Commentary

Haploinsufficiency for Tumor Suppression: The Hazards of Being Single and Living a Long Time

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By and large, gene expression levels in diploid organisms reflect the combined transcription of both copies, or alleles, of that gene. Notable exceptions include genes on the X chromosome and imprinted genes. The presence of genes in only one copy or in more than two copies can have major effects on the development and fitness of an organism. Many examples of these gene dosage effects can be found in model genetic organisms such as *Drosophila* and in inherited disorders in humans. Examples include the well-known effects of trisomy and contiguous gene deletion syndromes. It is now a well-established fact that inheritance of one mutant copy of a tumor suppressor gene (TSG) may predispose an individual to cancer because of loss of the remaining wild-type allele in somatic cells, resulting in cells completely devoid of the TSG product (for a review, see reference 1). However, more recent data suggest that some genes, when expressed at half normal levels, i.e., from only one functional allele, cannot fully suppress tumor growth (2, 3). In this issue, Rego et al. (4) report data showing that promyelocytic leukemia protein (*PML)* gene dosage has a major impact on the development of acute promyelocytic leukemia (APL) induced by transgenic overexpression of the PML-retinoic acid receptor α (RARα) fusion oncoprotein. The authors went on to show that heterozygous *PML* loss controls the sensitivity of PML-RARα–positive cells to apoptosis induction and differentiation by vitamin D3. In an accompanying report in this issue by Kogan et al., a role for apoptosis suppression in APL progression was also suggested by experiments showing that a *Bcl2* transgene dramatically synergized with a PML-RARα transgene in tumor induction (5). Together, these results show that APL development involves overcoming apoptosis sensitivity, which likely occurs in part due to loss of one normal copy of the *PML* gene. Indeed, another class of TSGs may exist, perhaps with very context-specific effects, which are haploinsufficient for tumor suppression and will be found mutated in only one copy in cancer cells. This idea has important basic science and clinical implications.

It should come as no surprise that a complex process, such as multistep cancer development, would not be subject to gene dosage effects. Indeed, literature on cancer genes is full of evidence that gene dosage plays a determining role in whether a given mutation can exert its oncogenic effect. Many oncogenes become amplified in cancer cells (6). Examples include *NMYC* amplification in neuroblastoma and *HER2/NEU* amplification in breast carcinoma. Indeed, *RAS* genes activated by point mutation are also usually overexpressed in cancer cells, sometimes as a result of gene amplification (7, 8). Substitution of only one *Ras* allele with a point-mutated, activated version using homologous recombination does not by itself cause morphological transformation of fibroblasts (9). Instead, this substitution increases the likelihood of morphological transformation after some other mechanism has increased expression levels from the mutated allele. Moreover, cancer cytogenetic studies, and more recently comparative genome hybridization studies, show that cancer cells have major, recurrent chromosome gains and losses that may be selected for because they result in too little or too much expression of whole sets of genes (10, 11). Identifying such genes is likely to be very difficult. Classical TSGs can be identified because both alleles are inactivated in cancer cells and often cause hereditary cancer predisposition syndromes when inherited in mutant forms. The prototypical TSG is the retinoblastoma gene, *RB1* (for a review, see reference 12). Patients who inherit one inactive copy of the *RB1* gene are predisposed to develop multiple retinoblastomas after somatic inactivation of the wild-type allele. Inactivation of the wild-type allele can occur by loss of the whole chromosome carrying the wild-type allele, mitotic recombination, large or small chromosomal deletions, or point mutation of the wild-type allele (Fig. 1). Most of these events result in loss of heterozygosity (LOH) for polymorphic markers within and near the affected TSG. Therefore, consistent LOH is used as a tool for narrowing the region of interest when positionally cloning new TSGs. But such an approach will of course exclude identification of genes that require two functional copies to adequately suppress

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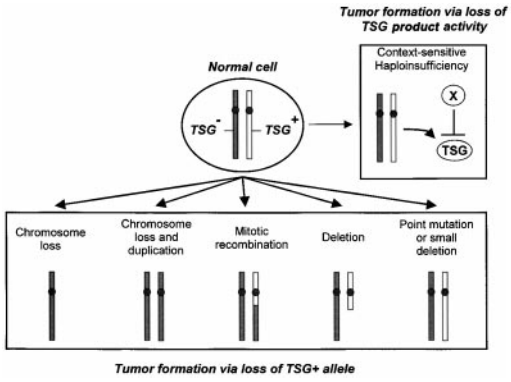

Figure 1. Mechanisms for loss of tumor suppression activity. A normal cell with one mutated TSG allele (TSG^-) on the dark chromosome and one wild-type allele (TSG^+) on the white chromosome is shown in the oval. Classical TSGs are somatically inactivated by one of the mechanisms shown in the bottom box, resulting in tumor formation. A class of TSG genes, such as *PML,* may be unable to fully suppress tumor cell growth in the presence of a factor, shown here as protein X, which can partially inhibit TSG protein function. In APL, factor X is the PML-RARα fusion oncoprotein itself. In this model, the order of events could be switched, they could occur simultaneously, and the inhibition of TSG function could be more indirect than shown here.

tumor cell growth and thus will have only one inactivated allele in tumors.

Although dominant, activated protooncogenes can be identified in many ways, creation of one class of protooncogenes consistently results in monoallelic loss of two genes: fusion genes created by chromosomal translocation or inversion. Most notably in leukemia, but also in other tumor types, oncogenes may be created by the fusion of two genes to create a chimeric fusion oncoprotein (for reviews, see references 13 and 14). It has been long appreciated that such an event also inactivates one copy of each gene involved in the translocation or inversion that creates the fusion oncoprotein. Does the loss of one normal copy of a gene involved in such a translocation contribute to leukemia development? This is the question Rego et al. (4) set out to answer in their study.

APL is closely associated with expression of a chimeric fusion oncoprotein, PML-RARα, composed of PML and RARα protein sequences (for a review, see reference 15). Alternative RARα fusions are present in <1.5% of APL (16). In the vast majority of APL patients, a balanced reciprocal translocation, t(15;17)(q22;q11.2), results in the generation of the *PML-RAR*α fusion oncogene (16). This translocation not only creates a *PML-RAR*α fusion, it also creates a *RAR*α-PML fusion gene, and causes haploinsufficiency for both *PML* and *RAR*α. These additional changes could play important roles in leukemia pathogenesis.

A *PML-RAR*α transgene can initiate leukemia with promyelocytic features, indicating that the additional changes wrought by the translocation are not essential for leukemia formation (17–19). Nevertheless, additional changes may have a permissive role, promoting the transition from a *PML-RAR*α initiated disturbance of myelopoiesis to acute leukemia. The RARα-PML fusion is not expressed in ~30% of APL (16), and a *RAR*α*-PML* transgene did not initiate leukemia in mice (20). Nevertheless, this reciprocal fusion did increase the penetrance of leukemia in mice expressing *PML-RAR*α (20). Similarly, $Pml^{-/-}$ mice do not have an increased incidence of spontaneous malignancies (21), but Rego et al. have now demonstrated that reduction in *Pml* gene dosage cooperates with a *PML-RAR*α transgene to decrease latency and increase the penetrance of acute leukemia (4). These findings make apparent that the combination of genetic changes effected by chromosomal translocations may be critical to the ability of these events to cause malignancies. Recent efforts to create chromosomal translocations in mice, including reciprocal fusion genes, haploinsufficiency for both partners, and expression under native promoter elements (22), may provide additional insights into the effects of such aberrations.

The results of Rego et al. definitively demonstrate that abrogation of PML function promotes myeloid leukemogenesis (4). Their study goes on to show that loss of PML likely contributes to leukemogenesis by enhancing survival of immature myeloid cells and by making these cells resistant to differentiating stimuli. Furthermore, these results strongly suggest that haploinsufficiency for *PML* contributes to leukemia formation in humans. In the mice, as in humans, PML function was impaired through two distinct mechanisms: functional impairment by PML-RARα and reduction in *Pml* gene dosage. Decreasing PML function by increasing PML-RARα expression and/or by reducing the number of intact *Pml* genes resulted in decreased survival and increased leukemia incidence in *PML-RAR*α transgenic mice. Given this dose effect, it appears likely that haploinsufficiency for *PML* does contribute to human APL. Additional evidence in support of a role for *PML* haploinsufficiency in leukemogenesis comes from previous work from the Pandolfi laboratory showing that loss of one allele of *Pml* could combine with PML-RARα expression to suppress cell death (23). One formal caveat to the hypothesis that *PML* haploinsufficiency is relevant to human APL must be noted. Although it is clear that the levels of PML-RARα expressed in the mice (even those homozygous for the transgene) do not phenocopy the effects of homozygous *Pml* gene loss, it remains possible that the levels of PML-RARα expressed as a result of the t(15;17) translocation in human cells are sufficient to completely abolish PML function.

PML has appropriately been added to the list of genes with tumor suppressor activity. As a tumor suppressor, *PML* has several interesting features. First, loss of *Pml* in mice does not itself initiate malignancies. Second, in human leukemia the decrease in PML function is brought about by dominant-negative activity of a gene fusion facilitated by accompanying haploinsufficiency. Additional aspects of PML's ability to inhibit tumor formation remain to be explored. Many tumor suppressors only make themselves apparent upon homozygous gene inactivation (as with *RB1*) or upon creation of a dominant-negative allele that is sufficiently strong as to render cells functionally null (as with many *TP53* mutations). Haploinsufficiency pro-

moted leukemic transformation in *PML-RARα* transgenic mice. However, the authors do not present data on whether or not the remaining *Pml* allele is expressed in the leukemic cells. It is possible that the effect of haploinsufficiency on leukemia formation was due primarily to reduction to homozygous gene loss or to transcriptional silencing in somatic cells. The demonstration that normal PML is actually expressed in leukemias that arise in *PML-RARα/Pml*$^{+/-}$ mice would provide additional evidence that *Pml* is not a typical TSG. Demonstration of PML tumor suppressor activity has been limited to few cell types, including myeloid leukemia, lymphoma, fibrohistiocytoma, and skin (21, 24). As with other TSGs, the tissue specificity of *Pml* remains an area of opportunity for novel investigations. The suggestion of Rego et al. (4) that this specificity is related to the relative importance of proliferation and apoptosis in tumor expansion appears a good point of departure for future work. Indeed, experiments reported by Kogan et al. in this issue demonstrate that inhibition of apoptosis by expression of the *Bcl2* oncogene can cooperate with the *PML-RARα* transgene in APL generation (5). Interestingly, the authors also presents data suggesting that, like reduction of the dose of *Pml*, expression of *Bcl2* at high levels can partially block differentiation. A role in blocking differentiation is a relatively understudied activity of the *BCL2* protooncogene. Whether this differentiation block also depends on the well-known role of Bcl2 in mitochondrial permeability is unknown.

PML can be placed, at least presumptively, into a category of TSGs in which haploinsufficiency is sufficient to contribute to tumorigenesis. Other genes, which may also share this characteristic, are *P27KIP1* and *AML1/RUNX1* (2, 3). The *AML1*, or *RUNX1*, gene encodes a sequence-specific DNA binding transcriptional repressor protein (for a review, see reference 25). Homozygous loss of *AmL1* in knockout mice causes embryonic lethality due to an impairment in definitive hematopoiesis (26). In people, inheritance of one mutant copy of the *AML1* gene causes a thrombocytopenia with predisposition to acute myeloid leukemia (AML) development (3). Interestingly, AML which develop in these patients does not show loss of the *AML1*$^{+}$ allele, suggesting that this gene may predispose cells to leukemic transformation without biallelic inactivation. *AML1* is a partner in several translocations commonly found in human leukemia and so, like *PML*, is reduced to hemizygosity by the same events that fuse it to other genes to generate the fusion oncoprotein. This family of fusion oncoproteins may also cooperate with loss of one copy of the *AML1* gene, and in fact biochemical and genetic evidence suggests that AML1-ETO antagonizes AML1 function (27). The *P27KIP1* gene encodes a cyclin-dependent kinase inhibitor that maps to human chromosome 12p12. Deletions in this region are common in human B cell acute lymphoblastic leukemia (ALL) and consistently include the *P27KIP1* gene. However, the wild-type allele is not mutated in these *ALL*, suggesting that *P27KIP1* is haploinsufficient for ALL tumor suppression (2). Similar results were obtained with mice heterozygous for the *p27kip1* gene (28). Mice heterozygous for *p27kip1* were found to be predisposed to gamma irradiation or chemically induced tumors. Furthermore, the tumors that developed in these heterozygous animals showed neither mutation of nor silencing of the wild-type allele.

Are haploinsufficient TSGs commonly involved in the development of human cancer? Some well-known, recurrent chromosomal deletions or monosomies may actually be selected for due to loss of a haploinsufficient TSG. Only one copy of the TSG would be lost in these cases, hindering attempts to identify the gene(s) involved. Indeed, although biallelic deletions can be observed in solid cancer with loss of *RB1* and other TSGs, such biallelic deletion has not been reported in 5q- or monosomy 7 syndrome, common forms of myeloid leukemia. Perhaps this is because a haploinsufficient TSG resides in these regions. Such a situation would necessitate a new strategy for finding these TSGs. One might choose tumors which share clinical features with 5q- or Mo7 syndrome, but which lack large deletions in these regions, hoping to identify monoallelic inactivation of a gene. It may also be possible to test for the presence of haploinsufficient TSG in the syntenic regions of the mouse genome by generating large chromosomal deletions in mouse embryonic stem (ES) cells and looking for cancer in mice subsequently generated from these cells. Several methods exist for generating large deletions in specific chromosomal regions in mouse ES cells (29, 30). Such deletions could be expected to contribute to tumor formation without loss of genetic material or gene mutations on the wild-type chromosome. As Rego et al. show (4), the effects of this sort of TSG loss may be very context specific and so such models may have to accommodate the right tissue, target cell, and presence of the right additional genetic events. In the case of PML, its heterozygous inactivation may contribute to APL induced by PML-RARα specifically because the fusion oncoprotein also partially blocks PML function. However, one could imagine less direct reasons for context-specific effects of heterozygous TSG inactivation. Partial suppression of TSG protein function could be mediated by intrinsic factors such as the expression of viral genes, specific oncogene mutations, or loss of other TSGs. Alternatively, extrinsic factors such as growth factors, cytokines, or the presence of specific cell types could compromise TSG protein function. For haploinsufficient TSGs, these other factors represent a new way of thinking about loss of tumor suppression activity quite different from the traditional loss of the wild-type tumor suppressor allele in a heterozygous cell (Fig. 1). Understanding why these context-specific effects occur will be very important because they could suggest very specific, new routes for therapeutic intervention. Related to this idea, if some TSGs are insufficient to fully suppress tumor cell growth in one copy and one wild-type copy of the gene remains in tumor cells, it may be possible to boost its expression or the activity of its protein product to achieve a therapeutic effect.

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