The role of selected molecules in biologic behaviour of human breast cancer

Abstract

Ondřej Gojiš

In human breast cancer the rate of immunohistochemical discordance between primary tumour and secondary deposits were reported to range from 28% – 42% for ER and was 17% for PgR. Depending on the study and techniques utilized, discordance rates of Her2 ranged between 0% – 37%. It has been showed that PAX2 competes with SRC-3 for binding and regulation of HER-2 transcription. Human breast cancers that were PAX2 positive and SRC-3 negative had the lowest recurrence rate, and the relationship between PAX2 and SRC-3, with regard to levels that determine relapses, were found to be inversely dependent. This suggests a transcriptional link between the two subtypes of breast cancer, namely ER positive and HER2 positive tumors. Immunohistochemical profiles were performed for 30 metastatic deposits using antigens for SRC3, Pax2, ER, PgR, Her2, CK5/6, Ki67 and EGFR. SRC-3 was positive in 66.6% of cases and Pax2 in 73.3%. No correlation was observed between the patient's age, cancer grade, histotype, lymphatic node status, and protein expression. Using all seven antigens, the primary tumor's protein expression differed from that of the brain deposits in 13 cases out of 14 (93%). Constant expression of SRC3 and Pax2 was seen in 71% and 50%, respectively. The biggest gain was observed with EGFR in 7 cases; and the biggest loss of protein was observed with ER in 8 cases.

CEBPδ study discovered that the gene is a frequent target for down-regulation in primary breast carcinomas as a result of methylation in the CpG island. The data we present are consistent with hypothesis of a tumour suppressor and metastasis suppressor function in human breast cancer. The data showed correlation between methylation and down-regulation of expression in both breast cancer cell lines. This initial study was consequently extended to

investigate the possible role of down-regulation of CEBPδ in breast cancer metastasis. Analysis of paired primary metastatic lesions showed clear down-regulation in the metastases in 50% of cases. The data are clearly consistent with selective pressure for loss of CEBPδ during acquisition of a metastatic phenotype. The presence of methylation in the CEBPδ CpG island in primary breast carcinomas is associated with an increased risk of relapse and of distant organ metastasis. CEBPδ CpG island is frequently methylated in CNS metastases originating from primary breast carcinomas. Methylation in CEBPδ may be acquired during the process of metastasis to the CNS. The current data in early breast cancer is consistent with CEBPδ being a tumour suppressor. Fewer metastasis when CEBPδ is not methylated. Methylation of CEBPδ in the primary tumour is associated with metastasis in the liver, lymph node and skin, while metastases in bone and lung are not significantly influenced by the methylation status of CEBPδ. These data give evidence that CEBPδ is not the only gene contributing to a metastatic profile, multiple additional genes must be at play. We have previously shown the importance of one such candidate CACNA2D3 in the metastatic process. It is also known HER2 positive breast cancer has a predilection to metastasize to the lung and also to the brain and liver. A key question in the management of early breast cancer continues to be risk stratification to identify patients likely to relapse despite being deemed to be at low risk by clinico-pathological parameters. Methylation in the CEBPδ CpG island correlates with a significantly increased risk of metastatic relapse at distant organ sites including brain and liver. In conclusion, the study demonstrated that transcriptional silencing of CEBPδ is associated with metastasis in breast cancer.

As mentioned before, calcium ion channels mediate many biological processes relevant to the malignant potential including metastasis. CACNA2D3 in particular has a number of properties consistent with a tumour and/or metastasis suppressor function as demonstrated by its ectopic expression inhibiting cell growth and adhesion. With regard to breast cancer,

CACNA2D3 lies in 3p21 a region implicated in sporadic breast cancer development. The study showed that CACNA2D3 is a subject to epigenetic regulation in breast cancer cell lines and primary and metastatic lesions via aberrant methylation in the CpG island located in the regulatory elements of the gene. This suggests a tumour suppressor function in the breast cancer and it is consistent with the previous data in other tumour types such as gastric and lung cancer. In the clinical series analysed, the CACNA2D3 CpG island was methylated in both ER-positive and ER-negative breast cancers. The results of the study showed methylation-dependent transcriptional silencing being the mechanistic basis of CACNA2D3 downregulation in breast cancer. CACNA2D3 methylation in three well-characterised clinical series of breast cancers was analysed. The first comprised a panel of CNS metastases derived from patients with breast cancer and all confirmed by histopathology to be metastatic deposits of breast cancer. The methylation at CpG9 within the amplified fragment was most strongly associated with brain metastatic lesions. The presence of a relatively high frequency of methylation in CACNA2D3 in CNS metastases prompted determination whether methylation in primary breast carcinomas is associated with increased risk of recurrence and/or metastasis. In a series of 100 predominantly ER-positive primary breast carcinomas treated adjuvantly with tamoxifen was demonstrated that in primary cancers with CACNA2D3 CpG methylation, there was a significantly increased risk of recurrence, particularly at visceral sites of liver and lung, whereas there was no increased risk of nodal metastases. This finding suggests downregulation of CACNA2D3 is associated with clinically more aggressive disease. Further work is required to understand more closely physiological role of CACNA2D3 in a non-excitable cells such as breast epithelium, and improve understanding of the molecular effects of perturbation or loss of these calcium channel subunits. Interestingly, chromatin immunoprecipitation-based assay in MCF-7 cells has shown that, following treatment with estradiol, CACNA2D3 is negatively regulated by the co-

activator steroid receptor co-activator-3(SRC-3) via ER. In summary, the association of methylation in the CACNA2D3 CpG island with breast cancer metastasis and in particular visceral disease implies that analysis of this gene may be utilised as a biomarker for metastasis and warrants evaluation in larger independent clinical series.

Conclusions:

According to the immunohistochemical results, the studies suggest that not all distant metastases are biologically equal. It gives evidence that re-assessment of protein expression may be useful to optimize oncological treatment. Such patients could then be offered appropriate systemic therapy such as adjuvant chemotherapy.

The CEBPδ may have utility as a biomarker predictive of relapse and metastasis and thus in the identification of patients who may derive greater benefit from adjuvant treatment. Validation of these results in larger, independent cohorts of patients is required.

Further work is also required to understand the physiological role of CACNA2D3 in a non-excitable cells such as breast epithelium, and the molecular effects and mechanisms underlying CACNA2D2 role in malignant breast disease.