These results are consistent with numerous reports indicating that ErbB receptors are capable of mediating signaling from a variety of heterologous stimuli (Carpenter, 1999). Heterologous ErbB receptor activation can involve either growth factor ligand-dependent or -independent mechanisms, but result in MAP kinase activation via processes that are most often dependent on receptor tyrosine kinase activity. For example, transactivation of EGF receptors by G protein-coupled receptors (GPCRs) such as the lysophosphatidic acid (LPA) receptor has been demonstrated to induce the shedding of membrane-tethered EGF-like ligand precursors by metalloproteases (Gschwind et al., 2001). Cytokine receptors such as those for growth hormone and IL6 can interact with the EGF receptor and ErbB2, respectively, to mediate MAPK activation in response to their ligands. Finally, membrane depolarization and cellular stresses such as exposure to UV irradiation or oxidants can also lead to MAP kinase activation through the EGF receptor (Weiss et al., 1997).

The observations of Liu et al. are also consistent with the emerging theme that overexpression of some cell surface proteins could unmask or augment their ErbB activation activities. For example, the mucins MUC1 and MUC4 play roles in the normal protection and lubrication of epithelial surfaces, but are commonly found overexpressed in a variety of malignant tumors. Because their size and highly negative charge disrupt cell-cell and even cell-protein interactions, mucins are thought to contribute to tumor cell evasion of immune surveillance. However, both MUC1 and MUC4 have been shown to interact with ErbB receptor family members and to potentiate signaling (Schroeder et al., 2001) and cellular growth properties (Komatsu et al., 2001). While the physiological significance of this functional duality in normal tissue development or maintenance remains to be determined, aberrant ErbB activation by overexpressed mucins could actively contribute to the growth or progression of tumor cells (Carraway et al., 2001). Like uPAR, the mucins appear to exert their effects on ErbB receptors in the absence of RTK overexpression.

The results described above encompass dozens of individual reports and emphasize that there is remarkable plasticity in the activation of the ErbB RTKs. ErbB activation mechanisms appear to be more prevalent in tumor cells that overexpress some heterologous cell surface proteins, but ErbB receptor overexpression is not necessary. Hence, it is quite likely that aberrant ErbB receptor activation plays a more far-reaching role in tumor growth and progression than is represented by overexpression. Moreover, most of the described mechanisms involve the activation of receptor tyrosine kinase activity itself, as opposed to crossphosphorylation of receptors by other kinases to serve as a scaffold for the initiation of signaling events. These observations suggest that ErbB-directed tyrosine kinase inhibitors such as the small molecule EGF receptor inhibitor Iressa, which in preclinical studies exhibits some growth inhibitory effects toward EGF receptor-overexpressing non-small cell lung cancers (Raben et al., 2002), could possibly impact a wider subset of tumors than those that overexpress receptors. For the future it will be important to determine the extent to which ErbB receptors are aberrantly activated in tumors where overexpression is not observed. The development of highly sensitive phospho-specific antibodies directed toward active receptors could help alleviate some of the technical barriers in this regard.

Kermit L. Carraway III[1]
and Colleen Sweeney

University of California, Davis,
Cancer Center
Sacramento, California 95817
[1]E-mail: klcarraway@ucdavis.edu

Selected reading

Andreasen, P.A., Kjoller, L., Christensen, L., and Duffy, M.J. (1997). Int. J. Cancer *72*, 1–22.

Carpenter, G. (1999). J. Cell Biol. *146*, 697–702.

Carraway, K.L., Price-Schiavi, S.A., Komatsu, M., Jepson, S., Perez, A., and Carraway, C.A. (2001). J. Mammary Gland Biol. Neoplasia *6*, 323–337.

Gschwind, A., Zwick, E., Prenzel, N., Leserer, M., and Ullrich, A. (2001). Oncogene *20*, 1594–1600.

Komatsu, M., Jepson, S., Arango, M.E., Carraway, C.A., and Carraway, K.L. (2001). Oncogene *20*, 461–470.

Liu, D., Aguirre Ghiso, J.A., Estrada, Y., and Ossowski, L. (2002). Cancer Cell *1*, this issue, 445–457.

Ossowski, L., and Aguirre-Ghiso, J.A. (2000). Curr. Opin. Cell Biol. *12*, 613–620.

Preissner, K.T., Kanse, S.M., and May, A.E. (2000). Curr. Opin. Cell Biol. *12*, 621–628.

Raben, D., Helfrich, B.A., Chan, D., Johnson, G., and Bunn, P.A., Jr. (2002). Semin. Oncol. *29*, 37–46.

Schroeder, J.A., Thompson, M.C., Gardner, M.M., and Gendler, S.J. (2001). J. Biol. Chem. *276*, 13057–13064.

Shawver, L.K., Slamon, D., and Ullrich, A. (2002). Cancer Cell *1*, 117–123.

Weiss, F.U., Daub, H., and Ullrich, A. (1997). Curr. Opin. Genet. Dev. *7*, 80–86.

Myc—Is this the oncogene from Hell?

A new paper implicates the Myc oncoprotein in the direct induction of DNA damage and consequent genome instability in cultured cells. However, it is less clear whether Myc induces the same genetic pandemonium in vivo.

The corrupted genomes of most human epithelial cancers are, like the Yucatan crater formed by the meteor that wiped out the dinosaurs, unambiguous relics of some catastrophic calamity within the tumor cell, a salient reminder of the genomic abyss that opens when the mechanisms that maintain chromosomal integrity fail or are overridden. Indeed, so

common is karyotypic mayhem in tumor cells that many regard it as indispensable for the tumorigenic process, feeding inchoate tumor cells with a never-ending repertoire of selective advantages that foster outgrowth of ever more malignant and drug-resistant clones. It is now widely accepted that loss of genome integrity represents the failure of checkpoint machineries that would otherwise have censored the offending cell. Moreover, new genomic approaches have revealed that differing tumor cells exhibit a bewildering array of genetic lesions (Gray and Collins, 2000), lending credence to the notion that genome instability is an obligate feature of tumorigenesis.

Vafa et al. (2002) present a highly provocative study offering a direct link between activation of the ubiquitous Myc oncoprotein and genome instability. They show that ectopic activation of the Myc in fibroblasts in vitro induces the rapid onset of DNA damage, possibly through the induction of reactive oxygen species (ROS), a seeming by-product of Myc activation. Although Myc can induce apoptosis, an inherent ability thought to be an important restraint to Myc oncogenic potential, they see little sign of this. Instead, Myc not only induces DNA damage but, rather foolishly it seems, thwarts the ability of p53 to induce an effective checkpoint response: the combined effect is to drive cells into an ineluctable cell cycle with damaged DNA. In terms of genome instability, this makes for a heady brew, especially in vertebrates, where the favored way of dealing with broken pieces of DNA is to stick them back together again by nonhomologous end joining and hope for the best. The ensuing loss of genome integrity, Vafa et al. argue, propels the cell and its progeny down the evolutionary road to neoplastic Armageddon.

Evidence that Myc, and certain other oncoproteins, might induce genome instability has been slowly accumulating. Early studies indicated that elevated Myc promoted gene amplification in cell lines (Mai et al., 1996), but Felsher and Bishop were the first to show that Myc activation directly induced the rapid accumulation of chromosomal defects and aberrant copy number in Rat-1 fibroblasts (Felsher and Bishop, 1999). The lack of an operational ARF/MDM2/p53 pathway in such immortalized rodent cells left open the possibility that such genome havoc was a peculiarity of established cell lines. However, the dramatic data of Vafa et al. confirm that Myc activation can induce significant DNA damage in genetically normal, primary human fibroblasts. If this holds in vivo, it would provide a powerful mechanism by which oncogene activation could accelerate tumor evolution and progression.

However, this paper raises two thorny but important issues. The first has to do with how applicable these in vitro data are to the situation in vivo. The second has to do with how important genome instability really is for tumor progression.

The reason why in vitro models (yes, all of them!) need to be considered with some caution is that there is clear evidence that "standard" tissue culture conditions exact a severe punishment on cellular genomes. Perhaps the best evidence for this is the rapid genome disorganization that accompanies propagation of p53-deficient cells in vitro. Within only a few passages, the vast majority of explanted p53 KO fibroblasts exhibit gross karyotypic abnormalities, including polyploidy, aneuploidy, and chromosomal abnormalities (Harvey et al., 1993). This contrasts sharply with the stability of genomes in somatic cells of p53 KO mice in vivo, which underscores that loss of p53 does not in itself cause genome damage. We are left with the unpalatable but inescapable conclusion that growth in culture exposes cellular genomes to a sleet of genotoxic insults that, without the protective surveillance afforded by p53, rapidly leads to degeneration of genomic integrity. In rodent cells, the genotoxic depredations of such "culture shock" are widely acknowledged to be the mechanism underlying "crisis," the growth barrier emplaced by upregulation of the ARF tumor suppressor, which triggers p53 stabilization and growth arrest (Sherr and DePinho, 2000) and was long conflated with human replicative senescence. Unexpectedly, this tumor-suppressive barrier seems not to arise in human cells which, instead, encounter the more formidable proliferative backstop of telomere erosion (DePinho, 2000).

There are several candidate culprits for this culture shock including growth on plastic, the high blue content of white fluorescent light, and the unremitting mitogenic onslaught of fetal serum. But of them all, the most probable suspect is that pervasive, corrosive, and genotoxic gas, oxygen. In vivo, somatic cells are generally exposed to an oxygen tension in the range 1%–2%, which is, incidently, precisely the oxygen range over which stabilization of the hypoxia response transcription factor HIF1α is observed. Standard culture conditions exceed normal oxygen exposure by 10-fold, so it is rather provocative that Vafa et al. are able to attribute their Myc-induced DNA damage to reactive oxygen species and specifically suppress the damage with the oxido-protective reagent N-acetylcysteine. Thus, although they attribute their DNA damage specifically to Myc, it is conceivable that any proliferative process, whether induced by oncogenes or normal mitogens, could elicit the same DNA damage in such unphysiological oxygen levels. It is also acknowledged that c-Myc is a potent trigger of apoptosis, both in vitro and in vivo, and that this apoptotic proclivity is greatly exacerbated by p53-dependent damage signals (Evan and Vousden, 2001). This means that even were the p53 growth arrest response abrogated, the invariable fate of such cells could nonetheless be their expeditious p53-dependent apoptosis.

The only available studies of the effects of activated Myc in vivo come from mouse transgenic models, which exhibit little evidence of Myc-induced genomic instability. The recent elegant lymphoma model of Schmitt et al. (2002) demonstrates that the combination of c-Myc and apoptosis suppressor Bcl-2 is sufficient to drive advanced *E*μ-*myc* lymphoma without ostensible genomic instability. Moreover, although loss of p53 confers similar acceleration of c-Myc-induced lymphomagenesis, the genome instability that arises seems to be an irrelevant sideshow to the critical role of p53 loss, which is to curtail c-Myc-induced apoptosis. Likewise, switchable c-Myc transgenic tumor models in both skin and β cells (Pelengaris et al., 1999, 2002) indicate that despite acquiring many aspects of advanced neoplasia, such tumors remain absolutely dependent upon sustained c-Myc for their maintenance, suggesting no dramatic acquisition of secondary lesions. One important inference from such in vivo studies is that apoptosis is a powerful and inherent tumor-suppressive mechanism that acts as a major fetter to the oncogenic potential of c-Myc (Green and Evan, 2002). As already discussed above, therefore, it seems reasonable to assume that even were c-Myc able to inflict significant DNA damage, the almost invariable outcome would be

death of the affected cell.

More generally, there is now a fertile and sometimes fractious dispute as to the importance of genome instability in tumorigenesis. On the one extreme, some have argued aneuploidy is the fundamental mechanism of tumorigenesis, with activated oncogenes and wrecked tumor suppressors merely a sideshow to distract gullible molecular biologists (Duesberg, 1999). More mainstream, however, is the important debate over whether genome instability is a pervasive and sustained attribute of tumor cells, perhaps driven by oncoproteins like Myc, or rather a relic of some past genomic mayhem (such as telomere erosion) that has long since stabilized (DePinho, 2000). In this latter case, genome instability would be the sideshow to the underlying proliferative and antiapoptotic lesions that drive the inexorable proliferation and enforced survival of the evolving tumor cell. Defining a direct link between oncogenic lesions and genomic instability would certainly keep both camps happy: the testing of intriguing studies like those of Vafa et al. in animal models may settle the matter once and for all.

Laura Soucek and Gerard Evan[1]

Cancer Research Institute
University of California, San Francisco,
Cancer Center
2340 Sutter Street
San Francisco, California 94143
[1]E-mail: gevan@cc.ucsf.edu

Selected reading

DePinho, R.A. (2000). Nature *408*, 248–254.

Duesberg, P. (1999). Science *284*, 2091–2092.

Evan, G.I., and Vousden, K.H. (2001). Nature *411*, 342–348.

Felsher, D.W., and Bishop, J.M. (1999). Proc. Natl. Acad. Sci. USA *96*, 3940–3944.

Gray, J.W., and Collins, C. (2000). Carcinogenesis *21*, 443–452.

Green, D., and Evan, G. (2002). Cancer Cell *1*, 19–30.

Harvey, M., Sands, A.T., Weiss, R.S., Hegi, M.E., Wiseman, R.W., Pantazis, P., Giovanella, B.C., Tainsky, M.A., Bradley, A., and Donehower, L.A. (1993). Oncogene *8*, 2457–2467.

Mai, S., Hanley-Hyde, J., and Fluri, M. (1996). Oncogene *12*, 277–288.

Pelengaris, S., Littlewood, T., Khan, M., Elia, G., and Evan, G. (1999). Mol. Cell *3*, 565–577.

Pelengaris, S., Khan, M., and Evan, G. (2002). Cell *109*, 321–334.

Schmitt, C., Fridman, J., Yang, M., Baranov, E., Hoffman, R., and Lowe, S. (2002). Cancer Cell *1*, 289–298.

Sherr, C.J., and DePinho, R.A. (2000). Cell *102*, 407–410.

Vafa, O., Wade, M., Kern, S., Beeche, M., Pandita, T.K., Hampton, G.M., and Wahl, G.M. (2002). Mol. Cell *9*, 1031–1044.

Thinking beyond the tumor cell: *Nf1* haploinsufficiency in the tumor environment

Deletion of both copies of the *Nf1* gene in Schwann cells combined with *Nf1* heterozygosity in the tumor environment promotes neurofibroma formation in mice.

Tumor cells in vivo do not grow in isolation and are intimately associated with nonneoplastic cells such as endothelial cells, fibroblasts, and inflammatory cells. Together, tumor cells and their neighbors form a complex tissue mass in which a network of heterotypic cell interactions occurs (Hanahan and Weinberg, 2000). The net balance between growth-promoting and growth-inhibitory interactions likely determines whether a given host environment is permissive or resistant to tumor formation. A thorough understanding of these heterotypic cell interactions may therefore provide the basis for novel anticancer therapies aimed at increasing the resistance of the host environment.

In a recent paper, Zhu and coworkers focused on neurofibromas, benign tumors of the peripheral nerve sheath, as models for study of heterotypic cell interactions (Zhu et al., 2002). Neurofibromas are unique among tumors with respect to the extent of their cellular heterogeneity. Neurofibromas invariably contain all of the cell types found in normal peripheral nerves (axonal processes of neurons, Schwann cells, perineurial cells, fibroblasts, and mast cells) (Figure 1). The cellular heterogeneity of neurofibromas is so striking that some in the past argued that these lesions were actually hyperplasias, not tumors.

Neurofibromas are the major feature of the common familial cancer syndrome neurofibromatosis type 1 (NF1). Patients with NF1 inherit a germline mutation in one copy of the *NF1* gene, which encodes the protein neurofibromin, a member of the Ras-specific GTPase-activating protein (RasGAP) family (Buchberg et al., 1990; Xu et al., 1990). When a somatic mutation eliminates the remaining wild-type *NF1* gene copy, tumor formation is initiated. The fundamental problem with *NF1*-deficient tumor cells is thought to be that Ras, a key component of many growth factor signaling pathways, is constitutively activated, resulting in increased cell proliferation and/or cell survival.

The first obstacle that Zhu and coworkers had to overcome was to create a tractable mouse model of neurofibromas. While $Nf1^{-/-}$ mice die in midgestation, $Nf1^{+/-}$ mice are cancer prone, developing two tumors associated with NF1 (pheochromocytoma and myeloid leukemia) but not neurofibromas (Brannan et al., 1994; Jacks et al., 1994). Based on the hypothesis that somatic *Nf1* mutation is the rate-limiting step in neurofibroma formation in $Nf1^{+/-}$ mice, Cichowski and coworkers created chimeric mice partially composed of $Nf1^{-/-}$ cells (Cichowski et al., 1999). Nearly all of these mice develop numerous neurofibro-