Plasma DNA as a biomarker of breast cancer micrometastases

KEYWORDS: Breast Neoplasms; Neoplasm Metastasis; DNA

The main objective of our study is to outline comparative advantages of plasma DNA, relative to the conventional diagnostic tools, in an early detection of breast cancer (BC) metastases. This evaluation includes the published results from selected papers based on literature survey for BC and other prevalent solid tumors.

Detection of minimal residual disease in BC patients may become critically important in order to identify those patients who, after primary therapy, have a high risk of developing distant metastases. As shown recently, patients in this early stage of tumor progression have the best chance to benefit from adjuvant treatment modalities. Although immunocytochemical analysis may demonstrate the prognostic significance of disseminated tumor cells in the bone marrow, successful introduction of this quite cumbersome technique into the clinical setting is rare. As an alternative, recombinant DNA technology has been explored to identify cancer-related biomarkers, thus providing high-speed testing, efficacy, specificity, and sensitivity. However, at the present time the majority of genetic markers in BC have not demonstrated a specific clinical marker role. These markers pertain to the use of intracellular DNA derived from tumor tissue and include genetic changes, such as the activation of oncogenes and inactivation of tumor suppressor genes.

In the last decade, an increasing number of studies have suggested a potential biomarker role of extracellular DNA (1), which circulates freely in plasma of both healthy and diseased people. Although the source of plasma DNA remains enigmatic (2), the presence of its increased concentration in cancer patients may be explained, in part, by micrometastatic tumor shed and subsequent lysis of circulating cancer cells. Recent findings support a potential use of neoplastic genetic alterations in plasma DNA as a new staging tool for detection of minimal residual disease in solid tumor patients.

In general, the methodology is based on PCR technology applied to plasma DNA or RNA samples extracted from venous blood (volume range: 4-12 ml) of healthy donors versus cancer patients (taken preoperatively and post-operatively). The experimental approach includes multimarker analysis of DNA-based biomarkers. Those include oncogene mutations, oncogene amplifications, microsatellite instability and methylation analysis, as monitored by classical PCR, RT-PCR, real-time quantitative PCR, etc.

The following two examples are given as pioneering plasma DNA papers for BC patients. They describe alterations of several plasma DNA genes (3) and presence of tumor-related mRNA in plasma of BC patients (4).

The former report demonstrates the detection of increased concentrations of plasma DNA (mean value: 122 ng/ml) in BC patients (n=41) and describes neoplastic DNA changes before and after mastectomy in both tumor tissue and plasma of patients with no disseminated disease (3). Relationship between histopathological parameters and neoplastic DNA alterations, such as microsatellite markers D17S855, D17S854, D16S541, TH2, D10S197, D9S161; point mutations in the p53 gene, and aberrant methylation of p16 (INK4a), was also analyzed (3). The results revealed persistence of the aberrant DNA after 3 - mastectomy in the subgroup of poor-prognosis BC patients (with vascular invasion, more than three lymph node metastases and higher histological grade at diagnosis), thus implicating undetectable micrometastatic disease (3). The later report (4) has detected increased concentrations of circulating epithelial mRNA derived from the tumor (beta-actin mRNA, CK19 RNA and mammaglobin RNA) in plasma of BC patients (n=45). This was further correlated with poor prognostic features (increased tumor size, proliferative index, and histological stage) and with presence of circulating tumor cells of the patients (4).

Taken together, the described results obtained for BC, as well as for many different cancers, have opened a new research area indicating that circulating nucleic acids might eventually be used for the development of noninvasive diagnostic, prognostic and follow-up tests for detection of minimal residual disease.

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