apoptosis may complement images of energetics and proliferation. One may also be able to image cell stress using FMAU [1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-thymine] which tracks thymidine kinase 2. The best use of this array of tracers will require detailed studies to determine the best approach for different tumors and treatments. This is particularly important given the advent of many newer targeted drugs, which require new ways to determine their efficacy.

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Hypoxia imaging

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Hypoxia, a condition of insufficient O2 to support metabolism, occurs when the blood supply is inadequate. It occurs when a tumor grows so fast that immature blood vessels supply the tumor. Increased diffusion distances to tumor cells and reduced O2 transport exacerbate this problem. When tumor cells become hypoxic they adapt by up-regulating the production of numerous proteins that promote their survival. These proteins slow the rate of growth, switch the mitochondria to glycolysis, stimulate growth of new vasculature, inhibit apoptosis and promote metastatic spread, processes that enable tumor cells to survive or escape the O2-deficient environment. The consequence is that hypoxia represents a significant challenge to the curability of human tumors; patients with hypoxic tumors invariably experience overall diminished therapeutic response, malignant progression, increased recurrence, loco-regional spread and distant metastases. Strategies are being developed to surmount the cure-limiting consequences of hypoxia but methods are needed to select patients most likely to benefit from these approaches. Even though hypoxia is a common tumor phenotype, it is by no means universal and is often heterogeneous within a patient. This report considers several novel methods for imaging regional hypoxia and how information about the oxygenation of tumors might impact treatment. Tumor hypoxia can be detected by invasive and non-invasive techniques. Regional measurement of O2 partial pressure can be made using electrodes placed under CT guidance. While this provides an absolute measure of PO2 (mm Hg), it is inherently invasive and can only be applied to accessible sites. Nuclear medicine approaches have been developed with radiopharmaceuticals that accumulate with an inverse relationship to PO2. 18F-MISO and Cu-ATSM-PET, and BOLD-MRI are the lead contenders for human application based on their non-invasive nature, ease of use, robustness, validity, ability to demonstrate heterogeneity and general availability. This presentation will discuss where developments are required for hypoxia imaging to become clinically useful and explore potential new uses for hypoxia imaging. In conclusion, invasive methods to measure regional PO2 are now being replaced by noninvasive imaging methods using radionuclides or magnetic resonance detection. The most widely studied imaging procedure, FMISO PET, has been shown to have independent predictive value for outcome. The clinical role of hypoxia imaging will probably be less to detect disease and more to improve radiation treatment planning and selection of appropriate patients for treatment with hypoxia-selective cytotoxins. Hypoxia imaging has become an important success story over the last decade and provides some important lessons for development of new imaging probes or biomarkers.

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Apoptosis imaging

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Background: Over 4 decades ago John Kerr described an unusual form of cell death in rats undergoing experimental portal vein ligation. Kerr observed that some cells appeared to shrink and disappear without producing any inflammation. This observation contrasted with the findings of typical necrosis, where cells lose membrane integrity, spill their contents into surrounding tissues, and cause inflammation. Although it was clear that these cells were dying, the process was different from necrosis, since it was not associated with inflammation, the mitochondria and ribosomes remained intact, and extracellular bodies, occasionally occurring in clusters, suggested budding from the surface of the cells. Similar observations were made in histologic specimens of basal cell carcinoma. The incidence of 'shrinkage necrosis' was increased by treatment with radiotherapy. Professor Curry, while a visiting professor working with Kerr in Brisbane, described a similar observation made by his colleague Dr. Wyllie in the adrenal cortex of rats treated with prednisolone (to suppress ACTH). In a seminal article, Kerr, Wyllie and Curry coined the term apoptosis, derived from two greek words, 'apo' which means from and 'ptosis' which means falling, to describe this type of cell death.

Biochemical modifications of apoptotic cells include: (A) Activation of a series of cysteine protease enzymes (i.e. caspases), to crosslink and cleave specific intracellular proteins. Each of the caspases is associated with a specific inhibitor, allowing the system to be strictly regulated by a number of positive and negative feedback mechanisms. (B) DNA molecules are degraded into 50 to 300 kilobase-sized pieces. (C) Leakage of potassium and chloride from the intracellular environment, which results in loss of intercellular water and an associated decrease in cell size. Pieces of the cell undergoing apoptosis are packaged in small vesicles derived from the cell membrane. These cell pieces are called "apoptosomes" (D) Cells undergoing apoptosis signal their neighbors by expressing phosphatidylserine (PS) on the external leaflet of the cell membrane (as well as apoptosomes). PS is one of the 4 major phospholipids that make up the cell membrane (the other three are phosphatidylethanolamine, sphingomyelin, and phosphatidylcholine). Normal cell polarization confines PS to the inner leaflet of the cell membrane. Cells undergoing apoptosis lose this polarization, resulting in the expression of PS on the outer leaflet of the cell membrane. The expression of PS on the outer leaflet of the membrane signals neighboring cells that the expressing cell is undergoing apoptosis. A combination of macrophages and adjacent normal cells then phagocytize the remaining components of the cell carcass.

Annexin V (MW ~ 36,000) is an endogenous human protein that is widely distributed intracellularly, with very high concentrations in the placenta and lower concentrations in endothelial cells, kidney, myocardium, skeletal muscle, skin, red cells, platelets and monocytes. The precise physiologic function of annexin is uncertain. Annexin V binding to rafts of PS exposed on a cells surface with internalization via a newly discovered unique pathway of pinocytosis. Other investigations of annexin V binding have found that PS can be expressed at low levels in a reversible fashion under condition of cell stress that does not necessarily commit a cell to apoptotic cell death.

PET and SPECT Imaging with Radiolabeled Annexin V: Annexin has been radiolabeled with Iodine 125, Iodine 124, Fluorine 18, Technetium-99m and gallium-68. The half lives of the tracers limit the choices of imaging time. Typically, the tracer clears from the blood rapidly. When labeled with I-123, there was <4% residual in the blood 40 minutes after injection. However there is significant non-specific distribution in soft tissue, which has much slower clearance. Based on sequential imaging studies, once annexin binds to sites expressing PS, it appears that the signal persists, suggesting that imaging should be performed several hours after injection to maximize contrast while maintaining a reasonable count rate. Annexin imaging has been used to detect response to therapy in patients undergoing chemo and or radiotherapy.

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Applications of nanotechnology and molecular probes in cancer imaging

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Fundamental biological and clinical questions have driven technological advances in a number of diagnostic techniques. In vivo molecular imaging has demonstrated the ability to profoundly change our understanding of developmental and pathological events. One technique that has been a powerful tool in both experimental and clinical settings is magnetic resonance imaging (MRI). The intrinsic contrast in acquired MR images can be augmented by the use of paramagnetic contrast agents in both clinical and experimental settings.

The direct observation of ongoing developmental events in living embryos, and the descendants of individual precursors in an intact embryo, has been dominated by optical microscopy. We have been developing MR probes to augment this analysis in whole animals. Since a complete time-series of high-resolution three-dimensional MR images can be analyzed forward or backward in time, it is possible to reconstruct the cell divisions and cell movements responsible for any particular descendant(s). Unlike previous methods, where labelled cells are identified at the termination of the experiment, this technique allows the entire kinship relationships of a clone to be determined.

In order to understand signal transduction mechanisms of gene expression in whole animals we have developed a library of molecular MR probes that are biochemically activated in-vivo. The lanthanide chelates modulate fast water exchange with the paramagnetic center, yielding distinct "strong" and "weak" relaxivity states. The modulation is triggered by two types of biological events: i. enzymatic processing of the contrast agent and, ii. the reversible binding of an intracellular messengers (e.g., Ca^{2+}, Zn^{2+}).