Comment

Triggering p53 after cytokinesis failure

P. Todd Stukenberg

Department of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, VA 22908

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Cells that fail to divide during cytokinesis often arrest in the next G1 phase by a mysterious mechanism that depends upon p53. What triggers this arrest is unclear. New studies, including a report in this issue (Uetake and Sluder, 2004) suggest that this arrest does not occur because cells are polyploid, are binucleate, have multiple centrosome, or have failed cytokinesis, making this phenomenon even more puzzling.

A hallmark of most cancer cells is that they are highly aneuploid, whereas most somatic cells have stable ploidy. Polyploidy has even been postulated to generate genetic instability (Lengauer et al., 1998). It is unclear if normal somatic cells maintain their ploidy simply by faithful mitotic segregation of their chromosomes or if they have mechanisms to detect aneuploidy and either correct this problem or block aneuploid cells from further division cycles. A growing body of work suggests that cells that fail to undergo cytokinesis activate a "tetraploid checkpoint" that arrests them in the following G1 in a p53-dependent manner. However, recent papers suggest that polyploidy per se cannot trigger the p53 network, and the in vivo relevance of this arrest is still unclear.

It is well established that p53 blocks cell cycle progression in cells that fail cytokinesis, as many researchers have independently generated polyploid cells that arrest in the following G1 (Fig. 1). The original observation of this phenomenon preceded the discovery of p53. Hirano and Kurimura (1974) found SV40-infected cells did not arrest in G1 when treated with cytochalasin, a drug that poisons actin and, hence, prevents contraction of the cytokinetic furrow (Fig. 1 B). It is now known that SV40 infection inactivates p53. Reid and colleagues (Cross et al., 1995) incubated mouse embryo fibroblasts (MEFs) in nocodazole or colcemid, two different microtubule-depolymerizing drugs, for 22 h, and found that wild-type MEFs arrested with 4N ploidy, but P53$^{-/-}$ MEFs had rereplicated their chromosomes and become 8N (Cross et al., 1995). Further studies demonstrated that even though the cells were in nocodazole, the 4N cells did not arrest in mitosis but escaped the spindle checkpoint and arrested in the subsequent G1 phase in a state that had

many hallmarks of a p53 checkpoint arrest induced by DNA damage (Fig. 1 C) (Lanni and Jacks, 1998; Minn et al., 1996). It is worth pointing out that these experiments were first seen in mouse cells that have a functional spindle checkpoint but cannot maintain the mitotic arrest in nocodazole for nearly as long as human cells. Margolis's group generated binucleate cells with dihydrocytochalasin B (Fig. 1 B) (Andreassen et al., 2001), and once again p53-positive cells arrested in the subsequent G1 phase whereas p53-minus cells rereplicated their DNA to become 8N. While exploring how overexpression of the oncogene Aurora A generated multiple centrosomes, Erich Nigg's group found that excess Aurora A expression blocked cytokinesis (Fig. 1 B) (Meraldi et al., 2002). They went on to show that these cells also arrested in the following G1 in a p53-dependent manner. Although it still has to be formally established, it is likely that a common mechanism is activating p53 after each of these treatments.

Since cancer cells often have extra chromosomes, it has been postulated that there is an initial event causing cancer cells to become polyploid and then reduced fidelity of chromosome segregation results in subsequent aneuploidy that drives the loss of heterozygosity of tumor suppressors. Thus, the notion that p53 blocks the progression to S-phase in the cells that are polyploid is satisfying, as it further explains the almost universal loss of the p53 pathway during cancer progression. However, deeper thinking suggests that "normal" somatic cells are often polyploid, and the initial models may be naïve. Polyploidy, both autopolyploidy and allopolyploidy, is common among higher (angiosperm) plants but relatively rare among animals and not restricted to any particular genus. Muller (1925) was the first to suggest that polyploidy is rare in animals because of the evolution of sex chromosomes and a chromosomal basis for sex determination. Importantly, there are polyploid animals. A variety of frogs and toads are tetraploid, most famous among them is *Xenopus laevis*. The brine shrimp (*Artemia franciscana*) is tetraploid, whereas the pine sawfly (*Diprion similie*) has diploid males but tetraploid females. Increased ploidy has also been reported in humans. Triploid and tetraploid fetuses often die and are aborted in the first trimester, but there are many cases of fetuses that survive to the third trimester and a small number of cases of tetraploid live births (Edwards et al., 1994; Nakamura et al., 2003). There are certain cell types in humans that are polyploid; for example, megakaryocytes increase in ploidy as part of their differentiation (Queisser et al., 1971).

Address correspondence to P. Todd Stukenberg, Department of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, VA 22908. Tel.: (434) 924-5252. Fax: (434) 924-5069. email: Pts7h@virginia.edu
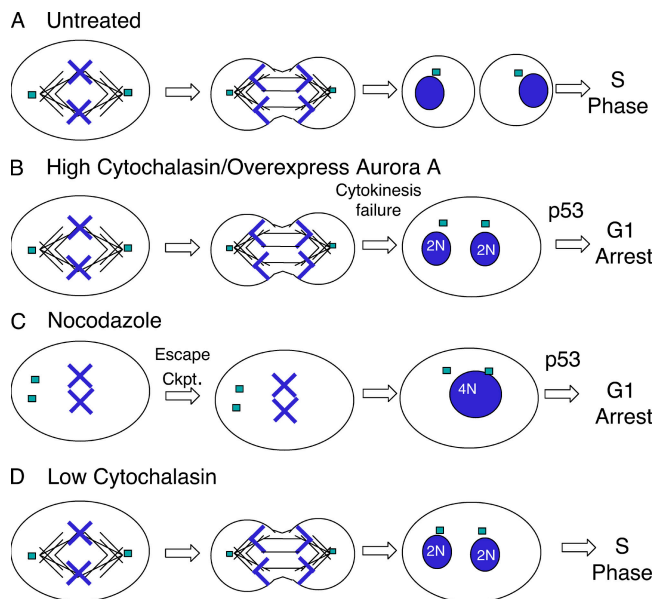
Figure 1. **A summary of the experiments addressing the p53-dependent G1 arrest after cytokinesis failure.** See text for details.

Although it is possible that polyploid organisms and cells undergo adaptive events, these observations suggest that polyploidy per se is not lethal at the cellular level.

A report in this issue provides new insight into the cause of p53-dependent arrest. Uetake and Sluder found that transient treatment with very low concentrations of cytochalasin D can block cytokinesis to generate binucleate cells but cells treated this way did not arrest at G1 (Fig. 1 D) (Uetake and Sluder, 2004). Using video microscopy, they followed binucleate cells formed in these low cytochalasin D concentrations and showed that they underwent mitosis and another round of cytokinesis. The lack of the arrest was not caused by the loss of the p53 pathway, since the same cells arrested at the higher concentrations of cytochalasin D. Similarly, Wong and Stearns fused human diploid foreskin fibroblasts (which can also arrest as binucleates with high concentrations of cytochalasin) and showed that the resulting binucleate hybridomas entered S-phase without a prolonged arrest (Wong, C., and T. Stearns, personal communication). These simple experiments argue strongly that p53-dependent arrest is not triggered by binucleation, polyploidy, multiple centrosomes, or failure of cytokinesis.

What is triggering the p53 network in tetraploid cells has become the central enigma in this field. One clue comes from the observation that there may be some cell type specificity. Margolis's group originally used rat embryonic fibroblasts (Ref52 cells) (Andreassen et al., 2001) and Uetake and Sluder found that these cells arrested even at the lower concentrations of cytochalasin D that did not block S-phase progression in hTert-RPE1 cells or human primary foreskin fibroblasts. Interestingly, the arrest in Ref52 cells could be relieved by plating the cells on fibronectin rather than directly on glass (Uetake and Sluder, 2004). It is unclear why fibronectin suppresses the arrest, but it is interesting that the binding of integrins to fibronectin can regulate the actin and microtubule cytoskeleton. Perhaps the disruption of the cytoskeleton during a failed cytokinesis generates a "dead end" cytoskeletal complex that is activating p53 and the pathways downstream of integrins can resolve these cytoskeleton network problems.

To understand if this p53-dependent arrest actually prevents cancer progression, not only does the signal need to be determined but the conditions by which the arrest is normally triggered must be described. Most studies have used drugs to trigger the arrest, with one exception from Brian Reid's group who found an increase in ploidy specifically in $p53^{-/-}$ mice. 25 d after birth, the pancreases of $53^{-/-}$ mice have ~23% of 4N cells as compared with 7% in wild type. Moreover, in transgenic mice that blocked p53 and other proteins by expressing SV40 T-antigen under the elastase promoter the number of polyploid cells in the pancreas was >45% (Cross et al., 1995). This report of p53 preventing polyploidy in vivo suggests that this mysterious pathway may still have an important role in preventing cancer progression.

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