Two additional issues merit further comment. First, how does SUMO ligation promote TopoII enrichment at *CEN*s? A straightforward model is that SUMO-conjugated forms of TopoII bind receptors within *CEN* chromatin. However, as is the case with most SUMO substrates, only a small fraction of TopoII is actually sumolyated at any one time. As suggested by Dawlaty et al., SUMO modification may only be required for an initial step in recruitment, allowing TopoII to remain associated with *CEN*s following SUMO deconjugation (Figure 1). Alternatively, sumolyation may promote a different aspect of TopoII dynamics, such as enzyme turnover on chromatin, which then indirectly facilitates *CEN* targeting through a different pathway. Further analysis of the genetic requirements for TopoII *CEN* localization, as well as determining if there are factors that bind sumolyated TopoII, should help clarify the recruitment mechanism.

Second, previous studies have shown that TopoII inhibition (or PIASy knockdown) during mitosis can activate a preanaphase checkpoint that exhibits considerable overlap with the SAC (Diaz-Martinez et al., 2006). The lesion inducing this response is unclear, but one idea is that a catenate-counting mechanism delays anaphase until chromatid entanglements fall below a threshold level. In the current study, it is notable that failure to completely decatenate *CEN*s did not appear to activate such a checkpoint response. Similarly, yeast *top2* mutants that exhibit a lethal decatenation defect proceed into anaphase with normal cell-cycle kinetics. In yeast, TopoII SUMO modification is not required for efficient chromatid disjunction (Bachant et al., 2002) and is largely, but not completely, dispensable for chromosome segregation (Takahashi et al., 2006). One phenotype observed in yeast SUMO-resistant *top2* mutants, however, is a failure to maintain *CEN* compaction under tension as chromatids biorient on the spindle (Bachant et al., 2002). Could TopoII be mediating additional functions within *CEN* chromatin, and might these activities, rather than decatenation, be influencing the SAC? The newfound connections between TopoII, SUMO, *CEN*s, and cancer suggest the answers may prove interesting.

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VHL Inactivation: A New Road to Senescence

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Loss of the *VHL* tumor suppressor gene promotes cancer in several ways, including activation of the HIF transcription factors. HIF overexpression is associated with increased malignancy in many tumor types. So why is the spectrum of tumors associated with *VHL* loss restricted only to a few specific organs? In a recent paper in the March issue of *Nature Cell Biology*, Kaelin and colleagues provide a possible explanation, suggesting that *VHL* loss can also trigger senescence, a potent tumor suppressor mechanism.

pVHL and HIF

von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome characterized by a spectrum of benign and malignant tumors including retinal hemangiomas, cerebellar hemangioblastomas, pheochromocytomas, and renal cell carcinomas (Kaelin, 2002). Patients with VHL disease inherit a faulty allele of the ubiquitously expressed *VHL* tumor suppressor gene, and emergence of pathology in these patients follows the inactivation of the remaining wild-type allele.

The gene product of *VHL*, pVHL, acts as the substrate recognition component of an E3 ubiquitin ligase complex (Kaelin, 2002). While several proteins have been identified as pVHL-binding proteins that are subject to ubiquitin-mediated proteolysis, the best characterized putative substrates are the alpha subunits of the hypoxia-inducible factor (HIF1α, HIF2α, and HIF3α). The HIFs function as heterodimeric transcription factor

complexes consisting of an unstable alpha subunit and a stable beta subunit (called HIF1β or ARNT) (Semenza, 2003). When oxygen is available, HIFα subunits are rapidly hydroxylated by members of the EglN prolyl hydroxylase family creating a high affinity pVHL binding site and subsequent HIFα polyubiquitylation and destruction (Kaelin, 2002). Under low oxygen conditions, HIFα subunits are not hydroxylated, escape recognition by pVHL, heterodimerize with HIF1β, and transactivate target genes. Many of the genes regulated by HIF are involved in the adaptation to acute or chronic hypoxia and include genes involved in the uptake and metabolism of glucose (*Glut-1*), angiogenesis (*VEGF*, *PDGF*), control of extracellular pH (*CA9*), mitogenesis (*TGFα*), and erythropoiesis (*erythropoietin*) (Semenza, 2003).

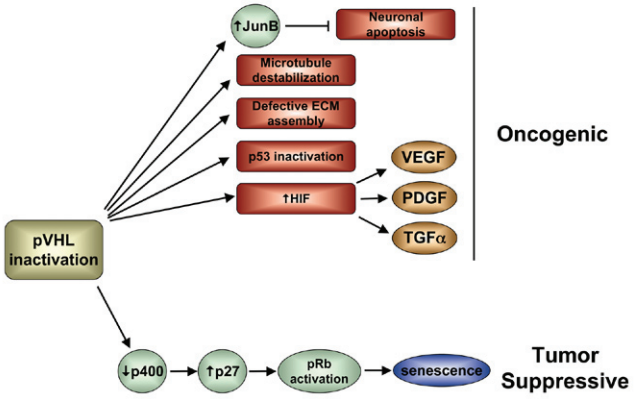

Figure 1. pVHL Inactivation Results in Both Pro- and Antitumorgenic Effects

pVHL loss induces both oncogenic signals and tumor suppressive responses. Oncogenic sequelae of pVHL inactivation include Jun-B-dependent inhibition of neuronal apoptosis, destabilization of microtubules, defective assembly of the extracellular matrix (ECM), p53 inactivation, and the upregulation of HIF. HIF stabilization results in the transactivation of protumorigenic genes including *VEGF*, *PDGF*, and *TGFα*. Kaelin and colleagues now show that pVHL loss also suppresses p400, resulting in $p27^{KIP}$ and pRb activation and promotion of the tumor suppressive response, cellular senescence.

In addition to HIF degradation, pVHL has been implicated to be involved in HIF-independent cellular processes (Figure 1). pVHL can bind to and direct the proper deposition of fibronectin and collagen IV within the extracellular matrix (Frew and Krek, 2007). In addition, it works to stabilize microtubules and foster the maintenance of primary cilium. pVHL has also been reported to promote the stabilization and activation of p53 in a HIF-independent manner and, in neuronal cells, promote apoptosis by downregulation of Jun-B (Frew and Krek, 2007). While in all of these roles, pVHL loss would be predicted to be protumorigenic, the extent to which these functions are fully HIF-dependent or HIF-independent is still unclear (Figure 1).

VHL Loss and Senescence

Against this framework, this recent study by Kaelin and colleagues revealed an additional and rather unexpected function for pVHL. The authors report that pVHL inactivation can result in the induction of an unusual form of cellular senescence (Young et al., 2008). Cellular senescence is a state of irreversible growth arrest and an important in vivo tumor suppressor mechanism (Campisi and d'Adda di Fagagna, 2007). Senescence has been reported to result from several cellular stresses, oncogene activation, and loss of certain tumor suppressor genes such as *NF1* and *PTEN* (Chen et al., 2005; Courtois-Cox et al., 2006). In this new work, the authors show that, relative to wild-type mouse embryo fibroblasts (MEFs), MEFs harboring *VHL* inactivation showed increased rates of growth arrest and the accumulation of markers associated with senescence such as expression of senescence-associated beta-galactosidae (SABG) and senescence-associated heterochromatic foci (SAHF). Additionally, when subjected to an in vitro immortalization assay, only MEFs without *VHL* inactivation were noted to undergo escape and immortalization. Surprisingly, the induction of senescence in the setting of *VHL* inactivation was not dependent upon HIF. Likewise, in wild-type MEFs neither hypoxia induction nor treatment with prolyl hydroxylase inhibitors was sufficient to induce senescence. Collectively, these results suggest that pVHL loss affects a senescence program that is independent of its ability to downregulate HIF.

Through a variety of approaches, including the clever use of viral oncoproteins, the authors demonstrated that the senescence associated with pVHL loss is not dependent upon activation of $p16^{INK4a}$ or p53, perhaps the two proteins most commonly associated with senescence. Instead, they showed that pVHL loss affected senescence through activation of the *RB* pathway in association with stabilization of $p27^{KIP}$, a cyclin-dependent kinase inhibitor. The authors show that *VHL* inactivation led to decreased mRNA levels of *Skp2*, the ubiquitin ligase for $p27^{KIP}$, and that the resulting accumulation of $p27^{KIP}$ will in turn lead to hypophosphorylated (activated) pRb. Intriguingly, the authors also noted that a mutant form of the viral oncoprotein adenovirus E1A unable to bind and inhibit pRb was still able to prevent senescence, implying that an additional target of E1A played a role in the senescence of *VHL*-defective cells. Armed with the knowledge that p400, a SWI2/SNF2 chromatin remodeling family member, is stabilized by E1A and that downregulation of p400 can induce senescence in human cells, the authors then turned to investigating the relationship between pVHL and p400 (Chan et al., 2005; Fuchs et al., 2001). pVHL inactivation resulted in diminished levels of p400 and the induction of senescence in a $p27^{KIP}$ and pRb-dependent manner (Figure 1). These experiments suggest the model that in MEFs, acute loss of pVHL can activate senescence by decreasing levels of p400 with subsequent activation of pRb (Figure 1). Finally, in accordance with this model, the authors demonstrate that acute somatic inactivation of *VHL*, in vivo in the murine kidney, is sufficient to upregulate levels of $p27^{KIP}$ and induce SABG as well as other markers of senescence.

The authors suggest that this interesting link between pVHL loss and senescence may explain the tissue specificity of VHL-associated disease. It is possible that in most tissues, loss of *VHL* induces senescence and is, therefore, not associated with cancer, while in other tissues (e.g., certain kidney cells), the oncogenic effects of *VHL* loss are more potent than the senescence promoting effects. In these tissues, pVHL inactivation is most likely to produce neoplasia.

Future Questions

This study by Kaelin and colleagues suggests that, like *PTEN* loss or *RAS* activation, an oncogenic event such as *VHL* inactivation can also induce tumor suppression via induction of cellular senescence in certain contexts. This finding raises several additional questions. First, although not HIF-dependent, exactly how pVHL plays a role in maintaining p400 expression is not clear. Whether this involves one of the known HIF-independent biochemical activities of pVHL (Figure 1) or a novel mechanism remains to be determined. Also, there appears to be a difference in the effects of pVHL loss in human and murine cells. While Kaelin and colleagues evidenced that pVHL loss results in decreased p400 expression in MEFs, they do not note this relationship in human cells. Indeed, in human diploid fibroblasts, either HIFα expression or pVHL loss appears to bypass senescence through the induction of telomerase (Bell et al., 2007). It is not readily clear how to reconcile these findings, although they may reflect species or cell type differences in the response to pVHL loss.

Lastly, in our view, telomere-independent senescence remains an enigma. While critical for day-to-day tumor suppression in mammals, their stimuli and precise effector pathways are only partially understood. Several stimuli relating to cellular metabolism (excess AKT/RAS signaling, oxidative stress, and pVHL loss in the present work among others) have now been shown to facilitate cellular senescence. While it is possible that these different events share a common promoting feature and pathway to senescence (e.g., DNA damage), it is equally plausible that they represent different flavors of senescence, induced by independent modes of stimuli associated with neoplasia.

The present work would appear most consistent with the latter possibility, which is a senescence mechanism that appears to occur independently of p53 and $p16^{INK4a}$ activation but relies on $p27^{KIP}$ stabilization. Moreover, senescence associated with pVHL loss does not seem to entail a DNA damage response, and in these ways, this senescence induced by pVHL inactivation in MEFs appears quite different from that induced by RAS activation or ionizing radiation in human diploid fibroblasts (Campisi and d'Adda di Fagagna, 2007). Nonetheless, these different stimuli all produce a growth arrest that bears the experimental hallmarks of senescence (SAHF and SABG). One continues to hope that determining how these seemingly unrelated genetic events converge to produce the common end point of cellular senescence might allow this most potent anticancer mechanism to be harnessed for the therapy of established cancers.

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