fibroblasts also proliferated much more slowly than either their wild-type or hemizygous counterparts; however, bruce-null embryonic fibroblasts were also more susceptible to diverse pro-apoptotic stimuli, again supporting the view that Apollon is important for setting a threshold for apoptosis. Alternatively, cells lacking Apollon may simply be more fragile for reasons not directly related to the ability of this protein to neutralize components of the cell death machinery. Clearly, additional analysis is required to resolve whether Apollon exerts its influence primarily during division or death, or both. □

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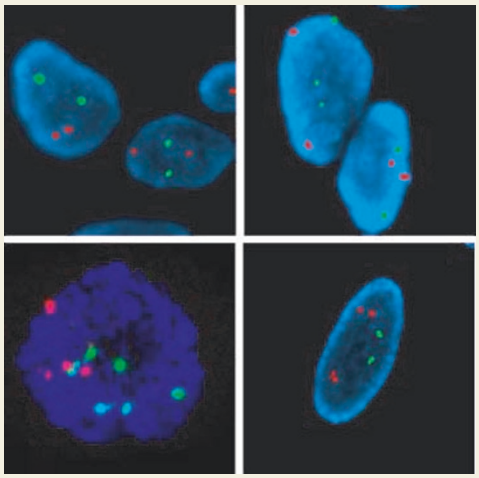
Spindle checkpoint protein links Rb pathway to aneuploidy

Aneuploidy is a hallmark of many human cancers, although its causal involvement in cellular transformation remains a matter of debate. Mutations debilitating the Rb pathway are commonly observed in cancers, and result in derailed cell-cycle regulation as a consequence of unchecked activation of the E2F family of transcription factors. How this is linked to the characteristic genomic instability of such cancers has remained unclear. Now, Hernando *et al.* report in *Nature* (**430,** 797–802; 2004) that the spindle checkpoint protein Mad2 is a transcriptional target of E2F, and that elevated Mad2 expression contributes to aneuploidy.

The spindle checkpoint senses aberrant kinetochore microtubule attachment and inhibits chromatid separation, cytokinesis and mitotic exit by inhibiting the ubiquitin ligase APC/C. Experimental inactivation of two checkpoint components, Mad2 and Bub3, results in chromosome instability in mice. However, these genes are rarely targeted in spontaneous tumours.

Hernando *et al.* observed constitutive Mad2 expression in fibroblasts with compromised Rb (knockout mouse embryonic fibroblasts, RNAi against *Rb* or *E1A* expressing cells). Correlating with E2F activity during the cell cycle, Mad2 is not expressed in normal quiescent cells and is induced in S phase, peaking in G2/M. Furthermore, constitutive Mad2 expression is observed in carcinoma and neuroblastoma cell lines, as well as primary human retinoblastomas, bladder carcinomas and neuroblastomas with deregulated E2F activity, where it correlates with poor patient prognosis. Consistent with previous global microarray and ChIP results, Hernando *et al.* found that the Mad2 promoter binds to — and is *trans*-activated by — E2F-1 in cells.

In order to address the somewhat paradoxical notion that overexpression of a spindle checkpoint protein may be causally involved in aneuploidy, Hernando *et al.* surveyed the genomes of fibroblasts with increased Mad2 levels by laser-scanning cytometry, fluorescence *in situ* hybridization and metaphase spreads. Frequent aneuploidy was observed in fibroblasts with constitutive Mad2 expression. This went hand-in-hand with delayed cytokinesis. Importantly, RNAi-mediated reduction of *Mad2* in cells with defective Rb reduced the appearance of aneuploidy.



Chromosomal instability in cells with elevated Mad-2 expression. FISH analysis of IMR90 human fibroblast cells infected with *E1A*, *shRB* or *Mad2* (top right, bottom left, bottom right, respectively) and stained with centromeric probes for chromosomes 1 and 17 (red and green) and DAPI (blue).

The E2F family of transcription factors function at the core of cell-cycle regulation, and their function is frequently derailed in cancers. Hernando *et al.* have uncovered that an unexpected corollary of this is super-induction of the spindle checkpoint protein Mad2 and, consequently, chromosome mis-segregation and aneuploidy — one of the major hallmarks of cancer. Although this data has uncovered a direct link from the regulation of cell-cycle progression to genomic instability, implying that aneuploidy is an early event in cancer, the question of chicken and egg stands. Nevertheless, as in life, the old adage that there can be too much of a good thing seems to apply also to the cell cycle.

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