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| **Differential expression analysis of miRNAs isolated from pleural fluid extracellular vesicles using the Nanostring nCounter® miRNA expression assay** |
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| **Introduction/Aim:**  Pleural fluid cytology involving cell block immunohistochemistry confers variable test sensitivity and specificity for malignant pleural effusions, especially in malignant pleural mesothelioma (MPM). Extracellular vesicles (EVs) in pleural fluids potentially enriched with disease-specific miRNAs could differentiate between non–malignant and malignant pleural effusions, and between specific types of malignant pleural effusions.  **Method:**  This study was approved by The Prince Charles Hospital ethics committee (LNR/2019/QPCH/52409). 16 mL of cell–free pleural fluid underwent two rounds of ultracentrifugation at 100,000 x g (w2t = 5.46e10) for 1 hour 40 minutes at 4 ⁰C, and the pellet resulting from the final ultracentrifugation was used for RNA extraction. EVs derived from pleural fluid were characterised by western blotting analysis and particle analysis by tunable resistive pulse sensing (TRPS) technology and transmission electron microscopy. The miRNAs extracted from pleural fluid extracellular vesicles (PFEV) were profiled by the Nanostring nCounter® miRNA expression assay which screens 827 miRNAs simultaneously.  **Results:**  Twenty–nine pleural fluid samples acquired from donors diagnosed with malignant pleural mesothelioma (MPM; n = 11), lung adenocarcinoma metastatic to pleura (LUAD; n = 11), or non-malignant conditions, including parapneumonic effusions, inflammatory pleuritis or benign asbestos-related pleural effusions (NM; n = 7) were studied. This proof-of-principle study demonstrated that some miRNAs extracted from PFEV expressed at different levels for each disease states of pleural effusion causes (non-malignancy, MPM, lung cancer metastatic to the pleura). Four miRNAs showed significances in differentiating pleural effusions between non-malignant and malignant causes, whereas seven miRNAs were significant in differentiating between MPM and lung cancer metastatic to pleura (*p-values* < 0.05).  **Conclusion:**  miRNAs from PFEV presents a potential and novel bioresource to distinguish pleural effusions due to malignant or non-malignant diseases. Further experiments in a larger study cohort will be necessary to confirm the diagnostic utility of miRNAs derived from PFEV.  **Key Words:**  Pleural fluid, extracellular vesicles, miRNA, Nanostring, lung, mesothelioma  **Nomination for New Investigator Award: No nomination**  **Grant Support:**  This work was supported by The Common Good (The Prince Charles Hospital Foundation) under The Innovation Grant (INN2018-17), The Emerging Research Grant (EM2018-08) and PhD Scholarship (PhD2014-01). |