Deathproof: New Insights on the Role of Skp2 in Tumorigenesis

Steven I. Reed[1,*]
[1]Department of Molecular Biology, MB-7, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: sreed@scripps.edu
DOI 10.1016/j.ccr.2008.01.023

The F box protein Skp2 is frequently overexpressed in human tumors and is capable of transforming cultured cells in vitro. It has been assumed, quite reasonably, that this oncogenic property of Skp2 is directly related to its role, as part of an SCF ubiquitin ligase complex, in the ubiquitin-mediated proteolysis of negative cell cycle regulatory proteins, notably $p27^{Kip1}$. However, building on earlier results indicating that silencing of Skp2 promotes apoptosis in some tumor-derived cell lines, Kitagawa and coworkers in the February 1 issue of *Molecular Cell* have elucidated an alternative mechanism for promotion of tumorigenesis by Skp2, specifically the suppression of p53-mediated apoptosis.

Evolution at the molecular level works in strange ways. There is abundant evidence that a protein perfected by evolution for a particular molecular role can suddenly assume a completely unrelated function. Presumably, all that's required is a mutation that creates a novel protein-protein interaction. If an advantage is gained, this interaction will be optimized by further evolutionary pressure. If distinct surfaces of the protein are involved in the respective interactions, the new function might arise without even detracting from the efficiency of the original function, creating a truly bifunctional protein. The recent report of a proteolysis-independent role for the F box protein Skp2 may be a case in point (Figure 1).

Skp2 (S phase kinase-associated protein 2) has been well characterized as one of several substrate-binding adapters for SCF family ubiquitin ligases (reviewed in Deshaies, 1999). F box proteins such as Skp2 contain two critical functional domains, an F box for integration into the SCF ligase core and a substrate-binding domain composed of any of a number of protein-protein interaction motifs. In the case of Skp2 the protein-protein interaction domain contains a series of iterations of a motif known as a leucine-rich repeat that, along with a small cofactor protein Cks1, forms the substrate-binding surface for the ubiquitin ligase (Hao et al., 2005). In this manner SCF^{Skp2} targets a number of important negative regulators of the cell cycle: $p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$, and a member of the retinoblastoma protein family, p130 (reviewed in Nakayama and Nakayama, 2006; Reed, 2006). It has therefore been proposed that the oncogenicity of Skp2 is implemented by depriving tumor cells of multiple critical cell cycle braking mechanisms, allowing for unchecked proliferation. It is certainly likely that Skp2 does contribute to oncogenesis via this mechanism in at least some cancers. However, another dark side of the cancer phenotype is the stubborn refusal of malignant cells to die. The impetus for the current work derives from the observation made in a number of laboratories that silencing of Skp2 by RNAi led specifically to killing of some cancer-derived cell lines by programmed cell death (apoptosis) (Lee and McCormick, 2005). Since this dependency on Skp2 for survival could not easily be explained simply by accumulation of cell cycle inhibitors, Kitagawa and coworkers (Kitagawa et al., 2008) searched for a mechanism integrally linked to apoptotic pathways. Remarkably, they found that Skp2 interferes directly with activation of p53, the transcription factor effector of the stress-responsive apoptotic program (Vousden and Lu, 2002). Equally remarkably, this function appears to be independent of SCF-mediated proteolysis, as an F box-deleted version of Skp2 that can't associate with SCF core is equally potent as a p53 attenuator.

Kitagawa and coworkers have carried out some nice molecular detective work to elucidate the mechanism. Specifically, Skp2 binds the transcriptional coactivator p300, thereby competitively blocking its interaction with p53. Since acetylation of p53 by p300 is critical for p53 activation (reviewed in Grossman, 2001), Skp2 is a dosage-dependent inhibitor of p53 transactivation function. Consistent with this being an acquired proteolysis-independent mode of action of Skp2, it is the amino terminus of the protein that binds p300, upstream of the F box and leucine-rich repeats required for ubiquitin ligase activity. On the other hand, Skp2 binds to the CH1 and CH3 domains near the amino terminus of p300, which are not surprisingly also involved in p53 binding.

This is not a new strategy for disabling p53. The adenoviral oncoprotein E1A binds to the CH3 domain of p300, preventing activation of p53 during viral infection. Mdm2, another antagonist of p53 function, also binds to p300, thereby blocking interaction with p53. In each of these cases, it is easy to rationalize p53 inhibition within the expression context of the respective protein. Neutralization of p53 by E1A is required to facilitate viral DNA replication and to preserve the integrity of the host cell during viral infection. Mdm2 is part of a negative feedback loop that limits the duration of the p53 response. This then raises the question of what the biological role of p53 antagonism by Skp2 is. Unfortunately, no completely compelling model is forthcoming, particularly when it comes to explaining Skp2-mediated protection from apoptosis.

At this point, the best explanation for the Skp2-p53 regulatory relationship derives from observations on the regulation of Skp2 expression through the cell cycle. Transcription of Skp2 is under control of the Rb-E2F system, and therefore Skp2 is only expressed at and after the G1/S phase transition, when Rb becomes hyperphosphorylated by cyclin-dependent kinases (CDKs). Activation of p53 by a variety of stresses has been shown to inhibit G1 CDK activity, thereby preventing Rb phosphorylation and E2F-dependent transcription, ultimately conferring G1 arrest. Activation of p53 in G1 would therefore promote a "low-Skp2" environment stabilizing and reinforcing the p53-active state. However, increased Skp2 synthesis would be indicative of decay of the p53-active state, as it would reflect loss of Rb-mediated inhibition of E2F-dependent transcription. Perhaps, then, Skp2-mediated inactivation of p53 serves as a mechanism for generating a decisive G1 to S phase transition by accelerating and stabilizing an alternative p53-inactive state. The parallel role of Skp2 in ubiquitin-mediated proteolysis of CDK inhibitors p21, p27, p57, and p130 also fits this "two metastable states" paradigm.

Whatever the intended role of Skp2 in the regulation of p53, it is evident that this becomes subverted during the course of oncogenesis. As is the case of Mdm2 (Hdm2 in humans) another p53 antagonist, Skp2 is frequently overexpressed in tumors, and overexpression correlates with particularly aggressive disease (e.g., Shapira et al., 2005). The link between Skp2 and suppression of p53-mediated apoptosis provides a plausible explanation for Skp2 selection during oncogenesis. p53 action poses a formidable barrier to malignancy, as is evidenced by the observation that 50% of human tumors are mutated for p53. Presumably the other 50% need to abrogate p53 function by alternative means. The requirement to neutralize p53 during oncogenesis is best explained by the recently developed concept known as "oncoprotein-induced stress" (reviewed in Bartek et al., 2007). Cells possess mechanisms that sense abnormally strong proliferative or replicative signals. These are

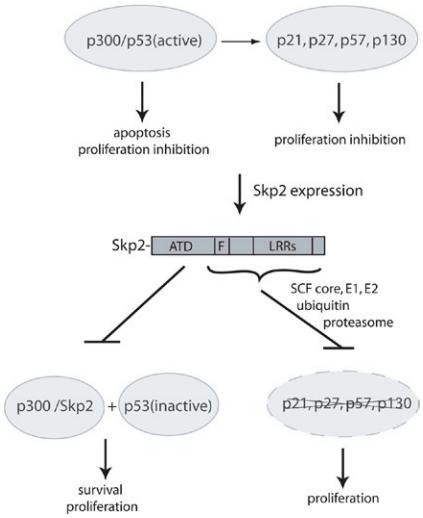

Figure 1. Dual Function for Skp2

In the absence of Skp2, p300 associates with and acetylates p53, creating an environment permissive for p53 activation, which leads to apoptosis and cell cycle arrest in response to stress. CDK inhibitors, p21, p27, p57, and p130 are also stable in the absence of Skp2, also promoting cell cycle arrest. Crosstalk between these systems is indicated, as p53 activation leads to upregulation of p21. Upon expression of Skp2, either in the context of cell cycle progression or during the course of oncogenesis, both of these pathways are negatively regulated. The amino-terminal domain (ATD) of Skp2 binds to p300, preventing association with p53, thereby preventing its activation. This activity of Skp2 promotes survival and proliferation in response to stress. Skp2 also serves as the substrate-binding subunit of an SCF ubiquitin ligase that along with E1, E2, and ubiquitin promotes the ubiquitin-dependent proteolysis of CDK inhibitors p21, p27, p57, and p130, stimulating proliferation. Skp2 binds the SCF core via its F box (F) and binds substrates through its leucine-rich repeats (LRRs).

then processed via a p53-dependent pathway to promote either apoptosis or a senescence-like state, depending on the cell type or the signal amplitude. Once p53 is lost, or its function attenuated, the probability of a cell becoming malignant is greatly increased. It is likely, therefore, that overexpression of Skp2 is one means to this end. Skp2-mediated attenuation of p53 function may also come into play at another key juncture in the life a tumor. Virtually all successful strategies of chemotherapy depend on a robust apoptotic response in the targeted tumor. Clearly, based on the current work, overexpression of Skp2 in p53-positive tumors is likely to promote resistance to therapy. Indeed, Kitagawa and coworkers show that ectopic expression of Skp2 renders tumor-derived cells resistant to killing by genotoxic drugs. It is therefore reasonable to ask whether targeting Skp2 or the Skp2-p300 interaction might enhance the efficiency of chemotherapy for p53-positive cancers. With respect to the Skp2-p300 interaction, the possibility of developing a small-molecule inhibitor depends on the nature of the interface between the two proteins, which has not yet been determined. Current dogma dictates that only contacts involving deep pockets or clefts are accessible to small-molecule interference. On the other hand, recent advances in the application of RNAi technology to tumors (reviewed in Masiero et al., 2007) may eventually permit a strategy of direct targeting of Skp2. This approach would have the advantage of eliminating all of the potentially pro-oncogenic functions of Skp2, including its role in the proteolysis of cell cycle inhibitors. Whatever the approach to be employed, the findings of Kitagawa and coworkers increase the profile of Skp2 as a therapeutic target worthy of further investigation.

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