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Pathophysiologic Significance of Host Reactions in Human Cancer Tissue: Desmoplasia and Tumor Immunity

HARUO OHTANI

Department of Pathology, Tohoku University School of Medicine, Sendai 980-8575

Invasive growth of malignant cells, particularly carcinoma cells, induces host reaction within and around tumor tissue. Representatives of them are desmoplasia, angiogenesis and immune reactions. Desmoplasia, a process of fibrosis, is induced by activation of fibroblasts with increased production of matrix proteins and matrix degrading enzymes. Angiogenesis is prerequisite for the growth of solid tumor. Inhibition of this is now a target of cancer therapy. The present author has proposed a concept that tumor vessels are composed of nutrient vessels and immune/inflammatory vessels. The latter is similar to venules in inflammatory lesions expressing the cell adhesion molecules to facilitate the transmigration of inflammatory cells to the tissue. In colon cancer, venules distributed along the invasive margin correspond to these vessels, which express E-, and P-selectins, and ICAM-1. These venules are considered to be an entry site of immune/inflammatory cells to cancer tissue. To further analyze immune mechanism, the present authors have confirmed that macrophages distributed along the invasive margin of colon cancer express costimulatory molecules B7.1/B7.2, which are required for the proliferation of T-cells. T-cells were co-localized with these cells. Clinicopathologic analysis confirmed that CD8+ T-cells distributed within cancer cell nest (intraepithelial) have the most significant impact on the patients' survival in colorectal cancer. These data suggest that various host reactions take place in the stroma of cancer tissue, which modulate the biologic behavior of cancer.

Key words--- stromal reactions; cancer invasion; immune reaction; desmoplasia

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Inhibitory Effects of Transforming Growth Factor-β1 Pretreatment on Experimental Pulmonary Metastasis of MCS-1 Chinese Hamster Mesenchymal Chondrosarcoma Cells

Nobuyoshi Fujisawa, Norimitsu L. Sato and Tei-Ichi Motoyama

Institute for Laboratory Animals, Niigata University School of Medicine, Niigata 951-8510, and The Second Department of Pathology, Yamagata University School of Medicine, Yamagata 990-9585

Recent studies have suggested that transforming growth factor(TGF)-β1 acts as a multifunctional regulator of cell growth, and also modifies tumor progression and metastasis. In the present study, we investigated the effects of TGF-β1 on the proliferation and experimental pulmonary metastasis of MCS-1. MCS-1 are undifferentiated type cloned tumor cells established from a mesenchymal chondrosarcoma which spontaneously occurred in the soft tissue of a female Chinese hamster. MCS-1 cells were pretreated with TGF-β1 (0, 0.05, 0.5, 2, 10 ng/ml) for 72 hours in a medium containing 1% fetal bovine serum, then tested for in vitro growth by the MTT method, in vivo growth by subcutaneous inoculation into athymic nude mice (1×10^6 cells/mouse) and experimental pulmonary metastasis by injection into the lateral tail vein of athymic nude mice (5×10^4 cells/mouse). TGF-β1 significantly inhibited in vitro growth of MCS-1, depending on its concentrations, and also experimental metastasis with maximal inhibition at 0.5 or 2 ng/ml treatment compared to untreated controls. TGF-β1, however, was ineffective for in vivo subcutaneous growth of MCS-1. These results indicated that TGF-β1 might be an inhibitor of metastasis of mesenchymal chondrosarcomas including other types of non-epithelial cartilage or bone formation tumors.

Key words--- experimental metastasis; TGF-β1; mesenchymal chondrosarcoma; Chinese hamster

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Address for reprints: Nobuyoshi Fujisawa, Institute for laboratory animals, Niigata University School of Medicine, 1 Asahimachi-dori, Niigata 951-8510, Japan.

E-mail: arinomi@med.niigata-u.ac.jp

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Better-Surviving Liver Grafts by the Injection of Anti-CD2 Antibody: The Important Roles of Host CD8+ and CD2+CD28+ T Cells in Chronic Graft Rejection and β Type Platelet-Derived Growth Factor Receptor (PDGFR-β) Expression on Apoptotic Liver Grafts

TADAKO NAKATSUJI

Department of Transfusion, Hamamatsu University School of Medicine, Hamamatsu 431-3192

Syngeneic liver grafts were implanted in the livers of 22 LEW/Sea strain rats. To prolong the graft survival, anti-CD2 monoclonal antibody (MAb) or anti β type platelet-derived growth factor receptor (PDGFR-β) antibody (Ab) was injected, or splenectomy was performed in the rats which were then followed until 10 to 11 weeks posttransplantation. The 22 rats with chronic graft rejection showed increased CD8a-like antigen (probably Fas ligand) on the peripheral blood T cells. All the liver grafts had both necrosis and apoptosis. The liver graft apoptosis was indicated by histopathological abnormalities, and by DNA strand breaks and hemosiderin depositions in the cytoplasm. PDGFR-β expression in the apoptotic liver graft was demonstrated immunohistochemically. Among the 17 rats injected with anti-CD2 MAb, CD2 signaling on host T cells was effectively suppressed by the injection of anti-CD2 MAb in 4 rats with better-surviving liver grafts. In these 4 rats, CD28 antigen on thymic lymphocytes was down-modulated and high numbers (136-233-positive cells per lobe) of the epithelial reticular cells with apoptotic lymphocytes were counted. Anti-PDGFR-β Ab caused high pulmonary secretions of growth factors and reticular fibrosis in the lungs of 5 rats injected with the Ab. Anti-PDGFR-β Ab injection reduced the host cell apoptosis in the lung and thymus, but did not prolong the survival of liver grafts. In the 9 rats with both splenectomy and anti-CD2 MAb injection, pulmonary apoptosis was induced with the 6-16% reductions of CD4+ lymphocytes. Prolonged graft survival was observed in only one of the 9 rats. Anti-CD2 MAb was effective for prolonging the liver graft survival with suppressed CD28 antigen, but anti-PDGFR-β Ab and splenectomy were not.

Key words--- anti-CD2 MAb; anti-PDGFR-β Ab; splenectomy; liver graft; apoptosis

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Address for reprints: Tadako Nakatsuji, M.D., Department of Transfusion, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-3192, Japan.

e-mail: nh80415@hama-med.ac.jp

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Administration of Nerve Growth Factor, Brain-Derived Neurotrophic Factor and Insulin-Like Growth Factor-II Protects Phosphate-Activated Glutaminase in the Ischemic and Reperfused Rat Retinas

HIROSHI TOMITA, SEI-ICHI ISHIKURO, TOSHIKAI ABE and MAKOTO TAMAI

Department of Ophthalmology, Tohoku University School of Medicine, Sendai 980-8574

Phosphate-activated glutaminase (PAG) activity decreases markedly in the early period of ischemia. The decrease of the enzyme activity is reversible if the ischemic period is relatively short, but it becomes irreversible after 90 minutes of ischemia. The deterioration is a functional damage of the retinas caused by ischemia. We studied effects of growth factors and neurotrophic factors on protection of PAG in the ischemic and reperfused rat retinas. Before ischemia, 1 μl of growth factors or neurotrophic factors (0.1 μg/μl for insulin-like growth factor-I [IGF-I], insulin-like growth factor-II [IGF-II], brain-derived neurotrophic factor [BDNF], nerve growth factor [NGF]; 1 μg/μl for basic fibroblast growth factor [bFGF]) were injected into the vitreous cavity of the left eyes of anesthetized Sprague Dawley rats. As a control, phosphate buffered saline was injected to the right eyes. To induce ischemia, we clamped left eyes for 90 minutes after bulbar conjunctival incision all around limbus. The rat retinas were homogenized with distilled water 1 day after reperfusion and used for PAG assay. Retinal ammonia concentration was also determined as a ischemic marker. About 80% decrease of retinal PAG activity and 50% increase of retinal ammonia concentration were observed after 90 minutes of ischemia and 1 day of reperfusion as compared with unoperated normal eyes. IGF-II, BDNF and NGF had protective effects on the retinal PAG activity, whereas IGF-I, bFGF, stable bFGF were less effective. In addition, IGF-II and BDNF suppressed elevation of retinal ammonia concentration. BDNF, NGF and IGF-II have marked effect on the protection of PAG activity in the ischemic and reperfused rat retinas, whereas bFGF, which is very effective for the protection of ischemic cell death, shows moderate effect.

Key words--- ischemia; phosphate-activated glutaminase; ammonia; growth factors

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The Effect of Spinal Instrumentation on Lumbar Intradiscal Pressure

EJI ABE, TROY NICKEL,1 GLENN R. BUTTERMANN,1 JACK L. LEWIS1 and ENSOR E. TRANSFELDT1

Department of Orthopaedic Surgery, Akita University School of Medicine, Akita 010-8543, and 1Bioengineering Laboratory at the Department of Orthopedic Surgery, University of Minnesota, Minneapolis, MN 55455, USA

The purpose of this study was to investigate the effect of spinal instrumentation on the intradiscal pressure (IDP) within the fixed motion segment. In vitro biomechanical testing was performed in six single functional spinal units of fresh calf lumbar spines using a pressure needle transducer. Various loads were applied by a materials testing system device. In addition to intact spine (control), anterior spinal instrumentation (ASI) and pedicle screw fixation (PS) constructs, as well as destabilized spine were tested. Relative to the control, the destabilized spine tended to have an increased IDP; by 15% in axial compression and by 9-36% in flexion-extension. Compared to the control, PS decreased the IDP by 23% in axial loading and 51% in extension loading and increased it by 60% in flexion for each loading. ASI decreased the IDP by 32% in flexion and 1% in extension. Lateral bending produced symmetrical changes of IDP in the control and destabilized spine, but no change in the PS construct. The IDP of the ASI construct was decreased by 77% in ipsilateral bending and increased by 22% in contralateral bending. These results demonstrated that eccentric loading from the spinal instruments increased IDP and significant disc pressure may still exist despite an increase in motion segment stiffness after lumbar stabilization.

Key words--- intradiscal pressure; load sharing; pedicle screw fixation; anterior spinal instrumentation

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Address for reprints: Eiji Abe, Department of Orthopedic Surgery, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan.

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Localization of PDK-1 mRNA in the Brain of Developing and Adult Rats

SHUICHI YOSHIDA,1,2 HIROYUKI SAKAGAMI,1 YUJI OWADA,1 SHOICHI KOKUBUN2 and HISATAKE KONDO1

1Division of Histology, Department of Cell Biology, and 2Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Tohoku University, Sendai 980-8575

Gene expression for 3-phosphoinositide-dependent protein kinase-1 (PDK-1) in developing and adult rat brains was examined by in situ hybridization histochemistry. In embryonic days, the mRNA was evident throughout the entire neuraxis. The expression remained evident throughout the entire gray matters until postnatal day 7, and thereafter it decreased overall in the mantle and ventricular zones except for the cerebellar Purkinje and granule cell layers, the olfactory and hippocampal neuronal layers. The pattern of this gene expression is similar to those of for protein kinase B and class I phosphoinositide 3-kinases. 

Key words--- 3-phosphoinositide-dependent protein kinase-1; Rat; Brain; Development; in situ hybridization

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Address for reprints: Shuichi Yoshida, Division of Histology, Graduate School of Medical Sciences, Tohoku University, Sendai 980-8575, Japan.

E-mail: yoshida@gonryo.med.tohoku.ac.jp

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Sera of 20 children falsely identified as positive for hepatitis C virus antibody (Anti-HCV) by a second generation anti-HCV-assay kit (Imucheck-HCV Ab “Kokusai”) were re-tested using a new third generation anti-HCV-assay kit (Imucheck•F-HCV C50 Ab “Kokusai”). Seventeen of the samples were reclassified as negative and only three remained positive. Changing well solids in the anti-HCV-assay kit from casein to bovine serum albumin appears to have improved the false-positive rate, most likely as a result of decreased non-specific adsorption of casein antibodies.

Key words --- Anti-HCV; false positive; casein antibodies

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Address for reprints: Kohachiro Sugiyama, Department of Pediatrics, Nagoya City University Medical School, Kawasumi-cho, Mizuho-ku, Nagoya 467-8601, Japan.

e-mail: sugiyama@med.nagoya-cu.ac.jp
Point Mutations in the Steroid-Binding Domain of the Androgen Receptor Gene of Five Japanese Patients with Androgen Insensitivity Syndrome

NOBUO YAEGASHI, SHIGEKI UEHARA, MASATO SENOO, JUNKO SATO,¹ JUNKO FUJIWARA,¹ TADAO FUNATO,¹ TAKESHI SASAKI and AKIRA YAJIMA

Department of Obstetrics and Gynecology and ¹Department of Laboratory Medicine, Tohoku University School of Medicine, Sendai 980-8574

We analyzed the androgen receptor (AR) gene in five Japanese patients diagnosed with androgen insensitivity syndrome (AIS). All AR genes from the five patients had single-nucleotide substitutions, which introduced a premature termination codon in three patients (Gln640, Arg752, and Gln640 and Trp751), and a single amino acid substitution in two patients (Arg831 to Gln, and Leu812 to Phe). All the mutations occurred in the steroid-binding domain, comprising exons D through G. The three patients with the premature termination codon(s) and the one patient with Arg831Gln were clinically diagnosed as having complete AIS, while the patient with Leu812Phe had a partial form of AIS. Pubic skin fibroblasts from four of the five patients did not show detectable androgen binding. These data on mutations that have not been reported previously, provide valuable information for the further characterization of structural and functional relationships in the steroid-binding domain of the AR protein.

Key words--- androgen receptor; nucleotide substitution; nonsense mutation; missense mutation

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Address for reprints: Shigeki Uehara, M.D., Department of Obstetrics and Gynecology, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan.
e-mail: uehara@ob-gy.med.tohoku.ac.jp

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Long-Term Azathioprine Therapy in Two Children with Steroid-Dependent Minimal-Change Nephrotic Syndrome

HIROSHI TANAKA,¹,² NORIO ONODERA¹ and SHINOBU WAGA²

¹Division of Pediatrics, Iwate Prefectural Kitakami Hospital, Kitakami 024-0063, and ²Department of Pediatrics, Hirosaki University School of Medicine, Hirosaki 036-8562

Long-term azathioprine therapy as an alternative treatment to cyclophosphamide was done in 2 children with steroid-dependent minimal-change nephrotic syndrome (MCNS). They had already been treated with prednisolone, intravenous methyl-prednisolone pulse therapy, cyclophosphamide and mizoribine. Although cyclophosphamide had been proved to be effective in maintaining their remission, the cumulative dose of the agent limited another course of cyclophosphamide therapy. Since ciclosporine therapy is much expensive, a trial of azathioprine (2 mg/kg per day) was started, and the therapy resulted in inducing sustained remission and reducing prednisolone. The patients were well tolerated the long-term azathioprine therapy over a year. Although the efficacy of azathioprine in the management of childhood MCNS might be restricted, we therefore suggest that this agent should be reconsidered as an alternative treatment to cyclophosphamide.

Key words--- alternative treatment; azathioprine; childhood; long-term treatment; steroid-dependent minimal-change nephrotic syndrome

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Infantile Pulmonary Alveolar Proteinosis with Interstitial Pneumonia: Bilateral Simultaneous Lung Lavage Utilizing Extracorporeal Membrane Oxygenation and Steroid Therapy

TADAHIKO ITO, MASAMITSU SATO,1 TADASHI OKUBO,2 IWAO ONO3 and JINZO AKABANE

Department of Pediatrics, 1Department of Anesthesiology, 2Department of Cardiovascular Surgery, 3Department of Pathology, Nakadohri General Hospital, Akita 010-8577

An infant with refractory pulmonary alveolar proteinosis (PAP) associated with severe interstitial pneumonia is described. Although she was treated by bilateral simultaneous lung lavage utilizing extracorporeal membrane oxygenation and steroid therapy, she died of progressive respiratory failure 28 days after admission. Histologic examination of lung autopsy specimen showed only partial alveolar spaces to be filled with a dense PAS positive granular eosinophilic material and showed severe interstitial pneumonia with marked fibrosis of alveolar walls and interstitium. The lung lavage seemed to be effective for PAP because the effluent fluid sufficiently became clear and the PAS positive material was detected only in partial alveoli. The full venoarterial cardiopulmonary bypass with extracorporeal membrane oxygenation seemed to be very useful to support bilateral lung lavage for small infants. The refractory symptoms and failure of treatment were resulted from the association of severe interstitial pneumonia. In neonates or infants with PAP and severe interstitial pneumonia with poor response for steroid therapy, the lung transplantation should be considered.

Key words--- pulmonary alveolar proteinosis; interstitial pneumonia; lung lavage; extracorporeal membrane oxygenation

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Address for reprints: Tadahiko Ito, M.D., Department of Pediatrics, Nakadohri General Hospital, 3-15 Misono-cho, Minami-dori, Akita 010-8577, Japan.
Prenatal Confirmation of the Translocation between Chromosome 15 and Y-Chromosome by Fluorescence in situ Hybridization

YUKIHITO FUKADA, TAKEHIKO YASUMIZU, ATSUHITO AMEMIYA, KEIKO KOHNO, MOTOI TAKIZAWA and KAZUHIKO HOSHI

Department of Obstetrics and Gynecology, Yamanashi Medical University, Yamanashi 409-3898

A 30-year-old woman and her husband visited our hospital with habitual abortion as the complaint. Chromosome examination revealed a normal 46, XX for her and 46, XY, -15, der (15) t (Y; 15) (q12; p12) for him. After her pregnancy amniocentesis was performed. The karyotype was 46, XX, -15, der (15) t (Y; 15) (q12; p12) pat. ish der (15) (DYZ1+). A female baby was delivered. The growth of the baby was normal at 12 months of age.

Key words--- chromosome 15 and Y translocation; fluorescence in situ hybridization (FISH); uncultured amniotic fluid cells; prenatal diagnosis

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