ABSTRACT

Exosomes are small vesicles (30-150 nm) found in abundance in human body fluids, which function as carriers of different species of RNA and proteins between diverse locations in the body. The spectrum of current scientific interest in exosomes is wide and ranges from studying their functions and pathways to utilizing them in diagnostics and therapeutics development. As such, there is a growing need for quick and easy methods for both isolation of exosomes and analysis of their RNA. We present herein a workflow for isolation of exosomes from serum, plasma, and urine of both healthy donors and patients with prostate cancer. The protocol described herein lays the groundwork for the development of a standardized operating procedure (SOP) for isolation of exosomes and downstream analysis of their constituents, using clinical samples. We demonstrate that cancer-specific RNA signatures residing within the exosomes can be isolated from different patient cohorts. This is the first time this workflow has been tested against the clinical samples. We present herein a workflow for isolation and analysis which entails: (i) Isolation of exosomal RNA and Protein utilizing Exosomal Isolation Reagents; (ii) Characterization of their size distribution and count with NanoSight (© 2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries) analysis (Single-Particle Tracking) (NanoSight Ltd. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license. © 2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries). Exosomal RNA was profiled using the Ion Torrent™ PGM™ (PGM) for mRNA and miRNA profiling.

RESULTS

Simple and efficient recovery of exosomes with Total Exosome Isolation (serum) reagents

- Transfer the required volume of clarified serum to a new tube. The required volume can be calculated as follows:
  - 9 mL of serum can be recovered from 10 mL of serum
  - 6 mL of serum can be recovered from 5 mL of serum

EM analysis of exosomes recovered from cell culture media with Total Exosome Isolation reagent. Immunostaining with COX2 and CD61 antibodies

- Rats were sacrificed and the prostate tissues were collected. The tissues were fixed in 10% formalin and embedded in paraffin. 5-μm thick sections were cut and stained with hematoxylin and eosin. The sections were then examined under a light microscope. The sections were then examined under a light microscope.

RNA sequencing results: exosomes isolated from cell culture media, serum, urine

- Randomly selected exosomal RNA cargo is isolated from cell culture media, serum, and urine using Total Exosome Isolation reagents. The RNA is then analyzed using qRTPCR for mRNA and miRNA expression.

CONCLUSIONS

- Based on our results, exosomes isolated from cell culture media, serum, and urine provide a valuable resource for studying the functional role of exosomal RNA and proteins in various disease states.

REFERENCES


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TRADEMARKS/LICENSES

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