Microarray analysis of the effect of Feridex-labeling on gene expression in neural stem cells

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Introduction:

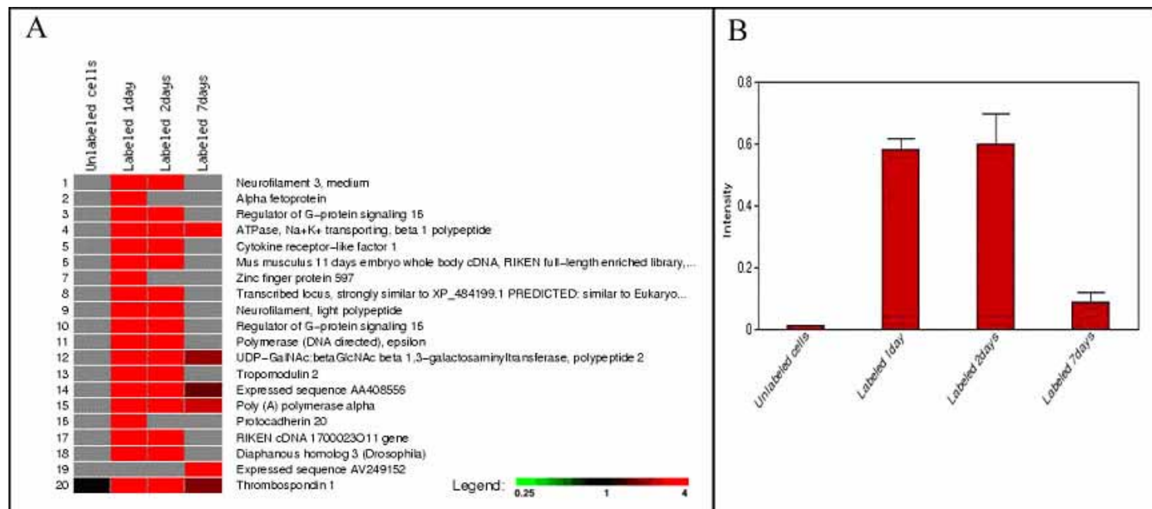
MRI cell tracking using Feridex has found many biological applications and has recently entered the clinic (1). Several techniques have been developed for efficient magnetic labeling of a variety of cells using superparamagnetic iron oxides (SPIOs). A potential cellular toxicity or change in cell function following labeling has since long been considered (e.g., reviewed in 2), but other than inhibition of chondrogenic differentation of mesenchymal stem cells (3) no obvious detrimental effects of SPIO-labeling have been reported. So far, biological endpoints such as cellular viability, proliferation, cytokine secretion, migration, cell surface marker expression, apoptosis, and free oxide radical formation have been compared to unlabeled cells. While for animal studies these assays are generally accepted and were found to have no significant changes, for clinical applications and approval by regulatory agencies a more detailed and comprehensive meta-analysis is required in order to assure patient safety. One such an approach is microarray analysis, allowing instant digitized statistical analysis of overall gene expression profiles. We report here on microarray studies of Feridex-labeled neural stem cells (NSCs).

Methods:

We used a prototype NSC line that has been widely used in transplantation studies. C17.2 cells (an immortalized cell line derived from neonatal mouse cerebellum) was cultured in standard culture medium. Cells were magnetically labeled with the FDA-approved ferumoxide formulation Feridex and poly-L-lysine (PLL) for 24 hours (4). Control cells were cultured without neither Feridex nor PLL. The total RNA was extracted from three separate labeling experiments for each time-point (1, 2, and 7 days post labeling). RNA was then purified using TriReagent, (MRC, Cincinnati, OH). Gene expression profiling was performed with a GeneChip® Mouse Expression Set (430 2.0, Affymetrix). An analysis of 39,000 transcripts was performed for the entire mouse genome. For identifying significant changes in expression levels, the acquired data were analyzed using Genesifter® software and filtered using a cutoff for changes >1.5 fold and with $p<0.05$.

Results:

Of the 39,000 genes analyzed, 101 were upregulated (most notably ATPase-Na/K transporter and neurofilament 3) and 335 were downregulated (most notably leucin-rich repeat LGI), all at 1-2 days after labeling when compared to unlabeled controls (Fig. 1A,B). Fewer affected genes were identified at 7 days (Fig. 1A, B), indicating that the changes in expression were transient. Hierarchical cluster analysis identified the altered expression of genes to be associated with embryogenesis, cell cycle control, cell metabolism, cell migration, and neurodifferentiation.



Conclusion:

Feridex-labeling altered the expression of over 400 genes in neural stem cells: these results are important for future FDA-approval of SPIO-based cell tracking. However, this effect appears to be transient, with a peak at 1-2 days post labeling. Follow-up studies at more different time points are required to determine how significant these alterations are for the long term. As it is anticipated that the alteration of gene expression differs dependent on cell type with neural stem cells possibly being more sensitive, further studies are clearly warranted.

References:

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