Intrinsic Susceptibility MRI Investigation of Acquired Resistance to EGFR Therapy in a Xenograft Model of Squamous Cell Carcinoma of the Head and Neck.

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Introduction: Overexpression of the epidermal growth factor receptor (EGFR) has been identified as a negative prognostic factor in squamous cell carcinoma of the head and neck (SCCHN). Despite the development of EGFR-targeted tyrosine kinase inhibitors (TKI’s), patient response rates are variable, and in those that initially respond, acquired resistance frequently occurs [1]. Tumor hypoxia is known to adversely affect loco-regional tumor control and disease-free survival in patients with head and neck cancer [2]. Furthermore, colocalization of EGFR expression and tumor hypoxia in SCCHN were recently associated with poor outcome, suggesting a role for hypoxia in drug resistance [3]. Imaging strategies to identify and monitor patients with emerging resistance to EGFR-TKI therapy are urgently required. Quantification of tumor R2* and hyperoxia-induced ΔR2* using intrinsic susceptibility MRI are being evaluated and qualified as non-invasive imaging biomarkers of tumor hypoxia [4,5]. In this study, intrinsic susceptibility MRI was used to investigate functional tumor vasculature and oxygenation in vivo in human xenograft models of SCCHN with either acquired resistance or sensitivity to EGFR-TKIs. Histopathological correlates of tumor vascular perfusion and hypoxia were also sought.

Methods: Tumor model: Xenografts were established from human CAL27 SCCHN cell lines, with either acquired resistance or sensitivity to multiple EGFR TKIs (gefitinib, erlotinib and afatinib). Female NCr nude mice were injected subcutaneously with either 5x10^6 resistant (R) cells (n=8) or 5x10^6 sensitive (S) cells (n=7).

MRI: When tumor volumes reached approximately 250mm^3, the hypoxia marker pimonidazole (60mg/kg) was injected i.p. Mice were then imaged on a 7T Bruker horizontal bore Microimaging system, using a 3cm birdcage coil, with a nosepiece positioned for gas delivery. During air-breathing, multi gradient-echo (MGRE) images were acquired from three contiguous 1mm thick axial slices through the tumor, with T\_R=200ms, T\_E=6-28ms, 4ms echo spacing and 8 averages. After 45 minutes, to allow for full bioreduction of pimonidazole, 100% O\_2 was delivered @ 1l/min and a second set of MGRE images were acquired. Following MRI, the perfusion marker Hoescht 33342 (15mg/kg) was injected via a lateral tail vein. After 1 minute, tumors were excised and snap frozen over liquid nitrogen. Apparent R2* maps were calculated on a voxel-by-voxel basis [6]. Median R2* for each slice was determined from a region of interest drawn over the whole tumor. Tumor R2* was derived from subtracting oxygen-breathing R2* maps from air-breathing R2* maps.

Histology: Whole tumor sections, cut approximately in the same plane as for MRI, were processed for Hoescht 33342 uptake and pimonidazole adducts using fluorescence microscopy. Hypoxic fraction and vessel perfusion were quantified as a % of the whole tumor section area, with 2 to 3 sections analysed per tumor.

Results: Baseline R2* maps of resistant tumors revealed regions of relatively fast R2* primarily in the tumor periphery, while sensitive tumors exhibited a more heterogeneous distribution of R2* across the whole tumor (Fig 1A). There was no significant difference in mean baseline R2* between resistant (64 ± 4s^-1) and sensitive tumors (76 ± 5s^-1, p=0.07) (Fig 2A). Oxygen-breathing resulted in a reduction in R2* for both resistant and sensitive tumors, consistent with an overall increase in tumor oxygenation. A significantly greater ΔR2* was observed for sensitive tumors (-9.1 ± 2.03s^-1) compared to resistant tumors (-2.4 ± 1.4s^-1) (*p<0.05) (Figs 1B & 2B). Resistant tumors had significantly (**p<0.01) lower levels of Hoescht 33342 uptake which was restricted primarily to the tumor periphery (Fig 1C, blue), and significantly (**p<0.01) increased pimonidazole adduct formation (Fig 1C, green), compared to the sensitive tumors (Figs 2C & 2D).

Conclusions: EGFR TKI-resistant tumors demonstrated peripheral regions of fast R2* compared to a more heterogeneous distribution observed in size-matched sensitive tumors. Of significance, regions of relatively fast R2* were spatially related to Hoescht 33342 perfusion, whilst slower R2* corresponded to regions of hypoxia. There was no significant difference in baseline R2* between resistant and sensitive tumors, however hyperoxia-induced ΔR2* was significantly smaller with resistance. This attenuated hemodynamic response was associated with lower levels of Hoescht 33342 uptake and an increased hypoxic fraction. ΔR2* therefore informs on both hemodynamic/functional response and tumor oxygenation. Overall, these findings suggest that resistance to EGFR-TKIs in the CAL27 model could result from a poorly perfused/hypoxic phenotype. Intrinsic susceptibility MRI revealed spatial and functional differences in tumor vasculature and oxygenation, providing a useful non-invasive imaging strategy for the investigation of EGFR-TKI drug resistance and tumor hypoxia.

References:

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