論文の内容の要旨

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論文題目

To purify and identify the novel androgen receptor cofactors in prostate cancer cells
(前立腺癌における新規アンドロゲン受容体転写共役因子群の精製及び機能解析)

Chapter 1 Introduction

The male sex hormone androgens are critical for expression of male phenotype including development and maintenance of secondary male characteristics. The physiological effects are outputted via diverse gene expression that are mainly mediated by its cognate receptor, androgen receptor (AR) which is a ligand-inducible transcription factor. Due to the extensive physiological role of AR, abnormal AR signaling is implicated in a wide variety of disorders including prostate cancer.

AR signaling start from the circulating androgens directly penetrate across cell membrane of target cells and bind to the inactive cytoplasmic AR. The androgen-bound AR undergoes conformational change, dimerization and translocates from cytosol to the nucleus where it binds to specific DNA sequences known as androgen-responsive elements (AREs) and recruits a series of coregulators (also termed cofactors) that exhibit distinct functions to regulate a vast number of target gene transcription.

More than 150 AR coregulators have been identified through basic molecular and

biochemical approaches. Recent studies revealed the crucial role of chromatin architecture in gene regulation and also emerged many of these AR coregulators mediate transcription through building appropriate chromatin environment. On the other hand, it has implicated that the posttranslational modifications of AR or AR complex components have a critical role for quantitative /qualitative regulation of AR. However, the mechanism by which these coregulators regulate AR posttranslational modifications and affect target gene expression is ill-defined.

In addition, given the advance of global genome-wide mapping technology, the function of AR coregulators as well as their specific target genes have been analyzed and brought new insights into AR transcriptional regulation.

The aim of these study is to reveal a novel AR transcriptional regulation mechanism by identifying AR coregulators. For this purpose I adopted biochemical purification of the AR-associated proteins, and investigated the AR quantitative /qualitative regulation as well as the global genome-wide transcript profiling by which these coregulators.

Chapter 2 Identification of novel AR transcriptional coregulators in prostate cancer cells

Identification of the AR-associated proteins using antibody affinity purification

To purify the AR-associated proteins in prostate cancer cells, I performed the anti-AR antibody affinity protein purification. The endogenous AR-associated proteins were subjected to LC-MS/MS analysis. In addition to the known AR coregulator PRMT5, several proteins were identified as AR coregulator candidates. Among these candidates, Ubiquitin Specific Protease 7 (USP7) was focused because of its high score identified by LC-MS/MS.

Ubiquitination is a reversible posttranslational modification that provides a tag either marking the labeled protein for degradation or modulates its function. Ubiquitination can be reversed by deubiquitinating enzymes (DUBs) such as USP7 through removing ubiquitin from target proteins. It is known that ligand-dependent ubiquitination of AR plays an important role in AR transcriptional regulation, including act as a scaffold for coactivators recruitment or transcriptional activation-coupled proteasomal degradation. However, the precise mechanism by which these ubiquitin network mediate AR transcriptional activity is poorly understood. To investigate the role of USP7 in AR transcriptional regulation may be helpful to clear the intricate ubiquitin network in AR signaling.

USP7 is a novel AR-associated protein in prostate cancer cells

To validate the interaction between USP7 and AR, co-immunoprecipitation was performed in potent androgen DHT-treated prostate cancer cells LNCaP and 22Rv1. The result confirmed the LC-MS/MS identification and revealed that the interaction of USP7 and AR was DHT-dependently enhanced. To investigate whether USP7 modulates the ubiquitination of AR, *in vivo* ubiquitination assay was carried out. As a result, overexpression of USP7 attenuated ubiquitinated AR produced by DHT, suggesting USP7 may participate in AR transcriptional activity regulation through control of AR ubiquitination status.

Chapter 3 The elucidation of USP7 functional role in AR transcriptional regulation

USP7 associates with AR on AREs upon rapid DHT stimulation

Previous studies indicated that USP7 mainly localizes in nucleus, hence the investigation was focused on chromatin region. The time course recruitment of USP7 to AREs was assessed using ChIP-qPCR. The recruitment of USP7 to AREs increased upon 1 hr DHT treatment and declined rapidly, suggesting USP7 was involved in the early stage of AR transcriptional activation. In addition, the interaction of USP7 and AR on AREs was confirmed using re-ChIP. This result exhibited that the association and disassociation of USP7 and AR on AREs upon rapid DHT stimulation.

USP7 regulates AR transcriptional activity and specificity

Given the gene specific regulation manner of AR coregulators, I profiled the global effect of USP7 knockdown on androgen-dependent gene transcript using RNA-seq analysis.

Approximately 45% of androgen-dependent gene expression were affected by USP7 knockdown, indicating the essential role of USP7 in AR signaling. In addition, USP7 functioned as a coactivator in DHT up-regulated genes, whereas it acted as a corepressor in DHT down-regulated genes. This result suggested that USP7 is able to mediate AR transcriptional activity and specificity.

USP7 regulates liganded AR protein stability and chromatin recruitment

To investigate the mechanism by which USP7 regulates AR transcriptional activity, I observed the effect of USP7 knockdown on AR chromatin recruitment. The result indicated that AR binding to AREs was diminished by USP7 knockdown. Since USP7

attenuated ligand-induced ubiquitinated AR, it was expected that USP7 facilitates AR chromatin binding through receptor stabilization. To corroborate this speculation, I observed the effect of USP7 overexpression on liganded AR protein degradation rates. The result revealed that the liganded AR protein half-life was prolonged by USP7.

Chapter 4 Conclusion and Discussion

To decipher the new AR transcriptional regulation mechanism, the endogenous AR-associated proteins in prostate cancer cells were affinity purified, and the DUBs USP7 was identified as a novel AR coregulator.

USP7 facilitates AR binding to AREs and activates gene transcription through attenuating ligand-induced ubiquitinated AR and receptor stabilization. By strictly controlling the dynamic USP7 association and disassociation, the AR transcriptional activity can be precisely fine-tuned.

In addition, the global profiling of USP7 knockdown effect on androgen-dependent gene transcripts supposed the gene specific regulation manner of USP7. Given that AR-mediated gene repression versus activation via distinct mechanisms, suggesting USP7 may regulate AR target gene expression through multiple pathways.

To conclude, this study biochemically identified USP7 as a novel AR coregulator which is required for mediation of AR transcriptional activity and specificity, thereby broadens the view of ubiquitin network in gene regulation.