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USP10 regulates the stability of the EMT-transcription factor Slug/SNAI2

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ABSTRACT

Epithelial-to-mesenchymal transition (EMT) is a fundamental mechanism governing the switch of cells from an epithelial to a motile mesenchymal-like state. This transdifferentiation is regulated by key transcription factors, including Slug. The stability and function of Slug can be regulated by multiple mechanisms, including ubiquitin-mediated post-translational modifications. Here, by using a genome wide siRNA screen for human deubiquitinating enzymes (DUBs), we identified USP10 as a deubiquitinase for Slug in cancer cells. USP10 interacts with Slug and mediates its degradation by the proteasome. Importantly, USP10 is concomitantly highly expressed with Slug in cancer biopsies. Genetic knockdown of USP10 leads to suppressed Slug levels with a decreased expression of the mesenchymal marker Vimentin. Further, it reduces the migratory capacity of cancer cells. Reversely, overexpression of USP10 elevates the level of both Slug and Vimentin. Our study identifies USP10 as a regulator of the EMT-transcription factor Slug and cell migration.

1. Introduction

Epithelial-to-mesenchymal transition (EMT) represents a fundamental mechanism governing the transdifferentiation from an epithelial to a motile mesenchymal-like state associated with cancer cell survival and development of tumors with highly invasive and migratory properties [1]. Accordingly, EMT has been shown to occur in wound healing studies and during the initiation of metastasis of cancer cells [1,2] by reversibly changing the expression of proteins involved in cell polarity, cell–cell contact, cytoskeleton structure and extracellular matrix degradation, including the repression of key epithelial genes [3]. Cells undergoing EMT are characterized by key hallmarks that include the combination of the gain of mesenchymal markers (e.g., Vimentin) and loss expression of the cell-to-cell adhesion molecule, E-cadherin (*CDH1*) [4], which are primarily regulated transcriptionally by ZEB1, SIP1 *(ZEB2)*, Snail/Snail1, Slug/Snail2 and TWIST (*TWIST1*) transcription factors [5], or by specific microRNAs [6]. These EMT transcription factors (EMT-TFs) are under tight regulation of multiple layers of control. The predominant mechanism is transcriptional activation, which can be induced by oncogenes. In addition, EMT-TFs are subjected to poly-ubiquitination and degradation by the UPS mediated by E3 ubiquitin ligases [7]. Accordingly, deubiquitinating enzymes (DUBs) are emerging as potential new regulators of the protein stability for several EMT-TFs [8].

DUBs are commonly mutated in human cancers suggesting their role as potential tumor suppressors or oncogenes. Although the transdifferentiation program of EMT depends on the contextual network of signaling, the action of different TFs in EMT regulation can be cellular or tumor specific [9]. Accordingly, in certain carcinomas the effect of one single TF can be predominant in regulating EMT markers. This is particularly relevant for lung and breast carcinomas in which the expression of Slug has been shown to display a strong correlation with loss of E-cadherin [10–13], compared to other EMT-TFs. This suggests that Slug may promote invasion.

In this study, we undertook a siRNA screen in order to identify DUBs responsible for the control of Slug protein stability. We identified USP10 as a DUB that directly regulates Slug and cell migration in multiple cancer cells of different origin.

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2. Materials and methods

2.1. Cell culture

The ovarian cancer ES2, OVCA433, OVCAR3, SKOV-3, the non-small cell lung carcinoma NCI-H838, NCI-H1792, A549, and the fibrosarcoma HT1080 cell lines were cultured in RPMI (Sigma-Aldrich) medium. The breast cancer MDA-MB-231 and the melanoma cancer MDA-MB-435 cell lines were cultured in DMEM (Gibco) medium. The RPMI and DMEM medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco), 100 U/ml penicillin and 100 U/ml streptomycin (Sigma-Aldrich) and 1% (w/v) glutamine (Sigma-Aldrich). The breast cancer cell line, SUM159, was cultured in Ham's F12 medium supplemented with 5% (v/v) heat-inactivated FBS (Gibco), 100 U/ml penicillin and 100 U/ml streptomycin (Sigma-Aldrich), 5 mg/mL insulin and 1 mg/mL hydrocortisone. All cells were grown in a humidified 5% CO_2 atmosphere at 37 °C allowing exponential cell growth.

2.2. Compounds

The following compounds were used for treatment of cells in indicated experiments: 50 μM Chloroquine (Sigma-Aldrich), 10 μM MG132 (Sigma-Aldrich), 1–6 μM PR619 and 1–6 μM WP1130 (Degrasyn) (Selleckchem).

2.3. siRNAs and plasmids

Transient transfections were performed with ViaFect (Promega) for overexpression, and Lipofectamine 2000 reagent (Invitrogen) was used for siRNA knockdown, according to the manufacturer's recommendations. The Human ON-TARGETplus siRNA Library,

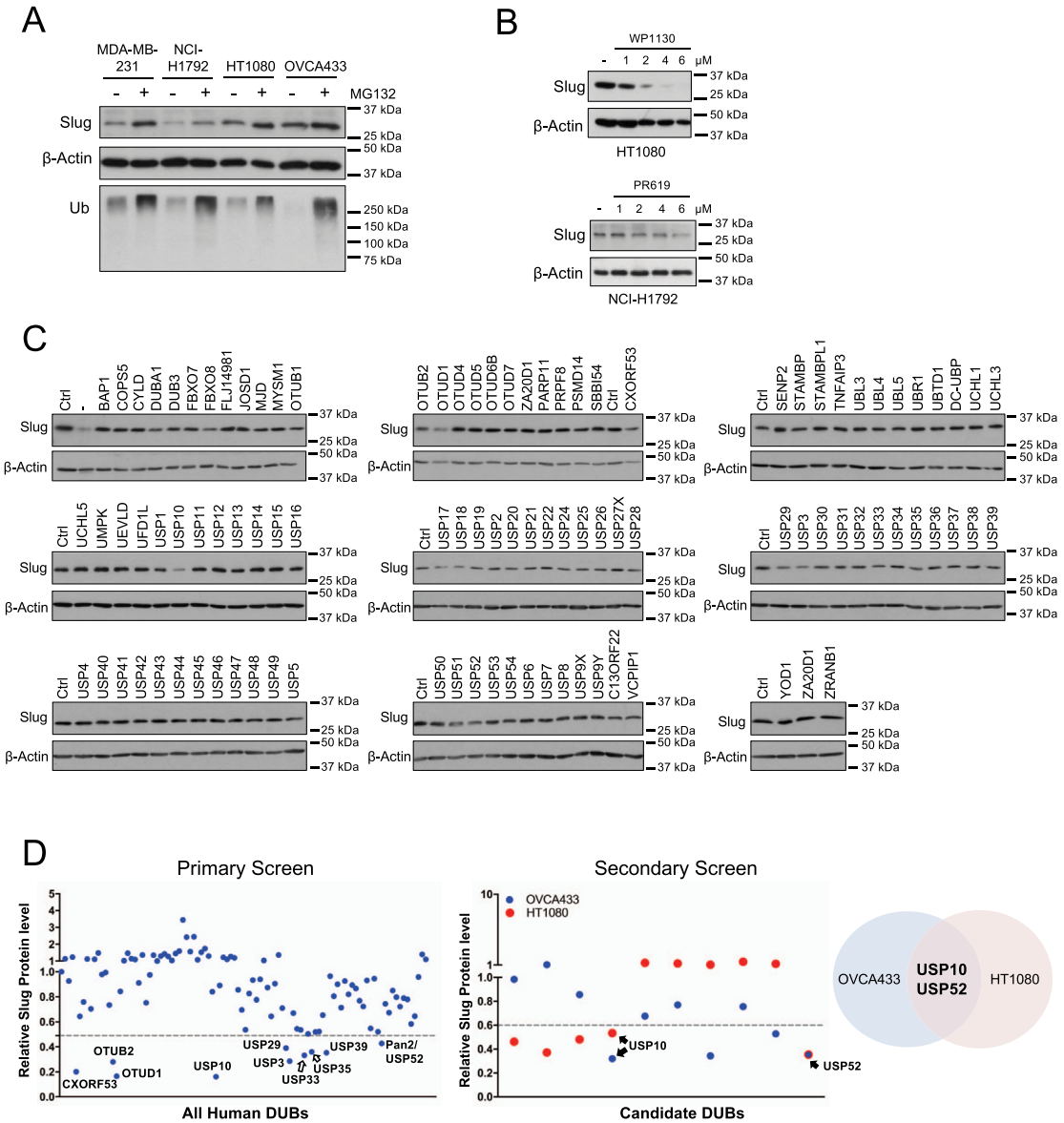

Fig. 1. *Identification of DUBs regulating Slug expression level in cancer cells.* (A) Analysis of Slug expression and accumulation of polyubiquitinated proteins upon 10 μM MG132 treatment for 6 h by western blotting in indicated cancer cells. (B) Assessment of the Slug levels in response to treatment with broad spectrum DUB inhibitors. HT1080 cells were treated with 1–6 μM WP1130 and NCI-H1792 cells with 1–6 μM PR619 for 24 h. (C) Primary siRNA DUB screen in OVCA433 cells to identify regulators of Slug. (D) The relative Slug levels in primary and secondary screening using HT1080 and OVCA433 cell lines with indicated potential DUB candidates. β-actin levels were used for loading control and for normalization.

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