Characterization of small non-coding RNAs in plasma of breast carcinoma patients by next-generation sequencing

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Background: Circulating small RNAs, including microRNAs, have the potential to be used as non-invasive biomarkers for diagnosis, prognosis assessment or monitoring the response of malignant tumors to therapy.

Objectives: The aim of this preliminary study was to characterize small RNA profile in plasma samples of breast carcinoma patients with the help of two next-generation sequencing kits.

Methods: The RNA was extracted from plasma samples obtained from 8 breast carcinoma patients. NEXTFLEX Small RNA-Seq kit v3 and QIAseq miRNA UDI Library kit were used for the preparation of small RNA-Seq libraries.

Results: The second kit was unsuccessful in preparing the libraries with the sufficient concentrations. With the NEXTFLEX kit we obtained an average of about 16.9 million reads per library. The fragments in length corresponding to miRNA molecules (from 16 - 28 bp in length) accounted for 71.6 % of all raw reads. Sequence analysis showed a diverse collection of the small RNA species among which miRNAs were the most abundant, making at least 50.3 % of all raw reads. The most represented miRNAs were hsa-miR-451a, hsa-miR-486-5p, hsa-miR-92a-3p, has-miR-26a-5p, hsa-let-7f-5p, andhsa-let-7a-5p.Selected miRNAs with high, medium, and low expression were subsequently reanalyzed with QIAcuity Digital PCR System. We obtained strong positive correlations of both methods for miRNA with high expression in plasma, but only weak or no correlation in case of miRNAs with medium or low expression.

Conclusion: Our results demonstrate the importance of careful selection of library preparation kits, optimization the sequencing data analysis procedure, and validation of the results obtained from small RNA sequencing.

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