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## Gene expression signature of putative Cancer Stem Cells in Retinoblastoma Y79 cell line

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## **Abstract**

<u>Purpose:</u>Gene expression studies in Cancer provide an insight into the global functioning of tumor and their pathways. In our previous studies on Retinoblastoma tumors, using a two parameter analysis (size and phenotype), we observed a FSClo/SSClo population that was CD133-CD44+ CD90- and expressed primitive stem cell markers, lacking the expression of differentiation markers (Balla et al. 2009). Since, CD44 and CD90 expression was absent in Y79 cell line, we used CD133 to sort the cells and analysed for various stem cell assays. This study highlights gene expression signature specific to putative cancer stem cells in Y79 cell line.

Methods: Cultured Y79 cells were analysed after doublet discrimination and sorted based on the expression of CD133 marker using BD FACS Aria. Total RNA was isolated and quantified. Microarray was performed in duplicates using human whole genome (4x44K) cDNA arrays (Agilent technologies, USA) as per the manufacturer's instructions. The data was analyzed and normalized using GeneSpring and Lowess algorithm. Data validation was done using semi quantitative PCR. Pathway and interaction studies were analyzed using GeneMania and String database.

**Results:** In comparison to CD133+ cells, the CD133- cells of Y79 cell line showed 2945 upregulated genes (≥ 1.5 fold) and 4531 downregulated genes (≤ 1.5 fold). There was down regulation of Purine metabolism

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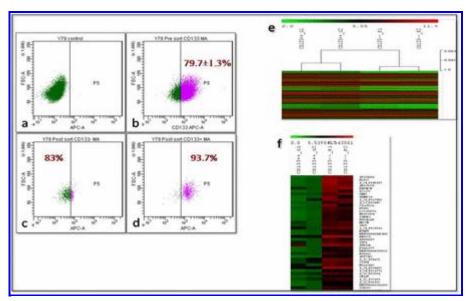
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pathway (p=0.009), TGF-beta pathway(p=0.009), p53 signaling (p=0.017), and oxidative phosphorylation pathway (p=0.012) in CD133- cells. Pathways upregulated in CD133- cells were involved in cell migration (3.53e-1), and cytokine signaling (3.69e-6). Stem Cell genes such as BMI1, ABCB1, CD69, HOXA11, KLF17 were found to be upregulated.

<u>Conclusions:</u> The gene expression data supports our hypothesis that the FSClo/SSClo cells lacking CD133 expression are more undifferentiated and possibly putative cancer stem cells in Y79 cell line. Further studies to validate these pathways are warranted to understand the role of cancer stem cells in Retinoblastoma.

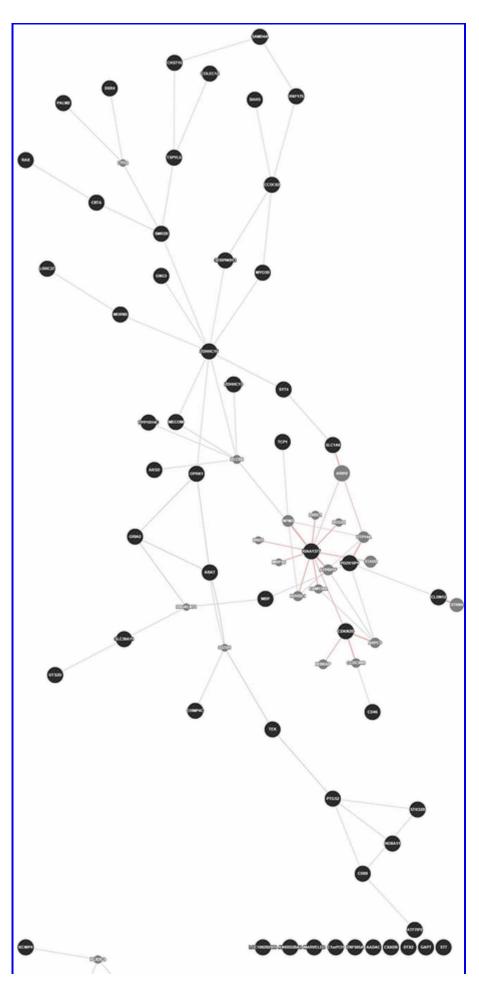


a-d) FACS profile of CD133 sorted Y79 cells e,f) Heat map of sorted CD133 cells

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Network analysis of upregulated Genes in CD133- Y79 cells

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