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Molecular Characterization of Estrogen Receptor Beta Variants; Cancer Cell Proliferation and Invasion

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ABSTRACT

Estrogen plays crucial roles in the pathogenesis of breast cancer. Most of the known effects of estrogen signaling are mediated by estrogen receptors (ERs), ERα and ERβ. ERα is explored for breast cancer molecular classification and is a target of endocrine therapy. The discovery of the second ER (ERβ) including its variants led to a need for re-evaluation of the biology of estrogen. This thesis aims to characterize molecular aspects of ERβ variants and provide knowledge to elucidate roles of ERβ variants in tumorigenesis with focus on breast cancer.

In **PAPER I**, we determined the frequency of a novel human ERβ isoform, human ERβ548 (hERβ548), which had been demonstrated to display different functional characteristics than wild-type ERβ, in several populations including African (n = 96), Caucasian (n = 100), and Asian (n = 128) subjects. We did not detect any alleles that correspond to hERβ548 in these samples or in additional samples of heterogeneous origin. This study concluded, for the first time, that hERβ548 is not a common variant in Africans, Caucasians, or Asians.

In **PAPER II**, we identified five novel polymorphisms in the ERβ gene in an African population. Two of these variants, I3V and V320G were expected to change the amino acid sequence of the ERβ protein. Compared to the wild-type ERβ, the V320G variant showed significantly decreased maximal transcriptional activity in the ERE mediated reporter assay. A pull-down assay and surface plasmon resonance analysis revealed that the decreased transcriptional activity of the novel ERβ variant hERβV320G was associated with weaker interaction with a co-factor, TIF2.

In **PAPER III**, we assayed the interaction of several known ligands with mouse ERβ1 (mERβ1) and mouse ERβins (mERβ2). A significant difference in ligand binding properties was observed. Our results suggest that ligand selectivity and co-activator recruitment of ERβ isoforms constitute additional levels of specificity that influence the transcriptional response in estrogen target cells in mouse.

In **PAPER IV**, 202 clinical patient specimens, different non-small cell lung cancer (NSCLC) cell lines and transgenic mouse models were used to investigate the role of the EGFR signaling pathway for tumorigenesis of NSCLC. We showed that activation of the EGFR pathway or hypoxia could promote cell invasion but not survival. Furthermore, we demonstrated that the HIF-1α/MET axis is involved in both EGFR and hypoxia induced signaling pathways, leading to cancer cell invasiveness.

In **PAPER V**, a breast cancer cell line BT549 that endogenously expresses the hERβ variant hERβ2 in the absence of hERα and hERβ was used to study the effects of hERβ2 signaling on breast cancer cell behavior and associated molecular mechanisms. Our data indicate that hERβ2 promotes proliferation and invasion in this cell line. A total of 263 genes were identified as hERβ2-upregulated genes and 662 identified as hERβ2-downregulated genes. hERβ2-regulated genes were involved in cell morphology, DNA replication and repair, cell death and survival. Based on our data, we hypothesize that effects of hERβ2 on proliferation and invasion were mediated via repression of prolyl hydroxylase 3 (PHD3) gene expression and induction of protein levels of the hypoxia induced factor 1 (HIF-1α) and MET.

In conclusion, the studies presented in this thesis contribute to the knowledge of the function of ERβ variants, and give additional insight into the molecular mechanisms underlying cancer cell proliferation and invasion.

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