Mutant p53 succumbs to starvation

Comment on: Rodriguez OC, et al. Cell Cycle 2012; 11:4436–46; PMID:23151455; http://dx.doi.org/10.4161/cc.22778
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While the wild type form of p53 possesses strong tumor-suppressive activities, the p53 proteins that are commonly mutated in cancer often endow more malignant properties to the cancers they inhabit.1,2 There are several lines of evidence supporting such oncogenic gain of function of mutant p53. Compared with p53-null mice, knock-in mice harboring mutant p53 proteins display different and more metastatic tumor spectra. Such mutant proteins are frequently present at far higher levels than the wild-type protein in tumors; in fact, the p53 protein present in the knock-in mice accumulates in tumors despite being inherently unstable in normal tissues,3 suggesting that stabilization of mutant p53 protein is required for its oncogenic activity. Consistently, knockdown of mutant p53 protein in human cancer cell lines leads to reduced cell proliferation, invasion, motility, tumorigenicity and resistance to anticancer drugs.1,2 Since epidemiological studies indicate that high levels of mutant p53 proteins correlate with tumor aggressiveness and poorer outcomes, it is important to understand how mutant p53 is stabilized in tumors and how it can be eliminated.

The Avantaggiati group in a recent issue of Cell Cycle have recently provided important new insight into this question.4 They demonstrated that glucose restriction (GR) results in deacetylation and destabilization of endogenous mutant p53, but not of wild-type p53 protein. As protein degradation is mediated primarily by two pathways; the 26S proteasome and autophagy, the authors sought to identify which pathway is responsible for the degradation. They found that while the proteasome inhibitor MG132 treatment does not abolish GR-induced mutant p53 degradation, knockdown of autophagy genes such as Beclin-1, ATG5, ATG7 or pharmacological inhibition of autophagy prevents the degradation. Further, mutant p53 physically interacts with components of the autophagy machinery in a GR-dependent manner, suggesting that mutant p53 is a substrate for autophagic degradation. Interestingly, a C-terminal acetylation-mimicking mutant version of p53 (G245A-6KQ) is resistant to GR-dependent degradation. Taken together, these findings suggest that GR induces posttranslational modifications of lysines within mutant p53 proteins, which subsequently target them for autophagy-dependent degradation.

The authors next examined the effects of GR-induced degradation of mutant p53 on autophagy and cancer cell death. As indicated by two markers of autophagy (LC3 conversion and p62 degradation), GR activates this process, and the subsequent mutant p53 protein degradation leads to a maximal induction of autophagy and cell death. Consistent with their previous observations, expression of G245A-6KQ mutant p53 confers at least partial resistance to GR-induced cell death. Next, the authors used two mutant p53 mouse models to investigate the effects of a low carbohydrate (LC) diet on p53 stability and tumorigenicity in vivo. In line with their ex vivo data, in knock-in mice harboring the tumor-derived p53 mutation (A135V) placed on an LC diet, p53 protein is destabilized in mammary glands, ovaries and adipose tissues, while p53 in wild-type mice is stabilized. In xenografted mice, mutant p53 expressing cancer cells show enhanced tumorigenicity compared with those that are either p53-null or bearing wild-type p53.2 Strikingly, Rodriguez et al. found that an LC diet leads to a marked decrease in size of xenografted tumors with mutant p53, while this diet does not decrease tumors arising from the GR-resistant mutant p53G245A-6KQ expressing cells—it actually increases their growth.

The findings of Rodriguez et al.4 raise several interesting questions. First, virtually every residue with the ~200 amino acid DNA-binding domain of p53 has been found to be mutated in different tumors, albeit with differing frequencies. Autophagy is activated when the proteasome fails to eliminate misfolded and aggregated proteins,4

\[ \text{Glucose Restriction} \rightarrow \text{Ac-mutant p53} \rightarrow \text{Ub-mutant p53} \rightarrow \text{mutant p53} \rightarrow \text{cancer cell death} \]

Figure 1. Glucose restriction induces post-translational modifications of mutant p53 (ubiquitination, Ub-mutant p53; acetylation, Ac-mutant p53), which, in turn, leads to its degradation by activated autophagy and ensuing autophagic cell death.
Can liberating p53 from E6 free patients from HPV-related head and neck tumors?

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Over half a million patients are diagnosed with head and neck squamous cell carcinoma (HNSCC) each year in the world. Most of these patients present with locally advanced tumors, and less than 50% will live for 5 years after treatment. Concurrent platinum-based chemoradiation protocols improve loco-regional control and have become a standard therapeutic strategy. However, they are associated with acute, high-grade toxicity that includes neutropenia, mucositis, xerostomia and swallowing impairment.

Consumption of alcohol and tobacco smoke is the major risk factor for HNSCC. However, compelling evidence has accumulated over the last decade for infection of head and neck epithelium by high-risk human papillomaviruses (HPVs) as an emerging etiology for HNSCC (about 25% of all HNSCC cases). The prevalence of HPV-related HNSCC is increasing rapidly in North America and North European countries. Patients with HPV-positive tumors constitute a distinct clinical HNSCC subpopulation, who respond better to therapy and have improved relapse-free and overall survival.

HPV-driven carcinogenesis is mainly a consequence of deregulation of the E6 and E7 viral oncoproteins. E6 binds to the p53 tumor suppressor protein and the E6AP cellular E3-ubiquitin ligase (Fig. 1A), leading to p53 ubiquitination, proteasomal degradation and impaired function. The functional consequences include loss of p21-mediated control of the G1/S and G2/M checkpoints, reduced DNA-damage repair and cell cycle inhibition as well as decreased expression of the pro-apoptotic Bax and Puma factors and consequent diminished cell death. E7 binds to the Retinoblastoma protein (pRb) and induces its degradation (Fig. 1B). pRb inhibits cell cycle progression via inhibition of transcription factors of the E2F family. Loss of pRb activates E2F and promotes cell proliferation.

Most HPV-related HNSCC contain wild type TP53, and their better response to genotoxic therapies could be due to activation of wild-type p53. Interestingly, inhibition of E6 in HPV-related HNSCC cell lines in culture leads to p53 stabilization and increases cell death. However, there was no direct evidence that p53 is responsible for this effect. Proof is now provided by Li and Johnson. They have confirmed that inhibition of E6 and E7 by siRNA in HPV-positive cell lines leads to p53 stabilization and triggers apoptotic cell death. In addition, they elegantly demonstrate the direct implication of p53 in this induced cell death by additional inhibition of p53. The authors further observe that Bortezomib, a proteasome inhibitor (Fig. 1A), increases p53 and p21 levels and results in dose-dependent death of HPV-positive cells. This effect is partially impaired by anti-p53 siRNA treatment, showing that p53 is implicated to some extent downstream of Bortezomib. Interestingly, the use of sublethal doses of Bortezomib leads to cell cycle arrest of HPV-positive cell cultures at either G0 or G2/M to various extents, depending on the cell line. The authors provide convincing evidence that this effect is also dependent on both p53 and p21.

The impact of HPV on improving the survival of patients with HNSCC is now clearly established, and there is a debate about de-escalating and/or modulating standard therapies in order to both better manage HPV-related HNSCC and spare patients treatment-related toxicities. In this context, the work by Li and Johnson provides evidence for a direct role of p53 in the increased sensitivity of HPV-positive lesions to genotoxic agents. It also suggests that liberation of p53 from HPV E6 oncoprotein-mediated degradation, by inhibition of E6 or stabilization with the proteasome inhibitor bortezomib, could be interesting therapeutic options. Further work is required to assess the efficacy of bortezomib on irradiated HPV-positive and -negative cell cultures. A recent phase I clinical trial has demonstrated that the use of bortezomib with concurrent cisplatin-based chemoradiotherapy is well tolerated by HNSCC patients.

In the light of these new findings, it will be interesting to evaluate tumor response with respect to HPV-status.
Cenexin1 and Odf2: Splice variants with diverged cilium functions
Comment on: Chang J, et al. Cell Cycle 2013; 12:655–62; PMID:23343771; http://dx.doi.org/10.4161/cc.23585
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The primary cilium is an essential sensory “antenna” jutting from the cell surface of animal cells. Once thought to be a vestigial structure, the cilium has emerged in the last decade as a vital sensory organelle that impacts a broad spectrum of human diseases named ciliopathies.

The cilium contains a microtubule-based axoneme composed of nine doublet microtubules arranged in a radially symmetric pattern that grows from the distal end of the basal body. The basal body is a modified centriole, a structural component of the centrosome that contains a mother-daughter centriole pair. At ciliogenesis, the mother matures into a basal body for cilium assembly. The mother centriole is distinguished from the daughter by distal and subdistal appendages that are involved in basal body anchoring to the membrane prior to cilium assembly. Loss of Cenexin1, a component of the distal appendages, disrupts cilium assembly.1 Therefore, dissecting the components of basal bodies and determining how they work will unravel the molecular mechanisms of ciliogenesis, providing a deeper understanding of ciliopathies.

Outer dense fiber protein 2 (Odf2), discovered in the sperm tail cytoskeleton,2 is encoded by odf2, and is essential for several aspects of centrosome and cilium function. While odf2 knockout in mouse F9 cells did not display obvious mitotic spindle assembly or cell division defect, RNAi knockdown in HeLa cells disrupted mitotic spindle organization in a Polo-like kinase 1 (Plk1)-dependent manner.3 Moreover, knockout mice show a very early pre-implantation embryonic lethality.4 The odf2 gene encodes at least ten proteins by alternative splicing. Among these, at least two classes emerge: those that have a ~190 amino acid C-terminal extension and those that do not have this domain. Isoform 9, which has the C-terminal extension, is referred to as Cenexin1, whereas isoform 6, without the extension, is Odf2.5 This distinction is important, because the isoforms have different functions and patterns of subcellular localization. Odf2 is produced predominantly in the testis, where it localizes within flagella (cilia) at structures called outer dense fibers. Cenexin1, on the other hand, is a broadly expressed mother centriole-specific protein6 that localizes to the distal/subdistal appendages and is required for their assembly.7 These differences

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Figure 1. Model representing the role of the E6 and E7 viral oncoproteins in HPV-driven carcinogenesis. (A) The p53 tumor suppressor regulates cell cycle arrest at G1 and G2/M and apoptosis induction via the regulation of its target genes, such as p21, and Puma and Bax respectively. HPV E6-dependent proteasomal degradation of p53 is blocked by Bortezomib. (B) pRb controls the G2/M cell cycle checkpoint via the inhibition of E2F. Binding of pRb to HPV E7 induces its proteasomal degradation, the activation of E2F and stimulation of cell proliferation.
Akt destabilizes p57Kip2: Akt at the converging crossroad?

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Cell cycle progression through the G1 phase is tightly controlled by cyclin-dependent kinases (CDKs). Activity of CDKs is negatively regulated by two unrelated families of CDK inhibitors (CDKIs), namely, INK and Cip/Kip. The Cip/Kip family of CDKIs consists of three members, p21Cip1/WAF1, p27Kip1 and p57Kip2. In the context of human cancers, these three proteins are considered suppressors of tumorigenesis and tumor progression. Therefore, their levels of expression in both normal and cancerous cells are under complex transcriptional and post-translational regulations, including ubiquitination and proteasomal degradation.1

The upstream regulators leading to degradation of p21Cip1/WAF1 and p27Kip1 proteins have been extensively studied. For both p21Cip1/WAF1 and p27Kip1 proteins, Ser/Thr phosphorylation serves as a pivotal event that exports them out of nucleus and promotes their degradation in both cytoplasm and nucleus. Although multiple Ser/Thr kinases can phosphorylate p21Cip1/WAF1 and p27Kip1, Akt has attracted substantial attention because of its frequent activation in many types of cancer and its close association with several oncogenic receptor tyrosine kinases (RTKs), such as EGFR and HER2. In HER2-overexpressing breast cancer cells, Akt is often hyperactive and phosphorylates p21Cip1/WAF1 at Thr143 and p27Kip1 at Thr157 and Thr198, resulting in their nuclear export and proteasomal degradation. Akt-mediated destabilization of p21Cip1/WAF1 and p27Kip1 underlies the aggressive growth and progression of HER2-driven breast cancer.

Emerging evidence suggests that p57Kip2 plays an important role in embryonic development, hematopoietic stem cell quiescence and inhibition of cell cycle progression. Unlike p21Cip1/WAF1 and p27Kip1, the upstream pathways leading to p57Kip2 intracellular trafficking and stability are still not well understood. Nevertheless, it has been reported that TGF-β1 stimulates p57Kip2 phosphorylation at Thr310, leading to its ubiquitination and proteasomal degradation. Stress-activated protein kinase p38 phosphorylates p57Kip2 at Thr143 and enhances p57Kip2 association with and inhibition of CDK2.2 Subunit 6 of the COP9 signalosome complex (CSN6) associates with p57Kip2 and Skp2, a component of the E3 ligase. This association, in turn, leads to Skp2-mediated p57Kip2 ubiquitination and subsequent degradation.3 Interestingly, CSN6 can be phosphorylated by Akt at Ser60, which renders CSN6 more stabilized.

The elegant study by Zhao et al. showed for the first time that Akt interacts with and phosphorylates p57Kip2 at Ser282 and Thr310, resulting in p57Kip2 nuclear export, ubiquitination and proteasome-mediated degradation.4 HER2-overexpressing breast cancer cells were found to express lower levels of p57Kip2 than those with normal HER2 expression. Constitutively active Akt induced p57Kip2 degradation, whereas a dominant-negative Akt mutant and PI3K inhibition led to p57Kip2 stabilization. The authors further showed that Akt-mediated phosphorylation and stabilization
of CSN6 contributed to p57Kip2 degradation. Consistent with these observations, restoration of p57Kip2 in HER2-amplified, p57Kip2-deficient breast cancer cells led to reduced cell growth in vitro and the inability to form xenografts in nude mice, indicating that p57Kip2 antagonizes HER2-mediated breast cancer cell growth and possibly HER2-related tumorigenesis. Analysis of patient breast carcinomas revealed for the first time that in patients with HER2-overexpressing tumors, low p57Kip2 levels correlated with poor overall survival.

A significant implication for the novel Akt→p57Kip2 signaling axis is that it may play an important role in embryonic development in addition to cancer. Unlike p21CIP1/WAF1 and p27KIP1, p57Kip2-knockout mice uniquely displayed developmental defects and a phenotype that resembles the Beckwith-Wiedeman syndrome, a childhood overgrowth syndrome. The potential involvement of the Akt→p57Kip2 link in embryonic development is worthy of investigations in future studies.

In light of the new findings reported by Zhao et al. combined with those published previously, we now know that Akt phosphorylates all three members of the Cip/Kip family of CDKi (Fig. 1). This important discovery indeed revised the signaling landscape for the HER2→Akt signaling module, in that Akt plays a central role in lifting CDKi-mediated cell cycle arrest and therefore facilitates proliferation of HER2-amplified breast cancer. An immediate significant implication of these findings is that the newly discovered Akt→p57Kip2 signaling axis may have a broad impact on different subtypes of breast cancers, such as triple-negative and basal-like breast cancer, given the fact that Akt can also be activated by other RTKs besides HER2, such as EGFR, IGF-1R, VEGFR, c-Met, PDGFR and several others. It is also known that there are a number of proteins serving as the downstream effectors of Akt, such as, mTOR and two CDKi, p21CIP1/WAF1 and p27KIP1. Importantly, the study by Zhao et al. provided the first evidence that defines p57Kip2 as the substrate of Akt, thus making Akt a central common Ser/Thr kinase that negatively regulates all three members of the Cip/Kip family of CDKi that contribute to cell cycle arrest. Consequently, these reported findings potentially place Akt at the converging point that connects multiple RTKs to all three members of the Cip/Kip family of CDKi, in order to unblock cell cycle arrest and support uncontrolled cell proliferation in cancer cells.

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