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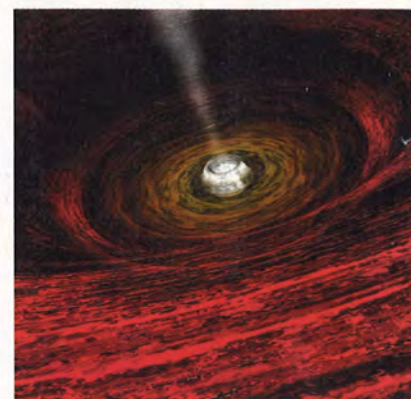
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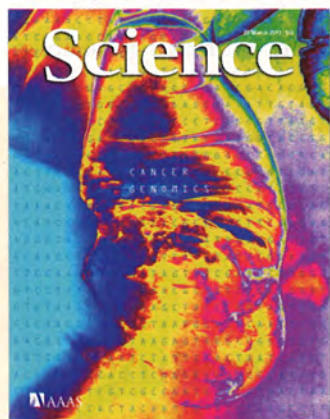
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Human colon cancer (at lower center) identified by a colored barium x-ray shown overlaid with a representation of a genetic sequence. Genome sequence analysis of human tumors has uncovered an array of genetic alterations that help drive tumor growth—information that may lead to more effective cancer therapies. See the special section beginning on page 1539.

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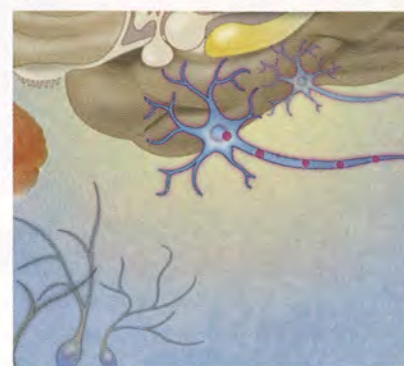
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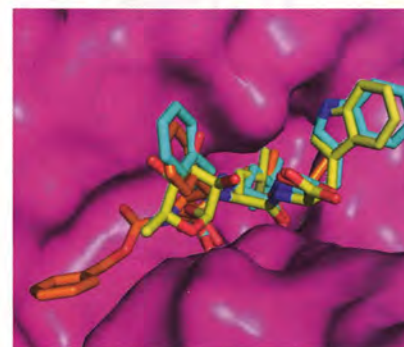
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REVIEW

Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shibin Zhou, Luis A. Diaz Jr., Kenneth W. Kinzler*

Over the past decade, comprehensive sequencing efforts have revealed the genomic landscapes of common forms of human cancer. For most cancer types, this landscape consists of a small number of "mountains" (genes altered in a high percentage of tumors) and a much larger number of "hills" (genes altered infrequently). To date, these studies have revealed ~140 genes that, when altered by intragenic mutations, can promote or "drive" tumorigenesis. A typical tumor contains two to eight of these "driver gene" mutations; the remaining mutations are passengers that confer no selective growth advantage. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance. A better understanding of these pathways is one of the most pressing needs in basic cancer research. Even now, however, our knowledge of cancer genomes is sufficient to guide the development of more effective approaches for reducing cancer morbidity and mortality.

Ten years ago, the idea that all of the genes altered in cancer could be identified at base-pair resolution would have seemed like science fiction. Today, such genome-wide analysis, through sequencing of the exome (see Box 1, Glossary, for definitions of terms used in this Review) or of the whole genome, is routine.

The prototypical exomic studies of cancer evaluated ~20 tumors at a cost of >\$100,000 per case (1–3). Today, the cost of this sequencing has been reduced 100-fold, and studies reporting the sequencing of more than 100 tumors of a given type are the norm (table S1A). Although vast amounts of data can now be readily obtained, deciphering this information in meaningful terms is still challenging. Here, we review what has been learned about cancer genomes from these sequencing studies—and, more importantly, what this information has taught us about cancer biology and future cancer management strategies.

How Many Genes Are Subtly Mutated in a Typical Human Cancer?

In common solid tumors such as those derived from the colon, breast, brain, or pancreas, an average of 33 to 66 genes display subtle somatic mutations that would be expected to alter their protein products (Fig. 1A). About 95% of these mutations are single-base substitutions (such as C>G), whereas the remainder are deletions or insertions of one or a few bases (such as CTT>CT) (table S1B). Of the base substitutions, 90.7% result in missense changes, 7.6% result in nonsense changes, and 1.7% result in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons (table S1B).

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Certain tumor types display many more or many fewer mutations than average (Fig. 1B). Notable among these outliers are melanomas and lung tumors, which contain ~200 nonsynonymous mutations per tumor (table S1C). These larger numbers reflect the involvement of potent mutagens (ultraviolet light and cigarette smoke, respectively) in the pathogenesis of these tumor types. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers (4). Tumors with defects in DNA repair form another group of outliers (5). For example, tumors with mismatch repair defects can harbor thousands of mutations (Fig. 1B), even more than lung tumors or melanomas. Recent studies have shown that high numbers of mutations are also found in tumors with genetic alterations of the proofreading domain of DNA polymerases POLE or POLD1 (6, 7). At the other end of the spectrum, pediatric tumors and leukemias harbor far fewer point mutations: on average, 9.6 per tumor (table S1C). The basis for this observation is considered below.

Mutation Timing

When do these mutations occur? Tumors evolve from benign to malignant lesions by acquiring a series of mutations over time, a process that has been particularly well studied in colorectal tumors (8, 9). The first, or "gatekeeping," mutation provides a selective growth advantage to a normal epithelial cell, allowing it to outgrow the cells that surround it and become a microscopic clone (Fig. 2). Gatekeeping mutations in the colon most often occur in the *APC* gene (10). The small adenoma that results from this mutation grows slowly, but a second mutation in another gene, such as *KRAS*, unleashes a second round of clonal growth that allows an expansion of cell number (9). The cells with only the *APC* mutation may persist, but their cell numbers are small compared with the cells that

have mutations in both genes. This process of mutation followed by clonal expansion continues, with mutations in genes such as *PIK3CA*, *SMAD4*, and *TP53*, eventually generating a malignant tumor that can invade through the underlying basement membrane and metastasize to lymph nodes and distant organs such as the liver (11). The mutations that confer a selective growth advantage to the tumor cell are called "driver" mutations. It has been estimated (12) that each driver mutation provides only a small selective growth advantage to the cell, on the order of a 0.4% increase in the difference between cell birth and cell death. Over many years, however, this slight increase, compounded once or twice per week, can result in a large mass, containing billions of cells.

The number of mutations in certain tumors of self-renewing tissues is directly correlated with age (13). When evaluated through linear regression, this correlation implies that more than half of the somatic mutations identified in these tumors occur during the preneoplastic phase; that is, during the growth of normal cells that continuously replenish gastrointestinal and genitourinary epithelium and other tissues. All of these pre-neoplastic mutations are "passenger" mutations that have no effect on the neoplastic process. This result explains why a colorectal tumor in a 90-year-old patient has nearly twice as many mutations as a morphologically identical colorectal tumor in a 45-year-old patient. This finding also partly explains why advanced brain tumors (glioblastomas) and pancreatic cancers (pancreatic ductal adenocarcinomas) have fewer mutations than colorectal tumors; glial cells of the brain and epithelial cells of the pancreatic ducts do not replicate, unlike the epithelial cells lining the crypts of the colon. Therefore, the gatekeeping mutation in a pancreatic or brain cancer is predicted to occur in a precursor cell that contains many fewer mutations than are present in a colorectal precursor cell. This line of reasoning also helps to explain why pediatric cancers have fewer mutations than adult tumors. Pediatric cancers often occur in non-self-renewing tissues, and those that arise in renewing tissues (such as leukemias) originate from precursor cells that have not renewed themselves as often as in adults. In addition, pediatric tumors, as well as adult leukemias and lymphomas, may require fewer rounds of clonal expansion than adult solid tumors (8, 14). Genome sequencing studies of leukemia patients support the idea that mutations occur as random events in normal precursor cells before these cells acquire an initiating mutation (15).

When during tumorigenesis do the remaining somatic mutations occur? Because mutations in tumors occur at predictable and calculable rates (see below), the number of somatic mutations in tumors provides a clock, much like the clock used in evolutionary biology to determine species

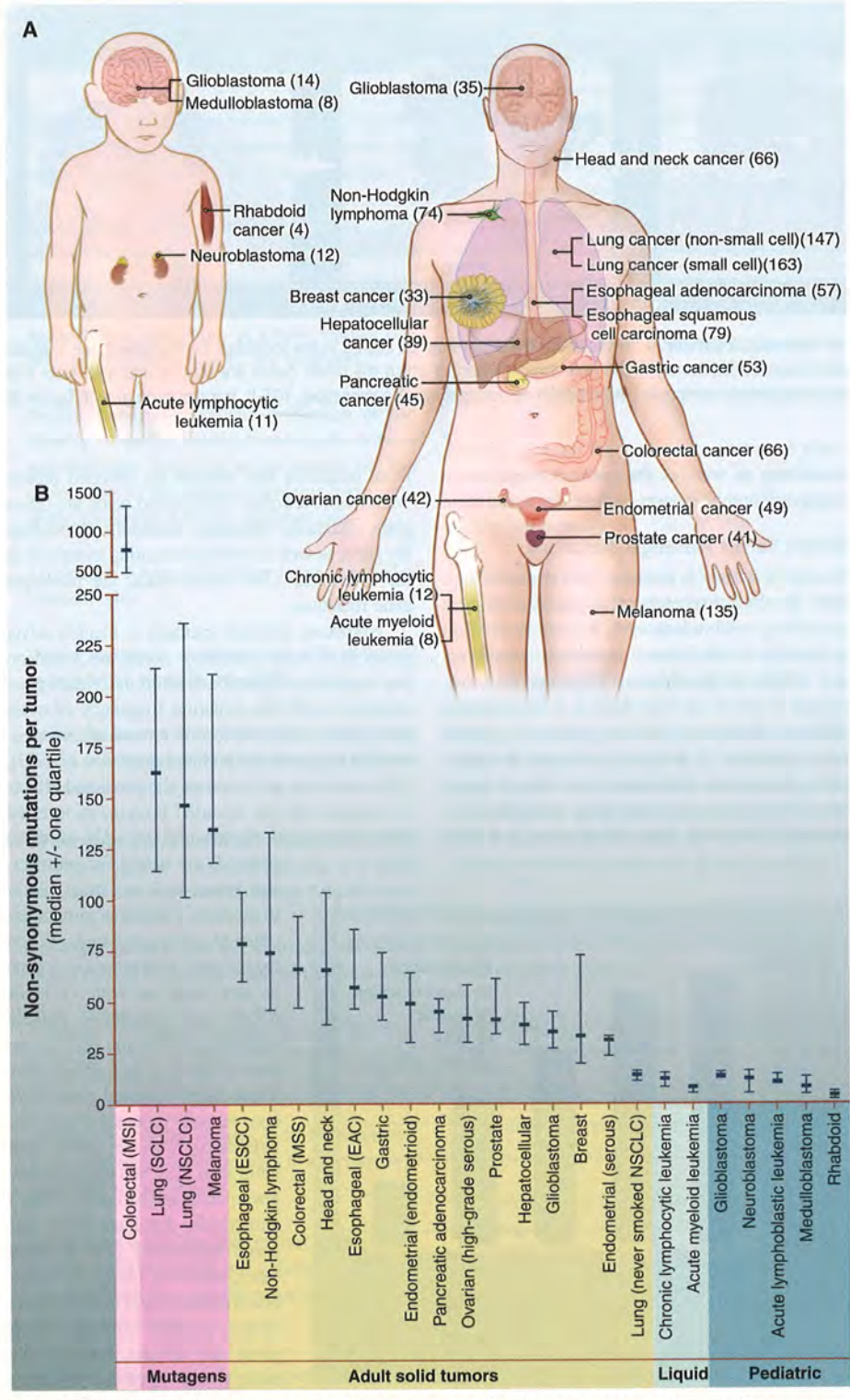


Fig. 1. Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies. (A) The genomes of a diverse group of adult (right) and pediatric (left) cancers have been analyzed. Numbers in parentheses indicate the median number of nonsynonymous mutations per tumor. (B) The median number of nonsynonymous mutations per tumor in a variety of tumor types. Horizontal bars indicate the 25 and 75% quartiles. MSI, microsatellite instability; SCLC, small cell lung cancers; NSCLC, non-small cell lung cancers; ESCC, esophageal squamous cell carcinomas; MSS, microsatellite stable; EAC, esophageal adenocarcinomas. The published data on which this figure is based are provided in table S1C.

divergence time. The number of mutations has been measured in tumors representing progressive stages of colorectal and pancreatic cancers (11, 16). Applying the evolutionary clock model to these data leads to two unambiguous conclusions: First, it takes decades to develop a full-blown, metastatic cancer. Second, virtually all of the mutations in metastatic lesions were already present in a large number of cells in the primary tumors.

The timing of mutations is relevant to our understanding of metastasis, which is responsible for the death of most patients with cancer. The primary tumor can be surgically removed, but the residual metastatic lesions—often undetectable and widespread—remain and eventually enlarge, compromising the function of the lungs, liver, or other organs. From a genetics perspective, it would seem that there must be mutations that convert a primary cancer to a metastatic one, just as there are mutations that convert a normal cell to a benign tumor, or a benign tumor to a malignant one (Fig. 2). Despite intensive effort, however, consistent genetic alterations that distinguish cancers that metastasize from cancers that have not yet metastasized remain to be identified.

One potential explanation invokes mutations or epigenetic changes that are difficult to identify with current technologies (see section on “dark matter” below). Another explanation is that metastatic lesions have not yet been studied in sufficient detail to identify these genetic alterations, particularly if the mutations are heterogeneous in nature. But another possible explanation is that there are no metastasis genes. A malignant primary tumor can take many years to metastasize, but this process is, in principle, explicable by stochastic processes alone (17, 18). Advanced tumors release millions of cells into the circulation each day, but these cells have short half-lives, and only a minuscule fraction establish metastatic lesions (19). Conceivably, these circulating cells may, in a nondeterministic manner, infrequently and randomly lodge in a capillary bed in an organ that provides a favorable microenvironment for growth. The bigger the primary tumor mass, the more likely that this process will occur. In this scenario, the continual evolution of the primary tumor would reflect local selective advantages rather than future selective advantages. The idea that growth at metastatic sites is not dependent on additional genetic alterations is also supported by recent results showing that even normal cells, when placed in suitable environments such as lymph nodes, can grow into organoids, complete with a functioning vasculature (20).

Other Types of Genetic Alterations in Tumors
 Though the rate of point mutations in tumors is similar to that of normal cells, the rate of chromosomal changes in cancer is elevated (21). Therefore, most solid tumors display widespread changes in chromosome number (aneuploidy), as well as deletions, inversions, translocations,

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