Magnetically Labeled Neural Stem Cells Exhibit Differential Gene Expression of Zinc (Finger) Binding Proteins

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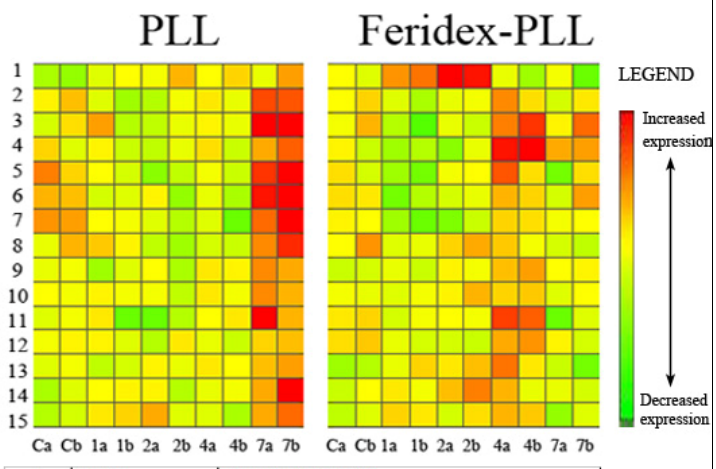
Introduction: Superparamagnetic iron oxides (SPIOs) are widely applied to render cells magnetic in order to track them in vivo using MR imaging. There are a variety of methods that can be used to incorporate SPIO particles into cells. One of these is by complexing negatively charged Feridex particles to cationic transfection agents, e.g. poly-L-lysine (PLL), through electrostatic interactions [1]. So far, few studies have reported on the biological/cellular events associated with intracellular SPIO uptake [2,3]. Now that MR cell tracking using SPIO-labeling has recently entered the clinic [4, 5] further studies on the biological effects and safety of cellular magnetic labeling are warranted. Because gene expression is the primary and most sensitive mechanism by which induced aberrations are converted into functional and structural adjustments, understanding the extent and patterns of gene regulatioin in response to SPIO incorporation is of fundamental importance. We therefore assessed time-dependent changes in gene expression for Feridex-labeled neural stem cells.

Methods: The mouse neural stem cell line C17.2 was cultured in standard medium. For Feridex-labeling, 25 µg Fe/ml ferumoxide (Berlex Laboratories Inc.) and poly-L-lysine hydrobromide (Sigma) at a concentration of 375 ng/ml were mixed together in culture medium and incubated at room temperature for an hour to create complexes, which were added to the cells for 24 hours, following which cells were cultured in standard medium. As a control for changes induced by the transfection agent (PLL) itself, cells were incubated with PLL only (375 ng/ml). Unlabeled controls (neither Feridex nor PLL) were included as a baseline reference. The cells were labeled on days 0, 3, 4 and 5 with the total RNA isolated on day 7. The design of this study thus created 4 time points: 1 day, 2, 4, and 7 days post labeling. Two independent experiments were performed, and for each time point, samples were analyzed in duplicate. Total RNA isolation was achieved using TRI reagent (Promega, Madison, WI) and Phase Lock Gel Heavy (Eppendorf) under RNA-se free conditions. The RNeasy Mini Kit (Qiagen) was used to purify the RNA and the RNA purity and quality were determined on Agilent 2100 Bioanalyzer - Bio Sizing.

The microarray was performed with oligo mouse 430 2 arrays (Affymetrix). The quality of the microarray data was assessed with the AffyPLM package, and the data were processed using a Robust MultiArray Analysis (RMA) algorithm which includes a Quantile normalization procedure. A batch effect on the probe set signal levels was identified by Multi-Dimensional Scaling (MDS) analysis and adjusted with a ComBat package based on an empirical Bayes method. The time-dependent, differential gene expression analysis between FePLL and PLL was made with the EDGE package. Finally, the pattern visualization was made with heat maps created using GeneSpring GX 7.3.

Results: For the genes that had the highest difference in expression between cells labeled with Feridex-PLL and PLL, we decided to further analyze the "top" 300 genes. Out of these 300 genes, 188 (63%) encode for proteins regulating catalytic activities, as well as developmental, metabolic, and molecular binding processes. Among this group, the largest subpopulation of genes (35/188, $P<0.05$) were found to be zinc-binding proteins, with 13 of them regulating zinc-associated DNA binding and protein transcription. Zinc finger proteins exhibited the highest differential expression (Fig. 1). These genes regulate several essential molecular functions, such as iron binding (Ppp3ca, Jmid1c), central nervous system development (Zeb1), and cell differentiation (Suv39h1) (Fig. 1). The effect of Feridex-PLL labeling is most pronounced on days 2-4 post labeling and is less than treatment with PLL alone, peaking around day 7.

Conclusion: We conclude that labeling of cells with Feridex-PLL has a significant effect on the gene expression of zinc binding proteins. The subgroup of transcription regulators (proteins binding to DNA and RNA), are primary responsible for maintaining homeostasis following magnetic labeling. The pronounced difference in the patterns of gene expression between neural stem cells labeled with Feridex-PLL and PLL alone suggests that the impact of Feridex is not just an addition to the effect of PLL, but rather that Feridex-PLL forms complexes with a different effect on the time-dependent changes of gene expression.



1	Rnf138	ring finger protein 138
2	Gatad2a	GATA zinc finger domain containing 2A
3	Zdhhc6	Zinc finger, DHHC domain containing 6
4	Zeb1	Zinc finger E-box binding homeobox 1
5	Rc3h1	negative regulation of T-helper cell differentiation
6	Jmid1c	jumonji domain containing 1C
7	1190005F20Rik	
8	Zfat1	ZFAT zinc finger 1
9	Vps18	vacuolar protein sorting 18 (yeast)
10	Ppp3ca	protein phosphatase 3, catalytic subunit, alpha isoform
11	Rfwd2	Ring finger and WD repeat domain 2
12	Zfp414	ZFAT zinc finger 1
13	Suv39h1	suppressor of variegation 3-9 homolog 1 (Drosophila)
14	Trim41	tripartite motif-containing 41
15	Lhx2	LIM homeobox protein 2

Figure 1: Heatmap overview of gene expression level over time for the "top 15" altered genes, nearly all of which encode for zinc (finger) binding proteins. C= untreated; a,b: independent experiments.

References:

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