Abstract:

In current days, examination of circulating tumor DNA (ctDNA) finds new use across different cancers. It is directed at tumor-derived short fragments of DNA present in peripheral blood of patiens (mainly in advanced stages). Due to its minimal invasivity, almost 100 % specificity and relatively high sensitivity in stage IV patients, this approch found its main potential clinical utility especially in early detection of disease relapse or progression after tumor resection (i.e. post-operative follow-up), prediction and monitoring of therapy response and estimation of prognosis. As a result of minute levels of ctDNA on a high background of other non-tumor DNA fragments present in plasma, a suitable method exhibiting highest sensitivity is the key for proper detection of this marker. The approach is predominantly based on initial identification of a mutation in tumor tissue and its subsequent detection in plasma.

The present work is aimed at optimization of ctDNA isolation and method of its detection based on PCR amplification followed by heteroduplex analysis by denaturing capillary electrophoresis (DCE) to achieve highest sensitivity for detection of mutated fraction in plasma sample. I have applied the optimized protocol to examine ctDNA in three types of cancers, namely colorectal cancer (122 plasma samples), non-small cell lung cancer (30plasma samples) and pancreatic adenocarcinoma (45 plasma samples). Based on obtained results in colorectal cancer patients, I have evaluated a relation between ctDNA levels and surgical radicality, disease relaps or progression and I have also correlated with data from imaging techniques and tumor markers. In adenocarcinoma patients I have detected presence of ctDNA in relation to the disease prognosis and in non-small cell cancer I have tested use of ctDNA for plasma testing of mutations in *EGFR* for decision on targeted biological therapy.

Keywords:

Circulating tumor DNA (ctDNA), cancer, mutation, DNA isolation, PCR, denaturing capillary electrophoresis (DCE), heteroduplex, colorectal carcinoma, non-small cell lung carcinoma, pancreatic adenocarcinoma, radicality of surgical resection, imaging methods, tumor markers, disease prognosis, protooncogene, tumor supressor gene, *KRAS*, *TP53*, targeted biological therapy