Single-dose X-Ray Irradiation Changes Significantly Tumor Perfusion, as Measured by *In Vivo* DCE ^{1}H MRI

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Introduction: Radioresistant tumors are often characterized by hypoxia and have been associated with reduced patient survival [1]. Applying a priming dose of radiation to radioresistant tumors may be associated with reoxygenation of previously hypoxic areas and increased tumor radiosensitivity [2]. Single-fraction radiotherapy has been shown to control murine tumor xenografts via a mechanism dependent on endothelial cell apoptosis [3]. Thus, we assess here in the radioresistent mouse mammary tumor MCa the effects of single high-dose irradiation on tumor perfusion as a function of time after irradiation.

Materials and Methods: The preclinical tumor model was the mouse mammary cancer MCa implanted in the right hind foot of C3H/He mice which we have studied previously. Tumor blood flow and perfusion was evaluated by DCE MRI before and after a single dose of 15 Gy irradiation. The MR experiments were performed using a home-built solenidal MR coil on a Bruker 7T BioSpin (Bruker, Germany) MR spectrometer. Spin-density MR images (FLASH, TR=500 ms, TE=3.1 ms, NR=1, NA=1, st=1 mm, FOV=1.1 to 1.5 cm x 1.1 to 1.5 cm (depending on tumor size), 128x128, slices=16; flip angle=45^o) were acquired to facilitate subsequent slice alignment of the post-radiation DCE MRI experiment with the pre-irradiation MRI. T_1-weighted DCE MRI (FLASH, TR=33 ms, TE=3.1 ms, NR=312, NA=1, st=1 mm, slices =4, FOV=1.1 to 1.5 cm x 1.1 to 1.5 cm, 128*128, flip angle: 45^o) was performed at 5.47s temporal resolution. The contrast agent Gd-DTPA was injected via tail vein after 2 min of baseline acquisition followed by 20 min of dynamic acquisition. After the pre-irraditition DCE MRI, the animal recovered for at least 3 hours to ensure complete clearance of the injected Gd-DPTA from the tumor before the post irradiation DCE MRI. The catheter was kept patent by injection of heparinized saline. To monitor the perfusion change as a function of post irradiation time, respective delays of 30 min, 100 min, 6 hrs and 24 hrs were used before the commencement of a second DCE MRI experiment. Each animal was used only for a single time point. After the post irradiation DCE-MRI scan, the tumor was excised, fixed in formalin and paraffin embedded. Tumor tissue sections were stained for apoptosis using TUNEL; the vasculature was marked with the pan-endothelial marker MECA32. The time-signal curves, obtained by DCE MRI, were normalized with respect to the baseline signal (initial 2 min of acquisition without contrast agent), fitted voxel-by-voxel using the Hoffman model [4] and Akep maps were generated for the corresponding tumor slices before and after irradiation. The Akep value is analogous to the slope of the MR signal enhancement at the initial time and is considered an approximate measure of vascular flow/perfusion of the tumor tissue. Akep value consists of an amplitude (A), which reflects the degree of relative MR signal enhancement and an exchange rate (kep), which characterizes the velocity of MR signal increase. To quantify the prefusion changes due to the irradiation, histogram analysis was performed for Akep values before and after irradiation. For pre and post irradiation separately, Akep values of all MR slices (four) for each animal were combined into one histogram and the median Akep value of each tumor calculated.

Results: Despite the necessary repositioning of the animal for the post irradiation DCE MRI, the MRI slices before and after irradiation could be aligned reproducibly within 0.5mm with only a small angular rotation (Fig. 1). For each tumor, pre irradiation Akep values (Fig. 2A-1, 2) were significantly higher than Akep values 30-min and 100-min post-irradiation (Fig. 2B-1, -2). Histogram analysis of Akep values for 30 min and 100 min post-irradiation showed a distinctive shift to lower values compared to pre-irradiation Akep values, consistent with the Akep maps (data not shown). The median Akep values for each tumor pre-irradiation dropped significantly at 30-min (Fig. 2C-1) and 100-min (Fig. 2C-2) post-irradiation. In tumors measured 6 hrs after irradiation, Akep values pre and post-irradiation (Fig. 2A-3, B-3) did not change significantly and no significant changes in median Akep values were observed (Fig 2C-3). Akep values, 24 hrs after irradiation increased slightly from pre-irradiation values (Fig. 2A-4), corresponding to a significant shift of Akep values to higher values in the histogram analysis (data not shown) and to increased median values of Akep distribution after 24h post irradiation (Fig. 2C-4). Figure 3 shows the change of Akep median values, normalized with respect to pre-irradiation Akep values, as a function of time elapsed after X-ray irradiation. Significant drops in median normalized Akep values from the pre-irradiation value (normalized Akep = 1) were observed at 30 min and 100 min after irradiation, whereas the median values were recovered, or even exceeded normalized Akep values at 6 hrs and 24 hrs after irradiation. Microvascular damage was evident on histology at 24 hrs post-irradiation, characterized disruption of endothelial staining and scattered TUNEL positive endothelial nuclei (Fig. 4). **Discussion:** Single high-dose X-ray irradiation reduces tumor perfusion in the short term whereas after 6 hrs tumor perfusion appears to increase which may be indicative of reoxygenation. Our results are in agreement with the increase of median tumor pO_2 found 24 hrs after single-dose irradiation of MCa tumors with 32 Gy [2]. DCE MRI may be a valuable tool to evaluate the window of increased radiation sensitivity after priming radioresistant tumors with a single high dose of radiation.

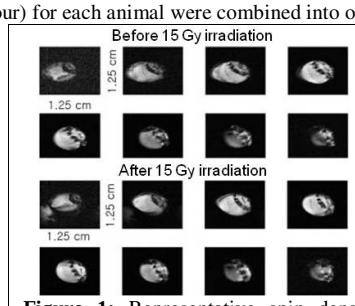

Figure 1: Representative spin density MR images (8 slices each) of a MCa foot tumor before and after 15 Gy single-dose X-ray irradiation. Realignment of tumors was achieved reprocudibly between pre and post irradiation DCE MRI.

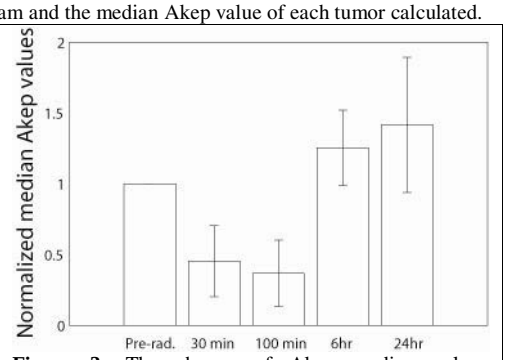

Figure 3: The change of Akep median values (mean±SD), normalized with respect to pre-radiation values, as a function of time elapsed after X-ray irradiation.

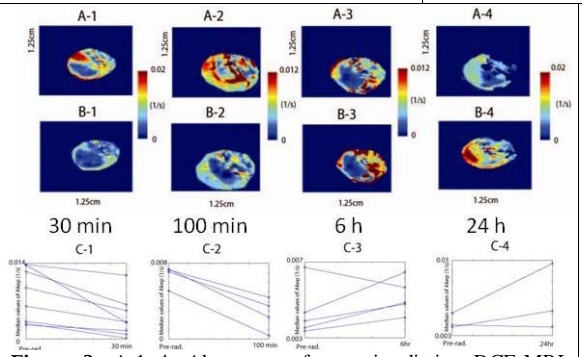

Figure 2: A-1~4: Akep maps for pre-irradiation DCE-MRI experiment. B-1~4: Corresponding Akep maps for irradiated tumors after 30 min, 100 min, 6 hr and 24 hr after irradiation, respectively. C-1~4: The change of median values of Akep distribution before and after irradiation.

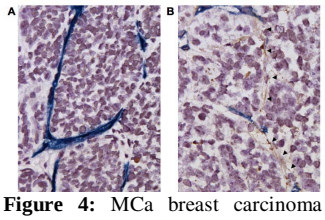

Figure 4: MCa breast carcinoma stained with TUNEL (brown) and MECA32 (blue). A. Intact microvasculature in un-irradiated specimen. B. Damaged vessels in specimen 24 hrs after 15 Gy; arrows show course of disrupted vessel.

References: [1] P. Vaupel (2004) *Semin Radiat Oncol* **14**:198-206, [2] J.A. Koutcher *et al.* (1992) *Cancer Res* **65**, 4620-4627. [3] M. Garcia-Barros *et al.* (2003) Science **300**, 1155-1159 [4] U. Hofmann *et al.* (1995) *Magn Reson in Med* **33**, 506-514. **Acknowledgements:** Supported by NIH grants PO1 CA115675, R24 CA83084 and NCI P30 CA0874 (Cancer Center Support Grant). We like to thank Dr. Richard Kolesnik and Dr. Zvi Fuks for their helpful discussion.