Molecular Cell
Article

Skp2 Suppresses p53-Dependent Apoptosis by Inhibiting p300

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DOI 10.1016/j.molcel.2007.11.036

SUMMARY

The F box protein Skp2 is oncogenic, and its frequent amplification and overexpression correlate with the grade of malignancy of certain tumors. Conversely, depletion of Skp2 decreases cell growth and increases apoptosis. Here, we show that Skp2 counteracts the transactivation function of p53 and suppresses apoptosis mediated by DNA damage or p53 stabilization. We demonstrate that Skp2 forms a complex with p300 through the CH1 and the CH3 domains of p300 to which p53 is thought to bind and antagonizes the interaction between p300 and p53 in cells and in vitro. As Skp2 antagonizes the interaction between p300 and p53, Skp2 suppresses p300-mediated acetylation of p53 and the transactivation ability of p53. Conversely, ectopic expression of p300 rescues the transactivation function of p53 in cells overexpressing Skp2. Taken together, our results indicate that Skp2 controls p300-p53 signaling pathways in cancer cells, making Skp2 a potential molecular target for cancer therapy.

INTRODUCTION

The tumor suppressor protein p53 responds to genotoxic and oncogenic stress by inducing cell-growth arrest or by promoting apoptosis (reviewed in Vousden and Lu, 2002). The tumor suppressor function of p53 depends on its activity as a transcription factor. Posttranslational modifications of p53 such as phosphorylation and acetylation, as well as binding of cofactors such as Mdm2 and CBP/p300, play important roles in regulating p53 stability and activity.

p300 binds to the p53 transcriptional activation domain, stimulates p53 transcriptional activity, and functions as an acetyltransferase for p53 (Gu and Roeder, 1997; Lill et al., 1997; Avantaggiati et al., 1997; Espinosa and Emerson, 2001; Ito et al., 2001). In contrast, Mdm2, a negative regulator of p53, binds to p300 and so inhibits p53 acetylation (Grossman et al., 1998; Ito et al., 2001). Furthermore, Mdm2 blocks the ability of p300 to stimulate sequence-specific DNA-binding and transcriptional activity of p53 (Kobet et al., 2000).

S phase kinase-associated protein 2 (Skp2) is an F box protein required for substrate recognition of the SCF^{Skp2} ubiquitin ligase complex. Skp2 targets several proteins for ubiquitination and subsequent degradation, including the cyclin-dependent kinase inhibitors p21, p27, and p57; tumor suppressors such as p130; and oncoproteins such as c-Myc (reviewed in Nakayama and Nakayama, 2006). Although Skp2 mediates ubiquitination and degradation of c-Myc, ubiquitination of c-Myc by Skp2 enhances c-Myc-induced G_1/S transition and c-Myc transactivation activity, suggesting that Skp2 is a transcriptional cofactor for c-Myc (Kim et al., 2003; von der Lehr et al., 2003). Consistent with its substrate specificity, Skp2 is oncogenic, and its frequent amplification and overexpression correlate with the grade of malignancy in certain tumors. Furthermore, the oncogenic potential of Skp2 has also been shown in vivo (Gstaiger et al., 2001; Signoretti et al., 2002).

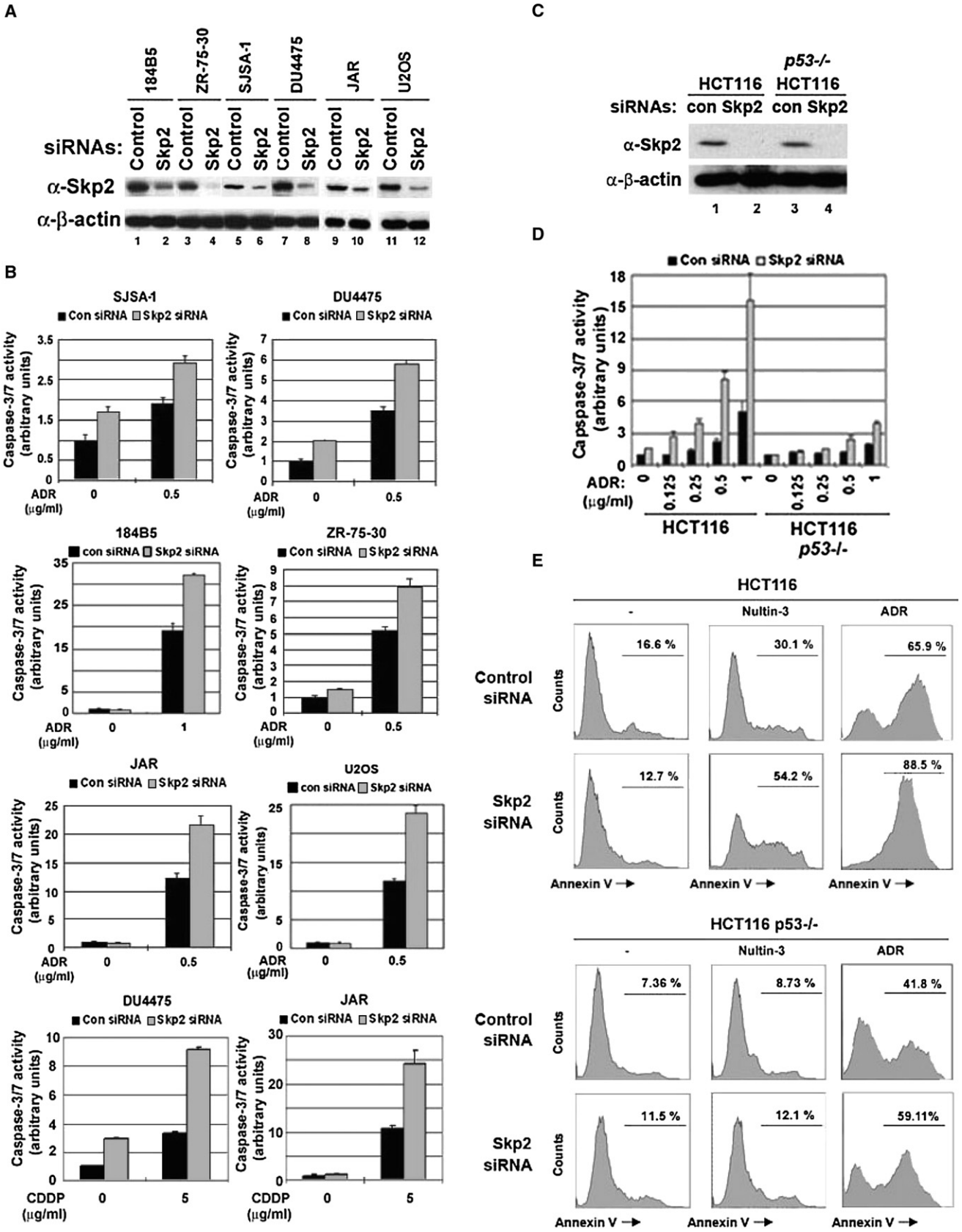
Skp2 has also been implicated in the regulation of apoptosis. Skp2-transfected gastric cancer cells are resistant to induction of apoptosis by actinomycin D, for example (Masuda et al., 2002). On the other hand, downregulation of Skp2 by small interfering RNA (siRNA) or antisense oligonucleotides decreases cell growth and increases apoptosis (Jiang et al., 2005; Lee and McCormick, 2005; Harada et al., 2005). However, the molecular mechanism by which Skp2 suppresses apoptosis in cancer cells remains unclear.

In this study, we demonstrate that reducing the expression of Skp2, together with the DNA-damaging agents or with Nutlin-3, a selective small-molecule antagonist of Mdm2, further induces apoptosis. Conversely, ectopic expression of Skp2 counteracts DNA damage and Nutlin-3-induced apoptosis by suppressing the transactivation function of p53. We show that Skp2 forms a complex with p300 and suppresses p300-mediated acetylation of p53 by antagonizing p300 binding to p53, whereas ectopic expression of p300 rescues the transactivation ability of p53 from Skp2-mediated inhibition. Taken together, our results implicate Skp2 as an important factor controlling p53-dependent signaling pathways by inhibiting p300 in cancer cells, and we suggest that Skp2 may be a new molecular target for cancer therapy.

RESULTS

Reducing the Expression of Skp2 Increases DNA-Damage-Mediated Apoptosis in Cancer Cells

Exposing cells to DNA-damaging agents that result in cell-cycle arrest or apoptosis is a successful strategy to treat cancers.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Con siRNA Skp2 siRNA
Caspase-3/7 activity (arbitrary units)
ADR (μg/ml)
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C
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HCT116 HCT116
siRNAs: con Skp2 con Skp2
α-Skp2
α-β-actin
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D
Con siRNA Skp2 siRNA
Capspase-3/7 activity (arbitrary units)
ADR: (μg/ml) 0 0.125 0.25 0.5 1 0 0.125 0.25 0.5 1
HCT116
HCT116 p53-/-
E
HCT116
Nultin-3
ADR
Control siRNA
Skp2 siRNA
Counts
16.6 %
30.1 %
65.9 %
12.7 %
54.2 %
88.5 %
Annexin V
HCT116 p53-/-
7.36 %
8.73 %
41.8 %
11.5 %
12.1 %
59.11%