Radiation induced hypoxia in TRAMP tumor detected using BOLD MRI

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Target audience: Researchers who are interested in exploring treatment response of tumor using MRI

Purpose: To monitor the changes of microvasculature and hypoxia after radiotherapy in transgenic adenocarcinoma of the mouse prostate (TRAMP)-C1tumors using gas challenge blood oxygen level dependent (BOLD) MRI.

Methods: Transgenic adenocarcinoma of the mouse prostate (TRAMP)-C1 tumors were grown in C57BL/6J mice (n=6) by i.m. inoculation of 3×10^6 viable cells into the thigh. Irradiation was performed on the peripheral half of the tumor using a 6 MV Novalis system with a 2-cm stereotactic radiosurgery cone [1]. A single dose of 15Gy was delivered on the 10th day after tumor implantation. Six days after irradiation, MR images were acquired using a 7 Tesla MR scanner (ClinScan, Bruker). BOLD MRI was performed using carbogen challenging (95% O_2 , 5% CO_2) with a block design experiment, which consisted of two stimuli: 5 minutes of room air inhalation and 5 minutes of carbogen challenge, with two cycles of this regimen. A series of quantitative R2* measurements were obtained using a multiple gradient-echo sequence with 12 echo times ranging from 2.4 to 36 ms and temporal resolution of 60 seconds. A dynamic time series of images was obtained for 20 minutes. The BOLD response to carbogen was quantified using $\Delta R2^* = (R2^*_{air} - R2^*_{carbogen}) / R2^*_{air}$, where $R2^*_{carbogen}$ is the R2* measurement acquired during the carbogen inhalation and $R2^*_{air}$ is that during room air breathing. The ΔR2*s were compared between the tumor regions with and without irradiation. Animals were sacrificed and the tumor tissue were obtained immediately after MRI acquisition. For detecting hypoxia, pimonidazole hydrochloride was administered in 0.1 mL, which was detected with mouse antibody and goat anti-mouse IgGg1 Alexa 488. For endothelial cells, rat anti-CD31 antibody was used, followed by goat anti-rat Alexa 594. The hypoxia fraction (HF) was defined as the area positive for pimonidazole divided by the total tumor area. Microvascular density (MVD) was determined as the number of pixels positive for CD31 divided by the total tumor area. The necrosis areas were excluded in the calculation of HF and MVD. The Wilcoxon signed-rank test was used to assess the difference in each metric between the non-irradiated and irradiated regions of the tumor

Results: Figure 1 shows the comparison of ΔR2* in the irradiated site and non-irradiated sites. A significantly decreased ΔR2* was observed in the irradiated site compared with that in the non-irradiated site (6.7±1.17% and 22.39±2.82% respectively, P<0.01). The histology shows a significantly reduced MVD and HF in the irradiated region of the tumor (5.71±1.6% and 13.5±3.1, respectively) compared with that in the non-irradiated region (12.5±3.1% and 21.3±1.5%) of the tumor (P<0.01) (Fig 2a). By overlaying the immunohistochemistry images of CD31 and pimonidazole, it is noticed that the vasculature in the hypoxic areas in the irradiated site disappeared (Fig 2b).

Discussion: Partial irradiation results in significantly differential responses within a single tumor, which can be detected by BOLD MRI with carbogen challenge. The decreased ΔR2* in the irradiated site was consistent with the decreased HF and MVD, suggesting that the microvascular functionality damaged in response to radiotherapy. The hypoxic area with absent microvasculature in the irradiated region suggests that radiotherapy eliminates the microvasculature associated with acutely hypoxic regions, making such regions chronically hypoxic [2]. We speculate that because breathing carbogen during a short period could change the oxygen status more prominently in the regions with acute hypoxia, the resulting BOLD response in the non-irradiated site (acute hypoxia) is more notable than that in the irradiated site.

Conclusion: Carbogen-challenging BOLD MRI can be used to detect the hypoxic change within a tumor in response to radiotherapy, which appears a shift of acute hypoxia in the non-irradiated region to chronic hypoxia in the irradiated region

Reference: [1] Lin YC et al. Int J Radiat Oncol Biol Phys 2013;85:1367-74. [2] Chen FH et al. Clin Cancer Res. 2009;15(5):1721-9.

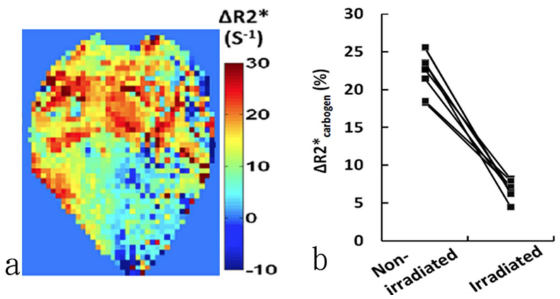

Fig.1 (a) ΔR2* map of a mouse following partial irradiation in peripheral half of the tumor. (b) Comparison of ΔR2* at non-irradiated and irradiated regions of the tumor from six mice.

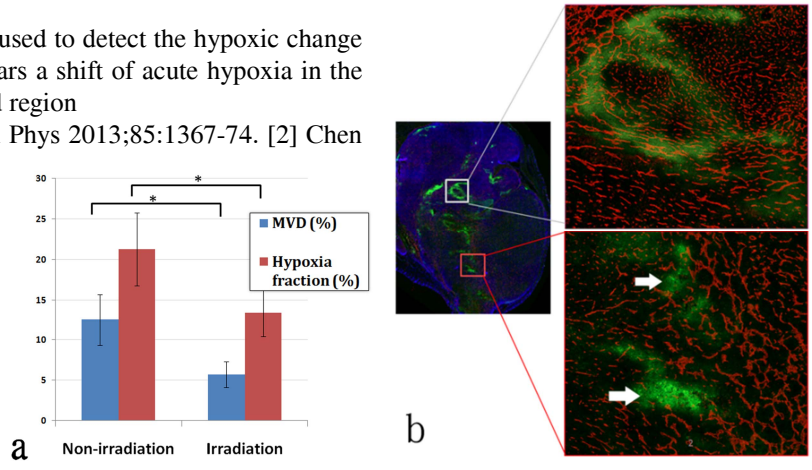

Fig.2 (a) Comparisons of MVD and hypoxia fraction at non-irradiated and irradiated regions of the tumor. (b) Overlaid immunohistochemistry images of CD31 and pimonidazole in a tumor. White box: non-irradiated; Red box: irradiated region. The white arrow indicates the hypoxic region where microvasculature disappeared.