation in the lung. The responses of pulmonary lesions and lung weights were dose-dependently increased.

P-37

Pulmonary Toxicity of Intratracheally Instilled Multiwall Carbon Nanotubes in Male Fischer 344 Rats

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In order to assess pulmonary toxicity of multiwall carbon nanotubes (MWCNT), male F344 rats were intratracheally instilled with MWCNT(MWCNT-7) suspension at a dose of 0, 40 or 160 µg/rat or alpha-quartz particles(MIN-U-SIL 5) as a positive control at a dose of 160 µg/head and sacrificed for lung histopathology and bronchoalveolar lavage (BAL) fluid analyses on Day 1, 7, 28 or 91 after instillation.

Well-dispersed MWCNT brought dose- and time-dependent changes in lung weight, total proteins, albumin, lactate dehydrogenase and alkaline phosphatase in the BAL fluid. Phagocytosed MWCNTs were found in alveolar spaces and walls, and bronchus-associated lymphoid tissue. The MWCNT deposition in the bronchus-associated lymphoid tissue gradually increased after instillation. Persistent infiltration of macrophages, microgranulomas, Type II cell hyperplasia and fibrosis with alveolar wall thickening increased dose and time-dependently. The MWCNT-induced lesions were more potent on Day 91 than the alpha-quartz-induced ones at an equal mass dose. These results indicate that MWCNT shows fiber toxicity *in vivo*.

P-38

Investigation for Thrombogenic Mechanisms in the Lungs of Phenylhydrazine-treated Rats

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Phenylhydrazine (PHZ) is well known for its capacity to induce oxidative hemolysis in animals and human. We previously reported acute fibrin thrombus formation in the alveolar capillaries in the lungs of PHZ-treated rats (J. Toxicol. Pathol. 2008; 21:249-51), however, the thrombogenic mechanisms remain unclear. In this study, we evaluated time-dependent changes hematologically and histologically in an attempt to reveal the pathogenesis of thrombus formation in lungs caused by a PHZ administration.

Six-week-old Sprague-Dawley male rats (n=6/each group) were injected intraperitoneally with 40 mg/kg/day of PHZ for treated groups or saline for control groups for 1, 2, 3 and 4 days. Necropsy was conducted 24 hours after each final administration, and collected blood samples were examined for erythroid and coagulation parameters. The lungs were examined by light microscopy and electron microscopy.

Hematologically, decrease in RBC count and hematocrit were observed after 1st administration, and the anemic condition continued until 24 hours after 4th administration. Prolonged PT and APTT, and decreased fibrinogen were observed after 4th administration. Level of thrombin-anti thrombin complex (TAT) was increased after 4th administration, indicating hypercoagulable state of blood.

Light microscopically, slight congestion in the alveolar capillaries was observed in all treated rats after 2nd administration and it progressively worsened with repeated administration. After 3rd administration, slight thrombus formation in the alveolar septa was observed in 3 out of 6 treated animals. The lesions were expanded and developed in all treated animals after 4th administration, and edema in alveolus were observed in some of the animals.

Electron microscopically, denatured erythrocytes including electron-dense spots congested in the alveolar capillaries after 2nd administration. Most endothelial cells of the capillaries maintained the lining on the basement membrane and their intercellular boundaries even after 4th administration.

The following three factors are generally known to be major causes of thrombus formation; endothelial injury, abnormality of blood flow and blood hypercoagulability. PHZ-treated rats showed congestion with denatured erythrocyte in alveolar capillaries from the early period after dosing, followed by blood hypercoagulability (or minimal endothelial injury). The present results indicated that the abnormality of blood flow in alveolar capillaries in lungs might be a trigger of acute thrombus formation in PHZ-treated rats.

P-39

A Study on the ultrastructure of spontaneous changes in the urinary tract of cynomolgus monkeys

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(Background) In histological studies on the urinary tract of cynomolgus monkeys, multinucleated epithelial cells in the renal papillary ducts and eosinophilic globules in the superficial cells of urinary bladder are often observable unrelated to drug administration. However, the detail or nature of the changes has not been clarified.

(Materials and methods) The tissue blocks from the kidneys and urinary bladders of cynomolgus monkeys, 28 males and 26 females, 3 years old, in the control groups in several toxicity studies were routinely processed. In addition to HE staining, the kidney sections were stained for PCNA and the bladder sections were stained with PAS, and immunostaining for hemoglobin using anti-human hemoglobin antibody. Ultra-thin sections prepared from the formalin-fixed kidneys and the bladders were observed under a transmission electron microscope.

(Results) In the kidneys of all cynomolgus monkeys of both sexes, multinucleated epithelial cells were scattered in the papillary ducts but no other abnormalities were observed. The nuclei of papillary duct showed no accelerated positive reaction for PCNA. In electron-microscopy, any morphological changes in organelles other than multiple nuclei were not observed. In the urinary bladder, many eosinophilic globules were seen in the superficial cells of most of the males and females (100 and 96%, respectively). The globules were PAS negative and showed negative reaction for hemoglobin by the immunohistochemistry. In the electron-microscopy, the globules were composed of aggregation of microtubules (each tubule: 15 nm in diameter) locating in the cytoplasm. No other abnormalities were seen in any other organelles.

(Conclusion) Nuclei of the papillary duct epithelial cells seemed to have no accelerated proliferative activity, and the pathognomic meaning of the change was obscure. The eosinophilic globules observed in the urinary bladder superficial cells were identified as globules consisting of microtubule aggregate. No abnormalities were seen in the fine structure in any other organelles and the pathogenetic process of the changes could not be elucidated.

P-40

Morphological Study of Deposits Associated with Accumulation of α_{2u} -Globulin in Renal Tubular Epithelium

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α_{2u} -globulin (α_{2u} -G) is a male rat specific protein synthesized in the liver and filtered through the glomerulus. Certain chemical binds to α_{2u} -G and the resulting complex accumulates in the renal tubular epithelium. If the level of accumulation intensifies, it becomes detectable as eosinophilic deposit with a light microscope, which deposit is generally called hyaline droplet. In Japan, the term of eosinophilic body has been also used for a certain type of the deposit but there has been no report describing the details of that and hyaline droplet. We therefore examined the morphological characteristics of these two types of deposits.

Male 11-week-old SD rats were orally treated with d-limonene (d-L) at 300 mg/kg/day up to 20 days for inducing α_{2u} -G nephropathy and their kidneys were collected. Comparative control was the kidney with spontaneously occurring eosinophilic bodies. Histopathology and electron microscopy were performed.

The d-L administration induced an accumulation of eosinophilic deposits which were small round or crystalloid forms (hyaline droplets) in proximal tubular epithelium and the degree became marked with increasing of the administration times. Large and round to occasionally irregular-shaped deposits (eosinophilic bodies) simultaneously appeared and proportionally increased with hyaline droplets. Hyaline droplets were stained brilliant red with HE, negative for PAS, and positive with Azan-Mallory (AM) and immunostaining for α_{2u} -G and LAMP. Most eosinophilic bodies were similarly stained with these stainings to hyaline droplets, although there were some variations with AM and immunostaining for LAMP. It was often difficult to distinguish them from hyaline droplets. Spontaneous eosinophilic bodies showed similar staining properties to those in d-L induced ones but a less variation. With electron microscopy, hyaline droplets were easily detected as highly electron dense substances in lysosomes, while eosinophilic bodies only in formalin fixed samples.

D-L induced eosinophilic body simultaneously appeared with hyaline droplet and showed similar staining properties to hyaline droplet. In addition, eosinophilic body and hyaline droplet were certainly associated with α_{2u} -G accumulation, although there was a slight apparent difference between them. It was considered not to be necessary to distinguish strictly eosinophilic body from hyaline droplet, because of regarding them as a sequence of changes associated with α_{2u} -G accumulation.

P-41

Thy-1 Expressing Mesenchymal Cells in Rat Nephrogenesis, in Correlation with Cells Immunoreactive for α -Smooth Muscle Actin and Vimentin

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Myofibroblasts play important roles in progression of fibrosis. Thy-1 expression on mesenchymal cells may influence myofibroblastic differentiation in fibrosis.

In this study, we investigated Thy-1 expression in nephrogenesis in F344 rats in correlation with vimentin and α -SMA expressions. In early nephrogenesis, loosely-arranged blastemal cell-derived mesenchymal cells in the cortex and medulla exhibited Thy-1 and α -SMA. Vimentin expression coincided with Thy-1. These findings indicate that the derivation of α -SMA-expressing myofibroblastic cells may be related to blastemal cells expressing both Thy-1 and α -SMA. Interestingly, there was a difference in Thy-1 expression between cortical and medullary tubulointerstitial cells in late nephrogenesis (neonates on days 12-18) and adults, in that cortical interstitial cells reacted negatively to Thy-1 except for pericytes, whereas medullary interstitial cells reacted strongly to Thy-1; additionally, bundle-arranged mesenchymal cells seen simply in neonates on days 1-12 reacted strongly to α -SMA but faintly to Thy-1. Collectively, blastemal cell-derived mesenchymal cells seem to alter immunoexpressions for Thy-1 and α -SMA, depending on developing conditions. Thy-1 immunoexpression would be useful to pursue the origin of myofibroblasts in fibrotic kidneys.

P-42

Mechanisms of testicular toxicity of acrylamide in rats: the interaction with a nuclear protein protamine.

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Acrylamide is utilized to synthesize polyacrylamides, which is used for wastewater treatment, papermaking manufacture and as a sealant. Acrylamide is also produced in many heated starchy foods, such as potato chips and French fries, which leads us the toxicities of acrylamide. Neurotoxicities, reproductive toxicity, genotoxicity and carcinogenicity due to acrylamide have been reported in human and animals. We previously reported that testicular toxicity was more severe in young rats compared to adult rats (Takahashi et al. 2009). Acrylamide binds to motor protein kinesins which interact with microtubules of sperm tail, and results in a reduction of sperm motility. Acrylamide also binds to a nuclear protein protamine that condenses sperm nuclei, causing the abnormal sperm nuclei. In this report, we examined the distribution of ^{14}C -acrylamide in the testis and epididymis by microautography and compared with the immunohistochemical distribution of protamine in both young rats and adults.

Four, 6 and 10-week-old male F344 rats were used. After 3 or 6 hrs of administration of acrylamide (0.1 mCi/mL/kg bw, p.o.), rats were sacrificed by bleeding under ether anesthesia. Testis and epididymis were dissected for microautoradiography. Exposure period was 90 days. Immunohistochemical study was performed using anti-protamine-1 antibody (sc-30174).

Semi-microautoradiograph of ^{14}C -acrylamide showed that radioactivity was rather higher in the testis and the head of epididymis of 10-week-old rats. In the microautoradiographic studies, radioactive grains were accumulated on elongated spermatids in the seminiferous tubules and on sperms in the epididymis. The distribution of grains seems to be uniform in both head and tails of elongated spermatids or sperms. The specific accumulation of radioactive grains was not observed in the testis and epididymis in 4-week-old rats. Immunofluorescence against protamine-1 appeared only in the head of the elongated spermatids (stage VII or VIII) in the testis.

The result that distributions of ^{14}C -acrylamide were all over the elongated spermatids and mature sperms is in agreement with the testicular and reproductive toxicity of acrylamide caused by its binding to both protamine and kinesin. The reason why testicular toxicity was stronger in young rats, however, remains obscure.

P-43

Lipofuscin Deposition in the Retinal Epithelial Cells of Aging Cynomolgus Monkey (*Macaca fascicularis*)

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To accumulate the ocular histopathologic data of aged monkey, we examined both eyes of seven monkeys, eight to 10 years old, obtained from a contracted laboratory. The monkeys have been kept there for over one year since they were used for a toxicokinetic study. One eye was embedded in paraffin for the routine histopathology. Another eye was embedded in an epoxy resin, sectioned and stained with toluidine blue to be able to examine in transmission electron microscopy (TEM). At the results, PAS and Schmorl reactions—positive lipofuscin deposited in the retinal pigment epithelium (RPE) around the optic nerve disk (OND). Other regions, including the macula were normal. In TEM, the RPE cells near the OND contained abundant lipofuscin granules in their cytoplasm. The basal folding of the cytoplasmic membrane regressed and other organelle was not observed. Homogenous and amorphous substances deposited in the extracellular space over the basal membrane. The substance was suggested to be an early phased drusen.

With reference to the results, we examined ophthalmoscopically 28 male monkeys around 10 years old, kept in our laboratory. Then, 20/28 (71%) monkeys revealed granular shaped auto-fluorescence around the OND. Lipofuscin deposition in the RPE was strongly suspected.

Excessive deposition of lipofuscin affects the RPE function and finally damages the photoreceptor cells. These retinal disturbances are detected at the macular area of a patient with early stage, age-related macular degeneration (ARMD). Aging cynomolgus monkeys might be a suitable animal model of early staged ARMD.

P-44

Histopathological Changes in Cerebrums of Middle-aged Monkeys

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In the increasingly ageing society of today, the problem of the neurodegenerative condition Alzheimer's disease (AD) has become global in scope. In humans, AD is a progressive neurodegenerative disease, characterized by pathological findings in the cerebrum such as senile plaques (SPs), neurofibrillary tangles (NFTs), cerebral amyloid angiopathy (CAA), and neuronal loss. There is great interest in the question of whether such changes also occur in monkeys. Accordingly, we studied histopathological changes in middle-aged monkeys.

The cerebrums of twelve middle-aged female cynomolgus monkeys (aged 18 or 19 years) were studied and compared with those of 12 young monkeys (aged 3 to 5 years). Specimens stained with Hematoxylin-Eosin (HE), Periodic Acid-Methenamine-silver (PAM), Prussian Blue, and Thioflavine S (Thio-S) were studied. Specimens stained immunohistologically with 4G8 for β -amyloid, NeuN for neurons, Iba1 for microglial cells, and S100 for astrocytes were also studied. In addition, numerical cell density was evaluated quantitatively with morphometric analysis for NeuN-positive neurons, S100-positive astrocytes, and Iba1-positive microglial cells.

In histopathological examination of the cerebrums, SPs were observed mainly in the cortex and hippocampus in all middle-aged monkeys. In contrast, CAA was observed only in one middle-aged monkey. Furthermore, accumulations of reactive astrocytes and microglial cells, and CAA with β -amyloid in the walls of blood vessels were also observed. Neither NFTs nor microhemorrhages were found in any middle-aged monkey. The results of morphometric analysis of the hippocampus were compatible with the findings stated above. It was concluded that the histopathological findings in middle-aged cynomolgus monkeys were similar to those in humans with Alzheimer's disease.

P-45

Quantifying Huntingtin Protein Aggregates in a Transgenic Mouse Model of Huntington's Disease

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Huntington's Disease (HD) is a progressive neurodegenerative disease that causes debilitating motor and cognitive deficits in those afflicted with the condition. The phenotypic impairments caused by HD are associated with the intracellular and extracellular aggregation of the protein, Huntingtin (HTT), in the striatum and cortex of the affected brain. HD has been well studied in recent years due in large part to the development of transgenic rodent models of the disease.

Historically, analyzing neurohistological preparations from a glass slide have been difficult from a quantification standpoint due to the size of tissue sections. However, digital whole-slide imaging systems and image analysis software allow researchers to qualitatively and quantitatively evaluate entire tissue sections using a single system, dramatically enhancing the speed and accuracy of such research studies. In this report, we show how to analyze brain sections and pathological markers using the Aperio system.

P-46

Investigation of a new pituitary tumor classification marker in rat

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Steroidogenic factor-1 (SF-1) is known as a transcriptional factor in the development of adrenal cortical cells and other steroid hormone producing cells and is also expressed in anterior cells of gonadotroph lineage in the pituitary of several animal species. We investigated its potential as a classification marker in rat pituitary tumors.

Double immunohistochemical staining of luteinizing hormone (LH) and SF-1 on normal pituitary of 13 weeks old male Crl:CD(SD) rats (n=3) revealed that 86.4% of LH cells are positive for SF-1. Proliferative lesions of the adenohypophysis including hyperplasia (n=3) and adenomas (n=41) from 40 to 115-week old male Crl:CD(SD) rats were examined immunohistochemically for LH, prolactin (PRL) and SF-1 single staining.

In LH-only positive proliferative lesions, 12 of 13 cases were also positive for SF-1, while 24 cases of PRL-only positive lesions were completely negative for SF-1. In addition to those, 9 lesions which were negative for both LH and PRL were positive for SF-1. It was confirmed that these 9 lesions were also negative for other anterior hormones by additional immunohistochemical stainings of GH, FSH, TSH and ACTH. These are called null cell tumors.

In conclusion, SF-1 could provide an aid for conventional immunohistochemical classification of pituitary tumors in rat and would yield a clue as to the origin of pituitary null cell tumor.

P-47

A Case of Thoracic Fungal Granuloma Caused by *Aspergillus* spp. Observed in A *Cynomolgus* Macaque (*Macaca fascicularis*).

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A male cynomolgus macaque at the age of 3 years and 11 months suffered sudden cardiac arrest during a surgical operation. This animal had been clinically asymptomatic from the acclimatization period to the death. At necropsy, a whitish mass approximately 5 cm in diameter was found at the base of the heart. Histopathologically, the mass consisted of a granuloma with a number of multinucleated giant cells and focal necrotic lesions. In periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS) stains, fungal hyphae with distinct septa and branches at a sharp angle were observed. The granuloma extended into the thoracic lymph nodes and the epicardium of the left atrium. It partially compressed the adjacent bronchioli while it was separated from the pulmonary parenchyma by a thick fibrous layer. In addition to the morphological characteristics of the fungal hyphae, polymerase chain reaction (PCR) assays of DNA extracts from paraffin sections of the mass demonstrated that the pathogen was *Aspergillus* spp. This case is the first case of spontaneous fatal aspergillosis in monkeys for laboratory use.

P-48

Spontaneous Myocardial Necrosis and Ischemic Lesions of the Brain in a Beagle Dog

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A 20 month-old female beagle dog used as a control animal in a toxicity study fell into a deep unconscious state (coma) with convulsion and it was euthanized on humane grounds.

Macroscopically, discolored foci were observed in the heart. White foci in the left ventricle including the apex and papillary muscle and multiple scattered red foci on the endocardial surface of the left ventricle were observed.

Microscopically, the heart showed contraction band necrosis of the myocardium and endocardial hemorrhage in the left ventricle. Contraction band necrosis was seen in the left ventricular wall including the papillary muscle, and was partially accompanied by hemorrhage but inflammatory cell infiltration was not found. In the thoracic aorta and right coronary artery, hemorrhage was noted in tunica media. In the brain, necrosis of the oligodendrocytes, cerebral neurons and Purkinje cells were also observed.

We thought that the marked deterioration of the beagle's condition was induced by the cardiac hypofunction due to the spontaneous contraction band necrosis of the myocardium and lesions of the brain were ischemic change responsible for the cardiovascular failure. In the toxicity study, there are few reports describing the spontaneous contraction band necrosis of the myocardium in the beagle dog. Additionally, ischemic lesions of the brain were observed in this case. Therefore, this case was considered to be very rare case.

P-49

Pathological Features of Paraprostatic Cyst in a Dog

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Paraprostatic cyst is a rare disease of middle-aged male dogs, which is considered to be a developmental anomaly and arise from the prostatic utricle (the uterus masculinus), vestiges of the paramesonephric duct. Here we report the histological findings of a paraprostatic cyst in a 9-year-old male Welsh corgi dog. The dog was presented to the Veterinary Medical Center of Osaka Prefecture University with oliguria and abdominal distention. CT and ultrasonography revealed a large abdominal cyst, extending from the caudal part of the liver to the pelvic cavity, displacing the bladder to the right. The cyst attached to the bladder, right ureter and prostate; however, no direct communication was observed between the cyst and prostate. Grossly, the cyst was 18 × 9 × 9 cm, had a wall 2-3 mm in thickness, and contained sterile serosanguinous fluid. The cyst wall had a bony consistency, and cauliflower-like bony lesions extending into the lumen. Histologically, the cyst wall was composed of fibrous tissue with abundant bone and occasional cartilage tissues. The cyst was lined by single or multilayered, cuboidal to flattened epithelia; ciliated epithelia were occasionally seen. Consistent with the cauliflower-like bony lesions, osteoid formation was diffusely found. Based on the anatomical location and histological findings, the cyst was diagnosed as paraprostatic cyst, which may derive from vestiges of the paramesonephric duct.

P-50

A case of the spontaneous cholelithiasis in young female Wistar-Hannover rat.

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We encountered a case of marked dilatation of the common bile duct and gall stones in a female Wistar-Hannover rat used in a toxicity study. This animal, one of 42 16-week-old female Wistar-Hannover rats (Br/Han; Wist@Jcl(GALAS)) maintained on the basal diet, was normal with regard to clinical observations and body weight. At necropsy, however, the common bile duct was observed to be markedly dilated, and many soft and yellowish gallstones were found in the lumen. On analysis of the infrared absorption spectrum, it was demonstrated that the gallstones were composed of fatty acid calcium and bilirubin calcium, and were thus designated as fatty acid calcium stones. Histopathologically, marked fibrosis, inflammation and proliferation of epithelial cells were observed in the common bile duct. Inflammation was also apparent in the head region of the pancreas and periportal areas of the liver, but bile pigment deposition was lacking. Thus, an extremely rare case of marked dilatation of the common bile duct with gall stones found in a young female rat is reported in the present paper.

P-51

Enterocyst in Rat Ileum

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As a well-known congenital abnormality of the vitelline duct (also known as vitello-intestinal duct or omphalomesenteric duct), Meckel's diverticulum, a remnant resulting from closure of the embryonic vitelline duct at the umbilical end, is often found in animals and humans. On the other hand, enterocyst is a congenital abnormality resulting from closure of the vitelline duct at both ends. This abnormality has been reported in dogs and humans, but not in rats. In this study, we report the cyst considered to be derived from the vitelline duct in rat ileum.

We are reporting on a female Crl:CD(SD) rat used for an in-house preliminary study and subjected to scheduled sacrifice at 10 weeks of age. Macroscopically, a cyst with a diameter of 8 mm was found adhering to the antimesenteric serosa in the ileum. The cyst had a white area (about 4 mm in diameter) and was found to contain viscous and clear fluid with no communication with the intestinal lumen. The tissue specimens were fixed in 10% neutral buffered formalin solution, embedded in paraffin, and stained with hematoxylin and eosin, periodic acid-Schiff, Alcian blue (pH 2.5), Masson's trichrome, and Anti-Human Smooth Muscle Actin.

Microscopically, the cyst wall had 3 layers consisted of mucosal epithelium with goblet cells, lamina propria mucosa, and muscle layer (smooth muscle), which was similar to the adjacent ileal wall. In addition, the grossly visible white area showed formation of granulation tissue and mineralization with exuding mucus in the muscle layer and serosa, necrosis/defluxion, and regeneration of the mucosal epithelium.

The cyst had a similar histological structure as the ileum, with no communication with the intestinal lumen. Moreover it was seen at the position in which Meckel's diverticulum is often observed. Therefore, we considered that this case was an enterocyst originating from the vitelline duct. This is the first report of an enterocyst in rat ileum.

P-52

Automated Analysis of Islet Morphology in Diabetes Research

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Diabetes results when insulin-secreting beta cells within the islets of the pancreas are lost or functionally compromised. An increase in the number of glucagon-secreting alpha cells within the pancreatic islet is also a histological finding in some animal models of diabetes. Whole slide scanning allows for quantitative monitoring of pancreatic changes in rodent diabetes models. We describe 3 novel techniques for identifying islets and quantifying cell morphology that combine IHC staining with image analysis across whole pancreas sections. We demonstrate automated identification of islet cells in H&E or IHC sections using tissue typing technology. Biomarkers can then be quantified within the islets, excluding exocrine tissue from the analysis. By calculating % area of insulin and glucagon staining, one is able to calculate alpha and beta cell mass. These technologies have wide application for understanding the pancreatic environment in diabetes research.

P-53

Pathological Characteristics of Streptozotocin and Alloxan-induced Diabetic Dogs

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Many diabetic animal models produced by administration of streptozotocin (STZ) and/or alloxan (ALX) are known. In this study, diabetes was induced in dogs by administration of a mixture of STZ and ALX, and evaluated pathologically after maintaining hyperglycemia for 7~8 years to determine the induction of pathological abnormalities comparable to those of diabetic complications in human patients.

Three female CSK beagle dogs received a single intravenous injection containing a mixture of STZ and ALX to induce diabetes mellitus. The dogs were subjected to a pharmacology study, and sacrificed 7~8 years after the administration, at the age of 8~9 years. Persistent hyperglycemia (150~300 mg/dL) was observed during a period of one year after the administration to necropsy. At necropsy organs were fixed in 20% formalin and embedded in paraffin. The sections were stained with HE and special stains (e.g. PAS, PAM, Masson's trichrome) for light microscopic examination. Three age-matched female dogs were also subjected to pathological examination as controls.

Macroscopically, paleness, scarring and recession in kidneys, opacity of the lens was observed in STZ/ALX treated diabetic dogs. Histopathologically, islet cells of the pancreas were decreased in diabetic dogs. Vascular lesions including fibrin-caps, nodular lesions, hyaline arteriosclerosis of afferent/efferent arterioles in kidneys, thickening of the basement membrane and hemorrhage in the retina was occasionally observed. In addition, degeneration and mineralization in lens fibers was also observed. In peripheral nerves (tibial and vagal nerve), a decrease of myelinated/unmyelinated nerve fibers and an increase of interstitium was observed. These histopathological lesions were not observed in control animals.

In conclusion, STZ/ALX induced diabetic dogs with persistent hyperglycemia developed lesions comparable to those of major complications in human diabetes; diabetic nephropathy, retinopathy, cataract, and neuropathy.

P-54

Dental Caries and Caries-related Periodontal Disease in Alloxan-induced Diabetic Rats

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Background: We have previously detected dental caries and periodontal disease in diabetic model rats. However, whether or not periodontal disease is directly induced by diabetes in these rodent models remains unknown. Thus, we tried to inhibit caries in diabetic rats by fluoride treatment, and confirmed whether periodontal disease is directly induced by diabetic conditions.

Methods: A total of 80 female F344 rats were divided into five groups. Sixty rats were treated a single dose of alloxan (35 mg/kg i.v.) at 7 weeks of age, and then given a drinking water containing 0, 10 and 50 ppm sodium fluoride (AL group, AL+F10 group and AL+F50 group; n=20 each group) for 13 weeks. During that same period, untreated rats were also given a drinking water containing 0 and 50 ppm sodium fluoride (Control group and F group; n=10 each group). All rats were sacrificed at 20 weeks of age and their upper and lower jaws including molar teeth were examined for caries and periodontal lesions macroscopically, radiographically and histopathologically.

Results: Macroscopically, the incidence of caries was much higher in alloxan-induced diabetic rats compared to that in non-diabetic rats. In fluoride-treated groups, there was a dose-related decrease of the frequency and severity of dental caries. Corresponding to the macroscopic dental caries, radiolucent area of molar teeth was observed by soft X-ray photography. In addition to the molar radiolucency, alveolar bone radiolucency was often observed in diabetic animals with caries. Contrary, diabetic rats without caries showed no change. Histopathological findings showed that dental caries made further progress toward the root within molars and caused bacterial infections followed by pulp necrosis and pulpitis. Furthermore, periodontal inflammation was adjacent to teeth with progressed caries, and the alveolar bone resorption correlated well with that of molar caries.

Conclusion: These results suggest that periodontal lesions in diabetic rats with caries may result from apical periodontitis secondary to dental caries.

P-55

Enhanced Tumorigenesis of Forestomach Tumors Induced by *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine in Rats with Hypoinsulinemic Diabetes

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Hyperinsulinemia and hyperglycemia in prediabetic and diabetic patients are thought to increase the risk of developing neoplasms because insulin is a growth factor with preeminent metabolic but also mitogenic effects.

To determine the effect of hypoinsulinemic diabetic conditions on carcinogenesis, we examined MNNG-induced forestomach carcinogenesis in hypoinsulinemic diabetic WBN/Kob rats of about 45 weeks old (DM) compared with non-diabetic younger WBN/Kob rats (C1), non-diabetic Wistar rats age-matched to DM (C2), and non-diabetic Wistar rats age-matched to C1 (C3).

All rats were treated with MNNG by gavage and were killed at 40 weeks after dosing. Various-sized tumors were disseminated throughout the forestomach of all rats, and the ratio of the area of tumors to the whole forestomach area was 23.3% in the DM group and was higher than in the C1-3 (4.2-14.3%) groups. The incidence of carcinoma was much higher in the DM group (36.8%) than in the C1-3 (7.1-16.7%) groups, and the incidence of papilloma was also significantly higher in the DM group (84.2%) than in the C1-3 (28.5-50.0%) groups. The average thickness of the squamous epithelium in the non-neoplastic mucosa was significantly greater in the DM group (50.8 μ m) than in the C1-3 (29.6-37.9 μ m) groups. Immunohistochemically, the Ki-67-positive index in the non-tumorous mucosa of the DM group (42.0%) was significantly higher than that of the C1 group (33.3%).

These results suggest that prolonged hyperglycemic conditions without hyperinsulinemia enhance tumorigenesis of MNNG-induced tumors by enhanced proliferative activity of the squamous epithelium in the rat forestomach.

P-56

Establishment of Invasive Prostate Cancer Model of TRAP Rat

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【Background】 In order to study prostate cancer metastasis and develop therapies to combat this disease, animal models resembling human prostate cancer are necessary. We have established a short-term high-incidence invasive prostate cancer model using the TRAP (Transgenic Rat for Adenocarcinoma of Prostate) rat.

【Experiments】 Two sets of experiments were conducted. In Experiment 1, TRAP rats were divided into 4 treatment groups: (1) no treatment; (2) 3,2'-dimethyl-4-aminobiphenyl (DMAB) plus Testosterone propionate (TP); (3) bilateral orchiectomy (Ox) plus TP; and (4) Ox plus DMAB and TP. In Experiment 2, TRAP rats were divided into 3 groups and the effects of continuous and intermittent application of TP on prostate carcinogenesis were compared.

【Results】 Ex.1: Enhancement of invasive prostate carcinogenesis was seen each of the three treatment groups compared with the untreated control. There were no clear effects of DMAB or Ox on invasive prostate carcinogenesis. Ex.2: The incidence and numbers of invasive adenocarcinoma in the lateral prostates after intermittent or continuous application of TP were G1 (TP was applied 3 times): 89%, 4.22±2.59; G2 (TP was applied 2 times): 56%, 0.89±0.93; G3 (TP was applied continuously): 33%, 0.33±0.52.

【Summary】 The most effective means of eliciting invasive prostate adenocarcinoma in TRAP rats is by numerous intermittent applications of TP. Therefore, this model will be used to search for chemopreventive and chemotherapeutic chemicals in future research.

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P-57

LAT1 Antagonists Suppressed *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine Induced Rat Gastric Carcinogenesis

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It is well known that patients with gastric carcinomas show high mortality rates. Gastric carcinomas have usually been treated by surgical operation with oral anticancer chemotherapy. Cancer cells require high abundance amino acids for preservation high neoplastic cellular activity, and L-type amino acid transporter 1 (LAT1) is especially higher present at neoplastic cells including gastric carcinoma, in vitro and in vivo. Then we investigated that in vivo anti-cancer effect of LAT1-antagonists for chemical induced gastric carcinomas in rats. As LAT1-antagonist compounds, we applied 2-amino bicycle (2,2,1) heptane - 2 - carboxylic acid (BCH) and a novel agent under development (Compound X). Male 8 weeks old Wistar rats were given *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) 100mg/L or tap water for 40 weeks. Forty weeks after each agent administration, rats were intragastrically administrated 20mg/kg BCH, 40mg/kg BCH, or 1mg/kg Compound X for 14 days. TUNEL analysis revealed all LAT1-antagonist administration groups showed significantly higher apoptotic index compared to that of vehicle administration group. BCH administration groups showed dose dependent increase apoptotic index. Compound X administration group showed significant higher apoptotic index than that of BCH administration groups. Moreover, expression levels of apoptosis induced proteins were similar to those of TUNEL analysis. In MNNG induced rats gastric carcinogenesis, LAT1-antagonists administration might enhanced the apoptosis induction of neoplastic cells.

P-58

Male-Female Rats Gastric Carcinogenesis Induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine phthalate

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Epidemiological studies have been demonstrated that incidences of gastric carcinoma in male are about two-times higher than those in female. Mechanism for the evidences of male-female difference for gastric carcinogenesis is, however, still unclear. Then we investigated that male-female difference in rat gastric carcinogenesis induced by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG). Eight weeks old male and female Wistar (slc) rats were given 100ppm MNNG in drinking water (MNNG-group), or tap water (Vehicle group) for 40 weeks, and 40 weeks after end of each treatment, rats were autopsied. Incidence of gastric carcinomas and weight of stomach in MNNG-group of male were higher than those of female, but histopathological types of gastric carcinomas were similar between those in male and female. Expressions of proliferating cell nuclear antigen, cyclin D1, cyclin dependent kinase 1, cyclin dependent kinase 2, cyclooxygenase-2, and vascular endothelial growth factor in gastric carcinomas of male were significantly higher than those of female. However, expressions of estrogen receptor beta and androgen receptor in gastric carcinomas of male were significantly lower than those of females. It was revealed that MNNG-induced gastric carcinogenesis was predominant in male compared to in female. The present study suggested that the mechanism of male-female difference in rat MNNG induced carcinogenesis might be involved in the expressions of estrogen receptor beta and androgen receptor in gastric carcinoma cells.

P-59

Preventive Effect of Caffeic Acid Phenethyl Ester on Gastric Carcinogenesis in *Helicobacter pylori*-Infected Mongolian Gerbils

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Nuclear factor- κ B (NF- κ B) plays important roles in host inflammatory responses and carcinogenesis and as such is an attractive drug target for adjuvant therapy. In the present study, we examined the effect of caffeic acid phenethyl ester (CAPE), a potent NF- κ B inhibitor, on *Helicobacter pylori* (*H. pylori*)-induced condition *in vitro* and *in vivo*. In AGS human gastric cancer cells, CAPE significantly inhibited *H. pylori*-stimulated NF- κ B activation and mRNA expression of several inflammatory factors such as TNF- α , iNOS, and IL-8 in a dose-dependent manner, and prevented degradation of I κ B- α and phosphorylation of NF- κ B p65 subunit. To evaluate the effects of CAPE on *H. pylori*-associated gastric disorders, specific pathogen-free 6-week-old male Mongolian gerbils were intragastrically inoculated with *H. pylori*, administered 0 or 10 ppm *N*-methyl-*N*-nitrosourea in drinking water, fed diet containing CAPE (0-0.1%), and sacrificed after 12 or 52 weeks. 0.1% CAPE alleviated infiltration of inflammatory cells, and attenuated translocation of NF- κ B p50 subunit and phosphorylation of I κ B- α in gastric mucosa. CAPE also reduced transcription of inflammatory factors including TNF- α and iNOS in pyloric mucosa. Furthermore, 0.1% CAPE significantly decreased the incidence of gastric adenocarcinomas (7/26, 26.9%) compared with that of the control group (29/54, 53.7%). These results suggested that CAPE has inhibitory effects on *H. pylori*-induced gastritis and carcinogenesis in Mongolian gerbils through the suppression of NF- κ B activation.

P-60

Hepatocarcinogenicity mechanism of piperonyl butoxide in mice

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Piperonyl butoxide (PBO) is a pesticide synergist used with pyrethroids as a domestic insecticide, and it act as a non-genotoxic hepatocarcinogen in rats and mice. PBO can generate reactive oxygen species (ROS) via a metabolic pathway in the liver of rats and induce oxidative stress including oxidative DNA damage that is possibly related to the hepatocarcinogenesis in rats. However, there is no data demonstrating that oxidative damage resulting from ROS generation is involved in the liver tumor promotion of mice. In order to clarify the possible mechanism of hepatocarcinogenesis induced by PBO, male ICR mice were subjected to a two-third partial hepatectomy, followed by DEN treatment, and given a diet containing 0 % PBO (DEN-alone) or 0.6 % PBO (DEN+PBO) for 25 weeks. The incidences of CK8/18-positive foci, adenomas and carcinomas significantly increased in the DEN+PBO group compared with the DEN-alone group. PBO increased ROS production in liver microsomes but did not increase oxidative DNA damage, as assessed by 8-hydroxydeoxyguanosine (8-OHdG). In real-time RT-PCR, PBO did not alter the expression of Xrcc5 and Ogg1. Thus, PBO can generate ROS via the metabolic pathway without any induction of oxidative DNA damage, activate cell growth, increase c-Myc and E2F1 related pathways, and act as a liver tumor promoter in mice.

P-61

Molecular Pathological Analysis on the Hepatocellular Tumor Promoting Effect of Indole-3-Carbinol (I3C) in Hepatectomized Rats

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Indole-3-carbinol (I3C) is the major alkaloid in cruciferous vegetables and is used as a supplement because of their tumor suppressing effect. On the contrary, I3C is reported to have a liver tumor promoting effect in rats, and also known as a CYP1A inducer. Our laboratory demonstrated that CYP1A inducers such as β -naphthoflavone and fenofibrate had a liver tumor promoting effect by the production of reactive oxygen species (ROS). However, its precise correlation between the ROS generation and tumor-promoting effect is unclear. To clarify the mechanism of hepatocellular tumor-promotion of I3C and to determine the threshold dose, partial hepatectomized rats were fed diets containing 0, 0.25, 0.50 or 1.0% I3C after an i.p. injection of N-diethylnitrosamine (DEN). The number and area of glutathione S-transferase placental form (GST-P)-positive foci significantly increased in rats given 0.5% I3C or more. The formation of liver microsomal ROS significantly increased in all I3C treatment groups. DNA microarray and real-time RT-PCR analyses showed that the expression of CYP1A1 and Yc2 levels significantly upregulated in rats of all I3C treatment groups. Ugt1a6, Gpx2 and γ -GT1 also upregulated in rats given 0.5% I3C or more. These results suggest that ROS is involved in the liver tumor-promotion of I3C and the threshold dose is 0.5% .

P-63

Epigenetic Alteration in the Gene Expression Involved in the Enhancing Effects of Chromated Copper Arsenate (CCA) on Skin Carcinogenesis

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Chromated copper arsenate (CCA) contains arsenic and chromium which are known as carcinogens, and has generated public concerns over possible risk of skin cancer. In a previous study, female SKH-1 mice were fed diets containing 0, 1, 10 and 90 ppm CCA for 28 weeks, and exposed to UVB 3 times a week from 2 weeks of CCA treatment onward. The results suggested that CCA enhances the skin carcinogenesis induced by ultraviolet B (UVB) radiation. In addition, tissues of squamous cell carcinoma in the UVB+CCA 0 and 90 ppm groups were sampled using laser capture microdissection (LCM), and microarray analysis was conducted. The results revealed that up-regulation of genes related to cell proliferation and malignant transformation and down-regulation of genes related to DNA repair, apoptosis and antioxidant may be involved in the enhancing effects of CCA on skin carcinogenesis. To elucidate the involvement of epigenetic alteration in the gene expression contributed to the enhancing effects of CCA, we conducted quantitative methylated DNA immunoprecipitation (MeDIP)-PCR analysis in frozen skin masses in the UVB+CCA 0 and 90 ppm groups. Real time RT-PCR analysis was also conducted using the LCM samples. In RT-PCR analysis of the enhancing effect-related genes, p53, Nrf2, Ogg1 and Msra mRNA levels were significantly decreased in the UVB+CCA 90 ppm group compared to the UVB+CCA 0 ppm group. Among these genes, MeDIP-PCR analysis revealed that CpG islands nearby transcription starting site in Nrf2 and Ogg1 were significantly hypermethylated in the UVB+CCA 90 ppm group. Furthermore, gene expressions of maintenance or *de novo* DNA methyltransferase (Dnmt1, 3a, 3b), Dnmt1-related genes (Ctcf, Parp1, Uhrf1), methylated DNA binding protein (Mbd2, Mecp2), histone deacetylase Hdac1 and transcriptional co-repressor Sin3a were examined, and mRNA levels of Dnmt3b, Ctcf, Parp1 and Hdac1 were significantly decreased in the UVB+CCA 90 ppm group. These results suggest that both the malfunctions of DNA methylation machinery and DNA methylation changes may play important roles in the enhancing effects of CCA on the skin carcinogenesis.

P-64

Involvement of Bone Marrow Derived Cells in *Helicobacter pylori*-infected Mouse Gastric Carcinogenesis

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Helicobacter pylori (*H. pylori*) infection is closely linked with gastric carcinogenesis. Recently, it has been reported that bone marrow derived cells (BMDCs) could be the origin of *H. pylori*-associated gastric tumors. To reveal its possibility, we examined the presence of BMDCs in gastric mucosa and tumors by using mice transplanted with green fluorescence protein (GFP)-labeled BM cells.

Five to six week-old female C57BL/6J mice were X-irradiated with 11.5 Gy and transplanted with 2×10^6 BM cells isolated from male GFP-transgenic mice of the same strain. Two to three weeks later, all mice were evaluated for settlement of GFP-positive BMDCs under a fluorescence stereomicroscope and divided into four groups: *H. pylori*-infected, *N*-methyl-*N*-nitrosourea (MNU)-treated, *H. pylori*-infected + MNU-treated, and control groups. The mice of *H. pylori*-infected groups were inoculated with *H. pylori* (SS1). After 1 week, the mice of MNU-treated groups were administered with 120 ppm MNU on alternate weeks (total exposure = 5 weeks). All surviving mice were sacrificed at 40 weeks. The mice prematurely sacrificed as moribund after experimental week 24 were considered as effective animals. Immunohistochemical analysis for GFP was performed in the excised stomachs.

The results showed that GFP-positive BMDCs infrequently replaced gastric epithelium irrespective of *H. pylori* infection in both fundic and pyloric glands, but only infiltrated into gastric stroma. Similarly, there were no gastric tumors composed of GFP-positive tumor cells both in MNU-treated (0/7=0%) and *H. pylori*-infected + MNU-treated mice (0/12=0%). These results indicated that BMDCs were rarely incorporated in the gastric epithelial cells. Thus, gastric cancers were considered to develop from primary gastric epithelial cells irrespective of *H. pylori* infection.

P-65

Effect of Angiotensin II Receptor Antagonist Losartan in Preventing Mouse Colorectal Cancer

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Angiotensin II (Ang II) is a key effector of the renin-angiotensin system, acting through AT1 and AT2 receptors. Recent studies have shown that AT1 signaling also functions in cell proliferation and protooncogene expression but AT2 works inversely. *In vivo* AngII function in colonic epithelial cells, however, is remained to be analyzed. In this study, we investigated the preventive effect of Ang II receptor 1 blocker, losartan (LOS), in suppressing intestinal carcinogenesis using two colon cancer models including a carcinogen-induced tumor model and DSS-treated *Apc^{min/+}* model. Six week-old ICR male mice were received AOM and 2% DSS, and treated with LOS in combination with aspirin (ASP) to observe the effect in preventing colorectal cancers (groups A, control; B, 200 ppm LOS; C, 1000 ppm ASP; D, 200ppm LOS + 1000 ppm ASP). The tumor volume bigger than 30mm³ in groups B and D were significantly decreased compared with group A (A vs. B, P<0.01). Invasive tumors in group D were less developed than in group A (A vs. D, P<0.05) at week 14. In the second model, *Apc^{min/+}* mice treated with LOS extended their survival period and decrease the multiplicity and volume of the tumors bigger than 5mm³ compared with the control group (P<0.05). Expression of MMP2, FGF2, and Survivin mRNA were suppressed in LOS groups. In conclusion, our study suggested that LOS in combination with ASP inhibited progression and invasion of colorectal tumors in the murine models. Pathways including the above molecules could be possible signal transduction pathways from Ang II toward tumorigenesis.

P-66

Sensitivity of a hypercholesterolemic mice model to AOM/DSS-induced colon carcinogenesis

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[Background and Aim] Hypercholesterolemia is associated with increased risk of atherosclerosis that causes ischemic heart disease and apoplexy. Some epidemiological investigations suggested that hypercholesterolemia reduce the risk of colon cancer but statins, cholesterol-lowering drugs, were also suggested to reduce the risk of colon cancer. To evaluate the association between hypercholesterolemia and the risk of colon cancer, we investigated the inflammation-associated colon carcinogenesis in low density lipoprotein receptor (LDLr) deficient mice.

[Methods] Male LDLr-deficient and wild type (WT) mice (C57black/6J) were treated with a single i.p. injection of azoxymethane (10 mg/kg bw) and then given 2% dextran sulfate sodium in drinking water for 7 days. They were sacrificed to determine the incidence and multiplicity of tumors at week 20.

[Results] The incidence and multiplicity of colonic adenocarcinomas were significantly lower in LDLr-deficient mice (21%, 1.43±0.13) than in WT mice (80%, 3.10±2.38). Mean volume of colon tumors was lower in LDLr-deficient mice (52.3 mm³) than in WT mice (170.4 mm³). Serum total cholesterol, triglyceride and adiponectin levels were significantly higher in LDLr-deficient mice than in WT mice.

[Conclusions] These findings suggest that LDLr-deficient mice show reduced susceptibility to inflammation-associated colon carcinogenesis. Serum adiponectin levels may be associated with this susceptibility.

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Subsequent Report on Spontaneous Colon Carcinoma of Wistar-Furth Rat

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In 1964, one male and two female rats of Wistar-Furth strain(W/F) were given by Dr. Kenjiro Yokoro, Hiroshima University, Institute of Atomic bomb. They have been bred by brother-sister mating up to now. During the course of breeding, descendants had been in Machidol University, Bangkok in Thailand where rats were fed rat chaws made in New Zealand. A report of massive occurrence of spontaneous colon carcinoma, localized in ascending colon, was published by Heslop, though studies did not reveal any case afterwards. In 1975, we reported numbers of colon carcinoma localized in the same region in W/F strain. To obtain a large number of descendants, primipara litters were used for brother-sister mating. Numerous colon carcinomas were found in these descendants and gastric adenocarcinoma in glandular stomach as well. With the establishment of transplantable carcinomas of the colon and stomach, recipients also developed the same type of carcinoma as well as occasional uterine adenocarcinoma. Intra-peritoneal injection of the serum from cancer carrying W/F rats induced the colon carcinoma in Wistar/Shionogi and LE strain rats. Electron microscopic study on supernatant of tissue culture medium of gastric carcinoma revealed particles with the size of 50 nm. The same particles were discovered in the cytoplasm of gastric carcinoma cells. Following standard brother-sister mating, the incidence of colon carcinoma has been gradually reduced. However, the incidence of colon cancer has been increased in descendants with repeated primipara mating. In addition, those descendants were not able to tolerate as recipient rats for successive transplantation of cancer due to development of severe diarrhea which was fatal.

Based on these findings, more research will be needed to maintain descendants from closed-colonies for transplantation. Materials will be offered to the investigators who are interested in this research.

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Lack of Effects of Isoleucine and Leucine at Early Stage of BBN-induced Rat Bladder Carcinogenesis

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We previously demonstrated the promotion effects of isoleucine and leucine on BBN-induced rat bladder carcinogenesis in a 29-week experiment. However, little is known about the mechanism underlying the promotion effects. The aim of the present study is to evaluate the effects of isoleucine and leucine at early stage of rat bladder carcinogenesis. In experiment 1, rats were fed with AIN-93G or MF basal diet, 2% leucine or isoleucine in basal diet for 2 weeks. In experiment 2, rats were administered 0.05% BBN in drinking water for the first 2 weeks, and then fed with basal diet, 2% leucine or isoleucine diet for 4 weeks. No treatment-related pathologic changes in bladder were observed in both experiments. There were also no differences in proliferative activities and expressions of 12 genes which were previously found to be up-regulated in BBN-treated bladder epithelium. These findings indicated the possibility that isoleucine and leucine had no modifying effect at early stage of rat bladder carcinogenesis.

P-69

Effects of Dimethylmonothioarsinic Acid on Bladder Urothelium

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[Background] Epidemiological studies have shown that arsenic exposure is associated with increased bladder cancers. We previously have demonstrated the bladder carcinogenicity of dimethylarsinic acid (DMA^V) in rats. Dimethylmonothioarsinic acid (DMMTA^V) is an organic metabolite of DMA^V in rats, and has been found in the urine of humans exposed to arsenic. However, little is known about its toxic and carcinogenic effects. The purpose of the present study is to evaluate the *in vitro* and *in vivo* effects of DMMTA^V on bladder urothelium.

[Methods] Experiment 1: To determine the *in vitro* cytotoxicities, human and rat urothelial cells were treated with various concentrations of arsenicals for 48 hours. Experiment 2: DMA^V was injected into bladders of SD rats after ligation of the urinary duct and urethra. Two hours later, arsenic concentrations of urinary contents and urothelium were measured. Experiment 3: Bladders of SD rats were exposed directly to DMA^V and DMMTA^V by osmotic pumps for 2 weeks. Concentrations of urine arsenicals and gene expression in urothelium were evaluated.

[Results] 1: *in vitro* LC₅₀ of DMMTA^V, arsenate, arsenite, monomethylarsonic acid, DMA^V, and dimethylarsinous acid were 4.6μM, 47.9μM, 3.2μM, 7.7mM, 2.7mM and 1.1μM respectively, in rat urothelial cells, and were 5.4μM, 81.7μM, 25.5μM, 6.1mM, 1.4mM and 4.1μM respectively, in human urothelial cells. 2: Both *in vitro* and *in vivo*, DMMTA^V was uptaken by urothelium and converted into DMA^V. 3: *in vivo*, oncomodulin expression was increased in urothelium treated with DMMTA^V but not in DMA^V.

[Conclusion] DMMTA^V is much more toxic than DMA^V, and may be involved in DMA^V-induced toxicities and carcinogenicities in bladder.

P-70

Tumor modifying activity in the urinary bladder of horseradish extract in F344 rats

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Horseradish extract (HRE) obtained from steam distillation of milled root of *Armoracia rusticana* is used as a food additive for antioxidant and ready-to-use wasabi paste in Japan. The main component of HRE is allyl isothiocyanate, which slightly increased the incidence of transitional-cell hyperplasias and papillomas of the urinary bladder in male rats administered 12 or 25 mg/kg b.w. in a previous rat carcinogenicity study (gavage study). HRE contains other isothiocyanates such as phenethyl and pentenyl homologues. In our previous study, HRE induced simple or papillary and nodular (P/N) hyperplasias in the urinary bladder of F344 rats by drinking water through bottle treatment for 2 weeks (up to 0.1 %) or 13 weeks (up to 0.05 %). However, a 52-week chronic toxicity study at concentrations of 0.005, 0.01 and 0.04 % through nozzle and tube from water feed tank revealed no obvious effects on proliferative lesions in urinary bladder. Therefore, to examine carcinogenic effects of HRE, a 2-year carcinogenicity study in 6-week old male and female F344 rats was performed at concentrations of 0.005, 0.01 and 0.04 % in drinking water. In addition, to clarify promotion activities of HRE on urinary bladder carcinogenesis, a medium-term bioassay was conducted in 6-week old F344 male rats initiated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in drinking water followed by HRE treatment at concentration of 0.005, 0.01 and 0.04 % for 13 or 32 weeks. In the carcinogenicity study, no significant differences in final survival rate and mean survival time were observed among the groups. Daily water consumption in 0.04% group of male rats was slightly decreased, but a good correlation between the dose and daily intakes were observed. In histopathological assessment, simple and PN hyperplasias, papillomas and transitional cell carcinomas were observed in the urinary bladder, but HRE did not increase the incidences of these lesions. In the other organs or tissues, none of the treated groups showed significant increase in the incidence of any specific tumor over the control group. In the medium-term bioassay, at week 13, all treated groups developed PN hyperplasia (p<0.01), and 0.01% and 0.04% groups showed significantly (p<0.05 or 0.001) high incidence of papilloma. At week 32, 0.01% and 0.04% groups showed significantly (p<0.05 or 0.01) high incidence of papilloma and transitional cell carcinoma. Tumor volumes were significantly increased in the HRE 0.04% group (p<0.01) at week 32. These results indicate that HRE promotes urinary bladder carcinogenesis at higher doses but does not exert carcinogenicity *per se* in rats.

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Immunohistochemical localization of Breast Cancer Resistance Protein (BCRP) in normal tissues of Rats and Mice

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[Introduction]

Breast cancer resistance protein (BCRP) is an efflux transporter that has been shown to contribute to the resistance of tumor cells to various types of anticancer drugs, and thus is a promising target molecule in the development of drugs to overcome anticancer drug resistance. Elucidating the localization of the BCRP molecule is thus important in determining the mechanism of toxicity caused by anticancer drugs excreted by BCRP and the combined use of BCRP inhibitors and anticancer drugs. The expression of BCRP in normal tissues of humans and rodents has primarily been examined by quantitative analysis of mRNA by RT-PCR, and its localization has only been studied in limited tissues in mice. This report describes the immunohistochemical localization of BCRP in normal tissues of rats and mice, which are commonly used for toxicity study.

[Materials and Methods]

Male and female SD rats and ICR mice, both at 10 weeks of age, were used. Organs and tissues that are used for histopathological examination in common toxicity study were fixed in formalin, paraffin embedded and cut into 4 μ m sections according to the standard procedure. The localization of BCRP, immunohistochemical staining was performed using an anti-mouse BCRP rat monoclonal antibody (BXP-53).

[Results]

In rats and mice, BCRP localization was detected in the surface of intestinal mucosa and the luminal surfaces of bile capillaries in the liver, proximal renal tubules in the kidney, efferent ductules in the caput of the epididymis, acini in the mammary and Harderian glands, and blood capillaries in all tissues of the body. BCRP expression in the choroid plexus in the brain was only observed in mice, indicating a species-specific difference in its expression.

[Conclusion]

Immunohistochemical analysis of BCRP expression revealed the localization of the BCRP molecule at the cellular level in rats and mice and a species-specific difference in its expression, which had not been elucidated by RT-PCR. These findings will provide useful information for analyzing toxicity caused by anticancer drugs excreted by BCRP and the combined use of BCRP inhibitors and anticancer drugs.

P-72

Inhibitory effects of pentachlorophenol, a sulfotransferase inhibitor, on *in vivo* mutagenicity and DNA damage in the kidneys of *gpt* delta mice treated with lucidin-3-*O*-primeveroside

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Lucidin-3-*O*-primeveroside (LP) is one of the components of madder root (MR), which is carcinogenic to the rat kidney and liver. Since lucidin (Luc) and rubiadin, metabolites of LP, showed obviously genotoxicity, it is likely that these metabolites play a key role in MR carcinogenesis. In fact, after incubation of Luc with calf thymus DNA, Luc-*N*²-dG and Luc-*N*⁶-dA were reported to be formed possibly via sulfotransferase (SULT) metabolic pathway. In the present study, we examined the reporter gene mutations in the kidneys of *gpt* delta mice given 0.3% LP in the diet for 13 weeks along with quantitative measurement of Luc-specific DNA adducts. Additionally, inhibitory effects of combined treatment with pentachlorophenol (PCP), a SULT inhibitor, were also investigated.

Ten-week-old male B6C3F₁ *gpt* delta mice were randomly divided into four groups consisting of five animals each and treated with basal diet, 0.3% LP, 0.02% PCP or 0.3% LP plus 0.02% PCP in the diet. Animals in each group were given basal diet and/or 0.02% PCP in the diet for one week prior to LP treatment. All mice were sacrificed at 13 weeks after LP treatment. Kidneys were used for the examination of histopathology, *gpt* mutation assay and Luc-specific DNA adduct formation by LC-MS/MS.

Histopathologically, karyomegaly in the proximal tubule epithelium in the outer medulla was observed in mice given LP with significantly increased incidence as compared with basal diet. *Gpt* mutation frequencies in mice treated with LP were significantly elevated. Also, analysis of the mutation spectra revealed that AT-TA and AT-CG transversions were significantly increased. On the other hand, combined treatment with PCP effectively inhibited these changes caused by LP administration.

LP caused *in vivo* mutagenicity with increased incidences of AT-TA and AT-CG transversions in the kidneys of mice. The fact that co-treatment with PCP inhibited histopathological changes and *in vivo* mutagenicity supported SULT participation in these changes. In addition, to Luc-specific DNA adducts will be quantitatively analyzed by LC-MS/MS.

P-73

Prediction of early detection of radiation-induced renal tumors in fetuses and children

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Previously, as part of an evaluation of radiation risk related to renal tumors in fetuses and children, we investigated the occurrence of renal tumors in the F1 generation of a cross between the hereditary renal tumor rat (Eker rat) and the F344 rat; the F1 generation rats were exposed to gamma-radiation from the fetal to the adult stage, and they were examined for the occurrence of renal tumors at the postnatal age of 27 weeks. The result showed that rats exposed to gamma-irradiation at gestation day 19 are highly susceptible to developing renal tumors. In this study, we investigated whether it is possible to detect radiation-induced renal tumors in a short period of time.

Male Eker rats (*Tsc2* mutant) were mated with female F344 rats. The F1 generation rats were exposed to 2 Gy of ¹³⁷Cs gamma radiation at gestation days 15 and 19, or at postnatal days 5 and 20. The experimental animals were housed and maintained under SPF conditions, including during the gamma-irradiation procedure. In this animal model, early proliferative lesion occurs in the renal tubule around the postnatal age of 8 weeks. Thus, dissections were performed at the age of 8 weeks. During the dissections, the kidneys were weighed and then fixed with 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with HE. The prepared paraffin sections were used to determine the incidence of proliferative lesions in the renal tubular epithelium.

In both male and female F1 generation rats with a heterozygous *Tsc2* genotype, gamma-irradiation—except for irradiation at gestation day 15—caused an increase in the number of atypical tubules. Particularly, the animals exposed to gamma-radiation after birth had a marked increase in the number of atypical tubules. Compared with the control group, the irradiated animals had a greater number of atypical hyperplasia, irrespective of irradiation time points. Renal tumors were not found in the control group. On the other hand, renal tumors were observed in the irradiated animals; however, the incidence of renal tumor was comparable between the different irradiation time point groups. The pattern that proliferative lesions appeared in the kidney was different from the pattern observed when irradiated animals were dissected and examined at the postnatal age of 27 weeks; however, the result of this study suggests that radiation-induced renal tumor can be detected in a short period of time after irradiation.

P-74

Chemopreventive Effects of Capsaicin and Silymarin in F344 *gpt* Delta Transgenic Rat.

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In vivo genotoxicity study using rodent model is useful to assess mutagenicity and carcinogenicity in target tissues. The integrations of mutagenicity and carcinogenicity tests may contribute to the reduction of the number of experimental animals. The *gpt* delta transgenic rat has transgene lambda EG10 DNA with the *gpt* gene of *E. coli* used as a reporter for detection of point mutations. In this study, we examined chemopreventive effects of two natural products against liver and colon carcinogenesis utilized 7 week-old male transgenic rats. Capsaicin from the red chili pepper and silymarin from the milk thistle are prospective chemopreventive agents. In the experiment 1, rats were given drinking water containing 20 ppm *N*-diethylnitrosamine (DEN) for 5 weeks. One week before this regimen, diets containing 0, 100 or 500 ppm capsaicin were supplied for 7 weeks. While GST-P positive foci were detected in DEN treatment groups, any effects of capsaicin were not observed. In the experiment 2, rats were given a single subcutaneous injection of 1,2-dimethylhydrazine (DMH, 40 mg/kg b.w.) followed by 1.5% dextran sodium sulfate (DSS) in their drinking water for a week. One week before this regimen, diets containing 0, 100 or 500 ppm silymarin were supplied for 4 weeks. Aberrant crypts foci (ACF) were detected in DMH/DSS treatment groups and the number of ACFs was significantly decreased in the silymarin treated groups. The results of mutant frequency and carcinogenicity in the target tissues were also discussed.

P-75

Modifying Effects of Co-treatment with Tocotrienol on *in vivo* Mutagenicity and Preneoplastic Lesion Development in the Liver of F344 *gpt* delta rats Exposed to Diethylnitrosamine

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Tocotrienols have higher antioxidant activity than tocopherols and may therefore be promising candidates for use as chemopreventive agents. In the present study, to clarify the protective effects of tocotrienol-rich oil (TTE) against diethylnitrosamine (DEN)-induced *in vivo* mutagenicity and preneoplastic lesion development in the livers. Male 7-week-old F344 *gpt* delta rats were given 1% dietary dose of TTE and intraperitoneally injected with 20 mg/kg body weight of DEN once a week for 13 weeks. Control groups were fed only basal diet with or without DEN treatment during the experimental period. At necropsy, the livers were removed for measurement of 8-hydroxydeoxyguanosine (8-OHdG) levels and *gpt* mutations in liver DNA, and immunohistochemical analyses for glutathione *S*-transferase placental form (GST-P) and proliferating cell nuclear antigen (PCNA) in liver tissues were performed. Significant increases in 8-OHdG levels and *gpt* mutant frequencies were observed in the DEN-treatment group compared to the control values, but they were not affected by TTE treatment. On the other hand, significant increases in both the number (/cm²) and area (mm²/cm²) of GST-P positive liver cell lesions (>0.2 mm in diameter) were found by TTE treatment, in line with observations that labeling indices for PCNA-positive hepatocytes and levels of *cyclin E1* were significantly enhanced compared with the DEN-treatment group. In conclusion, TTE did not effectively protect against DEN-induced oxidative DNA modification and *in vivo* mutagenicity in the livers. To the contrary, our data further suggest that TTE may enhance the development of liver preneoplastic lesions induced by DEN possibly due to acceleration of cell cycle progression.

P-76

Occurrence of spontaneous inflammation of molar gingiva in rats

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【Background】 There are limited reports about spontaneous lesions in oral tissues of laboratory animals. This is presumably because these tissues, except for the tongue, are often not examined routinely in general toxicity studies. Histopathological analysis on molar gingiva in various aged rats was conducted, with evaluation of characteristics and incidences of inflammatory lesions in molar gingiva at several ages.

【Materials and methods】 Upper and lower jaw were collected from female and male CrI:CD(SD) rats at 12, 16, 21, and 34 weeks of age and were fixed in formalin, decalcified in formic acid mixture, and embedded in paraffin. The paraffin-embedded sections of maxillary and mandibular gingiva with molars were stained with hematoxylin-eosin and examined histologically.

【Results】 Spontaneous inflammation was frequently in molar gingiva. The incidence of inflammation at 12, 16, 21, and 34 weeks in rats was 8/20(40%), 18/59(31%), 24/79(30%), and 50/95(53%), respectively. The incidence was similar irrespective of age. The inflammation was noted most frequently in the gingiva between first and second molar teeth. Mild lesions consisted of focal infiltration of inflammatory cells in mucosal epithelium or submucosal tissue, with occasional inflammatory exudate in the interdental gingiva. On the other hand, more severe cases consisted of an abscess in deep submucosa accompanied by absorption of adjacent alveolar bone. In 80% of rats with the inflammatory lesions, hair shaft was observed in the lesion. Bacterial colony was also observed around the hair shaft in the most severe cases.

【Conclusion】 Inflammatory changes in the molar gingiva were common in rats irrespective of age. The most common cause of inflammation is considered to be accidental piercing of hair shaft into the gingiva. Cases of systemic bacterial infection experienced in long-term toxicity studies or with immuno-suppressants occasionally suggest infection originating in oral cavity. The results indicate the presence of inflammation by piercing of hair shaft into the molar gingiva in rats as a common background change. This should be taken into consideration as a possible cause of systemic bacterial infection in safety evaluation of drugs.

P-77

Effect of Olopatadine Hydrochloride on Allergic Ear Auricular Dermatitis by Repeated Topical Application of Oxazolone in Mice

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【Object】Olopatadine hydrochloride (Allelock®, hereinafter called olopatadine) is anti-allergy agents widely used allergic rhinitis, urticaria, itch accompanying cutaneous pruritus and various skin diseases. The skin lesion induced by repeated application of hapten similar in that of the patient with atopic dermatitis. In this study, we investigated anti-inflammatory effect of olopatadine using a mouse dermatitis model induced by repeated hapten application.

【Materials and Methods】As sensitization, 10 µL of 0.5 w/v% oxazolone dissolved in acetone was treated on the inner surface of right ear auricle in male BALB/c mice at the age of 6 weeks. At 7 days after sensitization, the same region was challenged 3 times per week with the same dosage and composition of oxazolone, and then dermatitis was induced. In non-sensitization group, acetone was used instead of oxazolone. Olopatadine (10 mg/kg/day, p.o.) and betamethasone (0.12 w/v%, 10 µL/day, topical) were administered simultaneously from the day of the first oxazolone challenge and once a day for 17 days. On the day of following the last administration of olopatadine and betamethasone, the right auricles were removed, and routinely fixed with formalin, embedded in paraffin and sectioned. Sections from each animal were stained with H&E method, toluidine blue (visualized mast cells) and Fast Green FCF (visualized eosinophils), and examined histopathologically.

【Results】Repeated topical application revealed epidermal thickness, inflammatory cell infiltration and abscess, erosion in epidermis, and crust formation. The dermis thickened markedly with inflammatory cell infiltration (neutrophils, eosinophils and mononuclear cells), fibrosis and increased number of mast cells. Betamethasone as well as olopatadine ameliorated the severities of the above changes equally.

【Conclusion】Olopatadine exhibited the anti-inflammatory effect on the mouse dermatitis model induced by topical repeated hapten application. From this result, olopatadine was expected to improve the pathological condition of atopic dermatitis in humans.

P-78

Morphological effects by Treatment with Compound X, a Possible Sterol Synthesis Inhibitor, in Rat Palatogenesis.

(II).

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We have reported that cleft palate was found in rat fetuses of dams treated with compound X, a possible sterol synthesis inhibitor, and that cleft palate caused by inhibition of palatal shelf elevation without an abnormality of shelf components (2007). The present study was conducted to examine contribution of the tongue and submaxilla. Dams were treated once orally with 100mg/kg of compound X on gestation day (GD) 13, and fetuses were extracted on GD 15-17. Transverse and longitudinal tissue sections of the head were stained with HE, the TUNEL method and desmin immunohistochemistry. The fetuses from non-treated dams were used as controls. On GD 16, tongue descent was arrested in the treated group and the tongue persisted between palatal shelves on GD 17, as palatal shelves fused in the control group. There were no abnormalities in glossal tissues stained with TUNEL and desmin. In the treatment group during GD 16-17, distances from the hyoid to the tip of Meckel's cartilage and from the pituitary to the tip of Meckel's cartilage were shorter than those of the control, and necrosis/degeneration of cartilage cells and TUNEL positive reaction in Meckel's cartilage were observed. These results suggest that inhibition of tongue descent was not due to injury or developmental delay of the tongue. Injury of Meckel's cartilage was found to be a potential pathogenesis in compound X-induced cleft palate.

P-79

Observation of neonatal brain in a rat valproate-induced autism model: comparison with changes in fetal and neonatal brain

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We have already examined the direct effects of prenatal sodium valproate (VPA) exposure on the fetal brain (gestational day (GD) 14, 16, or 20). VPA-induced neurotoxicity was detected in the cortex, midbrain and pons. Hypoplasia of cortical plate and abnormal traveling of the neural fasciculus at the isthmus, and disturbance of the migration of tyrosine hydroxylase (TH)-positive and serotonin (5-HT) neurons at VTA and raphe were observed on fetal brain observations.

In this study, neonates on postnatal day 11 (PD11), which the DNT guidelines recommend to conduct neuropathological analyses, were examined histopathologically after dams were administered 800 mg/kg of VPA on GD11, and we examined the reliable and sensitive endpoints to detect DNT among GD16, GD20 and PD11 brain observations.

PD11 male neonates were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PFA), embedded in 10% gelatin, and coronal and/or sagittal serial sections were cut.

The hypoplasia of cortical plate observed in VPA-treated fetal brain was shown as the thinner cerebral cortex, but the construction of cortical layers was similar in the VPA and control groups.

Whereas no remarkable changes in the midbrain and pons were observed in the VPA-treated PD11 brain by nissl stain, immunohistochemical examination revealed the disturbance of the migration and/or distribution of TH-positive and serotonin neurons in the ventral tegmentum (A10 and VTA) and median raphe. These changes were also noted in the fetal brain. In the cerebellum, patchy loss of Purkinje cells in folia VI and VII was observed.

These results indicate that brain morphological changes in neonates treated with prenatal VPA, except the cerebellum, were predicted on the basis of morphological changes in the fetal brain. Thus, fetal brain observation combined with neonatal cerebellar analysis is useful to detect chemical-induced DNT in an early developmental stage.

P-80

N,N'-bis(2-chloroethyl)-*N*-nitrosourea (BCNU)-induced apoptosis of neural progenitor cells in the developing fetal rat brain

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N,N'-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) is one of the major drugs used in chemotherapy against malignant gliomas due to its effects, such as induction of bifunctional alkylation of DNA and formation of interstrand DNA cross-linkages, and induces cortical malformations in the fetal and neonatal rat brain. In this study, pregnant rats were treated with 7.5 mg/kg of BCNU on gestational day 13 (GD 13), and their fetuses were collected from 12 to 72 hours after BCNU treatment in order to examine the timecourses of morphological and immunohistochemical changes in neural progenitor cells in the developing brain. The number of pyknotic cells in the telencephalon peaked at 24 h and then gradually decreased until 72 h. The majority of these pyknotic cells were positive for cleaved caspase-3, a key executioner of apoptosis. The pyknotic cells showed the ultrastructural characteristics of apoptosis. The number of p53-positive cells began to increase prior to the appearance of apoptotic cells and p21-positive cells. The number of phosphorylated-histone H3-positive cells (mitotic cells) decreased from 24 to 36 h. The number of Iba1-positive cells (microglial cells) in the telencephalon increased from 12 to 48 h. These results suggest that BCNU induces p53-dependent apoptosis and reduces proliferative activity, resulting in reduction of the weight of the telencephalon and the thickness of the telencephalic wall in the fetal brain. This study will help to clarify the mechanisms of BCNU-induced fetal brain toxicity.

P-81

Chronological Changes of 2', 3'-Cyclic Nucleotide 3'-Phosphodiesterase (CNPase) in Spongy Change of Central Nervous System Induced by Repeated Oral Dose of Hexachlorophene (HCP) and Cuprizone (CPZ) in Rats

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Spongy change was observed in the central nervous system white matter in weanling rats given HCP and CPZ. This study was conducted to identify the mechanism of the spongy change induced in rats after repeated dose of HCP and CPZ. <Materials & Methods> Study 1: Twenty-two 21-day-old female Crj:WI(Wistar) rats were treated orally with 35 mg/kg HCP for 5 days and recovered for 7 days. Study 2: Thirteen 21-day-old male Crj:(CD)SD rats were fed powdered chow containing 1w/w% CPZ for 10 days and received normal diet for 14 days. The brain and spinal cord were removed and were stained with HE, followed by immunostaining. <Results> Study 1: Spongy change in the cerebrum, cerebellum, medulla oblongata, and spinal cord white matter and degeneration of oligodendroglia was found from day 2. The severity of both lesions increased prominently on day 5. Some oligodendroglia on day 12 revealed strong immunostaining of CNPase extending into the whole cytoplasm. Study 2: Spongy change in the cerebrum and cerebellum white matter and cell death of oligodendroglia were found from day 3. The severity of both lesions increased prominently on day 8. Some oligodendroglia on day 8 revealed strong immunostaining of CNPase extending into the whole cytoplasm. Compared to the controls, the expression of MBP immunostaining decreased in the white matter on days 3 to 10. It is suggested that the strong positive reaction in CNPase expression may be associated with regeneration of myelin and oligodendroglia induced by HCP and CPZ intoxication.

P-82

Effect of Busulfan on Neonatal Cerebellar Peduncle in Rats

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[Aim] Busulfan, an antineoplastic alkylating agent, is known to induce developmental anomalies and fetal neurotoxicities. We reported that busulfan-induced damages of fetal central nervous system and neonatal cerebellar granule cell layers in rats. In the present study, we demonstrate histological damages of the cerebellar peduncle in neonatal rats administered with busulfan.

[Methods] Animals were male Crj:CD(SD) rats, 6 days of age, and were treated subcutaneously with a single dose of Busulfan at 0, 10, 20 and 30 mg/kg. A total of 25 animals in each group were euthanized on days 1, 2, 4, 7 and 14. Four animals at 30 mg/kg and one animal at 20mg/kg died or were sacrificed moribund due to severe myelosuppression on day 12 and 13. Therefore these animals were excluded from this examination. The brains were weighed, fixed with neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, Kliver-Barrera and TUNEL method. In addition, an immunohistochemical staining using various antibodies was performed.

[Results] In the control group there were no histological changes in the cerebellar peduncle. In the Busulfan-treated groups, cell death characterized by pyknosis and karyorrhexis was sporadically observed in the cerebellar peduncle on days 1 and 2. These cells demonstrated positive reaction for TUNEL method and cleaved caspase-3 immunohistochemistry, and it was considered to be apoptosis. The number of apoptotic cells was peaked on day 2 and decreased thereafter. On the contrary, mitotic figures sporadically appeared on days 4 and 7 in the Busulfan-treated groups. Immunohistochemistry for phospho-histone H3 revealed that mitotic activity was elevated on days 4 to 7 and returned to the control level on day 14. These changes were more prominent in animals dosed at 20 and 30 mg/kg. Morphologically, the mitotic cells observed in animals treated with Busulfan were characterized by pale cytoplasm, locating near nerve cells. The feature of these cells was not clear in the immunohistochemical examination.

[Conclusion] The present data demonstrates that Busulfan induces apoptosis in the cerebellar peduncle of neonatal rats, and it may be a transitory effect with immediately recovery by cell renewal.

P-83

Lung and Testicular Development in Rats with Prenatal Perfluorooctane-sulfonate Exposure

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【Background】 Perfluorooctane sulfonate (PFOS) is fluorinated organic compounds that have been used for surfactant in applications ranging from oil and water repellent to specialty chemical applications such insecticides and fire fighting foams, and is suggested to widespread in wild life and humans. Although PFOS have been reported to induce developmental and reproductive effects, the detail is unclear. **【Materials and Methods】** Pregnant SD (slc) rats were given intragastrically 1mg/kg PFOS (1mg PFOS group), 0.5mg/kg PFOS (0.5mg PFOS group) or vehicle (Vehicle group) on day 6th though 15th after conception. We investigated the several biological parameters of offspring rats, and they were autopsied at 7, 10, 13 weeks old.

【Results】 Number of male offspring showed increase in 1mg PFOS group, but body and liver weights were decrease at birth. Male and female in 1mg PFOS group and 0.5mg PFOS group showed significant delay the day of eye opening. Female 1mg PFOS group and 0.5mg PFOS group showed significant increase of AGD. PFOS group showed hyperplasia of pulmonary alveolar type II epithelial cells and increase frequency of testicular seminiferous stage II & III, and decrease that of stage V.

【Conclusion】 The present study revealed that prenatal PFOS exposure induced pulmonary hyperplasia and disruption of testicular spermatogenesis.

P-84

Characterization of Increased Reelin-expressing Cells in the Hippocampal Dentate Gyrus Induced by Developmental Hypothyroidism in Rats

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Reelin is an extracellular matrix protein that plays an important role for neuronal migration and correct positioning during neuronal development. We have recently demonstrated the availability of measuring Reelin-expressing cells in the hippocampal dentate gyrus for detection of impaired neuronal differentiation induced by developmental neurotoxicants utilizing a rat hypothyroidism model with anti-thyroid agents. In the present study, to characterize the increased Reelin-expressing cells in the dentate hilus, immunohistopathological analysis was performed on rat offspring exposed maternally to 200 ppm methimazole, or 3 or 12 ppm propylthiouracil (PTU) in the drinking water from gestation day 10 until weaning at postnatal day (PND) 20. At PND 20 and postnatal week 11 (PNW11; adult stage), Calbindin (Calb)-D-28K or Glutamic acid decarboxylase (GAD)-67-immunoreactive cells suggestive of GABAergic interneurons and NeuN-positive postmitotic mature neurons were counted as with that of Reelin-expressing cells in male offspring. At PNW 11, double staining of Reelin and NeuN was also performed. In addition, apoptotic cells detected with Cresyl violet and PCNA-positive proliferating cells in the subgranular zone (SGZ) were also counted at both PND 20 and PNW 11. Reelin-expressing cells showed a sustained increase in the dentate hilus until the adult stage by exposure to anti-thyroid agents, in parallel with Calb-D-28K-expressing cells at PND 20 and with GAD67-positive cells at PNW 11, confirming an increase in GABAergic interneurons. At the adult stage, increase of NeuN-positive cells was also observed in the hilus; however, the increased population of Reelin-producing cells at this stage was either weakly-positive or negative for NeuN, indicative of immature neurons. In the SGZ, increased apoptotic cells and decreased proliferating cell suggestive of impaired neurogenesis occurred only at PND 20. These results suggest that sustained increases of an immature population of GABAergic interneurons synthesizing Reelin in the hilus revealed here could be a signature of compensatory regulation for impaired neurogenesis in the SGZ and mismigration due to developmental hypothyroidism. Notably, it was suggested that sustained production of Reelin-positive interneuron was highly associated with neuronal mismigration.

P-85

Pathological Investigation of The Rat Pituitary Gland on The Low Dose Estrogenic Agent-Induced Delayed Effect

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It has been well known that fetal or neonatal exposure of unphysiologically high dose estrogenic agents (i.e. ethinyl estradiol, p-t-octylphenol) causes androgenization in female rats. This effect is characterized by an early reproductive failure, namely abnormal estrous cycle (delayed effect), after undergoing of normal sexual maturation. It is suggested that the sexual abnormality is not attributed to the direct effects on genitals, but to the act on central nervous system (CNS) by these chemicals. In the present study, we treated 0.2, 2.0 or 20 ng/kg/day of diethylstilbestrol (DES, peroral) to mother rats from 6th day of pregnancy to 20th day of lactation for investigation of the delayed effect elicited by low dose of estrogenic agents. After treatment, their daughters' pituitary glands, exposed by the low dose of DES via placenta or lactation, were collected on 29th week after birth, and were morphologically examined by immunohistochemical staining of ER alpha, FSH or prolactin. The daughters which observed continuous aberrations in estrous cycles, revealed decreased number of pituitary type-I cells and lower staining intensity for ER alpha and FSH in both of pituitary type-I and -II cells. Although the daughters in the DES-treated groups showed transient aberration of estrous cycle, which recovered normal cycle at the time of necropsy did not exhibit the morphological abnormalities in the pituitary gland. These results indicate that a responsible site for the aberration of estrous cycle (delayed effect) is not pituitary gland, but presumably upper nervous system. Currently, we are investigating influences of low dose DES on upper CNS, especially on the hypothalamic-pituitary system.

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Sequential Morphological Changes of Small Follicles in the Ovary Damaged by Prepubertal Exposure to Gamma Ray

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Small follicle damage is irreversible and its detection is very important. Radiation targets small follicles, especially in immature animals. We investigated sequential morphological changes of small follicles damaged by prepubertal exposure to radiation in comparison with those in adults. The ovaries and major organs were examined 6 hrs, 1 day, 1 week or 9 weeks after the exposure to gamma ray at 0.2 or 1.0 Gy in Donryu female rats aged at 2 or 10 weeks. The treated effects on the ovary were found in rats prepubertally exposed at the high dose only. The effects were detected as apoptotic changes of the oocytes in the primordial or primary follicles at 6 hrs after the exposure. Apoptotic changes were also found in the granulosa layer cells in the secondary, preantral or antral follicles at the same point, but slight. At 1 day after the exposure, most of oocytes immediately disappeared in small follicles but the granulosa layer cells looked intact. At 1 week after the primordial or primary follicles were depleted, while any abnormalities were detected in preantral or antral follicles. Timing of the vaginal opening was not different from that same aged controls. Interestingly, estrous cyclicity was normal during 7 weeks after the exposure, at 9 weeks of age. At 11 weeks of age, most of rats showed persistent estrus in the vaginal smear. The ovary at 11 weeks of age showed severe atrophy without normal follicles or corpora lutea. No abnormalities were detected in serum gonadotropin and ovarian hormone levels at 4 weeks after the exposure in both prepubertal and matured animals in an additional study.

These results indicate that small follicles in the immature ovary are very sensitive to the damage of radiation. Apoptotic oocytes in the small follicles and consequent loss of small follicles are early occurring characteristics of the damage. However it took 1 month and more for the damage to be easily recognized the damage using other indicators such as the atrophic ovary or disruption of estrous cycle. Morphological investigation is one of the useful tools to detect ovarian toxicity including small follicle damage.

P-87

Luteal Function-Related Gene Analysis of New and Old Corpora Lutea in Normal Cycling Rats using Laser Capture Microdissection

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Detection of ovarian toxicants targeting the corpora lutea (CL) or follicles is very important for toxicologic pathologists. In normal estrous cycling rats, CL regress over several cycles but their function of progesterone (P4) production is insufficient and very limited compared to other species. In the present study, we clarified relationship between the function and morphology of new and old CL in normal cycling rats as a basic approach for detection of ovarian toxicants targeting CL. We especially focused on steroidogenic and luteolytic gene expressions in new CL of each estrous stage.

The ovaries of female SD rats at each estrous cycle (estrus, metestrus, diestrus, and proestrus) were obtained from normal cycling SD rats. Using frozen sections of the ovary, new and old CL were separated with laser capture microdissection and analyzed for mRNA expression of steroidogenic genes (P450scc and 3 β -HSD), and luteolytic gene (20- α HSD) with real-time PCR. The paraffin sections were also prepared for immunohistochemistry of P450scc and 3 β -HSD. The CL are morphologically classified into new and old CL, and new CL are defined as newly formed CL by the current ovulation.

In new CL, the expression of P450scc mRNA reached the highest level at metestrus and gradually decreased from this stage, and 3 β -HSD mRNA was gradually increased from estrus to diestrus but decreased at proestrus. Meanwhile, 20 α -HSD mRNA expression was remarkably low at estrus and metestrus and gradually increased from diestrus to proestrus. Immunohistochemically, both of P450scc- and 3 β -HSD-positive luteal cells were observed in all CL throughout the estrous cycle, and their reactions were more intensive in new CL than old CL. The steroidogenic and luteolytic gene expressions of new CL were relevant to serum P4 levels during estrous cycle. These results provide the clear evidence that new CL at metestrus, which have function of P4 production, show high steroidogenic gene expression and low luteolytic gene expression in cycling rats.

P-88

Investigation of Biomarkers using Drug-Derived Cardiotoxicity-Induced Rat

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Recently, biomarkers for prediction of cardiotoxicity, such as cardiac troponin-I (cTnI), brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP), has been reported. Since those markers are recognized as an effective marker for detection of cardiotoxicity, it has not been clearly revealed the relevancy between the elevation of those markers and the alteration of heart in histopathology. In this study, we confirmed whether those cardiotoxicity markers were correlated with histopathological changes in the heart using the cardiotoxicity model rats induced by administration of isoproterenol (ISO), doxorubicin (DXR) or monocrotaline (MCT).

Dose-dependent degeneration and/or necrosis of myocardia and infiltration of inflammatory cells were verified at left ventricle in the ISO-treated group. However, no significant alterations were confirmed in the DXR and MCT-treated group. In the plasma concentration of cTnI, BNP, and ANP using enzyme-linked immunosorbent assay, the concentration of cTnI was significantly elevated with slight histopathological changes at 6 hours after administration in the ISO-treated group. But the level of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were not elevated. On the other hand, although ANP was elevated at 24 hours after administration, BNP was not significantly elevated. In the DXR-treated group, the concentration of cTnI was elevated at 9 days after administration, though no significant histopathological changes were revealed. In MCT-treated group, cTnI was not elevated, though ANP and BNP were significantly elevated without significant changes in histopathology at 10 days and 14 days after administration, respectively.

These results suggested that plasma concentration of cTnI sensitively reflected myocardial injury, such as degeneration and/or necrosis, and all markers were able to elevate with very slight pathological changes which showed no significant changes in histopathological analysis. Although those cardiotoxicity markers are sensitive, but the variation is depend on drug, and grade or pattern of cardiotoxicity. Therefore, it is important that several markers should be measured at the correct schedule and be evaluated comprehensively for decision the cardiotoxicity.

P-89

Possible Genomic Biomarkers for Chemically-induced Cardiotoxicity in Rats Explored Using a Toxicogenomics Approach

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In this study, we aimed to explore potential genomic biomarkers for cardiotoxicity in rats using a toxicogenomics approach.

We induced cardiac lesions in rats by the following three cardiotoxic compounds, isoproterenol (0.01 mg/kg; s.c.), doxorubicin (20, 40 mg/kg; i.p.) and carbofuran (1.5 mg/kg; i.p.). We then conducted histopathological examination and microarray analysis using the heart sampled at 8 or 24 h after single dosing.

Isoproterenol and carbofuran induced necrosis and infiltration of inflammatory cells at both time points, while doxorubicin induced only edema in the 40 mg/kg group at 24 h after dosing. There were no histopathological changes in doxorubicin 20 mg/kg group. In the toxicogenomics analysis, we detected 32 genes which were commonly up-regulated following treatment with the three cardiotoxic compounds. Although most of these genes showed transient up-regulation, *Spp1*, *Fhl1*, *Timp1*, *CC17* and *Reg3b* showed consistently high up-regulation. In addition, some of the five genes showed up-regulation in doxorubicin 20 mg/kg group which showed no histopathological change.

In conclusion, the 32 genes, which were commonly up-regulated by cardiotoxic compounds, might be candidates of genomic biomarkers for cardiotoxicity in rats. In these genes, *Spp1*, *Fhl1*, *Timp1*, *CC17* and *Reg3b* showed consistent up-regulation and seemed to be especially hopeful.

P-90

Gene Expression Analysis in the Skeletal Muscles of Dysferlin-deficient SJL and A/J Mice

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[Object] We have previously reported that endoplasmic reticulum stress (ER-stress) was unlikely to participate in the pathogenesis of skeletal muscle lesions in SJL and A/J mice as model animals of limb girdle muscular dystrophy 2B in human. In this study, the expression of 33 genes including ER-stress markers at the age of 10 and 30 weeks were investigated by quantitative real-time PCR using TaqMan[®] probes to pursue further the involvement of ER-stress with the pathogenesis of skeletal muscle lesions in both mice.

[Methods] The rectus femoris and longissimus lumborum muscles were collected from 3 males each of BALB/c, SJL and A/J mice at the age of 10 and 30 weeks. First-strand cDNA was synthesized by reverse transcription reaction from total RNA extracted from these tissues. The quantitative real-time PCR using specific TaqMan[®] probes for 33 genes including ER-stress markers and these cDNA as templates was conducted. The comparative threshold cycle (CT) method was applied for relative determination of targeted genes to reference *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase) gene among three strains. Changes in mRNA expression level were calculated on the basis of CT value in BALB/c mice at the age of 10 weeks.

[Results and Conclusions] There were no significant differences of patterns in gene expression of the rectus femoris and longissimus lumborum muscles among all strains at the age of 10 and 30 weeks. As for ER-stress markers, the obvious change of BiP/GRP78, ATF6 and CHOP/GADD153 expression levels in SJL and A/J mice was not observed at both ages. In BALB/c mice, the expression level of heat shock protein (HSP) 70 at the age of 30 weeks was 30 to 40 times higher than that at the age of 10 weeks. In SJL mice, the expression level of HSP70 at the age of 10 and 30 weeks remained at 10 to 20-folds higher than that in BALB/c at the age of 10 weeks. In A/J mice, the expression level of HSP70 at the age of 30 weeks lowered compared to that in BALB/c at the age of 10 weeks. The expression of HSP40 known as co-chaperon of HSP70 was similar in pattern of HSP70 expression in three strains. Because HSP70 has a protective effect against various stresses, induction of HSP70 expression in SJL mice at the age of 10 weeks may reflect a part of protective mechanism against early damage of the skeletal muscles.

P-91

Reducing the Toxicity of Therapeutic Antibodies by Epitope Modification — Histopathological Evaluation in Mice Administered Anti-Desmoglein-3 Antibodies —

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Antibodies raised against different epitopes can initiate different biological responses, so the selection of a suitable epitope is thought to be effective for the reduction of toxicity when the target molecule of a therapeutic antibody is expressed in non-diseased tissue. Desmoglein-3 (Dsg3) is a promising target for anticancer therapy of squamous cell carcinoma, but is also related to human pemphigus vulgaris, and an anti-Dsg3 antibody (clone Ak23c directed against 1-87 of the N terminus) induces blisters in the skin of mice.

We have attempted to separate the toxic effects from the beneficial effects of anti-Dsg3 therapy by constructing an antibody (clone 18-1m) directed against an epitope (161-490 of the N terminus) that differs from that of the Ak23c antibody. Three weekly intravenous administrations of the 18-1m antibody to female SCID mice caused no clinical abnormalities and induced no significant changes in the skin and esophagus. A single dose of the Ak23c (provided by Forerunner Pharma Research Co., Ltd.) caused morbidity with a severe decrease in body weight. Necrosis of the whole layer of esophageal tissue with inflammatory cell infiltration, and single cell necrosis of the hair follicle were observed.

In conclusion, the changes induced by the 2 anti-Dsg3 antibodies differed, indicating that epitope selection is effective for reducing the toxicity of therapeutic antibodies.

P-92

Background variation of histopathological changes of the SPF pigs

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【Purpose】 Recently, it has been thought that pigs are useful animals as experimental animals, since they have biological characters which are similar to those of human. However, reports of histopathological background data are less. Our test facility has performed safety studies of animal vaccines using SPF pigs. Therefore, we report the histopathological background data of the SPF pigs.

【Methods】 Animals which were totalized were male and female animals of the control groups of safety studies. Examined organs were brain, spinal cord, liver, kidney, heart, lungs, trachea, stomach, duodenum, jejunum, colon, pancreas, spleen, thymus, submandibular lymph nodes, mesenteric lymph nodes, skin and the sex organs. Paraffine-embedded sections of each organ were routinely prepared and stained with hematoxyline and eosin for the light microscopic observation.

【Results】 Changes were observed in liver, lung, kidney and digestive organs. Extremely slight infiltration of mononuclear cells and neutrophils was seen in the liver. In the lung, extremely slight infiltration of mononuclear cells and lymphocytes also was seen in bronchus. Regarding changes of the kidney, extremely slight infiltration of mononuclear cells and lymphocytes was observed in interstitial tissues. In the digestive organs, infiltration of lymphocytes also was seen in lamina propria.

【Conclusion】 In the pigs which are bred in the conventional environment, pneumonia, atrophy of the villus of intestine, hyperplasia of the goblet cells, and hyperplasia of lymphatic nodule are often observed. However, these changes were not seen in the SPF pigs which were bred under the clean environment. There were few changes, and the changes which were observed in the each organ were slight.

P-93

Characterization of spontaneous neoplastic lesions in long term maintained BRIHan:WIST@Jcl (GALAS) rats; especially hematopoietic neoplastic lesions

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[Background] Recently, Wistar rats have been frequently used in chronic and carcinogenicity studies with agrichemicals in addition to the existing strains, F344 and SD. For appropriate estimation of toxicity of chemicals, understanding of historical data in each strain is considered to be important. Furthermore, adequate selection of rat strain for toxicity estimation is considered to be ideal. Historical data in Wistar hannover rats, however, is not sufficient when compared to other 2 strains because of the recent strain establishment. Even in the NTP, historical database was provided only in the strains of F344 and SD, not in Wistar Hannover. In the present study, we did retrospective study on neoplastic lesions, especially hematopoietic tumors in Wistar hannover rats used in the long term studies conducted in the IET to point out the characteristics of these tumors in the Wistar Hannover rats. **[Method]** Historical data of neoplastic lesions were collected from 5 carcinogenicity studies conducted in the IET from 2005 to 2007. Furthermore, on rats diagnosed as malignant lymphoma, the incidence of the lesion and involved organs were calculated for each sex. As no induction of hematopoietic tumors was suspected in any studies referred, the incidences were picked up from both of the control and high dose groups (systemic organs/tissues were examined histopathologically in these groups). **[Result]** Forty-four per 816 rats, 17/408 (4.2%) males and 27/408 (6.6%) females, were diagnosed as malignant lymphoma. Thymus was most frequently involved organ (100%). Detailed examination showed two types of tumors by developing site; tumor cells proliferated only in the thymus, or the liver, spleen and lymph nodes were also involved. Detailed histopathological examination including immunohistochemistry has been conducted on these lesions and differential diagnosis of lymphocytic thymoma and thymic lymphoma is also discussed.

P-94

Morphological Study of Lymphoid Organs in Cynomolgus Monkeys: Quantitative Analyses for Immunotoxicity Evaluation

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[Introduction] In recent years, the cynomolgus monkey has been widely used in toxicity studies; however, the state of morphological research on the immune system is insufficient for an evaluation of immunotoxicity in pathological examinations. In the present study, we quantitatively analyzed data on the lymphoid organs from cynomolgus monkeys.

[Materials and Methods] We sampled the spleen, major lymph nodes across the whole body, and Peyer's patches from naive male and female cynomolgus monkeys (*Macaca fascicularis*, 3 to 8 years old) imported from China, and examined them with H.E. staining, anti-CD3 immunostaining, and anti-CD20 immunostaining. Imported images were analyzed with analySIS software. Additionally, the spleen and lymph nodes were weighed and measured.

[Results and Discussion] It was considered that submandibular and mesenteric lymph nodes were suitable for reliable histopathological evaluation, because the sampling and the specimen preparation were easily accomplished, and the variations in weight and size were slight. For histopathological evaluation of the Peyer's patches, it was considered that almost all their compartments were clearly visible when sectioned multiply along the minor axis. The diameters of the lymphoid follicles in the spleen, submandibular lymph nodes, mesenteric lymph nodes, and Peyer's patches were $515.7 \pm 124.3 \mu\text{m}$, $279.7 \pm 60.7 \mu\text{m}$, $209.1 \pm 40.5 \mu\text{m}$ and $305.2 \pm 46.2 \mu\text{m}$, respectively (mean \pm S.D.). Germinal centers were clearly observed in all of the lymphoid follicles of the spleen, submandibular lymph nodes, mesenteric lymph nodes, and Peyer's patches. No sex difference was noted in any item. It was considered that the lymph nodes are useful to evaluate the changes in T cells, and the spleen and Peyer's patches in B cells.

P-95

Investigation of Age- and Sex-differences in the Thymus and Spleen with Immunohistochemistry in Crl:CD(SD) Rats

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The effect on immune development and sex differences in immune system changes need to be considered as part of any evaluation of immunotoxicity in neonatal or long-term toxicity studies.

The thymus and spleen were sampled from male and female Crl:CD(SD) rats (aged 1 day, and 1, 2, 3, 4, 9, 19, 23, 46, and 57 weeks), weighed, and stained immunohistologically with CD3 for Pan-T cells, CD4 for helper/inducer T cells, CD8 for suppressor/cytotoxic T cells, CD45R for B cells, ED1 for macrophages, NKR for natural killer cells, and PCNA for mitotic cells. For each antibody, the positive cells in the cortex and medulla of the thymus, and the periarterial lymphoid sheath (PALS), marginal zone (MZ), and follicle of the spleen were counted with morphometric analysis software. The numbers of positive cells were compared by age and sex.

The relative weight of the thymus versus the brain and body weight increased until 9 and 3 weeks of age, respectively, and decreased with age thereafter. The number of CD3-positive cells was higher in the cortex than the medulla at each week of age, and higher in males than females. CD45R-positive cells appeared with age in the cortex and medulla. PCNA-positive cells showed a similar pattern to CD3-positive cells.

The relative weight of the spleen versus the brain gradually increased with age, and its relative weight versus body weight increased until 2 weeks of age, and decreased with age thereafter. The number of CD3-positive cells was markedly high in the PALS and higher in males than females. CD45R-positive cells increased with age in the PALS, MZ, and follicle in males and females.

In general, chronological changes in thymic and splenic weights and cell counts had similar tendencies in males and females from birth to approximately 1 year old. However, sexual differences were expressed at some measurement points.

P-96

Comparison of Historical Control Data in Carcinogenicity Studies amongst 3 Strains (NMRI, CD-1, B6C3F1) of Mice.

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To select the most appropriate strain, historical control histopathology data of carcinogenicity studies performed at Harlan Laboratories Ltd Switzerland were compared amongst 3 common used mouse strains (NMRI, CD-1 and B6C3F1).

NMRI Mice:

(1)High Mortality (♂ : 56.9% ♀ : 76.8%). (2) Regarding common tumors in mice, malignant lymphoma, alveolar/bronchiolar adenoma, Harderian gland adenoma, and other tumors were recorded at highest incidences. (3)Granular cell tumors, rare in other strains, occurred in seminal vesicles, ovaries and uterus. (4)Liver cell necrosis and Kupffer's cell proliferation were recorded at high incidences. (5)Dermatitis was also present at a high incidence.

CD-1 Mice:

(1)High Mortality (♂ : 52.2% ♀ : 61.1%) . (2) The total occurrence of common tumors was at lowest incidences amongst the 3 strains of mice. (3)Amyloidosis occurred systemically in several organs that are deemed to be depending on suppliers, food or husbandry conditions. (4) The non-neoplastic lesions at high incidences included: peri-/arteritis, chronic progressive nephropathy including glomerulosclerosis, diffuse hyperplasia of the glandular stomach, cardiomyopathy, inflammation and dilation of preputial gland/clitoral glands, dermatitis, granulopoiesis (bone marrow), and deposition of an eosinophilic substance in the nasal cavities.

B6C3F1 Mice:

(1)Low Mortality (♂ : 17.7% ♀ : 26.3%) . (2)Hepatocellular tumors and/or altered cell foci were recorded at highest incidence. (3) Amongst non-neoplastic lesions, uterine cystic endometrial hyperplasia and fibro-osseous lesion in sternum or femur which were considered to be caused by estrogen or imbalance hormonal dyshomeostasis, and mineralization of the brain (thalamus) were recorded at highest incidences.

P-97

A Case of Malignant Mesothelioma Showing Sarcomatoid Growth Pattern in Abdominal Cavity in a Female F344 Rat.

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【Background】 Malignant mesothelioma is spontaneously tumor mainly arising from retroperitoneum and adipose tissue surrounding spermatic cord in male F344 rats. In the historical data in the IET, malignant mesothelioma had been observed in males only and epithelial type showing complex tubulopapillary growth pattern was dominant. We obtained a rare case of malignant mesothelioma which observed in a female F344 rat and showed sarcomatoid growth pattern. In this presentation, we introduce its histopathological characteristic. **【Animal case】** The case was observed in a female F344/DuCrjCrj rat which died at 92 weeks of age belonged to the high-middle dose group in a carcinogenicity study and was considered spontaneous one because no induction of mesothelioma was suspected in the study. At necropsy, retention of bloody ascites and multiple white nodules (less than 5 mm in diameter) in abdominal cavity were observed. Tumor samples were fixed in phosphate buffered 10% formalin, routinely processed, and stained with H&E. In addition, special stains using Oil Red O, PAS, Alcian blue and immunostaining including anti-keratin, vimentin, mesothelin, podoplanin, calretinin, α -smooth muscle actin, desmin and S-100 protein antibodies were provided. Using the part of the nodule fixed in formalin, electron microscopic examination was performed. **【Result】** The tumor consisted with cells which possessed notable atypical nucleus and eosinophilic cytoplasm including vacuoles. Some tumor cells showed signet-ring-like structure by large cytoplasmic vacuoles. The tumor cells proliferated in solid or nodular pattern with some amount of fibrillar connective tissues dominantly, though nest like proliferation was also observed partially. Disseminated metastasis was observed in the liver, pancreas and ovary. The vacuoles in the tumor cells were negative for Oil Red O stain. Granules observed in some tumor cells were positive for PAS reaction. Fibrous stroma was alcian blue positive and hyaluronidase pretreatment was markedly diminished the positive reaction. Immunohistochemically, keratin was strongly positive in almost tumor cells, while vimentin was mainly positive in spindle-shaped cells. Mesothelin and podoplanin were positive in many tumor cells. Electron microscopic examination revealed intercellular desmosome-like structure, basement membrane, and microvillus. **【Conclusion】** Because sarcomatoid growth pattern and abundant cytoplasmic vacuoles observed in the case, liposarcoma was suspected as a diagnosis. However, as detailed examinations revealed its mesothelial nature, we diagnosed the case as malignant mesothelioma.

P-98

A Case Report of the Malignant Ameloblastoma in a Fisher 344 rat

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Malignant ameloblastoma is a rare odontogenic tumor in rats. A case of malignant ameloblastoma spontaneously developed in the maxilla of a female F344/DuCrjCrj rat was presented in this report. At the age of 82 weeks, a hard mass with a diameter of 10mm was found in the perioral area of the rat. At the age of 92 weeks, the rat died.

Macroscopically, an elevated hard nodule with a diameter of 60mm extended from the head to the tip of nose.

Microscopically, a tumor developed in the region corresponding to the incisor of left maxilla. It invaded the surrounding tissues, and thereafter the neighboring bones. The tumor was composed of many enamel-organ like tissues that were various in size and form.

In the peripheral area of enamel-organ like tissues, tall and columnar cells were aligned in palisade arrangement. In the central area of the tissues, polygonal to spindle shaped cells loosely arranged like the stellate reticulum and squamous cell metaplasias with keratin formation were often observed. Solid proliferation of tall and columnar cells was also seen in some parts of the tumor.

The tumor had the characteristic of ameloblastoma that was formed of the enamel organ-like tissues and not formed of the enamel matrix or dentin. Furthermore, it showed high proliferative potential and destroyed the bone and the surrounding tissues. Based on these evidences, we diagnosed this present tumor as a "malignant ameloblastoma."

In addition, it may be possible to consider the diagnosis of "ameloblastoma, acanthomatous" based on the description in the International Harmonization of Rat Nomenclature (2000)."

No similar case has been found in the historical control database of our research center.

P-99

Spontaneous Thyroid Follicular Adenoma with Oncocytic Features in a Cynomolgus Monkey

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There were several reports of thyroid follicular adenoma in nonhuman primates, but only a few of them referred to its detailed histopathological characters. We present a case of spontaneous thyroid follicular adenoma with oncocytic features in a macaque. The animal was a 5-year-old male cynomolgus monkey in a 13-week toxicity study. No abnormality was found in the animal in clinical, clinicopathological, and gross pathological examinations. Formalin-fixed, paraffin-embedded tissues were stained with HE. Immunohistochemistry for thyroglobulin and prohibitin was also conducted for the thyroid. Histologically, a nodular lesion of 2 mm in diameter was found in the thyroid. The lesion was well demarcated without encapsulation showing mild compression of adjacent parenchyma. The lesion was composed of follicles filled with colloid-like materials. The follicles were lined by columnar to polygonal cells which had abundant eosinophilic cytoplasm and one to two nuclei with prominent nucleoli. The cells and colloid-like materials were immunohistochemically positive for thyroglobulin. Most of the cells exhibited diffuse finely granular immunoreactivity in cytoplasm for prohibitin, suggesting the abundant accumulation of mitochondria which is the feature of oncocyte. Invasive growth and metastasis were not observed. In conclusion, this case was diagnosed as thyroid follicular adenoma with oncocytic features.

P-100

Histological identification of unspecified large cells with rich cytoplasm observed in the small intestine of common marmosets.

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The objective of this study is to identify mucosal unspecified large cells with rich cytoplasm observed in the small intestine of common marmosets in negative control groups.

Ileac samples removed from 2 males (3 years old) were routinely fixed in 10% phosphate-buffered neutral formalin solution, embedded in paraffin and sectioned. Sections from each animal were stained with hematoxylin and eosin, Periodic acid-Schiff (PAS) and immunohistochemically visualized with anti-lysozyme (Clone : LYZ-Z, PROGEN Biotechnik GmbH, Heidelberg, Germany) and anti-CD68 (Clone : PG-M1, Code : N1576, Tokyo, Dako Japan). Ileac tissues from the animal stored in fixative solution were frozen with OTC compound, sectioned using a cryostat and stained with Oil red O. All above sections were examined under a light microscope. In addition, small ileac tissues stored in fixative solution from one animal were embedded in epoxy resin and examined under an electron microscopy.

A large number of cells with rich cytoplasm were mainly accumulated in lamina propria and sometimes in submucosal layer. They had weak positive microgranules/droplets of PAS and Oil red O stainings in the cytoplasm. In immunohistochemistry, the cross of the antibodies for common marmoset was not confirmed. Electron microscopic examination revealed that the unspecified cells were divided into two types by size of cytoplasmic vesicle. One had some of large vesicles (almost large as nucleus), which showed a medium and homogenous electron density and considered to be lipid droplets. The other contained small vesicles enclosed in single membrane and having small droplets with different electron density. This small vesicle was considered to be a lysosome and contain the neutral lipid corresponding Oil red O staining.

In conclusion, the unspecified cells were considered to be macrophage-like cells with lysosomes containing some lipids.

P-101

So-called Buphthalmos Developed in Albino Rabbits : Congenital Glaucoma

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Buphthalmos is a type of developmental glaucoma with early onset. Although few cases have been reported in rabbits, details of the pathogenesis have not been clarified. A breeder in Japan has made an animal model of the disease by successive mating of rabbits with naturally occurring buphthalmos with their siblings. We obtained some animals of the model from this breeder. In this study, ophthalmological and histopathological examinations were conducted in 4 animals: Japanese white (JW) rabbits at 76 and 86 weeks of age, and New Zealand White (NZW) rabbits at 93 and 68 weeks of age.

In the outward appearance of the rabbits, protrusion of the eye was found in 5/8 eyes. In intraocular pressure (IOP) measurements, abnormal changes were observed. Fundus examinations showed a depression of the optic disc, and slitlamp observations showed corneal opacity cornea and irregular corneal surface. In histopathological examinations, swelling of the eyeball, expansion of the anterior chamber, and hypoplasia of the trabecular meshwork (TM) were observed. By transmission electron microscopy, fine fibrillar-like material, basal lamina-like material and fine granular-like material were seen to have accumulated in the TM. As a secondary change caused by chronically high IOP, retinal atrophy, optic disc cupping, axonal atrophy with increased microglia and thinning of the myelin sheath were detected. There were no differences in such lesions in the JW and NZW rabbits.

P-102

Keratoconus in a Cynomolgus Monkey

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In a seven-year-old male cynomolgus monkey, erythema of the upper eyelid and forehead, and corneal opacity, edema, and conical protrusion in the eye were observed.

At necropsy, ophthalmological and serological examinations revealed binocular corneal opacity and conical protrusion, and a high IgE level, respectively.

In histopathology, thinning of the epithelium and stroma of the cornea was noted. At the center of the corneal epithelium, the number of epithelial cells was reduced, their cytoplasm was poorer, and the basal cells were flatter than at the periphery. Bowman's membrane was folded with partial loss or breakage. Collagen fibers were compacted or disarranged and the keratocytes were increased in the stroma, with focal pyknosis or loss of the endothelium and folding of Descemet's membrane. Electron microscopical examination revealed atrophy of the corneal epithelial basal cells.

This is the first report on a case of keratoconus in a cynomolgus monkey.

P-103

Bilateral Cataract in a Cynomolgus Monkey

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The incidence of cataract is known to increase with age in macaque monkeys, similarly to human cataract. However, almost all reports on cataract in the cynomolgus monkeys are on cases where it was slight, and reports on related histopathological changes are very few. Here, we report on histopathological changes in a case of severe bilateral cataract in a 7-year-old cynomolgus monkey.

The animal was a naïve female cynomolgus monkey (*Macaca fascicularis*) imported from China, in which bilateral cataract had been confirmed 3 months before necropsy. The formalin-fixed, paraffin-embedded eyeballs were sectioned and stained with Hematoxylin-Eosin (HE), Periodic Acid-Schiff (PAS), and Masson Trichrome (MT) for histopathological examination. For immunohistochemical examination, specimens were stained with vimentin, keratin, and α -smooth muscle actin (α -SMA).

In the macroscopical examination, severe opacity of the lens was observed in both eyes, and thinning of the lens was observed at the cut surface in both eyes (right: approximately 2 mm thick, left: approximately 1.5 mm thick) after formalin fixation. No abnormality was observed in any internal organs except for the eyeballs.

Histopathology revealed that lens nuclei and the majority of cortex lens fibers had disappeared or undergone cavitation, and a swelled lens fiber-like, irregular mass was observed in the subcapsular area. Various sized vacuoles were diffusely observed in the lens fiber. Remnants of cell nuclei were observed in the anterior and posterior lens fibers; however, mineralization of the lens fiber was not observed. Multiple vacuoles were clearly observed in the cytoplasm of lens epithelial cells. In the right eye, spindle cell proliferation with collagen fiber densely stained with MT was observed to continue to the lens epithelium under the anterior capsule. These spindle cells stained positive for vimentin, keratin, and α -SMA in immunohistochemical examination. In a part of the left eye, accumulation of collagen fibers without spindle cells was observed between the lens capsule and epithelium. No abnormality was observed in the lens capsule except for its partial distortion. Adherence of the lens to the ciliary body was not observed, and no abnormality was observed in the cornea or retina.

The cataract in this cynomolgus monkey was considered to be similar to hypermature cataract in humans, which is characterized by degeneration and dissolution of lens fiber. The spindle cells under the anterior capsule were considered to have derived from lens epithelial cells.

P-104

Spontaneous Erythroid Leukemia in a 7-Week-Old

CrI:CD (SD) Rat

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A young male CrI:CD (SD) rat with erythroid leukemia that presented with emaciation, abdominal distension and a pale visible mucosal membrane was euthanized at 7 weeks of age. At necropsy, enlargement of liver, spleen and pancreatic lymph node was noted. Analysis of blood smear samples revealed many mono- or binucleated erythroblasts that had PAS-positive vacuoles in the cytoplasm.

Histopathologically, neoplastic proliferation of atypical cells was observed in the hepatic sinusoids, splenic red pulp, bone marrow, pancreatic lymph node, kidney and lung. Neoplastic cells showed a round to spindle shape, and some neoplastic cells had deeply stained small nuclei and small cytoplasm and resembled erythroblasts.

Immunohistochemically, many neoplastic cells were positive for hemoglobin. To our knowledge, this is the first report of erythroid leukemia in a rat of this age. The observed features were similar to those of pure erythroid leukemia in humans.