Cancer Progression and Metastasis; Molecular and Physiological Imaging

Hadassa Degani
Edna Furman-Haran
Dalia Seger
Tamar Kreizman
Raanan Margalit
Nimrod Maril
Chidambaram Gunanathan
Galit Eliyahu
Maya Dadiani
Yaron Hassid
Gal Zahavi
Adi Pais
Erez Eyal
Gregory Ramniceanu

Our research is aimed at investigating hormonal regulation, angiogenesis and lymphogenic metastasis in the course of tumor progression. We also search for metabolic markers of breast malignant transformation and elucidate their molecular origin. In our studies we employ cancer cells of human origin, predominantly breast and lung cancer, and tumors developed from these cells growing in immuno-compromised mice. Our main research tools are non invasive; magnetic resonance imaging (MRI) and spectroscopy (MRS), as well as fluorescence imaging. In addition, we continue to develop the clinical application of dynamic contrast enhanced MRI of cancers and sodium renal imaging. In the following section we describe our ongoing projects:

Real-time imaging of lymphogenic metastasis
Metastasis to regional lymph nodes is one of the earliest events of tumor cell dissemination and presents a most significant prognostic factor for predicting survival of cancer patients. We used in vivo fluorescence microscopy and contrast enhanced MRI to monitor the progression of lymph node metastasis as well as the course of spontaneous metastasis through the lymphatic system of orthotopic MDA-MB-231 human breast cancer tumors in SCID mice. High-resolution, non-invasive visualization of metastasizing cancer cells in the inguinal lymph nodes was achieved using cells expressing high levels of red fluorescent protein. Sequential imaging of these lymph nodes revealed initial invasion of the tumor cells through the lymphatic system into the subcapsular sinuses, followed by intrusion into the parenchyma of the nodes. Microscopic MRI studies of lymph node metastasis also revealed invasion of the cells to the subcapsular sinus.

FITC-dextran injected intradermally in the tumor area enabled simultaneous tracking of lymphatic vessels, labeled in green, and disseminated red cancer cells within these vessels (Figure 1). Fast snapshots of spontaneous metastasizing cells in the lymphatic vessels monitored the movement of few tumor cells and the development of clumps clustered at lymphatic vessel junctions. The results suggested a mechanism for the intravasation into the lymphatic system invoked by the high interstitial fluid pressure in the tumors.

Mapping tumor transcapillary transfer rates and interstitial fluid pressure by contrast enhanced MRI
Tumor progression and response to blood borne drugs is critically dependent on the efficiency of vascular delivery and transcapillary transfer rates. However, increased tumor interstitial fluid pressure (IFP) forms a barrier to transcapillary transfer, leading

Fig. 1 Real-time fluorescence images of MDA-Mb-231 human breast cancer metastases in lymphatic vessels. A. Lymph node metastasis (LN) (red fluorescence) and lymphatic vessels (green fluorescence). B. High magnification of the region in A marked by a rectangle, revealing few tumor cells (arrows) and tumor cell clusters (arrowheads) within lymphatic vessels. The lymphatic vessels were marked by intradermal injection of FITC-dextran. The cells expressed red fluorescence protein and were inoculated orthotopically exhibiting spontaneous metastasis to lymph nodes, lungs, bones and abdomen cavities.
to impaired perfusion and drug resistance. We are developing non-invasive MRI methods for determining the transcapillary transfer rates as well as the IFP distribution in tumor models of human lung and breast cancers. New physiological models that account for tracer transfer between the intravascular and extracelluar compartments according to concentration and pressure gradients are developed. Figure 2 demonstrates the contrast agent concentration map and the derived IFP map of ectopic lung cancer tumor obtained by a new method based on slow infusion of the contrast agent and T1 reaxation rates measurements.

Molecular MRI of the estrogen receptor

The estrogen receptor plays a key role in the etiology of breast cancer and is important as a target for the treatment and prevention of this disease. We have recently designed and synthesized in collaboration with Professor D Milstein, Organic Chemistry, Weizmann a novel estrogen conjugated pyridine containing Gd(III) contrast agent (EPTA-Gd). This agent showed high affinity to the estrogen receptor and competed with 17β-estradiol on binding to ERα, with a competition equilibrium constant of 0.78 μM. It also markedly enhanced the T1 and T2 relaxation rates yielding a T1 relaxivity of ~8 (mMxsec)⁻¹ and T2 relaxivity of ~30 (mMxsec)⁻¹, serving as a molecular beacon for localizing ER by MRI. The biological activity of EPTA-Gd was in accord with an estrogen agonist, stimulating proliferation of ER positive human breast cancer cells as well as accelerating the volume and inducing water imbibition in the ovariectomized uterus. It also elicited a feedback response of ER degradation. EPTA-Gd induced MRI enhancement of uterine tissue indicating specific binding to the estrogen receptor. Further studies of various lanthanide complexes and antiestrogen based novel contrast agents are currently underway.

Choline as a metabolic marker of breast malignant transformation

Choline signal detected by magnetic resonance spectroscopy of breast lesions has been demonstrated to serve as a marker of malignancy. We recently revealed the principal molecular and biochemical steps associated with the induction of choline metabolism in breast cancer cells. The study was performed on primary cultures of human mammary epithelial cells and five different human breast cancer cell-lines by means of nuclear magnetic resonance, as well as biochemical and molecular methods. The expression levels of specific choline transporters: organic cation transporter-2 and choline high affinity transporter-1, as well as of the enzyme choline kinase α were found to be up regulated in the cancerous cells in comparison to normal mammary epithelial cells. These molecular changes accounted for the several fold increase in choline transport rate and choline kinase activity and formed the origin for the elevated levels of phosphocholine in the cancer cells.

Sodium imaging of the kidney

We developed sodium MRI of the intact rat kidney, and monitored distinct changes in the corticomedullary sodium profile under various physiological and pathological states. The corticomedullary sodium gradient increased linearly from the cortex to the inner medulla from a tissue sodium concentration of ~60 mM to ~360 mM. Changes in the gradient were related to the specific site and mechanism of diuretic drugs. Distinct profiles of the sodium gradient were observed in acute obstructed kidneys, as well as spontaneously obstructed kidneys. The changes in the sodium gradient correlated with the extent of damage and served to assess the residual function of the kidneys. Thus, we have shown that high resolution sodium MRI is a non invasive functional imaging method of renal physiology.

Progressive changes of tumor perfusion in benign and malignant breast lesions; parametric contrast enhanced MRI

Computer aided analysis of high-resolution dynamic contrast enhanced images indicated that progression from benign epithelial breast diseases to ductal carcinoma in situ (DCIS) lesions and to infiltrating ductal carcinomas is associated with distinct changes in the vascular properties, leading to the elevation of the influx and efflux transcapillary transfer rates. The benign breast fibroadenomas that originate from fibrous growth show influx transfer rates similar to that of DCIS, but differ in their efflux transfer rate due to their high extracellular volume space. This quantitative evaluation enabled us to define threshold values for the transfer rates which differentiate between the various lesions achieving high sensitivity and specificity of breast cancer diagnosis.

Selected publications


Fig. 2 Parameteric images of the steady state concentration of contrast agent (A) and the corresponding interstitial fluid pressure (B) in a typical ectopic H460 human lung cancer tumor. These images were obtained by analysis of T1 maps recorded before and at steady state infusion of GdDTPA.


Acknowledgements

H. Degani is the incumbent of the Fred and Andrea Fallek Professorial Chair for Breast Cancer Research, The work has been supported by NIH, USA; Israel Science Foundation; Moross Cancer Institute; Pasteur - Negri - Weizmann Institute grant; Lord David Alliance CBE, UK; The Estate of Julie Osler, USA