ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

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Abstracts

CORRELATION BETWEEN COPY NUMBER OF MITOCHONDRIAL DNA AND CLINICOPATHOLOGIC PARAMETERS OF HEPATOCELLULAR CARCINOMA

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Aims: In the current study, we investigated possible correlations of the mtDNA copy number in hepatocellular carcinoma (HCC) with the pathological findings and prognosis.
Methods: We studied 31 HCC specimens using quantitative real-time polymerase chain reaction analysis, and the correlation between the mtDNA copy number and the clinicopathologic parameters and mutations in the D-loop region of the mitochondrial genome.
Results: The mtDNA copy number was reduced in HCCs compared with the corresponding noncancerous liver tissues ($p = 0.002$), and significantly correlated with tumor size ($p = 0.014$) and cirrhosis ($p = 0.048$). Patients with a low mtDNA copy number tended to show shorter 5-year survival rates than patients with a high mtDNA copy number when assessed by Kaplan-Meier curves, but not a significant (overall survival rate, 63% vs. 83%; $p = 0.19$). The copy number of HCC with mtDNA D-loop mutation or deletion was lower, but not significantly so ($p = 0.656$, $p = 0.590$, respectively).
Conclusions: Our results indicated that a reduced copy number of mtDNA is correlated with HCC and associated with malignant potential.

SERUM TUMOR MARKERS IN SKELETAL METASTASIS

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Background: There have been no well-documented reports detailing the relationship between skeletal metastasis and tumor markers in a large series of patients. The purpose of our study was to assess the relationship between the clinical features of skeletal metastasis and serum tumor markers and to determine whether tumor markers are a useful modality in the differential diagnosis of skeletal metastasis.
Methods: We retrospectively reviewed consecutive 458 patients with skeletal metastasis and divided the patients into two groups according to six clinical presenting factors. We assessed whether these groups influenced the level of the tumor markers in univariate and multivariate analysis.
Results: Patients with skeletal metastasis of carcinoma had a higher level of markers CEA ($P < 0.0001$) and CA19-9 ($P = 0.0008$) than patients with primary bone tumors and hematological malignancies. Univariate analysis of clinical variables revealed that metastasis on axial skeleton, multiple skeletal metastases and visceral metastasis were associated with the serum CEA and CA19-9 levels. By multivariate analysis, metastasis on axial skeleton, multiple skeletal metastases

and visceral metastasis were found to be associated with the serum CEA and CA19-9 levels. At least one of the tumor markers was elevated in 73% of all patients.
Conclusions: The higher tumor marker level (CEA, CA19-9) is predictive of metastasis on the axial skeleton, multiple skeletal metastases and visceral metastasis. Tumor markers are useful as a screening test to distinguish skeletal metastases of carcinoma from primary bone tumors or hematological malignancy from primary bone tumor and hematological malignancy.

MELOXICAM INHIBITS OSTEOSARCOMA GROWTH, INVASIVENESS AND METASTASIS BY COX-2-DEPENDENT AND INDEPENDENT ROUTES

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Cyclooxygenae-2 (COX-2) inhibitors have been demonstrated to exert antitumor effect by many authors. We evaluated the antitumor activity of meloxicam, a preferential COX-2 inhibitor, in osteosarcoma. COX-2 expression in osteosarcoma cell lines MG-63, HOS and U2-OS was determined by real-time RT-PCR and western blotting. The inhibitory effects of meloxicam on osteosarcoma cell growth and invasiveness were assayed by MTT assays and matrigel invasion assays, respectively. Apoptotic activity was evaluated by TUNEL staining and semi-quantification of Bax and Bcl-2 expression by real-time RT-PCR and western blotting. PGE_2 production in the presence and absence of meloxicam was analyzed by EIA, and to determine whether the effects of meloxicam are COX-2-dependent or independent, PGE_2 was added to see if it reversed the effects of meloxicam. In addition, the antitumor effects of meloxicam were evaluated an *in vivo* mouse model using grafted LM-8 mouse osteosarcoma cells, together with immunohistochemical analysis for VEGF. Meloxicam inhibited PGE_2 production, proliferation and invasiveness especially in MG-63 cells, which express relatively high levels of COX-2. Only high concentration of meloxicam caused apoptosis and upregulated Bax mRNA and protein in MG-63 cell culture. These results suggested meloxicam may have both COX-2-dependent and independent inhibitory actions on osteosarcoma. In contrast, meloxicam didn't induce apoptosis in HOS and U2-OS cells, relatively low levels of COX-2. Exogenous PGE_2 reduced the effects of meloxicam on cell viability and invasiveness, but not its effect on Bax mRNA. *In vivo*, high doses of meloxicam suppressed LM-8 tumor growth and lung metastasis.

A ROLE FOR SHP-2 IN THE PRODUCTION OF MMP-2, TIMP-2, MT1-MMP AND INVASION OF TUMOR CELLS

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Matrix metalloproteinase (MMP)-2 is a matrix-degrading enzyme critical for tumor invasion and metastasis. MMP-2 is secreted from cells as an inactive zymogen and subsequently activated by proteolytic cleavage at cell surface. Tissue inhibitor of metalloproteinase-2 (TIMP-2) and MT1-MMP play a critical role in the activation of MMP-2. TIMP-2 binds to pro-MMP-2 and MT1-MMP via its C-terminal and N-terminal part, respectively and finally activates MMP-2. Production of TIMP-2 and MT-1 MMP as well as MMP-2 is, therefore, a critical step for tumor invasion and metastasis.

To search the signaling critical for MMP-2, TIMP-2 and MT1-MMP, we studied the role of SHP-2, a positive modulator of Ras/MAPK pathway, in v-Src-dependent signaling and Con A-dependent signaling in SHP-2-null fibroblast cells. We found that SHP-2 was necessary for the v-Src-dependent production of MMP-2, TIMP-2, MT1-MMP and for cell invasion. Similarly, SHP-2 is required for the Con A-dependent production of TIMP-2. In wild-type cells, v-Src bound with SHP-2 and induced complex formation of SHP-2 with Sos1 and Grb-2, which, in turn, activates Ras, Erk, Akt and p38, whereas activation of these signaling molecules was impaired in SHP-2 null cells. While MEK1 inhibitor could successfully suppress the v-Src-induced production of MMP-2 and TIMP-2, inhibitors for PI3K and p38 could not. Similar results were obtained in Con A-induced production of TIMP-2. Expression of exogenous SHP-2 in v-Src-transformed SHP-2-null cell could rescue the production of MMP-2, TIMP-2 and MT1-MMP as well as activation of Erk. Similarly, expression of exogenous SHP-2 in SHP-2-null cell could rescue the Con A-dependent production of TIMP-2 and activation of Erk. Finally, knockdown of SHP-2 with SR3Y1 and MDA-231, a breast cancer cell line, could suppress the production of MMP-2, TIMP-2, activation of Erk and invasion of the cells. Taken together our results strongly suggest that SHP-2 could be a good target for cancer therapy.

NEUROSTEROID ESTRADIOL RESCUES ISCHEMIA-INDUCED DEFICIT IN LONG-TERM POTENTIATION OF RAT HIPPOCAMPAL CA1 NEURONS

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Background: Increasing evidence indicates that the neurosteroid 17β-Estradiol (E2), one of the female sex hormones, has neuroprotective effects against cerebral injury. This study was designed to establish whether E2 can also protect hippocampal CA1 neurons from functional deficits in synaptic transmission and plasticity caused by ischemia.

Methods: Adult male Wistar rats were subjected to mild global cerebral ischemia created by the

four-vessel occlusion (4VO) for 10 min, and the effects of E2 administration against the ischemic injury were examined. Electrophysiological properties of Schaffer collateral-CA1 synapses were examined 7 days after ischemia with a real-time optical recording technique on the hippocampal slices stained with a voltage-sensitive dye RH482.
Results: The ischemic brain showed a decreased basal synaptic transmission and an impairment of LTP induction but no alteration in paired-pulse facilitation. Administration of E2 (1 mg/kg) 3h before ischemia was able to protect CA1 neurons from these ischemia-induced synaptic dysfunctions. The estrogen receptor-α (ERα) selective agonist propyl pyrazole triol (PPT, 2 mg/kg) exerted a similar protective effect, but the estrogen receptor β (ERβ) agonist diarylpropiolnitrile (DPN, 8 mg/kg) failed to do so. Histological examination revealed that transient global cerebral ischemia markedly reduced the density of pyramidal neurons in CA1 sub region. E2 and PPT significantly attenuated the cell loss due to ischemia in this region, but DPN did not.
Conclusions: These findings suggest that E2 possesses neuroprotective properties, i.e. protecting neurons not only from cell death but also from functional damages due to mild global cerebral ischemia; and this effect is mediated by ERα, but not by ERβ.

GENOTYPING OF CANDIDA ALBICANS ON THE BASIS OF POLYMORPHISMS OF ALT REPEATS IN THE REPETITIVE SEQUENCE (RPS)

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Purpose: Candida albicans is one of the most important etiologic agents causing superficial and deep fungal infections. For prevention of candidasis, it is important to develop a rapid system that discriminates C. albicans at the strain level. To develop a system that can identify C. albicans at the strain level.
Methods: Genomic DNAs were purified from 179 clinical isolates of C. albicans, and were used as templates for PCR amplification of 25SrDNA and ALT repeats in repetitive sequences (RPSs). PCR products generated from ALT repeats were digested with EcoR1 and /or Cla1 in order to study the relationships between restriction profiles and amplification profiles.
Results: 179 clinical isolates were grouped into genotypes A (92 isolates), B (38 isolates) and C (49 isolates) on the basis of their 25SrDNA, and each was further classified into five types (types 3, 4, 3/4, 2/3/4 and 3/4/5) by PCR amplification targeting ALT repeats. Type 3 C. albicans constituted the majority of isolates in any genotypes (66.3% for genotype A, 76.3% for genotype B and 73.4% for genotype C). Each C. albicans type showed several amplification patterns, indicating the existence of subtypes. RFLP analysis revealed that restriction profiles of PCR products corresponded to amplification patterns from PCR.
Conclusion: The present results indicate that PCR amplifications targeting 25S rDNA and ALT repeats are useful for rapid genotyping and distinction of C. albicans involved in superficial candidiasis.

EGFR POINT MUTATION IN NON-SMALL CELL LUNG CANCER IS OCCASIONALLY ACCOMPANIED WITH SECOND MUTATION OR AMPLIFICATION

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Activating mutations of *EGFR* are frequently found in a subgroup of patients with NSCLC and shown to be highly correlated with the response to gefitinib and erlotinib. In this study, we searched for mutations of *EGFR*, *HER2,* and *KRAS* in 264 resected primary NSCLCs from Japanese patients and determined whether there is a correlation between genetic alterations of these genes and clinicopathological factors, together with 85 tumors that we have reported previously. *EGFR* mutations were found in 102 of total 349 tumors, and 7 tumors had two missense mutations. RT-PCR of *EGFR* and subsequent subcloning analyses identified that the double mutations occurred in the same allele. Furthermore, in 202 NSCLCs analyzed with Southern blot, we identified 11 tumors with gene amplification of *EGFR,* with 8 tumors containing a mutation of *EGFR*. Sequence analysis detected only weak or no signals of the wild-type allele in the 8 tumors, strongly suggesting that the mutated allele was selectively amplified. These findings indicated that a dual genetic change of *EGFR* can occur in the same allele either with a possible second-hit mutation or amplification, which may imply a more selective growth advantage in a cancer cell. Meanwhile, *HER2* mutation and amplification were found in 6 of 349 tumors and 3 of 202 tumors, respectively, and *KRAS* mutation in 21 of 349 tumors. Mutations of the *EGFR* and *HER2* genes were more frequently found in female, never or light smoking patients with adenocarcinoma, and there were no tumors that have two or more mutations simultaneously among *EGFR*, *HER2,* and *KRAS* mutations. The current study further demonstrates that a double genetic event of *EGFR* can occasionally occur in lung cancer, thus providing new clues to the understanding of the involvement of EGFR signaling cascades in the pathogenesis of NSCLC.

COMPARISON OF CIRCULATING ADIPONECTIN AND PRO-INFLAMMATORY MARKERS REGARDING THEIR ASSOCIATION WITH METABOLIC SYNDROME IN JAPANESE MEN

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Background: Anti- and pro-inflammatory molecules purportedly play an important role in developing metabolic syndrome (MetS). However, little is known as to the relative importance of these molecules in the association with MetS.
Methods and Results: We studied 624 middle-aged Japanese men without medical history of

cardiovascular disease or cancer and investigated the associations of circulating tumor necrosis factor-α (TNF-α), interleukin-6, C-reactive protein (CRP), and adiponectin with MetS. We used the respective definitions proposed by the National Cholesterol Education Program's Adult Treatment Panel III (ATP-III), the International Diabetes Federation, and the Japanese Society of Internal Medicine. Decreased serum adiponectin was observed in those with any of the ATP-III-MetS components, while this was not the case with increased TNF-α, interleukin-6, or CRP. Adiponectin and CRP levels linearly deteriorated with an increasing number of ATP-III-MetS components (trend $P<0.001$, respectively). Significantly higher CRP and lower adiponectin levels were observed in those who met any MetS criteria, while increased TNF-α was observed in only those with ATP-III-MetS. Finally, odds ratios (ORs) for MetS prevalence of a 1-SD increase/decrease in log-transformed four markers were calculated with multivariate logistic regression analyses. Consequently, decreased adiponectin was associated most strongly with ATP-III-MetS (adiponectin: OR, 1.90 [95% confidence interval, 1.44–2.51], $P<0.001$, CRP: 1.33 [1.01–1.74], $P=0.03$, TNF-α: 1.25 [0.94–1.67], $P=0.12$, and IL-6: 0.87 [0.63–1.19], $P=0.37$). This result was not altered by using the other two criteria.

Conclusions: The present results raise the possibility that decreased serum adiponectin might be fundamentally involved in the development of MetS.

INVERSE CORRELATION BETWEEN SOLUBLE CD40 LIGAND AND SOLUBLE CD40 IS ABSENT IN PATIENTS WITH UNSTABLE ANGINA

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Background: The CD40/CD40 ligand (CD40L) system mediates inflammatory processes important in atherogenesis and plaque instability. The expression of CD40L on activated T cells was suppressed by soluble CD40 (sCD40) in vitro. However, the relationship between soluble CD40L (sCD40L) and sCD40 in unstable angina (UA) is still unknown.

Methods and Results: Thirty-seven consecutive patients with recent chest pain or discomfort were recruited. Patients with both Braunwald's class IB-IIIB and with coronary stenosis (or stenoses) of >75% were assigned to the UA group (n=19, aged 67.2±8.2 years), and the rest to the control group (n=18, aged 63.4±8.7 years). The serum levels of sCD40L and sCD40, and the plasma levels of matrix metalloproteinase (MMP)-9, were measured by enzyme-linked immunosorbent assays. A significantly inverse correlation between sCD40L and sCD40 was shown in the controls (r= –0.72, P=0.0007), but was absent in the UA group (r= –0.16, P not significant), although there was no statistical significance between sCD40L or sCD40. The difference of the regression slopes of these regression lines was statistically significant (P <0.01). Additionally, there was a significant correlation between sCD40 and plasma levels of MMP-9 in the patients with and without UA (r=0.58, P=0.0096), but no significant correlation between sCD40L and MMP-9 levels (r=0.00, P not significant).

Conclusions: The balance between CD40 and CD40L may be lost in patients with UA. Soluble CD40 expression may also be related to MMP-9 expression in atherosclerotic tissues.

ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS) AND METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE POLYMORPHISMS ARE ASSOCIATED WTH ENDOTHELIAL DYSFUNCTION IN YOUNG, HEALTHY MEN

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Background: Endothelial dysfunction is considered to be at the earliest stage of atherosclerosis and is thought to be associated with impairment of nitric oxide (NO) bioavailability and increased oxidative stress. eNOS gene Glu298Asp and MTHFR C677T polymorphism have been reported to be associated with atherosclerosis and cardiovascular disease through NO synthesis and homocystein metabolism.

Objectives: To examine whether these common eNOS and MTHFR gene polymorphisms are associated with endothelial dysfunction in young, healthy men without overt cardiovascular disease.

Subjects and Methods: Flow-mediated, endothelium-dependent vasodilation (FMD) and glyceryl trinitrate-induced, endothelium-independent vasodilation (GTN) were measured using high-resolution ultrasound of the brachial artery in 53 young, healthy men assigned to eNOS and MTHFR genotypes.

Results: Subjects with the eNOS Asp298 allele (n=15) showed significantly reduced FMD/GTN compared with those without this allele (n=38) (0.23+/–0.13 [mean +/–SD] versus 0.35+/–0.14, P=0.0072) whereas there was no significant difference in GTN between these two groups. Although subjects with the MTHFR T677 allele did not show significantly reduced levels of FMD/GTN, subjects with the eNOS Asp allele and who were carriers of the MTHFR T677 allele demonstrated markedly reduced levels of FMD/GTN compared with noncarriers (0.14+/–0.05 versus 0.28+/–0.13, P=0.04).

Conclusions: The data suggest that even in young men, the eNOS Asp298 allele may be involved in endothelial dysfunction before any overt vascular disease has occurred. Furthermore, a combination of the eNOS Asp298 and MTHFR T677 alleles may exaggerate endothelial dysfunction and may contribute to a comparatively earlier development of atherosclerosis.

DOBUTAMINE STRESS TEST UNMASKS CARDIAC SYMPATHETIC DENERVATION IN PARKINSON'S DISEASE

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Objective: The functional consequences of decreased cardiac uptake of ^{123}I-metaiodobenzylguanidine (MIBG) in Parkinson's disease (PD) are unknown. Our purpose was to detect denervation supersensitivity and elucidate the functional consequences on cardiac beta1 receptors in patients with PD.
Methods: We compared cardiovascular responses to dobutamine (DOB) in 48 PD patients and 16 age-matched normal controls. DOB was administered starting at 2 μg/kg/min and increased stepwise 4 to 6 μg/kg/min every 5 min. Changes in blood pressure (BP) were compared to determine the optimal dose to detect denervation supersensitivity. ^{123}I-MIBG scintigraphy was performed in all PD patients, and the ratio of the uptake in the heart to that in the mediastinum (H/M ratio) was calculated. Cardiac contractility was measured by DOB echocardiography in 26 consecutive PD patients and 7 normal controls based on peak aortic flow velocity.
Results: At 4 μg/kg/min, the systolic BP (SBP) increased more in the PD group than controls (ΔSBP: PD, 19.5 ±14.2 mm Hg; control, 6.4 ± 5.7 mm Hg, $p < 0.005$). ΔSBP correlated negatively with the delayed H/M ratio ($r = –0.51$, $p < 0.05$). The increase in cardiac contractility at 4 μg/kg/min also correlated negatively with delayed H/M ratio ($r = –0.65$, $p < 0.01$).
Conclusion: DOB 4 μg/kg/min can unmask cardiac sympathetic denervation in patients with PD. Since the H/M ratio correlated negatively with ΔSBP and the hyperdynamic cardiac response, reduced MIBG uptake reflects cardiovascular sympathetic dysfunction in PD.

FRACTIONAL ANISOTROPY AND APPARENT DIFFUSION COEFFICIENT VALUES DETECT EARLY INVOLVEMENT IN MULTIPLE SYSTEM ATROPHY

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Objective: To determine whether apparent diffusion coefficient (ADC) values and fractional anisotropy (FA) values can detect early pathological involvement in multiple system atrophy (MSA), and be used to differentiate MSA-P from Parkinson's disease (PD).
Methods: We compared ADC and FA values in the pons, cerebellum and putamen among 20 MSA patients, 21 age-matched PD patients, and 20 age-matched healthy controls using a 3.0T MR system.
Results: ADC values in the pons, cerebellum and putamen were significantly higher, and FA values lower in MSA than in PD or controls. These differences were prominent in the pons

even in MSA lacking dorsolateral putaminal hyperintensity (DPH) or hot cross bun (HCB) sign. To distinguish MSA-P from PD, we set cutoff points in the pons, cerebellum and putamen for FA and ADC values respectively. Using these cutoff points, we obtained equal sensitivity and higher specificity in the pons than in the putamen and cerebellum. In addition, all patients that had both low FA and high ADC values in all three areas were MSA-P cases, and some MSA-P cases had low FA and normal ADC values or normal FA and high ADC values. Furthermore all patients that had normal FA and ADC values in the pons were PD cases.
Conclusion: FA and ADC values could provide early pathological involvement in MSA. It was more useful to examine both FA and ADC values than only one or the other to distinguish MSA-P from PD. Low FA and high ADC values in the pons were particularly helpful.

ALTERED VENOUS CAPACITANCE AS A CAUSE OF POSTPRANDIAL HYPOTENSION IN MULTIPLE SYSTEM ATROPHY

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Patients with multiple system atrophy (MSA) often have clinically significant postprandial hypotension (PPH). To elucidate the cause of insufficient cardiac preload augmentation that underlies PPH, we recorded calf venous capacitance (CVC) by strain-gauge plethysmography, in 17 MSA patients and 8 healthy controls before and after oral glucose ingestion. Among 17 MSA patients, 9 who showed a decrease in systolic blood pressure exceeding 20 mm Hg and were diagnosed with PPH. MSA patients without PPH showed a significant decrease in CVC and a significant increase in cardiac output after oral glucose ingestion, as did controls; those with MSA exhibiting PPH showed a significant increase in CVC and no significant change in cardiac output. The change in CVC correlated positively with the decrease in systolic and diastolic blood pressure after glucose ingestion, and also correlated negatively with the increase in cardiac output. Physiologically, PPH is prevented by a decrease in venous capacitance, which increases circulating blood volume and cardiac output. In some MSA patients, failure of venous capacitance to decrease may induce PPH.

ARCHAEAL PROTEASOMES EFFECTIVELY DEGRADE AGGREGATION-PRONE PROTEINS AND REDUCE CELLULAR TOXICITIES IN MAMMALIAN CELLS

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Objectives: To treat familial ALS and other intractable neurodegenerative diseases caused by mutant and modified proteins, which are not properly degraded by endogeneous proteasomes and, thus, prone to toxic gain of function resulting in neurodegeneration, we generated archaeal proteasomes in mammalian cells and examined their effects.
Methods: We cloned the *Methanosarcina mazei* (Mm) proteasome alpha- and beta-subunits and generated a mutant beta-subunit (Thr1Cys) which loses proteasomal activities. The experiments were performed in both HEK293 and Neuro2a cells. To analyze turn over of substrates, cycloheximide and pulse chasing study were performed. We chose MTS assay to evaluate the cellular toxicities.
Result: We successfully reproduced the Mm proteasomes in a functional state in mammalian cells and show that the Mm proteasomes effectively accelerated species-specific degradation of mutant SOD1 and the mutant polyglutamine tract-extended androgen receptor, causative proteins of familial ALS and SBMA, respectively, and reduced the cellular toxicities of these mutant proteins by MTS assay. Further, we demonstrate that Mm proteasomes can also degrade other neurodegenerative disease-associated proteins such as alpha-synuclein and tau.
Discussion: We demonstrated that Mm proteasomes could effectively degrade neurodegenerative disease-related aggregation-prone proteins. Further studies are needed to determine whether archaeal proteasomes can be available to treat diseases in which toxic-gain of proteins are causative.

THE LATERAL SEMICIRCULAR CANAL AND VERTIGO IN PATIENTS WITH LARGE VESTIBULAR AQUEDUCT SYNDROME

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Objective: Compare patients with large vestibular aqueduct syndrome (LVAS) to controls evaluating the hypothesis that there are differences in the morphology of their lateral semicircular canal (LSCC); and to investigate the clinical implications of these possible differences.
Setting: University Hospital
Patients: Nine patients (2 men and 7 women; age range 8–54 years) with LVAS (all of the patients but one had bilateral LVAS; 4 patients (including the LVAS unilateral case) did not have vertigo).

Procedure: The area of the LSCC was raced on the MRI console and compared between the LVAS patients and 12 controls (without hearing loss). The LSCC fluid-containing area was divided by the sum of the LSCC inner area and the LSCC fluid-containing area for evaluation of the degree of the LSCC dysplasia.
Results: The LSCC fluid-containing ratio was significantly larger in LVAS patients than in the controls. Furthermore, the LSCC fluid-containing ratio was significantly larger in the ears with vertigo than in the ones without vertigo. There was no relationship between the hearing level and the LSCC fluid-containing ratio.
Conclusion: LVAS patients may have disturbed morphogenesis of both membranous and bony labyrinths. Our results reveal that the morphology of the semicircular canal is clinically associated with vertigo.

DIFFERENTIAL EFFECTS OF TOPICALLY APPLIED VOLATILE ORGANIC COMPOUNDS ON MICROVASCULAR LEAKAGE IN RAT SKIN

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Skin contact with volatile organic compounds (VOCs) frequently causes skin irritation and subsequent dermatitis. However, the irritation potential of VOCs remains to be completely evaluated. This study investigated the differential effects of topically applied VOCs on microvascular leakage in rat skin. Capsaicin, a compound that specifically causes skin response by endogenously released tachykinins, was tested. Evans blue dye extravasations served as an index for the increase in skin vascular permeability. Formalin, xylene, toluene, stylene, benzene, ethylbenzene, acetone, diethyl ether, hexane, heptane, cyclohexane, and capsaicin were applied to the skin of the abdomen, which was shaved. Skin samples were collected 40 min after the application of the compounds. Formalin and the aromatic VOCs produced significant microvascular leakage in the skin, similar to capsaicin, while the other compounds did not react. Furthermore, we investigated the effect of CP-99,994, a tachykinin NK1 receptor antagonist; capsazepine, a vanilloid VR1 receptor antagonist; and ketotifen, a histamine H1 receptor antagonist that stabilizes mast cells, on the skin response. The skin response induced by formalin and capsaicin was markedly inhibited by CP-99,994. On the other hand, the antagonist slightly but significantly reduced the response induced by xylene and toluene. Capsazepine and ketotifen did not alter the response induced by any of the VOCs, although capsazepine partly inhibited the response induced by capsaicin. Similar to capsaicin, formalin and the aromatic VOCs caused microvascular leakage in the skin, partly via NK1 receptor activation by tachykinins released from sensory nerve endings. However, the mast cells and VR1 receptors did not play an important role in the skin response.

MULTIPLE PATHWAYS FOR NOXIOUS INFORMATION IN THE HUMAN SPINAL CORD

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Objective: To investigate the pathways of noxious information in the spinal cord in humans.
Methods: We recorded cortical potentials following the stimulation of A-delta fibers using a YAG laser applied to two cutaneous points on the back at the C7 and Th10 level, 4 cm to the right of the vertebral spinous process.
Results: A multiple source analysis showed that four sources were activated; the primary somatosensory cortex (SI), bilateral parasylvian region (Parasylvian), and cingulate cortex. The activity of the cingulate cortex had two components (N2/P2). The mean peak latencies of the activities obtained by C7 and Th10 stimulation were 166.9 and 186.0 ms (SI), 144.3 and 176.8 ms (contralateral Parasylvian), 152.7 and 185.5 ms (ipsilateral Parasylvian), 186.2 and 215.8 ms (N2), and 303.0 and 332.3 ms (P2). Estimated spinal conduction velocities (CVs) of the respective activities were 16.8, 9.3, 8.7, 10.1 and 10.7 m/s. CV of SI was significantly faster than the others ($P < 0.05$).
Conclusion: Our results suggested that noxious signals were conveyed through at least two distinct pathways of the spinal cord probably reaching distinct groups of thalamic nuclei. Further studies are required to clarify the functional significance of these two pathways.

THROMBOPHILIC ABNORMALITIES AMONG PATIENTS WITH CRANIAL DURAL ARTERIOVENOUS FISTULAS

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Objective: Dural sinus thrombosis often accompanies or precedes development of dural arteriovenous fistulas (DAVF). As thrombophilic abonormalities can contribute to sinus thrombosis, we investigated prevalence of such abnormalities and venous sinus thrombosis in patients with DAVF.
Methods: Thrombophilic factors were measured in 27 patients with DAVF treated with embolization at our university hospital. Control data were obtained from patients with unruptured intracranial aneurysm. In addition to sinus thrombosis, we investigated prothrombin time, activated thromboplastin time, platelet count, fibrinogen, platelet, antithrombin III, protein C, protein S, anti-cardiolipin antibodies, anti-cardiolipin β2-glycoprotein-I complex antibody, and D-dimer.
Results: Of 18 patients with DAVF before treatment, 16 had abnormal D-dimer, while mean values for other thrombophilic factors were nealy normal. D-dimer was significantly higher in pre-operative DAVF patients than in controls. Importantly, the mean value of D-dimer was higher among patients with sinus occlusion than among those without occlusion (3.33 vs.1.19).

D-dimer rose after embolization in 8 of 10 serially tested patients, but on average the change was not significant. In patients in the chronic stage with cure of DAVF, D-dimer was lower than in pre-operative patients.
Conclusion: D-dimer is a very sensitive indicater of acute venous thrombosis, suggesting that elevations in patients with DAVF are likely to reflect sinus occlusion. Serial measurements of D-dimer may predict likelihood of spontaneous sinus thrombosis after incomplete embolization. D-dimer may be helpful in planning treatment and elucidating pathogenesis.

ASSOCIATION OF SOX10 WITH SCHIZOPHRENIA IN THE JAPANESE POPULATION

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Introduction: Microarray studies of schizophrenic brains revealed decreases in the expression of myelin and oligodendrocyte-related genes. Of these genes, sex-determining region Y-box 10 (SOX10) is a major transcription factor modulating the expression of proteins involved in neurogenesis and myelination, and SOX10 abnormalities are hypothesized to have a role in the pathophysiology of schizophrenia. The SOX10 gene is located on chromosome 22q13.1, a region repeatedly reported to show positive signals in linkage studies on schizophrenia.
Material and Method: We performed an association analysis of SOX10 in a Japanese population of 915 schizophrenic subjects and 927 controls. One SNP of the SOX10 gene (rs139887) was selected as a haplotype tag SNP using 96 controls.
Result: A significant association was observed in the genotype and allelic frequency of this SNP between schizophrenic patients and controls ($p = 0.025$ and $p = 0.009$, respectively). We also performed a mutational search of the whole coding region, branch site, and promoter region of SOX10 in 96 schizophrenia patients, but no potentially functional polymorphisms were detected.
Conclusion: The present study suggests that the SOX10 gene is related to the development of schizophrenia in the Japanese population.

ASSOCIATION STUDY BETWEEN SEROTONIN RECEPTOR 3B (*HTR3B*) AND TREATMENT-RESISTANT SCHIZOPHRENIA (TRS) IN THE JAPANESE POPULATION

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Introduction: *HTR3B* is located in 11q23 close to the possible susceptibility locus for schizophrenia. It is co-expressing with *HTR3A* in the neuron surface and increasing the channel conductance of HTR3A. Some antipsychotic drugs can inhibit the neurotransmitter release by HTR3A in brain. Therefore, we hypothesized the *HTR3B* might be one of the candidate genes of TRS.

In this study, we examined the association between *HTR3B* and the treatment response to antipsychotic drugs in Japanese schizophrenic patients by single-marker and haplotype analysis after linkage disequilibrium evaluation.

Material and Method: The subjects consisted of 345 Japanese schizophrenic patients who diagnosis by DSM-IIIR and had been hospitalized and receiving antipsychotic drugs for more than one year. The daily neuroleptic dosage was calculated from the most recent 1-year neuroleptic prescription history. TRS was defined to have been receiving at least 1,000 mg/day of chlorpromazine equivalents antipsychotic drugs for more than 1 year. Genotyping was performed using PCR-RFLP methods blind to the symptomatology of the subjects.

Result: The genotype frequency of polymorphism Del-AAG which located in the promoter of the *HTR3B* in the TRS group was significantly different from that in the NON-TRS group after correction for type I error rate using the SNPSpD (p=0.031).

Conclusion: The present results suggest that the *HTR3B* is likely to be related with the development of treatment-resistant schizophrenia.

PHOSPHORYLATION BY CDK1 INDUCES PLK1-MEDIATED VIMENTIN PHOSPHORYLATION DURING MITOSIS

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Several kinases phosphorylate vimentin, the most common intermediate filament protein, in mitosis. Aurora-B and Rho-kinase regulate vimentin filament separation through the cleavage furrow-specific vimentin phosphorylation. Cdk1 also phosphorylates vimentin from prometaphase to metaphase, but its significance has remained unknown. Here we demonstrated a direct interaction between Plk1 and vimentin-Ser55 phosphorylated by Cdk1, an event that led to Plk1 activation and further vimentin phosphorylation. Plk1 phosphorylated vimentin at ~ 1 mol phosphate/mol substrate, which partly inhibited its filament forming ability, *in vitro*. Plk1 induced the phosphorylation of vimentin-Ser82, which was elevated from metaphase and maintained until the

end of mitosis. This elevation followed the Cdk1-induced vimentin-Ser55 phosphorylation, and was impaired by Plk1 depletion. Mutational analyses revealed that Plk1-induced vimentin-Ser82 phosphorylation plays an important role in vimentin filaments segregation, coordinately with Rho-kinase and Aurora-B. Taken together, these results indicated a novel mechanism that Cdk1 regulated mitotic vimentin phosphorylation via not only a direct enzyme reaction but also Plk1 recruitment to vimentin.