



Molecular imaging of fibrin in tumors

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Introduction

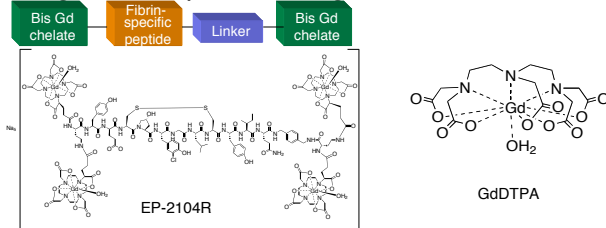
In 1865, Trousseau observed that thromboembolic complications were a common occurrence in cancer patients and suggested that there must be a correlation between the hemostatic system and cancer biology.¹ Since then, numerous studies have proved wounding to be an essential requirement for tumorigenesis.² Several clinical studies have shown that the expression of procoagulants and fibrinolytic factors by tumor cells and/or stromal cells correlates with advanced disease and poor outcome for cancer.³

Cellularly, both a tumor and a wound are characterized by the presence of a fibrin clot, inflammatory cells, newly formed blood vessels, and a large number of fibroblasts and myofibroblasts.² The fact that fibrin-specific MR contrast agents have shown success in identifying fibrin clots *in vivo* presents a possibility that they would be successful in identifying and characterizing primary and metastatic tumors.

The aim of this work is to compare EP-2104R and GdDTPA using steady-state and dynamic contrast enhanced imaging in a breast cancer xenograft mouse model.

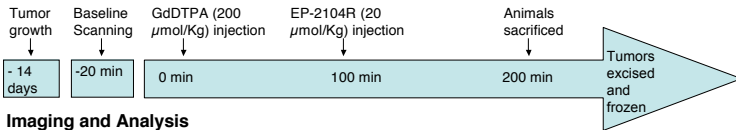
Methods

Contrast agents - Fibrin-specific contrast agent EP-2104R and GdDTPA



Animal model

- All animal studies were approved by the subcommittee for research and animal care at MGH.
- 5-6 week old nude mice (N=10) were used.
- Tumors were grown by subcutaneous injection of 3×10^6 human breast adenocarcinoma cells (BT-20) in 50 μ L of HBSS in the right flank of the mice.
- The tumors were allowed to grow for two weeks before imaging.



Imaging and Analysis

- Imaging was performed at 9.4 T.
- The imaging sequence included multislice FLASH at low resolution ($297 \times 297 \mu\text{m}^2$, $\text{acq} = 13 \text{ s}$) and higher resolution ($148 \times 148 \mu\text{m}^2$, averages = 16, $\text{acq} = 7 \text{ min}$) and inversion recovery T1 maps.
- Sections were cut from the frozen tumors and were stained with a fibrin-specific antibody (Mouse anti-human fibrin II β -chain antibody (clone NYBT2G1)).
- Images were analyzed using ImageJ by drawing ROIs in the tumor core, tumor periphery and muscle and signal intensity (SI) quantified for the same slice. Noise was quantified as the standard deviation (SD) of the signal measured in the air. Contrast to noise ratios (CNR) were calculated for the difference between tissue A and tissue B using the following equation.

$$\text{CNR}_{(\text{tissue A}/\text{tissue B})} = \frac{|S_{(\text{tissue A})} - S_{(\text{tissue B})}|}{\text{SD}_{(\text{air})}}$$

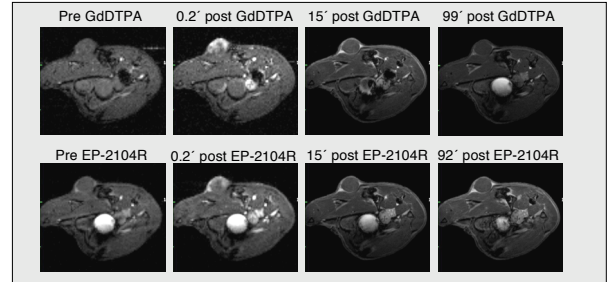


Figure 2: Serial FLASH images pre and post injection of GdDTPA (top) and EP-2104R (bottom) show transient tumor core enhancement following contrast administration. The tumor periphery remains enhanced at least 90 min after EP-2104R injection.

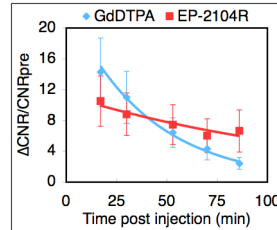


Figure 3: $[(\text{CNR}_{\text{post}} - \text{CNR}_{\text{pre}}) / \text{CNR}_{\text{pre}}]$ vs. time post injection of GdDTPA and EP-2104R for the tumor periphery. The clearance rate for EP-2104R was significantly slower than GdDTPA from the periphery.

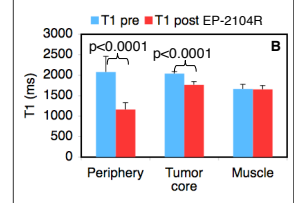
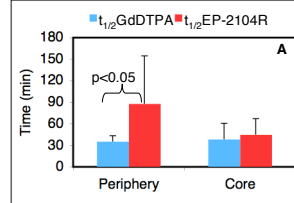


Figure 4: (A) Clearance half-life of GdDTPA and EP-2104R from the tumor periphery and tumor core. EP-2104R clears ~3 times slower as compared to GdDTPA from the tumor periphery. (B) T1 values measured 40 min post EP-2104R were lowest in the tumor periphery.

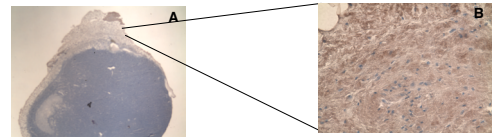
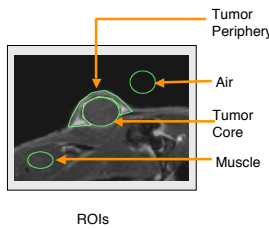


Figure 5: Immunohistochemistry of the tumor tissue (A) 20X magnification; (B) 400X magnification. Fibrin-positive areas stain brown and the tumor periphery appears to be rich in fibrin.

Conclusions

- The kinetics of contrast enhancement of tumor core are similar between GdDTPA and EP-2104R and EP-2104R may provide information on tumor permeability similar to that of GdDTPA.
- The persistent enhancement observed in the tumor periphery with EP-2104R may be a general steady state marker for solid tumors.
- The presence of fibrin may correlate with tumor aggressiveness and may guide response to therapy.
- Tumor periphery shows the greatest CNR increase and prolonged T1 reduction and this correlates with rich staining of fibrin in the periphery.

- Trousseau A. Phlegmasia alba dolens. In: Bailliere JB, editor, Clinique Medicale de l'Hôtel-Dieu de Paris, 2nd edition. 1865, 3, 654-712.
- Dolberg, D. S.; Hollingsworth, R.; Hertle, M.; Bissell, M. J. *Science* 1985, 230, 676-678. (B) Schuh, A. C.; Keating, S. J.; Monteclaro, F. S.; Vogt, P. K.; Breitman, M. L. *Nature* 1990, 346, 756-760.
- Palumbo, J. S.; Degen, J. L. *Thrombosis Res.* 2007, 120, S22-S28.

Results

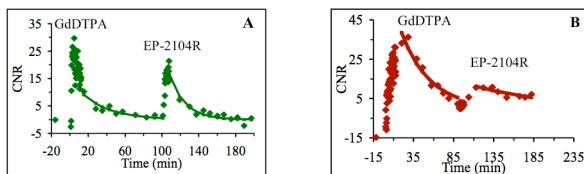


Figure 1. CNR relative to muscle for subsequent injections of GdDTPA and EP-2104R in the same animal. (A) Tumor Core. (B) Tumor Periphery.