A genome-based strategy uncovers frequent *BRAF* mutations in melanoma

Using a genome-scanning approach to search for oncogenes, a recent report identifies somatic mutations in the signaling gene *BRAF* that are particularly prevalent in melanoma.

The integrity of our organs and tissues is maintained by a complex network of genes that regulate cellular proliferation, differentiation, and death. Cancers arise when mutations in key genes cause this network to fail. Based on the age-dependent kinetics of cancer incidence, it has been estimated that human cancers require 4–6 cooperating mutations to achieve the fully malignant phenotype (Hahn and Weinberg, 2002). Cancer geneticists have developed a variety of clever strategies to identify and catalog the genes responsible for each type of cancer. A number of extraordinarily interesting genes have been discovered, and it is largely through the study of these genes that our current understanding of the cancer process has emerged. Although a few mutational targets cut across many cancer types (e.g., p53 and Ras), the catalog of target genes remains incomplete. The urgency of completing this process has been underscored by the development of drugs that target specific oncogenes, notably imatinib (formerly STI-571), a breakthrough drug that targets Bcr-Abl in chronic myelogenous leukemia and KIT in gastrointestinal stromal tumors.

Although current disease-specific strategies for identifying cancer genes are far from exhausted, the availability of the human genome sequence has created an opportunity that has been seized by Stratton and colleagues at the Sanger Institute. Taking advantage of the sequence and their expertise in high-throughput genomic technologies, these investigators are systematically combing the genome for genes that are mutated in human cancer. Knowing the importance of signal transduction in cellular growth regulation, the search has been initiated with genes in these pathways. The logic of this scheme was confirmed by the discovery of activating mutations in *BRAF* (Davies et al., 2002). Members of the Raf family (*RAF1*, *ARAF1*, and *BRAF*) encode serine/threonine kinases that act in the MAPK pathway to transduce regulatory signals from Ras to MEK1/2 (Figure 1). Davies et al. have identified activating mutations of BRAF with moderate to low frequency in a variety of cancers including colon (18% of cell lines and 12% of tumors) and ovarian (4% of cell lines and 14% of tumors) carcinomas. These numbers pale beside the stunningly high incidence of 59% in melanoma cell lines. Importantly, this high frequency is not the result of long-term cell culture, as the mutation frequencies in short-term cultures and melanoma tumors were also high. In an appealing parallel to activating mutations in Ras, which occur at characteristic sites, *BRAF* mutations are not scattered at random through the protein but cluster in specific regions of the B-raf protein. Remarkably, the preponderant mutation (80%) is a single phosphomimetic substitution in the kinase activation domain (V599E), leading to constitutive kinase activity (Davies et al., 2002).

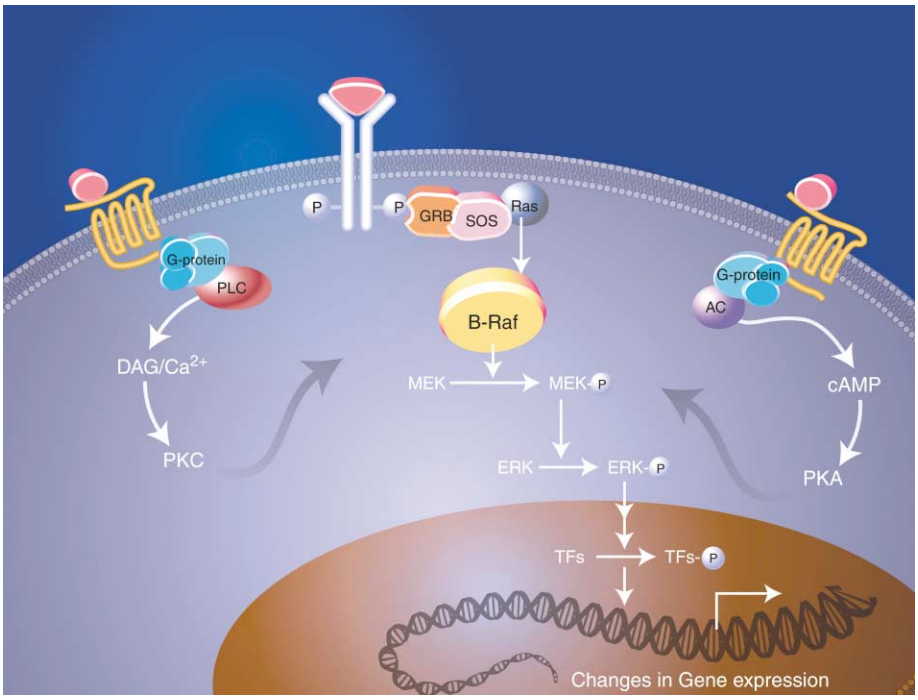

Figure 1. Melanocyte signal transduction

B-raf functions downstream of Ras in the MAPK cascade. Signals from extracellular growth regulatory factors (pink) transduced through receptor tyrosine kinases (blue) or G protein-coupled receptors (yellow) result in activation of the MAPK pathway. The mechanisms coupling GPCR signals to the MAPK pathway have not been fully elucidated in melanocytes but are known to involve B-raf in other cell types. The authors thank Darryl Leja for assistance with the illustration.

It is difficult to overemphasize the importance of the observation of *BRAF* mutations to melanoma research. With the exception of lung cancer in women, the incidence of cutaneous malignant melanoma (CMM) has increased more than any other cancer in the United States. In 2002 it is estimated that there will be 53,600 new cases of melanoma and 7,400 deaths. If caught early, melanomas can be cured by surgical removal. However, melanoma that has metastasized to distant sites has a dismal 5-year survival of only 6%, and there is little on the developmental therapeutics horizon that offers much hope for these patients. Epidemiologic evidence, supported by recent mouse models, indicates a role for UV light exposure as a risk factor for melanoma development, and efforts at prevention are directed at educating the public about the risks of sun exposure (Noonan et al., 2001).

Prior to the discovery of *BRAF* muta-

tions, somatic inactivation of the tumor suppressor gene, *CDKN2A*, was the most frequent genetic event reported in melanoma tumors. *CDKN2A* encodes two proteins, the cell cycle inhibitor p16, a molecule that inhibits the activity of the cyclinD/CDK4 (CDK6) kinase complex, and $p14^{ARF}$, an antagonist of the p53 regulator MDM2. It is inactivated by mutation, homozygous deletion, or methylation in ~25% of sporadic melanomas (Pollock et al., 2001). Further evidence of the importance of this cell cycle pathway comes from the observation of germline mutations in *CDKN2A* in ~20% of melanoma families worldwide and additional rare melanoma families with germline mutations in the p16 target *CDK4*. Less frequent somatic mutations have been reported in other well-known oncogenes and tumor suppressor genes: *NRAS* (~15%) and *PTEN* (~10%) (Herlyn and Satyamoorthy, 1996; Ali et al., 1999). Remarkably, in the Davies et al. study, *NRAS* mutations did not occur in the same melanoma cell lines that contained *BRAF* mutations. This observation (which was largely true of other cancers as well) suggests that activation of either one of these genes is sufficient to provide an oncogenic stimulus in melanocytes. Although confirmation of the *BRAF* mutation frequency in a larger panel of uncultured tumors from different stages of disease will be an important follow-up study, the available data lead to the speculation that an activating mutation in the Ras/Raf/MAPK pathway is an essential step in melanoma progression. The high frequency of B-raf activation may also help to explain a perplexing issue in melanoma biology, the stark contrast between the importance of activated Ras to tumor initiation and maintenance in melanoma mouse models and the low frequency of activated Ras reported in human tumors (Chin et al., 1999). This question will surely be addressed further by the development of an activated B-Raf mouse model. If this exhibits a similar phenotype to the Ras model, it would provide genetic confirmation of the importance of the MAPK pathway relative to other possible Ras effectors to melanoma tumorigenesis.

How does *BRAF* fit into melanocyte biology? In the skin, melanocyte homeostasis is controlled by the regulated production of mitogenic growth factors by keratinocytes (e.g., bFGF, endothelin, and MSH) and fibroblasts (e.g., bFGF, SCF, and HGF). During transformation to melanoma, autocrine production of growth factors probably contributes to autonomous tumor growth. The role of Raf is not limited to transducing RTK mitogenic signals through Ras but also includes mediation of growth factor stimulation through GPCRs (via cAMP/PKA and IP3/DAG/PKC). In melanocytes, B-Raf has been shown to mediate the MSH (cAMP) activation of the MAPK pathway (Busca et al., 2000). Endothelin-1, a strong melanocyte mitogen, is another candidate for signaling though B-raf. Et-1 can activate the MAPK pathway, although the role of B-raf in transducing this signal has not been demonstrated in melanocytes (Imokawa et al., 1996). Data from cultured human melanocytes demonstrates that synergistic growth factor stimulation of both RTKs and GPCRs is key to melanocyte proliferation. This synergy suggests the importance of the crosstalk between the AC/cAMP/PKA, the IP3/DAG/PKC, and the Ras/Raf/MAPK pathways in melanocytes. Possibly, mutational activation of B-Raf mimics the synergistic stimulation of multiple growth factors.

When considering possible causes of BRAF mutations in melanoma, it is important to bear in mind the constraints imposed by the gene sequence and the specific oncogenic amino acid substitutions that might be selected during tumor evolution. Nonetheless, it is remarkable that the most frequent mutation (T1796A) is not a common UV-induced base change; indeed, it is a relatively uncommon base change in carcinogenesis (Greenblatt et al., 1994). Both in vitro UV mutagenesis studies in endogenous mammalian genes and in vivo mutagenesis studies of the TP53 gene in squamous cell carcinomas (SCCs) and p16 in melanomas have shown that C→T transitions at dipyrimidine sites are the most common nucleotide change (Greenblatt et al., 1994; Pollock et al., 1996). The next most common changes are CC→TT and C→A mutations. Although the common UV-associated tandem mutation was not observed by Davies et al., a TG→AT tandem mutation was found in a melanoma cell line. Does this mean that the V599E is caused by a low-frequency but highly selected UV mutation, or could the known effect of UV on melanoma risk be mediated by mutation of another, as yet undiscovered gene?

One of the aims remaining in melanoma genetics is to identify the temporal sequence of genetic events required for melanoma pathogenesis. Most important is the identification of which mutational events initiate tumor growth in the easily curable radial growth phase, and which are the crucial events that govern the transition from this stage to the vertically growing invasive tumor with lethal metastatic potential. Follow-up studies should therefore address when, during melanoma pathogenesis, activation of B-raf occurs. Mutations in N-Ras have been reported in congenital melanocytic nevi and early radial growth phase melanoma, suggesting that B-raf might also be mutated in these early lesions (Demunter et al., 2001). Given that decoupling growth from extracellular signals is one of the earlier events in melanoma tumor formation, one might speculate that mutation of B-raf could be an early event. Interestingly, 4/5 ovarian cancers with *BRAF* mutations identified by Davies et al. were low-grade tumors, suggesting that at least in some tissues, *BRAF* mutation alone is insufficient to produce aggressive tumor growth.

Enzymes are attractive targets for small-molecule drug development, and the question arises as to whether B-raf is the ideal therapeutic target for melanoma. Hopes are raised by the prevalence of *BRAF* mutations in melanoma and bolstered somewhat by the demonstration that the ERK1/2 inhibitor U0126 causes decreased proliferation of melanoma cell lines bearing *BRAF* mutations. However, it also remains possible that other events in advanced melanomas might allow these tumors to become B-raf independent so that targeting B-raf would not provide adequate blockade of the mitogenic signal.

Uncovering BRAF mutations is an important step forward in melanoma research, but it is also an important indicator of the potential of high-throughput genomic approaches to cancer gene discovery. How many mutations are required for cancer to develop? Perhaps over the next several years, we will obtain a precise enumeration.

Pamela M. Pollock
and Paul S. Meltzer[1]

Cancer Genetics Branch
National Human Genome
Research Institute
National Institutes of Health
Bethesda, Maryland 20892
[1]E-mail: pmeltzer@nhgri.nih.gov

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Medulloblastoma: A problem of developmental biology

The identification of *SUFU* mutations in desmoplastic medulloblastoma provides new insights into vertebrate Hedgehog signaling and brain tumor formation.

Medulloblastoma, a primitive neuroectodermal tumor of the posterior fossa, is the most common central nervous system (CNS) malignancy of childhood. Despite aggressive multimodal therapy with surgery, radiation, and chemotherapy, 5-year survival rates have only recently approached >60% (Packer et al., 1999). In this preview we will address the genetic underpinnings of medulloblastoma and the role that Sonic hedgehog (Shh) signaling plays in this disease. Recent work by Taylor et al. (2002) extends our understanding of medulloblastoma oncogenesis and its relationship to normal developmental programs.

Medulloblastomas are thought to derive from immature granule cells of the cerebellum and comprise several histological and prognostic subgroups. The desmoplastic subtype is distinguished from "classic" medulloblastoma by the presence of relatively acellular nodules within the otherwise densely cellular sea of malignant cells. Expression of particular markers, such as TrkC, in tumor cells, has been shown to predict a relatively favorable outcome (Louis et al., 2002). A significant advance in our understanding of medulloblastoma tumorigenesis was the recognition that mutations of the Shh receptor, *Patched (PTCH),* were strongly associated with desmoplastic medulloblastoma. Germline *PTCH* mutations are etiologic in Gorlin's syndrome (also called Nevoid Basal Cell Carcinoma Syndrome), which is characterized by developmental anomalies and high rates of both basal cell carcinoma and medulloblastoma. Moreover, about 10%–20% of sporadic tumors, typically the desmoplastic variant, contain mutations within the Shh-PTCH pathway (Louis et al., 2002).

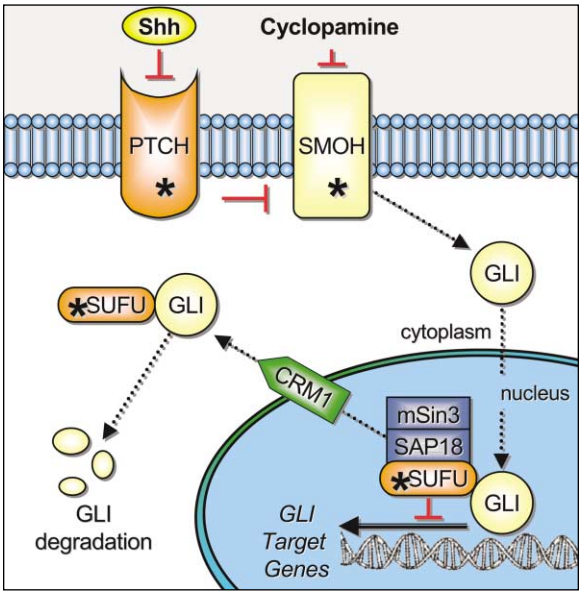

Figure 1. Mutations in Hedgehog (Hh) signaling predispose to tumorigenesis

Mutant alleles of *PTCH* and *SMOH* that result in activation of Hedgehog signaling are etiologic in basal cell carcinoma and medulloblastoma. Whether SMOH signaling directly regulates GLI activity is unclear. Recent work implicates *SUFU* as a functional repressor of GLI proteins that are thought to mediate activation of Hh transcriptional targets (Taylor et al., 2002). SUFU proteins in the nucleus repress GLI transcriptional activity by recruiting SAP18, a component of the mSin3 and histone deacetylase complex (Cheng and Bishop, 2002). Furthermore, SUFU exports GLI from the nucleus and the cytoplasm, where is targeted for degradation. Together, mutations in *PTCH, SMOH*, and *SUFU* (indicated by asterisks) account for the majority of cases of human desmoplastic medulloblastoma. Note that the cartoon represents a simplified scheme for vertebrate Hh signaling with particular emphasis on oncogenic mutations. For detailed summary of invertebrate Hh signaling, see Ingham and McMahon (2001).

Shh signaling is essential for the development of the mammalian CNS and is the major mitogenic pathway regulating proliferation of immature granule cells of the cerebellum (Wechsler-Reya and Scott, 2001). *hedgehog* was originally identified as a "segment polarity" gene by