RAS unplugged: Negative feedback and oncogene-induced senescence

Many normal cells respond to certain stresses, such as oncogene activation, by undergoing a permanent form of growth arrest known as senescence, an intrinsic tumor suppressor program. The predominant view has been that senescence is caused in some settings through a mutant oncogene's ability to induce activation of high levels of sustained MAP kinase and PI3 kinase signaling. A new study in this issue of *Cancer Cell* has challenged this model with the surprising finding that aberrant activation of the RAS/RAF pathway can induce a negative feedback loop that globally attenuates MAPK and PI3K signaling and that the reduction of signaling in these pathways is required for senescence.

The aberrant activation of the RAS/PI3K pathways is a potent oncogenic event that can result from activating mutations of oncogenes or inactivation of certain tumor suppressor genes. While these simple genetic events pose significant risks for cancer development, mammals have evolved an effective strategy to control such would-be cancer cells via a few mechanisms, including oncogene-induced senescence (OIS). While several recent reports have proven that OIS is not a mere artifact of cell culture and have established the importance of OIS in vivo in mammalian tumor suppression (reviewed in Narita and Lowe, 2005), the molecular mechanics of OIS, as opposed to other forms of growth arrest, are not fully understood. A new report in this issue of *Cancer Cell* (Courtois-Cox et al., 2006) suggests a surprising mechanism for this process involving a negative feedback network that significantly attenuates RAS/PI3K signaling in primary cells with mutations in this pathway.

The initial description of OIS came from a landmark study by Serrano and colleagues using activated RAS in cultured cells (Serrano et al., 1997). This report showed that mutant RAS expression in primary fibroblasts induced OIS and that, to achieve transformation, fibroblasts had to bypass OIS through genetic events leading to compromise of the $p16^{INK4A}$-RB and/or ARF-p53 pathways. A number of recent studies have supported the biological relevance of this process by showing that precancerous lesions of the lung, prostate, and melanocytes exhibit senescence-like features in humans and mice, thereby indicating that OIS traps cells with potentially cancer-causing mutations in a growth-arrested state in intact organisms (reviewed in Narita and Lowe, 2005; see also Gray-Schopfer et al., 2006). A particularly compelling demonstration is the finding that human dysplastic nevi (cutaneous moles) appear senescent, yet such lesions are found in children and persist in some cases through old age. Therefore, senescence appears to ward off cancer even in the youngest of humans, and senescent cells may persist a human lifetime.

The hallmarks of OIS include $p16^{INK4A}$ expression, which activates RB; p53 pathway activation; and the formation of characteristic nuclear structures known as senescence-associated heterochromatin foci (SAHF). The ability of MAPK and PI3K signaling to activate the ARF-p53 and $p16^{INK4A}$-RB tumor suppressor pathways, coupled with a role for RB in directing alterations in chromatin architecture, appears to explain, in part, these properties. Hence, aberrant high levels of MAPK or PI3K signaling were thought to be a driving force in senescence. In accord with this view, OIS can be blocked by inhibitors of MAPK signaling (Satyanarayana et al., 2004) or growth in low serum (which presumably reduces mitogenic drive) (Takahashi et al., 2006). Therefore, senescence appears to result from potent mitogenic stimulation in the face of a persistent $p16^{INK4A}$- or p53-mediated antiproliferative signal. The relationship of OIS to other forms of senescence—such as replicative senescence, which results from telomere dysfunction—is not completely understood, although these processes show both phenotypic and biochemical similarity.

The current report (Courtois-Cox et al., 2006) suggests a new model to explain OIS. The authors cleverly induce RAS activation in primary human fibroblasts by depletion of NF1, a protein that converts RAS to its inactive GDP-bound form. A strength of this approach is that it does not rely on the ectopic expression of RAS. In the setting of reduced NF1, RAS activation produced a marked, but transient, activation of the downstream ERK and AKT pathways followed by a profound shutdown of this signaling temporally accompanied by the appearance of senescence (Figure 1A). This global abrogation of ERK and PI3K signaling is associated with the activation of a broad transcriptional program of negative regulators of the RAS pathway, including Sproutys, RasGAPs, and DUSP6/MKP3 (an ERK phosphatase). The authors observed a similar response following ectopic expression of mutant RAS or of mutant B-RAF, a downstream target of RAS that activates ERK MAPK signaling, showing that these effects were not specific to NF1 deletion. Additionally, the authors confirmed many of their findings using small-molecule inhibitors of ERK or PI3K signaling. Curiously, the authors also show that abnormal *activation* of PI3K signaling also leads to senescence, a result suggesting that different circuitries mediate OIS depending on the inciting oncogene.

To document these processes in human patients, the authors turned to the study of tumors from human patients with neurofibromatosis type I, an autosomal dominant syndrome that results from inheritance of a single mutant allele of *nf1*. In such patients, characteristic tumors (e.g., neurofibromas) occur when there is somatic loss of the nonmutant *nf1* allele, which leads to RAS activation. In accord with the authors' hypotheses, however, such tumors generally have low malignant potential (e.g., the lifetime risk of a malignant nerve tumor in a patient with neurofibromatosis type I is $\sim$10%). Correspondingly, the authors show that tumors from neurofibromatosis type I patients exhibit features of senescence (e.g., increased $p16^{INK4A}$ expression and senescence-associated β-galactosidase activity). This result is consistent with the aforementioned studies showing OIS in intact organisms and is the first clear-cut demonstration of OIS in vivo in humans in a nonmelanocytic tissue. Therefore, these data suggest that OIS in response

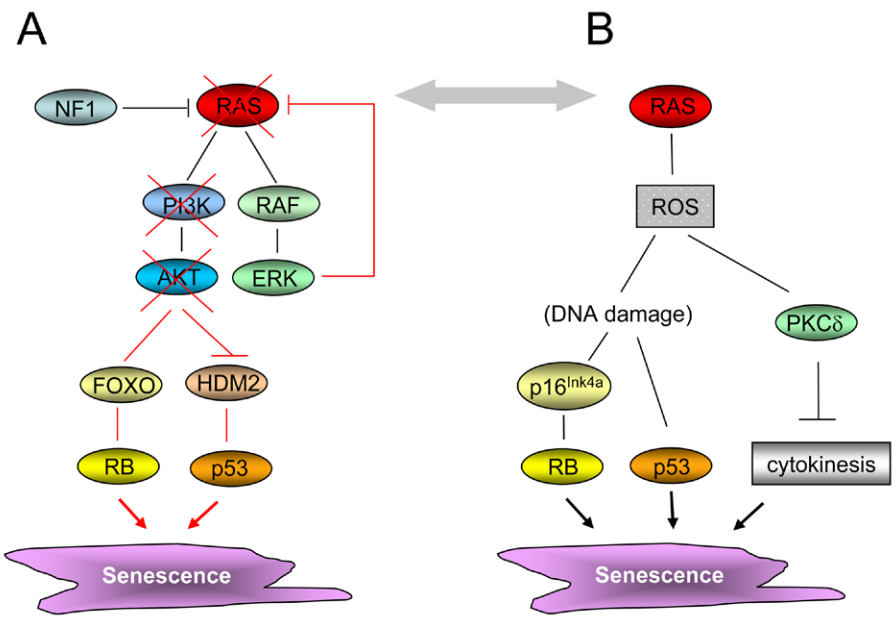

Figure 1. Two models of oncogene-induced senescence

A: Activated RAS leads to growth arrest as a result of potent negative feedback that abrogates ERK and PI3K signaling. The red lines indicate negative feedback signals.

B: RAS activation induces DNA damage via the production of ROS, which has been suggested to induce senescence through a few disparate mechanisms. These models differ in biochemical emphasis but are not mutually exclusive.

to acute *nf1* inactivation occurs in humans and is a barrier to malignancy in patients with neurofibromatosis type I. A caveat to this finding, however, is that many patients with neurofibromatosis type I suffer significant morbidity from persistent growth of neurofibromas, even in the absence of frank progression to malignancy. Therefore, reasonably common, but as yet unknown, mechanisms to escape OIS must exist in the setting of homozygous *nf1* inactivation.

It should be noted, however, that other models of OIS have also been advanced (Figure 1B). For example, several lines of experimentation have suggested that the generation of reactive oxygen species (ROS) (Lee et al., 1999), and possibly a persistent DNA damage response (reviewed in von Zglinicki et al., 2005), are crucial features of senescence, yet it is not obvious how attenuated ERK and PI3K signaling would produce high levels of ROS or an ongoing DNA damage response. Moreover, one wonders how lesions of tumor suppressors such as $p16^{INK4A}$ and/or p53 permit cells with oncogenic mutations to grow despite negative feedback. Such lesions indisputably render certain cells somewhat resistant to OIS (Serrano et al., 1997), but inactivation of these tumor suppressors would not be expected to restore ERK or PI3K signaling in an obvious way. In contrast to this work, Soengas and colleagues have recently suggested that certain forms of RAS activation induce a $p16^{INK4A}$/p53-independent senescence that requires sensors of stress in the endoplasmic reticulum (Denoyelle et al., 2006), and this form of senescence may differ entirely from that described by Courtois-Cox et al. Finally, Ferbeyre and colleagues have also reported a decrease in ERK signaling in the setting of RAS-induced senescence (Gaumont-Leclerc et al., 2004), but in their report, altered ERK localization rather than decreased phosphorylation appears to be the mechanism of attenuation in MAPK signaling. Several possible explanations exist that could reconcile these disparate findings (e.g., differences in type of senescence, activated oncogene, or cell type), and clearly further studies will be needed to integrate these various models of OIS.

In summary, Courtois-Cox et al. show that a profound attenuation of ERK and PI3K signaling after RAS activation is required for OIS. Additionally, they provide evidence that OIS occurs in vivo in response to *nf1* inactivation. This provocative report raises important future questions. For example, does negative feedback contribute to the persistence of senescence? It has been difficult to reverse OIS in human cells harboring SAHF (Beausejour et al., 2003; Takahashi et al., 2006), as such cells harbor widespread alterations in chromatin structure that prevent the transcription of genes needed for cell cycle traversal. Additionally, it has recently been suggested that ROS accumulate in senescent cells to produce a durable block in cytokinesis (Takahashi et al., 2006). Given these features, one doubts that OIS could be reverted merely by restoring ERK and/or PI3K signaling in truly senescent cells, but time will tell. More importantly, could therapeutic opportunity be derived from these findings? Perhaps the concomitant deprivation of ERK and PI3K signaling might induce senescence in established tumors harboring oncogenic lesions. With the advent of several potent, small-molecule inhibitors of these pathways directly, pharmacologic answers to this question in humans should be shortly forthcoming.

Acknowledgments

We would like to thank Drs. G. Ferbeyre and M. Narita for comments on the manuscript.

Nabeel Bardeesy[1,*] and Norman E. Sharpless[2,*]

[1]Department of Medicine, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts 02114
[2]Departments of Medicine and Genetics, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599
*E-mail: nelbardeesy@partners.org, nes@med.unc.edu

Selected reading

Beausejour, C.M., Krtolica, A., Galimi, F., Narita, M., Lowe, S.W., Yaswen, P., and Campisi, J. (2003). EMBO J. *22*, 4212–4222.

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, this issue.

Denoyelle, C., Abou-Rjaily, G., Bezrookove, V., Verhaegen, M., Johnson, T.M., Fullen, D.R., Pointer, J.N., Gruber, S.B., Su, L.D., Nikiforov, M.A., et al. (2006). Nat. Cell Biol. *8*, 1053–1063.

Gaumont-Leclerc, M.F., Mukhopadhyay, U.K., Goumard, S., and Ferbeyre, G. (2004). J. Biol. Chem. *279*, 46802–46809.

Gray-Schopfer, V.C., Cheong, S.C., Chong, H., Chow, J., Moss, T., Abdel-Malek, Z.A., Marais, R., Wynford-Thomas, D., and Bennett, D.C. (2006). Br. J. Cancer *95*, 496–505.

Lee, A.C., Fenster, B.E., Ito, H., Takeda, K., Bae, N.S., Hirai, T., Yu, Z.X., Ferrans, V.J., Howard, B.H., and Finkel, T. (1999). J. Biol. Chem. *274*, 7936–7940.

Narita, M., and Lowe, S.W. (2005). Nat. Med. *11*, 920–922.

Satyanarayana, A., Greenberg, R.A., Schaetzlein, S., Buer, J., Masutomi, K., Hahn, W.C., Zimmermann, S., Martens, U., Manns, M.P., and Rudolph, K.L. (2004). Mol. Cell. Biol. *24*, 5459–5474.

Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., and Lowe, S.W. (1997). Cell *88*, 593–602.

Takahashi, A., Ohtani, N., Yamakoshi, K., Iida, S., Tahara, H., Nakayama, K., Nakayama, K.I., Ide, T., Saya, H., and Hara, E. (2006). Nat. Cell Biol. *8*, 1291–1297.

von Zglinicki, T., Saretzki, G., Ladhoff, J., d'Adda di Fagagna, F., and Jackson, S.P. (2005). Mech. Ageing Dev. *126*, 111–117.

DOI 10.1016/j.ccr.2006.11.015

Integrated breast cancer genomics

Predicting survival and therapy responses of breast cancer patients is a significant challenge. Two studies in this issue of *Cancer Cell* present a novel integrated analysis of genomic and transcriptomic profiles of 145 primary breast cancers and 51 established cell lines. Data from clinical tumors highlighted mechanisms of disease and facilitated identification of potential therapeutic targets and prognostic biomarkers. An extensive well-characterized cancer cell line resource opens up opportunities to explore the determinants of cellular responses to existing and emerging therapies. Taken together, these studies illustrate how integrated molecular profiling may one day significantly impact diagnosis and therapeutic choice in human breast cancer.

Since the early days of microarray technology, numerous investigators have analyzed clinical cohorts of human breast cancers in order to better understand disease pathogenesis, and to provide a molecular explanation for the heterogeneity in the outcome and therapeutic response of breast cancer patients. A PubMed search identifies 827 publications with the keywords "microarray" and "breast cancer." Most of the research has focused on the characterization of transcriptional profiles of breast cancer (Table 1). For example, Perou et al. (2000) and Sørlie et al. (2001) established the classification of breast tumors into five different phenotypic subtypes. van 't Veer et al. (2002) and van de Vijver et al. (2002) divided breast cancer patients into those with favorable and unfavorable outcome with high accuracy, suggesting the potential of microarrays as a diagnostic test to select patients who would need adjuvant therapies. Many other studies have also identified gene signatures predictive of distant metastasis or survival (Wang et al., 2005; Naderi et al., 2006). Several authors have also studied profiles of genomic DNA copy numbers (Hicks et al., 2005; Bergamaschi et al., 2006; Fridlyand et al., 2006) using array-based comparative genomic hybridization (CGH). These studies have identified numerous specific genetic alterations and have also defined subtypes of breast cancers at the genomic level.

Few of the previous studies have integrated genomic and transcriptomics profiles from the same patient cohorts (Bergamaschi et al., 2006). This is a key contribution of the Chin et al. (2006) paper appearing in this issue of *Cancer Cell*. With 101 tumors comprehensively profiled at the DNA and RNA levels, the authors were able to better define the impact of specific genetic events on breast cancer phenotypes and clinical outcome. They indicate that genomic profiles provide additional prognostic information as compared to what is available from transcriptomics profiling alone. For example, patients whose tumors had one or more DNA amplifications had a poor prognosis independently of the previously defined five major gene expression classes (Sørlie et al., 2001). Interestingly, copy number imbalance (i.e., any deviation from diploidy) may be prognostically important for a specific region at 8p11-p12. Integrative DNA/RNA microarray profiling may also suggest novel therapeutic opportunities. Chin et al. list nine potential therapeutic targets, which, like the prototype HER2 oncogene, are activated by recurrent gene amplifications in breast cancer and may show an association with aggressive tumor types. Many of these are potentially druggable by small-molecule inhibitors. For example, two potential amplification targets, the GRB7 and PNMT genes, reside in the HER2 amplicon at 17q12 and are coactivated in breast cancer with HER2. Their targeting could provide synergistic therapeutic responses with Her2 inhibition or contribute to poor responses against Herceptin (Kao and Pollack, 2006).

Clinical tumor profiling is informative but at best associative in nature. In order to identify causative links between genes and tumor phenotypes, it is necessary to use cell lines. The Neve et al. (2006) paper describes the DNA and RNA microarray profiling data for a comprehensive resource of 51 different breast cancer cell lines,

Table 1. Selected previous microarray profiling studies of human breast cancer

Publication	No. of samples (gene expression)	No. of samples (array-CGH)
Chin et al., 2006	130	145
Neve et al., 2006	51	51
Bergamaschi et al., 2006	89	89
Fridlyand et al., 2006		67
Hicks et al., 2005		101
Naderi et al., 2006	135	
Perou et al., 2000	65	
Sørlie et al., 2001	78	
van 't Veer et al., 2002	98	
van de Vijver et al., 2002	295	
Wang et al., 2005	286	