

# Radiotracer-Based Angiogenesis Imaging

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PET and SPECT imaging has long played an important role in the clinical evaluation of patients with cancer and cardiovascular disease. Recently, radiotracer-based molecular imaging techniques are providing a unique opportunity to monitor molecular events of angiogenic and hypoxic tissue in such diseases. Angiogenesis represents the formation of new capillaries by outgrowth from existing microvessels, which occurs in cancer growth and as part of the natural healing process after ischemic injury. A large number of local and circulating angiogenic factors are involved in this process, including vascular endothelial growth factor, angiopoietins and basic fibroblast growth factor. Angiogenesis, whether excessive or deficient, underlies many clinical diseases, and imaging that directly targets angiogenic vessels may address important treatment issues such as selection of optimal targeted therapeutics, dose optimization, and early treatment response assessment.

The principal stimuli for angiogenesis are tissue hypoxia and inflammation, and its process includes local infiltration of immune cells and proliferation and migration of vascular endothelial cells.  $^{18}\text{F}$  labeled deoxyglucose ( $^{18}\text{F}$ -FDG), a radiolabeled glucose analog widely used for cancer evaluation, is now known to avidly accumulate in various non-cancer cells that are important components of angiogenic lesions. We are currently investigating the molecular mechanisms that regulate the enhancement of  $^{18}\text{F}$ -FDG uptake in activated inflammatory and endothelial cells that contribute to FDG accumulation of angiogenic tissue.

The angiogenic response is modulated by the composition of the extracellular matrix and intercellular adhesions including integrins, a family of heterodimeric cell surface receptors capable of mediating cell adhesion, migration and proliferation. Hence  $\nu\beta 3$  integrins have become a most successful target for angiogenesis imaging, and a number of radiotracers of arginine-glycine- aspartic acid (RGD) tripeptides, the binding motif for  $\nu\beta 3$  integrins, have been developed. These tracers have successfully shown specific and high affinity binding to its target in vitro and high quality tumor imaging in vivo. We have recently synthesized a novel [ $^{99\text{m}}\text{Tc}$ ] labeled RGD glycoprotein which demonstrates high affinity and specific binding to endothelial cells as well as purified  $\nu\beta 3$  integrin. This radiotracer allows clear visualization of tumors in animal models in an integrin-binding specific fashion. In addition to tumors, RGD tracers have been shown by our group to also allow imaging of angiogenic tissue within ischemic limb lesions.

A powerful technique to probe intracellular events is to exploit reporter gene systems controlled by inducible

promoters. We, and others, have verified that the sodium iodide symporter (NIS) gene can produce signals trackable with gamma cameras that faithfully reflect expression levels in target tissue of living animals. We are presently investigating the potential of this reporter gene strategy to image hypoxia, and preliminary experiments show that transfection of tumor cells with the NIS gene controlled by a potent artificial hypoxia responsive promoter results in significantly augmented  $^{125}\text{I}$  uptake when exposed to hypoxia.

In the future, cancer patients may be aided by angiogenesis imaging for selection of antiangiogenic agents, dose optimization, and early assessment of treatment response. In patients with ischemic heart disease, angiogenesis imaging could be complementary to perfusion imaging to guide the sites for local injection of angiogenic treatments, and to monitor for evidence of a therapeutic effect. Further studies in clinically relevant models will be required to validate the concept of angiogenic imaging as a clinically useful approach in cancer and cardiovascular disease.

## References

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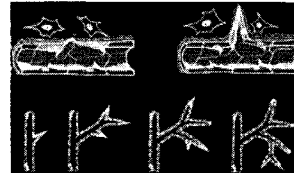
### Terminology and Definitions

- **Angiogenesis:** sprouting of new capillaries from preexisting postcapillary venules
- **Vasculogenesis:** the development of vessels de novo from primitive or precursor endothelial cells
- **Arteriogenesis:** formation of new collateral conduits

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### The Angiogenic Process

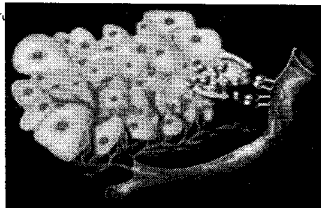
- Vascular epithelial cell stimulation
- Matrix metalloproteinase release
- Basal membrane degradation
- Endothelial cell migration (integrins)
- Endothelial cell proliferation
- New matrix formation



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### Angiogenesis in Cancer

- Tumors cannot grow beyond 1-2mm without formation of new blood vessels
- Tumors turn on the angiogenic switch by ↑ pro-angiogenic factors and ↓ anti-angiogenic factors



Judah Folkman

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### Antiangiogenic Tumor Therapy

- A single vessel provides the nutrition of thousands of tm cells and damage to only one point blocks flow
- Change in shape or initiation of local coagulation rather than endothelial cell killing may be sufficient
- Unlikely to acquire drug resistant mutations
- Easily accessible, without drug delivery issues

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### Angiogenesis in Ischemic Ds

- Angiogenic factors stimulate angiogenesis and collateral growth in the ischemic heart and limbs
- Angiogenic vessels in turn improve tissue perfusion and oxygenation.
- **Therapeutic angiogenesis** with VEGF or FGF is undergoing clinical trials as gene or protein therapy



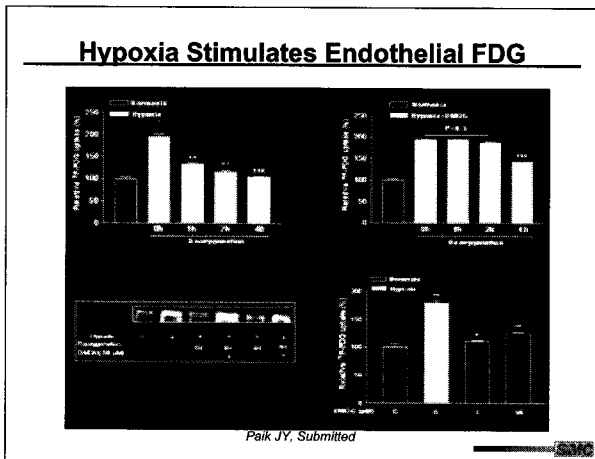
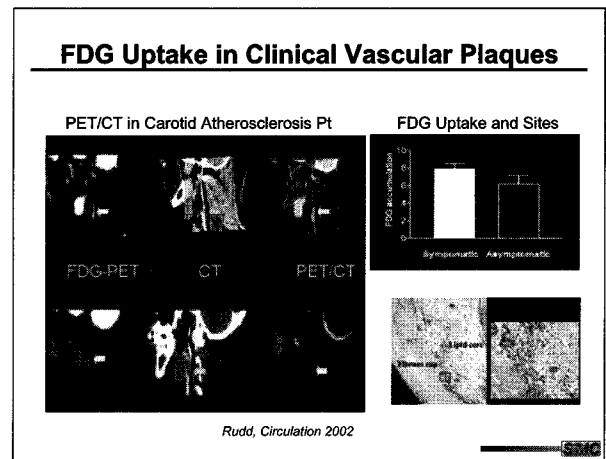
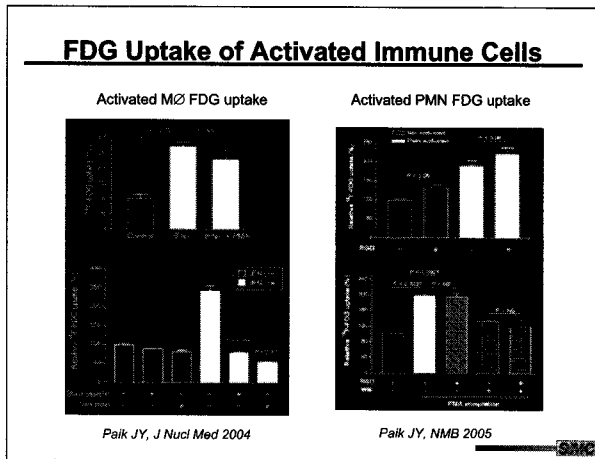
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### Need for Angiogenesis Imaging

- Angiogenesis-targeted therapies have been met with limited clinical success for uncertain reasons
- No currently available noninvasive biomarker approach to monitor angiogenic processes
- May provide information critical for assessing response to angiogenesis-targeted therapies

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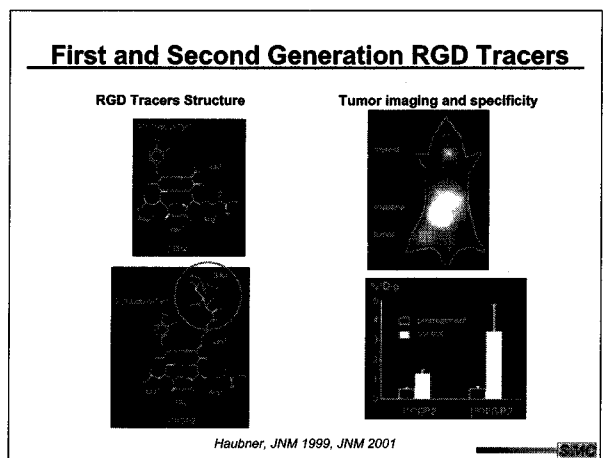


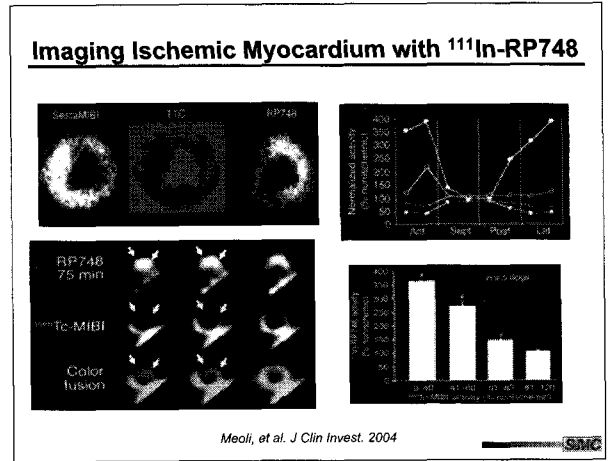
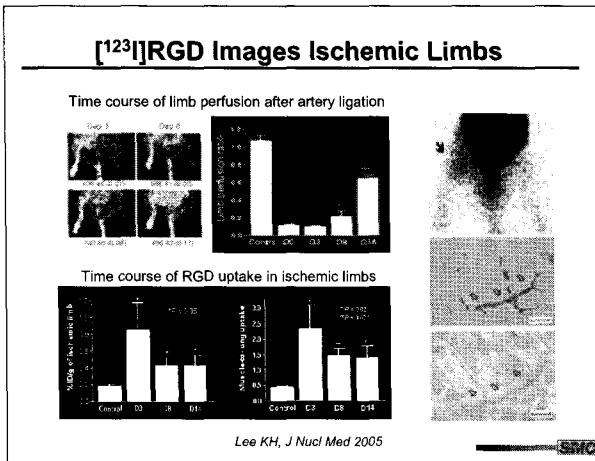
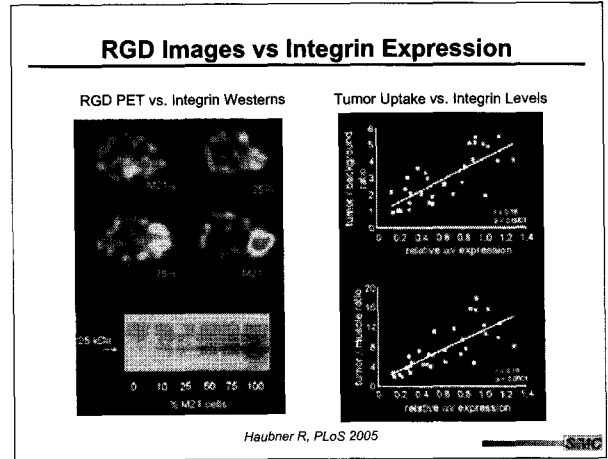
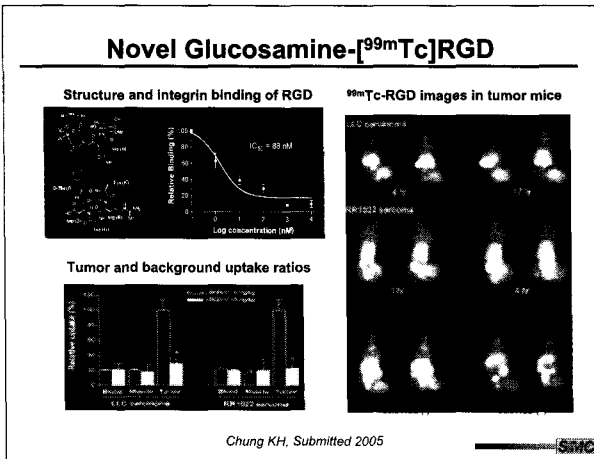
### Glu Metabolism Targeted Imaging

- FDG targets augmented glycolytic activity of such cellular components of angiogenesis as macrophages, neutrophils, and endothelial cells
- FDG-PET identifies activated vessel lesions of atherosclerosis and vascular plaques
- Thus, FDG-PET may provide information regarding the metabolic characteristics of angiogenic lesions

### Integrins in Angiogenic Vessels

- Integrins are heterodimeric adhesion receptors that regulate cell survival, proliferation, and differentiation through cytoskeleton interactions
- $\alpha v\beta 3$  Integrins are highly expressed in activated endothelial cells of angiogenic vasculature
- $\alpha v\beta 3$  Integrins bind to extracellular matrix proteins via RGD tripeptide motifs

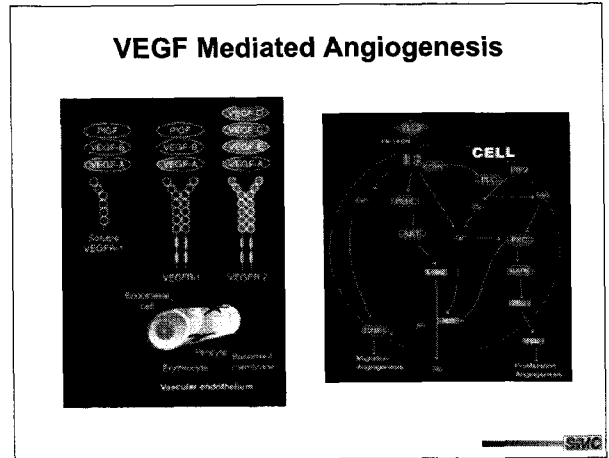


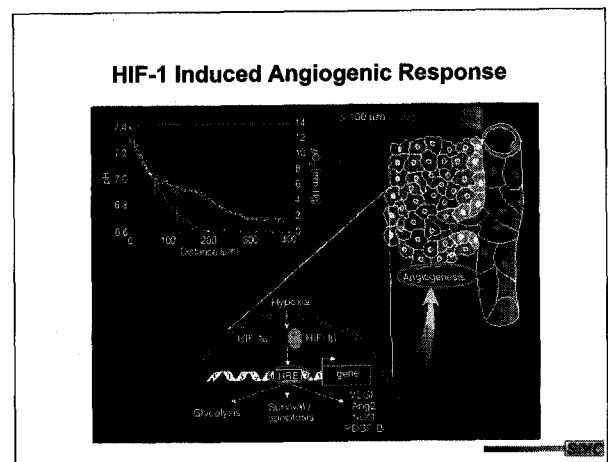
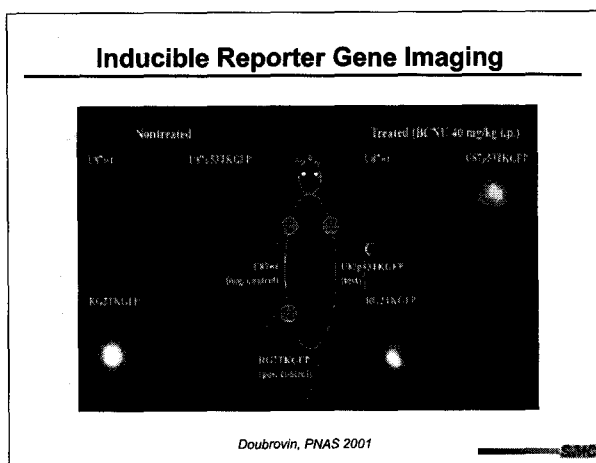
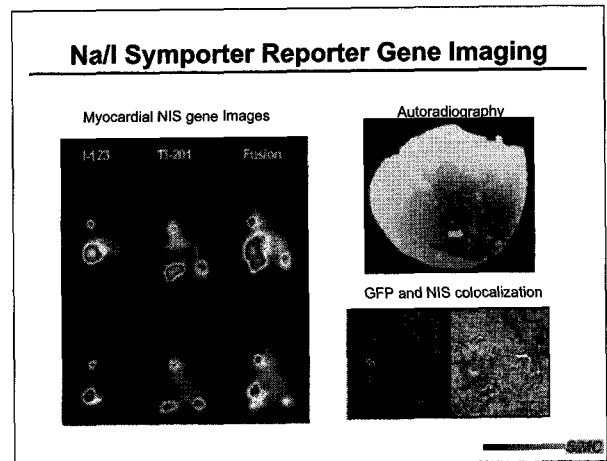
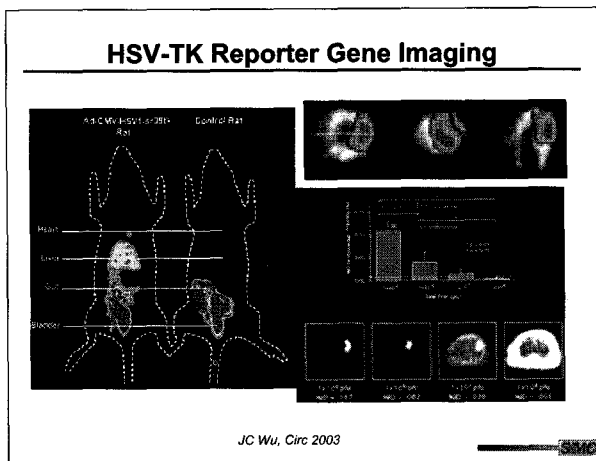
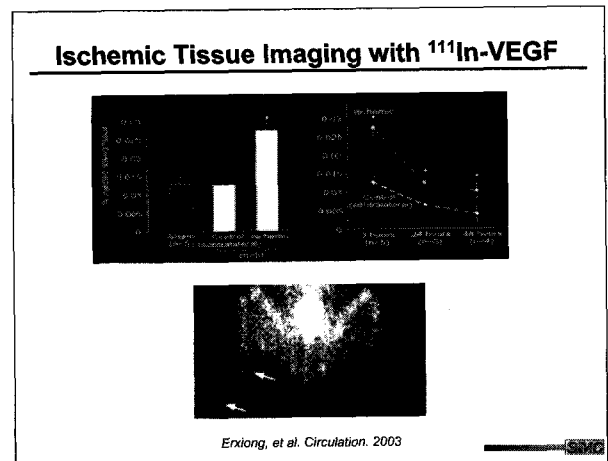
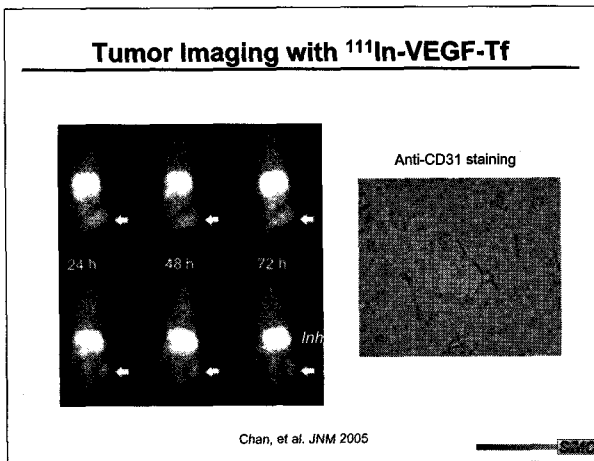


### Integrin Targeted Imaging

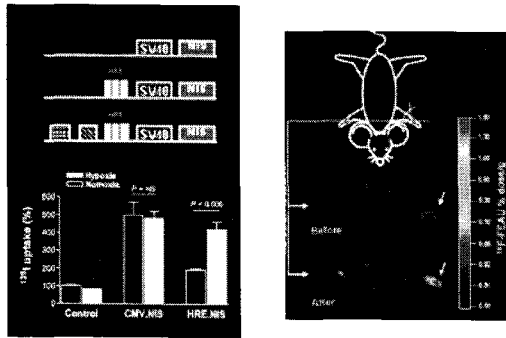
- $\alpha v \beta 3$  integrins are a key regulator of angiogenesis that are highly expressed in activated vasculature
- Radiolabeled RGD analogues are promising integrin-targeted tracers, and have entered clinical trials in cancer patients
- Studies are ongoing to validate the accuracy of RGD imaging to assess angiogenesis and predict response to molecular therapy

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### Hypoxia Inducible Gene Imaging



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### Hypoxia Targeted Imaging

- Reporter gene imaging is a powerful tool for preclinical studies of various disease models
- Inducible reporter genes may allow specific imaging of local milieu characteristic of angiogenic tissue
- HIF-1 is a transcription factor that mediates a major portion of angiogenic responses to hypoxia, hence HRE promoters may allow imaging of hypoxic tissue

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