Abstract

Title of the Thesis: Molecular Analysis of DNA Damage Response Pathways in Breast Cancer

It is evident that DNA damage response (DDR) pathways have important role in tumorigenesis. In this study, alterations of key regulatory genes of two important DDR pathways, i) homologous recombination repair (HRR) and ii) mismatch repair (MMR) pathway were analyzed in pretherapeutic and neoadjuvant chemotherapy (NACT) treated breast carcinoma (BC) samples and two BC cell lines MCF-7 and MDA MB 231 to understand the importance of these pathways in development and chemo-tolerance of BC. The alterations of these genes were correlated with clinicopathological parameters of pretherapeutic (n=133) and NACT treated (n=45) samples.

In pretherapeutic samples, prevalence of CD44 and PCNA positive cells were significantly high than NACT samples. In HRR pathway, high deletion/methylation (29-68%) and 64-78% overall alterations were found in BRCA1/BRCA2/FANCC/FANCD2 genes in both pretherapeutic and NACT treated samples. Interestingly, ER/PR negative tumors showed significantly high alterations in pretherapeutic samples even in NACT treated samples. Similarly, in MMR pathway, high frequencies of deletion/methylation (32-62%) and 58-71% overall alterations of MLH1/MSH2 genes were found in both pretherapeutic and NACT treated samples. The promoter methylation of HRR/MMR genes was validated in the BC cell lines by demethylating experiments. Reduced expression (mRNA/protein) of these genes showed concordance with their molecular alterations (deletion/methylation). Both HRR and MMR pathways were altered in 87% of the pretherapeutic samples and 78% of the NACT treated samples. This indicates that alterations of these pathways are necessary for development of BC.

To understand the importance of HRR/MMR genes in chemo-tolerance of BC, MCF-7 and MDA MB 231 cells were treated with two anthracycline anti-tumor antibiotics doxorubicin and nogalamycin. It was evident that these drugs could induce the expression this might be (mRNA/protein) of the HRR/MMR genes at different concentrations (< IC50, IC50, > IC50).

This might be due to the hypomethylation of these genes as seen in the cell lines as well as in the NACT treated samples. It seems that the hypomethylation might be due to reduced expression of DNA methyltransferase-1 as seen in our study. This indicates that epigenetic modifications might have important role in chemo-tolerance of BC.

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