Abstract

Breast cancer is the most frequent neoplasm in women and a reason of death of over half a million women in the world only in 2012. Development of metastases is related to especially poor prognosis, still the mechanism of metastases formation, both via lymphatic and hematogenous route, is not well understood. In order to improve treatment results new prognostic and predictive factors are needed. For this reason research has been intensified on cancer cells outside of the primary tumours, as these cells were able to pass stages of metastatic cascade. Circulating tumour cells (CTCs) present in blood and metastases to lymph nodes are a selected population of cells, which can differ in molecular markers status (molecular profile) from the primary tumour and provide additional information about the disease. Analysis performed within the submitted thesis concern cancer cells at different stages of dissemination, those present in primary tumour, blood (CTCs) as well in lymph node metastases, all isolated mostly from early breast cancer patients. In the collected material the role of invasion and metastasis markers e.g. those related to epithelial-mesenchymal transition process was analysed. Additionally, a CTCs isolation method was developed, which is independent of epithelial markers, thus allowing for isolation of CTCs which could have already gone through EMT.

The results showed that E-cadherin loss, characteristic for EMT process, in primary tumour was related to lymph node involvement and CTCs dissemination, especially those with mesenchymal phenotype. Expression of CTCs markers was more frequently found in patients with lymph nodes involved; similar detection rate of epithelial and mesenchymal markers was noted. Detection of mesenchymal CTCs markers was related to the increased expression of *CXCR4* and *uPAR* genes in CTCs and more lymph nodes involved. Preliminary results of survival analysis showed that death occurred only in patients who had CTCs detected. Sole expression of vimentin in the CTCs fraction was an independent risk factor of lymph node involvement. Lymph node metastases, in relation to primary tumours, re-expressed E-cadherin and at the same time had an increased expression of genes inducing/sustaining EMT process – *TWIST1, SNAIL, SLUG* and decreased proliferation rate of cancer cells. Elevated levels of *TWIST1* and *SNIAL* gene expression in lymph node metastases, but not in primary tumours, was related to decreased overall survival and disease free survival.

To summarize, molecular profiling allowing for characterization of cancer cells (at different stages of dissemination) for invasion and metastasis markers can deliver additional prognostic information in breast cancer.