Rapid screening of exosomal RNA cargo for therapeutic research applications.

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1. Introduction

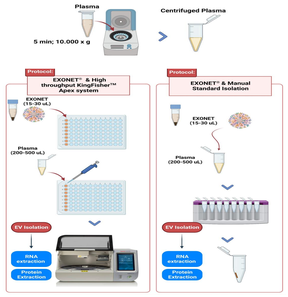
Exosome (EV) RNA therapy is emerging as a pivotal tool in cell and gene therapy, offering several advantages over traditional cell-based methods. Exosomes, as naturally occurring nanocarriers, provide a non-immunogenic, stable, and efficient means of delivering therapeutic nucleic acids. In addition, there are significant advantages in their manufacture, storage and distribution. Exosomes, when engineered through host cell transfection, can effectively deliver therapeutic nucleic acids such as mRNA, miRNA, siRNA, and circRNA. A crucial element in harnessing the full therapeutic potential of exosomes is the development of rapid, efficient methods for their isolation and the characterization of their RNA payload. The aim of this study was to validate a rapid and scalable exosomal miRNA isolation platform for characterizing nucleic acid payloads.

Aim 1: Analytical study: EV RNA and protein isolation and characterisation on high-throughput system vs manual isolation.

Aim 2: Clinical study: RNA-Seq and targeted RT-qPCR analysis : high-throughput EV and RNA isolation from clinical samples (60 ovarian plasma samples).

2. Methods

Fully automated exosomal miRNA isolated from 500µL of plasma (n=60) was performed using a magnetic bead-based affinity capture system (EXO-NET®, Inoviq Ltd) and a high-throughput miRNA extraction chemistry (Maxwell® HT miRNA Plasma and Serum, Promega Corporation) on an automated platform (KingFisher APEX, ThermoFisher). Western blot analysis was used to confirm the presence of canonical protein biomarkers. The EV-RNA content was assessed by RNA-seq using NovaSeq X Plus system (Illumina) and then targeted miRNAs were validated by RT-PCR.



3. High-throughput vs manual isolation

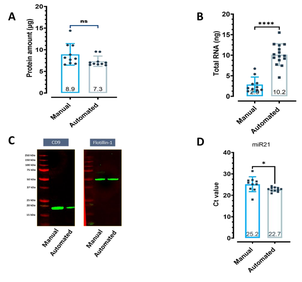
EVs were isolated from human plasma samples (500µL, n=10 each group) using EXO-NET (30µL each sample) manually or on high-throughput automated KingFisher Apex instrument. RNA or protein from isolated EVs were extracted on the same systems using the Maxwell® HT miRNA Plasma and Serum Kit or 1% SDS, respectively. For manual isolation, RNA was extracted using manual ReliaPrep™ RNA Miniprep Systems and protein using 1% SDS.

A. EV protein yield and recovery comparison from both manual and automated methods.

B. RNA yield and recovery comparison from both manual and automated methods. (Unpaired t-test (**** p-value < 0.0001). Data presented as Mean ± SD.)

C. Western blot analysis confirmed expression of CD9 and Flotillin-1 from EV isolated by both manual and automated methods.

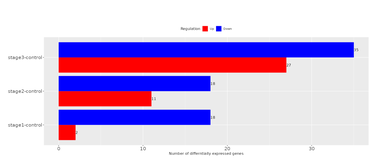
D. RT-qPCR analysis showed higher expression of miRNAs (miR-16 and miR-21) in EV isolated by automated method compared to manual isolation.

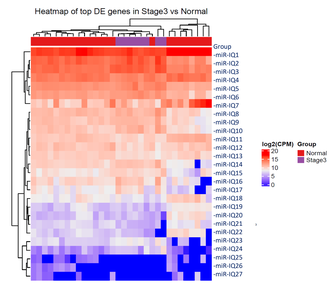


4. RNA-Seq analysis : High-throughput EV and RNA isolation

High-throughput EXO-NET EV isolation and RNA-seq analysis of normal human plasma and ovarian cancer samples. EV were isolated from 500µL plasma samples using EXO-NET (30µL) on automated KingFisher Apex system. Total RNA was isolated from captured EVs on same instrument using Promega Maxwell® miRNA Plasma and Serum kit and RNA-seq analysis was performed on Illumina NovaSeq X Plus instrument.

5. Differentially Expressed Plasma EV miRNA

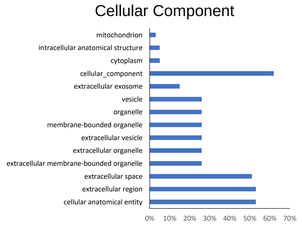


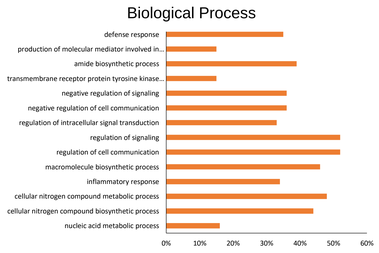


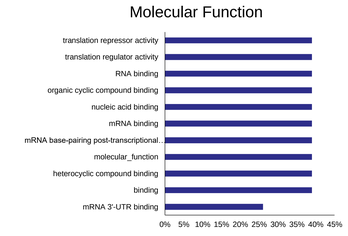
Comparisons	Up	Down
stage3-control	27	35
stage2-control	11	18
stage1-control	2	18

RNA-seq analysis identified 27 significant differentially expressed ovarian EV-associated miRNA from stage 3 cancer samples compared to healthy control plasma samples.

Top GO Pathway Analysis Between Stage 3&4 vs Normal







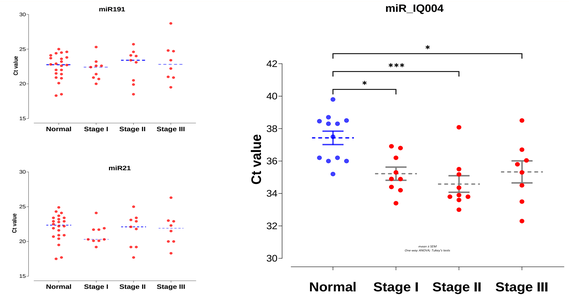
Data modelling including GO and KEGG pathway analysis identified molecular pathways associated with ovarian cancer and EV compartments.

Two thirds of the top abundant miRNAs across the samples were associated with vesicle gene ontologies.

7. Targeted RT-qPCR analysis from 60 ovarian plasma samples

High-throughput EXO-NET EV isolation and targeted miRNAs analysis from normal human plasma and ovarian cancer samples. EV were isolated from 500µL plasma samples using EXO-NET (30µL) on automated KingFisher Apex system. Total RNA was isolated from captured EVs on same instrument using Promega Maxwell® miRNA Plasma and Serum kit. RT-PCR analysis of 16 targeted miRNAs was perfumed (24 controls, 9 stage I, 9 stage II and 8 stage III)

8. RT-qPCR Analysis of Targeted microRNAs



- Expression of miR-191 and miR-21 was observed consistent and relatively similar in all samples which can be used as housekeeping mciroRNA controls for EV samples RT-PCR analysis.
- Out of 16 targeted microRNAs, miR-501 showed significantly upregulated in all stages of ovarian cancer compared to control samples. One-way ANOVA performed, * $p < 0.05$; *** $p = 0.0009$.

9. Conclusions

- miRNA obtained using EXO-NET® and Maxwell® HT miRNA Plasma and Serum Kit on a high-throughput, fully automated platform was fit-for-purpose for **miRNASeq analysis.**
- miRNA obtained using EXO-NET® and Maxwell® HT miRNA Plasma and Serum kit on a high-throughput, fully automated platform was fit-for-purpose for **RT-qPCR analysis.**
- Data modelling identified molecular pathways associated with ovarian cancer and EV compartments.
- We have established and validated a rapid, scalable and automated platform for exosomal RNA isolation for downstream analysis and characterization with an average **sample processing time of < 2 min.**
- The platform holds great promise for facilitating the development and translation of exosome-based companion diagnostics and therapeutics into routine clinical practice.
- Automated miRNA extraction can also be performed on Promega's Maxwell® RSC platform