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SciTechnol

Weronika Majer et al., J Clin Exp Oncol 2018, Volume 7
DOI: 10.4172/2324-9110-C1-009

Novel approach in monitoring of clear cell Renal Cell Carcinoma (ccRCC) using molecular biomarkers

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Renal Cell Carcinoma is one of the 10 most common cancers worldwide. There are three major subtypes of RCC; the most occurring is clear cell Renal Cell Carcinoma (ccRCC). The low recovery rate of ccRCC patients is related to late detection and diagnosis. The solution for earlier noninvasive detection might be biomarkers derived from body fluids (blood and urine). The example of potential biomarker is cell free DNA (cfDNA) short fragments of DNA released by tumor cells. According to the recent studies the concentration of cfDNA is significantly higher in cancer patients in comparison to healthy controls. The purpose of our project is to use NGS techniques to identify chromosomal aberrations characteristic for ccRCC in cell free DNA extracted from urine and plasma. cfDNA was isolated from 3 ml of plasma and from ccRCC patients and healthy individuals using QIAamp Circulating Nucleic Acid Kit (Qiagen). Libraries were constructed with NEBNext system and sequenced on HiScanSQ (Illumina). Comparison of cfDNA concentration showed its increased levels in body fluids of cancer patients. The mean concentration of patients cfDNA from plasma was 7.09 ng/ul with average fragments size 150 bp in comparison to healthy controls where amount of cfDNA was 0.05-0.4 ng/ul. DNA sequencing data analysis was performed using WISECONDOR method (WIthin-SamplE COpy Number aberration DetectOR), which detects small aberrations using low-coverage NGS. These results were further analyzed in relation to microarray data (Infinium SNP BeadChip). Comparison of cfDNA and tumor DNA showed differences in number and quality of aberrations. Our results revealed that cytogenetic analysis of cfDNA might enable more in-depth investigation of tumor aberrations. Therefore, there is a necessity to study relations between cytogenetic changes and clinical outcome in larger cohort.

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