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Comparison of Extracellular miRNA from Different Lymphocyte Cell Lines and Isolation Methods

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Abstract : The development of a panel of differential gene expression signatures has been of interest in the field of biomarker discovery for radiation exposure. In the absence of the availability of exposed human subjects, lymphocyte cell lines have often been used as a surrogate to human whole blood, when performing ex vivo irradiation studies. The extent of variation between different lymphocyte cell lines is currently unclear, especially with regard to the expression of extracellular miRNA. This study compares the expression profile of extracellular miRNA isolated from different lymphocyte cell lines. It also compares the profile of miRNA obtained when different exosome isolation kits are used. Lymphocyte cell lines were created using lymphocytes isolated from healthy adult males of similar racial descent (Chinese American and Chinese Singaporean) and immortalised with Epstein-Barr virus. The cell lines were cultured in exosome-free cell culture media for 72h and the cell culture supernatant was removed for exosome isolation. Two exosome isolation kits were used. Total exosome isolation reagent (TEIR, ThermoFisher) is a polyethylene glycol (PEG)-based exosome precipitation kit, while ExoSpin (ES, Cell Guidance Systems) is a PEG-based exosome precipitation kit that includes an additional size exclusion chromatography step. miRNA from the isolated exosomes were isolated using miRNEASY minikit (Qiagen) and analysed using nCounter miRNA assay (Nanostring). Principal component analysis (PCA) results suggested that the overall extracellular miRNA expression profile differed between the lymphocyte cell line originating from the Chinese American donor and the cell line originating from the Chinese Singaporean donor. As the gender, age and racial origins of both donors are similar, this may suggest that there are other genetic or epigenetic differences that account for the variation in extracellular miRNA gene expression in lymphocyte cell lines. However, statistical analysis showed that only 3 miRNA genes had a fold difference > 2 at $p < 0.05$, suggesting that the differences may not be of that great a significance as to impact overall conclusions drawn from different cell lines. Subsequent analysis using cell lines from other donors will give further insight into the reproducibility of results when difference cell lines are used. PCA results also suggested that the method of exosome isolation impacted the expression profile. 107 miRNA had a fold difference > 2 at $p < 0.05$. This suggests that the inclusion of an additional size exclusion chromatography step altered the subset of the extracellular vesicles that were isolated. In conclusion, these results suggest that extracellular miRNA can be isolated and analysed from exosomes derived from lymphocyte cell lines. However, care must be taken in the choice of cell line and method of exosome isolation used.

Keywords : biomarker, extracellular miRNA, isolation methods, lymphocyte cell line
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