



P3.361: Characterizing Exosomal and Free Circulating microRNAs as Biomarkers and Functional Mediators in Pleural Mesothelioma

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INTRODUCTION

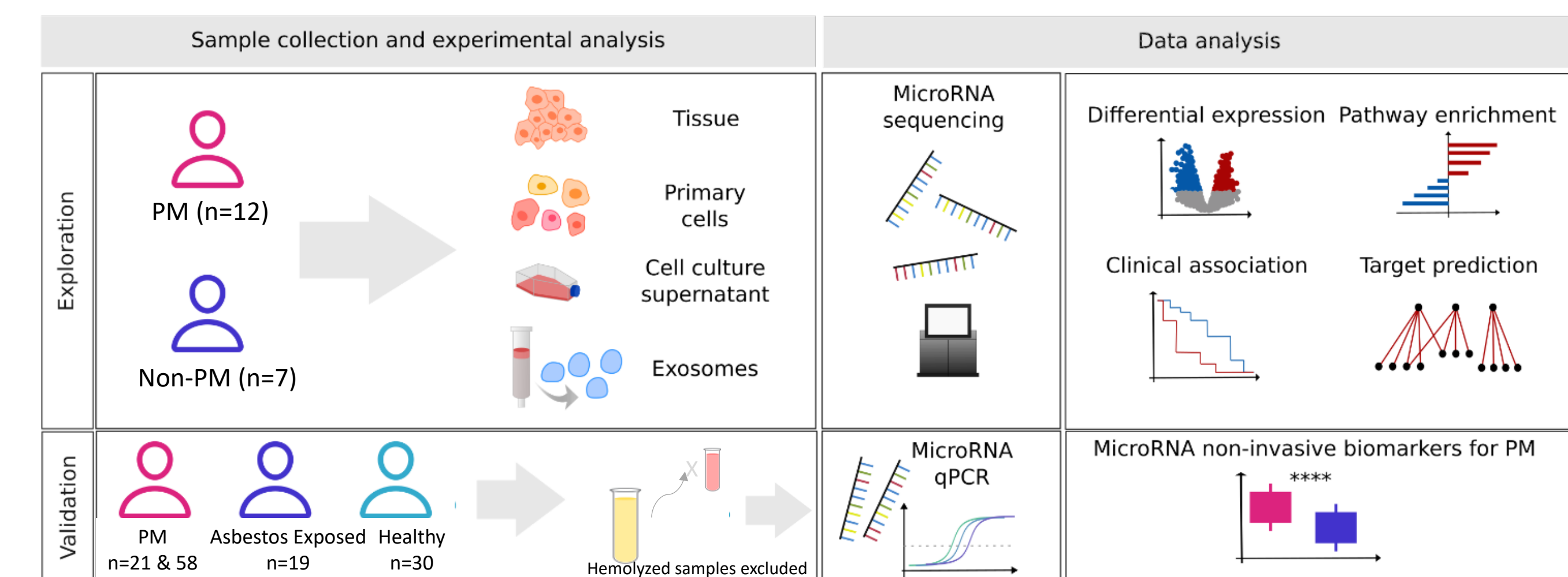
Early detection of pleural mesothelioma (PM) remains a major unmet clinical need. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and have emerged as promising biomarkers in cancer due to their stability in biological fluids. In PM, altered miRNA expression has been associated with disease development, progression, and patient prognosis¹.

Here, we aimed to identify PM-secreted miRNAs with potential utility as non-invasive diagnostic biomarkers. We further sought to compare the miRNA cargo of exosomes with that of freely secreted miRNAs and cellular miRNAs to better understand the mechanisms and patterns of miRNA release in PM. We further aimed to identify secreted miRNAs that may mediate intercellular communication and contribute to the regulation of the tumor microenvironment, thereby influencing disease progression.

METHODS

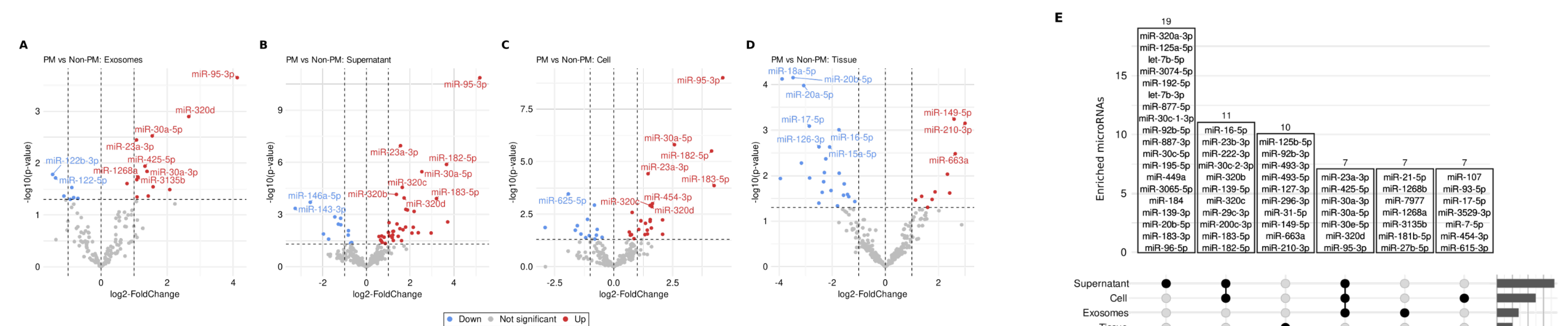
Primary cell cultures were established from pleural effusions of PM (malignant mesothelial cells) and non-PM patients (non-malignant mesothelial cells). Cells were seeded in exosome-depleted medium and cultured for 72 hours, after which the supernatant (200 ml) was collected. RNA was then isolated from 1.2 ml of the supernatant, and the remaining was used for exosomes isolation using iZON qEV columns. Cells and the corresponding tumor tissues were also collected. All RNA extractions were performed using the mirVana PARIS kit.

Sequencing reads were mapped to the human reference genome (GRCh38). Candidates that were either highly expressed or consistently upregulated across cells and the analyzed secreted fractions were selected. Candidate miRNAs were tested in plasma RNAs using miRCURY LNA miRNA PCR Assays (Qiagen) using geometric mean of miR103a-3p and miR23a-3p as normalizer. Cox-regression analysis was performed to identify prognostic significance of the miRNAs.



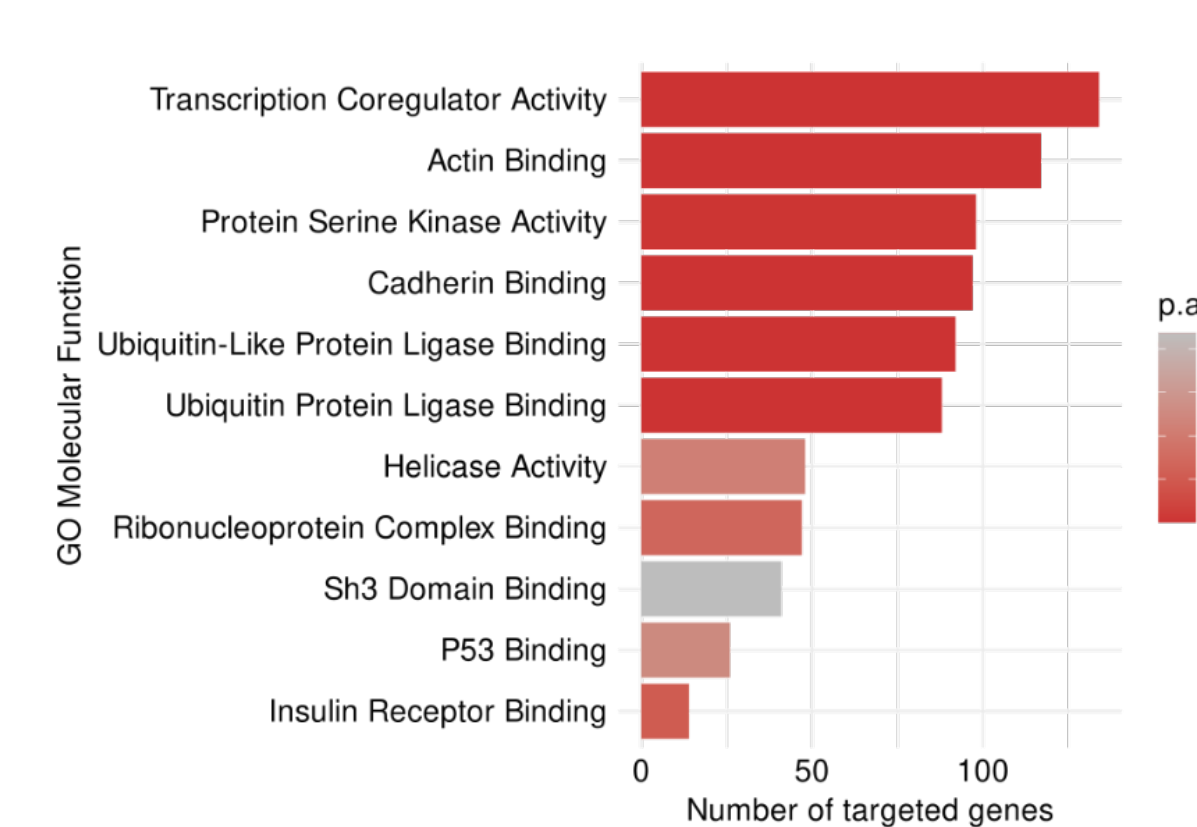
RESULTS

Result 1. Comparative miRNA Expression Between PM and Non-PM Samples



Volcano plots comparing miRNA expression profiles in exosomes (A), total secreted in supernatants (B), cells (C), and tissues (D) from PM and non-PM samples. (E) **Summary of differentially expressed miRNAs** across the analyzed compartments. Nineteen miRNAs were upregulated in the supernatant fraction and seven in exosomes. Seven miRNAs were exclusively upregulated in cells. Eleven miRNAs were commonly upregulated in both the supernatant and cellular fractions, while seven miRNAs were consistently upregulated across all three fractions.

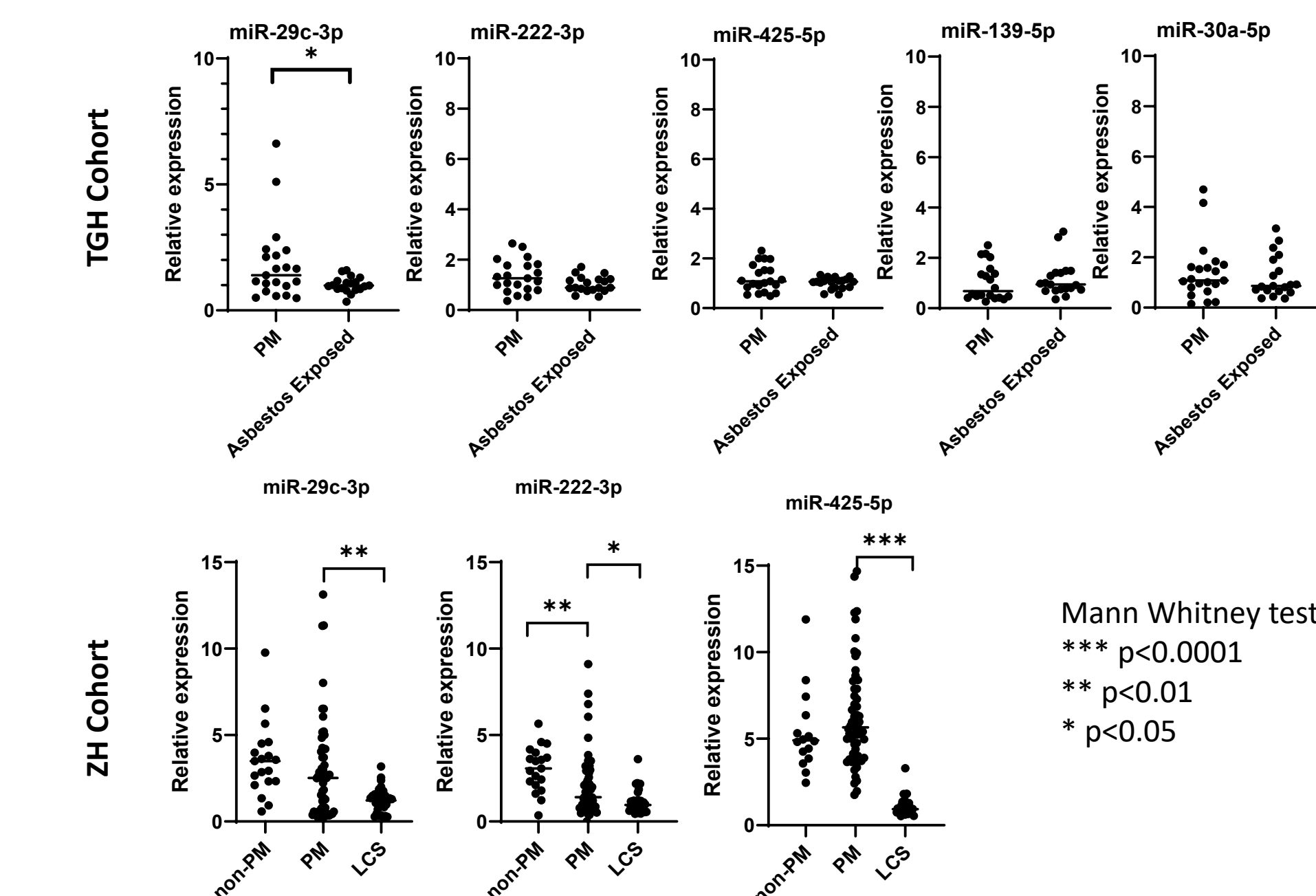
Result 3. Pathway Analysis



Gene Ontology (GO) enrichment analysis of molecular functions associated with experimentally validated target genes of exosomal, secretome, and cellular microRNAs.

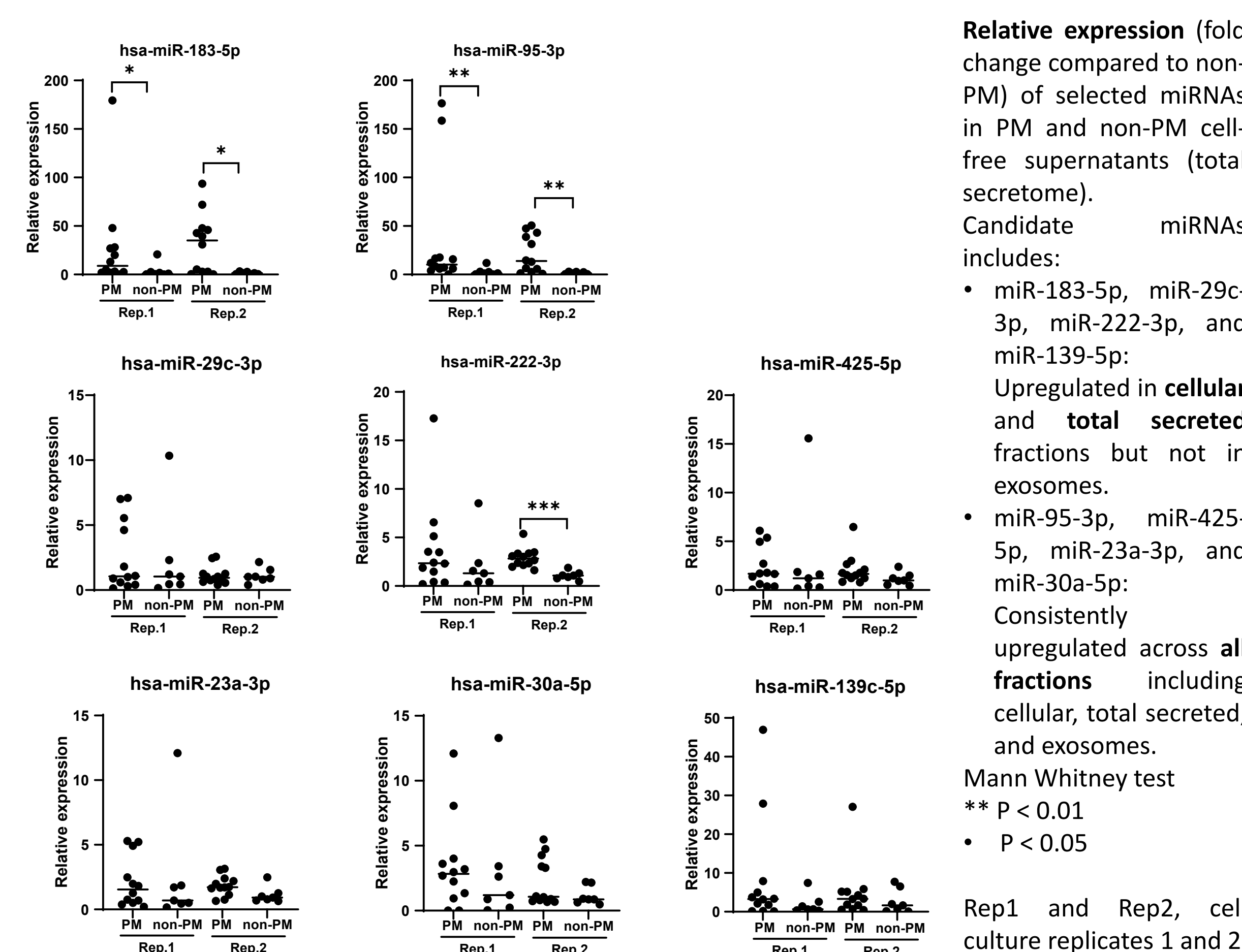
Reactome pathway enrichment analysis of predicted target genes of PM-upregulated miRNAs commonly identified in exosomes, supernatant, and cells. Network edges represent shared target genes between pathways.

Result 4. miRNA in Plasma Samples



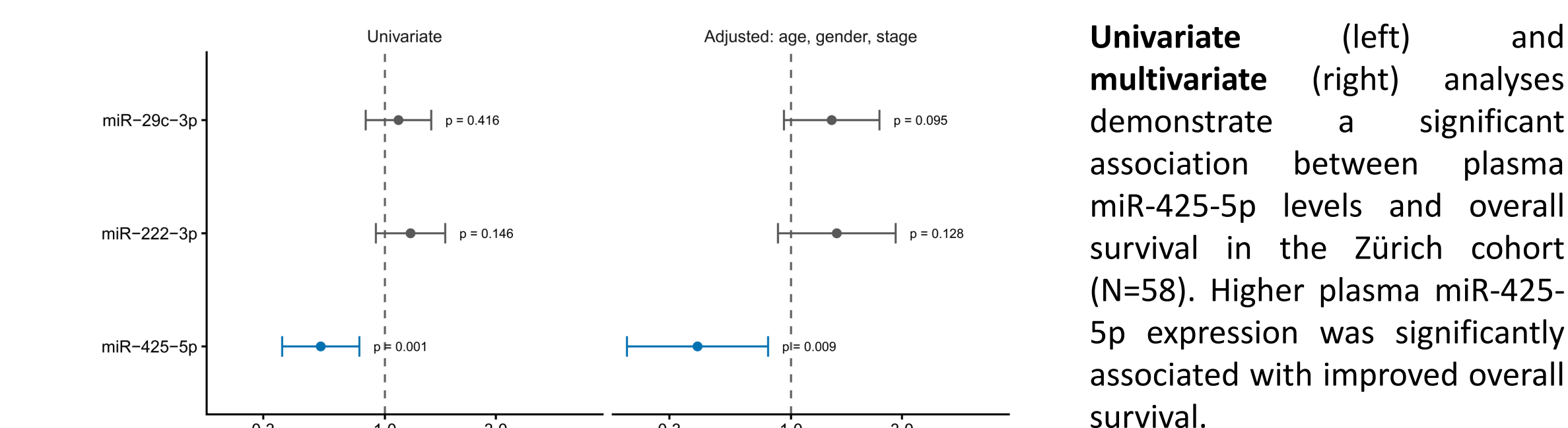
Relative expression (fold change relative to the control group) of selected miRNAs in total plasma. The Toronto General Hospital (TGH) cohort included 21 PM patients and 19 asbestos-exposed controls. The Zürich (ZH) cohort included 58 PM patients and was compared the control group, healthy non-cancer individuals enrolled in a lung cancer screening (LCS) trial (N = 30). Non-PM cohort comprises patients with pleural inflammatory diseases or other malignancies (non-PM; N = 19).

Result 2. Validation by RT-qPCR in Technical Replicates



Relative expression (fold change compared to non-PM) of selected miRNAs in PM and non-PM cell-free supernatants (total secretome). Candidate miRNAs includes: miR-183-5p, miR-29c-3p, miR-222-3p, and miR-139-5p: Upregulated in cellular and total secreted fractions but not in exosomes. miR-95-3p, miR-425-5p, miR-23a-3p, and miR-30a-5p: Consistently upregulated across all fractions including cellular, total secreted, and exosomes. Mann Whitney test ** p<0.01 * p<0.05 Rep1 and Rep2, cell culture replicates 1 and 2

Result 5. Associations Between Plasma miRNAs and Clinical Outcomes



Univariate (left) and **multivariate** (right) analyses demonstrate a significant association between plasma miR-425-5p levels and overall survival in the Zürich cohort (N=58). Higher plasma miR-425-5p expression was significantly associated with improved overall survival.

SUMMARY AND CONCLUSIONS

- We identified both compartment-specific and shared miRNA signatures across cellular, total secreted, and exosomal fractions, highlighting the regulated miRNA secretion mechanisms.
- We confirmed the role of miR-29c-3p as biomarker for the early detection of PM in plasma.
- miR-222-3p and miR-425-5p emerged as biomarkers for differentiating PM from healthy individuals. miR-425-5p demonstrated prognostic significance.
- Tissue miR-29c-3p was associated with clinical outcomes², while miR-222-3p was part of a prognostic miRNA signature used to stratify survival in PM³.
- miR-95-3p and miR-183-5p were among the most significantly upregulated miRNAs in PM cells and were actively secreted. miR-95-3p was also significantly enriched in exosomes.
- Although these miRNAs do not appear to be suitable circulating plasma biomarkers, their enrichment in secreted compartments suggests a potential role in mediating intercellular communication within the tumor microenvironment.
- Functional studies currently ongoing.

REFERENCES & ACKNOWLEDGEMENTS

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