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.

1. Jain et al. Dissecting tumours using intravital microscopy. *Nature Reviews Cancer* in press.
2. Fukumura, et al. Tumor induction of VEGF promoter activity in stromal cells. *Cell* 94: 715–725, 1998.
3. Tsuzuki et al. Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia inducible factor-1α-hypoxia response element—VEGF cascade differentially regulates vascular response and growth rate in tumors. *Cancer Res* 61: 6248–6252, 2000.
4. Fukumura, et al. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors *in vivo*. *Cancer Res* 61: 6020–6024, 2001.
5. Izumi et al. Herceptin acts as an anti-angiogenic cocktail. *Nature* in press.

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.

1. Tsukita, S. and Furuse, M. J. Cell Biol., 149: 13–16, 2000.
2. Tsukita, S., Furuse, M., and Itoh, M. Nature Rev. Mol. Cell Biol., 2: 285–293, 2001.

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