Oncogene-induced Senescence Pathways Weave an Intricate Tapestry

Paul Yaswen[1] and Judith Campisi[1,2]

[1] Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley CA 94720

[2] Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA 94945

Email addresses: P_Yaswen@lbl.gov

JCampisi@lbl.gov

The induction of cellular senescence by activated oncogenes acts as a barrier to transformation. Now, Sun et al. (2007) identify a key component of a senescence pathway that prevents tumorigenesis in a mouse model of skin cancer. They show that the p38-regulated/activated protein kinase (PRAK) induces senescence downstream of oncogenic Ras by directly phosphorylating and activating the tumor suppressor p53.

Cellular senescence suppresses the development of cancer by arresting the proliferation of damaged or stressed cells that are at risk for malignant transformation (Campisi, 2005). Of the many stimuli that induce senescence, DNA damage is the best understood. Genomic damage initiates a cascade of nuclear phosphorylation events, which in turn orchestrate DNA repair or—if the damage is severe or irreparable—cell death or senescence. The pivotal integrator and effector of these damage signals is p53, a tumor suppressor protein that controls the expression of numerous genes (Wahl and Carr, 2001). p53 activity is regulated at multiple levels, including degradation by H/MDM2, stabilization by ARF, and phosphorylation by the DNA damage response kinases. Many activated or overexpressed oncogenes, especially those that deliver strong mitogenic signals from plasma membrane receptors, also trigger p53-dependent senescence both in culture and *in vivo* (Braig and Schmitt, 2006). These oncogenes, of which (activated) Ha-RasV12 is the prototype, also trigger the MAPK (mitogen-activated protein kinase) pathway (Figure 1). In this issue, Sun et al. (2007) add a new dimension to oncogene-induced senescence. They show that PRAK, a downstream kinase in the MAPK signaling cascade is essential for Ras-induced senescence and its ability to suppress tumorigenesis *in vivo*.

Given that the MAPK pathway is initiated in the cytoplasm, how do activated RAS and similar oncogenes cause senescence? Recent findings show that these oncogenes cause

unbalanced DNA replication and a classic DNA damage response (Bartkova et al., 2006; Di Micco et al., 2006; Mallette et al., 2007). Although the mechanism is not fully understood, it may be due to increased expression of positive regulators of S-phase. Consequently, replicons refire or terminate prematurely, generating DNA breaks that initiate a DNA damage response and phosphorylation of p53 by DNA damage response kinases. Components of the MAPK cascade also increase the expression of the tumor suppressor ARF, and initiate a negative feedback loop that ultimately inhibits H/MDM2, resulting in p53 stabilization (Courtois-Cox et al., 2006) (Figure 1). Thus, oncogene-induced senescence appears to operate through known mechanisms that converge on p53.

In their new work**,** Sun et al. identify a downstream component of the MAPK pathway, PRAK, that is important for oncogene-induced senescence. Interestingly, PRAK–unlike other MAPK components immediately downstream of Ras, such as Raf—did not cause senescence when oncogenically activated or overexpressed (see Courtois-Cox et al., 2006),. Rather, PRAK overexpression alone only slightly suppressed cell proliferation. However, it substantially enhanced Ras-induced senescence, increasing both the kinetics and robustness of the response. In fact, PRAK was required for the increase in **p53** transcriptional activity that is stimulated by oncogenic Ras, but not by direct DNA damage. Sun et al. also show that PRAK directly phosphorylates p53 on a residue that is distinct from those targeted by the DNA damage response kinases.

These new findings provide insights into how some oncogenes engage the senescence program to ensure suppression, rather than promotion, of tumorigenesis. They indicate that oncogene-induced senescence and the DNA damage response have both common and unique

modes of action, and that both processes activate p53 (Figure 1). Are there additional layers of complexity yet to be uncovered? The answer almost certainly is yes.

In many cases, including those discussed above, oncogene-induced senescence is p53-dependent. This is not always the case, however. Oncogenic RAF, for example, induces senescence in both human fibroblasts and mammary epithelial cells independent of p53 function (Olsen et al., 2002; Zhu et al., 1998). In fibroblasts, oncogenic RAF increases the expression of $p16^{INK4A}$ (Zhu et al., 1998), a cyclin-dependent kinase inhibitor that prevents the phosphorylation and inactivation of the Retinoblastoma protein (pRB). pRB lies at the heart of a second major tumor suppressor pathway that regulates the senescence response (Campisi, 2005). In mammary epithelial cells, though, RAF induces senescence independently of both p53 and $p16^{INK4a}$ (Olsen et al., 2002). Given the well-established and crucial role that p53 plays in mediating DNA damage responses (Wahl and Carr, 2001), these findings suggest that oncogenic RAS, but not oncogenic RAF, drives unbalanced DNA replication and the subsequent DNA damage response —despite the fact that RAF is immediately downstream of RAS. Moreover, they suggest there are multiple pathways to the final growth-arrested senescent state (Campisi, 2005).

Additionally, there may well be both cell type- and oncogene-specific differences in how oncogenes induce senescence. Moreover, each component of signaling cascades, such as those governed by RAS, may have unique effects not shared by downstream components. For example, RAS and similar GTPases generate reactive oxygen species as signaling molecules, which in turn activate or inactivate redox-sensitive mediator proteins (Finkel, 2003). It is therefore possible that signaling by oncogenic RAS, but not all components of the MAPK pathway, initiates unique processes, such as the increased expression of genes that result in unbalanced DNA replication. Further, the high level of reactive oxygen species produced by an

overexpressed RAS oncogene might damage nuclear DNA, leading directly to a p53-dependent DNA damage response or might also exacerbate unbalanced DNA replication because of increased oxidative lesions that can retard or stall replication fork progression.

Although p53 controls multiple responses to stress and damage, including the permanent senescent growth arrest, a transient cell cycle arrest, and cell death (Wahl and Carr, 2001), p53 is dispensable at least for RAF-induced senescence of mammary epithelial cells (Olsen et al., 2002). What then are the ultimate mechanisms that establish and maintain the senescent growth arrest? One possibility is the pRb pathway, which can be indirectly activated by p53 via transcriptional upregulation of the p21 cyclin-dependent kinase inhibitor. Although $p16^{INK4A}$ does not appear to be required for **the** senescence growth arrest (Campisi, 2005), other means of activating the pRb tumor suppressor pathway may be crucial. Yet, p21 is induced similarly whether cells undergo a transient or senescent growth arrest, and RAF-induced senescence of mammary epithelial cells occurs even in the presence of viral oncogenes that inactivate pRb (Olsen et al., 2002). Thus, at this time, the available data cannot rule out the possibility of a senescence pathway that is independent of both p53 and pRb.

The mechanisms that regulate and implement oncogene-induced senescence, and their relationship to other inducers of senescence, may well be as myriad and complex as the data to date suggest (Figure 1). Understanding the signals and kinetics that establish and maintain **the** senescent growth arrest may only reveal that these pathways are woven in a complex tapestry. However, it is equally possible that future experiments will show that these pathways converge on a few critical threads.

References

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L. V., Kolettas, E., Niforou, K., Zoumpourlis, V. C., *et al.* (2006). Nature *444*, 633-637.

Braig, M., and Schmitt, C. A. (2006). Cancer Res *66*, 2881-2884.

Campisi, J. (2005). Cell *120*, 1-10.

Courtois-Cox, S., Genther Williams, S. M., Reczek, E. E., Johnson, B. W., McGillicuddy, L. T., Johannessen, C. M., Hollstein, P. E., MacCollin, M., and Cichowski, K. (2006). Cancer Cell *10*, 459-472.

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre', M., Nuciforo, P. G., Bensimon, A., *et al.* (2006). Nature *444*, 638-642.

Finkel, T. (2003). Curr Opin Cell Biol *15*, 247-254.

Mallette, F. A., Gaumont-Leclerc, M. F., and Ferbeyre, G. (2007). Genes Dev *21*, 43-48.

Olsen, C. L., Gardie, B., Yaswen, P., and Stampfer, M. R. (2002). Oncogene *21*, 6328-6339.

Sun, P., Yoshizuka, N., New, L., Moser, B. A., Li, Y., Liao, R., Xie, C., Chen, J., Deng, Q., Yamout, M., *et al.* (2007). Cell, *this issue*.

Wahl, G. M., and Carr, A. M. (2001). Nature Cell Biol *3*, 277-286.

Zhu, J., Woods, D., McMahon, M., and Bishop, J. M. (1998). Genes Dev *12*, 2997-3007.

Figure 1. Multiple pathways mediate oncogene-induced senescence.

Oncogenes that deliver strong mitogenic signals, typified by overexpressed oncogenic RAS, cause cellular senescence that arrests cell proliferation. Active RAS initiates two protein kinase cascades: the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol kinase (PI3K) pathway. The MAPK pathways includes the RAF and PRAK kinases, and increases the activity of p53, a tumor suppressor and pivotal regulator of the senescence response, by two distinct mechanisms. First, MAPK components induce the expression of ARF, which stabilizes p53 by antagonizing the degradative activity of H/MDM2. Second, PRAK, a downstream MAPK component, directly phosphorylates and activates p53. In addition, active RAF induces the senescence of some cells independent of p53 function. It is not known whether

this activity of RAF requires PRAK or other MAPK components. The PI3K pathway stabilizes p53 through the inactivation of H/MDM2. In addition to activating these kinase cascades, and possibly working through them, oncogenic RAS causes unbalanced DNA replication, resulting in DNA damage. The DNA damage response initiates yet another protein kinase cascade that activates p53, but in this case phosphorylation occur on sites distinct from that phosphorylated by PRAK. Finally, reactive oxygen species (ROS) are an intermediary in RAS signaling and may damage DNA directly.