Investigating the role of macromolecular transport in the formation of malignant ascites and metastases

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Introduction:

Ascitic fluid and metastasis are the main causes of morbidity and mortality in prostate and ovarian cancer patients. Orthotopic implantation models, where tumor xenografts are implanted directly into the prostate or ovary of immunodeficient mice, result in metastases, and frequently induce the formation of malignant ascites. Here we combine noninvasive MRI with optical imaging to characterize the relationship between tumor vasculature, interstitial fluid transport, malignant ascites formation and metastatic dissemination, using orthotopic human ovarian OVCAR3 and prostate PC3 tumor xenografts. By imaging the dynamics of the macromolecular contrast agent albumin-gadolinium-diethyltriaminepentaacetic acid (GdDTPA) labeled with fluorescent rhodamine (1), it is possible to quantify the transport of macromolecules from the tumor interstitium into malignant ascites. This enables us to gain insights into the role of tumor macromolecular transport, permeability, and vascularization in the formation of this devastating condition and to identify new therapeutic strategies.

Methods:

Ovarian OVCAR3 and prostate PC3 human cancer cell lines were used in the present study. Orthotopic implantations were performed as previously described (2). Briefly, we used a microsurgical method that avoids disseminating cancer cells during inoculation on the ovary or on the prostate. For the ovarian xenograft implantation, intact OVCAR3 tumor tissue (< 1mm^3) grown in the flank of severe combined immunodeficient (SCID) female mice is sutured on the ovary of anesthetized SCID female mice. A similar protocol is used for the PC3 tumor, using male mice, and suturing the piece of tumor on the prostate. By implanting tissues rather than injecting cells, the stromal tissue and the three dimensional cytoarchitecture, believed to play a critical role in tumor progression and metastasis, are maintained. All MR imaging was performed on a Bruker 4.7T spectrometer when tumors were approximately 300-400 mm^3. Tumor size was assessed non-invasively on T_1- and diffusion-weighted images, where the tumor appears hyperintense. We added 5(6)-carboxy-x-rhodamine N-succinimidyl ester, to react with the amine functional group of albumin-GdDTPA under weakly basic conditions to synthesize albumin-GdDTPA-rhodamine. Mice were anesthetized with a mixture of ketamine and acepromazine and the tail vein was catheterized before placing the animal in the magnet. Vascular volume, permeability surface area product, and interstitial fluid transport parameters were measured from quantitative T_1 maps obtained before and after intravenous administration of the contrast agent albumin-GdDTPA-rhodamine (500 mg/kg dose). As previously described, two different sets of MR images were acquired (3). Firstly, to characterize the tumor vasculature, MR images were obtained over the initial 30 min post-contrast injection. Then, to characterize macromolecular interstitial transport, MR images were acquired up to 123 min post-contrast. Macromolecular transport parameters derived included the number of draining and pooling voxels, draining and pooling rates and exudate volumes as previously described (3). Following MRI, we allowed a delay of 24 h for the contrast agent to diffuse into the ascitic fluid. Next, mice were sacrificed, and the red rhodamine fluorescence assessed in the tumor, lymph nodes and in the ascitic fluid. Lungs, liver, lymph nodes were excised and fixed in formalin to quantify the metastatic spread. The volume of ascitic fluid was measured, and the amount of rhodamine in the fluid relative to the injection volume quantified by spectrophotometry at 575 nm.

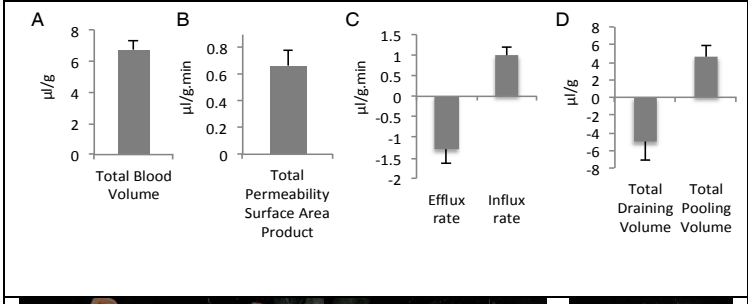

Figure 1: Total blood volume, total permeability surface area product, efflux and influx rates, and total draining and pooling voxels measured *in vivo* in OVCAR3 xenografts implanted orthotopically (mean ± sem, n = 4)

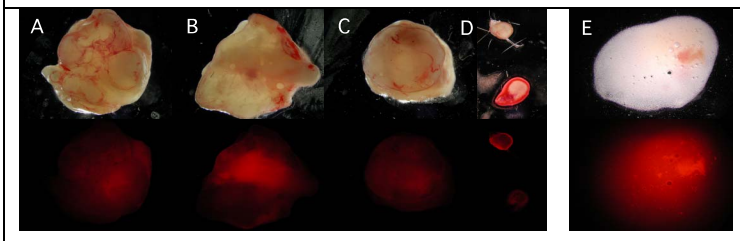

Figure 2: Rhodamine fluorescence in freshly excised orthotopic OVCAR3 tumor slices (A, B, C), lymph nodes (D) and in ascitic fluid smear (E) 24 h after injection of the contrast agent.

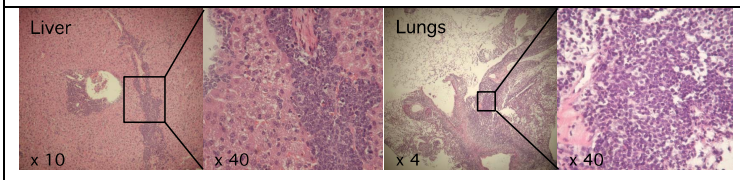

Figure 3: Metastatic nodules observed in liver and lung following OVCAR3 orthotopic implantation (H&E staining).

Results and Discussion:

Malignant ascites, a complication observed in terminal ovarian and prostate cancer, is a devastating condition that significantly contributes to poor quality of life and mortality. Treatment options for late-stage cancers are extremely limited and very invasive once malignant ascites develops. Ascitic fluid often contains free-floating cancer cells, which have been shed from the primary tumor and can lead to intraperitoneal metastases. New therapeutic strategies exploiting novel targets are urgently needed to minimize morbidity associated with this condition. The build-up of this fluid is due to the tumor secreting protein exudate into the intraperitoneal cavity. Here we relate ascitic fluid accumulation to vascular volume, total permeability surface area product, efflux and influx rates and total draining and pooling volumes (**Figure 1**). Rhodamine was detected 24 h post-injection in the tumor, lymph nodes and the ascitic fluid (**Figure 2**). Metastases were observed in the liver, lymph nodes and in the lungs (**Figure 3**). Ascites volume ranged from 150 to 500 µl and was independent of tumor volume, indicating that an active process determines this build-up. The ratio of fluid to injection solution rhodamine absorbance values were 0.02 to 0.035 for the tumors with the largest fluid build-up. We are currently characterizing the levels of VEGF in the ascitic fluid, since increased VEGF has been observed in malignant ascites of patients (4). The ongoing studies, as well as understanding the role of hypoxia in this condition using our hypoxia-driven fluorescence reporter cell lines (5), will provide new insights into malignant ascites build-up. Eventually, these findings may result in new strategies to reduce morbidity and mortality from prostate and ovarian cancer.

References: (1) Dafni *et al.*, Cancer Res (2002) 62:6731-9. (2) Penet *et al.*, Cancer Res (2009) 69:8822-9. (3) Pathak *et al.*, Cancer Res (2005) 65:1425-32. (4) Adam R *et al.*, J Am Coll Surg (2004) 198: 999–1011. (5) Raman *et al.*, Cancer Res (2006, 66:9929-36. **Acknowledgement:** This work was supported by the HERA Foundation, the Honorable Tina Brozman Foundation, NIH P50CA103175, and NIH R01CA73850.