SNPing Away at Cancer

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Excitement and controversy followed the discovery that a frequently occurring single nucleotide polymorphism in noncoding DNA affects cancer susceptibility. In this issue of *Cancer Cell*, Post et al. (2010) report using a mouse model to demonstrate directly that this subtle genetic variation significantly attenuates the p53 pathway and accelerates tumor formation.

The human genome shows a remarkable degree of variation, as revealed by whole-genome sequencing and high-throughput array-based methods. Polymorphisms include copy number variations, inversions, rearrangements, and single nucleotide polymorphisms (SNPs). SNPs have been particularly useful as markers of susceptibility to disease conditions such as cancer, diabetes, neurodegeneration, heart failure, and infection. As most SNPs are found in noncoding DNA or are synonymous (i.e., do not change encoded amino acids), they are usually thought to be merely genetic markers of regions of interest. However, in this issue of *Cancer Cell*, Post and colleagues provide in vivo and mechanistic evidence that a SNP in the promoter of the *MDM2* gene directly affects the p53 pathway of tumor suppression.

Either highly penetrant gene mutations or weaker genetic variants may confer increased cancer risk. For example, familial breast cancer is associated with mutations in the *BRCA1* and *BRCA2* genes (Balmain et al., 2003). Germline mutations of *TP53* (p53) cause Li-Fraumeni Syndrome (LFS), which is characterized by predisposition to various malignancies that usually arise at a relatively young age and the development of multiple primary tumors. p53 is normally induced in response to stress, resulting in gene transcription that culminates in cell-cycle arrest, senescence, or apoptosis, which in turn eliminates damaged cells. Mutation of p53 or the dysregulation of p53 pathway components inactivate this protective mechanism and can lead to malignancy. Somatic mutations in p53 are the most common specific genetic alteration in human cancer; therefore, one might anticipate that even subtle genetic variations that alter the function of p53 regulators would also affect the p53 pathway and, thus, tumor development.

Indeed, a SNP was identified in the promoter of the *MDM2* gene that affects cancer incidence in humans (Bond et al., 2004). *MDM2* encodes a ubiquitin ligase that binds and ubiquitinates p53, targeting it for degradation by the proteasome. MDM2 is a major negative regulator of p53, because the embryonic lethality of *Mdm2* null mice is caused by the uncontrolled activity of p53 and rescued by its deletion (Lozano, 2010). Furthermore, MDM2 is required to keep the spontaneous activity of p53 constantly in check, otherwise rampant apoptosis results in tissue damage and eventual death (Ringshausen et al., 2006).

The T-to-G SNP in the second promoter of the human *MDM2* gene has been associated with increased tumor susceptibility in humans. The presence of the G nucleotide at this position facilitates binding of the transcription factor Sp1, leading to increased *MDM2* transcription. Elevated levels of MDM2 suppress activation of the p53 pathway, including p53-dependent apoptosis (Figure 1). These findings are important because 40% of healthy people are heterozygous for the $MDM2^{SNP309G}$ allele (i.e., T/G) and 12% are homozygous (i.e., G/G) (Bond et al., 2004). The biochemical data predict that these people should be more prone to cancer because their ability to activate the p53 pathway in response to stress is suppressed. Indeed, analysis of LFS patients show that 75% of $MDM2^{SNP309G/G}$ individuals develop multiple tumors, in contrast to the 59% of $MDM2^{SNP309G/T}$ and 18% of $MDM2^{SNP309T/T}$ individuals who do so (Bond et al., 2004). However, various clinical studies investigating the association of the $MDM2^{SNP309G}$ allele with cancer susceptibility have yielded conflicting results.

To address this controversy, Post and colleagues replaced the mouse *Mdm2* alleles with humanized ones, $Mdm2^{SNP309G}$ and $Mdm2^{SNP309T}$. They also study this SNP in the context of a mouse model for LFS, in which the mice carry a knockin *p53* mutation ($p53^{515A}$) and, thus, express the mutant protein p53R172H (Lang et al., 2004). This study confirms that the $Mdm2^{SNP309G}$ allele has the same effect in mice that it does in humans on Sp1 binding, *Mdm2* transcription, and p53 pathway activation. $Mdm2^{SNP309G/G}$ mice develop tumors earlier and have shorter lifespans than $Mdm2^{SNP309T/T}$ mice. The same result was seen in $Mdm2^{SNP309G/G}$ $p53^{515A/+}$ and $Mdm2^{SNP309T/T}$ $p53^{515A/+}$ mice. In fact, 26% of the $Mdm2^{SNP309G/G}$ $p53^{515A/+}$ mice developed multiple primary tumors, whereas only 5% of the $Mdm2^{SNP309T/T}$ $p53^{515A/+}$ mice did so, mirroring the increased tumor burden of LFS patients with the $MDM2^{SNP309G}$ allele (Bond et al., 2004). Given that these mice have identical genetic backgrounds and are kept under identical environmental conditions, these observations prove conclusively that the $MDM2^{SNP309G}$ allele does indeed increase tumor susceptibility.

Although $MDM2^{SNP309G}$ only subtly modulates the p53 pathway, its biological consequences are profound, which is consistent with previous genetic and biochemical studies demonstrating the important role of gene dosage in the p53 pathway. For example, mice with an *Mdm2* hypomorphic allele, which express only 30% of the wild-type levels of MDM2, showed a delay in the development of intestinal tumors arising from a mutant

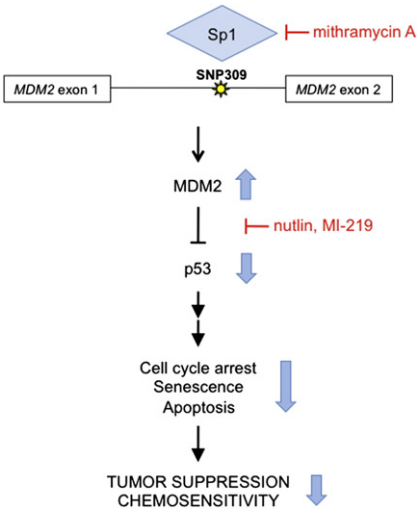

Figure 1. *MDM2*^{SNP309G} Suppresses p53 Pathway Activation and Accelerates Tumor Formation

The transcription factor Sp1 has increased affinity for *MDM2*^{SNP309G}, resulting in elevated MDM2 expression; the consequent inhibition of the p53 pathway impairs tumor suppression and chemosensitivity (denoted by the blue arrows). Potential therapeutic strategies for patients with the *MDM2*^{SNP309G} allele include treatment with mithramycin A, nutlin, and MI-219 (shown in red).

allele of the adenomatous polyposis coli gene (Mendrysa et al., 2006). This exquisite sensitivity of the p53 pathway to small changes in protein levels has important implications for the development of cancer therapeutic strategies, where a common approach is to activate p53 in order to induce tumor clearance. Post and colleagues showed that *MDM2*^{SNP309G} reduces p53-dependent apoptosis after low-dose irradiation. Similarly, this allele reduces the sensitivity of cells to chemotherapeutic drugs (Vazquez et al., 2008). Therefore, by suppressing p53 activation, *MDM2*^{SNP309G} may negatively affect the ability of a tumor to respond to radio- and/or chemotherapy (Figure 1).

This knowledge may contribute to the optimization of cancer treatment regimens according to a patient's genotype. Small molecules such as nutlin and MI-219 inhibit the interaction between p53 and MDM2 and are already in phase I clinical trials (Brown et al., 2009). In people carrying the *MDM2*^{SNP309G} allele, inhibition of increased levels of MDM2 could be a more effective therapeutic strategy. Alternatively, the Sp1 inhibitor mithramycin A, which is already in use as an anticancer drug, could be used to block *MDM2* upregulation in these patients and may lead to improved chemotherapeutic response (Bond et al., 2004) (Figure 1).

In conclusion, current sequencing technologies allow unprecedented genome analysis that has great potential to advance our understanding of pathologies. There is precedence for the role of SNPs as active contributors to disease mechanisms, because a SNP in factor V, known as factor V Leiden, causes a hypercoagulability disorder and an increased risk of thrombosis. Factor V Leiden carries an amino acid substitution making the protein resistant to inactivation (Bertina et al., 1994). Similarly, a SNP at codon 72 of p53 encodes either proline or arginine and affects the ability of p53 to induce apoptosis, thus affecting chemosensitivity (Vazquez et al., 2008). However, it is remarkable that a SNP in noncoding DNA should have such a significant effect on tumor formation, the proof of which has now been definitively provided by Post and colleagues. Since data from clinical studies are controversial because of genetic heterogeneity, environmental factors, and limited cohort sizes, this report also illustrates the importance of genetically engineered mouse models as tools for mechanistic studies. It will be important to identify and analyze similarly functional SNPs. Such information not only has both diagnostic and prognostic value, but will also allow us to develop novel treatment strategies and to optimize them for each patient, taking us one more step toward personalized medicine.

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