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The Influence of Polymorphisms in Oxidative Stress-related Genes on Breast Cancer Risk and Prognosis after Radiotherapy

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The contribution of genetic variants to breast cancer risk and their influence on breast cancer prognosis is still not entirely understood. Since oxidative stress induced by reactive species has been suggested to play a role in carcinogenesis, one hypothesis was that single nucleotide polymorphisms (SNPs) in oxidative stress-related genes may modify the ability to protect from oxidative damage, e.g. by altering functionality of relevant enzymes. As a consequence, these SNPs might have an impact on breast cancer susceptibility as well as on cancer prognosis because several cancer therapies utilize the harmfulness of oxidative stress to destroy tumour cells. In particular radiotherapy, which is routinely used in breast cancer treatment, leads to elevated levels of reactive species and to oxidative DNA damage which may also harm normal tissue. Identification of variants affecting breast cancer risk and prognosis may contribute to a better understanding of disease aetiology and the underlying biological mechanisms as well as may generate new treatment options. At time when this project was initiated, there were only few studies published which comprehensively investigated polymorphisms in oxidative stress-related genes with respect to breast cancer risk and survival, and they were often lacking appropriate adjustment for further relevant factors.

Therefore, the objective of this thesis was to assess associations between 178 SNPs in 38 oxidative stress-related candidate genes and (1.) breast cancer risk, (2.) survival in breast cancer patients, and (3.) the occurrence of late adverse effects of normal tissue after radiotherapy. These three objectives were investigated based on data from the German population-based case-control study MARIE and follow-up data of the patient cohort.

Specific aims of this thesis comprised the identification of common polymorphisms (mainly tagging SNPs) in oxidative stress-related candidate genes, the genotyping of polymorphisms in a subset of MARIE patients and frequency-matched controls, and the follow-up of the MARIE patient cohort (MARIE*plus* study, $MARIE_{RAD}$ study). For the statistical analyses of associations between SNP genotype and the three outcomes, potential risk factors, prognostic factors, and/or treatment modalities were taken into account. Validation of the results was attempted using genotype data from independent study populations.

Associations between 178 SNPs and postmenopausal breast cancer risk were assessed in a subgroup of 1,639 MARIE breast cancer patients and 1,967 controls. Eight polymorphisms with significant

associations were re-evaluated in further 3,726 MARIE participants. External validation was attempted using data from two studies with genome-wide data as well as by considering the results from a published candidate gene study. In conclusion, there was evidence for associations of six genetic variants in *CYBA*, *MT2A* and thioredoxin genes with postmenopausal breast cancer risk in the MARIE study. Two variants in *CYBA* (rs3794624) and in *TXN* (rs2301241) were confirmed summarizing evidence from up to four study populations. For *CYBA*_rs3794624, the odds ratio (OR) per allele was 0.93 (95 % confidence interval (CI) 0.87-0.99), while *TXN*_rs2301241 was associated with a moderate increase in breast cancer risk: OR per allele was 1.05 (95 % CI 1.00-1.10).

1,414 breast cancer patients (MARIE*plus* study) were included in the genotype association analysis regarding overall and breast cancer specific survival. After a median follow-up time of six years, 202 (14 %) of the 1,414 genotyped MARIE*plus* breast cancer patients deceased. 14 SNPs in *ATM, CCND1, CDKN2A*, *MT2A*, *NQO1*, *PRDX1*, *PRDX6*, and *TXN* were associated with overall survival in the MARIE*plus* study. Seven of these SNPs were re-evaluated in two Scandinavian study populations (HEBCS, SASBAC), but none of them were replicated. However, *PRDX6*_rs4916362 was consistently associated with an elevated risk of death from any cause in all three study populations with hazard ratios (HR) per allele in MARIE*plus* of 1.33 (95 % CI 1.06-1.66), in HEBCS: HR 1.01 (95 % CI 0.83-1.22), and in SASBAC: HR 1.08 (95 % CI 0.87-1.33). The lack of association in the replication studies may be due to the heterogeneity between the study populations.

Associations between genotype and late adverse effects of normal tissue were assessed in up to 363 $MARIE_{RAD}$ breast cancer patients treated with conventional radiotherapy after breast-conserving surgery. A study physician evaluated these late adverse effects by physical examination according to standardized EORTC/RTOG scoring after a median follow-up time of 5.6 years. Approximately 10 % of the $MARIE_{RAD}$ patient developed late adverse normal tissue reactions. SNPs with lowest *p*-values were re-evaluated in 390 patients from the ISE-2 study on radiosensitivity. Two SNPs in *NQO1* in high linkage disequilibrium (rs10517 and rs2917667) significantly decreased the risk for skin alterations in the $MARIE_{RAD}$ study and were replicated in the ISE-2 study: In $MARIE_{RAD}$, OR for carriers of the minor T allele of *NQO1*_rs10517 was 0.27 (95 % CI 0.08-0.79), and in ISE-2, $OR_{rs10517}$ was 0.44 (95 % CI 0.24-0.77). SNPs in *ATM, TNF, TXN*, and *TXN2* were found to be significantly associated with fibrosis in one of the two study populations. Since the analysis on radiosensitivity was based on low numbers of events, results have to be interpreted with caution until they are confirmed by larger independent studies.

In summary, this thesis provided evidence for SNPs in *CYBA, MT2A, NQO1*, peroxiredoxins, *TNF* and genes of the thioredoxin system to be associated with breast cancer risk, survival, and/or radiation toxicity. These findings may be a basis for further functional investigations which may elucidate the underlying biological mechanisms of our results.