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Molecular characterization of estrogen receptors with focus on breast cancer

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Milica Putnik

Huvudhandledare: Professor Karin Dahlman-Wright Karolinska Institutet Institutionen för Biovetenskaper och Näringslära

Bihandledare: Docent Chunyan Zhao Karolinska Institutet Institutionen för Biovetenskaper och Näringslära

Fakultetsopponent: Ph.D Jason S. Carroll Cambridge Research Institute Li Ka Shing Centre

Betygsämnd: Docent Svetlana Lagercrantz Karolinska Institutet Institutionen för molekylär medicin och kirurgi

Docent Annika Wallberg Karolinska Institutet Institutionen för miljömedicin

Docent Afshin Ahmadian Kungliga Tekniska Högskolan Institutionen för bioteknologi

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ABSTRACT

Estrogen signaling is mediated by estrogen receptors (ERs), ERα and ERβ. Aberrant estrogen signaling is involved in breast cancer development. ERα is one of the key biomarkers for diagnosis and treatment of breast cancer. Unlike ERα, ERβ is still not introduced as a marker for diagnosis and established as a target of therapy. Numerous studies suggest antiproliferative effects of ERβ, however its role remains to be fully explored. Albeit important, ERα is not a perfect marker, and some aspects of ERα function are still unclear. This thesis aims to characterize distinct molecular facets of ER action relevant for breast cancer and provide valuable information for ER-based diagnosis and treatment design.

In PAPER I, we analyzed the functionality of two common single nucleotide polymorphisms in the 3’ untranslated regions of ERβ, rs4986938 and rs928554, which have been extensively investigated for association with various diseases. A significant difference in allelic expression was observed for rs4986938 in breast tumor samples from heterozygous individuals. However, no difference in mRNA stability or translatability between the alleles was observed.

In PAPER II, we provided a more comprehensive understanding of ERβ function independent of ERα. A global gene expression analysis in a HEK293/ERβ cell model identified a set of ERβ-regulated genes. Gene Ontology (GO) analysis showed that they are involved in cell-cell signaling, morphogenesis and cell proliferation. Moreover, ERβ expression resulted in a significant decrease in cell proliferation.

In PAPER III, using the human breast cancer MCF-7/ERβ cell model, we demonstrated, for the first time, the binding of ERα/β heterodimers to various DNA-binding regions in intact chromatin.

In PAPER IV, we investigated a potential cross-talk between estrogen signaling and DNA methylation by identifying their common target genes in MCF-7 cells. Gene expression profiling identified around 150 genes regulated by both 17β-estradiol (E2) and a hypomethylating agent 5-aza-2’-deoxycytidine. Based on GO analysis, CpG island prediction analysis and previously reported ER binding regions, we selected six genes for further analysis. We identified BTG3 and FHL2 as direct target genes of both pathways. However, our data did not support a direct molecular interplay of mediators of estrogen and epigenetic signaling at promoters of regulated genes.

In PAPER V, we further explored the interactions between estrogen signaling and DNA methylation, with focus on DNA methyltransferases (DNMT1, DNMT3a and DNMT3b). E2, via ERα, up-regulated DNMT1 and down-regulated DNMT3a and DNMT3b mRNA expression. Furthermore, DNMT3b interacted with ERα. siRNA-mediated DNMT3b depletion increased the expression of two genes, CDKN1A and FHL2. We proposed that the molecular mechanism underlying regulation of FHL2 and CDKN1A gene expression involves interplay of DNMT3b and ERα.

In conclusion, the studies presented in this thesis contribute to the knowledge of ERβ function, and give additional insight into the cross-talk mechanisms underlying ERα signaling with ERβ and with DNA methylation pathways.

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