Correlation of Tumor Growth Kinetics, Cell Kill, and Radiotherapeutic Response in a RIF-1 Tumor Model Using Multispectral Analysis

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Introduction

Assessment of therapeutic efficacy is confounded by intra-tumor and inter-tumor heterogeneity. Variable pre-treatment tissue composition, tumor size, and growth kinetics, as well as post-treatment cell kill and tumor regrowth complicate dose-optimization and comparative treatment regimens in animal oncology studies. A multi-spectral (MS) analysis approach using ADC, T_2, and M_0 maps has been shown to aid in the differentiation between viable and necrotic tissue, as well as the identification of multiple compartments within necrotic tissue.[1] Here, we report on a single-dose radiotherapy study using RIF-1 tumors in which MS analysis, using k-means (KM) clustering, was used to identify multiple compartments in both viable and necrotic tissue. This methodology combined with the contributions of cell kill and tumor growth kinetics should provide a better understanding of the physiological dynamics of treated tumors.

Methods

Seven 6-8 week-old female C3H mice weighing 20-25g were anesthetized with an intraperitoneal injection of ketamine/zylazine (100mg/kg:10mg/kg). All mice were inoculated with 1×10^6 RIF-1 cells (0.15ml), delivered through a subcutaneous injection into the right hind leg. Tumors were allowed to develop for 3-4wks, yielding an approximate 1.0cc starting volume.

Data were acquired with a Bruker Biospin 2.0T/45cm imaging spectrometer operating at 85.56MHz for ^{1}H and equipped with ±20G/cm self-shielded gradients. Image acquisition was performed along the coronal plane [128×128, FOV=3cm, slices=8, slice thickness=1mm]. A DW-SE sequence was used to acquire the images at six b-values (15 → 760 s mm^{-2}) with TR/TE=2000/53ms, δ=4ms, Δ=35ms, resulting in an effective diffusion time t_{dif}=33.7ms. A T_2W-SE sequence was used to acquire images at six echo times (12.2 → 90 ms) with TR=2000ms. Tumors were irradiated with 1000cGy at a rate of 300cGy/min (Siemens Mevatron 77, 6 MeV electrons, Tufts University Veterinary School of Medicine). Imaging was performed 1d pre-treatment, 5hr, 1d, 2d post-treatment, and every 2d thereafter until tumor doubling (maximum 10d post-treatment).

ADC, T_2, and M_0 parameter-maps were generated using routines written in IDL® (RSI, Boulder, CO). Tissue classification was performed using the k-means (KM) clustering algorithm. KM was applied to segment data into two regions each of viable tumor (V1,V2) and necrosis (N1,N2), and one region of adipose tissue. Growth kinetic calculations and cell kill were determined using an exponential model of tumor growth[2] and the mathematical model proposed by Ross *et al*,[3] respectively.

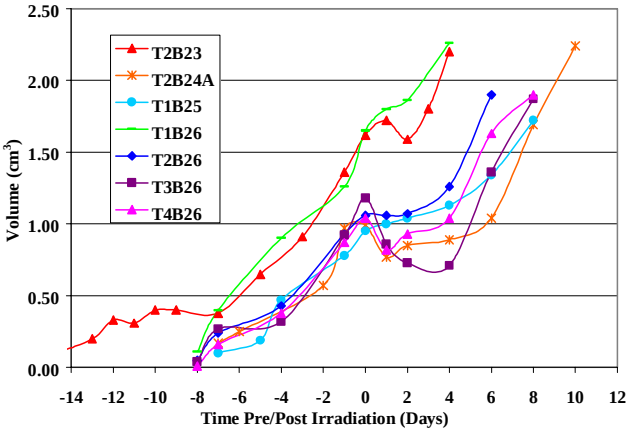

Fig.2: Plot of tumor growth on an animal-by-animal basis pre- and post-irradiation. Individual volumes were calculated from caliper measurements using the equation $V = (\pi/6)*(l \times w \times h)$ where l = length, w = width, and h = height.

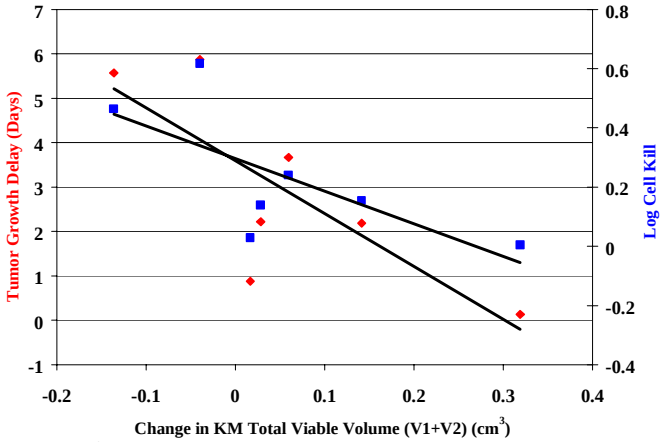

Fig.3: Plot of tumor growth delay and cell kill versus a change in KM viable volume. Volume changes were calculated based on the difference between pre-treatment V1+V2 and the minimum V1+V2 volume post-treatment on an animal-by-animal basis.

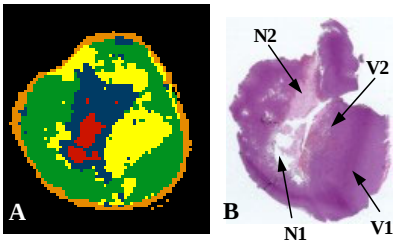

Fig.1: A multispectral (MS) image of a RIF-1 tumor. (A) KM map. (B) Hematoxylin-Eosin (H&E) Image. The map derived by k-means (A) depicts the segmentation of the tumor into two regions of viable tumor and two regions of necrosis. Tissue assignments are: Viable 1 (V1) = Green; Viable 2 (V2) = Yellow, Necrosis 1 = Red (N1); Necrosis 2 (N2) = Blue, Adipose Tissue = Orange.

Results

This method allows identification of tissue heterogeneity within viable tumor and necrosis. Fig.1 shows the cluster assignments for a representative RIF-1 tumor (Fig.1A) and the corresponding H&E image (Fig.1B) at 6d post-treatment. Fig.2 shows the tumor growth curves on an animal-by-animal basis pre- and post-irradiation. Fig.3 shows the correlation between the tumor growth delay (TGD), cell kill, and the change in KM total viable volume (V1+V2) pre-irradiation versus the minimum volume post-irradiation (1d or 2d post depending on the animal). There was a strong correlation between the change in V1+V2 and the resultant TGD (R = 0.78) and cell kill (R = 0.70). The relative contributions of pre-treatment V1 and V2 volumes versus post-treatment kinetic parameters were investigated. An increase in pre-treatment V1 correlated with a decrease in both TGD (R = 0.82) and cell kill (R = 0.83). There was no correlation between pre-treatment V2 volume and TGD or cell kill.

Discussion

These results suggest that V1 is well-oxygenated, radiosensitive tissue, while V2 is hypoxic, and therefore, radioresistant. The assignment of V1 and V2 will be validated using immunohistochemical (IHC) staining for hypoxic-inducible factor 1-alpha (HIF-1α). Since tumor oxygenation is a major factor for effective treatment and can be spatially heterogeneous across the tumor, MS methods should be helpful in monitoring the range of tissue viability as a function of time post-treatment.

References

[1] Carano RAD et al. Magn Reson Med 2004;51:542-551.

[2] Steele GG. Growth Kinetics of Tumors. Clarendon: Oxford; 1977.

[3] Ross BD et al. Proc Natl Acad Sci 1998;95:7012-7017.