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

their respective motifs in the bcl-2 promoter. Our results showed that ATF-1, CREB-1, ER, Sp1, and Sp3 do not specifically bound to their respective oligo domains from the bcl-2 promoter. However, specific bindings were observed for some of the AP-1 factors, i.e., c-jun, and c-fos, and ATF-1. Further experiments showed that an active ERE exists in c-jun promoter. The present results indicate that BIACORE technique is a powerful tool in screening candidate transcription factors. We suggest that AP-1 transcription factors such as c-Jun play an important role in transcriptional regulation of bcl-2, and the expression of c-Jun is controlled by estrogen receptor in normal human uterine endometrium.

MS3-3

Histochemical analysis of male germ cell apoptosis in mice

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Loss of germ cells is very common during various stages of mammalian spermatogenesis. Although cell death, particularly apoptosis, has been implicated in the loss, our understanding of the mechanism underlying germ cell death is still only limited. When we examined the frequency of degenerating germ cells in normal testes of fetal, neonatal and adult mice by electron microscopy and TUNEL staining, two peaks of the number of apoptotic germ cells, i.e. at 13 days of gestation and 10–13 days after birth, were observed. Thereafter, the occurrence of germ cell apoptosis was nearly constant, but mostly restricted to the stage XI to XII. Very interestingly, we found a significant number of degenerating germ cells of necrotic nature around birth. As a possible candidate of apoptosis inducers, we focused on the involvement of Fas and Fas ligand (FasL) in normal adult testis. However, our in situ hybridization and immunohistochemical study failed to find any significant spatial and temporal connections among TUNEL-, Fas- and FasL-positive cells. On the other hand, when we analyzed a torsion model of mouse testis, which was reperfused after 1 hr-ischemia, we found that the induction of germ cell apoptosis was strictly dependent upon the Fas system. The similar association of germ cell apoptosis with the Fas system was found in damaged testes treated with a high dose of environmental estrogenic compounds such as diethylstilbestrol and bisphenol A. More complicatedly, however, in an experimental cryptorchidism model, the germ cell apoptosis seemed to be associated with an elevated level of Bcl-2 and Bax, but not with the expression of Fas and FasL. Furthermore, in any cases including normal neonatal and adult spermatogenesis, Bax redistribution was always accompanied with TUNEL-positive cells. These histochemical results indicate that a set of different molecular pathways may be triggered to induce male germ cell apoptosis depending upon the physiological and pathological states of germ cells.

MS3-4

Use of fluorochrome-labeled inhibitors of caspases and serine (Ser) proteases to detect in situ activation of these enzymes during apoptosis

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Cysteine proteases (caspases) and serine (*Ser*) proteases are both involved in protein degradation during apoptosis. The objective of this study was to develop convenient assays to monitor activation of these enzymes. We applied fluorochrome labeled inhibitors of caspases (FLICA) and of *Ser* proteases (FLISP) as affinity labels of active centers of these enzymes. The FLICA ligands are fluorescein or sulforhodamine conjugated peptide-fluoromethyl ketones that covalently bind to enzymatic centers of caspases. The specificity of FLICA towards individual caspases is provided by the peptide sequence of amino acids. FLISP are 5(6)-carboxyfluoresceinyl-L-phenylalanylchloromethyl ketone (FFCK) or 5(6)-carboxyfluoresceinyl-L-leucylchloromethyl ketone (FLCK), the reagents that specifically tag active centers of the chymotrypsin-like enzymes that cleave at *Phe* (FFCK) or *Leu* (FLCK) site. Both FLICA and FLISP bind to the active centers with 1:1 stoichiometry. Exposure of live cells to FLICA or FLISP led to uptake of these ligands and binding to activated caspases and *Ser* proteases. The FLICA and FLISP-reactive proteins can be identified on immmunoblots. The FFCK- and FLCK-reactive proteins were of similar molecular weight ~58 and ~56 kD, respectively. The FLICA or FLISP labeled cells had characteristic features of apoptosis. Activation of caspases appears to be upstream of activation of FLISP-reactive sites. Detection of caspases or *Ser* proteases activation by FLICA or FLISP can be combined with other markers of apoptosis or cell cycle for multiparametric analysis by flow or laser scanning cytometry. Caspase inhibitors FLICA arrest the process of apoptosis preventing cell disintegration. They can be used, thus, in the stathmo-apoptotic assay to obtain cumulative apoptotic index over long period of time and estimate the rate of cell entry to apoptosis (cell death rate).

MS4-1

Studying Transcription in Real Time in Living Cells

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Activation of transcription in eucaryotic systems is associated in current models with the formation of a stable preinitiation complex. We previously reported the direct observation of steroid receptor binding to a tandem array of the mouse mammary tumor virus (MMTV) promoter in living cells. Through the use of photobleaching techniques, we unexpectedly found that the glucocorticoid receptor (GR) exchanges rapidly with regulatory elements in the continued presence of ligand [Science 287: 1262 (2000)]. Using fluorescence recovery after photobleaching (FRAP) we have now examined the dynamic behavior of several green fluorescence protein (GFP) tagged transcription factors at the MMTV tandem array. The glucocorticoid receptor interacting protein 1 (GRIP1) exhibits a half maximal time for fluorescent recovery (tR) of 5 sec., the same rapid exchange as observed for GR. In contrast, the large subunit of RNA polymerase II (RPB1) shows a very slow exchange, requiring thirteen minutes for complete fluorescence recovery. The quantification of cells showing a GFP-RPB1 fluorescent array at different time points after hormone treatment revealed that transcriptional activation of the MMTV is a very rapid and transient process, with maximum pol II loading between 15 and 30 minutes. This study is the first report on the dynamic behavior RNA polymerase II at an actual site of transcription in living cells. Our findings indicate that the formation of initiation complexes is a highly dynamic process.

MS4-2

Visulaization of PKC targeting in living cells and brain

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Protein kinase C (PKC) consisting of multiple subtypes plays an important role in various cellular signal transductions. It is important to identify the individual function of each PKC subtype for understanding the complicated roles of protein kinase C in various signaling pathways. Studies for the specific subcellular localization of each subtypes has been considered to be one of the most attractive ways to know the individual role of PKC subtypes. Activation of PKC,