In vivo cell transfer labeling of dendritic cells using Feridex–labeled GM-CSF tumor vaccines for evaluation of immune adjuvants

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Introduction: Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cancer cell vaccines have been demonstrated to elicit potent anticancer immune responses in a variety of animal models.[1] Paracrine production of GM-CSF by irradiated tumor cells recruits dendritic cells (DC) that capture tumor antigens released by cancer vaccines. However, to induce an effective immune response, DCs must not only be activated but also migrate to the lymph nodes where they prime antigen-specific naïve T cells. Quantifying this cellular migration in vivo is critical for evaluating strategies to improve the efficacy of GM-CSF based tumor vaccines. Here we describe a non-invasive *in vivo* cell transfer labeling method, which relies upon DC capture of dying, Feridex® labeled, cancer cells and their contents. We have used this method to test the efficacy of an FDA approved treatment for superficial basal cell carcinoma, Imiquimod (Aldara®), a Toll-Like Receptor 7 Agonist, in enhancing DC migration to the draining lymph nodes.

Materials and Methods: B16 melanoma cells and a GM-CSF producing "bystander cell" (B78H1/GM-CSF) were irradiated ($1x10^4$ rad and $5X10^3$ rad, respectively) and labeled with SPIO (Feridex) by magnetoelectroporation.[2] Iron uptake was determined by Prussian Blue as well as by Ferrozine assay. GM-CSF production after labeling was quantified by ELISA. $1X10^6$ labeled B16 cells along with $2X10^5$ labeled B78H1/GM-CSF cells were injected into the footpads of mice in a total volume of 20ul. In those groups where Aldara was applied, the right foot of each mouse was given 10ul of Aldara cream on days 0, 1, 3 and 5 and the cream was allowed to absorb into the footpad while the mouse was anesthetized and in the scanner (approximately 1 hour). The left foot was treated with a control lotion on the same schedule. MR imaging was performed on a Bruker 9.4T horizontal bore animal scanner. A T2 weighted coronal spin echo image was acquired for localization of the lymph nodes (slice thickness 1 mm, 24 slices, field of view 56 X 24 mm, matrix 128 X 128, TR = 3000 ms, TE = 50 ms, 1 average) using a rapid acquisition relaxation enhanced sequence (RARE) with a RARE factor of 8. Axial images were acquired using a multi-gradient echo (MGE) sequence (slice thickness 0.337 mm, 15 slices, field of view 23 X 23 mm, matrix 288 X 288, TR = 1000 ms, TE = 5 ms, number of echoes = 4, averages = 8, gauss512 fat suppression, scan time: 38 min) as well as a RARE sequence (slice thickness 0.337 mm, 15 slices, field of view 23 X 23 mm, matrix 288 X 288, TR = 2100 ms, TE = 60 ms, 4 averages, Rare factor 8, scan time: 4 min). RARE images were used for an accurate determination of lymph node size while MGE images were used to resolve SPIO labeled DCs.

Results and Discussion: In this study, we have shown that B16 melanoma cells as well as B78H1/GM-CSF producing tumor cells are excellent vectors for SPIO delivery. The tumor cells can be efficiently loaded with SPIO (~10pg/cell) while still maintaining their immunostimulatory behavior, as measured by GM-CSF production. To the best of our knowledge, we report the first study on indirectly labeling DCs with SPIO in vivo. Using this cell transfer labeling method, we have shown that it is possible to monitor the extent of dendritic cell trafficking from the site of vaccination to the popliteal or draining lymph node using magnetic resonance imaging. Additionally, we have preliminary evidence indicating that this imaging method may be sensitive enough to evaluate the efficacy of possible immune modulators. In mice treated with Aldara, the draining lymph node corresponding to the Aldara treated footpad saw a much larger decrease in signal as a result of more DCs trafficking (Figure 1, A-C). The volume of the Aldara treated lymph node was also observed to be larger than the control. All MR images corresponded well with the excised lymph nodes (Figure 1, D) in size and degree of iron content. As Feridex is clinically approved and DC MRI tracking has now been introduced in the clinic[3], this method may be readily clinically translatable as a means to determine vaccine activity in patients with and without immune adjuvants.

[1]Dranoff et al., Proc Natl Acad Sci USA 1993 (90) 3539
[2]Walczak et al., Magn Reson Med 2005 (54) 769-74
[3]de Vries et al., Nat Biotechnol 2005 (23) 1407-13

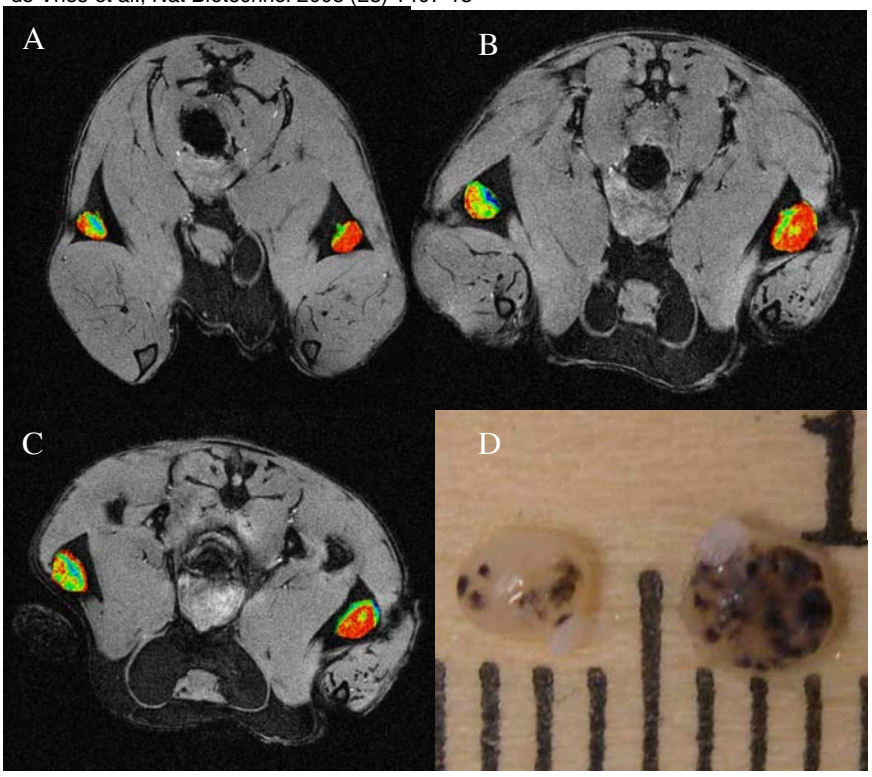

Figure 1. A-C) Merged images of popliteal lymph nodes. RARE images were used to determine the borders of each lymph node and MGE images were overlain using a pseudo-color scale. Red corresponds to a hypo-intense signal with blue being a hyperintense signal. The right lymph node of each image represents the Aldara treated footpad while the left lymph node of each image represents the SPIO labeled vaccine treated with control lotion. A) Day 3 B) Day 5 C) Day 7 post vaccination. D) Excised popliteal lymph nodes 9 days post vaccination with SPIO labeled GM-CSF vaccine. Lymph nodes are from the same mouse as that imaged in A-C. Left is vaccine treated alone while right is vaccine plus Aldara cream. Both MR images as well as excised lymph nodes show an increase in SPIO labeled DC trafficking after application of Aldara. Correspondingly, lymph node size is observed to be larger in the Aldara treated group.