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Modulation of Nuclear Receptor Signaling by RBR Ubiquitin Ligases

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ABSTRACT

Nuclear receptors (NRs) constitute a superfamily of transcription factors and play important roles in physiology. Transcriptional regulation by NRs can be modulated through interactions with various coregulators that activate or repress transcription through mediating receptor and chromatin modifications as well as communicating with the general transcription factor machinery. Coregulators also affect NR protein stability, which led to the discovery that the ubiquitin-proteasome system regulates transcriptional activity of certain NRs. However, only a few E3 ubiquitin ligases, that mediate the substrate specificity in the ubiquitin-proteasome system, have been identified as NR coregulators. The overall aim of this thesis was to identify novel RING-in-between-RING (RBR) E3 ubiquitin ligases that modulate NR signaling.

In the first study of this thesis, we provide evidence that the RBR ubiquitin ligase RNF31 acts as a novel coregulator for the NR DAX-1 in steroidogenesis. We demonstrate that RNF31 interacts with, and monoubiquitinates, DAX-1 and maintains DAX-1 stability. RNF31 is necessary for the formation of a ternary corepressor complex of RNF31, DAX-1 and SMRT on the DAX-1 target gene promoters CYP19 and Steroid Acute Regulatory protein.

In the second study, we identify the RBR ubiquitin ligase RBCK1 to be a novel cell cycle in breast cancer cells through modulating expression of the cell cycle regulators Cyclin B1 and Estrogen Receptor α (ERα). We demonstrate recruitment of RBCK1 to the breast-cancer associated ERα promoter B and find in several independent studies that RBCK1 mRNA correlates with ERα mRNA expression in breast cancer.

In the third study, we demonstrate that RBCK1 interacts with ERα and enhances ERα transcriptional activation of its own promoter. Further, we show occupancy of the RBCK1-interacting protein Protein Kinase C beta 1 ($PKC\beta_I$) at the ERa promoter B. Consistent with this, $PKC\beta_I$ modulates ERα expression. A ternary complex of ERα, RBCK1 and $PKC\beta_I$ on the ERα promoter B correlates with histone modifications associated with a permissive chromatin environment. Taken together, the two final studies suggest an ERα coactivator function of RBCK1 at the ERα promoter.

In conclusion, the papers included in this thesis demonstrate that the RBR ubiquitin ligases RNF31 and RBCK1 are novel NR-interacting proteins that modulate NR-dependent transcription through non-proteolytic coregulatory functions. Both the ligases are recruited to receptor target gene promoters and are necessary for formation of transcriptional complexes associated with repression and activation, respectively. These findings clearly support a coregulatory function of E3 ubiquitin ligases in NR signaling beyond degradation.

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