Skp2, the FoxO1 hunter

Skp2 is an oncoprotein that mediates the degradation of several negative regulators of the cell cycle to promote cell proliferation. A recent report by Huang and colleagues reveals that Skp2 directs the ubiquitylation and subsequent degradation of FoxO1, a member of the FoxO family of transcription factors. Since FoxO proteins possess tumor suppressor functions, this new finding suggests a new mechanism by which Skp2 may favor tumorigenesis.

FoxO1 is a member of the FoxO (Forkhead box-containing, O subfamily) family of transcription factors. The FoxO proteins FoxO1, FoxO3a, FoxO4, and FoxO6 are evolutionarily conserved transcription factors involved in a variety of cellular processes (Tran et al., 2003). In mammals, FoxO proteins have been shown to play a role in processes such as cell cycle control, differentiation, stress response, and apoptosis. The investigation of the signaling pathways that regulate the transcriptional activity of FoxO proteins, as well as the identification of FoxO target genes, has yielded evidence that FoxO proteins possess tumor suppressive functions.

The proapoptotic activity of FoxO proteins is mediated through the transcriptional regulation of several genes such as TRAIL, FasL, and Bim. The role of FoxO factors in inducing cell cycle arrest is achieved by transcriptional repression of positive cell cycle regulators such as cyclins D1 and D2, as well as transcriptional induction of negative cell cycle regulators such as p27 and p130 (Tran et al., 2003). FoxO proteins also induce stabilization of p27 protein by an as-yet-unknown mechanism (Nakamura et al., 2000). Significantly, FoxO-mediated cell cycle arrest is at least partially dependent on p27, as p27-deficient fibroblasts lose their ability to stop proliferating in response to FoxO protein expression (Medema et al., 2000).

FoxO family members are negatively regulated by the phosphoinositide 3-kinase (PI3K)-Akt pathway, which is induced by mitogenic and survival signals and is overactivated in a variety of tumors (Tran et al., 2003). Activation of the Akt kinase results in the phosphorylation of FoxO proteins on conserved residues, leading to their nuclear exclusion, inhibition, and degradation. In addition, FoxO3a is also phosphorylated by a different kinase, IKKβ, that, similarly to Akt, triggers the degradation of the transcription factor (Hu et al., 2004).

Several studies have demonstrated that the cellular abundance of FoxO1 and FoxO3a is regulated by the ubiquitin-proteasome system (Aoki et al., 2004; Hu et al., 2004). The degradation of FoxO1 was shown to be dependent on phosphorylation by Akt; however, the ubiquitination machinery targeting its degradation has remained elusive until recently. Both the dependency on phosphorylation and the role of FoxO1 and FoxO3a in cell cycle progression suggest that both factors may be substrates of one of many SCF (Skp1, Cul1, F box protein) ubiquitin ligase complexes (Cardozo and Pagano, 2004).

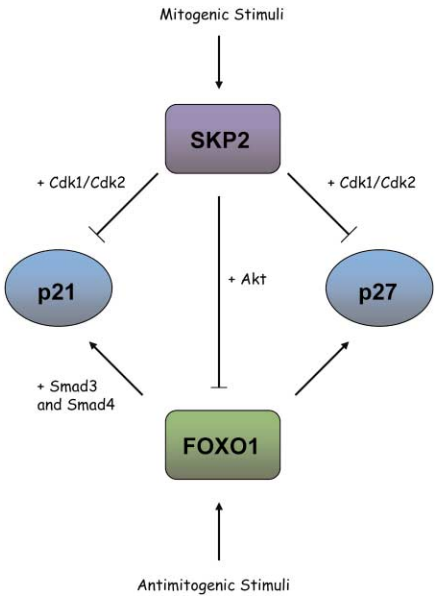

Figure 1. Skp2 directs the ubiquitylation and subsequent degradation of the cell cycle inhibitors p27 and p21 following their phosphorylation by CDKs

Huang et al. now demonstrate that Skp2 also promotes the degradation of FoxO1 following its phosphorylation by Akt. FoxO1 induces the expression of p27, and in response to TGFβ signaling, it forms transcriptional complexes with SMAD3 and SMAD4 to induce the expression of p21. Therefore, Skp2 controls the protein levels of p21 and p27 via two routes: directly by controlling p27 and p21 protein levels, and indirectly by controlling the protein levels of FoxO1. To add a further layer of complexity, the PI3K-Akt pathway appears to control all these players: it downregulates FoxO1, upregulates Skp2, and inactivates p21 and p27 by inducing their nuclear exclusion. Finally, it is possible that FoxO1 may negatively regulate Skp2 expression, since Skp2 is upregulated in cells expressing the dominant negative Pax3-FoxO1 chimeric protein.

A recent paper published in *PNAS* (Huang et al., 2005) now demonstrates that SCF^{Skp2} binds to FoxO1 and induces its ubiquitylation following Akt phosphorylation at Ser-256. The authors initially noticed that FoxO1 and Skp2 protein expression patterns inversely correlate during the cell cycle, an observation that provided a hint that FoxO1 may be a substrate of the F box protein Skp2. Using reporter gene assays, the authors show that Skp2-mediated degradation of FoxO1 inhibits its transcriptional activity. Furthermore, enforced expression of Skp2 eliminates the apoptotic effect of FoxO1 on prostate carcinoma cells, suggesting that Skp2 is able to inhibit the tumor suppressor activity of FoxO1. Conversely, Skp2 has no effect on the apoptotic response induced by a degradation-resistant mutant of FoxO1. In addition, the authors found that the levels of FoxO1 and Skp2 inversely correlate in a mouse lymphoma model. Downregulation of Skp2 by RNA interference resulted in the accumulation of FoxO1 protein in malignant T cells, suggesting that Skp2 upregulation is central to controlling FoxO1 protein levels in this type of tumor. Interestingly, the degradation of the related protein FoxO3a is dependent on the phosphorylation of a domain similar to that recognized by the F box protein βTrcp in its substrates (Hu et al., 2004). It is thus possible that the degradation of FoxO3a is also mediated by an SCF ubiquitin ligase.

Skp2 plays an important role in the timely degradation of several proteins involved in the negative control of the cell division cycle, such as p27, p21 and p130. In addition, Skp2-mediated ubiquitylation appears to stimulate the transcriptional activity of c-Myc and possibly b-Myb and E2F1. The new finding by Huang et al. reveals an additional mechanism by which Skp2 controls the levels of p27. In addition to directly regulating p27 protein stability by ubiquitylation, Skp2 is

now suggested to indirectly control p27 levels by promoting the degradation of FoxO1, thus inhibiting p27 transcription. Interestingly, FoxO proteins were recently demonstrated to cooperate with the SMAD tumor suppressor proteins in inducing the expression of p21, a protein closely related to p27 (Seoane et al., 2004). In response to TGFβ signaling, FoxO factors form transcriptional complexes with SMAD3 and SMAD4 that bind and activate the p21 promoter. Since Skp2 also promotes the degradation of p21 during S phase, the protein levels of both p27 and p21 are under the dual control of Skp2 and FoxO proteins (see Figure 1). Interestingly, as a further evidence of the connection among these different players, Skp2 mRNA expression is induced in response to PI3K signaling (Mamillapalli et al., 2001), and Akt can directly phosphorylate p21 and p27, resulting in their nuclear exclusion and thus eliminating their ability to inhibit the activity of CDKs (Bloom and Pagano, 2003).

Deregulation of FoxO factors seems to play a role in oncogenesis (Tran et al., 2003). In fact, FoxO genes were initially identified at sites of chromosomal translocations in several tumors such as leukemias and alveolar rhabdomyosarcomas (ARMS). In the latter, the translocation results in a fusion between the DNA binding domain of the transcription factors PAX3 or PAX7 to the transactivation domain of FoxO1. In light of FoxO's tumor suppressor function, it was suggested that the chimeric protein may act in a dominant negative manner and inactivate FoxO1 function. Accordingly, although haploinsufficiency of FoxO1 does not significantly increase cancer incidence, Pax3-FoxO1 homozygous mice develop ARMS in an Ink4a/Arf- or p53-deficient background (Keller et al., 2004). Interestingly, it was shown that in Pax3-FoxO1-expressing cells, Skp2 is upregulated, resulting in a decrease of p27 protein but not mRNA levels (Zhang and Wang, 2003). This shows that the fusion protein regulates Skp2 expression by a still-to-be-determined mechanism.

Another way to deregulate FoxO1 is via the inactivation of the tumor suppressor PTEN, an inhibitor of Akt that is commonly mutated in a variety of tumors. In cancer cell lines deficient in PTEN, FoxO1 is localized in the cytoplasm and displays decreased transcriptional activity (Nakamura et al., 2000). Although it has not been investigated, one would expect a destabilization of FoxO1 in PTEN-deficient cells due to Akt activation and upregulation of Skp2 (Mamillapalli et al., 2001).

The involvement of the Skp2-p27 axis in tumorigenesis is also well documented (Bloom and Pagano, 2003). Of all known Skp2 substrates, p27 is thought to be central to its oncogenic effect. This notion is supported by both animal models and examination of human tumors. For example, most of the cellular and cell cycle abnormalities present in Skp2-deficient mice are abolished in Skp2;p27 double knockout mice. Moreover, Skp2 expression inversely correlates with p27 levels in a variety of human tumors, including lymphomas, breast carcinomas, colorectal carcinomas, and lung cancers. Importantly, downregulation of p27 protein levels observed in human tumors does not correlate with a decrease in p27 mRNA levels. Instead, enhanced proteolysis represents a major mechanism to eliminate this tumor suppressor protein.

The finding by Huang et al. adds to accumulating evidence that strongly suggests an involvement of FoxO proteins in tumorigenesis. It will be of great interest to evaluate the expression of FoxO1 protein in human tumors and its correlations to the levels of Skp2 and p27, as well as to Akt activity and clinical outcome. A prediction is that in aggressive tumors overexpressing Skp2 and displaying increased Akt activity, levels of FoxO1 are low. It is possible that in these tumors, p27 expression is decreased not only at the protein level but also at the transcript level. Finally, it will be interesting to evaluate whether, in addition to the translocations described in ARMS, mutations in the FoxO1 gene are present in certain human tumors, and whether they correlate with high Skp2 levels. These studies will further enhance our understanding of the role of FoxO1 in tumorigenesis.

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