

In the third of a series of tutorials on “Molecular Imaging”, David Piwnica-Worms, MD, PhD, Washington University School of Medicine, described “Spying on Cancer with Genetically-Encoded Reporters”. Genetically-encoded imaging reporters introduced into cells and transgenic animals enable noninvasive, longitudinal studies of dynamic biological processes in intact cells and living animals. The most common reporters include firefly luciferase (bioluminescence imaging), green fluorescence protein (fluorescence imaging), Herpes Simplex Virus-1 thymidine kinase (positron-emission tomography) and variants with enhanced spectral and kinetic properties optimized for use in vivo. When cloned into promoter/enhancer sequences or engineered into fusion proteins, imaging reporters enable fundamental processes such as transcriptional regulation, signal transduction cascades, protein-protein interactions, oncogenic transformation, cell trafficking and targeted drug action to be temporally and spatially registered in vivo. A theme heard in this and earlier talks was the linking of anatomic and genetic information using a molecular imaging strategy; however no single molecular imaging strategy is optimal for all problems. One of the tools now used in molecular imaging of small animals is a micro-PET system which provides a spatial resolution of 1.5 mm. Micro-PET imaging of a rat which had had a ^{18}F -labelled adenovirus delivered to the lungs was shown. Spying on cancer with genetically-encoded imaging reporters provides new insight into cancer-specific molecular and regulatory machinery within the contextual environment of the whole animal.

Thomas Meade, PhD, Northwestern University, described a “Coordination Chemist -- Focused on Paramagnetic Chelates: New Chemical Strategies”. Fundamental biological and clinical questions have driven technological advances in an area of research known as biological molecular imaging. MRI has been a powerful tool in both settings; it offers a non-invasive means to map structure and function by sampling the amount, flow or environment of water protons in vivo. Such intrinsic contrast can be augmented by the use of paramagnetic contrast agents in both clinical and experimental settings. It is non-invasive and yields a true volume rendering of the subject with cellular resolution (~10 microns). Currently, microinjection of a stable, nontoxic, membrane-impermeable MRI lineage tracers allows the direct observation of ongoing developmental events in living embryos and of the labeled descendants of individual precursors in an intact embryo. 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 labeled 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 realize the potential of this technique a systematic means of delivering the charged MR contrast agents must be developed. Dr. Meade's lab has been investigating the development of molecular MR probes that are capable of crossing cell membranes and the blood brain barrier. In order to understand signal transduction mechanisms of gene expression in whole animals they have developed a library of molecular MR probes that are biochemically activated in-vivo. These agents report this information in the form of an acquired 3D-MR image. The lanthanide chelates modulate fast water exchange with the

paramagnetic center, yielding distinct "strong" and "weak" relaxivity states. This modulation is triggered by two types of biological events: i). enzymatic processing of the contrast agent and, ii). the reversible binding of an intracellular messenger. In order to direct the intracellular uptake of these agents, the lab has prepared a number of small molecule "chaperones" that are covalently attached to the macrocyclic skeleton of the agent. The chaperones are capable of transporting the agent inside a cell in relatively high yield and to not interfere with the activation of the agent by an enzyme or the binding of Ca^{2+} .