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Genetic variants in DNA repair genes, metabolizing & transporter genes and T regulatory cell genes, and the association with colorectal cancer prognosis

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Colorectal cancer (CRC) is one of the most common cancers. Even though screening programs and advances in treatment options have increased the 5-year relative survival in European countries to over 60%, there is still room for improvement. Part of this improvement could come from providing patients with individualized therapy; treatment based on the molecular and lifestyle characteristics of a patient. Although numerous studies have been conducted, no molecular markers have been approved for clinical use in CRC, except for KRAS mutational status. One commonly used chemotherapeutic agent for CRC is oxaliplatin. Oxaliplatin is a platinum drug that works through the formation of DNA inter- and intra- strand crosslinks which disrupt the replication process ultimately leading to cell death. Oxaliplatin needs transporter enzymes to cross the cell membrane and can be detoxified through metabolism in the cell. In addition, the DNA damage can be repaired by the DNA repair pathway and there are slight indications that oxaliplatin can cause immunogenic death. Regarding prognosis, different types of immune cells are present within the tumor microenvironment and several studies showed that both immune cell densities as well as the types present influence CRC prognosis. T regulatory cells (Tregs) are a main player in immunity as they are able to suppress activation, proliferation, and function of numerous immune cells.

The main aims of this thesis were therefore: to replicate previous findings regarding glutathione S-transferases (GSTs), which are involved in metabolism, and their predictive and prognostic potential in CRC patients treated with chemotherapy; to investigate the potential of genetic variants in DNA repair genes, genes involved with metabolism and transport, and genes associated with Tregs as predictive markers for oxaliplatin treatment in CRC patients; and to investigate the association between genetic variants in genes associated with Tregs and CRC prognosis.

Data was used from a German population-based prospective patient cohort included in the DACHS study. For the predictive marker analyses 623 CRC patients who received adjuvant or palliative chemotherapy (201 patients received oxaliplatin-based chemotherapy) could be included. Furthermore, 1742 CRC patients with follow-up data available could be analyzed in search for prognostic markers. Detailed treatment and lifestyle information were available. Furthermore, genotype data was available from two Illumina assays, the customized GoldenGate assay and the cytoSNP GWAS assay. The main analyses are all based on Cox regression models adjusted for age, sex, BMI, alcohol intake at diagnosis, TNM stage and grade. For the predictive analyses, a multiplicative interaction term between the SNPs and oxaliplatin was included and evaluated with the likelihood ratio test. Pathway and gene levels analyses were performed using the Gamma method and global test.

The replication analyses for GSTs showed that the GSTM1 copy number variant was differentially associated with overall survival (OS) by oxaliplatin treatment. CRC patients which had two copies of GSTM1 had a non-significant decreased survival when treated with oxaliplatin (hazard ratio (HR) 2.25 (95 confidence interval (CI) 0.93-5.44) compared to those without a copy of GSTM1. The opposite association was seen among patients who did not receive oxaliplatin (HR 0.65 (95% CI 0.46-0.92)). Four SNPs from two genes, MNAT1 and XPC, involved in the nucleotide excision repair (NER) pathway were significantly differentially associated with OS in CRC patients by oxaliplatin treatment after correction for multiple comparisons. The minor allele of rs3783819 (MNAT1) was independently associated with an increased survival (HR 0.51 (95% CI 0.36-0.73)) in patients who received oxaliplatin and a decreased survival (HR 1.36 (95% CI 1.07-1.71) in those who did not. A similar association was found for rs1043953 (XPC). In addition, pathway analyses showed that only the NER pathway was significantly associated with OS in patients who received oxaliplatin. Furthermore, it was shown that the inclusion of multiple marginally associated SNPs did increase the predictive ability of the model. Using a different analytical approach, which started with a screening step using the global test, nine genes involved in transport and metabolism were differentially associated with OS by oxaliplatin treatment. Model selection using backward elimination on the SNPs from these genes resulted in the inclusion of fourteen SNPs from eight genes in the model. Evaluation of the statistical process showed that this leads to a model that increases the integrated Brier score in patients who received oxaliplatin, but not in those who did not. There

were no significant differential associations in the Treg pathway after adjustment for multiple testing. However, several SNP * oxaliplatin interactions were association with p-values below 0.01. The most significant association was found for rs1957519 (GZMB), with similar effect size and direction of the associations as the DNA repair SNPs. Several SNPs from the Treg pathway were marginally associated with OS ($p < 0.01$). The most significant SNP was rs3181096 (CD28) which was associated with a small increase in survival. Replication of these results will be pursued using data from a large international consortium on CRC prognosis.

In conclusion, several SNPs from the investigated pathways were found to be differentially associated with OS by oxaliplatin in CRC patients. However, these results should be replicated in large independent studies before they can be used in clinical practice. Moreover, the discriminative power of the model could be improved by including multiple marginally associated SNPs in a polygenic score. A genome wide association study could be performed to identify further genetic variants as predictive markers for oxaliplatin treatment. The results suggest that genetic variants have importance in personalized medicine in CRC.