Chemical Biotinylation of Cells for Molecular Imaging and Cell Tracking

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Introduction

Chemical biotinylation of cells can be used for isolation and study of cell surface proteins [1]. Such relatively rapid methodology can also be used to biotinylate cells for conjugation to anti-biotin or anti-avidin/streptavidin antibodies, which could in turn be amenable to being conjugated with contrast agents e.g., superparamagnetic iron oxide nanoparticles (SPIOs). Thus, chemical biotinylation of cells raises the possibility of imaging such cells by MRI, following labelling with anti-biotin-SPIOs. Further, biotin could also be used to 'tag' cells to enable imaging of transgene expression using methodology based on magnetic-antibody-gene-imaging-contrast [2]. In this study, the feasibility of imaging chemically biotinylated cells *in vitro* and *in vivo* by MRI will be demonstrated

Method

In vitro experiment: IGROV1 cells (human ovarian epithelial cancer cell line) were obtained (Cancer Research UK) and chemically biotinylated, while a control population was treated only with phosphate-buffered saline (PBS, pH 8 – buffer used for the biotinylation reaction). After biotinylation, cell counts and viability was assessed by the trypan blue exclusion test. Both control and biotinylated cells were then washed with PBS (pH7, 2ml, x3). After washing, cells were reconstituted in PBS to the appropriate volume for FACS analysis of cell death (Sigma Aldrich kit, Poole, UK). Aliquots of cells were also retained for labeling (according to manufacturer's recommendations) with anti-biotin SPIOs (Miltenyi Biotec, Bisley, UK) and anti-biotin FITC (Miltenyi Biotec, Bisley, UK) for MRI and FACS, respectively. Following incubation with anti-biotin SPIOs, both non-biotinylated and biotinylated cells were washed with PBS (2ml, x3) and counted. 4.6 x 10^5 cells were suspended in 1% agarose (PBS, 50µl) and pelleted in 250µl tubes for MRI. The cell agarose pellets were allowed to set and the tubes filled with phosphate buffered saline. The tubes of cells were placed into a tube of water and then placed centrally inside a 40mm ID birdcage rf coil (Magnetic Laboratories, Oxford, UK) and MRI performed on a 4.7T horizontal bore Varian scanner (Palo Alto, CA). Standard spin-echo based methods were used to determine T2 of the control and biotinylated cell pellets (TR=3s, TE=6.5-300ms, FOV=100x100mm, matrix=256x128, 1 average and 1 transverse lice of 6mm thickness.)

In vivo experiment: For MRI, IGROV1 cells were biotinylated and then half the population incubated with anti-biotin SPIOs and the other, with basic microbeads (Miltenyi Biotec, Bisley, UK). Following washing off of unbound SPIO particles, the cells were suspended in PBS. SPIO-biotinylated cells (10^5, 15µl) were injected into the hindlimb of a B6 mouse and non-SPIO-biotinylated cells (10^5, 15µl) into the contralateral hindlimb. FLASH was performed using parameters: TR=50s, TE=1.8s, FOV=30x30mm, matrix=256x128, 8 averages and 13 transverse slices of 1.5mm thickness.

Results and Discussion

Chemical biotinylation of cells was confirmed by FACS following incubation of cells with anti-biotin FITC (Fig. 1). Viability of the control and biotinylated cells was 87% and 92%, respectively, by the trypan blue exclusion assay. Assay of apoptosis and necrosis using annexin V-FITC and propidine iodide by FACS also showed similar viabilities between the control and biotinylated cells (Fig. 2). MRI of the control and biotinylated cells following exposure to anti-biotin SPIOs and hence, labeling of the latter cells, showed negative enhancement in the biotinylated cells (Fig. 3), and consistent with the FACS data (Fig. 1). SPIO-labeled biotinylated cells were also readily observed *in vivo* using standard FLASH techniques (Fig. 4). Hence, we have been able to demonstrate the chemical biotinylation of cells for tagging with antibodies raised to biotin that are conjugated to SPIOs for the MRI of cells.

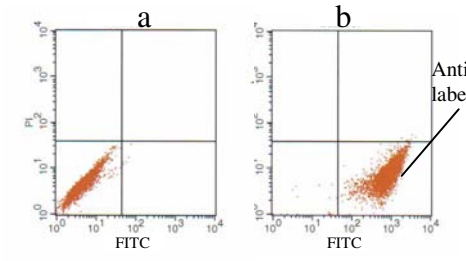

Fig. 1: FACS of (a) control cells and (b) biotinylated cells following incubation with anti-biotin FITC.

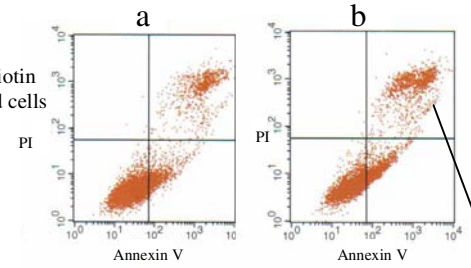

Fig. 2: FACS of (a) control cells and (b) biotinylated cells following incubation with Annexin V-FITC and propidine iodide.

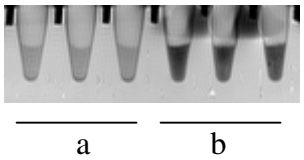

Fig. 3: Spin echo MRI images of (a) control and (b) biotinylated cells following incubation with anti-biotin SPIOs.

Conclusion

Preliminary data suggests that the chemical biotinylation of cells provides is a rapid and generic method for 'tagging' cells for *in vivo* imaging by MRI.

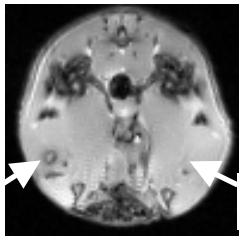

Fig. 4: Flash transverse MRI image of the hindlegs of a mouse in which control and SPIO labeled biotinylated cells were injected into side A and B, respectively.

References

1. Altin *et al.,* (1995), Anal. Biochem, 224, 382.
2. So *et al*., (2005), MRM, 54, 218.

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