QUANTITATIVE ASSESSMENT OF RNA AND PROTEIN TARGETS ON NBI-ISOLATED EXTRACELLULAR VESICLES FROM THE BLOOD OF METASTATIC COLORECTAL CANCER PATIENTS

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Extracellular vesicles (EVs) are secreted membranous particles intensively studied for their potential cargo of diagnostic markers. An efficient and high-throughput study of EVs is needed to detect somatic alterations of clinical utility at RNA and protein level. We recently designed the nickel-based isolation (NBI) procedure to rapidly isolate EVs preserving their integrity and dispersity in solution, while minimizing vesicle aggregation. Therefore, we combined this approach with a new protocol of droplet digital PCR (ddPCR), allowing for direct encapsulation of EVs into generated oil droplets, to ultrasensitively detect cancer biomarkers from liquid biopsy of oncological patients. From a retrospective cohort of 27 metastatic colorectal cancer (mCRC) patients, we identified somatic BRAF and KRAS transcript mutations matching 100% of concordance with tissue diagnostics and higher sensitivity and specificity compared with immunoenrichment of tumor-derived EVs. We also had a chance to validate the obtained liquid biopsy data in cases where further DNA analyses on additional matching FFPE tissues were available, proposing these strategies as a valuable approach to probe the tumor heterogeneity in advanced states of disease. Here we also show that a quantitative approach of RNA and protein analysis on circulating EVs can be exploited to detect EGFR expression levels in mCRC patients subjected to anti-EGFR therapy.