Hypoxia and elevated total choline are associated with ‘stem-like’ cancer cells in breast cancer xenograft in vivo: an MR, SPECT/CT, and optical study

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Introduction: The discovery of stem-like cell populations in several cancers, that are the most likely to be resistant to therapy and to lead to recurrence and metastasis, has generated tremendous excitement for understanding and treating tumor recurrence and metastasis. Stem-like breast cancer cells are usually identified by (i) CD44+/CD24- or low receptor expression with PET or SPECT in breast cancer patients will further validate these observations.

especially for therapies designed to target stem-like cancer cells. Future clinical evaluation of the relationship between elevated total choline using stem-like cancer cells may form a dynamic population that may transit between exhibiting stem- and non-stem-like phenotypic characteristics.

Results: CD44, VEGF and ABCG2 mRNA expression in MDA-MB-231 cells under normoxic and hypoxic conditions. Hypoxia was achieved by treating cells with 100 micromolar of the hypoxia mimetic cobalt chloride for 24 h. The discovery of stem-like cell populations in several cancers, that are the most likely to be resistant to therapy and to lead to recurrence and metastasis, has generated tremendous excitement for understanding and treating tumor recurrence and metastasis.

Methods: Studies were performed with MDA-MB-231 human breast cancer cells stably transfected with red-fluorescent tdTomato protein (RFP) expressed under control of the VEGF hypoxia response element (HRE). These MDA-MB-231 HRE-RFP tumors were grown orthotopically in female severe combined immunodeficient (SCID) mice. MR experiments were performed with a Bruker horizontal bore 9.4T dedicated animal MR scanner using a home-built RF resonator for the tumor. 3D MR imaging was performed with a fast spin echo sequence and 3D MR spectroscopic imaging (MRSI) of choline distribution with spin echo CSI sequence (TE/TR = 80/1000ms), 512 spectral points and 12x12x8 spatial matrix (1 mm resolution) with 4 averages. Fluorescence imaging of the tumor was performed in vivo with a Xenogen IVIS 200 system and endpoint fluorescence imaging was performed with a fluorescence microscope using fresh 2 mm tumor slices prepared with a tissue slicer.

For SPECT/CT imaging mice were administered intravenously with 0.616 mCi of [125I] labeled anti-CD44 antibody in 0.17 mL of saline. At 48 h post injection, SPECT images were acquired on a Gamma Medica X-SPECT scanner in 64 projections at 45 sec/projection (1 mm resolution). Following SPECT imaging, a CT scan using 512 projections was performed to obtain a co-registered 3D anatomical reference. Since CD44 is a transmembrane hyaluronic acid receptor that exists in several isoforms as a result of alternative splicing, we performed q-RT-PCR analyses of these isoforms as well as analysis of a region common to all isoforms (CD44 element (HRE)). These MDA-MB-231 HRE-RFP tumors were grown orthotopically in female severe combined immunodeficient (SCID) mice.

HRE-RFP tumor (~250 mm3).

Inset shows RFP expression map only.

Figure 1a. Overlay of total choline with RFP distribution in an MDA-MB-231 HRE-RFP tumor (~250 mm3). Inset shows RFP expression map only.

Figure 1b. Images from a representative MDA-MB-231 HRE-RFP tumor showing coregistration of M1 map of tumor (marked by square) obtained with MRI (gray), RFP expression from Xenogen (blue), and SPECT data (yellow) showing the overlap of a hypoxic region of high fluorescence with high CD44 antibody localization. L: lungs

Inset shows ex vivo SPECT (left) and optical (right) images of a fresh 2 mm thick slice from the tumor.

Figure 1c. CD44, VEGF and ABCG2 mRNA expression in MDA-MB-231 cells under normoxic and hypoxic conditions. Hypoxia was achieved by treating cells with 100 micromolar of the hypoxia mimetic cobalt chloride for 24 h. The discovery of stem-like cell populations in several cancers, that are the most likely to be resistant to therapy and to lead to recurrence and metastasis, has generated tremendous excitement for understanding and treating tumor recurrence and metastasis.

Discussion: Our data suggest that hypoxia and elevated total choline may serve as surrogate markers for tumor regions likely to contain stem-like breast cancer cells, and that radiolabeled anti-CD44 antibodies can be used to image stem-like cells in vivo. These preliminary data also suggest that stem-like cancer cells may form a dynamic population that may transit between exhibiting stem- and non-stem-like phenotypic characteristics depending upon the tumor microenvironment.

Noninvasive imaging becomes particularly important in tracking the dynamics of this population especially for therapies designed to target stem-like cancer cells. Future clinical evaluation of the relationship between elevated total choline using MRSI and CD44+/CD24- or low receptor expression with PET or SPECT in breast cancer patients will further validate these observations.

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References: