Identification of miR-X as a universal endogenous control for exosome cargo normalization in human cancer.

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Abstract

Exosomes study in liquid biopsy is a revolutionary new field in biomedical research. However, exosome’s investigations lack standardization measures that allow reliable comparisons between different contexts and pathologies including cancer. The number of exosomal particles circulating in the blood of cancer patients doubles the number identified in healthy individuals, which means much more information in this compartment about a large number of tumor biomarkers of different nature. Among these elements, miRNAs have great biomedical relevance due to their stability and feasible detection in all bodily fluids. To date there is no reliable endogenous control for the exosomal compartment, nor specific for miRNA content, available in the market. In the present study, we identify miR-X as a miRNA with stable levels in the exosomal content independently of many conditions tested. We have firstly identified miR-X from a panel of nine potential normalizers miRNAs arised from an integrative analysis comparing the global miRNA exosomal profile by miRNA-seq of six lung and ovarian cancer cell lines chemotherapy treatment. Its value as normalizer was also tested after radiotherapy and in the exosome miRNA content from eleven additional human cancer cell lines from different tumor types and 114 samples, 68 plasma samples from NSCLC patients, 10 from glioblastoma patients, 10 healthy donors and 13 paired samples from plasma and ascites fluid from ovarian cancer patients. We also analyze its variability and normalizing properties in comparison with the tissue-origin gold standard miR-16. Our results indicate that miR-X is constantly over-represented with minimal variability compared with miR16 in the exosomal content of all samples and conditions tested.