Subject Review

Mdm2 in the Response to Radiation

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Abstract

Murine double minute 2 (Mdm2) is a critical component of the responses to both ionizing and UV radiation. The level of Mdm2 expression determines the extent to which radiation induces an increase in the activity of the p53 tumor suppressor. Mdm2 acts as a survival factor in many cell types by limiting the apoptotic function of p53. In addition, expression of *mdm2* is induced in response to DNA damage, and the resulting high levels of Mdm2 protein are thought to shorten the length of the cell cycle arrest established by p53 in the radiation response. Increased levels of Mdm2 appear to ensure that the activity of p53 returns to its low basal levels in surviving cells. Decreased levels of Mdm2 sensitize cells to ionizing radiation. Thus, Mdm2 is a potential target for therapeutic intervention because its inhibition may radiosensitize the subset of human tumors expressing wild-type p53 such that radiotherapy is more efficacious.

Introduction

Murine double minute 2 (Mdm2)[1] is a critical survival factor in embryos and in a subset of adult tissues (1–3). *mdm2* was one of the first genes shown to be responsive to p53. Initially, a temperature-sensitive form of p53 was used to show that *mdm2* expression was induced under permissive conditions when wild-type p53 activity was high (4, 5). Shortly thereafter, both UV and ionizing radiation were found to induce *mdm2* expression in a p53-dependent manner (6, 7). Mdm2 is critical for preventing uncontrolled cell death in response to both types of radiation.

Both UV and ionizing radiation activate p53 to arrest the cell cycle and to stimulate apoptosis. However, they differ in the types of damage and signaling pathways they induce. UV radiation causes mainly pyrimidine dimers, which are repaired by the nucleotide excision repair pathway (8). In contrast, ionizing radiation induces double-strand breaks, which are repaired primarily by homologous recombination (8). Both types of radiation induce cell cycle checkpoints, processes that prevent or delay progression through the cell cycle when one stage of the cycle has not been properly completed (9). Both types of radiation can cause p53 to induce either cell cycle arrest in G_1 or apoptosis depending in part on the cell type irradiated (10, 11). The factors that determine whether p53 exerts a cell cycle arrest or an apoptotic response are just beginning to be defined (12–14). However, it seems clear that Mdm2 inhibits both responses (15).

Mdm2 can inhibit p53 in two general ways. One way is by directly interfering with the ability of p53 to induce gene expression (16). Mdm2 binds the amino terminus of p53 and blocks the interaction of p53 with the basal transcriptional machinery (17, 18). Mdm2 can also promote the degradation of p53 by adding ubiquitin to it (19–21). In addition to promoting p53's degradation, ubiquitination by Mdm2 promotes translocation of p53 from the nucleus to the cytoplasm, where it cannot function as a transcriptional activator (22). Mdm2 must bind directly to p53 to block transcription and to stimulate degradation (23). Thus, both the level of Mdm2 and its ability to bind p53 are critical for regulating the response to radiation.

This review will summarize the present state of knowledge regarding the regulation of *mdm2* expression and function in the response to UV and ionizing radiation. Gaps in understanding will be noted to encourage new experiments that advance our understanding of Mdm2's roles in the radiation response.

Induction of Mdm2 in the Response to UV Light

In cells exposed to UV light, *mdm2* expression is induced at the RNA level (6, 24). This induction is dependent on p53 because immortal murine fibroblasts lacking p53 do not induce *mdm2* following exposure to UV light (25). Moreover, UV-treated human fibroblasts expressing the p53-inhibitory oncoprotein E6 from human papillomavirus induce less *MDM2* than do normal human fibroblasts (24). The timing of the induction of *mdm2* is dose dependent and appears to be highly regulated (6, 25). For example, it has been reported that the induction of *mdm2* is delayed until hours after p53 has induced expression of the cell cycle inhibitor *p21*, which is critical for the G_1 arrest mediated by p53 in response to DNA damage (25, 26). This observation led to a model in which induction of *mdm2* is delayed to allow p53 to arrest the cell cycle such that DNA repair can occur prior to a resumption of normal rates of DNA synthesis. However, other experiments showed concomitant increases in *p21* and *mdm2* (27). Thus, the timing of *mdm2* induction may differ depending on several factors, including cell type. To date, the importance of the induction of *mdm2* is still unclear.

The *mdm2* gene has two promoters, only one of which responds to radiation (28). In both murine and human cells, the

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[1]The abbreviations used are *MDM2*, human gene and oncogene; MDM2, human protein and isoform; *mdm2*, mouse gene; Mdm2, mouse protein.

induction of *mdm2* RNA expression by both UV and ionizing radiation occurs through the p53-responsive P2 promoter without any concurrent induction of the upstream P1 promoter (Fig. 1; 24, 28). Two p53 binding sites are positioned in the first intron (5, 29, 30), and p53 stimulates only the downstream P2 promoter (28, 30). Different forms of *mdm2* mRNAs are expressed from the two *mdm2* promoters. The P1 promoter directs the synthesis of RNAs that contain exon 1 of *mdm2*, whereas the P2 promoter directs the synthesis of RNAs lacking exon 1 (28). In addition, there is a species difference in the exonic structure of the mRNA from the P1 promoter. In human cells, the major product of the P1 promoter lacks exon 2, whereas in mice, the message contains exons 1–12 (28, 30, 31). In contrast, in both species, the major product of the P2 promoter is a RNA containing exons 2–12 (28, 30, 31). Thus, promoter selection determines the exon structure of *mdm2* mRNAs.

The consequences of expression through the two different promoters are not clear because the first in-frame translation initiation codon for full-length Mdm2 is in exon 3 (Fig. 1; 28). In murine cell lines, the great majority of *mdm2* mRNA is synthesized from the P1 promoter (28). In addition, both diploid human fibroblast strains and murine tissues constitutively express 5–10-fold more RNA from the P1 promoter than from the P2 promoter (24, 32). Thus, the *mdm2* promoter that does not respond to p53 is more relevant physiologically. However, the basal levels of the two *mdm2* mRNAs may not reflect their respective contributions to Mdm2 function.

Promoter selection may influence the level of Mdm2 because full-length mRNA from the P1 promoter appears to be translated less efficiently than the mRNA from the P2 promoter, at least in some cell lines. When fused to a cDNA for the human growth hormone gene, the 5′ untranslated region (UTR) from the human P1 promoter product decreased translation of human growth hormone 40–60-fold (33). In contrast, the 5′ UTR from the *MDM2* P2 promoter product had very little effect on translation (33). The ability of the 5′ UTR from the P1 promoter product to inhibit translation was mapped to an open reading frame in exon 1 (33, 34). Similar results were obtained with an open reading frame from the murine *mdm2* exon 1 (34), leading to the suggestion that the RNA from the *mdm2* P1 promoter is translated less efficiently than RNA from the *mdm2* P2 promoter. Thus, induction of the P2 promoter by p53 in response to radiation would be expected to lead to more rapid synthesis of Mdm2 protein than if the P1 promoter were used. Such a regulatory step would limit p53 function and facilitate the return of low levels of p53 protein and activity following irradiation. However, further investigation into the mechanisms regulating Mdm2 translation is required because studies with full-length cDNAs have not yet supported this attractive model (35).

The products of the two *mdm2* promoters appear to differ in another aspect of translation. mRNAs from both murine P1 and P2 promoters can direct the synthesis of at least two Mdm2 proteins (28). The larger protein is p90Mdm2, the inhibitor of p53. The smaller protein is p76Mdm2, an activator of p53 that lacks the first 49 amino acids of p90Mdm2 (36). p76Mdm2 can be synthesized through internal initiation at AUG 50 in exon 4 of the p90Mdm2 coding sequence (Fig. 2). When murine mRNAs containing exons 1–12 or 2–12 are translated either in rabbit reticulocyte lysates or in cells, mRNA from the P2 promoter produces less p76Mdm2 than does RNA from the P1 promoter (28, 36). Thus, as transcription from the P2 promoter increases during the UV response, the ratio of p90Mdm2 to p76Mdm2 may rise. An increased ratio of p90Mdm2 to p76Mdm2 would be expected to promote recovery following

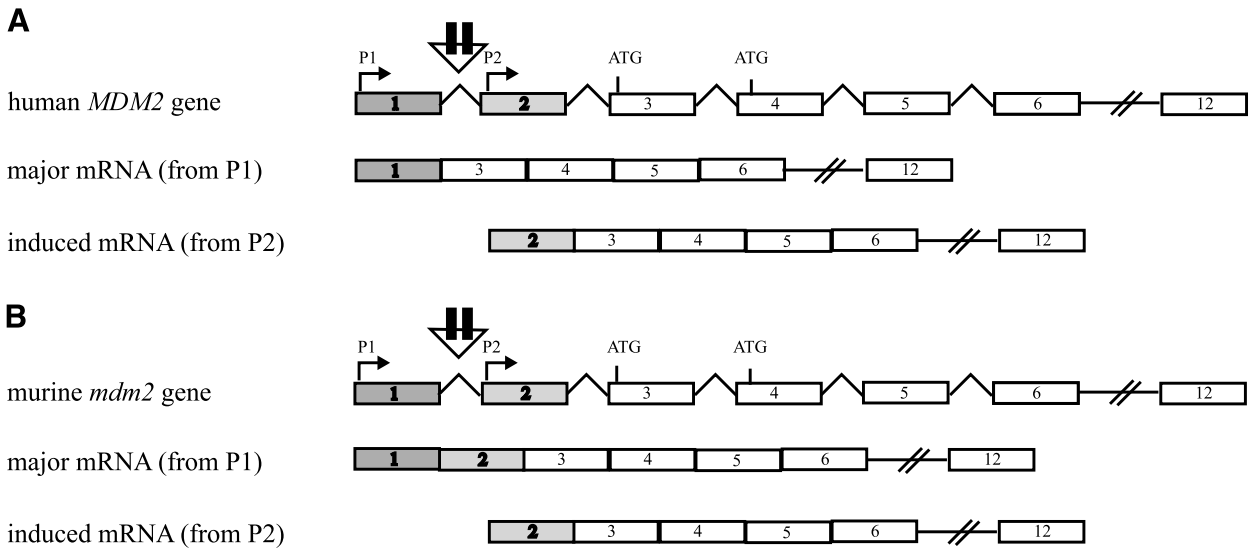

FIGURE 1. The *mdm2* gene. There are two p53 binding sites (*black boxes*) in intron 1 of the human and murine *mdm2* genes. Radiation stimulates the downstream P2 promoter but not the upstream P1 promoter. The first in-frame initiation codon is in *exon 3*. **A.** Human *MDM2* transcripts from the P1 promoter are spliced to lack exon 2. Those from the P2 promoter contain exons 2–12. **B.** Murine *mdm2* transcripts from the P1 promoter contain exons 1–12, whereas those from the P2 promoter contain exons 2–12.

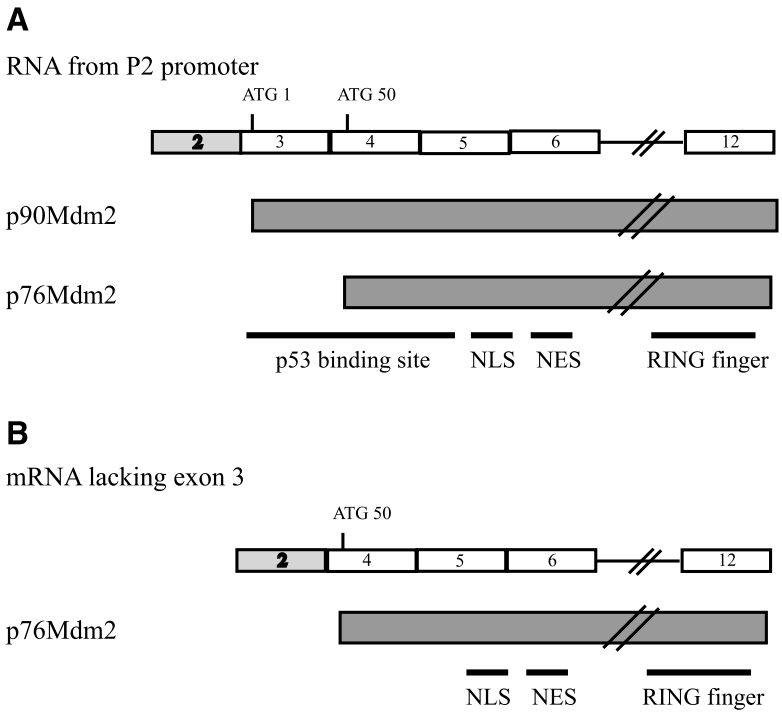

FIGURE 2. Two major Mdm2 proteins. **A.** Full-length mRNA from either the P1 or the P2 promoter can direct the synthesis of both p90Mdm2 and p76Mdm2. p90Mdm2 contains the entire p53 binding site and inhibits p53 function. p76Mdm2 has lost half of the site and cannot bind p53. Both proteins contain nuclear localization (*NLS*) and nuclear export signals (*NES*) as well as the RING finger domain responsible for Mdm2's ubiquitin ligase function. **B.** Alternatively spliced *mdm2* mRNA lacking exon 3 cannot direct the synthesis of p90Mdm2. However, p76Mdm2 can be translated because it initiates at an in-frame AUG codon in exon 4.

irradiation because p76Mdm2 acts as a dominant-negative inhibitor of p90Mdm2 and stabilizes p53 (36). Thus, p53's selective induction of the P2 promoter would maximize the chance that p53 would be neutralized by p90Mdm2 after *mdm2* expression is induced in response to radiation.

A second mechanism for expressing p76Mdm2 is through alternative splicing such that exon 3 is deleted (28). Such mRNAs cannot direct the synthesis of 90Mdm2 but can make p76Mdm2 because the start codon for p76Mdm2 is in exon 4 (Fig. 2; 28). Because the level of these alternatively spliced *mdm2* mRNAs rise in response to UV light (37), regulation of the splicing step may affect the activity of p53 in response to radiation. However, the mechanisms regulating the degree of splicing are unexplored. Both the alternative splice lacking exon 3 and p76Mdm2 have been detected in murine testes, indicating that they are *bona fide* products of the wild-type *mdm2* gene and therefore may be functionally relevant (36). However, as yet, there is no known role for p76Mdm2 in normal physiology or in the radiation response.

Although much is known about aberrant *MDM2* mRNAs expressed in human tumors (see review by Bartel *et al.* in this issue; 38, 39), a complete analysis of the wild-type human mRNAs and their protein products has not been carried out. One limitation of studies of MDM2 has been the lack of MDM2-deficient cell lines that would allow positive identification of MDM2 protein species introduced on mutant cDNAs. This limitation may now be overcome due to the advent of RNA interference protocols that can reduce expression of endogenous proteins to very low levels (40). Once the level of MDM2 protein is reduced to background levels, immunological characterization of exogenously expressed mutant proteins can establish criteria through which to identify those protein species expressed from various *MDM2* mRNAs.

The distinct functions of the mRNA and protein products of the *mdm2* gene may not be the reason for the existence of two *mdm2* promoters. Instead, it may be that intrinsic properties of the two promoters are important. As stated above, the upstream P1 promoter is not induced by p53 in response to DNA damage while the downstream P2 promoter is induced. The induction of the P2 promoter can occur even under extreme conditions in which expression from the P1 promoter is reduced due to DNA damage. For example, in fibroblasts from patients with a deficiency in repairing transcriptionally active genes [Cockayne syndrome (CS)], UV light causes a decrease in the amount of mRNA from the human P1 promoter (41). After a few hours, p53 induces expression from the P2 promoter although the level of RNA from the P1 promoter continues to decline (41). Thus, p53 appears to be able to stimulate transcription under conditions in which global transcription is inhibited. Indeed, such a function for p53 may be required for it to function as a stress response protein. It is not yet clear whether the ability of p53 to induce the P2 promoter under such conditions is a special characteristic of p53 or of the promoter. In sum, there may be many advantages for the irradiated cell to have two *mdm2* promoters under different regulatory controls.

Induction of Mdm2 in the Response to Ionizing Radiation

Ionizing radiation also induces *mdm2* expression in a p53-dependent manner (7). In murine tissues, all of the transcriptional induction of *mdm2* stimulated by whole-body ionizing radiation occurs through the P2 promoter (32). Indeed, a 400-bp fragment from the *mdm2* P2 promoter is sufficient to confer inducibility to a bacterial *β-galactosidase* transgene following whole-body irradiation of genetically engineered mice

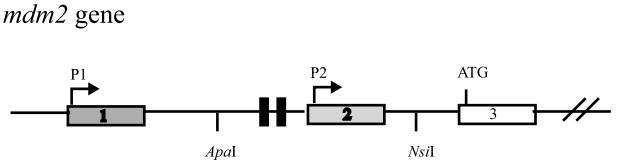

FIGURE 3. Control region for P2 promoter. The DNA sequences that permit the *mdm2* P2 promoter to be induced in response to radiation are contained within a 400-bp *Apa*I-*Nsi*I fragment surrounding the P2 promoter.

(Fig. 3; 42). In murine embryos carrying the *P2-β-galactosidase* transgene, induction is temporally and spatially regulated, becoming weaker and more tissue specific with age. For example, there is a posterior to anterior gradient of *β-galactosidase* expression down the caudal axis in embryos from dams exposed to whole-body ionizing radiation (42). The expression pattern becomes restricted over time in that embryos irradiated *in utero* at day 8.5 of embryogenesis (E8.5) show expression of β-galactosidase throughout the embryo, whereas those exposed at E12.5 show expression of β-galactosidase mainly in the extremities. Moreover, when irradiated at E8.5, embryos show expression throughout the developing neural tube, whereas those irradiated at E10.5 lack β-galactosidase expression in regions of the neural tube. Thus, the P2 promoter loses its responsiveness to radiation over developmental time.

The 400-bp *mdm2* P2 promoter region may lose its responsiveness because the level of p53 induced by radiation diminishes over developmental time (43). The magnitude of the induction of the *P2-β-galactosidase* gene is very sensitive to the level of p53. For example, heterozygous *P2-β-galactosidase* transgenic mice carrying one wild-type and one null allele of p53 show reduced induction of β-galactosidase following irradiation (42). Heterozygosity for p53 also diminishes the antiteratogenic effect of p53 following whole-body radiation. Fewer heterozygotes than wild-type embryos die in response to radiation *in utero*, whereas more heterozygotes suffer developmental defects of the hind limb and tail (44). It is intriguing that these same regions of the embryo show the greatest induction of the P2 promoter following whole-body irradiation of *P2-β-galactosidase* transgenic mice, leading to the interpretation that p53's transcriptional activation function is important for preventing these developmental defects (44). It is unlikely that the induction of *mdm2* acts to prevent the defects (44). Instead, the induction of *mdm2* may limit the number of damaged embryos killed by p53. It follows that the induction of *mdm2* expression must be highly regulated such that p53 is allowed to exert its antiteratogenic function without killing all irradiated embryos. However, a clear role for *mdm2* during post-implantation development is lacking due to the very early embryonic lethality of *mdm2*-null mice (1, 2).

The magnitude of the induction of *mdm2* in irradiated mice differs between tissues in both adults and embryos (32, 45). The induction of *mdm2* by p53 is much stronger in the spleen than in any other adult tissue (32). However, the P2 promoter of *mdm2* is induced 4–8-fold in all tissues tested. It is not known why the induction of *mdm2* is more robust in the spleen than in other tissues. One possibility is that p53 is particularly active in this tissue. In support of this notion, p53 does induce high levels of apoptosis in the spleen following whole-body ionizing irradiation (11, 46). Moreover, p53 induces some of its other transcriptional targets dramatically in the spleen. For example, both *p21* and *fas* are preferentially induced in spleen following whole-body ionizing irradiation (45). However, the proapoptotic *bax* gene is not induced to a high level (45). The selectivity in the magnitude of induction of p53 target genes implies that there are tissue-specific determinants of p53's ability to induce expression of subsets of genes. However, it is not yet known what factors determine the tissue-specific differences in the magnitude of *mdm2* induction following whole-body irradiation of either embryos or adult tissues.

There is evidence that the factors controlling the magnitude of induction of *MDM2* differ from those that control the magnitude of induction of other p53 target genes. For example, in a human tumor cell line, both *p21* and *gadd45* show clear dependency on the dose rate of ionizing radiation, whereas *MDM2* does not (47). While all three genes are induced by as little as 50 cGy of ionizing radiation, *MDM2* is not influenced by the rate at which the radiation is applied. Both *p21* and *gadd45* are induced to a greater extent when 50 cGy are delivered at higher dose rates. However, *MDM2* is induced to the same extent by 50 cGy delivered over 1.8 min or 3 h. Dose rate also affects the rate of apoptosis but not the degree to which the cells arrest in the cell cycle (47). Thus, the dose rate may play a role in determining the fate of cells following irradiation. In support of this idea, Kato *et al.* (48) found that the dose rate affected the frequency of developmental defects in wild-type murine embryos irradiated with 2 Gy *in utero*.

Experimentally, the magnitude of the induction of *mdm2* can be masked by techniques that do not differentiate between products from the two *mdm2* promoters (35). Because in some tissues the *mdm2* mRNA from the P1 promoter is 10 times more abundant than mRNA from the P2 promoter prior to induction, a 10-fold induction of the P2 promoter would appear to be only a 2-fold increase in *mdm2* expression if total *mdm2* mRNA were measured. In contrast, techniques that differentiate the products from the two *mdm2* promoters allow the magnitude of the induction to be measured. However, such techniques may exclude some alternatively spliced species of mRNAs because only a short region of the 5′ end of the mRNA is measured. Neither type of technique is ideal, and the choice of technique should depend on the question being asked. For example, if we were interested in how much Mdm2 is made, we should measure total mRNA or even Mdm2 protein. If we were interested in p53 activity, we should focus on the P2 promoter. It should be noted that techniques such as *in situ* hybridization and microarray analysis cannot distinguish the *mdm2* products from the P1 and P2 promoters because mRNA from the P2 promoter does not contain any sequence distinct from the P1 product (Fig. 1).

However, in *MDM2*, the product of the P2 promoter contains exon 2, whereas the product of the P1 promoter does not. Hence, Northern blots, *in situ* hybridization, and microarrays, with probes specific for exon 2 sequences, can be used to measure the induction of *MDM2* by p53.

Role of Mdm2 in Regulating p53 in the Absence of Exogenous DNA Damaging Agents

The observations that p53 stimulates *mdm2* expression while Mdm2 inhibits p53 led to the notion of the p53/Mdm2 autoregulatory feedback loop (5). In cultured cells, p53 stimulates *mdm2* expression constitutively and Mdm2 feeds back to inhibit p53 (49, 50). Thus, the p53/Mdm2 autoregulatory loop is constitutively active in cultured cells. However, it is not constitutively active in embryonic or adult tissues because p53 does not stimulate *mdm2* transcription in tissues unless they have been exposed to a stressor such as ionizing radiation (32, 42, 51). In itself, this observation is not surprising because the transcriptional activation function of p53 is undetectable in unperturbed homeostatic tissues. For example, there is no detectable influence of p53 gene status on the level of expression of *p21* or of several transgenes designed to report p53's transcriptional activation function in mice (32, 42, 46, 52). In contrast, when mice are subjected to whole-body ionizing irradiation, *mdm2*, *p21*, and the reporter transgenes are induced in a p53-dependent manner (32, 42, 43, 46). Together, these results indicate that a stressor is required for p53 to induce its target genes *in vivo*. Thus, the p53/Mdm2 autoregulatory loop is a special regulatory mechanism that does not operate in unstressed, homeostatic tissues.

It is not clear whether the p53/Mdm2 autoregulatory loop operates during early embryogenesis. It seems clear that Mdm2 must regulate p53 at some point in development because *mdm2*-null mice die unless p53 is also deleted (1, 2). However, an alternative explanation is that loss of Mdm2 results in a condition of stress that results in p53 activation. Likewise, there is no definitive evidence that p53 regulates *mdm2* expression during embryogenesis. Several studies show the same level of expression of p53-responsive genes in wild-type and p53-null embryos (42, 43, 51, 52), suggesting that p53 is not active as a transcription factor during embryogenesis. However, all of these studies were performed on embryos at a developmental stage later than that at which *mdm2*-null embryos die. Thus, an analysis of p53's transcriptional activation function in very early embryos, prior to E6.5, would be informative.

Although p53 does not regulate basal *mdm2* expression in any tissue tested to date, Mdm2 does regulate p53 function constitutively in all tissues tested (3). In genetically engineered mice in which the level of Mdm2 is reduced 3-fold from wild-type levels, the transcriptional activation function of p53 is readily detected in all tissues as a p53-dependent increase in the steady-state level of *p21* expression (3). A subset of tissues undergoes spontaneous, p53-dependent apoptosis in these mice. Thus, Mdm2 regulates p53 under conditions in which p53 does not regulate *mdm2* expression (Fig. 4). Because p53 does not stimulate *mdm2* expression constitutively in unstressed tissues, other factors must be critical for establishing the level and activity of Mdm2. None of these factors has yet been identified.

Role of Mdm2 Levels in Regulating p53 in the Response to UV Light

The extent of apoptosis in epidermal cells must be carefully controlled because limited apoptosis suppresses tumors, whereas widespread apoptosis causes sloughing of skin. p53 induces apoptosis in murine skin exposed to UVB light (53) and mice lacking p53 are prone to developing squamous cell carcinoma following exposure to UV light (54, 55). There is direct evidence that the ability of p53 to promote apoptosis prevents tumorigenesis in a genetically engineered line of mice expressing a fragment of the SV40 large T antigen (56). Moreover, there is evidence that the apoptotic function of p53 is critical for preventing human skin cancer. The level of p53 protein is elevated in human skin exposed to UV light (57); particularly, high levels of p53 are found in the apoptotic "sunburn" cells (53). The p53 gene is often mutated in human squamous cell carcinomas (58), suggesting that p53's apoptotic function prevents skin cancer in people.

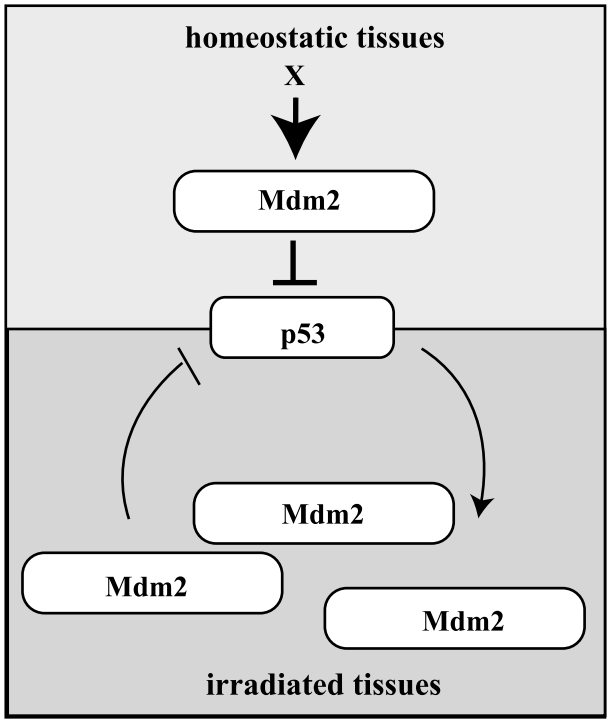

FIGURE 4. Regulatory interactions between Mdm2 and p53. In unstressed, homeostatic tissues, unidentified factors (*X*) determine Mdm2's level and activity. In the response to radiation, p53 induces *mdm2* expression, activating the p53/Mdm2 autoregulatory loop.

There is strong evidence that the level of Mdm2 is critical for regulating p53 during the response to UV light. Although it was known for some time that Mdm2 could block the ability of p53 to cause either cell cycle arrest or apoptosis in cultured cells (7, 15), more recently, it was demonstrated that Mdm2 inhibits p53's apoptotic function in UV-irradiated murine skin (59). Transgenic mice carrying a *mdm2* cDNA under the control of the epithelial-specific human keratin 14 promoter express 5–10-fold more Mdm2 protein in their basal skin cells than do their wild-type counterparts. When exposed to UVB light, 5–10-fold fewer skin cells undergo apoptosis in these *mdm2* transgenic mice than in wild-type mice (59). Moreover, the levels of induction of the p53-responsive *p21* gene are reduced in the *mdm2* transgenic mice (59). Thus, the basal level of Mdm2 can affect the magnitude of p53's apoptotic response to UV light.

The induction of *mdm2* expression by UV light also appears to be critical for regulating p53 during the UV response. For example, Conforti *et al.* (60) demonstrated that the induction of *MDM2* expression in UVC-irradiated human fibroblasts correlates with the return of low levels of p53 and prevention of widespread apoptosis. Fibroblasts deficient in certain nucleotide excision repair enzymes failed to induce *MDM2* and underwent rapid and extensive apoptosis, whereas normal fibroblasts induced *MDM2* and were less sensitive to the killing effects of UVC light (60). Thus, the induction of *MDM2*, in addition to its basal level, appears to be a critical determinant of p53's function during the UV response.

The ability of cultured human fibroblasts to induce *MDM2* correlates with their ability to repair photolesions on transcribed genes (60). Some DNA repair pathways, including the nucleotide excision repair pathway, can be stimulated to repair actively transcribed genes more rapidly than the rest of the genome. This ability is lost in cells from patients with CS (61), whereas the ability to repair UV-induced lesions in all regions of the genome is lost in patients with xeroderma pigmentosum (XP) subgroup A (XPA; 62). In contrast, XPC patients retain the ability to repair transcriptionally active genes but have lost the ability to repair the rest of the genome (62). Conforti *et al.* (60) showed that the induction of *MDM2* is inhibited in cells from patients with either CS or XPA but is normal in patients with XPC. Because both CS and XPA cells are prone to undergo p53-mediated apoptosis in response to UV exposure, these results support a model in which the induction of *MDM2* is critical for survival.

A subsequent study showed that both of the *MDM2* promoters are necessary to regulate the accumulation of MDM2 protein during the UV response. Michalowski *et al.* (41) confirmed that the induction of *MDM2* is reduced in both XPA and CS fibroblasts and is normal in XPC cells. However, this study used a lower dose of UVC light that allowed some induction of the P2 promoter of *MDM2* in CS fibroblasts but not in XPA cells (41). In these CS cells, transcription from the P2 promoter was induced while transcription from the P1 promoter was inhibited. Thus, p53 can induce the P2 promoter under conditions in which DNA damage interferes with transcription from the P1 promoter. This ability of p53 may ensure that MDM2 levels can increase in response to DNA damage that inhibits gene expression. Although the P2 promoter is induced in CS cells, the net increase in p90MDM2 protein remains lower in CS cells than in cells capable of repairing transcribed genes (41). These results indicate that while induction of the P2 promoter is critical for raising the level of MDM2, expression from the P1 promoter must be maintained for MDM2 to reach levels optimal for preventing cell death.

Like the repair-deficient human fibroblasts, epidermal cells of genetically engineered mice lacking the ability to repair transcriptionally active genes show a defect in *mdm2* induction following UV exposure and an increase in p53-mediated apoptosis (63). In this more physiologically relevant system, Brash *et al.* (63) found that low, suberythemal doses of UVB light induce Mdm2 protein expression in wild-type skin, whereas higher doses suppress Mdm2 below its normal level. Mice proficient for repairing transcriptionally active genes but selectively deficient in repairing other DNA lesions suppress Mdm2 expression at the same doses as do wild-type mice. However, mice unable to repair transcriptionally active genes suppress Mdm2 expression at lower doses than wild-type mice, confirming the notion that damage in transcriptionally active genes limits the induction of *mdm2*. Previously, Ljungman and Zhang (64) had proposed that inhibition of transcription triggers a signaling pathway leading to p53-mediated apoptosis. Brash *et al.* (63) modified this model slightly by suggesting that the transcriptional blocks induced by UV light lead to reduced expression of Mdm2, stabilization of p53, and induction of apoptosis. In this model, the apoptotic trigger could be the reduced level of Mdm2.

Although all of these studies demonstrate a correlation between induction of *mdm2* expression and prevention of apoptosis, none demonstrates conclusively that the induction of *mdm2* is required to control p53 during the UV response. The most insightful experiment may be one in which mice are genetically engineered to lack the P2 promoter and therefore the ability to increase Mdm2 levels following UV exposure. One would predict that if induction of the P2 promoter were critical for controlling p53 during the UV response, mice lacking the P2 promoter would stabilize p53 and undergo apoptosis at low doses of UV light as do repair-deficient mice. Of course, because *mdm2* is required for embryonic development, such mice may not be viable (1, 2). Thus, it would be best to generate a conditional allele of *mdm2* that allows the P2 promoter region to be excised in a time- and tissue-specific manner (65). A caveat to this experiment is that UV light may regulate the level of p90Mdm2 independently of transcription. For example, *mdm2* mRNA can be stabilized by UV light (25) and the level of MDM2 protein rises slightly even before the increase in *MDM2* mRNA in fibroblasts (41).

Role of Mdm2 Levels in Regulating p53 in the Response to Ionizing Radiation

Overexpression of Mdm2 can inhibit the ability of p53 to mediate cell cycle arrest in the G_1 phase of the cell cycle in response to ionizing radiation (7). Recently, it has been shown that mice expressing reduced levels of Mdm2 are very sensitive to whole-body ionizing radiation. For example, mice with only a 20% reduction in the level of Mdm2 all die when exposed to the same dose of ionizing radiation that allows 50% of wild-type mice to survive (3). The radiosensitivity of mice expressing decreased levels of Mdm2 is due to p53 (3). Thus, Mdm2 is a critical regulator of p53 in the response to ionizing radiation.

p53 induces apoptosis in a subset of tissues following whole-body ionizing irradiation, including the spleen, thymus, and small intestine (11, 43). Intriguingly, these same tissues undergo spontaneous p53-dependent apoptosis in mice expressing low levels of *mdm2* (3). In contrast, tissues such as the liver and kidney, which do not undergo p53-mediated apoptosis in irradiated mice, do not demonstrate an increase in spontaneous apoptosis when *mdm2* levels are reduced. They do, however, demonstrate an increase in p53 activity as measured by an increase in *p21* expression (3). It is not yet known whether p53 functions differently in those tissues that undergo apoptosis or whether downstream factors determine whether the cells

undergo apoptosis. Identification of the tissue-specific determinants of the propensity to undergo p53-mediated apoptosis may help identify those human tumors in which blocking the Mdm2/p53 interaction could increase susceptibility to radiotherapy.

In a subset of tumors expressing wild-type *p53*, the *MDM2* gene is amplified (for a review, see Ref. 66). The resulting high levels of MDM2 are thought to inhibit p53's function, allowing for tumorigenesis (67). Moreover, the high levels of MDM2 in these tumors may decrease the response to radiotherapy by inhibiting p53-mediated apoptosis. Indeed, antisense oligonucleotides against *MDM2* decrease *MDM2* expression and sensitize human tumor cells to radiation (68). It is hoped that drugs that block MDM2 function or expression will be useful cancer therapeutics (reviewed in Ref. 69). However, it should be noted that recent work indicates that the radiosensitivity of some tumors is mediated by the endothelial cells within the tumor (70). Thus, the genetic status of *MDM2* in the tumor cells proper may not be a reliable predictor of radioresistance. Obviously, much work remains to be done to answer the critical question of how to manage tumor radioresistance.

Factors That Regulate the Interaction Between p53 and Mdm2 During the Response to Radiation

The inhibitory effects of Mdm2 on p53 appear to require binding between the two proteins (23); thus, reducing the interaction between them may be a major strategy for stabilizing and activating p53 in the response to radiation. Reducing this interaction is clearly a strategy for developing drugs that reactivate p53 in tumors overexpressing Mdm2 (69). The interaction between Mdm2 and p53 can be inhibited via covalent modifications of either protein (for reviews, see Refs. 71–73). One of the most attractive arguments for the importance of covalent modifications of Mdm2 and p53 in the radiation response is the finding that Mdm2 and p53 are phosphorylated *in vitro* by kinases that establish cell cycle checkpoints and/or stimulate DNA repair (74–77). p53 is critical for some radiation checkpoints (10, 78), making this link satisfying intellectually. However, it is not clear whether these kinases must phosphorylate either p53 or Mdm2 to exert their checkpoint functions.

Insights into the mechanisms regulating the response of p53 to ionizing radiation have been obtained from experiments in genetically engineered mice. The cell cycle arrest, apoptotic, and tumor suppressor functions of p53 are readily detectable in mice (11, 79, 80). Because it is not clear that the transcriptional activation function of p53 is required for apoptosis (81–83), these physiological end points appear to be the most informative. It should be noted that different cell types respond to radiation differently even *in vivo*. For example, lymphocytes and epithelial cells of the small intestine rapidly undergo apoptosis following whole-body ionizing irradiation, whereas hepatocytes and osteocytes do not (11). Thus, cell type is another important consideration for any experiment designed to measure p53's response to radiation. Here, the discussion will be focused on what has been learned from T cells because these have been shown to undergo both cell cycle arrest and apoptosis mediated by p53 in response to whole-body ionizing radiation.

The ionizing radiation-induced cell cycle arrest and apoptotic responses of p53 are inhibited in some mice with mutations in the phosphoinositol 3-kinase (PI3K) family (reviewed in Refs. 84, 85). This family includes the DNA-dependent protein kinase (DNA-PK), the protein mutated in the human radiosensitivity disorder ataxia telangiectasia (ATM), and the ATM and rad 3-related protein kinase (ATR). Each of these kinases has been shown to phosphorylate p53 *in vitro* at sites critical for the interaction with Mdm2 (74–77). In addition, both DNA-PK and ATM have been shown to phosphorylate Mdm2 (86, 87). All three kinases respond to double-strand breaks such as those that occur in response to ionizing radiation (84). In addition, ATR responds to damage induced by UV light (75). These kinases appear to regulate p53 in the responses to radiation.

One of the first kinases proposed to play a critical role in activating p53 in response to radiation was DNA-PK (74). DNA-PK phosphorylates p53 on Ser^{15} and Ser^{37}. In some reports, mutation of these two amino acids to alanines blocks the ability of DNA-PK to enhance Mdm2's ability to inhibit p53's transcriptional activation function (74). Because DNA-PK is activated by DNA ends (88), these data suggested that DNA-PK might be part of a signaling cascade that activates p53 in response to ionizing radiation.

In support of this notion, Wang *et al.* (89) demonstrated that thymi from mice carrying a null allele of the DNA-PK catalytic subunit (DNA-PKcs) were deficient in p53-mediated apoptosis following whole-body ionizing irradiation (89). In contrast, the percentage of T cells in S phase was diminished in both wild-type and DNA-PKcs-null mice following whole-body ionizing irradiation (89). These data were interpreted to indicate that DNA-PK mediates the apoptotic function of p53, whereas another kinase could be necessary for the cell cycle arrest function. However, a different null mutation of DNA-PKcs resulted in a proportion of radiation-induced apoptotic thymic cells greater than that in wild-type mice (90), indicating that DNA-PK was not required for p53-mediated apoptosis. The apparent discrepancy between the two studies could be explained in several ways. For example, DNA-PK deficiency can result in a decrease in T cells (91), the thymic cell type known to undergo p53-mediated apoptosis in response to whole-body radiation (92, 93). Thus, the percentage of thymic cells that are T cells would be expected to influence the percentage of apoptotic thymic cells. The mice in the first study had 50–100-fold fewer T cells than wild-type mice (91), whereas it is not known how many fewer T cells the mice used in the second study had. It would be informative to isolate T cells from each mouse line before and after whole-body ionizing radiation and compare the percentages of apoptotic T cells. To date, it is not clear whether DNA-PK regulates the function of p53 following whole-body radiation.

There is strong evidence that the ATM kinase is critical for regulating p53 in the damage response (94). ATM-deficient T cells do not undergo cell cycle arrest in response to whole-body ionizing radiation as do wild-type T cells (80, 89), indicating that ATM is important for mediating this p53-dependent checkpoint. In addition, there is evidence that ATM may contribute to the induction of apoptosis by p53. In one study, the radiation-induced apoptotic response of *atm*-null

T cells was reduced (95). However, in another study, there was no difference in the percentage of apoptotic cells in wild-type and *atm*-null thymi or isolated T cells (80). Thus, the role of ATM in stimulating p53-dependent apoptosis remains tenuous. It is interesting to note that *atm*-null mice are more sensitive to the lethal effects of ionizing irradiation than are wild-type mice. Furthermore, p53 does not contribute to the radiosensitivity of *atm*-null mice (96). Thus, some of the important biological functions of ATM are not mediated through p53 or, presumably, Mdm2.

The role of ATR in regulating T-cell apoptosis and cell cycle arrest has not been established due to the early embryonic death of ATR-null mice (97). However, several cell culture experiments have established the concept that ATR regulates cell cycle checkpoints in response to both ionizing and UV radiation (78, 84). In response to ionizing radiation, ATR appears to play a complementary role to ATM in preventing mitotic entry (78). ATM is activated early on, whereas ATR is activated later in the time course (84). In the UV response, ATR appears to be the PI3K most critical for setting checkpoints (84). Like ATM, ATR can phosphorylate p53 on Ser^{15} (75, 78) and ATR is required for maximal phosphorylation of p53 in the response to UV radiation (75). However, the importance of this modification is controversial (for a recent review, see Ref. 73) as is the role of ATR in regulating p53 functions in the radiation response. For example, while a dominant-negative form of ATR inhibits phosphorylation of p53 Ser^{15} in response to UV light (75), it does not inhibit either the accumulation of p53 or the G_1 arrest in UV-treated cells (98). Thus, the importance of ATR-mediated phosphorylation of p53 in the response to either ionizing or UV radiation is not yet clear.

Several kinases appear to regulate Mdm2 because it is highly phosphorylated in cultured cells and the pattern of phosphorylation changes on irradiation (99). Several serines in the center of Mdm2 become hypophosphorylated, and this correlates with a decrease in the ability of Mdm2 to stimulate p53 degradation without affecting its ability to ubiquitinate p53 (99). Both DNA-PK and ATM are candidates for important MDM2 kinases because both phosphorylate MDM2 *in vitro* (86, 87). DNA-PK phosphorylates MDM2 at Ser^{17}, which is in the region that binds p53, and this modification reduces the amount of p53 that can bind MDM2 (86). ATM phosphorylates Mdm2 on Ser^{395} and this modification decreases the ability of Mdm2 to stimulate p53 degradation without compromising the ability of the two proteins to bind (100). The ability of MDM2 to stimulate p53 degradation is also inhibited through phosphorylation of an adjacent amino acid, Tyr^{394}, by the c-Abl tyrosine kinase (101). Moreover, substitution of Tyr^{394} with phenylalanine enhances the ability of Mdm2 to inhibit both transcriptional activation and apoptotic functions of p53 (101). It is intriguing that c-Abl is phosphorylated and activated by ATM during the response to ionizing radiation (102). Perhaps the redundancy and cross-talk between the different kinase cascades ensure that p53 is rapidly stabilized and activated in response to damage.

There is also evidence that PI3K can indirectly activate Mdm2's antiapoptotic functions by stimulating the activity of a kinase known as protein kinase B or Akt (reviewed in Ref. 103). Akt phosphorylates Mdm2 and promotes its translocation to the nucleus where it can inhibit p53 (104). Thus, PI3K enhances the survival function of Mdm2. A feedback loop ensures that p53-mediated apoptosis is not eliminated by PI3K activity. p53 stimulates cleavage of Akt in apoptotis-prone cells, suggesting that p53 can decrease Mdm2's antiapoptotic function by down-regulating Akt under appropriate circumstances (105). The cross-regulation between p53 and Mdm2 in the damage response is complex and only beginning to be understood.

One reason for the multitude of kinases that can modify p53 and Mdm2 is that different populations of p53 protein are regulated differently. For example, Woo *et al.* (106) found that DNA-PK is required to activate the apoptotic function of the population of p53 that exists prior to irradiation but not to activate p53 that is synthesized following irradiation. In addition, Buschmann *et al.* (107) found that subsets of p53 proteins associate with different regulatory proteins depending on the stage of the cell cycle. The majority of p53 associates with Mdm2, p300, and the c-Jun NH_2-terminal kinase (JNK) throughout the cell cycle, whereas the subset of p53 bound to DNA associates with JNK during the G_0 and G_1 phases but with Mdm2 and p300 during S and G_2-M phases. It is thought that JNK controls the stability of p53 during the G_0 and G_1 phases of the cell cycle, whereas Mdm2 controls p53 during S and G_2-M phases (108). Thus, both cell type and cell cycle stage influence the pathways that regulate p53.

Although several covalent modifications have been proposed as critical regulators of the p53/Mdm2 interaction, it should be noted that there is some evidence that the interaction between Mdm2 and p53 does not have to be diminished for p53 to accumulate during the UV response (27). Moreover, ubiquitination of p53 by Mdm2 is not sufficient to catalyze its degradation (99, 100). A recent report demonstrates that turnover of p53 is dependent not only on p90Mdm2 but also on the p300 protein (109). p300 adds multimers of ubiquitin to p53 molecules that are monoubiquinated by Mdm2. Only the polyubiquitinated p53 protein molecules are susceptible to proteolysis. Thus, factors that disrupt the function of p300 may contribute greatly to the accumulation of p53 in response to ionizing radiation and UV light. Investigation of this regulatory step is only beginning.

Concluding Remarks

Mdm2 clearly promotes radioresistance in mice (3) and is overexpressed in a subset of human tumors (31, 66, 67). However, the ability of MDM2 to confer radioresistance to tumors is only beginning to be assessed. For example, antisense oligonucleotides that decrease *MDM2* expression radiosensitize human soft tissue sarcoma cells in culture, suggesting that MDM2 may be a useful target for adjuvant cancer therapy (68). Now, it is important to determine whether increased levels of MDM2 correlate with poor clinical outcome and whether MDM2 can be targeted to increase the efficacy of radiotherapy.

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