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Regulation of angiogenesis: Vascular growth, maturation and regression, permeability and lymphatic remodeling

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Nutrient and oxygen supply and clearance of toxic waste products is a critical determinant in maintaining viability and biological function. Thus organisms spend a large fraction of their energy in regulating the exact activity of those pathways maintaining chemical homeostasis. At short distances, on the order of 1-100 micrometers, diffusion complemented with active transporters provides adequate solution for transfer of molecules through space and across barriers. However diffusion rapidly becomes inefficient at larger distances, and thus in large multicellular organisms life depends on the existence of coherent flow in which supply to the tissue matches local demands. The goal of our work is to study the regulation of interstitial diffusion, vascular expansion (angiogenesis), regression and remodeling. For that end we study a number of biological models ranging from the normal ovary to solid tumors. In vivo analysis of diffusion, perfusion and other characteristics of the vasculature are studied by magnetic resonance imaging (MRI). A large part of the effort is directed towards the development of novel methods so as to increase the array of vascular features that MRI can reveal.

Over the last year we studied the role of vascular remodeling in maintenance of ovarian tumor dormancy and in regulation of the exit from dormancy. Our studies demonstrate that angiogenesis per se may be necessary, but is not sufficient and thus cannot be the critical switch leading to tumor development. However during dormancy vascular instability was found, a feature that cannot be detected by conventional invasive methods. Vascular instability can be explained for example by fluctuation in VEGF level in response to changes in tumor oxygenation. The tumor vessels included immature endothelial capillaries and in addition also mature vessels, which have perivascular coating of smooth muscle cells and pericytes. These mature vessels can be detected by MRI through their capacity to respond to vasoactive challenges (e.g. hypercapnia). The immature vessels were found to be sensitive to acute VEGF withdrawal and thus vascular maturation was suggested as a mechanism for vascular stabilization (in collaboration with Laura Benjamin, Beith Israel).

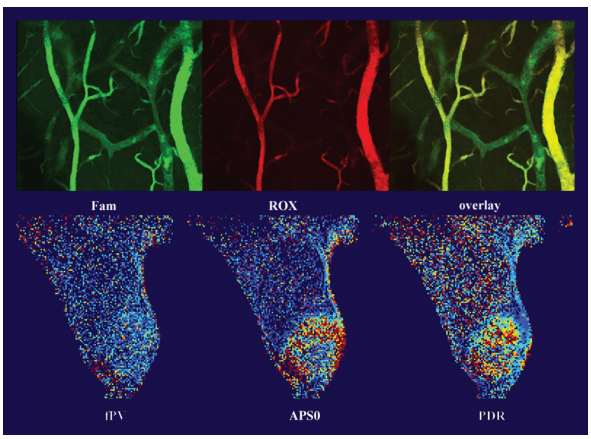

Fig. 1 *Monitoring the response to VEGF using macromolecular contrast material. Top) Biotin-BSA-GdDTPA-carboxyfluorescein (FAM) was administered i.v. to C6-pTET-VEGF tumor bearing mice and allowed to circulate and extravasate for 60 minutes. A second dye, BSA-carboxy-X-rhodamine (ROX) was injected intravenously just before tissue retrieval. In addition to blood vessels, the first dye was observed also in lymphatic vessels whereas, the second dye remained in the blood. Bottom) Leak of MMCM (biotin-BSA-GdDTPA) from blood vessels was induced by intradermal injection of VEGF165 in the hind limb of nude mice. The kinetics of the leak, quantified from MR images, fitted well to a monoexponent. From the exponential fitting 3 parameters were derived: fPV, the fraction of intravascular plasma volume, APS0, the initial permeability, and PDR, rate of inactivation of VEGF induced hyperpermeability.*

VEGF is one of the key positive regulators of angiogenesis. While prolonged exposure to VEGF results in vascular growth and withdrawal leads to vascular obliteration, VEGF also leads to a number of acute responses. Using albumin tagged with Gd-DTPA and fluorescent tags we showed that bolus administration of VEGF results in vascular dilation and hyperpermeability. The extravasated albumin is taken up into the lymphatics possibly due to VEGF induced increase in interstitial colloid pressure. This acute response to VEGF is short term and is terminated within 60-90 min by inactivation of the administered VEGF (Figures 1,2). The role of VEGF in

driving lymphatic uptake, lymphatic drain and remodeling of the lymphatics (lymphangiogenesis) is now being studied (in collaboration with Zaver Bhujwalla, Johns Hopkins). Analysis of the response to VEGF is critical not only because of its role in tumor angiogenesis but also because it is being tested for proangiogenic therapy.

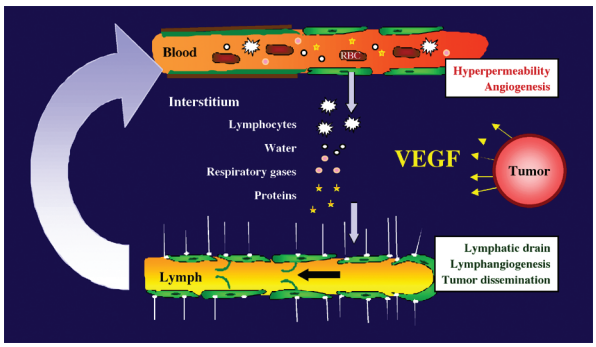

Fig. 2 *Schematic representation of the effect of VEGF on vascular permeability, lymphatic uptake and lymphatic drain.*

Proangiogenic therapy is proposed in many cases in which perfusion insufficiency are compromising the wellbeing of the tissue. Two such systems are now being evaluated by MRI, the generation of collaterals in ischemic limbs, and fertility preservation by ectopic ovarian transplantation (with Alex Tsafriri). Additional projects include MRI analysis of vascular damage following PDT (with Yoram Salomon and Avigdor Scherz), and spinal cord regeneration (with Michal Schwartz).

In summary, Vascular remodeling is a critical component of tissue remodeling and thus may dictate cell proliferation and cell death. Using non invasive MRI we are able to provide dynamic information on vascular changes and thus elucidate central regulatory elements in the control of homeostasis by modulation of the vascular structure and function.

Selected Publications

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Schiffenbauer, Y.S., Meir, G., Maoz, M., Cohen Even Ram, S., Bar-Shavit R., and Neeman, M. (2001) Gonadotropin regulation of alpha v-integrin and CD44-hyaluronate mediated adhesion in MLS human epithelial ovarian carcinoma cells. Gynecol Oncol. In press.

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