Development of MRI contrast material for in vivo mapping of transglutaminase activity

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Introduction

Transglutaminases form a family of enzymes that evolved for covalent cross-linking of proteins. The cross–linking activity can serve disparate biological processes depending on the location of the target protein. Extracellular activation of tissue transglutaminase (tTG) contributes to stabilization of the Extracellular matrix (ECM) and promotes cell – substrate interaction. To generate new matrix, tumors as well as newly formed angiogenic blood vessels, are known to elicit wound-healing responses from the host tissues resulting in formation of granulation tissue at the advancing margins of the tumors. In addition, the enzymatic activity of tTG is a critical component of clotting. The goal of this work is to develop a peptide substrate that would be cross-linked and thus highlight sites of TG activity. Here we report evaluation of a candidate peptide, labeled with GdDTPA and a fluorescent tag, tested for its cross linking to multicellular tumor spheroids derived from MCF7 human breast carcinoma cells.

Methods

Contrast materials: Biotin / Dansyl-TGS-GdDTPA, TGS (transglutaminase substrate) were synthesized as reported previously (1).

NMR/MRI experiments: R1 relaxivities of biotin -TGS-GdDTPA and dansyl-TGS-GdDTPA were measured on a 9.4 T horizontal Bruker (Germany) 400 MHz spectrometer. Spin echo: TR 100-2000ms, TE 8ms, 2 averages, matrix 128X128, FOV 5X5mm, flip angle 90^0. Inversion recovery: P_1 9 μs, P_2 18 μs. Inversion recover selective pulse: P_1 1ms, P_2 2 ms, 13 delay times 0.01-4s

MRI data were analyzed on an Indigo-2 workstation using Matlab (the Math works Inc.).

Optical imaging: Spheroids were incubated with biotin pentylamine or a biotinylated tTG peptide-substrate, and then were incubated with Avidin–FITC for detection.

Histology: MCF-7 spheroids were fixed, embedded in paraffin and sectioned (4 μm). In addition the slides were stained for tTG by anti tTG monoclonal antibody.

Results

Fluorescence microscopy of spheroid histological sections showed that the biotinylated pentylamine was cross-linked by tissue Transglutaminase (tTG) to the viable rim of the spheroid (Fig 1A). tTG was detected in the cells and in the ECM in the viable rim in correlation with the sites of cross-linking activity (Fig 1B). Biotin- TGS-GdDTPA showed strong signal enhancement and high R1 relaxivity. Signal enhancement in solution could be significantly attenuated by addition of avidin beads, which bound and precipitated the biotinilated contrast material (Fig 2). Thus the relaxivity was attributed to the peptide and not due to residual free GdDTPA. Selective inversion recovery significantly prolonged T1 relaxation, suggesting a role for magnetization transfer in relaxivity induced by TGS-GdDTPA.

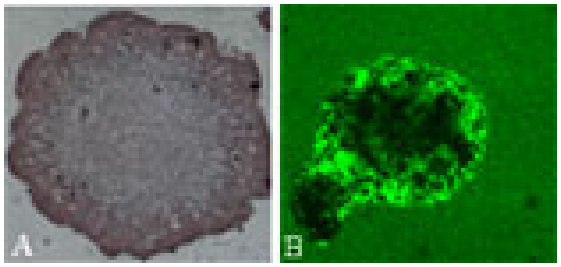

Fig 1. (A) MCF7 spheroids were stained with anti tTG mouse monoclonal antibody. Goat anti mouse, conjugated to alkaline phosphatase, was used as 2nd Ab and was visualized with fast red.
(B) MCF7 spheroids were incubated with Avidin-FITC or 45min after 48 hours incubation with 5-biotin amido pentylamine.

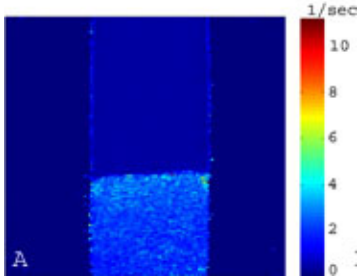

Fig 2. Pixel by pixel calculation of R1 resulted in R1 maps of Avidin beads bound to biotin-TGS-GdDTPA (bottom phase) and water with residual free Gd ions or GdDTPA (upper phase).

Discussion

We present here a novel peptide based MR contrast agent, with very high relaxivity, which functions as a substrate for cross linking by transglutaminase. This contrast material could potentially help map and delineate activity of transglutaminases in vivo in coagulation and clotting, as well as in angiogenesis and tumor progression.

References

(1) Dafni H, Landsman L, Schechter B, Kohen F, Neeman M. NMR Biomed. 15(2), 120-31

Acknowledgement: this work was supported by Varian Biosynergy