

## A miRNA signature identified by a chemoresistant cell line model is also found in advanced pancreatic cancer specimens

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Recent studies link cancer cells that possess an epithelial to mesenchymal transition (EMT) phenotype and cancer stem cell (CSC) properties with chemoresistance and metastatic potential. Here, we further investigated the molecular bases of the CRMP with emphasis on identifying a miRNA signature that may play role in maintenance of CRMP and whether this signature is found in advanced pancreatic cancers. We developed a gemcitabine resistant cell line model from BxPC3. This gemcitabine resistant cell line (BxPC3-GZR) showed a pronounced mesenchymal phenotype and expressed high levels of CD44 a marker found on stem cells and showed a highly significant increase in expression of nine miRNAs and decrease in eight miRNAs. Based on relevance to cancer and on validation by q-RT-PCR we chose six of the miRNAs (four up regulated and two down regulated) as a signature to determine whether they were similarly deregulated in 43 advanced PDAC tumor specimens previously analyzed as part of the Total Cancer Genome Atlas (TCGA). A strong correlation was observed for five of the six miRNAs in the tumor specimens compared to normal pancreas tissue. To begin to assess the functional relevance we focused on miRNA-125b for which target genes are known. Knockdown of miRNA 125b in BxPC3-GZR cells caused a partial reversal of the mesenchymal phenotype and enhanced response to gemcitabine. Moreover, a screen of additional PDAC cell lines revealed an association of miRNA-125b expression with expression of the mesenchymal marker vimentin and CD44 and with loss in expression of E-cadherin an epithelial marker. Moreover, RNA seq data from each of the 43 PDAC tumor specimens revealed a negative correlation between the expression of miRNA-125b and five potential miRNA-125b target genes (BAP1, BBC3, NEU1, BCL2, STARD13). The results of this study suggests that CRMP may be caused in part by deregulation of a specific set of miRNAs and that these miRNAs are also deregulated in advanced PDAC. These miRNAs and their molecular targets may serve as targets to enhance sensitivity to chemotherapy and reduce metastatic spread.

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## *In vitro* tumorigenicity and stemness characterization of the U87MG Glioblastoma cell line based on the CD133 cancer stem cell marker

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The cancer stem cells (CSC) hypothesis is currently the most widely accepted theory regarding tumour formation and self-renewal ability. The need to find pharmacological compounds or biological agents capable of eliminating CSC makes the search for methods of recognition and isolation of these cells a matter of great urgency.

The aim of the study was to separate CD133+ and CD133- cell sub-populations from the U87MG glioblastoma cell line by magnetic activated cell sorting (MACS), and to analyze the tumorigenic and stem cell differences among four sets of cells: the cell line grown in serum (monolayer group), the neurospheres of the same cell line, and the CD133+ and CD133- sorted cell fractions from the neurospheres.

Our results showed that the neurospheres and the CD133+ cells over expressed stem cell marker genes such as SOX2, nestin, musashi1 and CD133, and formed more colonies in soft agar than the cell groups with less CD133 expression (monolayer and CD133- group). On the other hand the CD133- and the monolayer groups had similar expression levels of CD133, while the CD133- cells expressed higher levels of nestin, SOX2 and musashi1 than the monolayer cells did. In addition, cell migration assessed by the scratching test showed that the CD133- and the monolayer cells migrated more than the neurospheres and the CD133+ cells did. Moreover, the CD133- cells had a higher proliferation rate than the CD133+ cells and the neurospheres, with all of these three groups being cultured in the medium for neurospheres.

In conclusion, we point out the presence of CSC in the CD133- group, as suggested by others. Therefore, CD133+ cells are not necessarily equivalent to CSC. Perhaps CD133+ cells present a higher probability of including CSC than CD133- do. Further functional and genetic analyses must be performed in order to reach optimal isolation of CSC.

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