Andrea Woltmann

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Pathway enrichment analysis of a genome-wide association study on breast cancer survival: Influence of genetic variation in genes of the adherens junction and the calcium signaling pathway

Fach/Einrichtung: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. Dr. Kari Hemminki

Breast cancer is the most common cancer among women worldwide. Further knowledge about the effects of genetic polymorphisms on BC survival can help to predict the patient's individual risk for disease progression and survival probabilities and to develop new and better therapies and prevention strategies. Genome-wide association studies (GWASs) may help to understand the effects of genetic polymorphisms on inherited breast cancer (BC) progression and survival. However, they give only a focused view, which cannot capture the tremendous complexity of this disease. Therefore, data from a previously conducted GWAS on BC survival was investigated for enriched pathways by different enrichment analysis tools using the two main annotation databases Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The goal was to identify the functional categories (GO terms and KEGG pathways) that are consistently overrepresented in a statistically significant way in the list of genes generated from the single nucleotide polymorphism (SNP) data. The SNPs with allelic p-value cut-offs 0.005 and 0.01 were annotated to the genes by excluding or including a 20 kb up- and down-stream sequence of the genes and analyzed by six different tools. Eleven consistently enriched categories were identified, the most significant ones relating to cell adhesion and calcium ion binding. As the gained pathways are based on genetic variations in the germ line, the comparison of the GWAS pathways with prognostic expression signatures was of interest to connect the germline mutations to somatic events. Therefore, the similarity between the results of the GWAS and the enrichment analyses of twelve published gene expression signatures for breast cancer prognosis was investigated. Five of them were commonly used and commercially available, five were based on different aspects of metastasis formation and two were developed from meta-analyses of published prognostic signatures. This comparison revealed a similarity between the GWAS data and the general metastasis gene signature. As metastasis formation is a strong indicator of a patient's prognosis, this result reflects the survival aspect of the conducted GWAS and supports cell adhesion and calcium signaling as important pathways in cancer progression.

In the conducted pathway enrichment analysis one of the overrepresented categories was the KEGG pathway "adherens junction", which was selected as a promising target for a further association study. There, the investigations concentrated on the nectin-afadin-ponsin (NAP) system, which forms adherens junctions in a calcium-independent manner. It is important for the recruitment of the cadherin-catenin system to the adherens junction and its stabilization. Moreover a role in formation of tight junctions has been suggested, supporting its importance in

cell adhesion and tumor proliferation. The aim of this study was to identify potentially functional SNPs in the genes of the NAP system, which are associated with BC risk or survival. From twelve genes, 35 potentially functional SNPs were chosen for genotyping in a Swedish study population. Twelve SNPs in six genes showed an association with BC risk or BC survival. Especially, the *VCL* gene was identified to play a central role in different cellular adhesion processes, as it harbored four significantly associated SNPs, which have the potential to deregulate the *VCL* expression and thus the whole cellular adhesion forming process which can alter a normal epithelial cell to the malignant, mesenchymal phenotype of a metastasizing BC cell.

The pathway enrichment analysis also revealed a connection of BC survival with calcium ion binding. Therefore, the consistently overrepresented pathway "calcium signaling" was selected as target for the second association study. The focus was laid on eleven genes involved in the storage operated calcium entry. This calcium entry pathway is characterized by the calcium release from the endoplasmatic reticulum into the cytosol and a following calcium influx from the extracellular space to refill the calcium stores. 41 potentially functional SNPs were selected for genotyping in a Swedish study population. Eight SNPs showed significant associations with BC risk or survival or the hormone receptor. The significantly associated SNPs were concentrated in five calcium signaling genes, which were identified as key players in BC progression. Together with a deregulated vinculin protein, they form a signal cascade. The result is the loss of tissue integrity and the transition of a cell of the primary BC tumor into the mesenchymal phenotype, which is able to migrate through the body by calcium regulated focal adhesion turnover.

Altogether, the presented thesis is able to show that cell adhesion and calcium signaling are essential processes in the BC progression from the primary tumor to the invasive phenotype. This is already manifested in the germ line of the BC patients by genetic alterations in the genes of the adherens junction and the calcium signaling pathway, which might add some prognostic value to the inherited BC risk and prognosis.