Hypoxic Environments and the Extracellular Matrix: MRI and Second Harmonic Generation Microscopy Studies

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Introduction. To understand the relationship between hypoxic environments and macromolecular transport and drain in solid tumors, we characterized collagen fiber distribution in hypoxic and non-hypoxic tumor regions using second harmonic generation (SHG) microscopy of human breast or prostate cancer xenografts genetically engineered to fluoresce under hypoxia (1). Intrinsic SHG microscopy is proving to be valuable for understanding the remodeling of the collagen matrix in intact tissue. In this approach, the SHG signal primarily arises from fibrillar collagen (2). While quantitation of collagen structure is challenging, an advantage of this approach is that differences in fiber structure can be determined in 3D with micrometer resolution. It is possible that hindrance to the movement of macromolecules due to abnormal extracellular matrix (ECM) deposition in hypoxic areas may lead to poor drainage. On the other hand, hypoxia is known to upregulate expression of matrix metalloproteinases (3), making the ECM more porous under hypoxic conditions. Consequently, both functional and molecular characterization are necessary to provide insight into the dynamics governing interstitial fluid drainage, ECM remodeling, and the mechanisms of clearance of macromolecules in tumors.

Methods and Results.

MR: MRI characterization of the macromolecular contrast agent albumin-GdDTPA was performed on a Bruker Avance 4.7T spectrometer, as previously described (4) to quantify draining and pooling voxels in tumors. As shown in Figure 1, distinct pooling and draining regions were typically identified in tumors, demonstrating the heterogeneity in the transport of macromolecules through the ECM of tumors.

Studies characterizing collagen fiber distribution in hypoxic and normoxic regions were performed on fresh tissue slices obtained from human cancer xenografts (MDA-MB-231 breast cancer or PC-3 prostate cancer), genetically engineered to express enhanced green fluorescent protein expression (EGFP) under the control of the hypoxia response element of VEGF (1). The fluorescence from EGFP expressing hypoxic cells was imaged by two-photon excitation at 880 nm. Collagen fibers from colocalized regions in the same slices were imaged using SHG microscopy at 880 nm. Fiber distributions in hypoxic and normoxic regions were visualized and quantified using a specialized 3D analysis tool that was developed to identify the collagen fiber distribution based on its tubular geometry, and quantify collagen fiber volume in hypoxic and normoxic regions of the tumor. Images from each channel were analyzed independently to avoid channel crosstalk and improve image quality. In this approach, we computed the 3D Euclidian distance maps and used the histogram profile to characterize the distribution of the collagen fibers. In addition, fiber volumes were computed for hypoxic and normoxic regions of interest (ROIs).

Discussion. Our data provides evidence that the ECM is significantly altered in hypoxic tumor regions. The analyses revealed that the amount of collagen fiber in hypoxic ROIs was lower than that of non-hypoxic ROIs (Figure 3), and the fiber distribution was significantly different. These data suggest that the ECM underwent structural changes in hypoxic regions. Several matrix metalloproteinases (MMPs) that degrade the ECM are upregulated under hypoxia (3). The observation of fewer fiber voxels in hypoxic regions is consistent with this MMP upregulation under hypoxia, and with the possibility that hypoxic environments may facilitate the movement of macromolecules as well as dissemination of cancer cells in the metastatic cascade. To further quantify the effect of hypoxia on macromolecular transport, we are currently relating macromolecular transport characteristics to the distribution of hypoxia within the same tumor.


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