Mouse models of Mdm2 and Mdm4 and their clinical implications

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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].

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...
Muscle and brain tissues are affected by the bone marrow and cerebellum. Although different restoration of p53 activity as the lungs, kidneys, brain, and liver, were not affected. However, some classical radio-insensitive tissues, such as the thymus, and intestines, the mice remained normal and healthy. 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

| Mouse model | Genotype | Phenotype | Reference(s) |
|-------------|----------|-----------|--------------|
| Mdm2 null | Smaller mice, increased apoptosis in lymphocytes and epithelial cells, and increased radiosensitivity | [16] |
| Mdm2KO.7-12 | Mice die within 2 weeks after birth due to p53-dependent cell cycle arrest. Severe impairment in postnatal hematopoiesis and cerebellar development | [17] |
| p53R172P | 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] |
| Mdm2null, p53R172P | Mice are predisposed to spontaneous tumor formation with a high incidence of sarcomas. | [40] |
| Mdm2 transgene | | |
| Mdm4 null | Embryonic lethal at 9.5–11.5 dpc (day post coitum) | [19,20,21] |
| Mdm4KO. p53R172P | Minor defects in radiosensitive tissues | [22] |
| Mdm4KO. Ring | Early embryonic lethal | [33] |
| Mdm4Δ54 | Early embryonic lethal | [34] |
| Mdm4KO, Mdm4R172P | 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-myel-induced tumor formation. | [42] |
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[^22^]. To understand the role of the Mdm2-Mdm4 interaction in regulating p53 activity, two knockin mouse models, Mdm2^C462A^ and Mdm4^C462A^, have been generated[^32-33^]. The Mdm2^C462A^ and Mdm4^C462A^ 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^∆neo^ when a hypomorphic p53^-dependent 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 Mdm2 and 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-myec 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.

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