Cell-free DNA distribution in bioliquids: exosome-associated vs. free circulating form

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Exosomes contain a variety of biomolecules including DNA. Knowledge of cfDNA distribution and localization in bioliquid is important for understanding both biological function of cfDNA and exosomes. Some publications state that a large proportion of plasma cfDNA is localized in exosomes. Salting-out technique is associated with aggregation of macromolecules and vesicles and thus leads to overestimation of exosome-associated polymers content, including cfDNA. Ultracentrifugation also may not separate DNA bound to the outer membrane surface from the exosomes. To quantify cfDNA content in free vs. exosomal form in human plasma, urine and saliva, we employed a proprietary compound SubX that has affinity to phosphates groups of the polynucleotide chain and to exosomes via membrane phospholipids. This property of SubX eliminates bias related to both AT/GC content and DNA fragments length during capture, thus improving extraction efficacy and accuracy of downstream applications. SubX technology allows for precipitation of the [SubX-DNA/ SubX-Exosomes] complexes without ultracentrifugation. Excess of SubX molecules in the solution results in oligomerization of up to 10-15 exosomes and formation of micron-size particles. Addition of magnetic silica beads enhances precipitation of [SubX-EV-DNA] conglomerates. SubX also separates cfDNA fragments non-specifically attached to the outer lipid layers of the exosome membrane from the true intra-exosomal cfDNA. Exosomes are easily extracted from the total pellet in exosome reconstitution buffer (ERB), followed by subsequent isolation of tightly bound cfDNA from the [SubX-DNA-Beads] pellet. ERB does not extract DNA form the [SubX -DNA] pellet and therefore does not contaminate reconstituted exosomes with cfDNA. DNA from exosomes is extracted also using SubX protocol. Thus, we can separate two distinct types of extracellular material – intact exosomes and purified cfDNA in a single protocol from the same sample. Our findings show that over 90% of DNA in plasma and urine exist as a free circulating pool, while in saliva up to 30% is associated with exosomes. Therefore, we conclude that cfDNA distribution is probably bioliquid-specific and must be evaluated by methods that eliminate cfDNA-outer exosomal membrane aggregation. SubX technology is suitable for simultaneous isolation of both cfDNA and exosomes from the same bioliquid sample.

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