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A new method of enhancing the sensitivity of tumor-specific extracellular nanovesicle detection in plasma

Nadezhda Nikiforova
National Medical Research Center of Oncology named after N.N.Petrov, Russia

Liquid biopsy is an attractive strategy for diagnosing cancer. Recent studies of the nano-components of circulating blood have opened up a new cancer markers class. For example, extracellular nanovesicles (ENV) are secreted by all types of cells into the extracellular space and circulate with plasma. ENVs retain a tissue-specific pattern of surface proteins. The ENVs secreted by malignant cells are thought to reflect specific characteristics of cancer, and their detection represents a promising approach for cancer diagnosis. Standard approaches to analyze tumor-derived ENVs (tu-ENVs) in plasma suppose consequent steps of ENVs isolation followed by immune-based labeling and quantification. However, this thecnique is not sensitive enough to detect small fraction of tu-ENVs in total population of plasma vesicles. To improve sensitivity of method, we explored 'inverse' technology of tu-ENVs quantification. Firstly, we colored membrane parts of plasma with lipofilic dye (CM-Dil). Secondly, we isolated population of ENVs by size-exclusion chromatography. Thirdly, we quantified tu-ENVs by flow cytometry with immune-beads. This approach allowed us to increase considerably sensitivity of detection of tu-ENVs in plasma. To confirm this statement we mixed plasma with different quantity of colon cancer cell Colo320 derived ENVs. ENVs were isolated by ultracentrifugation, quintified by NTA, and added to the plasma. Three samples of donor's plasma contained 20*1010, 10*1010 and 1*1010 tu-ENVs were processed by proposed method and amount of ENVs positive for specific colon epithelium marker GPA33 was estimated. We obtained gradient of signal (CV,%): 272, 177, 119. Since portion of artifically mixed tu-ENVs could be estimated in a range of 0,1 – 1% of total amount of plasma ENVs, proposed methods provides with exceptional sensitivity for tu-ENVs detection and might by applied for cancer screning.

niki2naden_ka@mail.ru

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