Unsupervised Analysis of miRNA Expression Patterns in Extracellular Vesicles (EV) Derived from Glioblastoma Multiforme (GBM) Cell Lines Yields Unique miRNA Signature Clusters With Potential Prognostic Importance

Brandon A. McCutcheon MD MPP; Tristan De Mooij MD; Timothy E Peterson MSc; Alexey A Leontovich PhD; Ian F. Parney MD, PhD

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Introduction

There is emerging interest in using tumor derived extracellular vesicles (EVs) from peripherally obtained biofluids for diagnosis, monitoring treatment response, and prognostication. EVs are also of pathophysiologic importance as they interact with the tumor microenvironment via cell-to-cell communication to facilitate oncogenesis.

Methods

Cell cultures from five adult GBM patients were established in stem cell (serum-free) media and subsequently differentiated with 10% fetal calf serum (FCS). EVs were harvested with serial ultracentrifugation using a previously validated protocol. Total RNA was harvested from EV's using the miRNeasy mini kit (Qiagen). Next generation RNA sequencing was performed using an Illumina HiSeq 2000/2500. Read mapping, transcript assembly, hierarchical clustering, and differential expression analyses were performed using validated algorithms on commercially available software.

Results

Unsupervised hierarchical clustering analysis demonstrated two distinct clusters as a function of miRNA expression pattern. Samples bt114, bt132, and bt165 were grouped into cluster A while bt116 and bt120 were in cluster B. When differential transcript expression analysis was performed, ZNF436-AS1 was significantly underexpressed in cluster B (log fold change -4.97, p = 0.0244) while mir100 was significantly overexpressed (log fold change 3.74, p=0.0366) relative to cluster A. An additional 22 RNA species including miRNA, mRNA, and snRNA were expressed at significantly different levels prior to Bonferroni correction. Survival analysis as a function of miRNA clustering was subsequently performed which demonstrated that Cluster A was associated with longer survival (32.8 months versus 11.0 months, p= 0.1402) and increased mean age at diagnosis (72 years vs. 56 years, p= 0.0583). However, these differences were not statistically significant given the current limited sample size.

Conclusions

We identified heterogeneity and unique clustering of miRNA expression patterns in EVs isolated from GBM cell lines. Differences in miRNA signature between distinct EV populations may provide insight into tumor pathogenesis and may be associated with patient survival.

Learning Objectives

1. Understand differences in hierarchical clustering of GBM derived EVs as a function of miRNA signature

2. Understand specific miRNAs that drive differences between GBM derived EV subpopulations

3. Understand potential differences in patient survival as a function of GBM derived EV miRNA expression signature