**Extracellular Vesicle-Associated Circular RNA Signatures to Facilitate an Improved Pleural Mesothelioma Diagnosis**

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**Background and Aims.** Pleural mesothelioma (PM) is an asbestos-induced thoracic cancer associated with poor prognosis, partly due to a lack of reliable diagnostic biomarkers that can facilitate an early and accurate diagnosis. Extracellular vesicles (EVs) constitute promising diagnostic biomarkers due to their abundance, enrichment with disease-specific cargo and stability in circulation [1]. We have previously identified circular RNAs (circRNAs) that are upregulated in PM cells. Here, we examined the expression of six circRNAs across three EV subpopulations to evaluate their potential as diagnostic biomarkers for PM.

**Methods.** Three EV subpopulations (10K, 18K and 100K) were isolated from the conditioned media of PM and non-PM cells using our established protocol [2]. EVs were characterized using transmission electron microscopy (TEM), western blot and ZetaView Advanced Nanoparticle Tracking Analysis (NTA). Total RNA was extracted from the EVs using TRIzol, converted to complementary DNA (cDNA) using a reverse transcription kit and then subjected to droplet digital PCR (ddPCR) analysis. Receiver operating characteristic (ROC) curves were generated to determine the area under the curve (AUC) values of each circRNA.

**Results.** ZetaView, western blot and TEM analyses confirmed the presence of distinct EV subpopulations. PM and other cancer cell types were found to secrete a higher number of EVs compared to the non-malignant controls. The ddPCR analysis revealed three circRNAs, CircC, CircD and CircE, were overexpressed in all three PM-derived EV subpopulations compared to the other cancer type controls (AUC ≥ 0.85). Two circRNAs, CircA and CircB, were enriched by as much as 30-fold in the PM-derived small EVs (100K) compared to the non-malignant controls (AUC > 0.95).

**Conclusion/Discussion.** Specific EV-associated circRNAs demonstrate significant potential to facilitate an accurate differential diagnosis of PM. This study effectively lays the foundation for future studies to evaluate their suitability for a first-of-its-kind liquid biopsy diagnostic technique for PM.

**References:**

1. Wang, X., et al., *Extracellular vesicles as biomarkers and drug delivery systems for tumor.* Acta Pharmaceutica Sinica B, 2025. **15**(7): p. 3460-3486.

2. Ahmadzada, T., et al., *Small and Large Extracellular Vesicles Derived from Pleural Mesothelioma Cell Lines Offer Biomarker Potential.* Cancers (Basel), 2023. **15**(8).