Immunohistochemical Study of [D-Ala²]deltorphin I in Rat Retina

Hiromichi ABE¹, Kazuo NONOMURA²,
Ikuro TOOYAMA³ and Hiroshi KIMURA³

¹Departments of Ophthalmology and ²Pediatrics, and

³Molecular Neurobiology Research Center,
Shiga University of Medical Science, Otsu

Immunohistochemical distribution of [D-Ala²]deltorphin I (DADTI), a highly selective ligand for δ opioid receptors, was investigated in rat retina by using a specific antiserum. The antiserum against DADTI was raised in a rabbit by immunizing with a synthetic peptide (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH₂) conjugated to poly-L-glutamate with water soluble carbodiimide. When examined by immunospot assay, the major epitope of the antiserum appeared to be in the C-terminal region containing Asp-Val-Val-Glu-NH₂, the sequence known to be responsible for δ opioid receptor selectivity. Immunohistochemically, DADTI-like-positive staining was found in some retinal amacrine cells situated in the inner nuclear layer. Their stained processes were distributed in the inner plexiform layer. In addition, a few positive cells, probably ectopic amacrine cells, were seen in the ganglion cell layer. Double immunostaining for DADTI and other neurotransmitters or neuropeptides revealed that 12.8% and 27.7% of DADTI-positive cells contained GABA and neuropeptide Y, respectively. However, DADTI-positive cells were seldom colocalized with somatostatin or tyrosine hydroxylase. The present study suggests that DADTI in retinal amacrine cells play role(s) functioning as an endogenous opioid peptide.

³H-Leucine Uptake of Pancreatic Peri- and Tele-Insular Acinar Cells in Mice.

Aughsteen A. ADIB and Katsuko KATAOKA

Department of Anatomy, Hiroshima
University School of Medicine,
Hiroshima

The uptake of ³H-leucine by pancreatic acinar cells of peri- and tele-insular acini was analysed quantitatively, 60 min after the intraperitoneal injection of ³H-leucine in normal and streptozotocin-diabetic mice. A large tissue sample was selected in an unbiased systematic procedure and the silver grain density was counted with a color image analyzer equipped with a computer. The radioactivity was expressed as number of silver grains per cell as well as per unit area of acinar tissue.

In normal mice, the radioactivity in peri-insular acinar cells was 39% greater than in tele-insular acini. The intercalated duct associated with the former also showed a higher density of silver grains. In streptozotocin-diabetic mice, the uptake of ³H-leucine was significantly lower than in normal mice, and the grain densities in both peri- and tele-insular acini were almost similar.

The estimate of mean acinar cell profile area was 22% larger in the peri-insular acini of normal mice, while no difference was observed between the estimates of peri- and tele-insular acinar cells of diabetic mice.

These results suggest that B cell secretion, probably insulin, plays a certain role in the protein synthesis of pancreatic acinar cells, mainly in the peri-insular acini.

Analysis of transgene products in transgenic mice with high copy numbers of Major Histocompatibility Complex class II gene

Sadakazu AISO,^{*} Masahide SHIOZAWA, Manabu OHYAMA, Yuhji TAKEUCHI & Toshihiko EZAKI
Department of Anatomy, Keio University
School of Medicine, Tokyo

We produced transgenic mice with transgenes of Major Histocompatibility Complex (MHC) class II I-A^b genomic clone DNA fragments by a microinjection method. Presence of transgenes and copy numbers of them were confirmed by Southern blot analysis. Transgenic mice lines with various copy numbers from a single to 65 copies of transgenes were established. Northern blot analysis revealed that the amount of mRNA transcribed from transgenes was correlated with their copy numbers. However, flow cytometry of spleen cells showed that the amount of the transgene product protein expressed on the cell surface was less in high copy number transgenic mice than that of the mice with 10 copies of transgenes. Moreover, the amount of endogenous I-A decreased with the increase of transgene copies. Histologically, the spleen from transgenic mice with high copy numbers showed remarkable decrease in numbers of lymphoid follicles and I-A (+) cells and increase in Mac-1 (+) cells. The number of CD4 (+) or CD8 (+) cells did not show significant change.

In summary, it is suggested that B-cell maturation process is impaired in the I-A^b transgenic mice with high copy numbers of transgenes.

Human colorectal carcinoma-specific glycoconjugates detected by pokeweed mitogen lectin

Toshihiro AOKI^{1,*}, Tsutomu OINUMA²,
Jun-ichi KAWANO², and Tatsuo SUGANUMA²
¹First Department of Internal Medicine and
²Department of Anatomy, Miyazaki Medical
College, Japan

Pokeweed mitogen (PWM) lectin, which is confirmed to bind branched poly-N-acetyllactosamine structures of glycoconjugates, possesses a highly selective affinity towards carcinoma tissues (Muramatsu H et al., J Biochem 1990; 107: 629-634). Lectin histochemistry with PWM was performed on paraffin sections of human colorectal normal mucosae, adenomas, and carcinomas.

Normal mucosae and adenomas with mild dysplasia showed negative staining (0/10, 0/13 respectively) with or without neuraminidase predigestion, while adenomas with moderate dysplasia showed a small increase in PWM lectin staining after neuraminidase predigestion (4/23). In contrast, high incidence of positive staining was found in colorectal carcinoma without neuraminidase predigestion (38/44). After neuraminidase digestion, colorectal carcinomas exhibited increased reactivity in carcinomas in situ (7/12) and invasive carcinomas (13/32).

These results demonstrate that PWM lectin-reactive branched poly-N-acetyllactosamine structures of glycoconjugates consistently increase in human colorectal carcinomas. Therefore PWM lectin histochemistry should be useful for the identification of carcinoma tissues and the analysis of carcinoma-associated antigens involved in the adenoma-carcinoma sequences.

**Immunohistochemical Studies on the
Localization of GAP-43 in Growth Cones of
Regenerating Nerves in Rat.**

Masahiko Arakawa, Akira Mizoguchi and
Chizuka Ide
Department of Anatomy, Kobe University School
of Medicine, Kobe

Localizations of GAP-43 (Growth-associated Protein-43) in growth cones of regenerating nerves in the rat were investigated immunohistochemically with monoclonal antibody which was purchased from Boehringer Mannheim and with horseradish peroxidase-linked secondary antibody.

In the transected median nerve, growth cones sprouted from nodes of Ranvier in the region 1 to 2 mm proximal to the lesion within 1 day postoperative. However, GAP-43 immunoreactivity (GAP-43-IR) was not obvious in these early growth cones. At 2 days following the lesion, GAP-43-IR was identified in growth cones of the regenerating axons extending in contact with the basement membrane. GAP-43-positive growth cones were increased in number 3 days after transection. GAP-43-IR was distributed homogeneously in the axoplasm, with occasional accumulations in the periphery of the growth cone.

These results indicate that GAP-43 might not be necessary for the initiation of sprouting. It appeared that GAP-43 accumulates gradually in the growth cones which have grown out for a certain distance.

**Fluorescence characteristics of ethidium bromide
bound to nuclear DNA *in situ* in cell nucleus
(Evaluation of the fluorescence characteristics
by nanosecond microscope fluorometry)**

Tsutomu ARAKI
Department of Production and Mechanical Engineering,
Technical College, University of Tokushima

Ethidium Bromide (EB) is intercalated into double stranded DNA, resulting in a DNA specific fluorochrome. When EB is used as the DNA probe in histochemical study, EB sometimes shows a non-specific binding to unexpected specimens. We evaluated the binding of EB using a nanosecond microfluorometry. For this, a time-resolved microscope fluorometer with a time resolution of 50 ps was devised.

Fluorescence lifetime of EB increased dramatically when forming a complex with DNA. Degree of the increase was enlarged as increasing the P/D ratio (a molar ratio of DNA-p to EB). Therefore, the fluorescence lifetime can be used as an effective indicator of the binding of EB. Cotton and paper fibers were stained with EB as the same manner as cell nuclei. There were no appreciable differences in the fluorescence spectra and fluorescence lifetimes among the cotton-EB, paper-EB and nucleus-EB. This indicated that EB can be intercalated into the cotton fiber (and paper fiber), showing a non-specific binding.

The P/D ratio was estimated in the cell nucleus from the decay curve of time resolved fluorescence. Resultant P/D ratio was 20. Thus EB was concentrated to 1.5 mM in the nucleus, though concentration of EB was as low as 10 μ M in the staining medium.

**Development of Langerhans Cells in Mouse Skin:
Examination by Zinc Iodine-Osmium Method**

Mari ASADA-KUBOTA and Shinsuke KANAMURA
Department of Anatomy, Kansai Medical
University, Moriguchi, Osaka

The development of Langerhans cells during the differentiation of skin was studied in mice from fetal day 14 to adult by zinc iodine-osmium (ZIO) method. In the skin at fetal day 14, no cells were stained. In dermis, some fibroblastic cells and cells located around capillary vessels were stained after fetal day 15. These cells were frequently observed immediately below the epidermis during the fetal period. Some of the cells may be the precursors of Langerhans cells. In epidermis, desmosome-free fibroblastic cells were initially stained at fetal day 16, and they increased in number in the differentiating epidermis during the late fetal period. These cells are considered to be immature Langerhans cells. Kobayashi *et al.* (1987) reported that immature Langerhans cells could be identified ultrastructurally in the mice epidermis from fetal day 16, with ATPase-positive cells appearing the same day at light microscopic level. The present results agree with their observations on the development of Langerhans cells in mice epidermis.

**Cytochemical glucose 6-phosphatase activity
in the oviduct of rat**

Youko Asaka, Jun Watanabe and
Shinsuke Kanamura
Department of Anatomy, Kansai Medical
University, Moriguchi 570, Osaka

For study of the origin of glucose in the oviduct fluid, glucose 6-phosphatase (G6Pase) activity was cytochemically examined in rat oviduct. For light microscopic observations, the sections (12 μ m) were incubated in a modified Wachstein-Meisel medium for 30 min at room temperature, immersed briefly in ammonium sulfide solution and mounted in glycerin jelly. For electron microscopy, tissues were fixed by immersion in buffered 2% glutaraldehyde (pH 7.4) for 30 min at 4°C, sectioned at 30 μ m and incubated in the reaction medium for 1 hr at room temperature. The sections were postfixed in buffered 1% OsO₄, dehydrated and embedded in Spurr. During the proestrous, estrous and metestrous phase, the staining reaction for the activity was weak in the epithelium of the ampulla and proximal isthmus and moderate in that of the distal isthmus and uterotubal junction. The staining reaction became strong in the epithelium of the distal isthmus and uterotubal junction in the diestrous phase. The reaction product for the activity was localized in the endoplasmic reticulum and nuclear envelope of the secretory and ciliated cells in the epithelium of distal isthmus. The reaction product in the secretory cells appeared abundant in the diestrous phase. The results show that G6Pase activity is high in the epithelium of the distal isthmus and uterotubal junction, and suggest that the role of the enzyme is to release glucose into the oviductal fluid.

**Exprssion and intranuclear distribution
of the Ki-67 antigen during the cell
cycle as analyzed by autostage
cytofluorometry**

Yuji Azumi, Hayazo Kubo, Atsushi Horii,
Eiichi Konishi, Youji Urata and Tsukasa
Ashihara

Department of Surgery, Akashi Municipal
Hospital. Department of Pathology, Kyoto
Prefectural University of Medicine

Ki-67 antigen has been thought as a proliferation-related factor which measures the growth fraction (G.F.) of human tumors. We analyzed the expression and intranuclear distribution of this antigen in ConA-stimulated lymphocytes. Multiparametric autostage cytofluorometry for DNA, RNA, and Ki-67 antigen in combination with ³H-thymidine autoradiography, has enabled the detailed analysis of the expression of Ki-67 antigen in relation to the cell cycle. In ConA-stimulated lymphocytes, Ki-67 antigen was found to appear in the granular pattern from the beginning of the first S phase. The amount of this antigen increased during the course from S phase to mitotic phase, where its expression became the maximum, with the dense distribution around chromosomes. After mitosis, its amount decreased gradually during the subsequent G₁ phase. The interphase cells displayed both granular and nucleolar staining patterns except for the first S phase cells, which revealed the finely granular pattern.

Immunohistochemical Study on the Localization of Alkaline
Phosphatase during Regeneration of Proximal Tubule in Rat
Kidney
Kohsuke CHIDA
Department of Pathology, Kitasato University School of
Hygienic Sciences, Sagamihara, Kanagawa.

Immunohistochemical localization of alkaline phosphatase was examined during regeneration of proximal tubule in the gentamicin-injured rat kidney.

Gentamicin was injected subcutaneously for 10 days at a dose level of 90 mg/kg/day into seven-week-old male Fischer 344 rats. Kidneys were excised 1 day, 3 days, 5 days and 8 days after final administration. Kidney fragments were fixed in periodate-lysine-paraformaldehyde fixative solution under microwave irradiation and then further immersed in the same fixative solution. After fixation, cryostat sections were cut and stained by means of peroxidase labeled antibody method using anti-rat alkaline phosphatase serum. As a specimen for electron microscope, after the immunohistochemical staining, frozen sections were fixed in OsO₄ and thereafter embedded in Quetol 812.

Three days and 5 days after gentamicin administration, several proximal tubules whose the form of epithelial cells was flat or cuboidal and lumen enlarged were seen. The areas showing positive reaction were scanty in those proximal tubules. Upon examination under a electron microscope, in the epithelial cells of those proximal tubules, microvilli shortened and decreased, and therefore the positive sites decreased.

**Cytochemical Investigation of 5'-Nucleo-
tidase Activity in Paramecium Caudatum**
Kensuke CHIKAMORI, Tsutomu ARAKI* and
Shinsuke YAMASHITA
Department of Chemistry, Naruto University
of Education, Naruto and *Department of
Mechanical Engineering, University of
Tokushima, Tokushima

5'-Nucleotidase catalyses the dephosphorylation of 5'-AMP and other 5'-nucleotides to nucleosides and inorganic phosphate. This enzyme widely distributes within many species and the variety of its function has been reported so far.

The aim of this study is to investigate cytochemically whether this enzyme activity could be detected in a ciliated protozoa, Paramecium caudatum or not. The activity is visualized with lead capture method and its staining intensity is measured with a microphotometer.

Using the substrate for alkaline phosphatase (8-glycerophosphate), the staining intensity is lowered to about one third of that using 5'-AMP as the substrate. Tetramisole, specific alkaline phosphatase inhibitor, does not afford any inhibitory effect in the presence of 5'-AMP. However, the specific competitive 5'-nucleotidase inhibitors, 2'-AMP and adenosine-5'-O-thiomono-phosphate do not lower the staining intensities in the presence of 5'-AMP.

It concludes that the phosphatase activity detected with 5'-AMP as the substrate is non-specific and its characteristics may be different from those of non-specific alkaline phosphatase in mammalian tissues.

Unfortunately, we did not detect cytochemically the 5'-nucleotidase activity in Paramecium caudatum.

**Application of confocal LASER scanning microscope
(CLSM) to human surgical pathology materials.**

Fumiko DATE, Hironobu SASANO and Hiroshi NAGURA
Dept. of Pathol. Tohoku Univ. of Med., Sendai

Recently developed confocal LASER scanning microscope (CLSM) has provided valuable informations for studies on functions and structures of cultured cells and those in vitro experimental specimens. However, little has been reported on the application of CLSM to human diagnostic pathology materials.

We applied CLSM to immunostaining of cytokeratin, c-erbB 2, PCNA and p53 and PI nuclearstain in diagnostic materials of cell smears and sections of carcinoma of esophagus, endometrium and stomach. We also used CLSM to double immunostaining of p53 c-erbB 2 and PCNA in tissue sections from stomach cancer. Clear immunolocalizations for these antigens in the tissue sections could not be obtained on the same screen by image processing of the whole cells. In smear cells, however, CLSM could provide detailed intracellular localization of reaction products by the immunostaining. That is, compared to non-CLSM, better resolutions were obtained in cell smear preparations. These studies have shown that CLSM can provide valuable informations in diagnostic pathology, particularly in cytology.

Histochemical Demonstration of Copper in LEC Rat Liver

Yukiko FUJII, Kazuko SHIMIZU, Mami SATOH,
Fumiyo TAKAHASHI, Kenichi NAKASE,
Yasunori FUJIOKA, Kazuo NAGASHIMA,
Yuji TOGASHI*, and Noritoshi TAKEICHI**

Department of Pathology, and **Laboratory of Cell
Biology Cancer Institute, Hokkaido University
School of Medicine, Sapporo, and *Research
Institute for Pharmacology, Tsumura & Co., Tsukuba.

Livers of LEC rats were histochemically stained for copper according to the modified Timm's method, which included trichloroacetic acid (TCA) treatment. TCA pretreatment was effective for removing zinc and iron, leaving copper as the major metal stained by Timm's method. Hepatocytes of 3-month-old rats were intensely stained both in frozen section and in paraffin-embedded specimens. The staining was intensified in the hepatocytes of 8-month-old LEC rats, in which high concentration of copper was detected. The copper staining was augmented in copper-loaded livers, but less intense in those treated with d-penicillamine. Hepatocellular carcinoma showed less copper deposits than the hepatocytes surrounding the tumor. The staining intensity was well correlated with the copper concentration which was determined by a spectrophotometer. Lysosomal deposition of copper in hepatocytes was also confirmed by electron-microscopic analysis for copper. Thus, the modified Timm's method was shown to produce valuable results in demonstrating copper in LEC rat livers.

Immunohistochemistry of cell adhesion molecules
in the adult and developing mouse kidneys
Kazushi FUJIMOTO

Department of Anatomy, Faculty of Medicine,
Kyoto University, Kyoto 606, Japan

We studied the expressions of cell adhesion molecules, cadherins and neural cell adhesion molecule (N-CAM) in the fetal, neonatal and adult mouse kidneys. During the development of the kidney, a mesenchyme is converted into an epithelium when it is properly induced. The uninduced mesenchyme did not express E- and N-cadherin. However, as the mesenchyme was transformed into the epithelial structure of the metanephric tubules, both N- and E-cadherin appeared in the epithelial cells. At the S-shaped stage of nephron development, anti-E- and N-cadherin antibodies reacted with the epithelial cells of the tubules, but not with the podocytes of the glomeruli. Interestingly, the podocytes were labeled for N-CAM but the surrounding tissues including the proximal and distal tubules were devoid of N-CAM. In adult kidneys, all epithelial cells of the proximal, distal tubules and collecting ducts were positive for cadherins except the first segment of the proximal tubules. The epithelial cells of distal tubules and collecting ducts were more weakly stained than the proximal tubule cells. Staining for N-CAM could be detected only in the parietal epithelial cells of Bowman's capsule. Electron microscopically, the immunogold particles for E-cadherin were found in the intercellular spaces of the basolateral infoldings of the proximal tubule cells. The results raise the possibility that cell adhesion mediated by cadherins and N-CAM plays a primary role in histogenesis of kidney.

Distribution of catecholaminergic and
peptidergic nerves in interscapular
brown adipose tissues of mice

Satoshi Fujimoto, Jun WATANABE
and Shinsuke KANAMURA

Department of Anatomy, Kansai
Medical University, Moriguchi

To study the relationship between nerve fibers and blood vessels, distribution of catecholaminergic and peptidergic nerve fibers in the interscapular brown adipose tissue of adult male ddY mice were examined. Noradrenaline (NA) fibers were visualized by the glyoxilic acid condensation method, and peptidergic fibers were detected by the indirect immunofluorescence method or the peroxidase-antiperoxidase method using anti-neurotransmitter antibodies. Numerous NA fibers and a few neuropeptide Y (NPY) or substance P (SP) fibers were observed in the walls of interlobular or intralobular arteries and arterioles in the brown adipose tissue. NA and NPY fibers were also found around brown adipose cells in the parenchyme, whereas SP fibers were absent in the parenchyme. Although strong fluorescence or staining reaction due to NA, NPY, or SP was found in the walls of thin arteries and arterioles, the fluorescence and staining reaction was weak or absent in the walls of thick arteries. No fluorescence or staining reaction was seen in the walls of veins and venules. Neither somatostatin-positive nor vasointestinal polypeptide-positive fibers were seen in the brown adipose tissues.

Localization of Calcium Binding Proteins
in Mouse Kidney
Atsuko Fujioka and Mamoru Nagano

Department of Anatomy, Kinki University
School of Medicine, Osaka-Sayama

A large fraction of Ca^{2+} filtered in the glomerulus of a kidney is reabsorbed in a nephron. It is interesting that how Ca^{2+} is transported across the epithelial cell layer in the nephron, because the changes of intracellular Ca^{2+} concentrations regulate or trigger various events in the cell. It has been reported that the Ca^{2+} binding proteins (CaBPs) participate in the regulation mechanism of the intracellular Ca^{2+} concentrations. In the present study, CaBPs (calbindin 28Kd, parvalbumin) were immunocytochemically detected in the mouse kidney. Positive reaction was seen in distal convoluted tubules, connecting and initial portions of collecting ducts, and sometimes in the collecting ducts of a medullary ray, under a light microscope by a PAP method. Some unstained cells coexisted in their collecting ducts. IgG-gold conjugates were applied to LR-white embedded ultrathin sections for the demonstration of intracellular localization of their proteins. The gold-particles were specially rich in the distal convoluted tubule cells and the connecting tubule cells. Intercalated cells adjacent to the positive connecting tubule cells showed few particles. The gold particles appeared in the nucleus and the cytoplasm, without particular reference to intracellular membrane structure or filaments. Their positive cells to the immunoreaction seem to correspond to them showing abundant precipitation of Ca^{2+} , which is demonstrated in the previous study. It is suggested that the CaBPs regulate intracellular Ca^{2+} concentrations, in the cells absorbing Ca^{2+} actively.

Lectin Staining Patterns in Normal Human Skin Appendages and Mammary Glands
Yoshiko FUJITA, Yoshio IZUNO, Airo TSUBURA and Sotokichi MORII
Department of Pathology, Kansai Medical University, Moriguchi

Lectin bindings in normal human skin appendages and a specialized skin appendage, the mammary gland parenchyme, were observed on formalin-fixed, paraffin-embedded sections with ABC technique using 5 different biotinylated lectins; peanut agglutinin (PNA), soybean agglutinin (SBA), Dolichos biflorus agglutinin (DBA), Ulex europeus agglutinin-1 (UEA-1) and Helix pomatia (HPA). Each lectin examined showed a characteristic binding site. In the secretory segment of eccrine glands, the superficial dark cells showed a membranous staining pattern with UEA-1, while plasma membranes of the basal clear cells were stained with DBA, SBA, HPA and UEA-1. Acinar cells of the apocrine gland revealed sporadically a luminal membranous staining pattern with all 5 lectins examined. In the hair follicle, inner root sheaths were labeled with all examined lectins except for PNA, and entire outer root sheath was labeled with PNA. Outer root sheaths at the level of sebaceous duct insertion and the sebaceous duct were stained by all 5 lectins. Mature sebocytes in the sebaceous lobules were labeled with PNA, SBA and HPA. In the mammary gland, resting lobules showed sporadic membranous stainings with examined lectins except for SBA, while lactating alveoles showed linear membranous stainings with PNA and UEA-1. Enzymatic digestion with α -amylase, trypsin or neuraminidase, in general, introduced no any alterations in these lectin staining patterns. Such recognitions of lectin staining patterns in normal tissues may be useful to search for the origin and to make the diagnosis in human skin appendage tumors.

Immunohistochemical staining of cancerous cell with anti-single-stranded DNA antibody
Masaru Fukuda, Yoshiaki Imamura, Sakon Noriki Kenichi Koizumi
Department of Pathology, Fukui Medical School, Fukui

Based on the finding that the nuclear DNA of cancer cell is much more unstable than that of normal cell, yielding a larger amount of single-stranded DNA at acid hydrolysis, we developed a new method of cancer cell detection in ordinary pathological section by immunohistochemical staining with anti-single-stranded DNA antiserum after acid hydrolysis. Ordinary paraffin-sections taken from 482 malignant tumors, 73 benign tumors of human epithelial or non-epithelial origin, and 82 border-line tumors, were stained with the antiserum after RNase digestion and DNA denaturation by hydrolysis with 2N HCl at 30°C. In all sections, the acid hydrolysis for 20-30min was found optimum for the specimens fixed with 10% buffered formalin at room temperature for 16-24h, and all cancer cells were specifically stained positive, in sharp contrast to the negative stainability of all normal cells including inflammatory cells. All colorectal border-line adenoma (moderately to severely dysplastic) and group IV gastric adenoma were also stained positive. In these lesions, abnormal mitoses, single cell necroses without acute inflammation, abnormal glandular buddings and bridgings were usually present indicating their malignant characters. The increased DNA instability seems to be the common and essential phenomenon accompanying even the early stage of malignancy progression, possibly due to deficient production of chromatin packing proteins, and membrane abnormalities inducing the production of active oxygens, reflecting the loss of cytodifferentiating capabilities in malignancy. This new method is useful for the diagnosis and research of malignancy.

Light microscopic radioautographic study of RNA synthesis in the kidneys of perinatal mice
Toru HANAI, Nobuteru USUDA, Takashi MORITA and Tetsuji NAGATA

Department of Anatomy and Cell Biology, Shinshu University School of Medicine, Matsumoto

We studied the changes of RNA synthesis in the kidney tissues of perinatal mouse by light microscopic radioautography using ^3H -uridine.

Normal ddY strain mouse kidney tissues of various ages: prenatal 15 and 19 days of gestation, postnatal 1 and 8 days, 1 month were used for this experiment. The kidney tissues were labeled with ^3H -uridine either in vitro (3.7 MBq/ml) or in vivo (370 KBq/g bw). The tissues were fixed with 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. They were dehydrated and embedded in Luveak 812. From all the tissue blocks, thick sections (2 μm) were cut with a Porter-Blum MT-2B ultramicrotome using glass knives. The sections were picked up onto glass slides and coated with Konica NR-M2 emulsion by dipping. After the exposure for 3 months (in vitro) or 6 months (in vivo), they were developed with SD-1 developer, fixed, stained with 0.1% toluidine blue and were observed with an Olympus Vanox AHB-LB light microscope. On the radioautograms, the number of silver grains per cell was counted in glomeruli and uriniferous tubules in the superficial and deep layers of the kidney cortex.

As the results, the number of silver grains per cell (grain counts) in glomeruli and uriniferous tubules in the superficial and deep layers of the kidney cortex decreased due to aging. The grain counts in the superficial layer were higher than those in the deep layer from 15 days of gestation to 1 day after birth. However, the grain counts in the superficial layer were nearly equal to those in the deep layer from 8 days to 1 month after birth.

From the results, it is concluded that RNA synthesis of mouse kidney tissues changes during development and aging of mice.

Effects of tannic acid on antigenicity in immunocytochemical study by the aid of microwave irradiation.

1. Distribution of immunogold-labeled anti-glucagon in rat pancreatic islets under light and electron microscopy.

Hiroshi HASEGAWA, Vinci MIZUHIRA and Mitsuru NOTOYA
Shionogi Co. & Ltd., Kanzakigawa Research Laboratory, Toyonaka, Osaka 561.

A fixation method using aldehyde containing a small amount of tannic acid (TA) has been found to be excellent for fixing soluble proteins and low molecular peptides, such as angiotensin II (8-) or enkephalin (5-amino acids) including glucagon, which are cell components ¹⁾. This method can be used to enhance membrane contrast and intra- and extracellular ultrastructural preservation. However, only few reports have appeared on the effects of TA on antigenicity. In this study, we evaluated the effects of TA using the immunogold technique.

Methods: Rat pancreas was cut into small blocks and a fixative containing 2.0% (para)formaldehyde with or without addition of 0.1% TA in 0.1 M cacodylate buffer and irradiated with a household microwave oven at 2.45 GHz for 25-30 sec at room temperature, as described previously ²⁾. For the microscopy, semithin sections were immunostained with gold-labeled anti-glucagon antibody, and then silver-enhanced with modified Timm's physical developer for 3 or 4 hrs. For electron microscopy, thin sections were immunostained by an indirect method using protein A-gold after embedding in LR-White.

Results: Under light microscopy, no significant differences were observed between the two methods, TA(+) or TA(-) fixatives. In the case of the electron microscopy, TA was very effective for preserving the ultrastructures including glucagon granules of A cells, and uniform distribution of immunogold particles was clearly observed. However, on the contrary, coarse glucagon granules and diffusion of immunogold particles were observed in the case of TA(-) fixation method.

In summary, using the TA-containing fixative led to good ultrastructural preservation of glucagon granules and prevented damage to the antigenicity.

(1) Notoya M. et al.: Acta Histochem. Cytochem. (in press).

(2) Mizuhira V. et al.: Acta Histochem. Cytochem. 23, 502-523 (1990).

Three dimensional reconstruction from the whole mount specimen with a confocal laser scanning microscope.
 Hisashi Hashimoto and Moriaki Kusakabe*
 Dept. Anat., Jikei Univ. Sch. Med., Tokyo and Lab. Cell Biol., Tsukuba Life Sci. Ctr., RIKEN, Ibaraki*

We have developed a novel methodology with confocal laser scanning microscope (CLSM) to investigate three dimensional localization of laminin (Ln), type IV collagen (IV Col) and tenascin (Tn) in the adult mouse small intestine and applied to examine the role of extracellular matrix during mouse lung morphogenesis. Adult male and fetal (Day12-14) mice of ICR strain were used in this study. The tissues were fixed in 4% paraformaldehyde (PFA) in 0.1M sodium phosphate buffer (SPB) for overnight at 4°C or in 0.5% PFA and 15% (v/v) of saturated picric acid (PFA-PA) in 0.1M SPB for 2 hr at room temperature. The fixed small intestine was sliced at the width of a villus with razor blades. The fetal lungs were handled as a whole organ. The tissues were pretreated with 3% aqueous sodium deoxycholate solution, and the 4% PFA fixed tissues were further treated with 0.1% pepsin in 0.01N HCl for none, 1 or 2hr. After the indirect immunofluorescence staining against Ln, IV Col and Tn, the specimens were mounted in SPB:glycerol (1:9) containing DABCO and observed with Carl Zeiss LSM-10 CLSM. Each of Ln, IV Col and Tn showed adequate immunoreactivity without pepsin treatment in both 4% PFA and PFA-PA fixed tissues. However, antibodies were prevented from achieving the antigens deep in the 4% PFA fixed tissue. Immunofluorescence of Ln and IV Col was enriched by pepsin treatment at capillary networks and nerve fibers in villi while it was extremely diminished at the epithelial basement membrane. Pepsin treatment significantly reduced the antigenicity of Tn. Pepsin treatment may partially break the epithelium, its basement membrane and the epitopes of antigens, so that antibodies would be able to achieve the antigen and that they lost the targets. The PFA-PA fixation without pepsin treatment gives us a better result than the 4% PFA fixation followed by pepsin treatment. A sheet of Ln expanding beneath the epithelium of villi was wavy with many porous defect. Tn was located as stripes beneath the epithelium of villi and the defect of Tn corresponded to the site of underlying capillaries. No tenascin was found in the basement membrane of the crypt. Capillary networks and nerve fibers expanding in villi were clearly showed up by the Ln or IV Col staining. In all lungs, Ln enveloped whole bronchial trees composed of the lobular bronchus, outgrowing segmental bronchi and expanding buds. Tn deposited in the basement membrane of the segmental bronchus like a cylinder in three dimensional morphology, but forming terminal buds were naked with Tn. With bifurcation of the terminal bud, the cylinder of Tn extended to the branching point although Tn never covered the distal end of the forming branches. The proximal end of the Tn cylinder was getting disappeared. The restricted localization of Tn suggests that Tn plays an important role in branching. In conclusions, 1) three dimensional reconstruction from the whole mount specimen is very useful to examine the distribution of extracellular matrices; 2) the whole mount specimen preserves more antigenicity than conventional sections; 3) this methodology will be very effective on the investigation of the morphogenesis of various organs.

**Immunohistochemical Study of Granular
 Convoluted Tubules in Rat
 Submandibular Glands**

Junji HASHIMOTO, Mayuko KUNIKATA
 and Masahiko MORI

Dept. of Oral & maxillofac. Surg. Asahi
 Univ. School of Dentistry, Gifu, Japan

The granular convoluted tubule(GCT) of rodent submandibular gland(SMG) consists of three different cell types :1) granular cell (GC), 2) pillar cell(PC) and 3) Transition cell(TC). In order to evaluate structure and functions of these cells, immunohistochemical identification of r-BGF, S-100 protein, calmodulin and NSE was studied with light and electronmicroscopy.

SMG of female Sprague-Dawley rats(200-250g) was removed under anesthesia, fixed in Bouin solution and stained with the ABC method. For electronmicroscopy, rats were perfused through the heart with 2.5% glutaraldehyde in 0.1M phosphate buffer(pH7.4), postfixated with buffered 1.0% OsO₄ and embedded in EPON 812.

The GC contains many granules located in the apical three-quarters of the cell. The PC is narrow and tightly located between two GC. The cytoplasm contains no granules and the surface of the apical side possess many microvilli. The TC located in between GCT and striated duct(SD), is characterized by properties of both the GC and SD cells. Immunohistochemically, reaction products of S-100 protein, calmodulin and NSE are observed in PC, TC and SD cell, and r-BGF is confined to granules of GC. Calmodulin has the function in regulatory process of secretion. The PC may participate in the regulatory process of granular secretion from adjacent or neighboring GC. The PC may have neurotransmitter-like functions. TC and SD might be involved in absorption of biologically active peptides secreted from GCs.

**Effects of a secretory inhibitor
 (brefeldin A) on intracellular
 structures of antibody producing cells:
 morphological and cytochemical studies**

Ryuju HASHIMOTO, Hiroki OTANI,
 Toshihisa HATTA, and Osamu TANAKA

Department of Anatomy,
 Shimane Medical University, Izumo

Effects of a secretory inhibitor, brefeldin A (BFA) on the intracellular structures were studied in an antibody producing cell line(H35). In our previous study, rough endoplasmic reticulum (RER) of BFA treated cells was dilated within 10 min. after the beginning of culture and the Golgi complex (Go) disappeared within 30 min. Activity of thiamine pyrophosphatase(TPPase), an enzyme associated with trans-element of Go trans-located to the dilated RER. When BFA was removed from culture, Go reappeared after 30 min, whereas TPPase activity remained the RER, suggesting a discrepancy between morphological and functional recoveries of Go from BFA effects. In our present study, to elucidate BFA-induced morphological and functional changes in Go and RER further in detail, we analyzed changes in localization of deposits Zinc-Iodine-Osmium (ZIO) staining and enzyme activity of nicotinamide adenine dinucleotide phosphatase(NADPase), markers of cis- and intermediate-elements of Go, respectively, in addition to TPPase activity. Within 10 min of BFA treatment, ZIO deposits and NADPase reaction products were detected in the vesicles which developed coincidentally with reducing Go, while TPPase activity was only in the dilated RER. Upon BFA removal from culture, within 30 min, ZIO deposit and NADPase activity were detected in cisterns of Go only after 120 min. These findings suggested that cis- and intermediate- elements recover from BFA effects sooner, whereas recovery of trans-elements, thus mature functional Go takes longer time.

**Study of qualitative and quantitative
 Changes in nuclear DNA and phenotypic
 Gene Expression in Human malignant
 Skin Tumors during their Progression**

Masahiro HATCHOH', Keiichi UEDA', Sakon
 NORIKI², Yoshiaki IMAMURA² and Masaru FUKUDA²

¹Department of Dermatology, and ²Department of
 Pathology, Fukui Medical School, Fukui

Qualitative and quantitative changes in nuclear DNA and phenotypic gene expression of human malignant skin tumors were examined during the course of progression. 1) The nuclear DNA contents of benign tumors, low-grade malignancies and high-grade malignancies were increased in this order; 2) the DNA-ploidies were largely shifted to the higher side with aneuploid stem-lines and polyploid cells in the invasive parts of all malignant tumors; 3) in one case of squamous cell carcinoma with invasion, the degree of chromosomal abnormalities was much greater in the invasive than in non-invasive parts, with marked topographical heterogeneities; 4) expression of "oncogenes" was found in about 60% of all malignant tumors examined. Thus, the progression of malignancy is accompanied by both qualitative and quantitative changes in nuclear DNA, resulting in abnormal gene expression.

Fundamental study for observation of cytokeratin fiber of tissue specimens using confocal laser scanning microscopy

Akihiro HEMMI, Koichi SUZUKI, Ryohei KATOH,
Akira KAWAOI

Department of Pathology,
Yamanashi Medical University

To evaluate fibrous structure of cytokeratin (CK), confocal laser scanning microscopic examination was performed using tissue materials from rat gastrointestinal tract, liver and kidney.

From each tissue, 4 μ thick frozen sections and whole-mount specimens were prepared. The whole mount specimens from the kidney and liver were cut in 80 μ thick small pieces by micro-slicer, and those from gastro-intestine were cut in 200 to 300 μ thick small pieces by hands using a razor. Some of these frozen sections and whole mount specimens were extracted with 0.5 % Triton X-100 for 5 min. before or after fixation (Group A and Group B, respectively), while the other were fixed without Triton treatment (Group C). All of these were stained by the indirect immunofluorescence method, using polyclonal anti-cytokeratin (Z622, DAKO) and monoclonal anti-cytokeratin 18 (ICN). For observation, confocal laser scanning microscopy (LSM-GB200, OLYMPUS) was used.

Using of confocal laser scanning microscopy, observation of whole-mount specimens was possible. Triton extraction was not effective in the distribution of CK positive cells and staining intensity. However non-specific staining reaction, which was occasionally observed in the whole-mount specimens, were diminished by Triton treatment. Moreover, CK bundle became clearly in order of Group C to A. Compared with the whole-mount specimens, destruction of CK fiber was prominent in the frozen sections. The use of Triton extracted whole-mount specimens seemed to be useful for the observation of more clear CK fiber, simplify of preparation procedure and prevention of artifact on the process.

Cytokeratin distribution and fibrous structure of rat gastro-intestine. Observation of confocal laser scanning microscopy.

Akihiro HEMMI, Koichi SUZUKI, Ryohei KATOH,
Akira KAWAOI

2nd Department of Pathology,
Yamanashi Medical University

To evaluate fibrous structure and distribution of cytokeratin (CK) of rat gastro-intestine, confocal laser scanning microscopic examination was performed.

Whole mount specimens for observation of cut surface, and mucosal or serosal surface, were prepared from stomach and small and large intestine. The tissues were cut in 200 to 300 μ thick small pieces by hands using a razor for cut surface observation, while 2x2 mm sized tissue blocks were trimmed for the mucosal or serosal surface observation. These were extracted with 0.5 % Triton X-100 for 5 min. before or after fixation. All of these were stained by the indirect immunofluorescence method, using polyclonal anti-cytokeratin (Z622, DAKO). For observation, confocal laser scanning microscopy (LSM-GB200, OLYMPUS) was used.

Positive CK reactivity were observed in epithelia of gastro-intestinal mucosa and mesothelium. Staining intensity, thickness of CK bundle and density of CK network were varied on the cell types. Endocrine cells of gastro-intestine and parietal cells were characteristic of thick bundle with coarse network, whereas most epithelial cells, such as foveolar epithelium and columnar cells of intestinal mucosa, revealed fine network in the cytoplasm. In goblet cells, intense CK staining were seen in apoptotic cells. Mesothelial cells showed various staining intensity and spike like CK projections were characteristic at the cell periphery.

Immunohistochemical Study of the BM-1/JIMRO Reactive Carbohydrate Antigen in Human Thymus and Other Tissues.

Katsuya HIRAISHI^{1,2}, Nobuhito KASHIWAGI²,
Toshinobu MIZUTA², Masakazu ADACHI²,
and Keiji SUZUKI¹.

¹ College of Medical Care and Technology,
Gunma University, Gunma.

² Japan Immunoresearch Laboratory Co., Ltd., Gunma.

BM-1/JIMRO mouse IgM monoclonal antibody detects the Lewis^x determinant (Le^x), which is a blood group related-antigen. Blood group related-antigens are present on various human organs, and are defined by the MoAbs against these carbohydrate antigens using immunohistochemical techniques. We have investigated the immunohistochemical localization of BM-1/JIMRO reactive carbohydrate antigen (BM-1 antigen) in the human tissues.

We found that PCNA negative cells (non-proliferating cells) in malignant and non-malignant tissues of human kidney and stomach expressed the BM-1 antigen. These BM-1/JIMRO positive cells exhibited cytoplasmic shrinkage, pyknosis, and nuclear fragmentation.

Hassall's corpuscles and thymocytes in the thymus medulla of infants were also positive for BM-1/JIMRO. The BM-1/JIMRO positive thymocytes in the thymus medulla decreased in number with aging of infant.

From these results, we suggest that the BM-1 antigen expression in these tissues related to apoptosis.

In situ hybridization for hard tissues

Seiichi HIROTA, Tohru IKEDA, Yukihiko
KITAMURA, Shintaro NOMURA
Osaka University Medical School, Dept. of
Pathology, Showa University Dental School,
Dept. of Dental Pathology

It has not been universally applicable to carry out in situ hybridization for hard tissues because of the difficulty in preparing the sections. We examined various methods about treatment before mounting and about probe preparation. Firstly, we compared paraffin-embedded decalcified tissues (by EDTA or formic acid) with plastic embedded undecalcified tissues. Both were applicable for mRNA detection. Secondly, we compared RI (³⁵S) labeled riboprobes with non-RI (digoxigenin labeled) riboprobes. Both were fully successful. Samples used were as follows:

1. mature femoral bone of rats.
2. fractured bone of rats.
3. ectopic bone induced by BMP.
4. chondroma-like tissues derived from mouse Shionogi carcinoma (SC115).
5. odontogenic tissues of mice (+/+ and mi/mi)
6. mouse inner ear.
7. cultured osteoblasts and osteoclasts of mouse calvaria

mRNA of bone and tooth matrix proteins (osteonectin, osteopontin, osteocalcin, matrix gla protein, amelogenin) was examined. Osteonectin mRNA was detected in the fibroblastic, chondrocytic, osteoblastic, and odontoblastic cells. Osteopontin mRNA was detected in the chondrocytic, osteoblastic, and osteoclastic cells, and marginal cells of the inner ear. Osteocalcin mRNA was detected in the osteoblastic and odontoblastic cells. Matrix gla protein mRNA was seen in the hypertrophic chondrocytes. Amelogenin mRNA was seen in the ameloblastic cells, even in odontoma-like tissues of the mi/mi mice.

Ultrastructural localization of antigens reactive with monoclonal antibodies to human tongue squamous cell carcinoma

Shinsaku HORI¹, Kenichi UOBE², Kazuya TOMINAGA², Seiji WADA², Masahiro WATO², Tetsunari NISHIKAWA² and Akio TANAKA²

¹Research Institute for Clinical Dentistry and ²Department of Oral Pathology, Osaka Dental University, Osaka

Mouse monoclonal antibodies (MoAbs: E8, P53 and P54) were produced using deparaffinized tongue cancer tissue from a formalin-fixed, paraffin-embedded specimen as the antigen. Using immunoelectron microscopy, we then studied the localization of antigens reactive with these MoAbs in oral cancers. In cancer tissues, cell membranes except keratinized and basal layer in cancer nests were positive for E8. At the ultrastructural level, E8 was circumferentially reactive with the cell membrane of non-neoplastic epithelium and cancer nest. P53 gave a positive reaction in the cytoplasm of non-neoplastic epithelium and cancer nest except keratinized cells. P54 produced positive immunostaining in the cell membranes of the basal two thirds of non-neoplastic epithelium and in the cell membrane of cancer nest. It was reactive with the cell membranes adjacent to desmosomes.

These MoAbs were thus shown to be useful for studies of non-neoplastic epithelium and squamous cell carcinoma.

Identification of Human Brainstem Neurons Expressing Substance P Receptor mRNA: An In Situ Hybridization Study

Takeshi HOUTANI, Kazuo SATO, Michiko IKEDA, Teizo UEYAMA, Tadami KUMAZAWA and Tetsuo SUGIMOTO
Departments of Anatomy and Otolaryngology, Kansai Medical University, Osaka

Localization of substance P receptor (SPR) messenger RNA to human brainstem neurons was examined by in situ hybridization which was carried out under stringent condition using digoxigenin-labeled RNA probes generated from human SPR cDNA. Postmortem brain tissue was collected from patients without neurological abnormality. Frozen sections of 10-µm thickness were prepared from frozen tissue blocks at the level of the midbrain, the inferior olive and the pyramidal decussation. Hybridization with antisense probes yielded distinct accumulations of hybridization signal in neuronal perikarya and also in cytoplasm of major cell processes of discrete brain sites, while hybridization with corresponding sense probes in adjacent sections gave no detectable amount of hybridization signal. In pigmented neurons, hybridization signal was only found in the cytoplasmic space that lacks lipofuscin granules. Among the brain sites examined in this study, highly specific hybridization signal for SPR mRNA was observed in the superior colliculus, the motor nuclei for oculomotor, vagus and hypoglossal nerves, the gracile and cuneate nuclei, the external cuneate nucleus, the lateral reticular nucleus, the dorsal and medial accessory olives, the solitary nucleus and the spinal trigeminal nucleus.

Detection of Glomerular Bleeding by the Real Time

Laser Microscope

Toru Hyodo

Department of Urology, Matsue Municipal Hospital

In 1979, Birch et al. reported that the morphology of erythrocytes in hematuria differs under the phase contrast microscope between patients with nephritis and those with urological diseases. However, a simple and objective examination method based on this morphological difference in erythrocytes has not been established. The objective of this study was to evaluate the usefulness of the laser microscope for the diagnosis of microhematuria. Characteristics of the test were evaluated in 88 patients with hematuria. Of the patients who were examined about 30 erythrocytes in urinary sediments, those in whom less than 20% of the erythrocytes were poikilocytes were considered to have urological diseases, and those in whom 80% or more of the erythrocytes were poikilocytes to have nephritis. According to these criteria, the sensitivity and the specificity of the examination to nephritis were 98.1% and 100%, retrospectively.

The time required for the examination was less than 3 minutes in samples containing 1-2 erythrocytes/HPF under ordinary light microscopy.

Thus, this real time laser microscopic urinalysis is considered to be a simple and objective examination method.

Sugar conformational change of rat parietal cells by feeding and development Kaori IHIDA, Shinichiro TSUYAMA, Nobuyuki KASHIO and Fusayoshi MURATA

Department of Anatomy, Faculty of Medicine, Kagoshima University, Kagoshima

It is well known that sugar components of surface membrane of parietal cells are affected by the acid secretion and development. In this study, we used *Dolichos biflorus* agglutinin (DBA) lectin which is specific to GalNAc residues and *Limax flavus* (LFA) lectin which is specific to sialic acid residues to study the feeding and developmental alterations of sugar conformation of rat gastric parietal cells in light and electron microscopic levels. We used Wistar rats from embryo 18 days to adult animals. Adult animals were divided into two groups: one is free of eating and another is 72h fasted. Tissues were stained by lectin CG method in the LM level, while preembedding staining method were used in EM level. Between ad lib. eating and 72h fasted animals, 72h fasted fundic gland showed strong staining by DBA and LFA lectins on parietal cells in LM and EM levels, while ad lib. eating fundic gland showed negative or very weak staining. Both DBA and LFA lectins showed characteristic changes during development and between two food conditions. During development, DBA and LFA strongly stained the parietal cells on whole length of the gland in LM and EM levels from gestation to weaning. After weaning, staining by both lectins gradually decreased with aging and finally almost disappeared in adult animals. From these results, conformational changes of the parietal cells surface closely related to the acid secretion and development of this cell.

Heterogeneous damage of intraventricular 6-hydroxydopamine treatment on nigrostriatal dopaminergic neurons in the adult rat

T. Ikeda¹, H. Okamura¹, C. Yokoyama², Y. Ichitani¹, I. Nagatsu³, Y. Ibata¹

Department of ¹Anatomy and ²Psychiatry, Kyoto Prefectural University of Medicine, Kyoto;
³Department of Anatomy, Fujita-Gakuen University, Toyoake

6-Hydroxydopamine (6-OHDA) is a chemical toxin, which exerts its toxic effect to catecholamine neurons by being selectively taken up into neurons by monoamine high affinity uptake system. In the previous study, we reported that neonatal intraventricular 6-OHDA treatment resulted in region specific damage on dopaminergic (DA) neuronal systems. In the present study, we examined its effect on adult rat. Pargyline (40 mg/kg) pretreated adult male rats were injected with 6-OHDA (200 µg) into the right lateral ventricle. After 2 weeks, animals were fixed and processed for immunocytochemistry using anti-tyrosine hydroxylase (TH) serum. In the injected side of the caudate-putamen (CP), most TH-immunoreactive (IR) fibers were disappeared, but a few beaded TH-IR fibers were detected in the periventricular area. In the contralateral side of CP, the degree of the damage was most prominent in the dorsolateral part, little affected in the periventricular part. TH-fibers were less affected in the nucleus accumbens and the tuberculum olfactorium compared with those of the dorsolateral part of the CP. In the midbrain, TH-IR cell bodies were completely abolished in the lateral part of the substantia nigra pars compacta (SNPC), but the significant number of TH-IR cells were still detected in the medial part of the SNPC and ventral tegmental area (VTA). No alternation of the TH-immunoreactivity was detected in the medial part of the VTA and the midbrain linear nuclei of raphe. These findings suggest that there are regional differences of the resistance of DA neurons against 6-OHDA toxicity in the adult rat brain.

Development of fluorescent NORs (FlnORs) and its application

Yoshiaki Imamura, Sakon Noriki, and Masaru Fukuda
Department of Pathology, Fukui medical school, Fukui

A new method of fluorescent staining of nucleolar organizer lesions (FlnORs) was reported. Aluminium ammonium sulfate was used instead of silver as the cationic metal ion for binding with NORs-proteins, and fluorescent morin was successively bound to aluminium by chylating. After bleaching the fluorescent staining of NORs by washing with water, ordinary AgNORs staining was performed on the same section taken from the uterine cervical squamous cell carcinoma (10 cases) and thyroid papillary carcinoma (10 cases), and both images of FlnORs and AgNORs were found to coincide with each other.

Using the thick sections, FlnORs staining of human malignant tumors was examined by 3-dimensional analysis of the fluorescent images under confocal laser scanning microscope (CLSM). Remarkable increase of FlnORs was found not only in number but also in volume with bizarre configuration in tumor cells in comparison with those in non-neoplastic cells, and the FlnORs-CLSM technique will be helpful for daily pathological diagnosis of malignancy.

The Detection of Tumor Suppressor Gene Product p53 in Japanese Cancers Using New Monoclonal Antibody BP53-12

Shunsuke IMAI¹, Satoshi HAGA², Toshihiko NISHINO², Kouko NAGANO², Hiroshi YAMAMOTO² and Yasuhiko KIYOZUKA³

¹Second Department of Pathology and ²Second Department of Anatomy, Nara Medical University and ³Department of Obstetrics and Gynecology, Kurume University.

The mode of arrangement of actin filaments in the perineurial cells of mammalian peripheral nerves---CLSM & TEM study---

Tetsuo INOKUCHI, Ryuji YOKOYAMA, Masao HAMASAKI and Satoshi MIYAJIMA

Department of Anatomy, Kurume University, Kurume

INTRODUCTION

Many researchers reported that the mutation of tumor suppressor gene is related to the oncogenesis in human. We examined the expression of tumor suppressor gene product p53 histochemically in tumors from three different tissues, breast, ovary and colon, using newly developed monoclonal antibody against p53.

MATERIALS AND METHODS

Operated breast, ovary and colorectal cancers were obtained from the Department of Surgery, National Nara Hospital and the Department of Obstetrics and Gynecology, Kurume University Hospital. These samples were fixed in formalin, embedded in paraffin and sectioned. Immunohistochemistry was performed using a new monoclonal antibody BP53-12 (Japan Tanners Co., Kobe).

RESULTS

The positive stainings of p53 were observed 44% of colon, 35% of ovary and 33% of breast cancers, respectively. Positive reactions were mainly seen in moderately and poorly differentiated adenocarcinomas of colorectal cancer, scirrhous and comedo carcinomas of breast cancer and serous type of ovarian cancer. No expression of p53, however, was seen in benign or normal tissues.

DISCUSSION

We reported herein the expression of p53 protein in formalin fixed and paraffin embedded samples routinely used in the hospital. The frequency of positivity was 30-40% in each carcinoma. The reaction of BP53-12 was specific to malignant lesion and no reaction was seen in benign and normal lesions. We concluded that this new monoclonal antibody is useful for the diagnosis between the normal and the cancerous lesions.

The perineurium of the mammalian peripheral nerve is composed of several lamellae of thin perineurial cells which are held together mainly with the zonula occludens. Viewing by the transmission electron microscope (TEM), the cytoplasm of individual perineurial cells contains closely aggregated filaments. After the treatment of the nerve fascicles, pieces of the sciatic nerves of animals, with FITC- or rhodamine-labelled phalloidin which have a property to combine strongly with actin filaments, the confocal laser scanning microscopic (CLSM) observation of the specimens exhibited remarkable positive fluorescence on the perineurial cell cytoplasm. Viewing at higher magnification under the CLSM, the cytoplasm contained numerous, fluorescent, thread-like fibers. The fibers tended to run parallel within the individual perineurial cells and run in different directions to those in the neighboring cells. Some of the specimens which were quickly dissected out from the animals were pretreated by glycerine and then reacted with heavy meromyosin (HMM). TEM observations of the specimens after the HMM reaction showed the arrowhead formation on the filaments which scattered in the perineurial cell cytoplasm. These actin filament bundles may play a role to maintain the relatively-high hydrostatic pressure within the endoneurial space.