In vivo measurements of tissue Transglutaminase activity using new Contrast Material for Magnetic Resonance Imaging.

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Introduction

Transglutaminases form a family of enzymes that evolved in 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. We previously reported tTG substrate as contrast agent for MRI (1). The goal of this work is to test a new peptide substrate that would be cross-linked and thus would highlight sites of TG activity. Here we report the evaluation of DCCP-16, a candidate peptide labeled with GdDOTA. The contrast material, DCCP-16 showed strong signal enhancement in MCF-7 spheroids as well as in a preliminary *in vivo* MRI analysis using 4T1-GFP-tTG mammary gland tumors. This contrast material could potentially delineate *In-vivo* activity of transglutaminases in coagulation, as well as in angiogenesis and tumor progression.

Materials and Methods

In vitro study: MCF7 human breast carcinoma cells were cultured as multicellular tumor spheroid. Spheroids were incubated with the contrast material DCCP-16 for 48h. T1 weighted was 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. R1 maps were generated using Matlab (the Math works Inc.).

In vivo/MRI experiments: 4T1-GFP-tTG mammary gland tumors cells were inoculated s.c into CD-1 nude mice (6 weeks old, female). Contrast material was administrated I.V. T1 weighted was measured on a 4.7 T Bruker (Germany) 200 MHz spectrometer. Spin echo: TR 100-2000ms, TE 8ms, 2 averages, matrix 256X256, flip angle 90^0. MRI data were analyzed on an Indigo-2 workstation using Matlab (the Math works Inc.).

Results

DCCP-16 showed strong signal enhancement in the NMR using 4T1-GFP-tTG multicellular spheroids (Fig 1). CD-1 nude mice bearing tumors showed signal enhancement in the tumor margins 8 hours post injection I.V of the contrast material, DCCP-16, consistent with covalent binding of the administered substrate to the ECM provisional matrix.

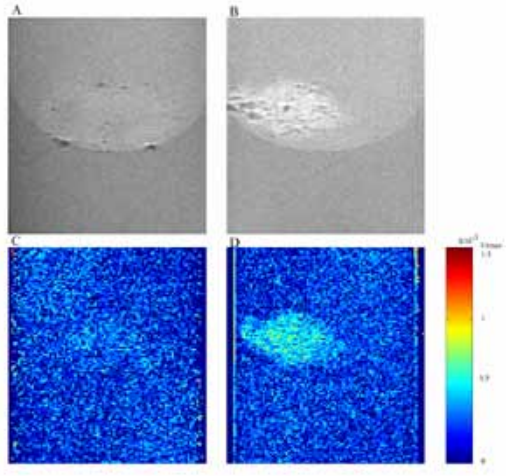

Fig 1. MRI detection of transglutaminase mediated binding of DCCP16. (A) An image of MCF7 spheroid in RPMI medium on agarose incubated with DCCP-16 for 48 hours (C) R1 maps of MCF7 spheroids in RPMI medium (D) R1 maps of MCF7 spheroids from the same suspension culture incubated with DCCP-16 in RPMI medium for 48 hours.

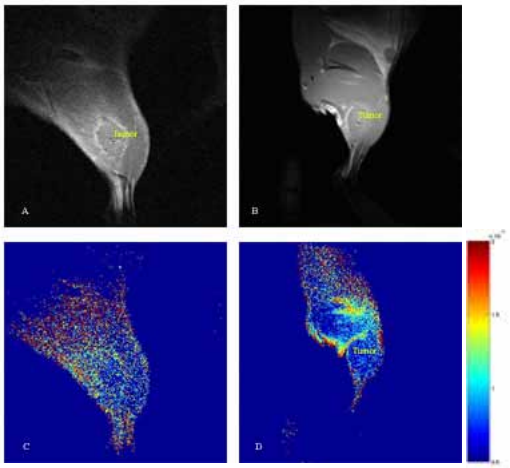

Fig 2. Subcutaneous inoculation of tTG over expressing mammary carcinoma cells (4T1-tTG-GFP; $5X10^6$ cells/ mouse) in CD-1 nude mice (female, 6 weeks old). Contrast material, DCCP-16, was administered 6 days after inoculation. (A) Gray scale image acquired 48h before injection of DCCP- 16, TR=800msec. (B) R1 maps derived 48h before injection of DCCP-16. (C) Gray scale image of 8h post I.V injection of DCCP-16. (D) R1 maps of 8h post I.V injection of DCCP-16.

Discussion

We present here a novel peptide based MR contrast agent, DCCP-16 with high affinity to tissue transglutaminase, which functions as a substrate for cross linking by transglutaminase. Detection of tTG in cells and in ECM in the viable rim of MCF7 spheroids correlated with the sites of cross-linking activity. The contrast material, DCCP-16 showed strong signal enhancement in MCF7 spheroids as well as in a preliminary *in vivo* MRI analysis using 4T1-GFP-tTG mammary gland tumors. 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.

Reference: 1. Mazooz, G., et.al (2005). Cancer Res. 65(4): 1370-1375.

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