Many genes originally identified because of their role in embryonic development are also important in postnatal control of cell growth and differentiation. Mutations in some of these genes have been shown to cause cancer. Basal cell carcinoma (BCC) of the skin is the most common cancer in humans. More than 750,000 new cases are diagnosed annually, and the incidence is rising. BCCs are slow-growing, locally invasive tumors that rarely metastasize but can result in extensive morbidity through local recurrence and tissue destruction. Epidemiologic studies suggest that sunlight (particularly UVB radiation) is a strong risk factor for BCC formation, although other factors are also involved. The nevoid basal cell carcinoma syndrome (NBCCS), a rare genetic disorder, is characterized by predisposition to BCCs and other tumors as well as to a wide range of developmental defects. NBCCS maps to chromosome 9q22.3, and loss of heterozygosity at this site in both sporadic and hereditary BCCs suggests that it functions as a tumor suppressor. The gene for NBCCS was recently cloned and is the human homologue of the Drosophila gene “patched.” Genetic studies in Drosophila show that patched is part of the hedgehog signaling pathway, which is important in determining embryonic patterning and cell fate in multiple structures of the developing embryo. Human patched is mutated in both hereditary and sporadic BCCs, and inactivation of this gene is probably a necessary, if not sufficient, step for BCC formation. Delineation of the biochemical pathway in which patched functions may lead to rational medical therapy for BCCs and possibly for other tumors associated with NBCCS. [J Natl Cancer Inst 1997;89:1103-9]

The fruit fly Drosophila melanogaster has served as a valuable genetic model in the field of developmental biology. Various developmental pathways first elucidated by genetic studies in flies appear to be conserved in vertebrates, and a number of key genes important in embryonic patterning in Drosophila have vertebrate homologues (1). Many of these genes continue to play an important role in cell growth and differentiation after embryogenesis, and there is increasing evidence that mutations in at least some of these genes can result in cancer.

Basal cell carcinoma (BCC) of the skin is the most common cancer in humans (2). More than 750,000 cases are diagnosed each year in the United States, and the incidence has been rising rapidly during the past several decades (3,4). The incidence of BCCs is very low before the fourth decade of life and peaks by the seventh decade. Although BCCs are slow-growing tumors that rarely metastasize or cause death, they can result in extensive morbidity through local invasion and tissue destruction (2,5). The cell of origin is probably a pluripotential stem cell, and there is a range of histologic subtypes, including the well-circumscribed nodular subtype (45%-60%), the superficial subtype (15%-30%), the pigmented subtype (1%-2%), and the aggressive-growth or infiltrative subtype (4%-17%) (5). Although there is no formal grading system for BCC, the infiltrative subtype is considered more aggressive than the nodular and superficial subtypes. Treatment for BCC is usually by local excision, but recurrence is common because of noncontiguous growth and difficulty defining surgical margins (2). Exposure to sunlight, particularly UVB radiation (wavelength, 280-320 nm), is a strong risk factor associated with BCC development (6). However, compared with the incidence of squamous cell carcinoma of the skin, which is associated with cumulative exposure to sunlight on the most sun-exposed regions of the body, BCC incidence has only a modest association with cumulative exposure and is more likely to occur on areas of the body moderately exposed to the sun, including the trunk in males and lower legs in females (7-12). Individuals at highest risk for BCC are Caucasians with light hair and eyes who had an early history of “recreational” exposure to the sun, i.e., intermittent, intense exposure that usually results in sunburn ([13-15]; reviewed in (16)]. Other known risk factors include exposure to ionizing radiation, arsenicals, polyaromatic hydrocarbons (6), transplantation (17,18), and psoralen-plus-UVA therapy (19).

Molecular Alterations in BCCs

Cancer arises from cells that escape normal growth regulation through a multistep process, involving activation of growth-promoting oncogenes and inactivation of tumor suppressor genes. Oncogenes, originally identified as retroviral genes essential for the transforming function of their parent virus, have...
been shown to be mutant forms of eukaryotic host genes. Their normal homologues, proto-oncogenes, encode proteins involved in the cascade of events by which growth factors stimulate cell division. Proto-oncogenes fall into four classes: 1) growth factors, 2) growth factor receptors, 3) transducers of growth factor receptor signals, and 4) transcription factors (20).

Activating mutations of proto-oncogenes are found in many types of cancer, but there is little evidence that oncogene activation plays a major role in BCC pathogenesis. Several investigators (21-23) have shown HRAS and KRAS point mutations in a small fraction of BCCs. The tumors with RAS mutations could not be distinguished clinically or histologically from BCCs in which no RAS mutations were found. Neither point mutations nor amplification of NRAS was seen in BCCs, and limited studies of other oncogenes (24) have shown no genetic abnormalities. These data suggest that RAS activation may play a secondary role in BCC pathogenesis but cannot be the primary genetic alteration leading to disordered growth in basal cells. Whether mutations in other oncogenes play a more important role remains to be seen.

Tumor suppressor genes normally exert a negative control on cell growth, and the paradigm for their role in cancer is that inactivation of both homologues ("two hits") is required for a growth-promoting effect (25). Several classes of tumor suppressors have been identified, including cell cycle regulators, intercellular adhesion and signaling molecules, and direct modulators of oncogene proteins and other elements of signal transduction pathways [reviewed in (26)]. TP53 is a tumor suppressor gene that plays a role in carcinogenesis in a wide variety of tissues (27). Direct sequencing of the TP53 gene in BCCs revealed mutation of at least one allele in approximately 50% of tumors (28-30). No trends have been identified that suggest a relationship between TP53 mutation and tumor size, histology, or recurrence (31). These data indicate that TP53 mutations contribute to the malignant characteristics of tumors but are probably not a necessary event in BCC pathogenesis, and mutations in additional genes must confer histologic and other biologic characteristics observed clinically.

Hereditary BCCs and a Novel Tumor Suppressor Gene

Constitutional tumor suppressor gene mutations underlie a large number of hereditary cancer predisposition syndromes (32), and key evidence that BCCs may arise through inactivation of a tumor suppressor gene came from studies of hereditary predisposition to BCCs. Approximately one half of 1% of BCC cases are attributable to the nevoid basal cell carcinoma syndrome (NBCCS) (33), an autosomal dominant disorder characterized by multiple BCCs. Other tumors associated with the syndrome include medulloblastoma, ovarian fibroma, cardiac fibroma, fibrosarcoma, rhabdomyosarcoma, and meningioma. Congenital malformations are also a striking feature of the syndrome and include pits of the palms and soles, keratocysts and other dental malformations, midline brain malformations, strabismus, spine and rib abnormalities, ectopic calcification, mesenteric cysts, macrocephaly with characteristic coarse facies (34), and generalized overgrowth (35). Given the clinical features, the gene for this disorder must play a prominent role in both normal development and continued control of cell growth and differentiation.

The behavior of neoplasms in NBCCS suggests that the underlying defect in this disorder may be mutation in a tumor suppressor gene. Like the cancers arising in hereditary retinoblastoma in which the RB1 tumor suppressor gene is mutated (36) and in Li–Fraumeni syndrome involving germ-line mutation of the TP53 tumor suppressor (37), BCCs in this disorder are multiple, occur relatively early compared with sporadic tumors of the same type, and tend to develop after exposure to ionizing radiation with a brief latent period. On the basis of the clinical behavior of these BCCs, several investigators suggested that the NBCCS fits a two-hit model ([38]; reviewed in [39]); i.e., tumors develop in cells sustaining two genetic alterations. The first alteration or hit is inheritance of a mutation in a tumor suppressor gene, and the second hit is inactivation of the normal homologue by environmental mutagenesis or random genetic rearrangement. Sporadic BCCs would arise in cells that underwent two somatic events resulting in inactivation of the NBCCS gene.

If the NBCCS gene functions as a tumor suppressor, then it should be inactivated in BCCs. Inactivation of a tumor suppressor gene frequently occurs through point mutation of one homologue and loss of the other homologue by nondisjunction, deletion, or mitotic recombination. The latter mechanisms result in loss of heterozygosity (LOH) for polymorphisms in the chromosomal region surrounding the tumor suppressor gene (40) (Fig. 1). A comprehensive analysis of each chromosome arm in a series of BCCs revealed consistent LOH for markers on chromosome 9q (41). A detailed tumor deletion map showed that the smallest area of overlap included the chromosome band 9q22.3 (30,42-45). Linkage analysis with multiple NBCCS kindreds confirmed that the gene mapped to the exact region lost in tumors (41,46-49).

Cloning the NBCCS Gene

Isolation of a disease gene based on its chromosomal location is termed "positional cloning." This method does not require any prior knowledge of the protein product of the gene. The NBCCS gene was cloned by first defining the smallest possible
critical region on chromosome 9q22.3 through tumor deletion studies and linkage analysis and then isolating candidate genes from the area. The human homologue of a Drosophila developmental gene, i.e., patched, was one of the sequences found in this region (50).

Drosophila patched is a component of the hedgehog signaling pathway, a developmental pathway important in early embryonic patterning [reviewed in (51)]. Patched specifically represses the action of hedgehog, a secreted protein that acts on target cells to increase the transcription of several other genes, including wingless (vertebrate Wnt family) and decapentaplegic or dpp (transforming growth factor-β [TGF-β] family) as well as patched itself (52-56) (Fig. 2). All are important in patterning and determination of cell fate within multiple structures of the developing fly. The hedgehog pathway appears to be conserved in vertebrates as a gene family with varying patterns of expression (57-59). Sonic hedgehog, the most common vertebrate homologue, is required for the correct patterning of the neural tube, the somites, and anterior/posterior positioning of the limb bud (60-63). Patched is expressed in all the target tissues of sonic hedgehog. Given the clinical features of NBCCS, which include abnormalities of the brain, ribs, vertebrae, and limbs, and the apparent role of patched in development of these structures, the gene was thought to be a reasonable candidate for NBCCS (Table 1). Screening of patched revealed a wide spectrum of mutations in NBCCS patients, most of which were predicted to result in protein truncation (50,64-67).

Like most autosomal dominant disorders, NBCCS shows significant phenotypic variability (68). Although the severity of the syndrome tends to breed true in families, genotype–phenotype association is very limited. Different kindreds with similar or identical mutations differ dramatically in the extent of clinical features, suggesting that genetic background may have an important role in modifying the spectrum of both developmental and neoplastic traits (67,69). In Drosophila, patched is one of at least 15 genes that play interacting roles in development (70,71), and the mammalian homologues of these interactors may be important modifiers of the NBCCS phenotype.

**Patched and Sporadic BCCs**

Inactivation of patched in BCCs may be a necessary, if not sufficient, event for carcinogenesis. The majority of neoplasms, both sporadic and hereditary, show allelic loss for chromosome 9q22. Minute BCCs are as likely as large tumors to have chromosome 9 allelic loss (44), and all histologic subtypes, whether primary or recurrent, have a high frequency of allelic loss on chromosome 9 (31). Tumors with allelic loss on chromosome 9 sometimes show additional areas of loss on other chromosomes, but no tumors have loss on other chromosomes without involvement of chromosome 9 (41).

Identification of patched has made possible direct mutation screening in sporadic tumors. Single-strand conformation polymorphism (SSCP) analysis of patched in 36 sporadic BCCs found 12 inactivating mutations of the gene. Nine mutations were in tumors with LOH confirming inactivation of both copies of the gene. Three mutations were in three tumors without LOH for this region. SSCP screening of the patched gene has not proven to be highly sensitive and is known to miss at least 50% of mutations. Two tumors without LOH for chromosome 9q and negative SSCP screens were directly sequenced, and each contained two separate mutations. With the assumption that these mutations were in trans (not in cis), these findings indicate that both alleles were inactivated. This sampling of tumors by direct sequencing implies that a high percentage of all sporadic BCCs probably has inactivated both copies of the gene (Fig. 3).

It has been proposed that patched may function as a ‘‘gatekeeper gene’’; i.e., a precursor cell acquires a survival advantage with inactivation of the gatekeeper gene, and inactivation would be necessary before clonal expansion and accumulation of other genetic hits would lead to BCC formation (73,74).

**Molecular Epidemiology**

For some tumors, particular agents have been associated with specific genetic alterations; e.g., aflatoxin B1 appears to lead to
mutation in codon 249 of the TP53 gene in hepatocellular carcinoma (75,76). UV radiation can cause several types of genetic damage, including formation of photodimers that most commonly result in a G·C to A·T transition at a dipyrimidine site (primarily UVB exposure) as well as single-strand breaks (primarily exposure to UVA, i.e., UV radiation of 320- to 400-nm wavelength) (77-79). UVB-related point mutations have been found in the TP53 gene of 40%-56% of BCCs (28-30). In addition, mutations in the ras family of proto-oncogenes are often of the type caused by UVB (21,22).

The role of UV radiation in the pathogenesis of the genetic alterations in patched on chromosome 9 is unclear. The data from NBCCS patients suggest that agents other than UVB may cause somatic alterations on chromosome 9. The first hit in hereditary tumors is a germline point mutation or submicroscopic deletion. Any allelic loss observed in these tumors must reflect the somatic second hit. Nearly all hereditary tumors have allelic loss, and UVB does not characterize cause this type of gross rearrangement of genetic material. Mutations in sporadic tumors also suggest the possibility of an etiologic agent other than UVB. As in hereditary tumors, allelic loss is frequent. In one third of sporadic tumors with allelic loss, the remaining allele has a mutation characteristic of UVB; however, in two thirds, the genetic alteration of the remaining allele is not a typical UVB-induced alteration (72).

The mutational spectrum in the patched gene is distinctly different from that in the TP53 gene, where most mutations can be linked to UVB. Other factors, in addition to UVB exposure, may play a synergistic role in many BCCs and may reflect an etiologic contribution of additional environmental agents.

**Hedgehog Pathway and Cancer**

Based on complementary DNA sequence, patched is predicted to encode a large glycoprotein with 12 membrane-spanning domains and two large extracellular loops (57,58,80,81) (Fig. 2). The patched gene product has no known homology to any other protein or protein motif but does have some characteristics suggestive of a receptor or transporter. Patched does not resemble any known tumor suppressor genes, although there are several membrane proteins involved in intercellular adhesion and signaling that function as tumor suppressors (82-85).

Based on epistatic relationships in *Drosophila* mutants, both patched and smoothened (a seven-pass membrane protein with characteristics of a G-protein-coupled receptor) have been proposed to be the hedgehog receptor (52,86-88). Recent work has shown that patched specifically binds hedgehog (89,90), whereas smoothened does not (89). However, smoothened does complex with patched, and the current theory is that, in the absence of hedgehog, smoothened and patched form an inactive complex. When hedgehog binds to patched, the complex is altered and smoothened is then free to transduce the signal (Fig. 2). In the absence of functional patched, smoothened may be constitutively active, independent of hedgehog control, and may induce overexpression of target genes including patched (89).

Analysis of BCCs by northern blot and in situ hybridization supports this theory. Expression of patched is barely detectable in epidermises or cultured keratinocytes, but patched is readily detectable in tumors both by northern blot analysis and in situ hybridization, which suggests up-regulation (i.e., an increase) of the mutant gene product (72) (Fig. 4). Up-regulation of other hedgehog target genes may play a role in tumors formation. Wnt1, the vertebrate homologue of wingless, is known to cause mammary tumors in mice when it is activated [reviewed in (91)]. Decapentaplegic is a member of the TGF-β superfamily, with closest homology to the vertebrate bone-morphogenetic-protein (BMP) subfamily. Members of the TGF-β family have complex roles in cell growth and differentiation [reviewed in (92)]. The mad (mothers against dpp) gene is a component of the signal transduction pathway of dpp in *Drosophila* (93), and one human homologue of the mad gene, DPC4, has been shown to act as a tumor suppressor in both pancreatic and colon cancers (94,95). Cubitus interruptus, a *Drosophila* transcription factor related to human GLI genes, is functionally activated by loss of patched. Human GLI1 is believed to act as an oncogene in brain tumors (96).
Future Directions

Analysis of genes whose products interact with patched may provide new insights into basal cell carcinogenesis. Several kindreds with early onset of multiple BCCs and no other features of NBCCS have been studied for patched mutations, and no germ-line mutations were found. The known interactions between patched, hedgehog, and smoothened suggest that overactivity of the latter two genes in susceptible tissues might result in carcinogenesis and that germline-activating mutations might lead to a syndrome similar to NBCCS. Hereditary melanoma provides an analogy because many melanoma kindreds have inactivating mutations of CDKN2, a negative regulator of CDK4 activity, but a few families have “activating” CDK4 mutations that release the gene product from CDKN2 regulation (97).

A clear prediction can be made about the phenotypic effect of germline-activating mutations of patched. Both mouse and human data indicate that inactivation of hedgehog results in major brain malformations known as holoprosencephaly (98). On the basis of the antagonistic effect of hedgehog on patched, it seems likely that germline activation of patched will result in holoprosencephaly. Positive and negative mutations of other members of this signal transduction pathway will no doubt cause overlapping phenotypes with NBCCS and holoprosencephaly.

Like many other cancer-predisposition syndromes caused by germline inactivation of a tumor suppressor gene, the spectrum of neoplasia is very limited in NBCCS. Multiple regulatory pathways that keep cell growth and differentiation under control interact to varying degrees in different tissues. While patched may have an important regulatory role in many tissue types, its function in the precursor cells related to tumors found in NBCCS must be critical to maintenance of cell growth.

Clinical Implications

Further studies into the function of patched and other interacting genes may suggest alternative methods for treatment or prevention of BCCs. Pharmacologic agents that mimic the effect of patched may form the basis for a topical medical treatment that would serve as an alternative to surgery. If patched truly acts as a gatekeeper gene, similar agents could be used in prevention of BCCs in susceptible individuals. More importantly, the hedgehog–patched signaling pathway may play a key role in many other types of tumors, and pharmacologic manipulation of the pathway might have wide-ranging implications for a rational medical therapy of cancer.

References


Note

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