temperature, time, molarity and chemical composition of retrieval solution are recognized to be important but despite empirical success the actual mechanism of antigen retrieval remains speculative. In some instances, different retrieval methods may act in a synergistic manner such as the application of both proteolytic digestion and microwave-stimulated antigen retrieval for the demonstration of cytokeratins and immunoglobulins. Until more novel methods of antigen retrieval are introduced we need to further explore and optimize the important variables identified in the procedure. For instance, the increase of both time and temperature appears to enhance the demonstration of most tissue antigens, to this end a machine has recently been developed. These variables and their manipulations will be discussed.

S4-2

Application of a monoclonal antibody against occludin, a marker for tight junctions, to paraffin-embedded human materials

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Tight junctions are intercellular junctions adjacent to the apical end of the lateral membrane surface. They have two functions, the regulation of the paracellular pathway and the maintenance of cell polarity. The former is relevant to edema, jaundice, diarrhea and diabetic retinopathy. The latter is deeply involved in cancer biology, in terms of de-differentiation. Of the proteins comprising tight junctions, integral membrane proteins claudin and occludin are key molecules regulating tight junction functions. In this study, we raised monoclonal antibodies against the C-terminal cytoplasmic domain of occludin, and stained paraffin-embedded human tissues using the Ab after autoclave-mediated epitope retrieval. In normal glands and well-differentiated adenocarcinomas of the colon, carcinoembryonic antigen (CEA) was exclusively detected on the apical cell membranes with the apical-most basolateral localization of occludin. In poorly differentiated adenocarcinomas, CEA was observed on all cell surfaces whereas no occludin was detected. Regarding the endometrial carcinomas, the amount of occludin decreased with the advance of grade determined by FIGO's classification. Furthermore, endometrial carcinomas with a low degree of occludin expression show high activities of invasion and lymphatic metastasis. These findings imply that fully developed tight junctions prevent cancer cells from acquisition of malignant properties such as loss of cell polarity, invasion and metastasis.

S4-3

Application of Laser Assisted Microdissection Technique To Diagnostic Surgical Pathology

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Laser assisted microdissection technique (LAMT) is a valuable tool for the precise analysis of gene expression and amplification in tumor cells or tissues. This technique has widely been accepted, and several kinds of instruments are now available. Although frozen tissue sections are most commonly used for the analysis of mRNA, routinely processed formalin-fixed paraffin embedded tissue sections are also available for DNA analysis in surgical pathology specimens. We describe herein an application of LAMT to surgical specimens, particularly focused on the RNA analysis in the cytology specimens handled routinely in pathology laboratories and the tissue sections processed for immunohistochemistry.
Materials and Methods: Pleural effusions from the patients with positive cytology; adenocarcinoma and mesothelioma were used for this study. Cultured Kato III cells, an established cell line of adenocarcinoma were used for control. Cells were smeared on a membrane film coated glass slide by cytospin, 700 rpm for 5 min. The cytosmears were stained with toluidine blue, Papanicolaou or Giemsa staining after light fixation in methanol, and then air-dried. For the immunomicrodissection, human breast cancer specimens were used. Immunostaining was done using EPOS (Enhanced Polymer One-step Staining)/HRP kit (DAKO). Microdissection was carried out using a laser scissors instrument: LS-337 (Cell Robotics Inc., USA, Meiwa Inc., Japan). RNA was extracted from the microdissected cells or immunostained tissue samples by phenol chloroform method and stored at Ð80 until use. The RNA pellet was resuspended in RNase free water, and then reverse transcription was performed. A total 35 to 45 cycles of cDNA amplification was carried out and the PCR products were separated on a 1 to 2% agarose gel stained with ethidium bromide or by a bioanalyser based on microcapillary electrophoresis.
Results: We obtained a satisfactory amplification of actin cDNA fragments by RT-PCR from the control cell sample (5 cells). Such a satisfactory amplification was also obtained from the pleural effusion, which was stored for 4 days at 4 . Although an amplification of actin cDNA was demonstrated from the effusion that was stored more than 10 days, approximately 10 times more cells (50 cells) were needed. Regarding the staining condition, toluidine blue brought constant and reliable results, though the cell samples stained with Papanicolaou or Giemsa staining were also available for the analysis. For the RNA analysis in the immunostained tissue sections, a satisfactory amplification of actin cDNA fragments was obtained in the microdissected samples consisting of approximately 1×103 cells. On the other hand, for the demonstration of satisfactory amplification of HER2 mRNA, roughly 5 times more cells (5×103 cells) were needed.
Conclusion: Our result showed that a combination of LAMT and RT-PCR can be used as a powerful and reliable tool for the analysis of tumor specific or related gene amplification in the routinely processed cytology specimens and also in the immunostained tissue samples.

S4-4

Visualization of Angiogenesis In Vitro

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An in vitro model has been developed to investigate the initial steps of spontaneous angiogenesis by living human dermal microvascular endothelial cells. Time lapse differential interference contrast microscopy, zymography, and immunohistochemical techniques have been employed to follow the migration of these cells and their morphogenesis into capillary-like networks on a reconstituted basement membrane in a defined culture medium. The temporal and spatial distribution patterns and expression of endogenous angiogenic modulators were analyzed in migrating and non-migrating cells. The model provides a cell-based endpoint within 24 hours, is sensitive to nanomolar concentrations of specific exogenous modulators, has a modifiable throughput, and can be followed in real time. The defined system allows variation of experimental conditions singly and in combination. The model has been used to investigate levels of secreted and cell membrane-bound matrix metalloproteinases and expression of integrins during migration, and to screen crude extracts and pure compounds in subcytotoxic concentrations as angiogenic inhibitors or enhancers.

S4-5

Noninvasive Imaging of Angiogenesis and Physiological Function *in vivo*

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A solid tumor is an organ comprised of cancer cells and host stromal cells embedded in an extracellular matrix and nourished by a vascular network. Intravital microscopy (IVM) has provided unprecedented molecular, anatomic and functional insight into the inner workings of

this organ as well as response to various therapies. IVM is a powerful optical imaging technique that permits continuous non-invasive monitoring of molecular and cellular processes in an intact living tissue with 1–10 μm resolution. Tumor vessels have tortuous shape, irregular surface and diameter, and heterogeneous spatial distribution. Tumor blood flow is often sluggish and static, and even changes the direction over time. Furthermore, some tumor vessels lack oxygen despite well perfusion. As a result, tumors are often hypoxic and acidic. Tumor vessels have high vascular permeability and low leukocyte endothelial interaction. We found that host stromal cells significantly contributed to the expression of angiogenic factors such as vascular endothelial growth factor by means of IVM, novel green fluorescence reporter gene system and targeted deletion of specific genes. Gene expression and physiological functions in tumors are spatially and temporally heterogeneous, depend on tumor-type and organs, vary during treatment, and influence the efficiency of various treatment modalities. A better understanding of tumor-host interaction especially during tumor growth and response to treatments should improve future tumor treatment strategies.

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S4-6

Non-Invasive Visualization of Fluorescent Tumors In Intact Animals

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Mouse models of metastatic cancer with genetically fluorescent tumor cells that can be imaged in fresh tissue, *in situ*, as well as externally have been developed. These models have opened many new possibilities including real-time tumor progression and metastasis studies on internal organs and real-time drug response evaluations. The green fluorescent protein (GFP) gene, cloned from bioluminescent organisms, has now been introduced into a series of human and rodent cancer cell lines *in vitro* to stably express GFP *in vivo* after transplantation to metastatic rodent models. Techniques were also developed for transduction of tumors by GFP *in vivo*. With this fluorescent tool, tumors and metastasis in host organs can be imaged down to the single cell level. GFP tumors on the colon, prostate, breast, brain, liver, lymph nodes, lung, pancreas, bone, and other organs have also been visualized externally, transcutaneously by quantitative whole-body fluorescence imaging. Real-time angiogenesis has also been imaged and quantified using GFP technology. The GFP technology enables a fundamental advance in the visualization of tumor growth and metastasis in real time *in vivo*.

PL2

The claudin family: A key player in the barrier function in multicellular organisms

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Multicellular organisms contain various compositionally distinct fluid compartments, which are established by epithelial and endothelial cellular sheets. For these cellular sheets to establish each compartment, the paracellular pathway between adjacent cells must be sealed to prevent the diffusion of solutes. Tight junctions (TJs) are composed of TJ strands, a polymer of integral membrane proteins, and these strands have been thought to be directly involved in the barrier function of TJs. Until recently, however, little was known of their constituents. Set against this situation, recently we identified two distinct types of TJ-specific integral membrane proteins, occludin and claudins. We are only just beginning to understand the molecular biology of TJs. Here, I will present an overview of our current understanding of the structure and functions of TJs, and discuss the relationship between TJs and the compartmentalization in multicellular organisms in molecular terms.

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MS3-1

Methods for Detection of Apoptosis in Cells and Tissues

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Cell death through apoptosis is a process of central importance in mammalian development and several pathologic conditions, including neurodegenerative diseases and neoplasia. The past decade has seen a rapid expansion of knowledge concerning the biochemical mechanisms that mediate apoptosis in cells. A fundamental component of the apoptotic process is the activation of intracellular caspases within the cytoplasm of cells, the proteolytic activity of which lead to the multiple morphologic and biochemical features seen in apoptotic cells. Distinguishing apoptosis from necrotic cell death is vital to the understanding of pathologic and developmental processes involving cell death. Methods for detecting apoptotic cells originally relied on morphologic changes, as well as the detection of breakdown of cellular DNA, through methods such as agarose gel fragmentation assays, and DNA fragment end-labeling in situ methods (ISNT and TUNEL). Many of these assays are difficult to interpret in intact cells or tissues. Recently, assays more specific to the biochemical mechanisms in apoptosis have been developed, including in situ methods that detect novel neo-epitopes in proteins that are substrates of active caspases. Such in situ immunocytochemical assays, utilizing commercially available antibodies, offer great promise for improving specificity of detection of apoptotic cells in both isolated cells and in intact tissue sections.

MS3-2

The study of transcription molecules of bcl-2 in human endometrium, using real-time Southwestern method

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Bcl-2 has been extensively characterized as an inhibitor of apoptosis. We have shown that there is a close relationship between the expression of Bcl-2 and appearance of apoptosis in normal human uterine endometrium. To elucidate how bcl-2 is transcriptionally regulated in the glandular cells of endometrium, we observed the immunohistochemical staining pattern for ATF-1, CREB-1, Brn-3a, Sp1, Sp3, and WT1 that have been implicated in regulation of bcl-2 transcription in various cell lines. We found that most of these molecules were expressed in both the glandular and stromal cells. Therefore, the results are not definitely indicative concerning the transcriptional effect of these molecules on bcl-2 in the endometrium. Next we employed a technique called biomolecular interaction analysis (BIACORE) to measure if any one of these transcription factors binds