separated from its abilities to inhibit cyclin-CDK complexes (*1*).

An early precedent for a double life for CKIs has come from the Far1p protein of the budding yeast *Saccharomyces cerevisiae*. Originally discovered as a CKI induced by mating pheromones, Far1p was later shown to have a distinct function: orienting the yeast cell toward its mating partner (*2*, *3*). Similarly, the mammalian p21 protein studied by Di Cunto *et al.* has another personality, a domain capable of binding to the PCNA (proliferating cell nuclear antigen) component of DNA polymerases, thereby affecting the process of DNA replication (*4*, *5*). This function of p21 outside of the core clock machinery provides an additional precedent for a multifunctional CKI that can affect cellular targets other than the core components of the clock machinery.

Other surprises of this sort have emerged recently. Cyclin D1 was initially portrayed as an important activator of the CDK4 and CDK6 complexes that phosphorylate pRB and related proteins in the G_1 phase (*6*). But reports from two groups indicate, totally unexpectedly, that cyclin D1 can bind and activate the estrogen receptor (ER) (*7*, *8*). Before this work, estrogen was thought to be the major physiologic activator of this receptor. The biological consequences of the cyclin D1–ER interaction remain unclear; given the wide-ranging actions of the ER, some of them might involve differentiation-like responses.

pRB has been portrayed exclusively as the brake shoe of cell cycle advance in the G_1 phase of the growth cycle; its absence or functional inactivation in many types of human tumors is compatible with this action (*9*). But new research indicates that pRb helps to direct the development of at least two distinct differentiation programs. Cultured myoblasts do not differentiate properly in the absence of pRB (*10*, *11*). This differentiation function appears to be associated with a domain of pRB that is distinct from those domains that directly control proliferation (*12*). Yet other work indicates an analogous role for pRB in programming adipocyte differentiation (*13*). Although these results stem from in vitro differentiation models, we suspect that they reflect processes operative in living tissues and that the differentiation programs in a variety of other tissues may be similarly dependent on pRB function.

A particularly intriguing example of an intrinsic cell cycle regulator moonlighting in another cellular function is the CDK-activating enzyme CAK, a kinase required for the full stimulation of CDK activity. In mammalian cells, CAK is also a critical component of the RNA polymerase holoenzyme (its TFIIH subunit), required for the transcription of most cellular genes (*14*–*16*). Whether this is an example of a cell cycle regulator being co-opted by evolution to perform a transcriptional function or the reverse is not known.

The portrait of the cell cycle clock as an apparatus focused exclusively on governing proliferation has become simplistic. It now seems clear that this apparatus, embedded in the heart of the eukaryotic cell for a billion years, has been exploited by the tinkering hand of evolution to control other important cellular functions, particularly those that are required for complex cellular differentiation. Evolution, always opportunistic, uses the hardware already lying around on the shelves to make clever new toys. The powers of the cell cycle clock apparatus are likely to be far broader than currently suspected.

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ONCOGENESIS

Landscaping the Cancer Terrain

Kenneth W. Kinzler and Bert Vogelstein

Few lines of investigation have taught us more about cancer than the study of inherited tumor susceptibility syndromes. Initially, the mutations responsible for these diseases were thought to promote malignancy in a straightforward manner, through inactivation of "tumor suppressor" genes, which directly modulate cell birth or cell death. More recently, however, susceptibility genes that work through less-direct mechanisms have come to light. The genes defective in patients with juvenile polyposis syndromes (JPSs), for example—one of which is described on page 1086 of this issue (*1*)—illuminate this principle and also raise fundamental questions about the relation between neoplastic cells and the "other cells" that together constitute a tumor mass.

A dozen tumor suppressor genes are known to prevent cancer through direct control of cell growth—including *p53*, *Rb*, *VHL*, and *APC*. Inactivation of these genes contributes directly to the neoplastic growth of the tumor; thus, they normally function as "gatekeepers" to prevent runaway growth (see the figure). Accordingly, restoration of the missing gatekeeper function to cancer cells leads to suppression of the neoplastic growth.

These traditional tumor suppressors are being joined by an ever-increasing number of susceptibility genes that indirectly suppress neoplasia (for example, *XPB*, *ATM*, *MSH2*, and *MLH1*). The prototypes for this class of genes encode DNA repair proteins that act as "caretakers" of the genome. Inactivation of a caretaker gene results in a greatly increased mutation rate and is equivalent to a constant exposure to mutagens. It is not surprising that such defects should lead to cancer, but restoration of caretaker function to a cancer cell will not affect its growth. As these indirectly acting genes are never *required* for neoplasia, most nonhereditary (sporadic) tumors will evolve without them.

A second class of indirectly acting cancer susceptibility genes is suggested by recent studies on JPS. Individuals with JPS have an increased risk of colorectal cancer, but the primary manifestation of this syndrome is the development of multiple hamartomatous polyps of the colon at a young age. These polyps are markedly different from the epithelium-rich adenomatous polyps that give rise to most cases of colorectal cancer. Polyps from patients with JPS have a low potential to become malignant and are composed largely of stromal cells, comprising a mixture of mesenchymal and inflammatory elements in which epithelium is entrapped, often forming dilated cysts. The epithelial cells within and surrounding the polyp are initially devoid of neoplastic features but nevertheless are at increased risk of becoming malignant.

It would thus seem that the increased cancer susceptibility due to inherited mutations in JPS is the product of an abnormal stromal environment. That an abnormal stroma can affect the development of adjacent epithelial cells is not a new concept. Ulcerative colitis

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Pathways to colorectal cancer. Colorectal cancer can be caused by genetic defects that interrupt "gatekeeper," "caretaker," or "landscaper" functions in the colon. The bold arrows indicate the step accelerated in that class of tumor.

Sporadic colorectal cancer. The probability that a colorectal epithelial cell will acquire the genetic changes leading to a benign tumor is low, but still half the Western population develops such a tumor by age 70. A fraction of these benign tumors (adenomatous polyps) progress to cancer, yielding a lifetime cancer risk in the general population of 5%.

Gatekeeper defects. In patients with familial adenomatous polyposis (FAP), for example, an inherited defect in the *APC* gene leads to the development of hundreds of adenomatous polyps (*10*). Because of their great numbers, some polyps are virtually guaranteed to progress to cancer.

Caretaker defects. Patients with hereditary nonpolyposis colorectal cancer (HNPCC) develop adenomatous polyps at about the same rate as the general population, but these polyps progress to cancer much more often because of defective mismatch repair, which results in an increased mutation rate (*10*).

Landscaper defects. Patients with juvenile polyposis syndrome (JPS) and ulcerative colitis (UC) develop hamartomatous polyps in which the proliferating defective population of cells appears to be derived from the stroma. Consequently, the epithelial cells associated with the polyps are more likely to undergo neoplastic transformation, as a result of an abnormal microenvironment. Likewise, the initially normal epithelial cells associated with the inflammatory process of UC are at increased risk of neoplastic transformation.

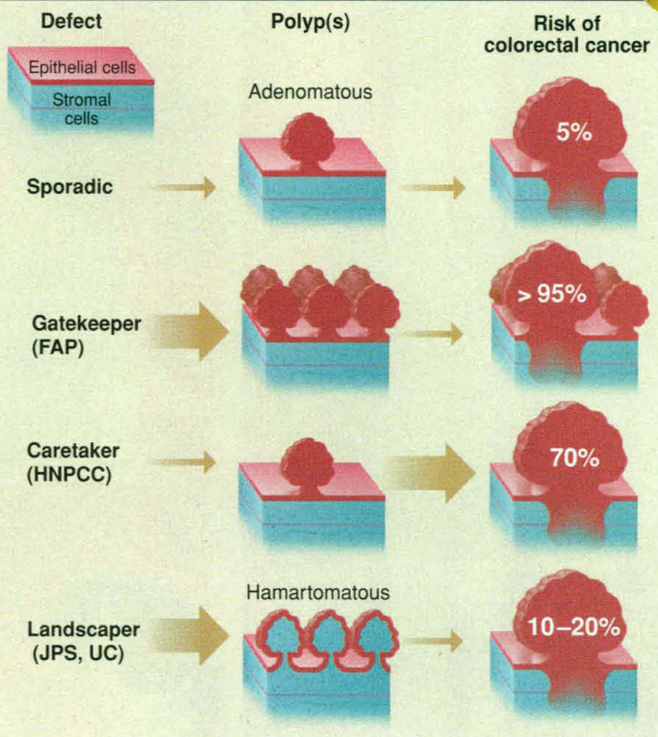

ILLUSTRATION: K. SUTLIFF

(UC) is an autoimmune disease that leads to inflammation and cystic epithelium in the mucosa of the colon. Initially the embedded epithelium shows no neoplastic changes, but foci of epithelial neoplasia and progression to cancer eventually develop in many cases. The regeneration that occurs to replace damaged epithelium may increase the probability of somatic mutations in this abnormal microenvironment. The increased risk of cancer in JPS and UC patients therefore seems primarily the result of an altered terrain for epithelial cell growth and can be thought of as a "landscaper" defect.

How can one test the landscaper hypothesis and demonstrate that the primary oncogenic effect of the mutation is on stromal rather than epithelial cells? This should be possible through careful genetic evaluation of the stromal and epithelial populations of the hamartomas. It is intriguing that the stromal cells, but not the epithelial cells, of most hamartomas from JPS patients contain a clonal genetic alteration (*2*). Similarly, clonal genetic changes have been demonstrated in the stroma, but not the epithelial cells, of endometrial polyps (*3*). In contrast, clonal genetic alterations have been demonstrated in epithelial cells, but not stromal cells, of the polyps arising in patients with familial adenomatous polyposis (FAP) (*4*) or Peutz-Jeghers syndromes (*5*)—which are morphologically distinct from those in JPS patients. Now that we know that inherited mutations in *PTEN* (*6*) or *SMAD4* (*1*) can lead to the development of the hamartomatous polyps, it will be informative to determine whether the stromal or epithelial compartment of hamartomatous lesions (and the cancers that arise within them) show inactivation of the wild-type copy of *PTEN* or *SMAD4* inherited from the unaffected parent.

Both *PTEN* and *SMAD4* directly control cell growth in other tumor types. Accordingly, somatic mutations of *PTEN* (*7*) and *SMAD4* (*8*) commonly occur in brain and pancreatic cancers, respectively, although mutations of these genes occur infrequently in colorectal cancer cells (<5% of colorectal cancers for *PTEN* and <15% for *SMAD4*). Could the same gene function as a gatekeeper in one tumor type or at one stage of tumor development and as a landscaper in another, violating the principle of Ockham's razor? Perhaps this is not unexpected given the functions of these genes. PTEN is a dual-specificity phosphatase that is likely to affect a plethora of processes in many cell types, and SMAD4 is a central player in the signal transduction pathway activated in response to the large family of TGF-β (transforming growth factor–β)–like ligands. The ligand that triggers the pathway containing SMAD4 in pancreatic epithelial cells may be entirely different from the one it responds to in JPS hamartomas.

These results add to the emerging realization that solid tumors are not simply composed of neoplastic epithelial cells. Historically, the search for drugs that can modulate neoplasia has focused on such epithelial cells. More recent results, however, have suggested that targeting specific stromal cells (such as those forming blood vessels) might be more valuable for therapeutic purposes (*9*). Could drug targeting of the paracrine factors and other features of the stromal-epithelial interaction be similarly useful? Although such drugs would be unlikely to affect advanced tumors, in which the neoplastic cells are largely autonomous, they might be efficacious in the early, benign stages of tumorigenesis, nipping them in the bud.

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