Identification of the USP15-binding proteins

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摘要

轉化生長因子（Transforming growth factor-β；TGF-β）的訊息傳遞在調控細胞功能上伴演一個重要的腳色。過去研究指出，TGF-β 訊息傳遞在早期癌細胞中扮演腫瘤抑制因子，反之在腫瘤細胞晚期則扮演腫瘤促進因子，本實驗室先前研究指出，細胞在 TGF-β 的刺激下，透過 PI3K/AKT 去促進 USP15（Ubiquitin specific protease 15）轉譯。大量表現 USP15 可以維持細胞中 p53 穩定性。我們先前研究指出，在 p53 默化的細胞中表達 USP15 會增加細胞的凋亡。所以 USP15 會調控與 p53 不相關的細胞凋亡機制。由於 USP15 在此作用與機轉尚未明瞭，本次研究透過免疫共沉澱分離出 142 種可能為 USP15 的結合蛋白，我們選用兩個 USP15 的結合蛋白分別為 Pyrroline-5-carboxylate reductase 1（PYCR1）與 Pyrroline-5-carboxylate synthase （P5CS），兩者為 Proline 代謝作用中的重要腳色。先前文獻指出癌細胞調控致癌蛋白(MYC) 或 腫瘤抑制蛋白(p53) 去改變 Glutamate-Proline 代謝以利癌細胞生長與避免細胞凋亡。我的研究將分析 USP15 是否會調控 PYCR1 以及 P5CS 的穩定進而參與細胞凋亡機制。首先我們利用 GST pull down assay 分析 USP15 是否直接與 PYCR1 以及 P5CS 結合。另外我們在默化 p53 的細胞中表達 USP15 ，觀察是否影響 PYCR1 與 P5CS 蛋白的穩定。

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Abstract

Transforming growth factor-β (TGF-β) plays a critical role in regulation of cancer progression. The previous studies indicated that TGF-β can act as a tumor suppressor in the early stages of progression. However, TGF-β can also function as a tumor promoter in the advanced stages of cancers. The recent study in our lab has demonstrated that TGF-β signaling can up-regulate the translation of USP15 via the PI3K/AKT pathway to promote p53 stability. In addition, USP15 can competitively recognize MDM2/p53 complex to form a stable complex USP15/p53, allowing that p53 suppresses the tumor progression. Our previous study demonstrated that overexpression of USP15 in p53-knockdowned cells can induce cell apoptosis, indicating that USP15 can trigger a p53-independent cell apoptosis. The mechanism for USP15 mediating the p53-independent cell apoptosis remained unknown. In my study, I carried out the co-immunoprecipitation of USP15 from cell crude extract, combined with proteomics to identify the USP15-binding proteins. 142 USP15 binding proteins have been identified. I focused two USP15-binding proteins, pyrroline-5-carboxylate reductase 1 (PYCR1) and pyrroline-5-carboxylate synthase (P5CS), for my study. Both proteins play a vital role in proline metabolism. Cancer cells are able to regulate their metabolism to supply energy and cellular building blocks for their growth. Previous studies have indicated that the oncogenic protein (MYC) and p53 dysregulation can promote tumor cell proliferation and prevent undergo apoptosis through regulation of glutamate-proline metabolism. USP15 regulation the stability of PYCR1 and P5CS may associate with cancer progression or cell apoptosis. I will use GST pull-down assay to analyze whether USP15 directly interacts with PYCR1 or P5CS. In addition, I will exam whether overexpression of USP15 can stabilize PYCR1 and P5CS in p53-knockdown HEK293 cells.