Rb Loss Causes Cancer by Driving Mitosis Mad

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Aneuploidy is a hallmark of most human cancers, but whether it is a cause or a consequence of cellular transformation remains a subject of debate. The spindle checkpoint functions to prevent aneuploidy and plays a central role in this discussion. The checkpoint gene *Mad2* is activated by E2F1 and overexpressed in cells lacking a functional Rb pathway. In this issue of *Cancer Cell*, Sotillo and coworkers report that *Mad2* overexpression leads to chromosomal instability and tumorigenesis, indicating that Mad2 contributes to cancer development after Rb mutation. In a second paper, Weaver et al. report that aneuploidy has both tumor-promoting and -suppressing properties.

Aneuploidy, a state of having an abnormal number of chromosomes, is a hallmark of most human tumors (Weaver and Cleveland, 2005). Aneuploidy results from missegregation of chromosomes during mitosis, but its molecular basis and role in the causation of human cancer remain largely unclear. The spindle checkpoint is an intricate multiprotein network that delays the onset of anaphase until all kinetochores are properly attached to the mitotic spindle and aligned in the metaphase plate. Since the discovery of this checkpoint, there has been much speculation that its malfunction plays a major role in the development of aneuploidy and cancer. Genes encoding key components of the spindle checkpoint are rarely mutated in human cancers, but some of these genes are quite often expressed at reduced or elevated levels. Mouse models mimicking these reductions have moderate to severe aneuploidy, and some of these develop carcinogen-induced tumors at increased rates, implying that at least certain mitotic checkpoint genes act to suppress malignant cell transformation (Baker et al., 2005).

Mad2 expression must be tightly regulated because both reduced amounts and overproduction of the protein induce aneuploidy (Dobles et al., 2000; Hernando et al., 2004; Michel et al., 2001) (Figure 1). However, whether Mad2 overexpression acts to drive tumorigenesis has remained an open question. A study by Sotillo et al. in this issue of *Cancer Cell* now addresses this issue (Sotillo et al., 2007). The authors created transgenic mice in which the overexpression of Mad2 can either be induced or repressed by doxycycline. Mice in which the transgene was constitutively "on" developed a wide variety of tumors, including liver and lung carcinomas, sarcomas, and lymphomas. Most of these tumors emerged in the second year of life and continued to grow even when the *Mad2* transgene was switched

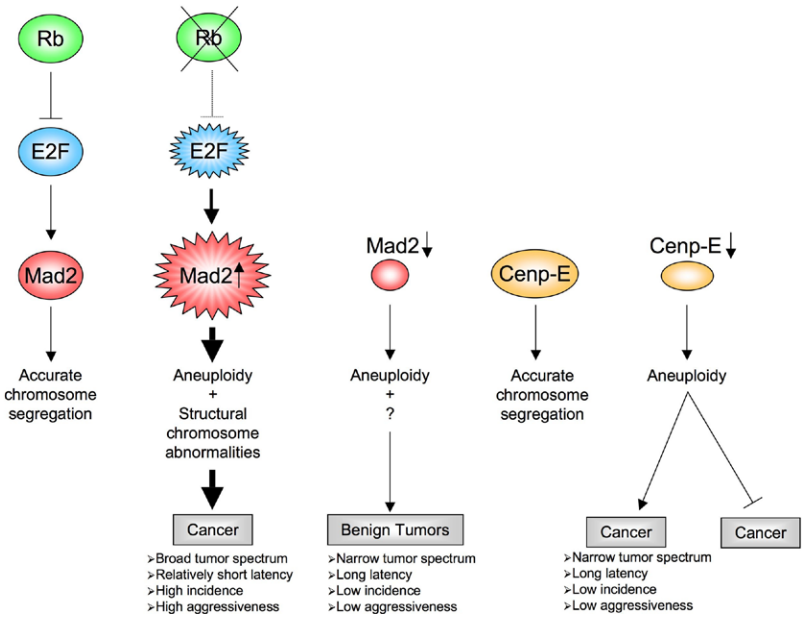

Figure 1. Mitotic Checkpoint Genes and Tumorigenesis
Mad2 is an E2F-regulated gene whose level of expression must to be tightly controlled during the cell cycle to maintain euploidy. In human cancer cells lacking a functional RB pathway, E2F is hyperactive, resulting in overexpression of *Mad2*, which, in turn, leads to aneuploidy, tetraploidization, and structural chromosomal damage, and formation of aggressive tumors in a broad spectrum of tissues and cell types. On the other hand, aneuploidy resulting from downregulation of Mad2 expression is linked to formation of nonaggressive lung tumors with long latency and low incidence. Because Mad2 presumably has functions outside the spindle checkpoint, it is not entirely sure that aneuploidy drives tumorigenesis in Mad2 haploinsufficient mice. In contrast, the expression and function of CENP-E seems to be restricted to mitosis. Aneuploidy arising from CENP-E haploinsufficiency causes a mild cancer phenotype in lymphocytes and lung epithelium, but not in other tissues and cell types. Surprisingly, in the presence of certain cancer-critical gene mutations, aneuploidy from CENP-E deficiency reduces tumor formation.

off, indicating that oncogenic levels of Mad2 are not required for tumor maintenance. Together, these elegant experiments firmly establish that Mad2 has oncogenic properties and imply that hyperactivation of Mad2 by E2F1 drives neoplastic transformation of cells in which the Rb pathway is disrupted.

Mice overexpressing Mad2 undergo frequent chromosome missegregation and accumulate aneuploid cells, as do mice with low amounts of Mad2 (Michel et al., 2001; Sotillo et al., 2007) (Figure 1). However, compared to mice with low Mad2 levels, mice with high levels of the protein have a much wider tumor spectrum than Mad2-haploinsufficient mice. In addition, tumors from Mad2 transgenic mice appear to be much more aggressive than those from Mad2-haploinsufficient mice. What might be the basis for these profound differences in tumorigenic properties? Besides aneuploidy, Mad2 transgenic mice also develop several structural chromosome defects such as chromosome breaks, end-to-end fusions, interstitial deletions, and chromosomal amplifications. Structural abnormalities or structural and numerical abnormalities combined might pose a much higher risk for neoplastic transformation than aneuploidy alone, perhaps explaining why Mad2 overexpression is more tumorigenic than Mad2 insufficiency. How structural chromosomal abnormalities arise in cells with high levels of Mad2 is currently unclear. Mad2 overexpression results in the incomplete destruction of cyclin B and securin in late metaphase, which, in turn, is likely to impair the activation of separase (Hernando et al., 2004; Sotillo et al., 2007). One possible scenario would therefore be that cells with high levels of Mad2 are driven into anaphase when cohesins that hold sister chromatids together are still incompletely cleaved, thereby promoting mechanical breakage of unresolved sister chromatids as they move toward opposite poles of the spindle. Another characteristic of cells that overexpress Mad2, not seen in cells with low amounts of the protein, is failure of cytokinesis, which results in tetraploidization. In p53 null cells, tetraploidy has been shown to accelerate numerical and structural chromosome instability and promote tumorigenesis (Fujiwara et al., 2005). Thus, an increase in structural rearrangements and tetraploidization may, at least in part, explain why Mad2-overexpressing mice are so much more susceptible to spontaneous tumors than Mad2-haploinsufficient mice.

With the causal involvement of Mad2 overexpression in tumorigenesis firmly established, it will be important to determine whether other mitotic checkpoint proteins also cause chromosomal instability and cancer when expressed at supranormal levels. An obvious protein to test would be the Mad2-interacting protein BubR1. Like Mad2, BubR1 is important for proper timing of cyclin B degradation at the metaphase-to-anaphase transition, and therefore its overexpression may also lead to incomplete activation of separase, and as a result, to numerical and structural chromosome abnormalities, and tetraploidization. On the other hand, BubR1 has also been implicated in induction of apoptosis following chromosome missegregation. It is possible that this function may be reinforced by BubR1 overexpression and perhaps act to prevent tumorigenesis. Notably, mitotic checkpoint genes including BubR1 are frequently overexpressed in human cancers (Weaver and Cleveland, 2006). Despite this, these studies are likely to provide underestimated rates because there may only be a transient need for mitotic checkpoint gene overexpression, as has been demonstrated for Mad2 by Sotillo et al. (2007).

Given that Mad2 transgenics develop structural chromosomal aberrancies in addition to aneuploidy, one could argue that this mouse model is unsuitable for testing the 100-year-old hypothesis of Theodor Boveri that aneuploidy drives tumorigenesis (Boveri, 1902). Others have previously attempted to test Boveri's hypothesis by using mice with reduced amounts of the checkpoint proteins Mad2, BubR1, or Rae1 (a Bub3-related checkpoint protein) (Baker et al., 2005). These mouse models accumulate aneuploid cells, and BubR1 and Rae1 are prone to carcinogen-induced tumors. However, since Mad2, BubR1, and Rae1 are expressed throughout the cell cycle and implicated in cellular processes other than mitotic checkpoint control, such as apoptosis, DNA replication, and nucleocytoplasmic transport, one could argue that these models are also not ideal for testing the Boveri hypothesis. CENP-E is a mitotic checkpoint protein whose expression and function seem to be narrowly restricted to mitosis. In this issue of *Cancer Cell*, Weaver and colleagues show that mice with reduced CENP-E levels develop aneuploidy and late-life splenic lymphomas and lung tumors, thereby providing the most compelling evidence yet that numerical chromosomal instability plays a causal role in tumorigenesis, at least in a subset of tissues (Weaver et al., 2007) (Figure 1). The authors further show that CENP-E mutant mice are protected against tumors caused by the DMBA carcinogen or by loss of the p19/ARF tumor suppressor. These are surprising findings because aneuploidy was expected to promote but not inhibit tumorigenesis. There is currently no straightforward explanation for these results, but Weaver et al. have proposed that DMBA and loss of p19/ARF cause moderate levels of DNA damage and/or chromosome missegregation that, when combined with aneuploidy caused by reduction in CENP-E, drive rates of genetic instability above a threshold that is compatible with cell viability. In any case, the work of Weaver et al. shows that aneuploidy can exert tumor-suppressive, tumor-promoting, or benign effects depending on tissue or cell type and the repertoire of additional cancer-critical gene mutations present in the cell. These findings stress the importance of identifying the genetic alterations that cooperate with mitotic checkpoint gene mutations in the development of cancer. It will also be critical to identify cancer gene mutations that negatively synergize with mitotic checkpoint defects or aneuploidy, as such efforts may contribute to the development of novel therapies for the treatment of a broad spectrum of human cancers.

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Hit 'Em Where They Live: Targeting the Cancer Stem Cell Niche

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Cancer stem cells (CSCs) are thought to be critical for initiation and propagation of many types of cancer. Because these cells are resistant to conventional therapies, they have been very difficult to eliminate. A study in this issue of *Cancer Cell* suggests that brain tumor CSCs live in a "vascular niche" that promotes their long-term growth and self-renewal. Disrupting this niche impairs CSC self-renewal and thereby significantly inhibits the growth of tumors. Targeting the unique microenvironment of CSCs may be the key to effective cancer therapy.

Once upon a time, cancer was viewed as a homogeneous mass of rapidly proliferating cells, and therapeutics were designed to eliminate highly proliferative cells. But recent studies have suggested that tumor cells are heterogeneous with respect to proliferation and differentiation, and that a cell's proliferative rate may be a poor indicator of its tumorigenic potential. In several malignancies, the capacity to initiate and maintain tumor growth has been found to reside in a small population of cells called cancer stem cells (CSCs) (Al-Hajj et al., 2004; Reya et al., 2001; Wicha et al., 2006). Like normal stem cells, CSCs have the ability to self-renew and to give rise to the variety of proliferating and differentiated cells that make up the bulk of a tumor. Importantly, CSCs are often relatively quiescent and therefore may not be affected by therapies targeting rapidly dividing cells. Elevated expression of transporters that pump out chemotherapeutic agents (Donnenberg and Donnenberg, 2005) and an increased capacity to repair DNA damage (Bao et al., 2006a) may also contribute to CSCs' ability to survive conventional modes of therapy.

The resistance of CSCs to conventional therapies may help explain why such therapies often fail: although they may destroy the bulk of a tumor, they cannot prevent the surviving CSCs from kicking into action and regenerating it again (Al-Hajj et al., 2004; Reya et al., 2001; Wicha et al., 2006). In this view, effective cancer treatment will require targeting CSCs themselves. But what are the signals that regulate CSC survival and function, and is there an effective way to subvert them?

One way to identify regulators of CSCs is to look for analogies with normal stem cells. An important characteristic of normal neural stem cells (NSCs) is that they are concentrated in regions that are rich in blood vessels, called "vascular niches" (Ramirez-Castillejo et al., 2006; Shen et al., 2004). These niches are thought to shelter NSCs from apoptotic stimuli and allow them to maintain a proper balance between self-renewal and differentiation. Endothelial cells (ECs), which line blood vessels, secrete factors that promote stem cell survival and self-renewal and are thought to be a key component of the NSC niche.

A study in this issue of *Cancer Cell* suggests that CSCs in brain tumors, similar to NSCs, reside in vascular niches, and that disrupting these niches may be the key to eliminating CSCs (Calabrese et al., 2007). By analyzing a large cohort of human brain tumors, Calabrese et al. dem-