Oncogene-Induced Mitotic Stress: p53 and pRb Get Mad Too

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Oncogenic signaling frequently results in unscheduled overexpression of cell cycle proteins and replicative stress. In this issue of *Cancer Cell*, Schvartzman et al. show that inactivation of p53 or pRb induces Mad2, and the overexpression of this mitotic checkpoint protein is a necessary event during oncogene-induced mitotic stress.

The mitotic checkpoint (also known as the Spindle Assembly Checkpoint) is a molecular pathway that ensures ordered chromosome segregation, thus preventing the generation of aneuploid cells with abnormal chromosome composition (Musacchio and Salmon, 2007). A critical component of this pathway is Mad2, a protein that inhibits the activity of the anaphase-promoting complex (APC/C). ACP/C is an E3-ubiquitin ligase that, when bound to the Cdc20 cofactor, targets Cyclin B and Securin for degradation, triggering chromosome segregation and mitotic exit. Upon incomplete biorientation of chromosomes, Mad2 inhibits APC/C-Cdc20, thus preventing chromosome miss-segregation.

Since the identification of the mitotic checkpoint, the search for tumor-associated alterations in the components of this pathway has been intense. Although some mutations have been reported (Pérez de Castro et al., 2007), clinical correlative data were insufficient to explain the widespread aneuploidy found in human tumors. These initial negative results led to the hypothesis that this molecular pathway may not be involved in tumor development. However, further work with cultured cells and genetically engineered mice provided some new insights on the relevance of the mitotic checkpoint in preventing aneuploidy. The pioneer analysis of mutant mice partially deficient in Mad2 showed a correlation between the presence of chromosomal instability and the development of lung tumors (Michel et al., 2001). However, Mad2 is not downregulated in human tumors, but is rather overexpressed as part of a transcriptional signature commonly found in chromosomally unstable tumors (Pérez de Castro et al., 2007). Further work identified Mad2 as an E2F-target gene linking the inactivation of the retinoblastoma (pRb) protein with Mad2 overexpression (Hernando et al., 2004) (Figure 1). Overexpression of Mad2 was then shown to generate chromosomal instability and to favor tumor development in vivo (Sotillo et al., 2007). Since E2F transcription factors induce multiple mitotic genes and Mad2 may have additional nonmitotic functions, it was not clear to what extent Mad2 was merely a marker of E2F deregulation or responsible for the chromosomal instability induced by the loss of pRb or other tumor suppressors.

In a study published in this issue of *Cancer Cell*, Schvartzman et al. (2011) show that upregulation of Mad2 is necessary for the aneuploidy induced upon inactivation of not only pRb but also p53 in tumor cells. Since all three pocket proteins—pRb, p107, and p130—may repress Mad2, Schvartzman et al. studied chromosomal instability in cells deficient in these three proteins. These triple mutant cells displayed a significant overexpression of Mad2 as well as BubR1, another component of the mitotic checkpoint. Specific normalization of Mad2 levels, using lentiviral vectors expressing specific short-hairpin interference RNAs, had no significant effect on cell proliferation but rescued chromosomal instability in these mutant cells. Similarly, Mad2 was overexpressed in mammary gland adenocarcinomas induced by the inhibition of pRb family members using the T121 fragment from the SV40 large T antigen. Schvartzman et al. reduced Mad2 levels close to wild-type levels by crossing these transgenic mice with *Mad2*(+/−) heterozygous mutants. Normalization of Mad2 levels resulted in more differentiated tumors and a dramatic decrease in anaplastic neoplasias, a tumor type characterized by high levels of aneuploidy (Schvartzman et al., 2011).

Inactivation of p53 is also known to induce chromosomal instability as a consequence of defective $p21^{Cip1}$ function. Knockin mice in which the wild-type p53 alleles have been replaced by $p53^{R172P}$, a p53 mutant that does not induce apoptosis but is competent in inducing $p21^{Cip1}$, developed lymphomas and sarcomas with stable diploid genomes. These tumors became aneuploid in a $p21^{Cip1}$-deficient background, suggesting that this CDK inhibitor is necessary for preventing chromosomal instability downstream of p53 (Barboza et al., 2006). Interestingly, $p21^{Cip1}$-deficient mice harboring $p53^{R172P}$ alleles overexpressed Mad2 and normalization of Mad2 levels using a *Mad2*(+/−) background also rescued chromosomal instability in these animals (Schvartzman et al., 2011). These elegant genetic data establish the relevance of the p53-$p21^{Cip1}$-Mad2 pathway in the control of the integrity of the genome. p53 is likely to repress Mad2 through the $p21^{Cip1}$-dependent inhibition of cyclin-dependent kinases (CDKs) and the subsequent activation of pRb. Given the known regulation of Mad2 by the pRb-E2F axis, these data establish a major role for the two major tumor suppressor molecules, p53 and pRb, in controlling genomic integrity through the E2F-dependent transcriptional regulation of Mad2 (Figure 1).

The widespread aneuploidy seen in human tumors has been frequently used to support a possible causal role of

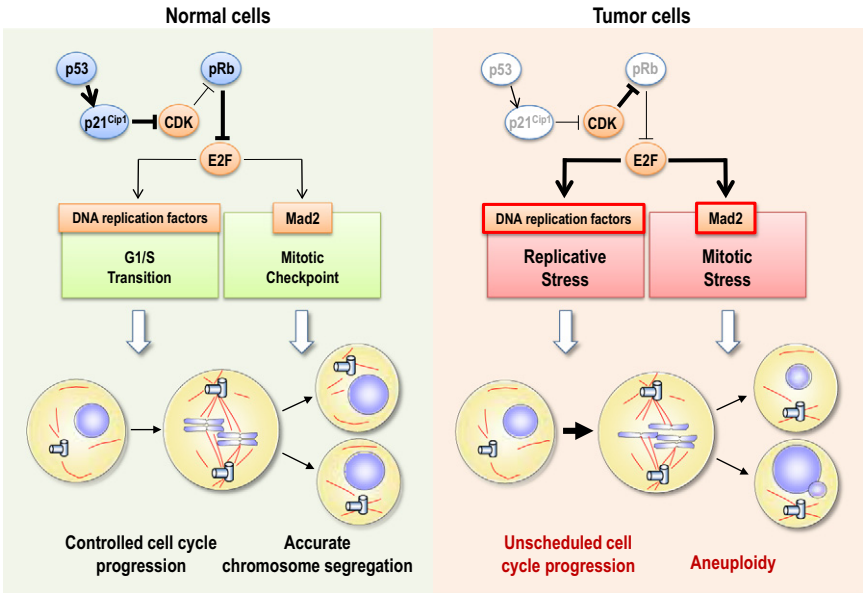

Figure 1. Oncogene-Induced Replicative and Mitotic Stress

In normal cells, pRb family members and p53 control the expression of critical regulators of DNA replication and mitosis, in part by regulating the activity of the E2F family transcription factors. Mad2 is one of the E2F targets involved in the mitotic checkpoint that controls chromosome segregation. Oncogenic alterations resulting in the inactivation of these major tumor suppressor pathways lead to deregulated E2F activity and the overexpression of multiple genes required for the G1/S transition. This results in replicative stress and eventually unscheduled cell cycle progression. In these conditions, several mitotic genes including Mad2 are also overexpressed, resulting in mitotic stress. The overexpression of Mad2 leads to deregulation of the mitotic checkpoint and aberrant chromosome segregation. Normalizing Mad2 levels in p53- or pRb-deficient mice rescues aneuploidy suggesting that Mad2 is a necessary target of these proteins to suppress chromosomal instability.

chromosomal instability in tumor formation. However, aneuploidy has clear antiproliferative effects, and the debate on its oncogenic or tumor suppression function is still open (Holland and Cleveland, 2009). How is it possible that most human tumors are aneuploid? Since virtually all human tumors display deficient p53 or pRb pathways, Mad2 upregulation is possibly a common event during specific stages of malignant transformation. This upregulation likely causes a mitotic stress that parallels the replicative stress caused by oncogenic signaling (Halazonetis et al., 2008); we propose here to use the term "oncogene-induced mitotic stress" to define this state (Figure 1). Similar to the defective DNA replication observed in the presence of replicative stress, the mitotic stress would have antiproliferative effects as a consequence of abnormal chromosome segregation. The frequent aneuploidy observed in human tumors may remain in some cases as a fingerprint of this original chromosomal instability. However, the fact that, despite the initial chromosomal instability generated by loss of these tumor suppressors, many cancers maintain relatively stable genomes (either diploid or aneuploid) actually favors the idea that chromosomal instability is initially detrimental for tumor development. The tumor cells may either downregulate Mad2 or, alternatively, acquire additional alterations (perhaps overexpression of Cdc20) (Pérez de Castro et al., 2007) to counteract the adverse effects of high levels of Mad2. Other tumors manage to survive with high levels of chromosomal instability, and this alteration usually correlates with poor prognosis. How these tumor cells deal with the adverse effects of this continuous instability is a major question to be solved in the future. Fortunately, chromosomal instability and aneuploidy can also be exploited for cancer therapy (Manchado and Malumbres, 2011), and p53- or pRb-deficient cells can eventually become a selective target for such treatments. The requirements for Mad2 in oncogene-induced mitotic stress may provide the first molecular clue to translate the inactivation of tumor suppressors into a lethal strategy against tumor cells.

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