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Academic Meeting for Foreign Researchers

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1. Laparoscopic Single-Port Cholecystectomy

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Introduction: Cholecystectomy is the surgical removal of the gallbladder. An estimated 80% of such operations are now performed laparoscopically. We report on the present status of cholecystectomy in Japan, especially laparoscopic single-port cholecystectomy with a multitrocar access system.

Indication: The indications for single-port cholecystectomy are symptomatic cholecystolithiasis and chronic cholecystitis.

Methods: The access system consisted of one 12-mm trocar and two 5-mm trocars. A 20-mm periumbilical incision was made, and the access system was inserted into the abdomen. The procedures were performed with a combination of straight and articulated instruments. Olympus 5-mm flexible laparoscopes were used for visualization. The cystic duct and artery were clipped with a clip applicator. Electrocautery was then used to remove the gallbladder from the liver bed, and the specimen was removed along with the port.

Discussion: Single-port cholecystectomy requires a technique modified from that of traditional laparoscopic cholecystectomy. Spatial conflict is unavoidable and must be compensated for with skillful technique. However, the single-port approach has many advantages, such as decreased pain, lower hernia rates, and cosmetic benefits. For these reasons, this procedure should become widespread in the future.

2. Preoperative Multidetector Row Computed Tomography Angiography for the Planning of Perforator Flap Surgery

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In perforator flap surgery, the course and territory of perforators are different in each region; thus, careful preoperative assessment is necessary with Doppler ultrasonography, color Doppler ultrasonography, or multidetector row computed tomography (MD-CT). In particular, preoperative mapping with MD-CT angiography is fast and simple and provides accurate information about the location, type, and course of perforators. It allows the preoperative selection of a sizable perforator with the shortest intramuscular and suprafascial course, leading to a safer and easier operation with an optimal outcome. We analyzed dominant perforators preoperatively by using Digital Imaging and Communications MD-CT data and then marked the location and course of perforators on the flap donor site. Flaps were elevated safely, and perforators could easily be found intraoperatively. In this paper, we will discuss the effectiveness of MDCT analysis for the planning of perforator flap surgery.
3. Acellular Adipose Matrix as a Natural Scaffold for Tissue Engineering

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Background: Scaffolds for tissue engineering can be natural matrices or synthetic matrices, which are less biocompatible. Acellular adipose matrix (AAM) might provide a natural, biocompatible scaffold for tissue regeneration.

Objectives: This study aimed to establish the optimal method for adipose tissue acellularization.

Methods: Discarded human adipose tissue from routine operations was used. As a first step, 4 previously described methods were compared to select the most suitable method for adipose tissue acellularization. The selected method was further optimized using adipose tissue from different parts of the body (abdomen, chest, forearm) with different weights (0.8 g, 25 g, 80 g). Routine histologic examination, immunohistochemical staining, and scanning electron microscopy were used for evaluation.

Results: With only 1 method, reported by Flynn et al in 2010, was complete acellularization achieved with some modifications. Although the 0.8-g specimens were completely acellularized, cell components still remained in the 25-g and 80-g specimens. Basic tissue architecture was also preserved in AAM, as demonstrated by immunohistochemical staining for collagen type IV and laminin. There were no differences between the different donor sites.

4. Transmission Blocking Vaccine Strategy

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Dirofilaria immitis is responsible for dirofilariosis, a widespread zoonosis that affects humans and many species of wild and domesticated animals, such as cats and dogs. Filarial chitinases have been considered as vaccine candidates, in particular against third-stage larvae and microfilarial stages, which are of fundamental importance to the transmission and continuation of parasite life cycles. However, detection of D. immitis chitinase activity in the microfilarial stage or any other larval stage has yet to be unequivocally demonstrated. In this study, a 1446-bp complementary DNA coding sequence isolated from D. immitis, encoding for a 481-amino acid protein with a general structure similar to that of other known filarial chitinases, was expressed with a baculovirus expression vector system and assessed for chitinase activity. D. immitis r-chitinase exhibited predominant activity with triacetylatedtrose, in addition to chitobioside, thus indicating exochitinase activity. Moreover, D. immitis r-chitinase pH-dependent enzyme activity exhibited a sharp peak, with an optimum at pH 6.5; whereas temperature-dependent enzyme activity exhibited a peak at 37°C, decreasing thereafter, until inactivation at 65°C. With preliminary plasmid-based immunization (gene gun and intramuscular routes), immunogenicity profiles of the protein will also be discussed as a lead in for future work.

5. Brain Injury Caused by Bamboo Penetration: A Case Report

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A man in his 60s was found dead in a bamboo forest. He was lying sideways on the ground. Some dirt and blood were on his face. There was a 2.2-m-high slope from the roadside down to the bamboo forest where the body was found. At the scene, the police found empty beer cans beside the body and a car belonging to the deceased stopped at the roadside over the slope. To determine the cause of death, a judicial autopsy was performed. At autopsy the body was 171 cm tall and weighed 64 kg. On the face, a 2.3-cm-long horizontal wound and a 3.8 × 0.8-cm abrasion were observed below the left lower eyelid. The left bulbar conjunctiva showed bleeding. There were minor abrasions and bruises on the head, chest, back, and both upper limbs. Internal examination showed a small subdural hemorrhage on the left side of the brain and a subarachnoid hemorrhage over the left and right frontal lobes. After the brain was removed, a comminuted fracture of the floor of the left anterior cranial fossa was found within a 2.5 × 3.0-cm area. The wound below the left lower eyelid penetrated the left anterior cranial fossa and reached the base of the left frontal lobe. The depth of the wound was 5.3 cm. Five pieces of bamboo, about 2.2 cm in length and 0.1 cm in diameter, were also found in the left anterior cranial fossa. No injuries were found in other organs. After the brain tissue was fixed, we found an intracerebral hemorrhage from the entrance of the wound at the basal aspect of the left frontal lobe to the left basal nucleus with perforation of the left lateral ventricle. On the basis of these findings, we determined that the cause of death was brain injury caused by penetration by bamboo.

6. Disaster Medicine Education System in Japan

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Background: China is subject to numerous natural disasters, as is Japan.

Objectives: To investigate the disaster medicine education system in Japan and to provide useful information for establishing a similar system in China.

Methods: Data were collected via questionnaires, interviews with disaster medicine specialists.

Results: The disaster medicine education system in Japan is composed of lectures for medical students; practical training, such as disaster medical assistance team training, for healthcare providers; and public education.

Conclusions: Although further improvements can be made, the disaster medicine education system in Japan is a well-constructed and effective system that has benefitted from past lessons learned. It would be helpful to understand the existing disaster medicine education system in Japan to establish a similar system in China.

7. The Relation of the Early Graying Phenomenon to the Nondevelopment of Melanoma Symptoms in Mongolian Native Gray Horse

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We have previously reported that the Mongolian native white-horse group includes a subgroup of gray horses having a white coat at birth. Moreover, this coat color does not change throughout the life of such horses, and the development of cutaneous melanoma symptoms was not observed. In the present study, we examined the relation of the early graying phenomenon to the nondevelopment of melanoma symptoms by syntaxin 17 (STX17) gene mutation in Mongolian native gray horses. Skin tissues were collected from the Mongolian native gray horses and thoroughbred gray horses. Also, melanoma lesions were removed from the multiple papules of the tails of thoroughbred gray horses. Histological examinations were then performed to determine the distribution of melanin granules in the skin tissues and the melanoma specimens. The distribution and the quantity of melanin granules in the skin tissues of Mongolian native gray horses
were compared with those in skin and melanoma specimens from thoroughbred gray horses. On the one hand, few melanin granules were found in the melanocytes of hair follicles within the subcutaneous tissue taken from the Mongolian native gray horses. On the other hand, a single nucleotide polymorphism, close to the TATA box in the promoter region of the STX17 gene, was detected in such tissue. Consequently, the early graying phenomenon in Mongolian native grey horses is thought to be mediated by a cis-acting abnormal regulator with a 4.6-kb duplication in intron 6 of the STX17 gene. Meanwhile, the abnormal transportation of melanin granules in melanosomes is facilitated by a transcriptional regulator with a single nucleotide polymorphism in the promoter region of the STX17 gene. Furthermore, the nuclear receptor subfamily 4, group A, member 3 (NR4A3) gene, which is a cell-cycle-regulatory gene, is closely associated with the STX17 gene in equine chromosome 25. Therefore, the expression level of the STX17 and NR4A3 genes were then analyzed with the real-time polymerase chain reaction and messenger RNA from the skin of Mongolian native gray horses and melanoma specimens from thoroughbred gray horses. The expression levels of STX17 and NR4A3 were found to be higher in melanoma specimens from thoroughbred gray horses than in skin tissues from Mongolian gray horses.

8. Establishing a Single Nucleotide Polymorphism Assay for Dihydropyrimidine Dehydrogenase Gene-related Cancer Chemotherapy

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Introduction: Personalized genetic medicine uses a patient’s genetic profile to guide decisions made in regard to the prevention, diagnosis, and drug treatment of disease. The effectiveness and toxicity of the drugs differ according to patient genotype. Some single nucleotide polymorphisms (SNPs) in dihydropyrimidine dehydrogenase (DPYD) and methylenetetrahydrofolate reductase (MTHFR) genes are associated with fluorouracil treatment-related toxicity. Last year, Anarkhhuu established the small amplicon genotyping (SAG) method for SNPs in MTHFR. In DPYD, 4 common coding region SNPs (cSNPs) and 1 splice site mutation are known to be associated with fluorouracil treatment-related toxicity.

Purpose: The purpose of this study was to establish a genotyping method for cSNPs in DPYD.

Methods: We used a SAG method to genotype 4 targeted cSNPs and 1 splice site mutation in DPYD using samples of genomic DNA from 51 Japanese subjects.

Results: We established SAG genotyping method for cSNPs c. 1627A>G and c. 496G>A. Each SNP genotype was successfully differentiated with the SAG method and confirmed by sequencing. The cSNPs heterozygous for c. 2194G>A and IVS14+1G>A were not found in this preliminary experiment. The SNP GG homozygote of c. 1896T>C was found in samples but has not previously been reported in Japan.

Conclusion: We established a method for 2 cSNPs of DPYD which can be screened for easily and rapidly and with high sensitivity and specificity.


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Objectives: MicroRNAs (miRNAs) are noncoding RNAs that bind to the 3’ untranslated region of target mRNA and regulate its translation. MiRNA derived from the chromosome 19 miRNA cluster (C19MC; e.g., miR-517a) is exclusively expressed in the human placenta. The expression of C19MC-derived miRNA has been considered to be highly
correlated with the methylation state of a CpG island (cytosine/guanine-rich regions of DNA) located about 18 kb upstream of the C19MC genes. In this study, we examined the methylation status of the CpG-island in human placentas, a human extravillous trophoblast cell line (HTR8/SVneo), and choriocarcinoma cell lines (BeWo and JEG3).

Methods: The expression levels of C19MC-derived miRNAs were analyzed by real-time PCR. For analysis of methylation status, genomic DNA was extracted from samples. After sodium bisulfite treatment, the DNA regions of C19MC were amplified and sequenced to identify the CpG methylation profile.

Results: In BeWo and JEG3 cell lines, the CpG-rich region located about 18 kb upstream of the C19MC genes was hypomethylated, and C19MC-derived miRNAs (e.g., miR-517a) were highly expressed. In contrast, in HTR8/SVneo cell line, the CpG-rich region was hypermethylated, and C19MC-derived miRNAs were hardly expressed. In addition, the CpG-rich region was hypomethylated in human placentas that highly expressed placenta-specific miRNAs.

Conclusion: This study provides new insights into the epigenetic regulation of C19MC-derived miRNAs in human trophoblast cells.

10. A Placenta Specific microRNA that Induces Mitochondria-mediated Apoptosis in Human Umbilical Vein Endothelial Cells

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Objective: The balance between endothelial cell survival and apoptosis is an important cellular process. It plays a key role during the development of many vascular diseases. MicroRNAs (miRNAs) are small noncoding RNAs that repress target mRNA posttranscriptionally. However, few studies have examined the effects of placenta-specific miRNAs in endothelial cells. In this study, we showed that miR-5XX, a placenta-specific miRNA, induces apoptosis in human umbilical vein endothelial cells.

Methods: Cell viability was evaluated with CellTiter-Glo reagent (Promega). The Caspase-Glo assay kit (Promega) was used to measure the activities of caspase-3, -7, -8, and -9. Released cytochrome c and cleaved caspase-3 were detected by Western blot.

Results: Multi-caspase assay revealed that miR-5XX activated caspase-3 and -9. Western blot analysis provided evidence for cytochrome c release from mitochondria and an increase in cleaved caspase-3 during apoptosis.

Conclusions: These findings suggest that miR-5XX induces apoptosis through a mitochondrial intrinsic pathway in human umbilical vein endothelial cells. Our studies may provide new insights into the role of placenta-specific miRNAs in the pathophysiological mechanisms of preeclampsia.

11. A Novel Production Method of Adeno-associated Virus Vectors

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Recombinant (r) adeno-associated virus (AAV) vectors are a promising system for clinical application in gene therapy. Traditionally, rAAV vector particles are commonly harvested from cell lysate on the basis of experience with AAV2-based vectors. Previously reported methods require time-consuming generation of vectors. However, we have observed that large amounts of vectors of several serotypes are found in culture medium, a source of vectors more pure than cell-derived material. Here we describe the high-yield production of rAAV vectors with the polyethylenimine transfection method as modified by Lock et al (Human Gene Therapy 221: 1259–1271, 2010). Type 1, 2, 8, or 9 rAAVs were harvested from the culture medium and the transfected-cell lysate carried out without fetus calf serum and purified with Iodixanol linear
gradient and stepwise gradient centrifugation, respectively. Finally size-exclusion chromatography was used to remove the small molecular weight contaminants and iodixanol on a Superdex 200HR filtration column with high-performance liquid chromatography. Vector titers were determined with the real-time polymerase chain reaction, and the final preparations of rAAVs were shown as only 3 capsule bands on sodium dodecylsulfate-polyacrylamide gel electrophoresis. The highest titer was $3.4 \times 10^4$ genome copies. This production method may be useful for a number of different AAV serotypes.

12. Development of Combination Therapy of Systemic Cancer Gene Therapy Using MDA-7/IL-24 and Gemcitabine for Pancreatic Cancer

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Aim: To investigate the efficacy of the combination therapy of gene therapy using AAV8 vector-mediated melanoma differentiation-associated gene-7 (MDA-7)/interleukin (IL)-24 and gemcitabine for pancreatic cancer.

Methods: In vitro (growth curve analysis): PGHAM-1 cells (hamster pancreatic cancer cells) were treated with various concentrations of gemcitabine (0.1, 1, 5, 10, and 100 ng/mL) and IL-24 (0.1, 1, 10, 20, 30, and 100 ng/mL). The number of cancer cells was calculated on 2, 4, 6, and 8 days. The effects of combination treatment on cell growth and apoptosis induction were also examined. In vivo (experimental intrapancreatic implantation model): $5 \times 10^5$ (groups A, B, and E-H) or $1 \times 10^5$ (groups C and D) PGHAM-1-Luc cells were implanted into the splenic lobe of the pancreas. Tumor growth was monitored with the IVIS imaging system once a week. Some animals in groups E-H received intraperitoneal injections of 50 mg/kg of gemcitabine every 3 days.

Results: In vitro: The proper concentrations of gemcitabine and IL-24 to inhibit cell growth of PGHAM-1 were 10 ng/mL and 20 ng/mL, respectively. In vivo: Half of the animals in each group had primary pancreatic tumors. Primary tumors (region of interest $>1.0E+06$), which had been detected with the IVIS imaging system 2 weeks after implantation, had developed until the animals’ deaths. All animals with primary tumors died of cancer within 3 months.

Conclusions: In the in vitro experiment, administration of gemcitabine and IL-24 inhibited the growth of PGHAM-1 cells. The experimental intrapancreatic implantation model was well established.

13. Glutamatergic Inputs to Gonadotropin-releasing Hormone Neurons during Pubertal Development

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Puberty is the developmental period when an individual attains reproductive capability. The onset of puberty is triggered by augmentation of gonadotropin-releasing hormone (GnRH) release from the hypothalamus. The present study aimed to quantify glutamatergic inputs, which are excitatory synaptic inputs onto GnRH neurons, during pubertal development. Brain sections were processed for double-label immunohistochemical studies for GnRH and vesicular glutamate transporter-2 (VGlut2), and close appositions between VGlut2-immunoreactive puncta and GnRH neurons were counted with confocal laser scanning microscopy. No difference was found between juvenile and adult female rats in the number of glutamatergic inputs to soma and dendrites of GnRH neurons. This result indicates that the glutamatergic inputs to GnRH neurons are not a major factor driving GnRH neurons during pubertal development. We are also performing a similar analysis for GABAergic inputs onto GnRH neurons.
14. Oxytocin Deprivation Eliminates Odor Preference without Diminishing Sexual Interactions in Male and Female Mice

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The preoptic area and medial amygdala are crucial for sociosexual behavior and are rich in oxytocin receptor-positive neurons, implying roles of oxytocin in the regulation of the behavior in these structures. We performed behavioral tests for olfactory preference for conspecifics and sexual interactions in a seminatural environment in male and female oxytocin-knockout mice. In the preference test, the experimental animals were exposed to 2 pairs of odors: intact males vs. receptive females, and intact males vs. castrated males. Wild-type females showed significantly longer investigation of the odor of intact males than of the odors of receptive females or castrated males; wild-type males showed an opposite pattern to that of females. Oxytocin-knockout mice of both sexes failed to distinguish the pairs of odors and spent equivalent times investigating all stimulus pairs. In the sexual interaction test, oxytocin-knockout females needed numerous trials to exhibit sexual behavior comparable to that of wild-type females, whereas no genotypic difference was observed in males. Lastly, neuronal activation in response to opposite-sex odor stimulation was examined by means of immunohistochemical staining for c-Fos in the preoptic area and the medial amygdala. The c-Fos-immunoreactive cells in both areas were fewer in oxytocin-knockout males and females than in wild-type mice. These results suggest that oxytocin regulates these social behaviors by activating neurons in the preoptic area and the medial amygdala.

15. The Role of the Brain-gut Corticotrophin-releasing Factor Family in Mucosal Inflammation

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To determine whether mucosal inflammation is associated with brain-gut interactions, in the present study we evaluated mucosal inflammation and expression levels of corticotrophin-releasing factor (CRF) receptors in lipopolysaccharide (LPS)-pretreated central urocortin 1-administered model rats. Intraperitoneal injection of LPS at a dose of 600 µg/kg was used to produce the inflammatory model. Inflammatory or control rats underwent stereotaxic surgery. After 6 to 7 days of recovery, rats were given intracerebroventricular injections of saline or of urocortin 1 at 0, 3.3, 10, or 30 µg/kg, followed 1 day later by sacrifice or evaluation of gastrointestinal motility. The stomach, ileum, and colon were collected first to evaluate the degree of inflammation by counting the numbers of inflammatory cells and measuring myeloperoxidase activity, and then to evaluate the accumulation of eosinophils. The distribution and expression of CRF receptors 1 and 2 in different parts of the gut were also investigated with immunohistochemical staining, Western blotting, and the real-time polymerase chain reaction. Finally, gastrointestinal motility was evaluated by measuring gastric emptying and small intestinal transit with Evans blue stain.

Results: In LPS-treated rats, many mucosal eosinophils were observed in the sigmoid colon. In contrast, in other parts of the colon and in the stomach, increased migration of eosinophils was not observed. In rats treated with urocortin 1, the migration of mucosal eosinophils was reduced compared with that in rats pretreated with LPS but not with urocortin 1.

Conclusions: Further studies will be needed to clarify whether urocortin 1 treatment is associated with the decreased migration of eosinophils in LPS-treated rats.
16. Immunohistochemical Expression of β-catenin by Endometrioid Adenocarcinoma in High-dose Progestin Therapy

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Objectives: Endometrial adenocarcinoma of the well-differentiated endometrioid histologic type shows changes from carcinoma to atrophic glands through degenerative swollen glands and squamous morules during high-dose progestin therapy. This study aimed to clarify the characteristics of morules through the immunohistochemical expression of β-catenin, which has various functions, and gene abnormalities of which are frequently found in endometrioid adenocarcinoma.

Methods: Immunohistochemical studies for β-catenin were performed with hematoxylin and eosin-stained 4-µm-thick sections of endometrial curettage specimens obtained from 6 patients at diagnosis and at 4 through 16 weeks of treatment with medroxyprogesterone acetate (600 mg/day). Immunohistochemical expression was compared among the 4 characteristic morphologic stages mentioned above which could be observed during treatment.

Results: The positivity rates for β-catenin in atypical glands of the carcinoma, degenerative swollen epithelium, and morular epithelium were 100%, 97.4%, and 95.5%, respectively. The rates of nuclear staining for squamous morules, carcinoma, and degenerative epithelium were 100%, 55%, and 50%, respectively. The positivity rate for benign-appearing atrophic epithelium was 20%. The stromal component was not stained, whether or not it was decidualized.

Conclusions: Although morules typically exhibit nuclear and cytoplasmic β-catenin expression, positivity for β-catenin is associated with a malignant character.

17. Production of Canine Insulin-Secreting Cells Using Bone Marrow-Derived Mesenchymal Stem Cells

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Bone marrow-derived mesenchymal stem cells (BMSCs) are a multipotent, undifferentiated population of cells capable of committing and differentiating into a number of mesodermal lineages. The transcription factors PDX-1, BETA-2, and MafA have been reported to be important for the normal production and secretion of insulin. Therefore, our laboratory cloned and transfected canine Pdx-1, Beta-2, and MafA genes into canine BMSCs to determine whether canine insulin-secreting cells can be produced from BMSCs. After transfected cells were cultured for up to 2 weeks, insulin gene expression was verified with the reverse transcriptase polymerase chain reaction (canine insulin messenger RNA expression) and insulin production by intracellular immunofluorescence staining. The results of the reverse transcriptase polymerase chain reaction indicated a correct, expected size band for canine insulin. In addition, preliminary results of immunofluorescence staining were positive, indicating that canine insulin was expressed in cells transfected with Pdx-1, Beta-2, and MafA.