In vivo imaging of angiogenesis

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Homeostasis of multicellular tissues relies on accurate match of vascular supply and drain to the needs of the tissue. Multiple pathways are involved in detection, signaling and execution of the required steps involved in remodeling of blood and lymphatic vessels during tissue development. Similar mechanisms are utilized for overcoming changes in tissue requirements also in adult tissues and in pathological processes. Angiogenesis and lymphangiogenesis are critical to pregnancy and fetal development, to graft implantation and to cancer progression and metastasis. The aim of our group is to reveal the regulatory checkpoints associated with vascular remodeling. Non invasive multimodality imaging methods developed in our group, provided an important platform for dynamic, structural, functional and molecular evaluation of multiple steps in vascular remodeling.

Vascular remodeling in reproduction

Upon implantation of the embryo into the uterine wall, maternal angiogenesis provides the necessary supply of nutrients to maintain the pregnancy at these early critical days. Non invasive imaging by MRI, revealed these early implantation sites as points of vascular hyperpermeability that can be detected using macromolecular MRI contrast material. One day later, rapid maturation of the blood vessels is accompanied by reduced permeability. The role of maternal angiogenesis in maintenance of early pregnancy could be revealed by MRI using genetically modified mouse models of infertility associated with failed embryo implantation (in collaboration with Nava Dekel).

At later stages of pregnancy, fetal survival depends on angiogenesis in the placenta. MRI showed the ability to quantify the function of blood vessels in the placenta. Surprisingly, maternal exchange of blood in the placenta was found to be very low. These methods are now applied for analysis of genetic models for intra-uterine growth retardation. The role of placental vascular deficiency was studied using PKB/Akt1 deficient mice (in collaboration with Brian Hemmings, Basel; and Alina Maizenberg, Rebecca Haffner and Alon Harmelin).

In vivo imaging using MRI and ultrasound provides an important tool for following angiogenesis and development during fetal implantation and pregnancy in mice (Figure 1).

The tumor vasculature

We previously showed that ovarian carcinoma tumors show prolonged dormancy, associated with futile cycles of angiogenesis and vascular regression. Vascular stabilization, associated with recruitment of perivascular stroma cells, and particularly myofibroblasts, provides an important signal for initiation of tumor progression. Non-invasive imaging was applied for tracking the recruitment of perivascular angiogenic support cells to tumors (in collaboration with Leoni Kunz Schughart, Dresden Univ.) Fibroblasts labeled with MRI or fluorescent reporter probes, administered intraperitonealy, showed directed recruitment to a remote subcutaneous tumor. Within the tumor, the cells organized in the peritumor vascular rim as detected by two photon microscopy. Recruitment of cells, vascular function and tumor blood flow can be independently measured either by MRI or by ultrasound (Figure 2).

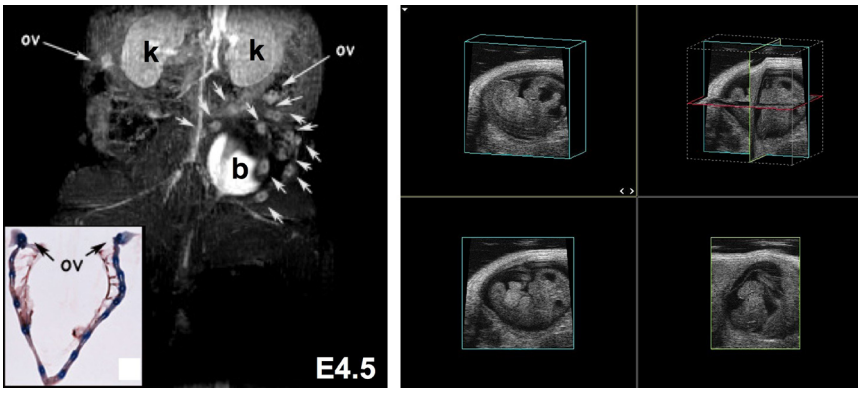

Fig.1 *In vivo imaging of the mouse pregnancy. Left) MRI of mouse embryo implantation sites at E4.5. Maximal intensity projections of 3D-GE MRI acquired 7.5 min post i.v. injection of biotin-BSA-GdDTPA (individual implantation sites are indicated by arrows). Insert: Ex vivo visualization of implantation sites highlighted by i.v. administration of Evans blue 10 min before tissue retrieval. k- kidney, b- bladder, ov- ovary (Plaks et al). Right) Micro-ultrasound 3D image of embryonic mouse E 12.5. In this figure the neural tube, the heart and the somites can be identified (Vandoorne et al).*

Our working hypothesis is that hormonal stimulation of dormant tumors, is one of the reasons for induced progression of disease at menopause. We previously reported hormonal regulation of angiogenesis and adhesion of ovarian cancer cells by the gonadotropin hormones LH and FSH. Our current studies aim to detect the role of LH and FSH in induction of lymphangiogenesis thus further affecting tumor metastasis.

In addition to angiogenesis the peritumor region is served by lymphatics, which drain interstitial fluids from the tumor and provides a route for escape of tumor cells towards sentinel lymph nodes. Over the last years we developed tools for in vivo imaging of the tumor lymphatic drain (in collaboration with Zaver Bhujwalla, Johns Hopkins University).

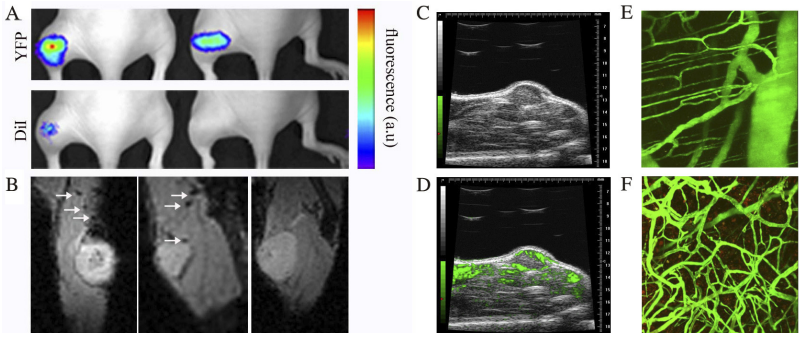

Fig.2 *Angiogenesis in ovarian carcinoma. Left) Recruitment of human primary fibroblasts by subcutaneous ovarian carcinoma tumors (Granot, Addadi et al). A) NIR fluorescence imaging of subcutaneous YFP expressing tumors following intra- peritoneum injection of WI38 fibroblasts labeled with DiI (left panel) or unlabeled cells (right panel). B) MRI analysis of tumors initiated by subcutaneous inoculation of MLS cells, followed by intra-peritoneum injection of PFN2 fibroblasts labeled with Feridex (left and middle panels) or unlabeled cells (right panel). Arrows- hypo-intense regions correlating with Feridex- labeled PFN2 fibroblasts. Center) Visualization of tumor micro-vasculature by ultrasound using microbubbles. C) subcutaneous tumor on the leg before injection with microbubbles. D) microbubbles are injected IV. Blood flow is colorized and quantified (Vandoorne et al). Right) Two photon intravital microscopy. E) Normal dermal vasculature. F) Vasculature in a subcutaneous ovarian carcinoma tumor (Addadi, Kalchenko et al).*

MRI studies and pharmacokinetic analysis of early and delayed contrast enhancement demonstrated the role of VEGF in providing the driving force for interstitial flow and lymphatic uptake, and the role of such flow in tumor metastasis. Our current work aims to reveal the microenvironmental molecular machinery controlling lymphatic remodeling.

Endothelial cells induced by VEGF in tumors show chronic activation of PKB/Akt1. The role of this signaling pathway in angiogenesis was studied in transgenic mice expressing contitutively active myrAkt in endothelial cells under tetracycline regulation (in collaboration with Laura Benjamin, Beth Israel Medical Center Harvard Univ). Dynamic contrast enhanced MRI experiments revealed systemic elevation of blood volume, increased blood volume fraction and vascular permeability, demonstrating that activation of PKB/Akt1 is sufficient for recapitulating the angiogenic phenotype of the tumor vasculature. Treatment with rapamycine reversed the angiogenic activity induced by PKB/Akt1. Ongoing experiments aim to reveal the role of this pathway in regulation of lymphangiogenesis.

Molecular and functional characteristics of the tumor vasculature can be utilized for targeted delivery of imaging probes and therapy. Image guided targeted delivery of functionalized nanoparticles to the tumor vasculature is being developed as part of the European FP6 MEDITRANS integrated project.

Remodeling of the ECM during tumor angiogenesis

The extracellular matrix undergoes extensive changes during angiogenesis. We developed molecular imaging contrast media for detection of two key enzymatic reactions altering the extracellular matrix. The first addresses the deposition of a provisional fibrin matrix, induced by extravasation of fibrinogen from leaky blood vessels. Fibrin clotting is stabilized by covalent crosslinking catalized by tumor derived tissue transglutaminase. The activity of tissue transglutaminase could be detected by MRI and by NIR imaging using contrast media that are low molecular weight substrate analogs (in collaboration with Mark Dewhirst, Duke University; and Silvio Aime, Univ of Torino). The same materials can also be used for analysis of the transglutaminase reaction catalized by Factor XIII during blood clotting.

Another important constituent of the extracellular matrix affecting tumor angiogenesis is hyaluronan. Ovarian tumor cells express CD44, the hyaluronan receptor and show hormonally regulated adhesion to hyaluronan coated surfaces. High molecular weight hyaluronan is potently antiangiogenic leading to expulsion of blood vessels, while its degradation products are proangiogenic. The activity of tumor derived hyaluronidase in converting antiangiogenic high molecular weight hyaluronan into low molecular weight pro-angiogenic fragments was revealed using an activatable smart MRI contrast agent (Figure 3).

Ferritin as a reporter gene for MRI

The iron binding protein ferritin accumulated in many organs as part of normal physiology (e.g. in the liver and spleen) and in pathology and aging (e.g. the brain). Such endogenous levels of ferritin are detectable by MRI as changes in the R2 relaxation rate. Thus we evaluated the possibility to utilize ferritin as an endogenous reporter gene that would allow detection of transcriptional activation of gene expression by MRI. After demonstrating feasibility in tumors, with the help of the transgenic facility at Weizmann we generated transgenic mice in which ferritin expression is regulated by tetracycline. Mating with mice expressing the tetracycline transactivator generates double transgenic offspring with tetracycline inducible promoter selective expression of ferritin. MRI depicted changes in R2 induced by liver and endothelial selective expression of the tetracycline transactivator. In utero analysis at day E13.5 of pregnancy allowed in vivo detection of the pattern of ferritin expression induced by the vascular endothelial cadherin promoter (in collaboration with Laura Benjamin; Figure 4). Ongoing studies aim to reveal the impact of prolonged over expression of ferritin by liver hepatocytes.

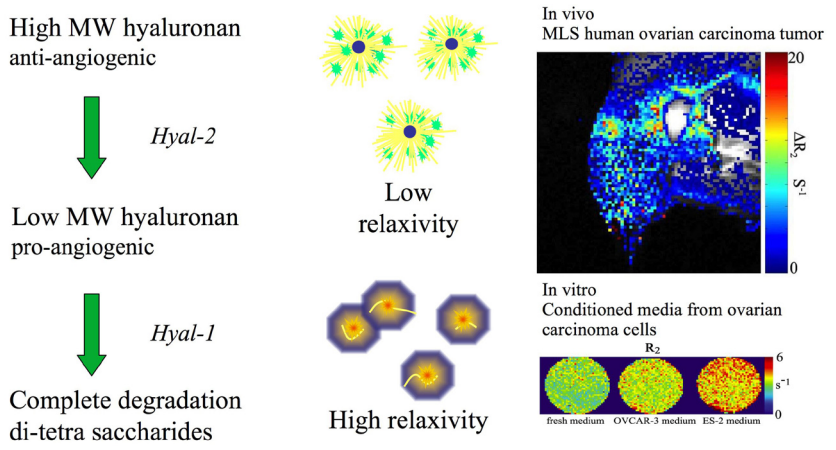

Fig.3 *Molecular imaging of hyaluronidase activity by MRI: Left) Conversion of the extracellular matrix from antiangiogenic to proangiogenic, through the activity of tumor derived hyaluronidase. Center) The activity of hyaluronidase can detected using quenched contrast media with low relaxivity that can be activated by enzymatic degradation. Right) Bottom- in vitro MRI analysis of the activity hyaluronidase secreted by human ovarian carcinoma cells. Top- In vivo analysis of the activation of the MRI contrast media by an ovarian tumor in a mouse (Shiftan et al).*

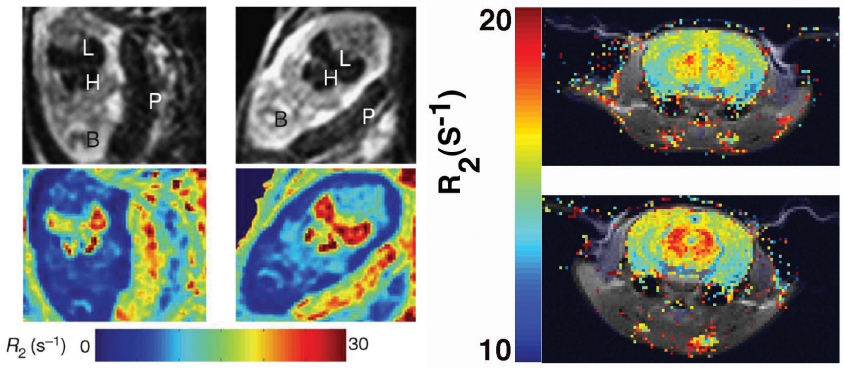

Fig. 4 *Ferritin as a reporter gene for MRI (Cohen, Ziv, Plaks et al). Left) Fetal activity of the VE-cadherin promotor detected by MRI. MRI images of a pregnant mouse, showing the embryonic liver, heart, brain and placenta. Bottom: Embryos genetically engineered to overexpress the ferritin reporter gene in the blood vessels (r) show higher ferritin induced contrast of the heart and liver compared with siblings that don't overexpress the gene (l). Right) MRI virtual section of adult mouse brain expressing the ferritin reporter gene in vascular endothelial cells (bottom row) shows high ferritin-induced contrast compared with that of a sibling mouse, in which expression of this gene is silent (top).*

Multimodality imaging of small animals

(in collaboration with Alon Harmelin and Ronen Basri)

The aim of this project, supported by the ISF converging technologies program, is to establish at Weizmann a platform for cross modality imaging of small animals. The imaging systems will be operated as a service facility by the Department of Veterinary Resources. Non invasive imaging tools will allow image guided intervention and functional, anatomical and molecular imaging from three dimensional whole body to microscopic resolution. The activity over the last year included installation of a small animal ultrasound system which allows functional imaging and image guided intervention and molecular and hemodynamic imaging using microbubble contrast media. Optical imaging units for small animals include a twophoton microscope for in vivo imaging, a new system for whole body bioluminescence, fluoroscence spectral and tomographic imaging and a system for endoscopic fluorescence imaging. Ongoing efforts include construction of home built fluorescence scanning tomographic system (in collaboration with Jorge Ripoll, Inst. of Electronic Structure & Laser, Crete; and Vyacheslav (Slava) Kalchenko) and generation of hardware and software solutions for cross modality registration and convergence of information.

A specific application for multimodality imaging is the development of a novel skin flap window chamber for high resolution MRI and two photon intravital fluorescence imaging of tumor angiogenesis (in collaboration with Fabian Kiessling, German Cancer Center).

In summary, development and functional activity of blood and lymphatic vessels are regulated by microenvironmental cues, which provide the required feedback to maintain tissue homeostasis and support changes in tissue requirements. The same building blocks used for vascular development in embryo implantation and fetal development, are frequently utilized, often out of their proper context, in tumor angiogenesis to support tumor progression and metastasis.

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