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A NEW NON-INVASIVE METHOD FOR ISOLATION OF CIRCULATING EXTRACELLULAR VESICLES AND EVALUATION OF ITS SUITABILITY FOR HEMATOLOGICAL MALIGNANCY BIOMARKER DISCOVERY

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Introduction and aim: Extracellular vesicles (EVs) are naturally secreted cellular lipid bilayer particles, which carry a selected molecular content. Due to their systemic availability in biological fluids and to their role in tumor pathogenesis, circulating EVs (cEVs) can be a valuable source of new biomarkers useful for tumor diagnosis, prognostication and monitoring of tumors, probably alternative to traditional biopsy.
However, a precise approach for isolation and characterization of cEVs as tumor biomarkers, exportable in a clinical setting, has not been conclusively established.
This study was conceived to demonstrate the feasibility/employment of serum cEVs as a source of tumor biomarkers by using simple, fast and sensitive procedures that can be complemented by other in-depth analyses.
Methods: We developed a novel and laboratory-made procedure performing a bench centrifuge step which allows the isolation of serum cEVs suitable for subsequent characterization of their size, amount and phenotype by nanoparticle tracking analysis (NTA), different microscopy and flow cytometry, and for nucleic acid assessment by digital PCR.
Results: Applied to blood from healthy subjects (HSs) and tumor patients, our approach, permitted from a small serum volume: i) the isolation of a great amount of EVs enriched in small vesicles, free from protein contaminants; ii) a suitable and specific cell origin identification of EVs, and iii) nucleic acid content assessment. In clonal plasma cell malignancy, like multiple myeloma (MM), our approach allowed to identify specific MM EVs, and to characterize their size, concentration and microRNA content allowing to significantly discriminate between MM and HSs. Finally, EV associated biomarkers correlated with MM clinical parameters.
Discussion and conclusions: EVs are highly stable and easily quantified in serum. Therefore, biological samples can be analyzed with minimal sample processing and the combined use of NTA, flow cytometry and digital PCR for EV routine screening could become a reality for monitoring tumor patients. To our knowledge, this is the first approach that, using only a bench centrifugation step, allows the efficient isolation of protein contaminant-free EVs suitable for subsequent characterization (size, count, surface antigens and nucleic acids).
Overall, our cEV based procedure can play an important role in malignancy biomarker discovery and then in real-time tumor monitoring using minimal invasive samples. From a practical point of view, it is smart (small sample volume), rapid (few hours), easy (no specific expertise required) and requirements are widely available in clinical laboratories.