Combined Magnetic Resonance Spectroscopy and Mass Spectrometry Imaging of Breast Tumor Hypoxia

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Target audience: Physicians and researchers who are interested in hypoxia detection in breast tumors *in vivo* and *ex vivo*.

Introduction: The abnormal tumor vasculature causes regions of tumors to be transiently or chronically hypoxic [1-4]. Hypoxia is associated with resistance to therapy through multiple mechanisms such as cell cycle arrest, resistance to apoptosis, HIF-1 stabilization, and extracellular acidification, among others [5]. In addition, clinical and pre-clinical experiments point to a fundamental role of hypoxia in metastatic progression [6, 7]. Hence, it is necessary to be able to measure tumor hypoxia to assess tumor aggressiveness and predict therapy outcome.

Aims: The aim of this work is to identify and employ a hypoxia probe in human breast cancer models that can be imaged *in vivo* by magnetic resonance spectroscopy (MRS) and *ex vivo* by mass spectrometry imaging (MSI).

Methods: We used Hypoxyprobe F6 (Hypoxyprobe, Inc, Burlington, MA, USA) as a probe for detecting hypoxia. Hypoxyprobe F6, a hexafluorinated derivative of 2-nitroimidazole, forms stable covalent bonds with proteins, peptides, and amino acids under hypoxic conditions (Figure 1A). Human MDA-MB-231-HRE-tdTomato breast tumor cells, which were genetically engineered to express red fluorescent tdTomato protein under hypoxic conditions [8, 9], were orthotopically grown in athymic nude mice. Ketamine/xylazine anesthesia was used to avoid overlapping ^{19}F signals from isoflurane. We intravenously injected 1.5 mg of F6 per mouse dissolved in DMSO/saline (1/10, v/v). MR experiments were carried out using a home build MRS coil on a Bruker 9.4T horizontal bore spectrometer. Tripilot and 3D RARE images were collected for tumor positioning inside the coil. ^{19}F MRS was started at 30 minutes post F6 injection (NSA=1024, BF=376.667, RG=3250, 90^0 pulse, dB=12). Following ^{19}FMRS *in vivo*, mice were sacrificed, and tumors were excised and cryo-sectioned. Sections were mounted onto indium tin oxide (ITO) coated slides. Prior to MSI analysis, sections were dried and sinapinic acid matrix was applied (10 mg/ml 1:1 ACN:H_2O/0.1%TFA). Matrix-assisted laser desorption ionization (MALDI) MSI was performed on a MALDI-TOF/TOF (Ultraflex III, Bruker Daltronics) instrument in MS mode detecting positive ions. A separate cohort of mice was injected with the hypoxia marker pimonidazole, another 2-nitroimidazole derivative with binding properties similar to those of F6, and was tested by MALDI MSI as well. F6 and pimonidazole were also detected by immunohistochemistry (IHC) as previously described [10].

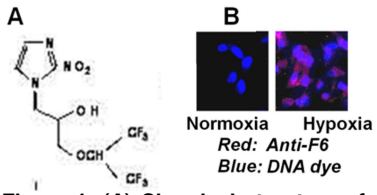

Figure 1: (A) Chemical structure of F6. (B) F6 probe is taken up by MDA-MB-231 cells under hypoxic conditions.

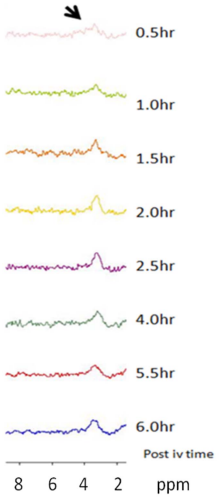

Figure 2: *In vivo* ^{19}F MRS spectra showing time course of F6 uptake in MDA-MB-231 tumors.

Results: F6 was taken up by wild-type MDA-MB-231 cells following exposure to 100 µm of F6 probe for 24 hours under hypoxic conditions of 1% O_2 in a hypoxia chamber (Figure 1B). *In vivo* ^{19}F MRS demonstrated that F6 was detected at 3.5 ppm as early as 30 minutes following injection, and peaked at 2.5 hours post-injection with a maximum signal-to-noise (SNR) ratio of 11.06 as shown in Figure 2. MALDI MSI of pimonidazole injected tumor sections showed that pimonidazole was detected at m/z 124.1, m/z 142.1, and m/z 223.2. The most prominent ion at m/z 223.2 was used to image the pimonidazole distribution as shown in Figure 3. Overlay with IHC detected pimonidazole from adjacent sections showed a good co-localization between MSI and IHC detected pimonidazole. An F6 fragment has also been detected by MALDI MSI in breast tumor sections at m/z 324.1, and is currently further being validated. Overlay with tdTomato fluorescent hypoxic regions generated by the use of the genetic model will also be performed.

Figure 3: *Ex vivo* detection of pimonidazole by MSI (left), IHC (middle) and overlay (right) of MSI and IHC showing good co-localization of pimonidazole in MSI and IHC.

Discussion: Our experiments indicate that hypoxyprobe F6 has the potential to be a multimodality imaging probe for MRS/MRSI, MSI, and IHC detection of hypoxia. Further experiments are underway to acquire ^{19}F MRSI and to register *in vivo* MRSI and *ex vivo* MS images with IHC staining of F6 probe in breast tumor xenografts. Combined MRS(I) and MSI detection of hypoxia will enable the discovery of novel *in vivo* and *ex vivo* markers of tumor hypoxia as well as signaling pathways activated under hypoxia.

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