Tracking recruitment of fibroblasts to ovarian carcinoma tumors by MRI

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Introduction

The tumor microenvironment has recently been established as a central participant in the biology of tumorigenesis /tumor angiogenesis and the invading fibroblasts as key modulators in the process. Interactions between fibroblasts and endothelial cells have been demonstrated for *in-vitro* tumor models (1, 2), and bone marrow derived fibroblasts were reported to be recruited to s.c/i.p tumors in mice (3). We previously reported that myofibroblast infiltration into implanted ovarian carcinoma spheroids was associated with exit of tumors from dormancy (4) and that these cells contributed to vascular stabilization in ovarian tumors by expression of angiopoietin 1 and 2 (5). However, gaining deeper insight regarding their interactions with the endothelium during tumor angiogenesis requires specific *in-vivo* monitoring. We recently demonstrated that fibroblasts which were labeled *in-vitro* with biotin-BSA-GdDTPA, were visible *in-vivo*, and that the *in-vitro* intracellular relaxivity of the contrast material increased with cell divisions/redistribution of the contrast material (6). Here we show that pre-labeled fibroblasts can be followed *in-vivo* by MRI over several days, thus allowing the study of their recruitment into the tumor stroma.

Material and Methods: Contrast material: Biotin-BSA-GdDTPA was synthesized as described (7)

Ex-vivo cell labeling: Sub-confluent cultures of cv-1 fibroblasts were incubated with 10 mg/ml contrast material for 48 h and labeling was terminated by 4 washes in serum free medium.

In-vivo model: Tumors were initiated in CD-1 nude mice by inoculation of human epithelial ovarian carcinoma MLS cells. After a tumor was formed, a second injection (s.c/i.p) of labeled/non labeled fibroblasts was performed. Mice were studied 1-14 days after tumor initiation.

MRI measurements: Measurements were performed on a horizontal 4.7 T Bruker Biospec spectrometer using an actively RF-decoupled 1.5-cm surface coil stabilized in a Perspex board, and a whole-body birdcage transmission coil. A series of T1-weighted 3D gradient-echo (GE) images, with pulse flip angles of 5°, 15°, 30°, 50°, and 70° were acquired to determine the R_1 values. The acquisition parameters were as follows: TR 10 ms; TE 3.561 ms; two averages; spectral width 50,000 Hz; FOV 4X4X2 cm, 128X128x64 pixels, resulting in a voxel resolution of 0.312 X 0.312 X 0.312 mm; and total acquisition time per frame 163 s.

Results:

Recruitment of labeled cells to the tumor was monitored by R_1 measurements of tumors before and after injection of pre-labeled fibroblasts. Follow up MRI studies revealed that 5 and 6 days after injection of labeled cells R_1 was elevated relative to 1 day after their injection (asterisks), indicating, that the signal during the initial days after injection of labeled cells is enhanced. This elevation decreased approximately a week after injection. Furthermore, the R_1 maps suggest that labeled cells migrated within the tumor (Figure 1A). Moreover, R_1 of tumors 6 days after s.c injection of labeled fibroblasts was significantly elevated compared to un-labeled fibroblasts (1.8 s^{-1} and 1.4 $^{s-1}$, respectively, $p<0.05$; figure 1B). These results encouraged us to perform a preliminary study of recruitment of fibroblasts to tumors after i.p injection of labeled/un-labeled fibroblasts. Remarkably, R_1 of tumors following i.p injection of labeled fibroblasts was greater than the one observed with un-labeled cells.

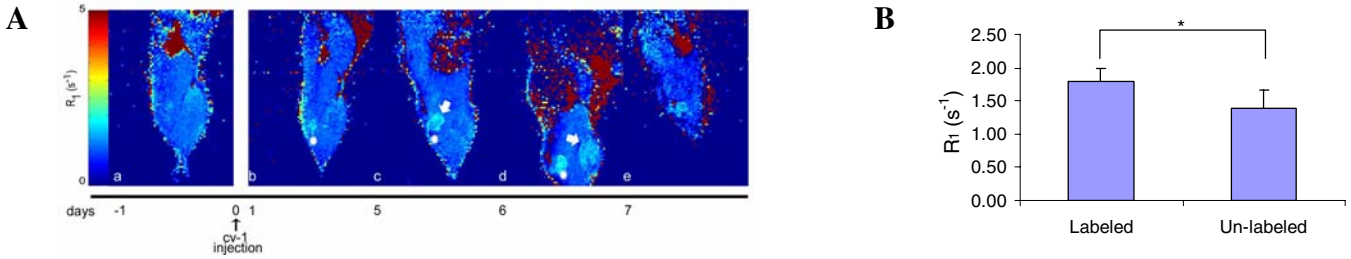

Figure 1. Recruitment of labeled fibroblasts. Tumors were initiated in the hind limb of CD-1 nude mice by inoculation of MLS cells (3 X 10^6) followed by a second injection of labeled cv-1 fibroblasts (0.3 X 10^6). A) Presented are R_1 maps of one representative tumor scanned over several days. (a) R_1 map of the tumor before injection of labeled cv-1. (b) R_1 map 1 day (c) 5 days (d) 6 days and (e) 7 days after injection of labeled fibroblasts. Note the migration of labeled fibroblasts from one side of the limb to the other (arrows). B) Average R_1 values obtained from ROI analysis of the tumor regions 6 days after s.c injection of labeled/unlabeled cv-1 cells. (n=3 for each group; * significant change in R_1 of labeled vs. unlabeled tumors; $P< 0.05$, *t*-test, unpaired, one-tailed).

Conclusions:

This study shows that *in vivo* MRI tracking of fibroblasts labeled with biotin-BSA-GdDTPA is feasible and can be used for mapping the recruitment of these cells during tumor progression. Furthermore, the increase in visibility of the contrast material with cell proliferation which we reported in vitro (6), can be detected also in vivo.

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Supported by: This work was supported by the Israel Science Foundation and by the USA NCI RO1 CA75334.