QUANTITATIVE ASSESSMENT OF MAGNETICALLY LABELED LUCIFERASE POSITIVE CELLS USING MULTIMODALITY IMAGING

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INTRODUCTION

Cellular imaging using a combination of MRI and bioluminescence imaging (BLI) has been used to trace live cells with high spatial resolution and sensitivity; more importantly, cell migration and survival can be correlated and detected[1,2]. Breast cancer metastasis[3] and cell-based therapy of demyelization[1] has been studied using magnetically labeled luciferase positive cells with multimodality imaging. However, these studies involve imaging dual-labeled cells, tracking their migrations without quantification of local density of the dual-labeled cells. Quantitative analysis of cells after transplantation may allow more accurate assessment of cell delivery and subsequent distribution and migration, which can lead to more effective monitoring and optimization of therapeutic paradigms. In this study, we show that parameters from imaging modalities, the photon flux from BLI and transverse relaxation from MRI, can be analyzed and correlated quantitatively for dual-labeled cells *in vitro*, suggesting that cell numbers quantification can be potentially done *in vivo*[4] using multimodality imaging with dual-labeling.

METHODS

Luciferase Labeling: Human breast tumor cell line transfected with Fluc gene expressed from the CMV promoter, MCF-7-luc-F5 (Xenogen), was grown in culture medium as specified by the company. Rat hepatoma cell line McA-RH7777 (CRL-1601, ATCC) was transfected with Fluc gene[5,6] (pGL3-Control Vector, Promega) and grown in standard culture medium.

Magnetic Labeling: Monocrystalline iron oxide nanoparticles (MION-47; CMIR, MGH, MA), and Poly-L-lysine (PLL; Sigma, St Louis, MO) were used for magnetic labeling of cells under the following labeling protocols. PLL of 2.0 μg/ml was mixed with MION-47 solution of 50 μg/ml for 60 minutes in serum-free cell culture medium at room temperature on a rotating shaker. These culture media were then added to the cells and kept overnight for 24 hours at 37 °C in a 95% air per 5% CO_2 atmosphere. Cells were washed twice with phosphate-buffered saline (PBS, pH = 7.4) to remove MION–PLL complex, trypsinized, washed, and re-plated on a 96-well plate with various cell concentrations (n = 4) for BLI with D-luciferase of 300 μg/ml. After the BLI, cells were fixed with 4% paraformaldehyde, uniform gel suspensions (1% agarose gel) were then prepared with same cell concentrations placing in separate 4-cm long, 1-cm diameter cylindrical phantom tubes for MRI measurement of relaxavities. Viability after labeling was determined by Trypan blue exclusion and the result was expressed as percentage live cells of total cells.

Bioluminescence Imaging: BLI measurements were acquired using Xenogen IVIS imaging System 100 within 5 – 15 minutes after adding of D-luciferase. Images were analyzed using LivingImage R (Xenogen). *In vitro* photon flux measurements were taken from four wells of same cell concentration by drawing circular region-of-interest (ROI) in the images.

MRI: MRI was performed on a 7 Tesla scanner (Bruker *PharmaScan*®). R_2* maps were acquired with multiple gradient echo sequences (TR/TE = 1500/4 ms, 16 echoes, 128 x 128 matrix, 13 slices, slice thickness = 1 mm, FOV = 3.84 cm, NEX = 2). Images were analyzed using a software toolkit developed in IDL (RSI, Boulder, CO). Values of R_2* were calculated by linear least-squares fitting of log signal intensity versus echo time. The R_2* measurements were taken from the average in five slices by drawing circular ROI in the R_2* maps. Values of relaxation rate enhancement ΔR_2* in each tube with labeled cells were calculated by subtracting R_2* of that tube by that of gel-only tube.

RESULTS

Dual-labeled MCF-7-luc-F5 and McA-RH7777 cells viability in culture after labeling, as determined by absence of uptake of Trypan blue stain, was about 81% and 98% respectively. Figures 1 and 2 illustrate the linear relationships of photon flux and average ΔR_2* with cell concentrations. Figure 3 shows the parameters from the two imaging modalities, photon flux and average ΔR_2*, of the same cell concentrations is linearly correlated for the two types of dual-labeled cells with coefficients of determination (R^2) greater than 0.98.

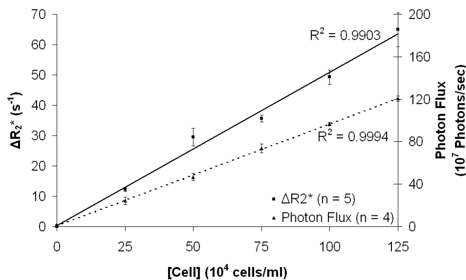

Figure 1 ΔR_2* and Photon Flux versus number of MION labeled MCF-7-luc-F5 cells. The error bars represent one standard deviation.

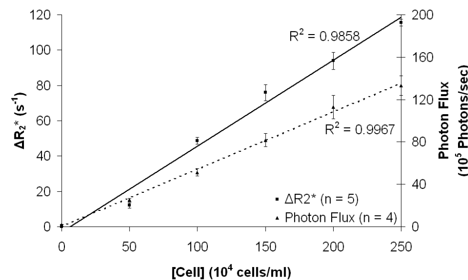

Figure 2 ΔR_2* and Photon Flux versus number of MION and Fluc labeled McA-RH7777 cells. The error bars represent one standard deviation.

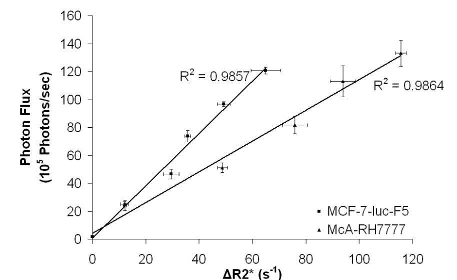

Figure 3 Photon Flux versus ΔR_2* of labeled MCF-7-luc-F5 and McA-RH7777 cells. The error bars represent one standard deviation.

DISCUSSIONS AND CONCLUSIONS

In this study, we demonstrated that the cells can be dual-labeled for BLI and MRI. The two types of dual-labeled cells exhibited excellent linear correlations between cell concentration with photon flux and ΔR_2* *in vitro*, illustrating that quantitative assessment of dual-labeled cells can be achieved. Under the same cell concentrations, excellent linear relationship was observed between photon flux and average ΔR_2*, showing that there are no interferences between luciferase labeling and magnetic labeling of cells. BLI has been used to measure cell number during tumor progression[7], while for MRI, it has been suggested by calibrating the transverse relaxivity of magnetically labeled cells *in vitro* and quantifying ΔR_2* *in vivo*, cell number quantification can be achieved[4]. Through combining previous demonstrated techniques, quantification of local cell density and number can be feasibly done by calibrating the photon flux and transverse relaxivity of dual-labeled cells *in vitro* and quantifying local photon flux and ΔR_2* *in vivo*. Living status of cells can be determined from BLI, whereas a more precise location of cells can be detected using MRI, in utilizing these two, survival rates, cell status and precise cell locations can be correlated quantitatively. *In vivo* quantification of transplanted cells is particularly useful and applicable for accurate assessment of local retention of donor cells in specific tissue or organ after systemic and local injection[8], and the spatial-temporal dynamics of their subsequent migrational activities[9]. This technique is applicable in studies of cell-based therapies for monitoring and correlation of cell migration and survival, as well as studies of cancer progression and metastasis.

REFERENCES

[1] Walczak P, et al., ISMRM 2007;604. [2] Song H-T, et al., SMI 2006;512. [3] Song H-T, et al., ISMRM 2007;2903. [4] Cheung JS, et al., ISMRM 2007;1195. [5] Caceres G, et al., Luminescence 2003;18(4):218-223. [6] Edinger M, et al., Eur J Cancer 2002;38(16):2128-2136. [7] Uhrbom L, et al., Nat Med 2004;10(11):1257-1260. [8] Tran N, et al., Cell Transplant 2006;15(4):351-358. [9] Imitola J, et al., Proc Natl Acad Sci U S A 2004;101(52):18117-18122.