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-

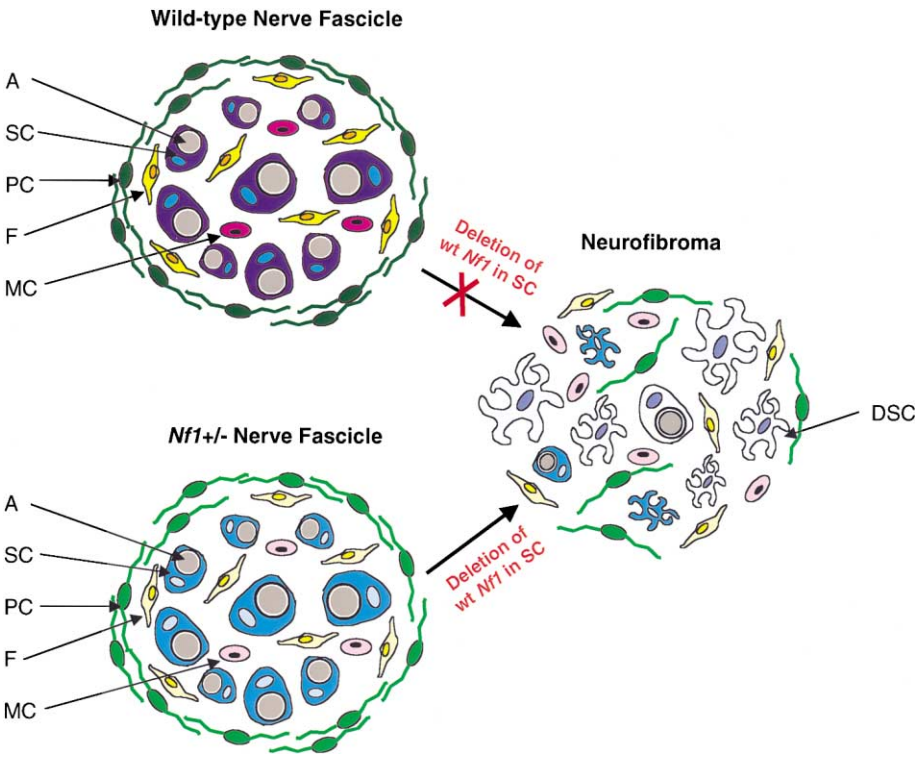

Figure 1. *Nf1* heterozygosity in the tumor environment promotes neurofibroma formation in mice

Peripheral nerves are composed of multiple nerve fascicles. Each nerve fascicle is divided into the endoneurium, which contains axons (A), Schwann cells (SC), fibroblasts (F), mast cells (MC), and collagen, and the perineurium, a layer of perineurial cells (PC), which forms the blood-nerve barrier. In a wild-type mouse, deletion of both copies of the *Nf1* gene in Schwann cells results in hyperplasia but not frank neurofibroma formation. In contrast, deletion of both copies of the *Nf1* gene in Schwann cells from an $Nf1^{+/-}$ mouse leads to widespread plexiform neurofibromas. Neurofibromas contain all of the cell types normally present in peripheral nerves as well as abnormal Schwann cells, which have become dissociated from axons (DSC).

mas that histologically resemble human plexiform neurofibromas. (In humans, neurofibromas of the plexiform subtype occur almost exclusively in NF1 patients, grow in the intraneural space of either a plexus of nerves or multiple fascicles of a medium to large nerve, and are prone to malignant change.) The chimeric mouse model, however, has its limitations: the cell type in which *Nf1* is deleted cannot be controlled and these animals cannot easily be crossed to other mutant mouse strains. Therefore, Zhu and coworkers generated a conditional *Nf1* mouse model in which a floxed *Nf1* allele is deleted by a Cre transgene under control of the Schwann cell-specific promoter, Krox-20. All progeny with the $Nf1^{flox/-}$;Krox20-cre genotype develop plexiform neurofibromas, confirming that the Schwann cell is the cell of origin.

Loss of *NF1* in Schwann cells provides a straightforward explanation for the hyperproliferation of Schwann cells within a neurofibroma, but it does not explain the excess numbers of perineurial cells, fibroblasts, and mast cells, nor does it explain how these neighboring cells may influence tumor development. To complicate matters further, not all neighboring cells are genetically equivalent. In NF1 patients and mice with a germline knockout of one *Nf1* allele, all of the cells in the body are heterozygous for *NF1*, raising the question of whether heterozygous neighboring cells can promote tumor growth more efficiently than wild-type neighboring cells. Implicit is the possibility that heterozygous inactivation of *NF1* has functional consequences (haploinsufficiency).

The importance of tumor suppressor gene haploinsufficiency in tumor cell biology has only recently begun to draw attention. Classically, a tumor suppressor gene has been defined as a gene in which mutation (or other functional inactivation) of both copies is required for tumor formation, implying that only complete loss of the gene product produces a cellular defect. However, there is a growing list of genes that encode proteins with tumor suppressor activities (*p53*, $p27^{Kip1}$, and *Dmp1*) in which mutation of one copy of the gene has functional consequences (Cook and McCaw, 2000; Quon and Berns, 2001). *p53*, for example, is a transcription factor and well-known tumor suppressor that plays a critical role in cell cycle arrest and apoptosis. In a subset of human tumors with *p53* mutations and in over half of tumors arising in $Trp53^{+/-}$ mice, loss of heterozygosity at the *p53* locus is not detected. Extensive analysis of the murine $Trp53^{+/-}$ tumors revealed that the remaining wild-type allele appears to be structurally and functionally intact, suggesting that *p53* haploinsufficiency may promote tumor formation (Venkatachalam et al., 1998). Importantly, loss of both *p53* alleles is more tumorigenic than loss of one, indicating that in the case of *p53*, haploinsufficiency is partial. Quon and Berns have recently proposed that all tumor suppressor genes that are frequently mutated in sporadic human cancers may show varying degrees of haploinsufficiency, with some genes showing strong effects and others showing partial or weak effects (Quon and Berns, 2001).

Since tumor suppressor gene haploinsufficiency can provide a selective advantage to tumor cells and/or their precursors, it is logical to consider whether tumor suppressor gene haploinsufficiency can alter the function of nonneoplastic cells in the tumor environment. To address this issue, Zhu and coworkers compared the size and frequency of neurofibromas occurring in $Nf1^{flox/-}$;Krox20-cre mice in which all neighboring cells are phenotypically heterozygous for *Nf1* and $Nf1^{flox/flox}$;Krox20-cre mice in which all of the neighboring cells are phenotypically wild-type. In striking contrast to the widespread plexiform neurofibromas of the $Nf1^{flox/-}$;Krox20-cre mice, the $Nf1^{flox/flox}$;Krox20-cre mice only developed small, infrequent hyperplastic lesions in the cranial nerves (Figure 1). Although the number of mice in this study was limited, the data supports the conclusion that *Nf1* heterozygosity in the tumor environment promotes neurofibroma formation.

What is the mechanism by which *Nf1* heterozygosity in the tumor environment exerts its effect on neurofibroma growth? The first step toward answering this question is to identify the relevant cell type(s) in

which *Nf1* heterozygosity is required to see frank tumor formation. One candidate cell type is the mast cell. $Nf1^{+/-}$ mast cells have been shown to hyperproliferate in vitro and in vivo in $Nf1^{+/-}$ mice (Ingram et al., 2000). In comparing peripheral nerves from $Nf1^{flox/-}$;Krox20-cre and $Nf1^{flox/flox}$;Krox20-cre mice, Zhu and coworkers noted a marked reduction in mast cell infiltration in the hyperplastic lesions from $Nf1^{flox/flox}$;Krox20-cre mice relative to the tumors of $Nf1^{flox/-}$;Krox20-cre animals. They also found that mast cells infiltrated preneoplastic peripheral nerves of $Nf1^{flox/-}$;Krox20-cre mice but not peripheral nerves of $Nf1^{flox/flox}$;Krox20-cre or $Nf1^{+/-}$ mice, suggesting that recruitment of mast cells to peripheral nerves requires both $Nf1^{+/-}$ mast cells and $Nf1^{-/-}$ Schwann cells. The presence of $Nf1^{+/-}$ mast cells in preneoplastic peripheral nerves as well as in the plexiform neurofibromas led Zhu and coworkers to speculate that $Nf1^{+/-}$ mast cells may play a central role in the initiation of neurofibromas.

In addition to infiltrating neurofibromas, mast cells have been identified at the periphery of several other human cancers such as melanoma, breast carcinoma, and colorectal adenocarcinoma. During an acute inflammatory response, mast cells release diverse factors capable of inducing angiogenesis, remodeling the extracellular matrix, and stimulating cell proliferation: effects that hasten wound healing but in a different context may promote tumor growth. Indeed, Hanahan's group has demonstrated that mast cells and the inflammatory cell product matrix metalloproteinase (MMP)-9 can contribute to tumorigenesis in a mouse model of squamous carcinoma (Coussens et al., 1999, 2000). Thus, there is precedent for implicating mast cells in tumor formation.

While the recruitment of mast cells in the conditional neurofibroma model may be influenced by their *Nf1* genotype, wild-type mast cells likely contribute to tumorigenesis in other settings. For instance, in the squamous carcinoma model noted above, the tumor-promoting mast cells were presumably wild-type. Furthermore, in humans the vast majority of neurofibromas (which are of the localized cutaneous subtype and grow in the extraneural tissue of the dermis and subcutis) are not associated with NF1, yet these sporadic neurofibromas contain abundant wild-type mast cells (Scheithauer et al., 1999). Perhaps there are species-specific differences that allow wild-type mast cells to home to neurofibromas in humans but not in mice.

The effects of *Nf1* heterozygosity in the tumor environment may not be due solely to *Nf1* haploinsufficiency in mast cells, but interactions with other $Nf1^{+/-}$ cell types may also be required. Prior work has shown that $Nf1^{+/-}$ Schwann cells are able to induce angiogenesis and are more invasive than their wild-type counterparts (Kim et al., 1997). In addition, embryonic fibroblasts from $Nf1^{+/-}$ mice hyperproliferate, respond abnormally to wound cytokines, and secrete higher amounts of collagen in vitro. In vivo in $Nf1^{+/-}$ mice, wounding incites an excessive proliferation of fibroblasts, leading to an increased amount of granulation tissue (Atit et al., 1999).

While focused on a model of an inherited cancer syndrome, the work from Zhu et al. raises the intriguing possibility that the genotype of the nonneoplastic cells comprising the microenvironment of sporadic tumors might also influence the properties of the tumor, or, indeed, might help determine whether a tumor arises in the first place. Like the tumor cells, such cells could undergo protumorigenic somatic mutations, or perhaps more likely, their particular constellation of inherited polymorphic alleles might make them inherently more (or less) capable of supporting tumor development. This might shed new light on how some cancer "modifier genes" might affect cancer susceptibility in the general population. Moreover, an increased understanding of the role of these support cells might lead to new directions for cancer therapy and prevention.

Margaret E. McLaughlin[1,2] and Tyler Jacks[1,3]

[1]Howard Hughes Medical Institute
Department of Biology and Center for Cancer Research
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
[2]Department of Pathology
Brigham and Women's Hospital
Boston, Massachusetts 02115
[3]E-mail: tjacks@mit.edu

Selected reading

Atit, R.P., Crowe, M.J., Greenhalgh, D.G., Wenstrup, R.J., and Ratner, N. (1999). J. Invest. Dermatol. *112*, 835–842.

Brannan, C.I., Perkins, A.S., Vogel, K.S., Ratner, N., Nordlund, M.L., Reid, S.W., Buchberg, A.M., Jenkins, N.A., Parada, L.F., and Copeland, N.G. (1994). Genes Dev. *8*, 1019–1029.

Buchberg, A.M., Cleveland, L.S., Jenkins, N.A., and Copeland, N.G. (1990). Nature *347*, 291–294.

Cichowski, K., Shih, T.S., Schmitt, E., Santiago, S., Reilly, K., McLaughlin, M.E., Bronson, R.T., and Jacks, T. (1999). Science *286*, 2172–2176.

Cook, W.D., and McCaw, B.J. (2000). Oncogene *19*, 3434–3438.

Coussens, L.M., Raymond, W.W., Bergers, G., Laig-Webster, M., Behrendtsen, O., Werb, Z., Caughey, G.H., and Hanahan, D. (1999). Genes Dev. *13*, 1382–1397.

Coussens, L.M., Tinkle, C.L., Hanahan, D., and Werb, Z. (2000). Cell *103*, 481–490.

Hanahan, D., and Weinberg, R.A. (2000). Cell *100*, 57–70.

Ingram, D.A., Yang, F.C., Travers, J.B., Wenning, M.J., Hiatt, K., New, S., Hood, A., Shannon, K., Williams, D.A., and Clapp, D.W. (2000). J. Exp. Med. *191*, 181–188.

Jacks, T., Shih, T.S., Schmitt, E.M., Bronson, R.T., Bernards, A., and Weinberg, R.A. (1994). Nat. Genet. *7*, 353–361.

Kim, H.A., Ling, B., and Ratner, N. (1997). Mol. Cell. Biol. *17*, 862–872.

Quon, K.C., and Berns, A. (2001). Genes Dev. *15*, 2917–2921.

Scheithauer, B.W., Woodruff, J.M., and Erlandson, R.A. (1999). Tumors of the Peripheral Nervous System (Washington, D.C.: Armed Forces Institute of Pathology).

Venkatachalam, S., Shi, Y.P., Jones, S.N., Vogel, H., Bradley, A., Pinkel, D., and Donehower, L.A. (1998). EMBO J. *17*, 4657–4667.

Xu, G.F., Lin, B., Tanaka, K., Dunn, D., Wood, D., Gesteland, R., White, R., Weiss, R., and Tamanoi, F. (1990). Cell *63*, 835–841.

Zhu, Y., Ghosh, P., Charnay, P., Burns, D.K., and Parada, L.F. (2002). Science *296*, 920–922.