

The Landmark Discovery That Paved the Way to a Mechanistic Understanding of P53 Gain of Function and Personalized Medicine

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In 1990, Baker and colleagues reported their seminal findings in *Cancer Research* focusing on the transition from adenoma to carcinoma of the colon. By sequencing the *TP53* locus in 58 colorectal tumors (25 adenomas and 33 carcinomas) and measuring its allelic deletions, they discovered that this transition requires the loss of one *TP53* allele and the mutation of the other one. Here, we discuss how this landmark discovery shed a new light on p53 mutations, prompting the generation of novel mouse models that definitively proved the

Despite the molecular and pathologic features distinguishing cancers originating from different organs, a crucial characteristic common to most epithelial tumors is the series of morphologic and anatomic changes collectively known as multistep tumorigenesis. This process was initially investigated in colorectal cancer, which represents an ideal model due to both its high incidence and its slow evolution from hyperplastic structures into adenomas and finally into carcinomas. Indeed, this slow progression typically spans decades, making colorectal cancer a prime candidate for early intervention and treatment. One of the most significant breakthroughs in the comprehension of colorectal cancer progression was achieved by Vogelstein and colleagues (1). They unequivocally demonstrated that each subsequent step of cancer progression is associated with the accumulation of specific genetic alterations (Fig. 1; refs. 1, 2). This paradigm, now generally referred to as the Vogelstein genetic model of colorectal tumorigenesis, clearly showed that the induction of the WNT pathway due to the loss of the tumor suppressor *APC* is pivotal for the transition of the normal colon epithelium into early lesions. Only if activating mutations in *KRAS* occur, these lesions will then aberrantly grow into large yet benign tumors, that is, late adenomas. Notably, Baker and colleagues in Vogelstein's group elegantly demonstrated that malignant tumors, that is, colorectal carcinomas, can only emerge as a result of the inactivation of both alleles of the tumor suppressor p53 (2). This crucial observation was made by analyzing the *TP53* locus in a series of 25 adenomas and 33 colorectal carcinomas. The authors' strategy comprised both the quantification of allelic deletions occurring in this locus and the sequencing of its exons 5 to 9, which correspond to its DNA-binding domain, where the vast majority of p53 mutations

mutant p53 gain-of-function hypothesis suggested by these results. Finally, we evaluate the implications that the Vogelstein model of cancer progression had on numerous aspects of cancer biology and cancer care, including the characterization of tumor evolution and the response to therapy, and how it ultimately contributed to the wider adoption of early detection screenings and personalized medicine.

See related article by Baker and colleagues, *Cancer Res* 1990; 50:7717–22

cluster in human cancers (3). Through these analyses, the authors found that in 90% of adenomas, both *TP53* alleles were retained and did not have any mutations. In contrast, approximately 70% of carcinomas had a mutant *TP53* allele. Even more impactful for the p53 field was the discovery that 87% of the mutant p53-expressing carcinomas also lost the remaining *TP53* allele. This observation led the authors to speculate a very intriguing hypothesis that would take almost 15 years before being confirmed by the p53 field with genetically engineered mouse models (4, 5). In fact, prior to this article by Vogelstein's group, mutations in *TP53* were believed only to have two main consequences: (i) a "loss of function," because the mutated protein cannot bind to the p53 response elements on the promoters of its targets; and (ii) a "dominant negative" effect, due to the interaction between the mutant protein and the wild-type counterpart, thus preventing the latter from acting as a tumor suppressor. However, if these are the only two consequences of mutations in *TP53*, a major unanswered question was the need for genetic pressure to mutate a *TP53* allele in colorectal carcinomas rather than losing both alleles. The answer, the authors argued, must be that the mutation of *TP53*—rather than its simple loss—endows cancer cells with advantageous properties allowing tumors to become invasive. This was formally proven to be correct in 2004, when two laboratories led by Guillermina Lozano (4) and Tyler Jacks (5) generated mouse models mimicking the Li-Fraumeni syndrome, a disease caused by the somatic mutation of *TP53* predisposing to the spontaneous onset of multiple tumor types, especially in children and young adults (3). Importantly, in contrast to *Trp53*^{-/-} mice, both *Trp53*^{R172H/-} and *Trp53*^{R270H/-} mice developed distinct tumors, including invasive and metastatic carcinomas (4, 5), thus confirming the hypothesis based on analysis of clinical samples. The characterization of these Li-Fraumeni mouse models also unveiled that the novel oncogenic properties associated with mutant p53, collectively referred to as mutant p53 gain of function, can partially be ascribed to the capability of mutant p53 to bind to and inhibit the other p53 family members—the transcription factors p63 and p73—that are crucial tumor and metastasis suppressors (6). Prompted by the observations from Vogelstein's group, further investigation of the mutant p53 gain of function has over the years paved the way toward the identification of multiple strategies to tackle aggressive tumors bearing mutant p53 with various degrees of success, such as

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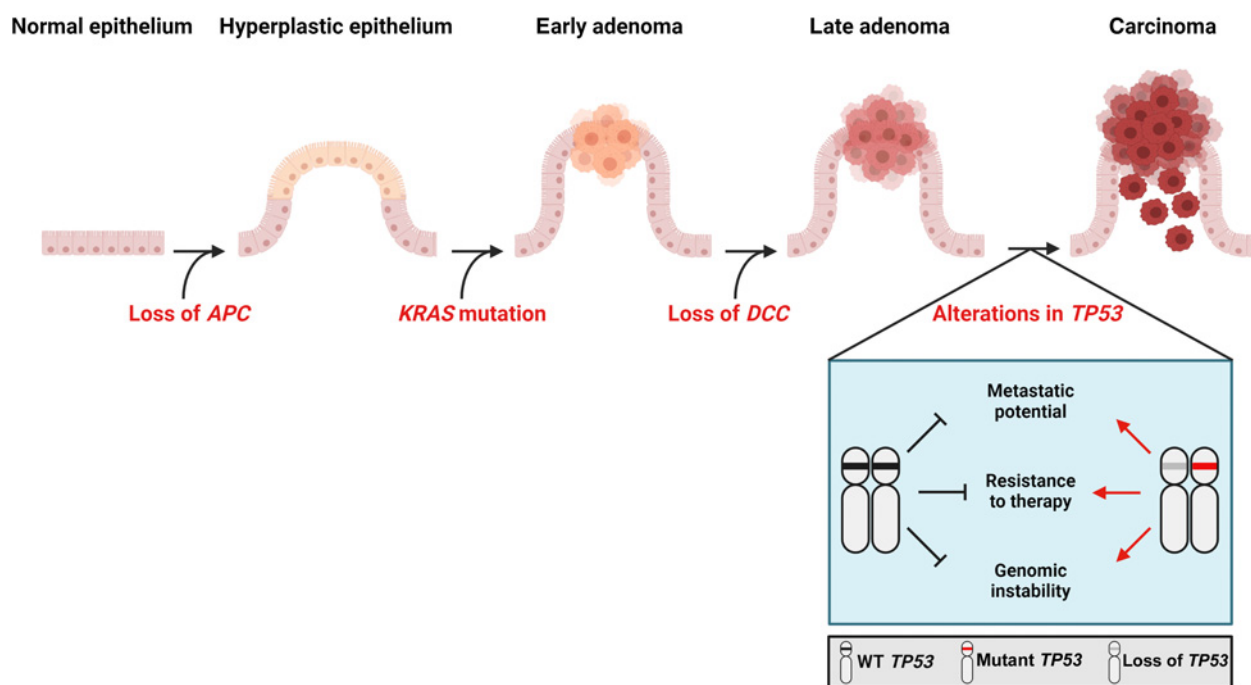


Figure 1. Schematic representation of the multistep tumorigenesis of colorectal cancers. The insert depicts the effects occurring during the transition between late adenomas and carcinomas, which are associated either with the presence of two wild-type *TP53* alleles or with the loss of one *TP53* allele and the mutation of the remaining one, respectively. Created with BioRender.com.

blocking mutant p53 activities, causing its proteasomal degradation, reactivating wild-type p53-like tumor suppressive functions, or compensating for p53 inactivation through the remaining p53 family members, p63 and p73 (3, 7, 8).

Beyond the p53 field, the seminal findings by Vogelstein’s group have resonated across the cancer research community and impacted our understanding of the process of cancer progression. Indeed, the Vogelstein model encompassing a specific chain of genetic alterations responsible for multistep tumorigenesis has since been successfully applied to other tumor types, particularly pancreatic ductal adenocarcinomas. However, because of the broad availability of rapid genome-wide sequencing, it has become clear that most cancers elude this linear sequence of mutations and are instead characterized by a plethora of low frequency alterations driving a high degree of heterogeneity. The realization of this incredible complexity has increased the level of sequencing granularity to the point of achieving single cancer cell resolution, which has been remarkably relevant for two distinct yet intertwined issues: tumor evolution and response to therapy (9). In fact, the genomic instability arising during multistep tumorigenesis underlies the development of numerous subclones adapting to the different microenvironments of the expanding tumor mass. The outcompeting clones can then branch out via the blood stream and reach distant organs, where metastases will form. Comparison of the clonality between the primary tumors and the respective metastases has shown that further genetic variation emerges in the latter (9), and this has profound implications for their diverse response to treatment. An ever-growing body of evidence has indeed indicated how monitoring the changes in the clonality of both tumors and metastases during treatment is beneficial to evaluate the efficacy of the treatment and the possible emergence of resistant clones (9).

One of the most revolutionizing consequences of the Vogelstein model in the clinic has undoubtedly been the steady improvement of the 5-year survival rate of patients with colon cancer, which is now beyond 90% in the case of localized tumors (10). Indeed, one crucial aspect of multistep tumorigenesis is that the detection of early lesions can prevent them from progressing into more deadly colorectal carcinomas. This has initially been accomplished with the wider adoption of colonoscopies that are now recommended as a screening method in the general population starting at age 45. Over the years, additional early detection methods have been designed, which include noninvasive alternatives focusing on the identification of enzymatic hemoglobin and the detection of DNA aberrations, such as fecal occult blood tests, fecal immunochemical tests, and multitarget stool DNA tests (10). The benefits achieved in colon cancer survival rates, thanks to early detection methods, have been extended to other organs, including breast (e.g., mammograms), cervix (e.g., Pap smears), prostate (e.g., digital rectal exams), and skin (e.g., screening of atypical nevi; ref. 10). Given the emphasis of the Vogelstein model on the accumulation of cancer mutations, after tumors are detected with these screening methods, they can undergo comprehensive genomic profiling for the identification of their unique cancer mutational landscape. If the sequencing unveils the presence of targetable mutations, patients will then be selected to receive the most suitable treatments, thus accomplishing a personalized medicine type of approach.

After more than 30 years since its publication, the article by Baker and colleagues continues to impart lessons about how observations in clinical samples are crucial not only to further our knowledge of the mechanisms controlling cancer initiation and progression, but also to provide the best possible strategies for the treatment of cancer patients.

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