Chinese Journal of Cancer

Review

Mouse models of *Mdm2* and *Mdm4* and their clinical implications

Shunbin Xiong

Abstract

Mdm2 and *Mdm4* are two key negative regulators of the tumor suppressor *p53*. Deletion of either *Mdm2* or *Mdm4* induces *p53*-dependent early embryonic lethality in knockout mouse models. The tissue-specific deletion of *Mdm2* induces *p53*-dependent apoptosis, whereas the deletion of *Mdm4* induces both *p53*-dependent apoptosis and cell cycle arrest. Compared to *Mdm4* deletion, *Mdm2* deletion causes more severe phenotypic defects. Disrupting the *Mdm2* and *Mdm4* interaction using knockin mice models causes embryonic lethality that can be completely rescued by the concomitant loss of *p53*, suggesting that *Mdm2* and *Mdm4* heterodimerization is critical to inhibit p53 activity during embryogenesis. Overexpression of *Mdm2* and *Mdm4* in mice induces spontaneous tumorigenesis, which clearly indicates that *Mdm2* and *Mdm4* are bona fide oncogenes. Studies from these mouse models strongly suggest that blocking Mdm2- and Mdm4-mediated p53 inhibition is an appealing therapeutic strategy for cancer patients with wild-type *p53* alleles.

Key words *p53* , knockout, knockin, transgene, Mdm2 and Mdm4 inhibitors

The *p53* tumor suppressor pathway is inactivated in approximately 50% of human cancers (www-p53.iarc.fr). The loss of p53 function in tumor cells allows increased proliferation, the inhibition of apoptosis, and cell metabolism switching, providing advantageous signals for tumor cell survival[1,2]. Tumor cells have multiple mechanisms for disrupting p53 activity. Missense mutations in particular account for 80% of the alterations at the ***p53*** locus[2]. In addition, several negative regulators of p53 are overexpressed in many tumors of diverse origins[3-8]. Thus, increased levels of p53 inhibitors in tumor cells are other mechanisms that inhibit p53 function in human cancer. In particular, Mdm2 can inhibit p53 through its p53-binding domain and its carboxyl terminal ring finger domain, which is an E3 ubiquitin ligase of p53. Mdm4, a homolog of Mdm2, also inhibits p53 activity by binding to the transcriptional activation domain of p53. The importance of Mdm2 and Mdm4 in the inhibition of p53 has been shown with several knockout, knockin, and overexpressing transgenic mouse models *in vivo*. Studies from these mouse models have suggested that blocking the interaction of Mdm2 and/or Mdm4 with p53 could be a potential therapeutic strategy for cancer patients with wild-type *p53* alleles. Several Mdm2 inhibitors have been published and are undergoing clinical trials[9-11]. Strategies to block p53 and Mdm4 interaction are also under intensive investigation[12,13].

Author's Affiliation: Department of Genetics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.

Corresponding Author: Department of Genetics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Tel: +1-713-834-6387; Fax: +1-713-834-6396; Email: sxiong@mdanderson.org.

doi: 10.5732/cjc.012.10286

Mouse Models of *Mdm2* and *Mdm4* Knockout

The ***Mdm2***-knockout mouse is the first mouse model of negative regulators of p53. Loss of ***Mdm2*** leads to embryonic lethality due to excess apoptosis, which is completely rescued by concomitant deletion of *p53* (Table 1). This demonstrates that p53 activity is strictly repressed by Mdm2 during the developmental stages[14,15]. The role of Mdm2 in the later stages of the mouse lifespan has also been investigated in two other mouse models. One mouse model contains a hypomorphic ***Mdm2*** allele, which only expresses approximately 30% of the wild-type ***Mdm2*** allele due to the insertion of a puromycin selection cassette in the ***Mdm2*** locus at intron 6. The mice with this hypomorphic allele show decreased lymphoid cells, increased

Table 1. Genetic mouse models of *Mdm2* and *Mdm4*

Mouse model	Genotype	Phenotype	Reference(s)
Mdm2	*Mdm2* null	Embryonic lethal around implantation	[14,15]
	$Mdm2^{Puro/\Delta 7-12}$	Smaller mice, increased apoptosis in lymphocytes and epithelial cells, and increased radiosensitivity	[16]
	$p53^{515C/515C}$	Mice die within 2 weeks after birth due to *p53*-dependent cell cycle arrest. Severe impairment in postnatal hematopoiesis and cerebellar development	[17]
	$Mdm2^{-/-}$		
	$Mdm2^{-/-}$, $p53^{ERKI/-}$	Mice die shortly after p53 restoration with defects in multiple radiosensitive tissues; other radio-insensitive tissues, such as the lung, kidney, brain, and liver, are not affected.	[18]
	Mdm2 transgene	Mice are predisposed to spontaneous tumor formation with a high incidence of sarcomas.	[40]
Mdm4	*Mdm4* null	Embryonic lethal at 9.5–11.5 dpc (day post coitum)	[19,20,21]
	$Mdm4^{-/-}$,$p53^{KI/-}$	Minor defects in radiosensitive tissues	[22]
	$Mdm4^{\Delta Ring}$	Early embryonic lethal	[33]
	$Mdm4^{C462A}$	Early embryonic lethal	[34]
	$Mdm4^{Tg1}$, $Mdm4^{Tg15}$	Spontaneous tumorigenesis and accelerated tumorigenesis with *p53* heterozygous background	[41]
	ROSA26–pCAGG–Mdm4	Mice with the *Mdm4* homozygous transgene die during embryogenesis, yet mice with the heterozygous *Mdm4* transgene are viable and not prone to spontaneous, radiation-induced or Eμ-myc–induced tumor formation.	[42]

radiosensitivity, and increased apoptosis in both lymphocytes and epithelial cells[16]. Another mouse model used to investigate the function of Mdm2 after birth is the ***Mdm2***-knockout mouse with a *p53* hypomorphic ***R172P*** allele background. Because ***p53R172P*** only modestly induces ***p53***-mediated cell cycle arrest, it rescues the embryonic lethality of the ***Mdm2*** null allele[17]. In this study, the loss of ***Mdm2*** caused neonatal death due to cell cycle arrest in multiple proliferating tissues, including the bone marrow and cerebellum. Although different tissues are affected by ***Mdm2*** reduction or deletion, both models demonstrate that Mdm2 inhibition of p53 is required for embryogenesis, after birth, and in adulthood.

To further investigate the role of ***Mdm2*** in adult tissues, Evan's lab used the tamoxifen-inducible $p53ER^{TAM}$ allele to restore p53 activity in the $Mdm2^{-/-}$, $p53^{KI/-}$ mice. This mouse model further confirmed the requirement of the Mdm2 protein to inhibit p53 activity in the adult stage, and that the phenotypes were more severe than 30% of the ***Mdm2*** hypomorphic allele. The mouse died shortly after the restoration of p53 activity and had defects in multiple radiosensitive tissues. However, some classical radio-insensitive tissues, such as the lungs, kidneys, brain, and liver, were not affected by the restoration of p53 activity[18], indicating that the effects of Mdm2 inhibition are tissue-specific. This tissue specificity may be due to the different levels of endogenous p53 that can be restored. Therefore, it will be interesting to compare the endogenous levels of p53 in radiosensitive and -insensitive tissues.

More recently, two different strategies have been used to target the ***Mdm4*** locus. Using the viral gene trap technique, *Mdm4* transcription was blocked, and the mouse died around E10.5 to E11.5 dpc (day post coitum)[19,20]. Another mouse model of the *Mdm4* conventional knockout led to *p53*-dependent embryonic lethality earlier than E9.5 dpc[21]. These studies show that the loss of ***Mdm4*** induces *p53*-dependent cell cycle arrest and apoptosis in different tissues, demonstrating that ***Mdm4*** is a non-redundant *p53* inhibitor of ***Mdm2 in vivo***.

To examine the role of ***Mdm4*** in the adult stages, p53 activity was restored in the $Mdm4^{-/-}$, $p53^{KI/-}$ mice. Although these mutant mice have only shown minor defects in radiosensitive tissues such as the spleen, thymus, and intestines, the mice remained normal and healthy[22]. This observation is consistent with previous reports suggesting that Mdm2 is a more potent inhibitor of p53 than Mdm4. However, there are caveats to these restoration strategies: 1) the efficiency of different tissues taking metabolized tamoxifen may vary; and 2) the mice start with only one allele of *p53* to be restored.

Based on the results from the ***Mdm2*** and ***Mdm4*** deletions in mice, the inhibitors targeting Mdm2 may

cause lymphocyte and epithelial defects when the inhibition reduces Mdm2 efficacy to 30%; therefore, Mdm4 inhibitors in cancer patients may be a more desirable choice due to the fewer deleterious effects on normal tissues. Certainly, it will also be important to investigate whether the effects of ***Mdm2*** and ***Mdm4*** inhibition are age- and tumor type-dependent.

Mouse Models for the Tissue-specific Deletion of *Mdm2* and *Mdm4*

To understand how Mdm2 and Mdm4 regulate p53 activity together in specific tissues or specific cell types, ***Mdm2*** and ***Mdm4*** conditional alleles have been generated[23,24]. Tissue-specific ***Mdm2*** deletion in the heart, intestine, testis, thymus, spleen, erythrocytes, adult smooth muscle cells, bone, and hepatocytes induces *p53*-dependent apoptosis[25-27]. The effects of ***Mdm4*** deletion are more complex. ***Mdm4*** deletion in the quiescent or fully differentiated adult smooth muscle cells does not cause obvious defects[28]. On the other hand, although the loss of ***Mdm4*** in the embryonic heart does not cause any obvious defect, it induces apoptosis in cardiomyocytes and leads to dilated cardiomyopathy in adult mice, suggesting that non-proliferating cardiomyocytes also require Mdm4 to inhibit p53 [29]. These data suggest that the Mdm4 inhibition of p53 is not cell cycle-dependent but depends on the cell type or the endogenous levels of Mdm2 and p53. The deletion of both ***Mdm2*** and ***Mdm4*** induces higher p53 activity than the deletion of ***Mdm2*** alone in the embryonic central nervous system, indicating that Mdm2 and Mdm4 cooperatively inhibit p53. These data indicate that a combination therapy by employing both Mdm2 and Mdm4 inhibitors to induce p53 activity in tumors may activate p53 more strongly and therefore have a better treatment outcome [30].

Mouse Models of *Mdm2* and *Mdm4* Knockin

The relationship of Mdm2 and Mdm4 in regulating p53 is very complex. Both Mdm2 and Mdm4 bind to the p53 transactivation domain with similar affinities; therefore, they may compete for the binding and inhibition of p53 activity. Additionally, Mdm2 and Mdm4 interact with each other through their respective RING finger domains[31]. Mdm2 is also an E3 ubiquitin ligase of Mdm4[32]. To understand the role of the Mdm2-Mdm4 interaction in regulating p53 activity, two knockin mouse models, $Mdm4^{C462A}$ and $Mdm4^{\Delta Ring}$, have been generated[33,34]. The $Mdm4^{C462A}$ *and* $Mdm4^{\Delta Ring}$ proteins fail to bind to Mdm2, but both of them bind to p53 and are more stable than wild-type Mdm4. However, both mouse models show ***p53***-dependent early embryonic lethality, indicating that Mdm2 and Mdm4 binding is essential for inhibiting p53 activity during embryogenesis. This implies that the Mdm2 and Mdm4 heterodimers may ubiquitinate p53 more efficiently than the Mdm2 homodimers. Interestingly, the p53 protein level and activity does not change in the adult tissues or mouse embryonic fibroblasts of $Mdm4^{\Delta Ring}$ when a hypomorphic $p53^{neo}$ allele is used to rescue the mice from early lethality, indicating that the interaction of Mdm2 and Mdm4 may not be important for normal tissues or during homeostatic conditions in later stages. Therefore, improving the interaction between Mdm2 and Mdm4 could be another strategy to activate p53 in cancer patients, but this needs to be further examined.

Mouse Models of *Mdm2* and *Mdm4* Overexpression

MDM2 and *MDM4* have been found to be overexpressed in many human cancers[35-39]. Overexpression of ***Mdm2*** in mice leads to tumorigenesis with a significantly higher percentage of sarcoma than the *p53* null mice, suggesting that ***Mdm2*** overexpression may have a ***p53***-independent function[40]. There are two transgenic mouse models of ***Mdm4***. One induced spontaneous tumors and accelerates tumorigenesis of *p53* heterozygosity in the mouse[41]. However, the other transgenic mouse model did not induce tumorigenesis within 50 weeks. When crossed with the ***Eμ-myc*** transgene, a p53 dosage-sensitive tumor model, it did not accelerate tumorigenesis[42]. Because the relative concentrations of Mdm2 and Mdm4 are important for their inhibitory effects on p53[43], the discrepancy may be due to different expression levels of Mdm4 in these two transgenic lines. These studies again demonstrate that Mdm2 and Mdm4 are critical negative regulators of p53. More importantly, they suggest that the overexpression of ***Mdm2*** and ***Mdm4*** is another mechanism for tumorigenesis in cancer patients. This strongly supports that blocking the inhibition of Mdm2 and Mdm4 with p53 can be a therapeutic strategy to treat cancer patients.

In summary, the mouse models of ***Mdm2*** and ***Mdm4*** have greatly improved and expanded our knowledge of their inhibitory function towards p53. They also clarify the conflicting results from different cell culture studies. Although the conclusions based on the loss of function mouse models of ***Mdm2*** and ***Mdm4*** were drawn from irreversible deletion, they still provide very valuable information for understanding the tissue specificity and sensitivity of Mdm2 or Mdm4 inhibition. The results from the mouse models strongly suggest localized treatment in cancer patients to avoid any unwanted damages could be caused by these inhibitors. Furthermore, mouse

models with ***Mdm2*** or ***Mdm4*** overexpression demonstrate that these two genes are truly oncogenes that can drive spontaneous tumorigenesis in the presence of wild-type *p53* alleles, and this further supports the idea of treating cancer patients with high levels of these two proteins by the inhibitors. The mouse models of ***Mdm2*** and ***Mdm4*** overexpression can also be used to test the efficacy of the preclinical Mdm2 and Mdm4 inhibitors ***in vivo***.

Received: 2012-11-22; revised: 2013-01-14; accepted: 2013-01-14.

References

[1] Levine AJ, Puzio-Kuter AM. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science, 2010,330:1340–1344.

[2] Sigal A, Rotter V. Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res, 2000,60:6788–6793.

[3] Watanabe T, Hotta T, Ichikawa A, et al. The MDM2 oncogene overexpression in chronic lymphocytic leukemia and low-grade lymphoma of B-cell origin. Blood, 1994,84:3158–3165.

[4] Zhou M, Yeager AM, Smith SD, et al. Overexpression of the MDM2 gene by childhood acute lymphoblastic leukemia cells expressing the wild-type p53 gene. Blood, 1995,85:1608–1614.

[5] Valentin-Vega YA, Barboza JA, Chau GP, et al. High levels of the p53 inhibitor MDM4 in head and neck squamous carcinomas. Hum Pathol, 2007,38:1553–1562.

[6] Wade M, Wahl GM. Targeting Mdm2 and Mdmx in cancer therapy: better living through medicinal chemistry? Mol Cancer Res, 2009,7:1–11.

[7] Duan W, Gao L, Druhan LJ, et al. Expression of Pirh2, a newly identified ubiquitin protein ligase, in lung cancer. J Natl Cancer Inst, 2004,96:1718–1721.

[8] Dornan D, Wertz I, Shimizu H, et al. The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature, 2004,429:86–92.

[9] Issaeva N, Bozko P, Enge M, et al. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nat Med, 2004,10:1321–1328.

[10] Vassilev LT, Vu BT, Graves B, et al. *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. Science, 2004,303:844–848.

[11] Shangary S, Qin D, McEachern D, et al. Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition. Proc Natl Acad Sci U S A, 2008,105:3933–3938.

[12] Bernal F, Wade M, Godes M, et al. A stapled p53 helix overcomes HDMX-mediated suppression of p53. Cancer Cell, 2010,18:411–422.

[13] Reed D, Shen Y, Shelat AA, et al. Identification and characterization of the first small molecule inhibitor of MDMX. J Biol Chem, 2010, 285:10786–10796.

[14] Montes de Oca Luna R, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. Nature, 1995,378:203–206.

[15] Jones SN, Roe AE, Donehower LA, et al. Rescue of embryonic lethality in mdm2-deficient mice by absence of p53. Nature, 1995,378:206–208.

[16] Mendrysa SM, McElwee MK, Michalowski J, et al. mdm2 is critical for inhibition of p53 during lymphopoiesis and the response to ionizing irradiation. Mol Cell Biol, 2003,23:462–472.

[17] Liu G, Terzian T, Xiong S, et al. The p53-Mdm2 network in progenitor cell expansion during mouse postnatal development. J Pathol, 2007,213:360–368.

[18] Ringshausen I, O'Shea CC, Finch AJ, et al. Mdm2 is critically and continuously required to suppress lethal p53 activity *in vivo*. Cancer Cell, 2006,10:501–514.

[19] Migliorini D, Denchi EL, Danovi D, et al. Mdm4 (Mdmx) regulates p53-induced growth arrest and neuronal cell death during early embryonic mouse development. Mol Cell Biol, 2002,22:5527–5538.

[20] Finch RA, Donoviel DB, Potter D, et al. mdmx is a negative regulator of p53 activity *in vivo*. Cancer Res, 2002,62:3221–3225.

[21] Parant J, Chavez-Reyes A, Little NA, et al. Rescue of embryonic lethality in Mdm4-null mice by loss of Trp53 suggests a nonoverlapping pathway with MDM2 to regulate p53. Nat Genet, 2001,29:92–95.

[22] Garcia D, Warr MR, Martins CP, et al. Validation of Mdmx as a therapeutic target for reactivating p53 in tumors. Genes Dev, 2011,25:1746–1757.

[23] Grier JD, Yan W, Lozano G. Conditional allele of mdm2 which encodes a p53 inhibitor. Genesis, 2002,32:145–147.

[24] Grier JD, Xiong S, Elizondo-Fraire AC, et al. Tissue-specific differences of p53 inhibition by Mdm2 and Mdm4. Mol Cell Biol, 2006,26:192–198.

[25] Lengner CJ, Steinman HA, Gagnon J, et al. Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. J Cell Biol, 2006,172:909–921.

[26] Kodama T, Takehara T, Hikita H, et al. Increases in p53 expression induce CTGF synthesis by mouse and human hepatocytes and result in liver fibrosis in mice. J Clin Invest, 2011,121:3343–3356.

[27] Wade M, Wang YV, Wahl GM. The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol, 2010,20:299–309.

[28] Boesten LS, Zadelaar SM, De Clercq S, et al. Mdm2, but not Mdm4, protects terminally differentiated smooth muscle cells from p53-mediated caspase-3-independent cell death. Cell Death Differ, 2006,13:2089–2098.

[29] Xiong S, Van Pelt CS, Elizondo-Fraire AC, et al. Loss of Mdm4 results in p53-dependent dilated cardiomyopathy. Circulation, 2007,115:2925–2930.

[30] Xiong S, Van Pelt CS, Elizondo-Fraire AC, et al. Synergistic roles of Mdm2 and Mdm4 for p53 inhibition in central nervous system development. Proc Natl Acad Sci U S A, 2006,103:3226–3231.

[31] Marine JC, Jochemsen AG. Mdmx and Mdm2: brothers in arms? Cell Cycle, 2004,3:900–904.
[32] Pan Y, Chen J. MDM2 promotes ubiquitination and degradation of MDMX. Mol Cell Biol, 2003,23:5113–5121.
[33] Pant V, Xiong S, Iwakuma T, et al. Heterodimerization of Mdm2 and Mdm4 is critical for regulating p53 activity during embryogenesis but dispensable for p53 and Mdm2 stability. Proc Natl Acad Sci U S A, 2011,108:11995–12000.
[34] Huang L, Yan Z, Liao X, et al. The p53 inhibitors MDM2/MDMX complex is required for control of p53 activity *in vivo*. Proc Natl Acad Sci U S A, 2011,108:12001–12006.
[35] Momand J, Jung D, Wilczynski S, et al. The MDM2 gene amplification database. Nucleic Acids Res, 1998,26:3453–3459.
[36] Onel K, Cordon-Cardo C. MDM2 and prognosis. Mol Cancer Res, 2004,2:1–8.
[37] Ramos YF, Stad R, Attema J, et al. Aberrant expression of HDMX proteins in tumor cells correlates with wild-type p53. Cancer Res, 2001,61:1839–1842.
[38] Riemenschneider MJ, Buschges R, Wolter M, et al. Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. Cancer Res, 1999,59:6091–6096.
[39] Laurie NA, Donovan SL, Shih CS, et al. Inactivation of the p53 pathway in retinoblastoma. Nature, 2006,444:61–66.
[40] Jones SN, Hancock AR, Vogel H, et al. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. Proc Natl Acad Sci U S A, 1998,95:15608–15612.
[41] Xiong S, Pant V, Suh YA, et al. Spontaneous tumorigenesis in mice overexpressing the p53-negative regulator Mdm4. Cancer Res, 2010,70:7148–7154.
[42] De Clercq S, Gembarska A, Denecker G, et al. Widespread overexpression of epitope-tagged Mdm4 does not accelerate tumor formation *in vivo*. Mol Cell Biol, 2010,30:5394–5405.
[43] Wang YV, Wade M, Wong E, et al. Quantitative analyses reveal the importance of regulated Hdmx degradation for p53 activation. Proc Natl Acad Sci U S A, 2007,104:12365–12370.