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Regulatory Pathways In Angiogenesis And Lymphangiogenesis

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The cardiovascular system is essential for survival of all multicellular organisms larger than 1 mm. Thus, vasculogenesis and angiogenesis are critical for embryonic development and wound repair and on the other hand can control progression of solid tumors. Progress in angiogenesis research led to the discovery of many molecules that participate in angiogenesis and recently also in lymphangiogenesis. A major bottleneck in research is the analysis of the impact of these molecules, which must be done *in vivo* due to the complex dependence and involvement of many cell types that build and interact with blood vessels, the impact of the microenvironment, and the dynamics of flow.

Regulation of vascular remodeling *in vivo* is the focus of our research. Angiogenesis and lymphangiogenesis are studied in an array of biological models including embryonic implantation (in collaboration with Prof. Nava Dekel), hypoxia and VEGF in the placenta, ovarian graft implantation for preservation of fertility (in collaboration with Prof. Alex Tsafriri), recovery of ischemic limbs, and vascular involvement during growth of solid tumors. *In vivo* analysis includes development of novel approaches for non-invasive imaging by MRI.

A novel multimodality contrast material (biotin-BSA-GdDTPA(FAM/ROX)) was developed for imaging angiogenesis. Intravenous administration of the contrast material allowed mapping vascular permeability in response to induced expression of VEGF in C6-pTET-VEGF tumors in mice, in which expression of VEGF can be modulated by administration of tetracycline in the drinking water. The role of tumor angiogenesis in modulating lymphatic metastatic spread was evaluated demonstrating changes in MR contrast enhancement in sentinel lymph nodes (in collaboration with Prof. Prof. Israel Vlodavsky).

Elevation of vascular permeability can increase

interstitial pressure and thus can affect interstitial convection and lymphatic drain (collaboration with Prof. Zaver Bhujwalla, Johns Hopkins University). Indeed increased permeability upon induction of VEGF expression resulted in increased convection and lymphatic drain, manifested in dynamic contrast enhanced MRI as delayed enhancement. In order to separate vascular permeability from lymphatic drain, we applied avidin chase, in which intravenously administered avidin chelates the contrast material and eliminates it from the circulation, while extravasated contrast material is not affected.

Vascular permeability and extravasation of plasma proteins, along with secretion of proteolytic enzymes and deposition of matrix proteins by tumor and stroma cells lead to extensive remodeling of the extracellular matrix (ECM). Collagen deposition in tumors and particularly the impact of LOR-1 expression on tumor invasion were studied (in collaboration with Prof. Gera Neufeld, Technion). Angiogenesis has been linked with deposition of provisional fibrin matrix, and crosslinking of fibrin by tissue transglutaminase (TG) was reported in regions of angiogenesis and in the advancing front of solid tumors. A low molecular weight TG substrate is being developed as an MR contrast material that would allow non-invasive imaging of TG activity (in collaboration with Prof. Mark Dewhirst and Prof. Charles Greenberg, Duke University).

An additional contrast material is being developed for non-invasive imaging of degradation of hyaluronan by hyaluronidase. While high molecular weight hyaluronan is antiangiogenic, the products of its degradation by hyaluronidase are pro-angiogenic. We have previously reported that ovarian carcinoma cells show CD44-hyaluronan mediated adhesion, where CD44 expression is induced by gonadotropins. By degradation of hyaluronidase, cancer cells can modulate the angiogenic activity in

their vicinity thereby facilitating tumor progression.

Additional aspect of the role of stroma to tumor progression is the infiltration of myofibroblasts. Infiltration of these cells marks the initiation of growth of dormant implanted ovarian spheroids. These cells contribute to vascular maturation and stability by expression of specific growth factors and by association with tumor blood vessels. In order to study the role of myofibroblasts in tumor angiogenesis and tumor progression we have developed protocols for vital staining of cells with MR contrast material. This approach would hopefully allow for non invasive tracking of cell migration and recruitment.

In summary, novel MR methods allowing non-invasive analysis of vascular remodeling, were used for revealing the role of VEGF as a driving force for peritumor interstitial convection and lymphatic drain. Vascular maturation and the role of tumor associated myofibroblasts were demonstrated to be important in regulating exit of ovarian carcinoma from dormancy. New generation of contrast agents are being developed that would allow insight on specific pathways regulating growth and regression of blood and lymphatic vessels.

Selected Publications

Dafni, H., Israely, T., Bhujwalla, Z.M., Benjamin, L.E. and Neeman, M. (2002) Overexpression of vascular endothelial growth factor 165 drives peritumor interstitial convection and induces lymphatic drain: magnetic resonance imaging, confocal microscopy, and histological tracking of triple-labeled albumin. *Cancer Res*, 62, 6731-6739.

Dafni, H., Gilead, A., Nevo, N., Eilam, R., Harmelin, A. and Neeman, M. (2003) Modulation of the pharmacokinetics of macromolecular contrast material by avidin chase: MRI, optical, and inductively coupled plasma mass spectrometry tracking of triply labeled albumin. *Magn Reson Med*, 50, 904-914.

Gross, S., Gilead, A., Scherz, A., Neeman, M. and Salomon, Y. (2003) Monitoring photodynamic therapy of solid tumors online by BOLD-contrast MRI. *Nat Med*, 9, 1327-1331.

Israely, T., Dafni, H., Granot, D., Nevo, N., Tsafirri, A. and Neeman, M. (2003) Vascular remodeling and angiogenesis in ectopic ovarian transplants: a crucial role of pericytes and vascular smooth muscle cells in maintenance of ovarian grafts. *Biol*

Reprod, 68, 2055-2064.

Akiri, G., Sabo, E., Dafni, H., Vadasz, Z., Kartvelishvily, Y., Gan, N., Kessler, O., Cohen, T., Resnick, M., Neeman, M. and Neufeld, G. (2003) Lysyl oxidase-related protein-1 promotes tumor fibrosis and tumor progression in vivo. *Cancer Res*, 63, 1657-1666.

Neeman, M. (2002) Functional and molecular MR imaging of angiogenesis: seeing the target, seeing it work. *J Cell Biochem Suppl*, 39, 11-17.

Neeman, M. and Dafni, H. (2003) Structural, functional, and molecular MR imaging of the microvasculature. *Annu Rev Biomed Eng*, 5, 29-56.

Gilead, A., Meir, G. and Neeman, M. (2004) The role of angiogenesis, vascular maturation, regression and stroma infiltration in dormancy and growth of implanted MLS ovarian carcinoma spheroids. *Int J Cancer*, 108, 524-531.

Ziv, K., Nevo, N., Dafni, H., Israely, T., Granot, D., Brenner, O. and Neeman, M. (2004) Longitudinal MRI tracking of the angiogenic response to hind limb ischemic injury in the mouse. *Magn Reson Med*, 51, 304-311.

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