

Extra View

MdmX regulates transformation and chromosomal stability in p53-deficient cells

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The cellular homologues Mdm2 and MdmX play critical roles in regulating the activity of the p53 tumor suppressor in damaged and non-damaged cells and during development in mice. Recently, we have utilized genetically defined primary cells and mice to reveal that endogenous levels of MdmX can also suppress multipolar mitosis and transformation in hyperploid p53-deficient cells and tumorigenesis in p53-deficient mice. These MdmX functions are not shared by Mdm2, and are distinct from the well-established ability of MdmX to complex with and inhibit p53 activity. Here we discuss some of the ramifications of MdmX loss in p53-deficient cells and mice, and we explore further the fate of MdmX/p53-double null embryonic fibroblasts undergoing multi-polar cell division using time-lapse video microscopy. We also discuss the relationship between chromosomal loss, cell proliferation, and the tumorigenic potential of p53-deficient cells lacking MdmX.

Introduction

The p53 transcription factor is activated by inappropriate cell growth stimulation or by certain types of DNA damage and it regulates the expression of other genes involved in cell growth and death, DNA repair, and chromosomal stability.¹ The importance of these p53 functions in preventing tumorigenesis is underscored by findings that mice deleted for *p53* form spontaneous tumors with 100% penetrance and that mutation of the *p53* gene or the p53-signaling pathway is the most common genetic alteration identified in human cancer.² The ability of p53 to arrest cell growth and induce cell death following genetic or metabolic insult or during development and in normal cell homeostasis is kept tightly in check by cellular proteins that bind with p53 and downregulate p53 activity. Chief among these p53 inhibitors are the cellular homologues Mdm2 and MdmX.

Mdm2 and MdmX in the Regulation of p53 Activity

Mdm2 was initially cloned as one of several genes present on a mouse double minute chromosome found in a spontaneously

transformed derivative of a NIH3T3 cells. Subsequently, Mdm2 was found to complex with p53 and negatively regulate p53-induced transcription of several target genes, including the *Mdm2* gene itself.³ Although *Mdm2* is ubiquitously expressed at a low level, p53 strongly upregulates *Mdm2* gene expression following DNA damage by binding to a p53 response element within the first intron of the *Mdm2* gene.⁴ Induction of increased Mdm2 protein levels leads to an increase in Mdm2-p53 complex formation that interferes with the ability of p53 to transactivate *Mdm2* expression. Thus, *Mdm2* is autoregulated through the ability of Mdm2 to negatively regulate p53.⁵ Mdm2 interferes with the ability of p53 to transactivate target genes by binding to the N-terminal activation domain of the p53 protein,^{6,7} or by promoting p53 protein modifications that inhibit p53 transcriptional activity.⁸ In addition, Mdm2 induces shuttling of p53 from the nucleus into the cytoplasm.^{9,10} Importantly, Mdm2 can also function as an E3 ligase to ubiquitinate and induce the degradation of p53 in the 26S proteasome.¹¹⁻¹⁴ The ability of Mdm2 to negatively regulate p53 activity is best illustrated by mouse studies wherein the early embryonic lethal phenotype of Mdm2-null mice was fully rescued by co-deletion of p53.^{15,16}

The *Mdm2* homologue, *MdmX*, is also a ubiquitously expressed gene that encodes a p53-binding protein that inhibits p53 transcriptional activation.¹⁷ However, *MdmX* expression does not appear to be regulated by p53, and MdmX does not function as an E3 ligase to direct the ubiquitination and destabilization of p53. Although there are distinct differences in the manner by which Mdm2 and MdmX inhibit p53 activity,¹⁸ deletion of MdmX can also induce an embryonic lethal phenotype in mice that can be rescued by either the concomitant deletion of p53 or by overexpression of Mdm2.¹⁹⁻²² These results indicate that MdmX, like Mdm2, is a key regulator of p53 activity in development. Subsequent analyses of various mouse models bearing conditional alleles of *Mdm2* or *MdmX* has further demonstrated that these MDM proteins play key roles in regulating p53 activity in organogenesis during later stages of development and in tissue homeostasis in adult mice.²³⁻²⁹

Given that the MDM proteins are key negative regulators of the p53 tumor suppressor, it is not surprising that *Mdm2* and *MdmX* have strong oncogenic potential. *MDM2* is overexpressed in a wide variety of human tumors, and the *MDM2* gene is amplified in approximately one-third of human sarcomas and in roughly 10% of all human cancers.^{30,31} Likewise, *MDMX* is amplified or overexpressed in 10–20% of all human cancers, and upregulation of

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MDMX was recently found to be an important mechanistic step in the formation of retinoblastoma.³²

p53-Independent Functions of Mdm2 and MdmX

While there is a large amount of evidence to substantiate that overexpression of *Mdm2* or *MdmX* induces tumorigenesis through the ability of these oncoproteins to suppress p53 activity, reports have also suggested that MDM proteins might have additional, p53-independent, roles in cell growth control and in tumorigenesis. Human tumors have been identified that overexpress *MDM2* and yet lack functional p53, a seemingly redundant set of mutations.^{33,34} Furthermore, patients that present with sarcomas or bladder cancers containing both mutations have a worse prognosis than patients with only one or the other mutation.^{33,35} Other genetic evidence for a secondary role for Mdm2 in tumorigenesis (in addition to p53 inhibition) has been provided by transgenic mouse studies. Overexpression of the *Mdm2* gene alters the spectrum of spontaneous tumors that arise in p53-deficient mice,³⁶ and expression of *MDM2* cDNA in the mammary epithelium of transgenic mice was found to inhibit mammary gland morphogenesis and uncouple DNA synthesis from mitosis in the presence or absence of p53.³⁷ In addition, *Mdm2* splice variants lacking the p53-binding domain have been isolated from human or mouse tumors that overexpress Mdm2. Expression of these Mdm2 isoforms has been reported to promote proliferation, inhibit apoptosis, and induce transformation in p53-deficient cells in culture, and promote tumorigenesis in vivo in mice.³⁸⁻⁴⁰

Transfection studies and biochemical analysis has indicated that Mdm2 can complex with a variety of cell growth regulatory proteins aside from p53, including the Rb tumor suppressor protein,⁴¹⁻⁴⁴ the E2F1 and DP1 transcription factors,⁴⁵ as well as with FOXO3a,⁴⁶ E-cadherin,⁴⁷ Numb,⁴⁸ MTBP,⁴⁹ SMADs,⁵⁰ Tip60,⁵¹ and β -arrestin.⁵² In addition, Mdm2 has been recently reported to complex with Nbs1, a component of the Mre11/Rad50/Nbs1 DNA repair complex and can inhibit DNA break repair in p53-deficient cells.⁵³ Although these various findings indicate that there may be additional roles for Mdm2 in regulating cell growth or transformation that are distinct from its crucial role as a negative regulator of p53, it is interesting to note that these additional roles have been uncovered mainly through experiments investigating the effects of Mdm2 overexpression in cells or in mice. As noted previously, mice lacking both Mdm2 and p53 undergo normal development and display the same spontaneous tumor formation rate and tissue spectrum as mice lacking p53 alone,^{15,36} suggesting that any functions possessed by Mdm2 aside from its ability to regulate p53 are dispensable for normal growth and development. Thus, normal physiologic levels of Mdm2 primarily serve to regulate p53 activity. Furthermore, primary fibroblasts lacking both Mdm2 and p53 are indistinguishable from p53-null fibroblasts in their growth characteristics, their rate of immortalization, and in their response to metabolic insult or DNA damage. Therefore, it is possible that any p53-independent roles for Mdm2 are of biological significance only when Mdm2 is expressed above normal physiologic levels, such as during tumorigenesis in cells that lack the Mdm2-inhibitor p14^{ARF} or that contain amplified copy numbers of the *Mdm2* gene.

Similar to Mdm2, MdmX has been found to complex with and/or inhibit the activity of growth regulatory transcription factors other than p53, including several of the Smad proteins and E2F1.^{50,54-56}

However, these molecular targets have yet to be confirmed in vivo, and the contribution of these interactions with MdmX to cell growth control is uncertain. However, our lab has provided data using primary cells and mice to document a role for MdmX in cell growth and transformation distinct from its well-established ability to inhibit p53 activity. We have previously noted that mouse embryonic fibroblasts (MEFs) that lacked p21 or that overexpressed Mdm2 proliferated faster when MdmX was also deleted in these cells.^{22,57} This is an unusual set of findings if the only function of MdmX was to inhibit p53. Thus, in order to establish whether a p53-independent role truly exists for MdmX in cell growth or in tumorigenesis, we characterized the growth and transformation potential of p53-null primary cells that were deficient in MdmX or contained physiologic levels of MdmX.⁵⁸

MdmX Promotes Bipolar Mitosis to Suppress Transformation and Tumorigenesis in p53-Deficient Cells and Mice

We have recently discovered that the presence or absence of MdmX did not alter the proliferation of low-passage p53-null MEFs, in agreement with data published previously by other groups.²⁰ However, we noted that deletion of MdmX drastically increased the rate of cell proliferation when the cells were at higher passages.⁵⁸ Differences in proliferation between MdmX/p53-null (DKO) cells and p53-null cells become apparent around the eighth or ninth passage, a time when wild type cells cultured under the same conditions typically enter senescence. MEFs lacking p53 often undergo centrosome amplification and display hyperploidy after several passages in culture, and both events were readily observed in our p53-null cells and in DKO cells. In addition to augmented growth at higher passages, p53-null MEFs co-deleted for MdmX had increased incidence of multipolar mitosis and reduced number of chromosomes relative to p53-null cells. We noted that these genetic events correspond to more rapid growth of the DKO cells during a 3T3 immortalization assay. Unlike p53-null MEFs at high passage, cells lacking both MdmX and p53 underwent a reduction in chromosome number and displayed a spontaneously transformed phenotype in culture. In agreement with these findings, mice deleted for both MdmX and p53 had a significantly increased rate of spontaneous tumorigenesis, though the spectrum of tumor types observed in the two populations of mice was unchanged. Tumor cells were subsequently isolated from the p53-null mice and from MdmX/p53-double null mice and grown in culture. The double-null tumor cells grew much faster than the p53-null tumor cells, and displayed reduced ploidy and increased incidence of multipolar mitosis. Conversely, transduction of an MdmX expression vector into the DKO tumor cells resulted in a decrease in the rate of cell growth and in the percentage of cells undergoing multipolar mitosis. Collectively, these results reveal that MdmX has an anti-proliferative, anti-transformation function and can promote bipolar mitosis and prevent chromosome loss in hyperploid p53-deficient cells (Fig. 1). We briefly discuss several aspects of this newly discovered MdmX functions.

Centrosome Amplification and Hyperploidy in p53-Deficient Cells

It has been known for some time that MEFs isolated from p53-deficient mice are genetically unstable and have amplified

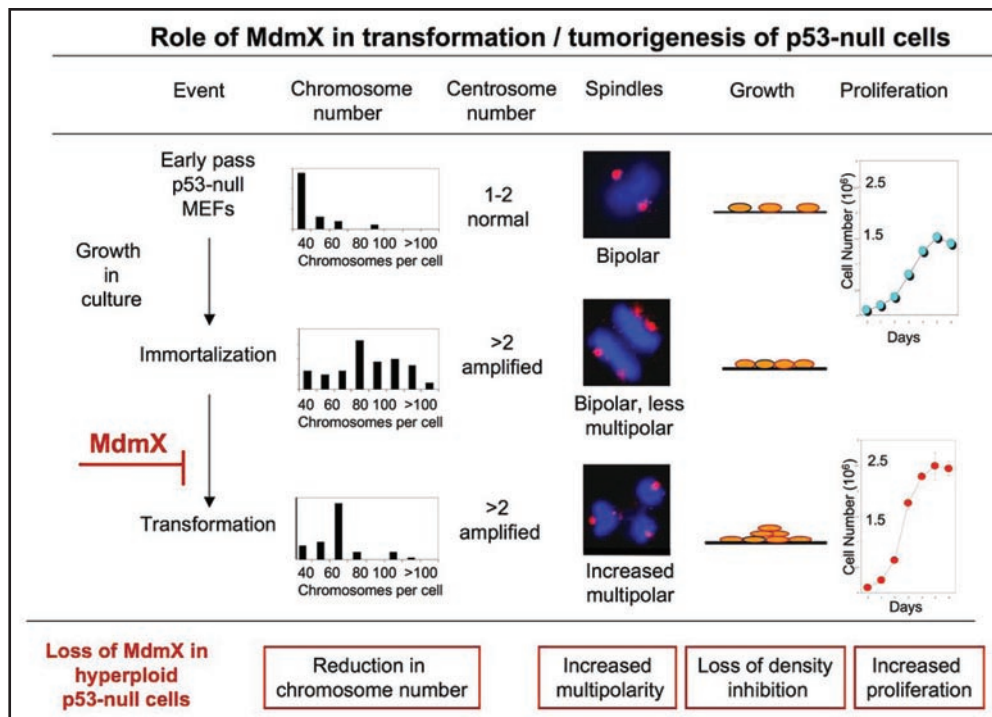


Figure 1. Summary of events observed in p53-null mouse embryonic fibroblasts triggered by co-deletion of MdmX. Examples of each event are provided using data from Matijasevic et al., 2008.⁵⁸ The net effects of MdmX loss are given in boxes at bottom.

centrosomes and increased ploidy.⁵⁹⁻⁶¹ Although several studies have explored p53 in control of centrosome number, the p53-dependent mechanisms that regulate these events have not been fully elucidated. One proposed mechanism for p53 regulation of centrosomes involves the p53 transcriptional target gene, p21. Cdk2-cyclin E activity appears crucial for centrosome reproduction,^{62,63} and inhibition of Cdk2-cyclin E activity by p21,⁶⁴ may provide coordination between centrosome duplication events and DNA replication. In addition, p53 has been proposed to inhibit centrosome amplification by regulating the spindle checkpoint protein BubR1, which can promote the death of cells with amplified centrosomes.⁶⁵ A role for p53 that involves direct p53 centrosomal localization has also been suggested.^{66,67} Regardless of the precise mechanism involved in the control of centrosome number, loss of p53 leads to generation of supernumerary centrosomes that facilitate the formation of multiple mitotic spindles leading to genetic imbalance and chromosomal instability.⁶⁸⁻⁷⁰

Immunofluorescence analysis of mitotic DKO MEFs revealed an increased incidence of multipolar spindles compared to p53-null MEFs at similar passages, suggesting that the loss of MdmX might further enhance centrosome amplification in p53-null cells.⁵⁸ The representative image of cell undergoing multipolar mitosis is shown in Figure 2A, left panel. However, closer inspection of mitotic cells revealed that the bipolar spindles of p53-null MEFs frequently contained multiple centrosomes clustered at opposite poles (Fig. 2A, right), indicating that p53-null cells with amplified centrosomes can still form bipolar spindles, and that MdmX might not affect centrosome amplification in p53-null MEFs. To confirm these observations, we have analyzed interphase p53-null MEFs and DKO MEFs by immunostaining for γ -tubulin (Fig. 2B) and determined that the rate of cells with amplified centrosomes

is very similar (around 20%) in DKO and p53-null populations. In contrast, spindle multipolarity is a much less frequent event in p53-null MEFs or in p53-null tumor cells that retain functional MdmX compared to the incidence of multipolarity observed in DKO MEFs or tumor cells.⁵⁸ Therefore, we conclude that MdmX does not affect centrosome amplification but rather the organization of amplified centrosomes, preventing formation of multipolar spindles and promoting bipolar mitosis and cell division.

Multipolar Cell Division in DKO-null MEFs can Generate Viable Daughter Cells

Normally, an individual diploid (2N) cell becomes tetraploid (4N) prior to mitosis, with bipolar mitosis resulting in the generation of two separate (2N) daughter cells. Only hyperploid cell (>4N) undergoing multipolar mitosis can divide into more than two viable daughter cells. Our previous DAPI-staining of hyperploid p53-deficient cells and examination of telophase figures indicated that the multipolar spindles that formed in DKO cells often led to multi-directional chromosome segregation, suggesting that multipolar mitosis in DKO cells can be followed by multipolar cell division.⁵⁸ As discussed previously, loss of p53 has been well established to promote centrosome amplification and hyperploidy in primary fibroblasts,⁶⁰ and our own data demonstrate that more than half of p53-null MEFs display a greater than triploid DNA content at higher cell passages. However, 90% of all mitotic p53-null MEFs display bipolar spindles and undergo bipolar mitosis. In contrast, multipolar mitosis is apparent in more than 20% of mitotic DKO MEFs. To determine whether multipolar spindles allow generation of more than two daughter cells per division, we employed time-lapse photography to document the fate of cells undergoing multipolar mitosis.

Multipolar mitosis was readily observed and filmed in DKO MEFs. A representative series of still pictures from the time-lapse video microscopy is presented in Figure 3. We have followed division of a single DKO cell into four viable daughter cells (Fig. 3, parts a and b), and subsequent division of one of the daughters (b; arrow) into three cells (c and d). Two of these three resulting cells continued to divide but generated only two daughter cells, and the third cell (d; arrow) became multinucleated (e) and eventually died (f). Thus, multipolar spindle formation and mitosis in DKO MEFs can result in the formation of multiple viable daughter cells. However, analysis of the time-lapse revealed that many of the resulting daughter cells die within one or two rounds of cell division. As the chromosomal content of the hyperploid DKO cells is rapidly reduced during cell passage,⁵⁸ we conclude that the formation of multipolar spindles in p53-null cells co-deleted for MdmX increases genomic instability, and that the unequal chromosomal distribution or loss of chromosomes during multipolar mitosis compromises survival in fraction of progeny. Thus, although more than two daughter cells are generated from some DKO cells undergoing multipolar mitosis, many of these cells are lost in subsequent divisions. Consequently, increased proliferative capacity of DKO compared to p53-null cells does not simply reflect an increase in the number of daughter cells generated through the multipolar division.

MdmX does not Affect the Duration of Mitosis

The decreased ploidy and increased proliferation rate of p53-null cells when co-deleted for MdmX suggest that differences in the growth rate between p53-null and DKO cells may reflect prolonged duration of mitosis in p53-null cells since they have to coordinate centrosome clustering and properly organize their large chromosome complement throughout mitosis. To explore this possibility, we measured the duration of bipolar and multipolar mitosis in p53-null MEFs, and compared it to the duration of bipolar and multipolar mitosis in DKO MEFs using time-lapse video microscopy (Fig. 4). We found that the duration of either bipolar or multipolar mitosis measured from nuclear envelope breakdown (NEB) to the cleavage onset is not impacted by the presence or absence of MdmX. In addition, the duration of multipolar mitosis was nearly twice that of bipolar mitosis, regardless of genotype. As the frequency of multipolar mitosis is higher in DKO population, the duration of mitosis in DKO cells is unlikely to account for the faster growth rate of DKO cells compared to p53-null cells.

Increased Tumorigenic Potential of p53-Null Cells Deleted for MdmX

Following plating and passaging in culture, MEFs lacking p53 display supernumerary centrosomes and hyperploidy regardless of MdmX status. However, unlike p53-null MEFs, DKO MEFs undergo a rapid reduction in ploidy, proliferate faster, and form numerous foci when plated in a cell transformation assay. Thus, MdmX suppress the transformation of immortalized p53-null cells. In agreement with these results, mice deficient for MdmX and p53 form tumors faster than p53-deficient mice. The increased transformation potential of DKO cells in vitro and in vivo

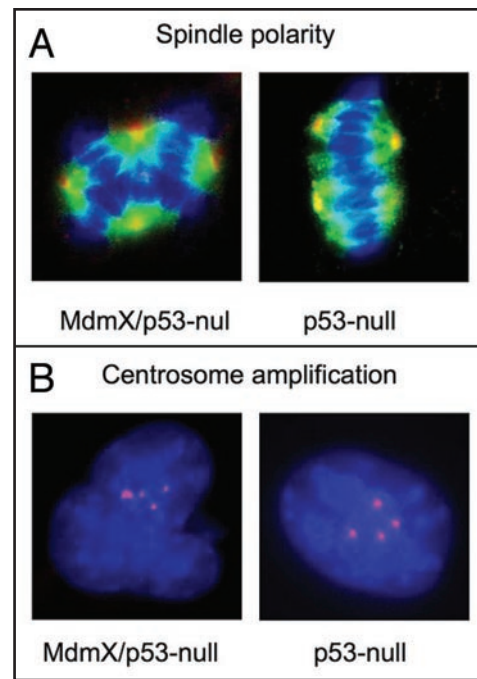


Figure 2. Spindle polarity and centrosome amplification in MdmX/p53-null and p53-null cells. (A) Representative merged immunofluorescent image of MdmX/p53-null (DKO) cell with amplified centrosomes undergoing multipolar mitosis (left) and p53-null cell with amplified centrosomes undergoing bipolar mitosis (right) stained with anti- α -tubulin antibodies, anti-g-tubulin antibodies and DAPI. More than 20% of mitotic DKO cells have multipolar spindles compared to 10% of p53-null cells with multipolar spindles [see: Matijasevic et al., 2008].⁵⁸ (B) Representative images of interphase DKO cells (left) and p53-null cells (right) with amplified centrosomes stained with anti-g-tubulin and with DAPI. The frequency of cells with amplified centrosomes in DKO and p53-null population is 21.9% and 19.3% respectively. At least 150 interphase cells from each cell line were scored.

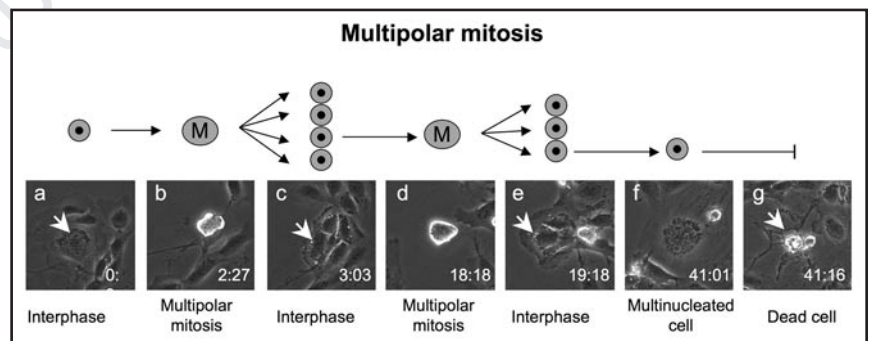


Figure 3. Fate of cell undergoing multipolar mitosis. Shown are selected images from time-lapse video microscopy of MdmX/p53-null mouse embryonic fibroblast (a) undergoing multipolar mitosis (b). Four viable cells were generated in first division (c). One of the daughter cells (c, arrow) was followed further, and found to also undergo multipolar mitosis (d) to generate three new cells, two of which continued to divide generating two daughter cells each (not shown). The remaining third cell (e, arrow) became multinucleated (f), and eventually died (g). Elapsed time in hours and minutes from the beginning of filming is shown in the lower right corner.

is most likely a function of the chromosomal instability of DKO cells. Multipolar mitosis and aneuploidy have been tightly linked to tumorigenesis,⁷¹⁻⁷⁵ and the reduction in chromosome number observed in hyperploid p53-deficient cells after prolonged culturing

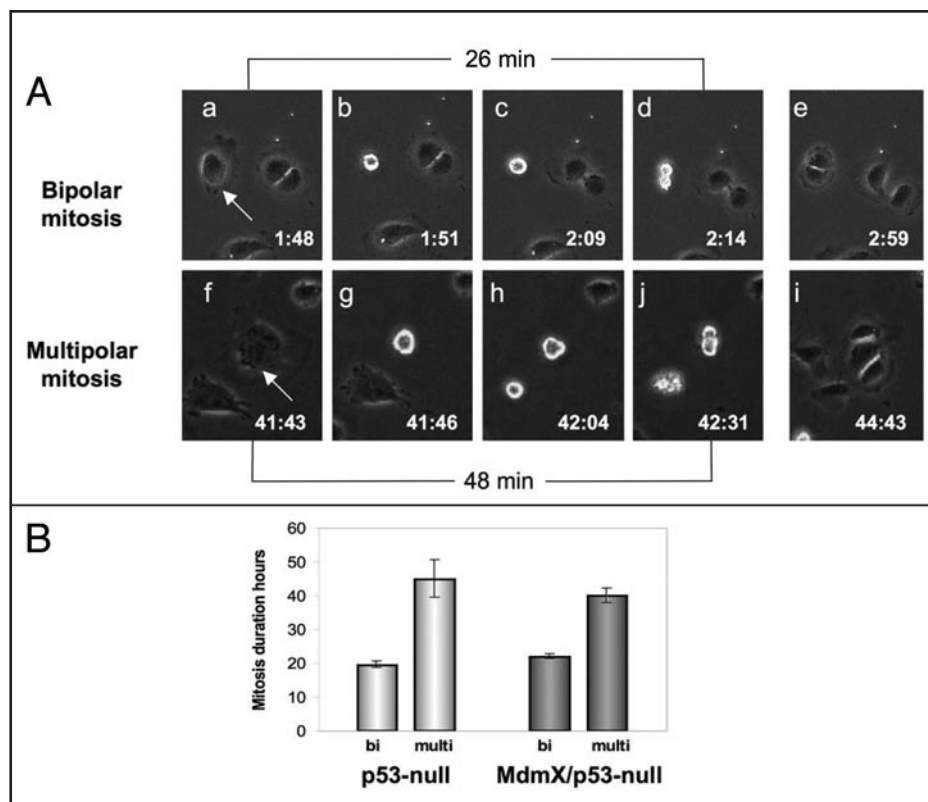


Figure 4. Duration of mitosis. Time-lapse video microscopy was used to measure the duration of mitosis in MdmX/p53-null and p53-null cells. (A) Selected images of bipolar mitosis and the resulting two daughter cells (a–e) or multipolar mitosis giving rise to three daughter cells (f–i) in a population of MdmX/p53-null MEFs. Elapsed time in hours and minutes from the beginning of filming is shown in the lower-right corner. Duration of mitosis is estimated for the cells indicated by an arrow in (a and f). Mitosis was measured from the nuclear envelope breakdown correlating with the rounding up of the cell to the cleavage onset or cell flattening. (B) Statistical analysis of the duration of bipolar and multipolar mitosis in p53-null and MdmX/p53-null cells. The duration of multipolar mitoses averages twice as long as the time required for bipolar mitosis in cells of either genotype. More than forty bipolar and ten multipolar mitoses were scored for each genotype. Error bars represent s.e.m.

in vitro has been proposed to result in aneuploid, but more stable karyotype that confers growth advantage to the cell.⁷⁶ This process resembles previously described genomic convergence that occurs during tumor progression in vivo.⁷⁷

The more rapid loss of chromosomes in DKO MEFs might facilitate cell transformation by promoting the loss of growth regulatory genes that inhibit cell proliferation. Conversely, the more rapid proliferation of DKO cells in culture may simply reflect their “more transformed” state. However, tumor cells cultured from DKO mouse tumors displayed more rapid proliferation than tumor cells from p53-null mice, and transduction of MdmX into these DKO cells increased their chromosome numbers and reduced their rate of proliferation, even though these DKO tumor cells were already fully transformed. Therefore, it is unlikely that the increased proliferation of DKO cells simply reflects their more transformed phenotype or the loss of a specific gene(s) during chromosomal reduction in culture. Rather, it would appear that MdmX alters the proliferation of p53-null cells through a more specific mechanism, one that might involve interaction with other cell cycle regulatory proteins. Further experiments are needed to distinguish between the ability of MdmX to suppress the proliferation of p53-null cells and to suppress the transformation of p53-null cells in vitro and in vivo. Interestingly, increased reduction in chromosome number during passaging in culture is also observed in MdmX-null cells that retain p53 but are

deleted for p21, as well as in MdmX/p53 DKO cells also lacking Mdm2.⁵⁸ These initial findings suggest that the ability of MdmX to promote bipolar mitosis is independent of these p53 target genes. Therefore, elucidation of the precise roles of MdmX in promoting bipolar mitosis and in altering cell proliferation will require additional studies to identify p53-independent binding partners and functions for MdmX. Since p53-null mice deleted for MdmX display increased tumorigenesis, and since approximately half of all human cancers are mutated for p53, a clearer understanding of the p53-independent roles of MDMX in inhibiting transformation is needed.

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References

1. Vousden KH, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol* 2007; 8:275-83.
2. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001; 1:233-40.
3. Iwakuma T, Lozano G. MDM2, an introduction. *Mol Cancer Res* 2003; 1:993-1000.
4. Juven T, Barak Y, Zauberan A, George DL, Oren M. Wild type p53 can mediate sequence-specific transactivation of an internal promoter within the mdm2 gene. *Oncogene* 1993; 8:3411-6.
5. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev* 1993; 7:1126-32.

6. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992; 69:1237-45.
7. Chen J, Lin J, Levine AJ. Regulation of transcription functions of the p53 tumor suppressor by the mdm-2 oncogene. *Mol Med* 1995; 1:142-52.
8. Xirodimas DP, Saville MK, Bourdon JC, Hay RT, Lane DP. Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. *Cell* 2004; 118:83-97.
9. Freedman DA, Levine AJ. Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6. *Mol Cell Biol* 1998; 18:7288-93.
10. Geyer RK, Yu ZK, Maki CG. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat Cell Biol* 2000; 2:569-73.
11. Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett* 1997; 420:25-7.
12. Kubbutat MH, Vousden KH. Proteolytic cleavage of human p53 by calpain: a potential regulator of protein stability. *Molecular & Cellular Biology* 1997; 17:460-8.
13. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997; 387:296-9.
14. Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W. Mono-versus polyubiquitination: differential control of p53 fate by Mdm2. *Science* 2003; 302:1972-5.
15. Jones SN, Roe AE, Donehower LA, Bradley A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* 1995; 378:206-8.
16. Montes de Oca Luna R, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature* 1995; 378:203-6.
17. Shvarts A, Steegenga WT, Riteco N, van Laar T, Dekker P, Bazuine M, van Ham RC, van der Houven van Oordt W, Hateboer G, van der Eb AJ, Jochemsen AG. MDMX: a novel p53-binding protein with some functional properties of MDM2. *EMBO J* 1996; 15:5349-57.
18. Toledo F, Krummel KA, Lee CJ, Liu CW, Redewald LW, Tang M, Wahl GM. A mouse p53 mutant lacking the proline-rich domain rescues Mdm4 deficiency and provides insight into the Mdm2-Mdm4-p53 regulatory network. *Cancer Cell* 2006; 9:273-85.
19. Parant J, Chavez-Reyes A, Little NA, Yan W, Reinke V, Jochemsen AG, Lozano G. Rescue of embryonic lethality in Mdm4-null mice by loss of Trp53 suggests a nonoverlapping pathway with MDM2 to regulate p53. *Nat Genet* 2001; 29:92-5.
20. Migliorini D, Denchi EL, Danovi D, Jochemsen A, Capillo M, Gobbi A, Helin K, Pelicci PG, Marine JC. Mdm4 (Mdmx) regulates p53-induced growth arrest and neuronal cell death during early embryonic mouse development. *Mol Cell Biol* 2002; 22:5527-38.
21. Finch RA, Donoviel DB, Potter D, Shi M, Fan A, Freed DD, Wang CY, Zambrowicz BP, Ramirez-Solis R, Sands AT, Zhang N. mdmx is a negative regulator of p53 activity in vivo. *Cancer Res* 2002; 62:3221-5.
22. Steinman HA, Hoover KM, Keeler ML, Sands AT, Jones SN. Rescue of Mdm4-deficient mice by Mdm2 reveals functional overlap of Mdm2 and Mdm4 in development. *Oncogene* 2005; 24:7935-40.
23. Lengner CJ, Steinman HA, Gagnon J, Smith TW, Henderson JE, Kream BE, Stein GS, Lian JB, Jones SN. Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. *J Cell Biol* 2006; 172:909-21.
24. Xiong S, Van Pelt CS, Elizondo-Fraire AC, Liu G, Lozano G. Synergistic roles of Mdm2 and Mdm4 for p53 inhibition in central nervous system development. *Proc Natl Acad Sci USA* 2006; 103:3226-31.
25. Grier JD, Xiong S, Elizondo-Fraire AC, Parant JM, Lozano G. Tissue-specific differences of p53 inhibition by Mdm2 and Mdm4. *Mol Cell Biol* 2006; 26:192-8.
26. Boesten LS, Zadelaar SM, De Clercq S, Francoz S, van Nieuwkoop A, Biessen EA, Hofmann F, Feil S, Feil R, Jochemsen AG, Zurcher C, Havekes LM, van Vlijmen BJ, Marine JC. Mdm2, but not Mdm4, protects terminally differentiated smooth muscle cells from p53-mediated caspase-3-independent cell death. *Cell Death Differ* 2006; 13:2089-98.
27. Francoz S, Froment P, Bogaerts S, De Clercq S, Maetens M, Doumont G, Bellefroid E, Marine JC. Mdm4 and Mdm2 cooperate to inhibit p53 activity in proliferating and quiescent cells in vivo. *Proc Natl Acad Sci USA* 2006; 103:3232-7.
28. Maetens M, Doumont G, Clercq SD, Francoz S, Froment P, Bellefroid E, Klingmuller U, Lozano G, Marine JC. Distinct roles of Mdm2 and Mdm4 in red cell production. *Blood* 2007; 109:2630-3.
29. Xiong S, Van Pelt CS, Elizondo-Fraire AC, Fernandez-Garcia B, Lozano G. Loss of Mdm4 results in p53-dependent dilated cardiomyopathy. *Circulation* 2007; 115:2925-30.
30. Leach FS, Tokino T, Meltzer P, Burrell M, Oliner JD, Smith S, Hill DE, Sidransky D, Kinzler KW, Vogelstein B. p53 Mutation and MDM2 amplification in human soft tissue sarcomas. *Cancer Res* 1993; 53:2231-4.
31. Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer* 2006; 6:909-23.
32. Laurie NA, Donovan SL, Shih CS, Zhang J, Mills N, Fuller C, Teunis A, Lam S, Ramos Y, Mohan A, Johnson D, Wilson M, Rodriguez-Galindo C, Quarto M, Francoz S, Mendrysa SM, Guy RK, Marine JC, Jochemsen AG, Dyer MA. Inactivation of the p53 pathway in retinoblastoma. *Nature* 2006; 444:61-6.
33. Cordon-Cardo C, Latres E, Drobniak M, Oliva MR, Pollack D, Woodruff JM, Marechal V, Chen J, Brennan MF, Levine AJ. Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. *Cancer Res* 1994; 54:794-9.
34. Watanabe T, Ichikawa A, Saito H, Horita T. Overexpression of the MDM2 oncogene in leukemia and lymphoma. *Leuk Lymphoma* 1996; 21:391-7, color plates XVI following 5.
35. Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C. Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. *Clin Cancer Res* 2002; 8:171-9.
36. Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci USA* 1998; 95:15608-12.
37. Lundgren K, Montes de Oca Luna R, McNeill YB, Emerick EP, Spencer B, Barfield CR, Lozano G, Rosenberg MP, Finlay CA. Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. *Genes Dev* 1997; 11:714-25.
38. Sigalas I, Calvert AH, Anderson JJ, Neal DE, Lunec J. Alternatively spliced mdm2 transcripts with loss of p53 binding domain sequences: transforming ability and frequent detection in human cancer. *Nat Med* 1996; 2:912-7.
39. Fridman JS, Hernando E, Hemann MT, de Stanchina E, Cordon-Cardo C, Lowe SW. Tumor promotion by Mdm2 splice variants unable to bind p53. *Cancer Res* 2003; 63:5703-6.
40. Steinman HA, Burstein E, Lengner C, Gosselin J, Pihan G, Duckett CS, Jones SN. An alternative splice form of Mdm2 induces p53-independent cell growth and tumorigenesis. *J Biol Chem* 2004; 279:4877-86.
41. Xiao ZX, Chen J, Levine AJ, Modjtahedi N, Xing J, Sellers WR, Livingston DM. Interaction between the retinoblastoma protein and the oncoprotein MDM2. *Nature* 1995; 375:694-8.
42. Sdek P, Ying H, Zheng H, Margulis A, Tang X, Tian K, Xiao ZX. The central acidic domain of MDM2 is critical in inhibition of retinoblastoma-mediated suppression of E2F and cell growth. *J Biol Chem* 2004; 279:53317-122.
43. Sdek P, Ying H, Chang DL, Qiu W, Zheng H, Toutou R, Allday MJ, Xiao ZX. MDM2 promotes proteasome-dependent ubiquitin-independent degradation of retinoblastoma protein. *Mol Cell* 2005; 20:699-708.
44. Uchida C, Miwa S, Kitagawa K, Hattori T, Isobe T, Otani S, Oda T, Sugimura H, Kamijo T, Ookawa K, Yasuda H, Kitagawa M. Enhanced Mdm2 activity inhibits pRb function via ubiquitin-dependent degradation. *EMBO J* 2005; 24:160-9.
45. Martin K, Trouche D, Hagemeyer C, Sorensen TS, La Thangue NB, Kouzarides T. Stimulation of E2F1/DP1 transcriptional activity by MDM2 oncoprotein. *Nature* 1995; 375:691-4.
46. Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X, Lang JY, Lai CC, Chang CJ, Huang WC, Huang H, Kuo HP, Lee DF, Li LY, Lien HC, Cheng X, Chang KJ, Hsiao CD, Tsai FJ, Tsai CH, Sahin AA, Muller WJ, Mills GB, Yu D, Hortobagyi GN, Hung MC. ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat Cell Biol* 2008; 10:138-48.
47. Yang JY, Zong CS, Xia W, Wei Y, Ali-Seyed M, Li Z, Broglio K, Berry DA, Hung MC. MDM2 promotes cell motility and invasiveness by regulating E-cadherin degradation. *Mol Cell Biol* 2006; 26:7269-82.
48. Juven-Gershon T, Shifman O, Unger T, Elkeles A, Haupt Y, Oren M. The Mdm2 oncoprotein interacts with the cell fate regulator Numb. *Mol Cell Biol* 1998; 18:3974-82.
49. Boyd MT, Vlatkovic N, Haines DS. A novel cellular protein (MTBP) binds to MDM2 and induces a G₁ arrest that is suppressed by MDM2. *J Biol Chem* 2000; 275:31883-90.
50. Yam CH, Siu WY, Arooz T, Chiu CH, Lau A, Wang XQ, Poon RY. MDM2 and MDMX inhibit the transcriptional activity of ectopically expressed SMAD proteins. *Cancer Res* 1999; 59:5075-8.
51. Legube G, Linares LK, Lemerrier C, Scheffner M, Khochbin S, Trouche D. Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. *EMBO J* 2002; 21:1704-12.
52. Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. *Science* 2001; 294:1307-13.
53. Wang P, Lushnikova T, Odvody J, Greiner TC, Jones SN, Eischen CM. Elevated Mdm2 expression induces chromosomal instability and confers a survival and growth advantage to B cells. *Oncogene* 2008; 27:1590-8.
54. Kadakia M, Brown TL, McGorry MM, Berberich SJ. MdmX inhibits Smad transactivation. *Oncogene* 2002; 21:8776-85.
55. Strachan GD, Jordan-Sciutto KL, Rallapalli R, Tuan RS, Hall DJ. The E2F-1 transcription factor is negatively regulated by its interaction with the MDMX protein. *J Cell Biochem* 2003; 88:557-68.
56. Wunderlich M, Ghosh M, Weghorst K, Berberich SJ. MdmX represses E2F1 transactivation. *Cell Cycle* 2004; 3:472-8.
57. Steinman HA, Sluss HK, Sands AT, Pihan G, Jones SN. Absence of p21 partially rescues Mdm4 loss and uncovers an antiproliferative effect of Mdm4 on cell growth. *Oncogene* 2004; 23:303-6.
58. Matijasevic Z, Steinman HA, Hoover K, Jones SN. MdmX promotes bipolar mitosis to suppress transformation and tumorigenesis in p53-deficient cells and mice. *Mol Cell Biol* 2008; 28:1265-73.
59. Harvey M, Sands AT, Weiss RS, Hegi ME, Wiseman RW, Pantazis P, Giovannella BC, Tainsky MA, Bradley A, Donehower LA. In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* 1993; 8:2457-67.
60. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF. Abnormal centrosome amplification in the absence of p53. *Science* 1996; 271:1744-7.

61. Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH, Reid BJ. A p53-dependent mouse spindle checkpoint. *Science* 1995; 267:1353-6.
62. Hinchcliffe EH, Li C, Thompson EA, Maller JL, Sluder G. Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. *Science* 1999; 283:851-4.
63. Hinchcliffe EH, Sluder G. Two for two: Cdk2 and its role in centrosome doubling. *Oncogene* 2002; 21:6154-60.
64. Minella AC, Swanger J, Bryant E, Welcker M, Hwang H, Clurman BE. p53 and p21 form an inducible barrier that protects cells against cyclin E-cdk2 deregulation. *Curr Biol* 2002; 12:1817-27.
65. Oikawa T, Okuda M, Ma Z, Goorha R, Tsujimoto H, Inokuma H, Fukasawa K. Transcriptional control of BubR1 by p53 and suppression of centrosome amplification by BubR1. *Mol Cell Biol* 2005; 25:4046-61.
66. Shinmura K, Bennett RA, Tarapore P, Fukasawa K. Direct evidence for the role of centrosomally localized p53 in the regulation of centrosome duplication. *Oncogene* 2007; 26:2939-44.
67. Tritarelli A, Oricchio E, Ciciarello M, Mangiacasale R, Palena A, Lavia P, Soddu S, Cundari E. p53 localization at centrosomes during mitosis and postmitotic checkpoint are ATM-dependent and require serine 15 phosphorylation. *Mol Biol Cell* 2004; 15:3751-7.
68. Brinkley BR. Managing the centrosome numbers game: from chaos to stability in cancer cell division. *Trends Cell Biol* 2001; 11:18-21.
69. Sluder G, Nordberg JJ. The good, the bad and the ugly: the practical consequences of centrosome amplification. *Curr Opin Cell Biol* 2004; 16:49-54.
70. Saunders W. Centrosomal amplification and spindle multipolarity in cancer cells. *Semin Cancer Biol* 2005; 15:25-32.
71. Boveri T. Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. *Vehr d phys med Ges zu Wurzburg NF* 1902; 35:67-90.
72. Hansemann D. Ueber pathologische Mitosen. *Arch Pathol Anat Phys Klin Med* 1891; 119:299-326.
73. Rajagopalan H, Lengauer C. Aneuploidy and cancer. *Nature* 2004; 432:338-41.
74. Storchova Z, Pellman D. From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 2004; 5:45-54.
75. Weaver BA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* 2007; 11:25-36.
76. Chiba S, Okuda M, Mussman JG, Fukasawa K. Genomic convergence and suppression of centrosome hyperamplification in primary p53^{-/-} cells in prolonged culture. *Exp Cell Res* 2000; 258:310-21.
77. Heim S, Mandahl N, Mitelman F. Genetic convergence and divergence in tumor progression. *Cancer Res* 1988; 48:5911-6.

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