A NOVEL MR-VISIBLE PERSISTENT APOPTOSIS MARKER VALIDATED WITH IMMUNOHISTOCHEMISTRY

Firas Moosvi[1], Jennifer H.E. Baker[1,2], Andrew Yung[1], Kenneth Curry[3], and Stefan A Reinsberg[1]

[1]Physics and Astronomy, University of British Columbia, Vancouver, BC, Canada, [2]Radiation Biology, BC Cancer Research Institute, Vancouver, BC, Canada, [3]RF Therapeutics, Vancouver, BC, Canada

Introduction

Target Audience: This work is intended for pre-clinical cancer researchers interested in a persistent MR-visible marker of necrosis.
Purpose: A new compound has been developed that binds only to necrotic cells after 24 hours of incubation in the systemic circulation (RF1106, RF Therapeutics Inc; Vancouver, Canada). RF1106 is MR visible through the T_1-shortening action of Gadolinium bound to the compound. This pilot study aims to assess the feasibility and utility of RF1106 in a preclinical tumour study in mice with validation using DCE-MRI and immunohistochemistry.

Methods

Animals: Eight (8) NOD/SCID mice were implanted with a human colorectal carcinoma (HT29) on the left flank and tumours were allowed to grow until they reached 300mm^3. Prior to the tumour cell implant, animals were implanted with a fiducial marker tube filled with wax and saline to facilitate the selection of corresponding MR and histology slices [1]. RF1106 was administered intravenously at a dose of 40 mg/kg, 24 hours prior to imaging.
MRI: Imaging was performed using a 7T scanner (Bruker Biospec 70/30, Germany) with an 86cm coil for transmit and a custom built surface receive coil. A high contrast anatomic image at high spatial resolution (0.2x0.2x0.1 mm) was acquired using a 2D RARE inversion recovery (RARE-IR) sequence (TR/TE = 5000/7.5 ms, TI = 850 ms, acceleration factor 4). Then a 0.2 mL bolus of Gd-DTPA-BMA (Omniscan, GE Healthcare; Milwaukee, WI) diluted to 0.05 mM/mL was injected manually and DCE-MRI data was acquired at a temporal resolution of 2.2s using a 2D spoiled gradient echo sequence (TR/TE= 35/2.75 ms, flip angle = 40, spatial resolution of 0.3x0.3x1.5 mm). Another RARE-IR image was acquired with the same parameters following Gd injetion for comparison.
Histology: Animals were euthanized following the second imaging session and tumours were immediately excised and frozen. Sequential sections 10μm thick were obtained every 0.5 mm and histology slices corresponding to MRI slices were identified [1]. Sections were stained with TUNEL to mark apoptosis and CD31 to mark blood vessels, and imaged using a robotic microscope and camera to obtain tiled images of whole tumour sections.

Results and Discussion

Results from a representative tumour are shown below (Fig.1) and RARE-IR images for RF1106 (Fig.1A) show a strong correlation with apoptotic regions stained using TUNEL in the immunohistochemical images (Fig.1D). Furthermore, following administration of Gd-DTPA-BMA, the rim of the tumour was highly enhancing relative to the central necrotic/apoptotic region (Fig.1B), indicating that RF1106 does indeed bind to necrotic cells in the tumour (Fig.1C). Voxel-wise time course (VTC) plots (Fig.1 H-J) of the DCE-MRI enhancement curve also show that heterogeneity in vascular distribution correlates remarkably well with immunohistochemical vessel staining (Fig.1 E-G) as well as intensities (higher intensities indicate shortened T_1s) in the RF1106 RARE-IR image (Fig.1A). Interestingly, some regions were not marked with RF1106 and also did not enhance after injection of Gd-DTPA (e.g., white arrow in Fig.1C). We think these areas are poorly perfused and cells are quiescent - neither apoptotic nor fully metabolizing. This leads to cells that are not yet necrotic, and thus may not have bound any RF1106. Biochemical analysis and validation of RF1106 (including binding specificity and mechanism of action) is currently underway.

Conclusions

The goal of this pilot study was to assess the feasibility of RF1106 as a marker of necrosis for use in pre-clinical models of cancer. We have shown that unbound RF1106 is cleared from the systemic circulation and, after 24h, only RF1106 bound to necrotic tissue remains (identified by regions of shortened T1 in Fig.1A). These regions were confirmed to be necrotic using immunohistochemical staining (TUNEL, red, Fig1D). RF1106 persists in the tumour microenvironment, bound to necrotic tissue and this characteristic can be exploited to screen animals for viable tissue without the need to assess perfusion by dynamic contrast enhanced MRI. Furthermore, RF1106 ha s the potential to provide information about the tumour microenvironment, differentiating cells that are at different stages on their path to necrosis (compare fully necrotic tumour core vs area marked by white arrow in Fig.1C). To further enhance the utility of RF1106, studies investigating the mechanisms of RF1106 and protocol optimization are underway.

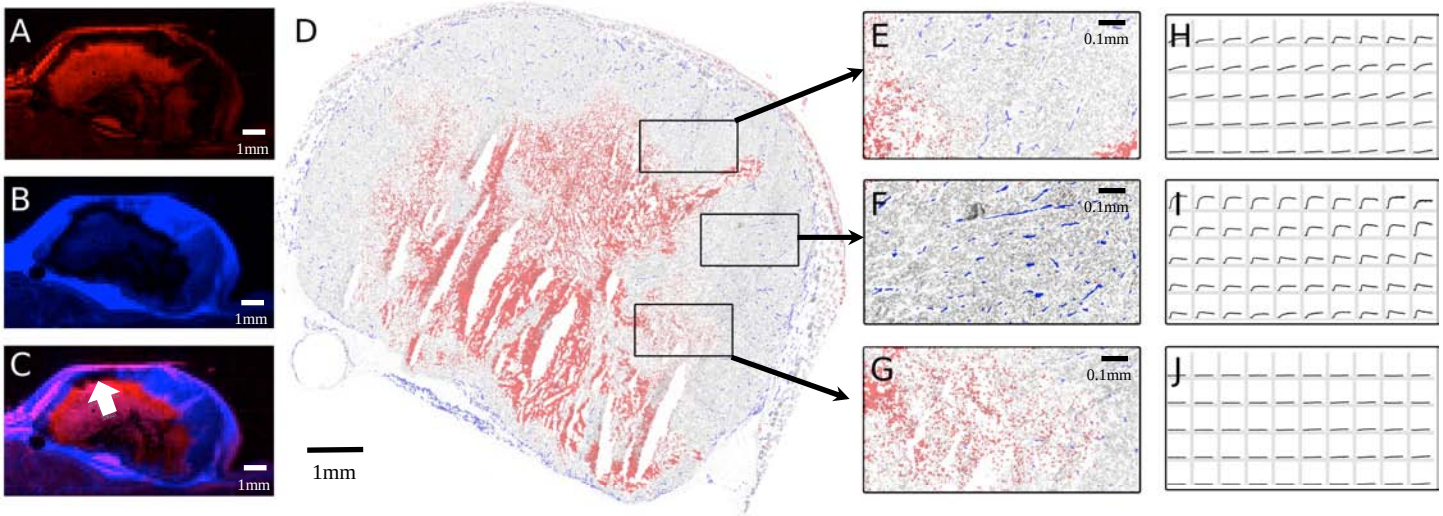

Fig 1. RARE-IR images are shown following 24 hours of RF1106 incubation (A), RF1106+Gd (B), and the overlay (C). The arrow marked in (C) indicates a region that is not enhanced by either RF1106 or Gd. A tissue section corresponding to the same MR slice is shown (D) with apoptosis staining marked in red, blood vessels marked in blue and nuclei marked in black. Three heterogeneous regions of vascular distribution - intermediate (E), vascular (F), and avascular (G) - are shown in the insets and VTC plots are shown for each region (H - intermediate, I - vascular, and J - avascular). Cells marked as apoptotic in the immunohistochemical slice (red) correlate well with regions of low enhancement using Gd (J) as well as high intensities in the RF1106 RARE-IR image (A).

References

[1] Bains, L.J., Baker, J.H.E. et al (2009). *International journal of radiation oncology, biology, physics*, *74*(3), 957–965.