INTRODUCTION
Glioblastoma (GBM), the most common and aggressive type of primary brain tumors, is also one of the most highly vascularized cancers (1). As a result, antiangiogenic treatment with the anti-VEGF monoclonal antibody Bevacizumab (Avastin; Genentech, CA) is now commonly used in the clinic in conjunction with surgical resection, radio- and chemotherapy. However, many GBM tumors develop resistance to Bevacizumab treatment through mechanisms not fully understood. In an effort to identify indicators of tumor resistance to antiangiogenic treatment, we used macromolecular dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) (2) at high field (14.1 T) to quantitatively measure the effect of treatment with B20 (a murine Bevacizumab analogue) in two GBM models: one responsive (U87) and one resistant (SF7796, obtained from a patient who developed resistance to Bevacizumab).

MATERIAL & METHODS

Tumor-bearing animals A total of 18 6-weeks-old athymic mice (Nu/Nu homozygous, Simonsen Laboratories, Gilroy, CA) were included in the study. For tumor implantation, animals were anesthetized using ketamine/xylazine (100/20mg.kg\(^{-1}\)) (respectively) and a suspension of U87 (n=10) or SF7796 (n=8) cells (~3x10\(^{5}\) in 2.5µl) was injected into the right caudate-putamen of mice brain (3).

B20 treatment Treated animals (n=5 for U87 group, n=4 for SF7796 group) received an ip injection of B20 (4) twice weekly (5 mg/kg, B20-4.1.1, Genentech, CA) for approx 2 weeks while controls (n=5 for U87, n=4 for SF7796) received the same dose of anti-ragweed antibody (isotype control).

MR system Experiments were performed on a 600 MHz wide bore vertical system (0.5~55 mm) equipped with 100 G. cm\(^{-1}\) imaging gradients (Varian Inc, Palo Alto, CA). Shimming and MR imaging were performed using a Varian millipede 3T coil (0=40mm, 5cm length).

In vivo MR acquisitions Mice were anesthetized using isoflurane (3% in O\(_2\), 1.5 L min\(^{-1}\)) and a 27G catheter was secured in the tail vein of the animal. Animals were positioned in the magnet using a custom built cradle. MR sessions were performed approx two weeks post B20 treatment initiation. Anatomical imaging was first performed to assess the location of the tumor (coronal SE, TE=20ms, TR=2000ms, FOV=32x32x2mm, matrix 256x256, slice thickness=0.5mm, gap=0.5mm, Tauc=8mm/32x, NT=2). Then six 3D gradient echo (GE) pre-contrast images with variable flip angles \((\text{FA}=2/5/10/30/45/70\text{deg})\) were acquired successively to determine the R1 maps (TE/TR=1.75/10ms, \(\text{FA}=45\text{deg}\) ) was then injected iv through the tail vein catheter and 3D GE post-contrast images (same parameters, \(\text{FA}=45\text{deg}\) ) were acquired every 3 min for 45 min.

Post-processing All 3D data sets were zero-filled to 256x256x64. Pixel-by-pixel analysis was performed using MATLAB software (MathWorks, Inglewood, CA, United States) to generate blood volume fraction (BV) maps from macro-molecular dynamic contrast images with variable flip angles (\(\text{FA}=2/5/10/35/45/70\text{deg}\)) was injected into the right caudate-putamen of mice brain for anesthetized using ketamine/xylazine (100/20mg.kg\(^{-1}\)). Shimming and MR imaging were performed using a Varian millipede 3T coil (0=40mm, 5cm length).

RESULTS & DISCUSSION

Figure 1 presents 3D GE pre and post contrast coronal slices, and PS and BV maps obtained from one animal from each group. Whereas no significant differences were observed prior to treatment, B20 treatment induced a significant decrease in the mean values of PS \((\text{PS}^\text{Control} = 4.5x10^{-3}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) vs PS \((\text{PS}^\text{Control} = 4.5x10^{-3}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) and BV \((\text{BV}^\text{Control} = 4.7x10^{-3}\text{mm}^3\text{s}^{-1}\text{min}^{-1})\) vs BV \((\text{BV}^\text{Control} = 4.7x10^{-3}\text{mm}^3\text{s}^{-1}\text{min}^{-1})\) for U87 animals, as illustrated in Figure 1 (upper rows) and a SF7796 (bottom rows) tumor bearing mouse. The tumor locations are circled in dashed lines.

In contrast, no significant changes were found between control \((\text{PS}^\text{Control} = 2.8x10^{-4}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) vs PS \((\text{PS}^\text{Control} = 2.8x10^{-4}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) and BV \((\text{BV}^\text{Control} = 2.5x10^{-4}\text{mm}^3\text{s}^{-1}\text{min}^{-1})\) for SF7796 animals, as illustrated in the bottom two rows of Figure 1 (PS \((\text{PS}^\text{Control} = 2.8x10^{-4}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) vs PS \((\text{PS}^\text{Control} = 2.8x10^{-4}\text{mm}^2\text{s}^{-1}\text{min}^{-1})\) and BV \((\text{BV}^\text{Control} = 2.5x10^{-4}\text{mm}^3\text{s}^{-1}\text{min}^{-1})\) ). Histograms of tumor PS and BV were then averaged for animals within each of the 4 groups (U87/SF7796 control/treated). Mean±SD values of PS and BV were also calculated for the 4 groups.

REFERENCES

Figure 2 – Averaged histograms of PS (A, B) and BV (C,D) tumor values for U87 (A,C) and SF7796 (B,D) controls (black) and treated (red PS, blue BV) animals.