

Subject Review**MDM2 and Its Splice Variant Messenger RNAs: Expression in Tumors and Down-Regulation Using Antisense Oligonucleotides**Frank Bartel,¹ Linda C. Harris,² Peter Würfl,³ and Helge Taubert¹¹Institute of Pathology, Faculty of Medicine, University of Halle-Wittenberg, Halle/Saale, Germany; ²Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis TN; and ³Surgical Clinic I, Faculty of Medicine, University of Ulm, Ulm, Germany**Abstract**

Alternative splicing has an important role in expanding protein diversity. An example of a gene with more than one transcript is the *MDM2* oncogene. To date, more than 40 different splice variants have been isolated from both tumor and normal tissues. Here, we review what is known about the alteration of *MDM2* mRNA expression, focusing on alternative splicing and potential functions of different *MDM2* isoforms. We also discuss the progress that has been made in the development of antisense oligonucleotides targeted to *MDM2* for use as a potential cancer therapy.

Introduction

*MDM2*¹ appears to play a role in many normal physiological and pathological pathways. It has been shown that *MDM2* not only acts as an oncogene (1–3) but also displays growth-inhibitory functions (4, 5). *MDM2* can also function independently of p53; for example, *MDM2* interacts with transcription factors of the E2F family and the human TATA binding protein (6, 7) and inhibits retinoblastoma growth-regulatory function (8). A question remains whether the same *MDM2* protein is responsible for the diverse functions reported or whether different isoforms or post-translationally modified *MDM2* proteins could be involved. We examine here the different *MDM2* variants that are likely translated from alternatively spliced *MDM2* pre-mRNAs and how antisense oligonucleotide (AS-ODN) therapies might be useful for down-regulating the expression of both full-length and alternatively spliced variants of *MDM2*. Down-regulation of full-length *MDM2* expression in tumors that overexpress *MDM2* would be predicted to result in p53 protein stability and sensitization to DNA-damaging agents (chemotherapeutic drugs and radiation) that act via

p53-dependent pathways (Fig. 1). In addition, p53-independent functions of *MDM2*, such as in regulating the cell cycle, would also be abrogated, thereby restoring growth control in the tumor (Fig. 1). Progress has been made in the *in vitro* and *in vivo* uses of *MDM2* antisense to down-regulate *MDM2* expression.

Splicing of the *MDM2* Messenger RNA

Three human *MDM2* mRNA transcripts of 6.7, 4.7, and 1.9 kb long were reported by Pinkas *et al.* (9) in breast carcinoma cells, with the 1.9-kb mRNA having lost exon 12. In addition, several truncated *MDM2* isoforms of 85, 76, and 57 kDa have been described in a panel of human breast carcinomas, together with the full-length 90-kDa protein (10). During the last few years, detailed expression analyses of the *MDM2* mRNA in various cancer types and in normal tissue have revealed alternative as well as aberrant splicing (Fig. 2; reviewed in Ref. 11). The tumor types investigated to date include ovarian and bladder cancer (12), glioblastomas (13), glioblastoma cell lines (14), breast carcinomas (15, 16), soft tissue sarcomas (17–19), giant cell tumors of the bone (20), and Hodgkin's lymphoma (21). The majority of the greater than 40 splice variants that have been detected to date lack sequences that encode at least part of the p53 binding domain, the nuclear localization and export sequences, the p300 binding domain, and the acidic domain. *In vitro* expression studies confirmed that the protein isoforms encoded by at least four of these splice variants (*MDM2*-A, -B, -C, and -D; 12) are unable to bind p53. The splice form *MDM2*-B, which is the most frequently detected transcript variant, has been described in various tumor types as well as in normal tissue, whereas many of the other variants have only been found in one particular cancer type.

A problem that has developed, however, is that some investigators have chosen to give different names to previously published isoforms, although most of the sequences of published splice variants are in Genbank. For example, the recently detected splice form *MDM2*-HD1 (accession no. AJ5505169) corresponds to the PM2 form (accession no. AJ278977), and another splice variant, *MDM2*-Del.G (15), which lacks the sequence between nucleotides 182 and 1432 of the coding region of the *MDM2* mRNA, exactly corresponds to the 219bp form

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

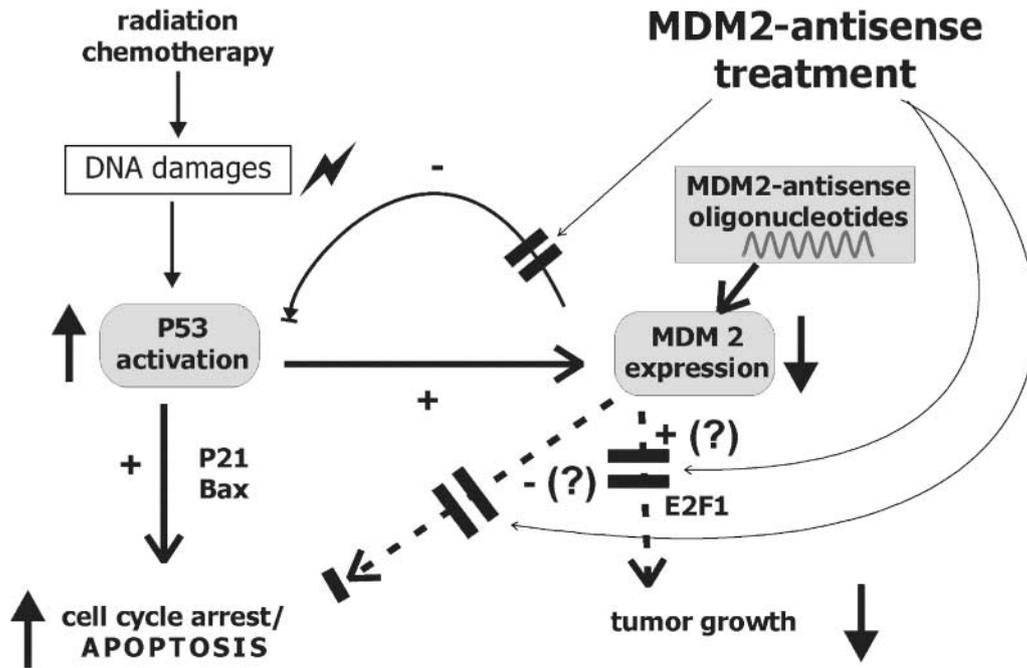


FIGURE 1. Proposed mechanism of action of MDM2-AS-ODNs (modified from Ref. 58). MDM2 and p53 form an autoregulatory feedback loop in which the MDM2 expression is up-regulated by p53 and MDM2 binds to p53, thereby promoting its degradation. In many tumors, however, MDM2 is overexpressed and can limit the effect of p53 activation by DNA-damaging drugs or radiation. By inhibiting the expression of MDM2 by targeting MDM2 mRNA with AS-ODNs, p53 should be activated to induce cell cycle arrest and/or apoptosis. In addition, it is predicted that MDM2-AS-ODN treatment will also inhibit p53-independent functions of MDM2.

described by Lukas *et al.* (16). This makes it difficult to determine the frequency of occurrence of specific isoforms and to compare the variants expressed within different tumor types.

A large proportion of the known *MDM2* transcripts is aberrantly spliced (*e.g.*, MDM2-PM2, -EU2, -KB3, and -219bp). These examples have a common splicing pattern that is illustrated in Fig. 3. Splicing occurs at cryptic splice donor and acceptor sites in regions with high sequence homology and that are present as many as four times within the coding region of the *MDM2* mRNA. For example, a 9-bp repeat sequence (AAGAGACCC in exon 3 and AAGAAACCC of exon 12) is involved in the splicing of the EU2 variant (a splice variant detected in soft tissue sarcomas; 17). Similar findings have been described by Hong and Li (22) for the retinitis pigmentosa GTPase regulator. Here, various portions of a purine-rich region were removed as introns. When analyzing the purine-rich sequences within the *RPGR* gene, several exonic splice enhancers that promote splicing through interaction with splicing factors were found, and Western blot analysis revealed many different sizes of RPGR proteins (22). Similar observations have also been made when MDM2 protein expression is evaluated in primary tumor samples (Bartel, unpublished observations) and in several normal tissues (23).

Given the fact that alternative splicing of the *Drosophila* Dscam pre-mRNA gives rise to over 38,000 transcripts (24), the splicing pattern of *MDM2* mRNA is still somewhat concise. However, the question remains as to why there are so many splice variants. We have described a mechanism by which a diverse collection of mRNAs can be generated from a single gene (Fig. 3), but it is unknown how many of the more than

40 splice variants described for *MDM2* are real and functionally relevant. It has been shown that many of the documented splicing errors can be caused by mutations in the genomic DNA, thereby creating new or destroying normal splice sites (25). Furthermore, mutations within binding sites of splicing regulatory proteins can cause “missplicing” (26, 27). However, whether this is also true for *MDM2* is not known. There are only a few reports describing analysis of mutations within the *MDM2* cDNA, but it is unknown whether these contribute to the diversity of the *MDM2* mRNA transcripts. Point mutations have been described in the zinc finger-encoding region of *MDM2* cDNA isolated from non-Hodgkin’s lymphomas, leukemias, and hepatocellular carcinomas (28) and in other domains in liposarcomas (19). However, the mutation frequency of the *MDM2* gene in general is rather low, as other reports detected no mutations in the *MDM2* cDNA isolated from other tumor types (29–31).

The fact that some splice variants have been detected only in particular tumor types suggests that they might contribute to the transformed phenotype of these tumors, whereas others (*e.g.*, *MDM2-A*) may be associated with tumorigenesis in general. However, it is important to note that a considerable number of *MDM2* splice variants have been detected in normal tissues (23), demonstrating that MDM2 isoforms do not always possess oncogenic properties.

Functions of MDM2 Isoforms

There are several variants that share great structural homology (Fig. 2), suggesting that they may perform similar cellular functions. In addition, because multiple isoforms with

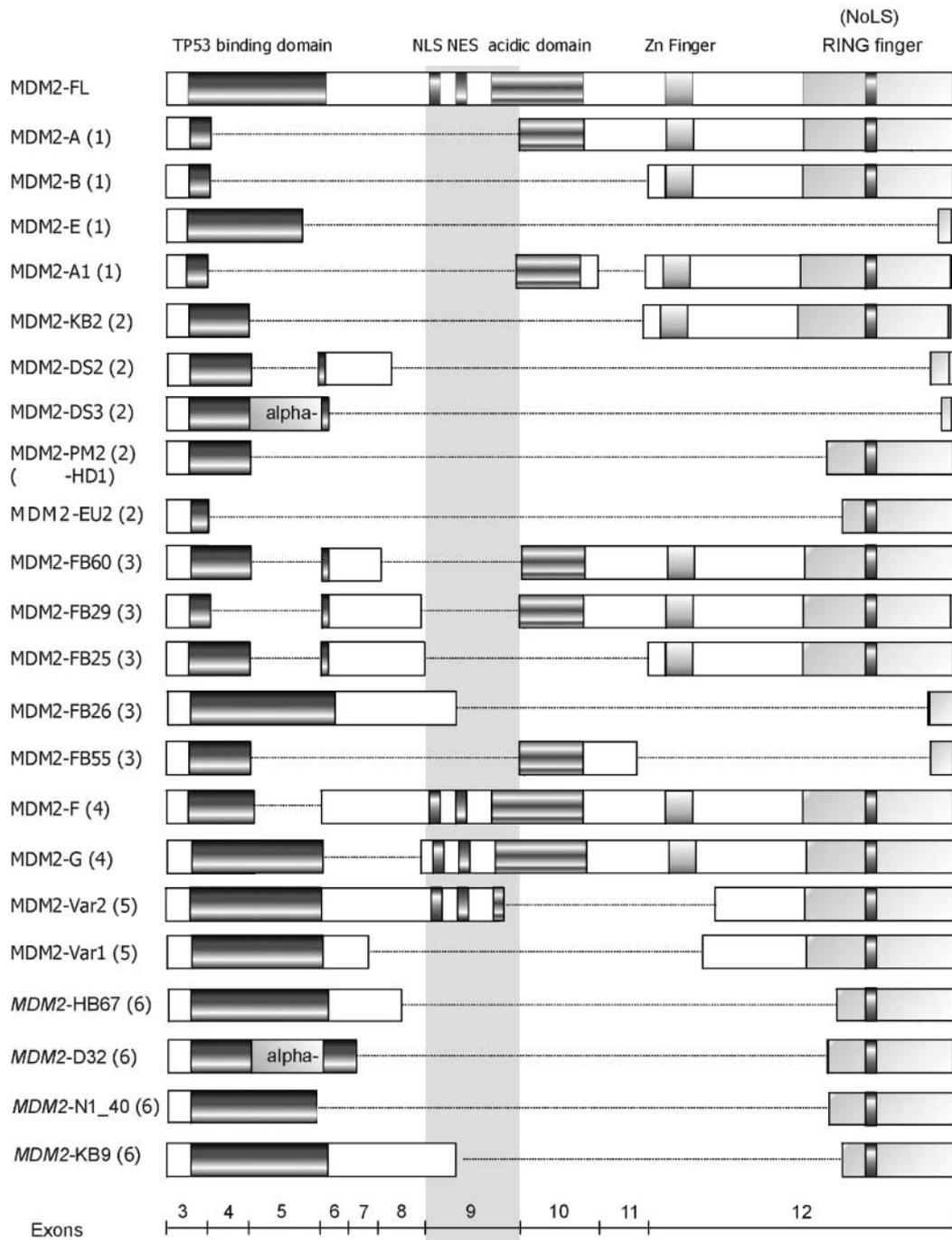


FIGURE 2. Summary of the most frequently expressed MDM2 splice variants and the domains that they encode. The sequence that is spliced out in most of the variants is highlighted with a *light gray box*. Numbers in parentheses, (1) Sigalas *et al.* (12), (2) Bartel *et al.* (17), (3) Bartel *et al.* (18), (4) Tamborini *et al.* (19), (5) Schlott *et al.* (59), and (6) Bartel *et al.* (unpublished observations).

similar sequences are often expressed within the same tumor (11), some isoforms are functionally redundant. Many examples of alternative splicing result in the region encoding the COOH terminus to be out of frame. This frame shift leads to the generation of novel amino acid sequences; however, because these new sequences differ in every case and are very short (only eight or nine amino acids), they are unlikely to contribute

to a common novel function. However, it is not without precedent that these unique sequences could contribute to a gain of function as has been shown for MDMX-S, an isoform of the MDMX protein (32, 33). The MDMX-S isoform is characterized by a short unique amino acid sequence, which increases 12-fold the affinity of MDMX-S binding to p53 compared with MDMX (33).

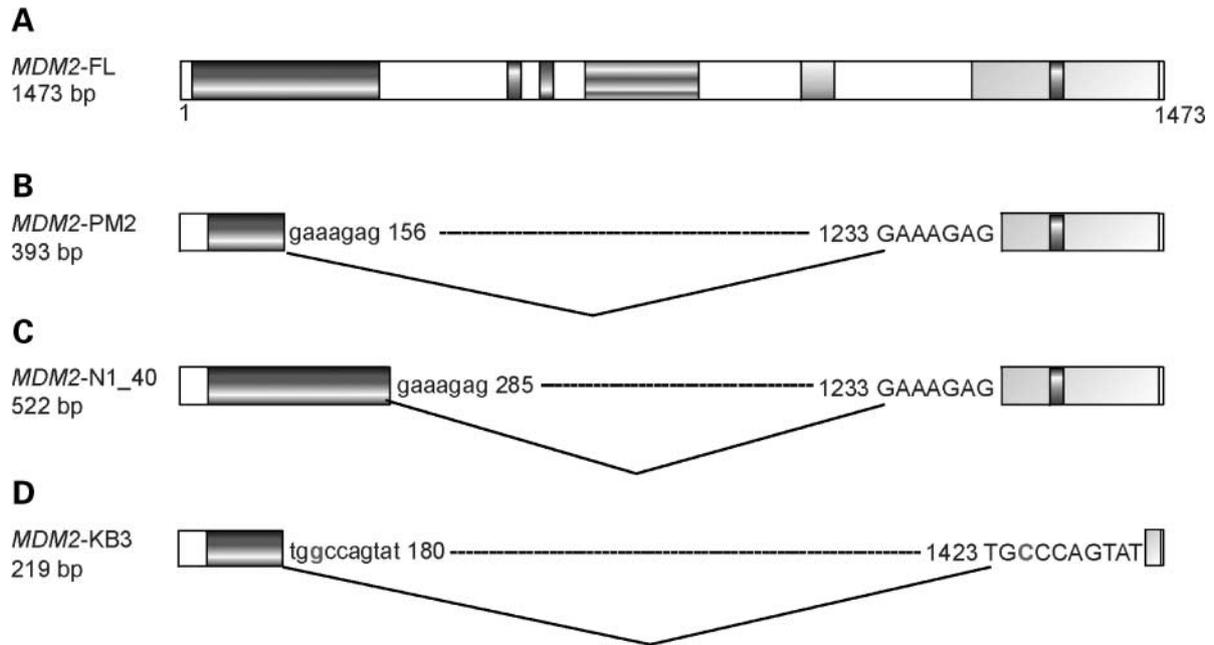


FIGURE 3. Mechanism of aberrant splicing at repetitive sequences. *MDM2-FL*, full-length *MDM2*; *capital letters*, sequence that is present in the splice variant. A detailed description of the splicing mechanism is given in the text.

Evans *et al.* (34) have shown that at least one splice variant (*MDM2-B* or *ALT1*) encodes a protein that binds full-length *MDM2*, resulting in sequestration of both proteins in the cytoplasm. This finding is important because alternatively and aberrantly spliced *MDM2* mRNAs are usually expressed together with full-length *MDM2* transcripts. *MDM2-B* is the most frequently expressed *MDM2* splice variant. It has been observed in numerous types of cancer, including ovarian and bladder cancers (12), breast cancer (13, 16), soft tissue sarcomas (17, 19), and giant cell tumors of the bone (20), but it also occurs in normal breast tissue (16).

Evans *et al.* (34) demonstrated that binding of *MDM2-B* to full-length *MDM2* increased wild-type p53 activity, an observation that was also described by Dang *et al.* (35) with some of the murine variants. On binding of *MDM2* splice variants with an intact COOH-terminal RING finger domain to full-length *MDM2* protein, p53 protein becomes stabilized, resulting in a growth-inhibitory phenotype.

A correlation between expression of *MDM2* splice variants and stabilization of wild-type p53 has also been described in glioblastoma cell lines despite an amplified *MDM2* gene (14). In soft tissue sarcomas, the expression of *MDM2* splice forms was also associated with an overexpression of mutant and wild-type p53 (17). These findings suggest that p53 accumulation arises as a consequence of alternative and aberrant *MDM2* splicing independent of the mutational status of p53. Although wild-type p53 overexpression induced by *MDM2* isoforms is inconsistent with tumor progression, it is conceivable that the stabilization of mutant p53 might contribute to transformation and tumor growth. It is also possible that *MDM2* splice variant-mediated p53 activation could result in enhanced selective pressure to inactivate the p53 apoptotic pathway, thereby increasing accumulation of other genetic defects and promot-

ing tumorigenesis. However, the association between *MDM2* splice variants and malignancy is controversial. Initial studies by Sigalas *et al.* (12) demonstrated that the expression of *MDM2* splice forms in transfected NIH3T3 cells could grow as colonies in soft agar. These findings have been supported by data from Steinman *et al.* (36) who demonstrated that *MDM2-B*, which is the most prevalent isoform identified in tumors, can cause tumors in a transgenic mouse model. Recent data from Fridman *et al.* (37) show that the murine equivalents of the human *MDM2-B*, *-D*, and *-E* splice forms, but not *MDM2-A*, significantly accelerated lymphomagenesis in an *Eμ-myc* transgenic mouse model. The lymphomas produced by the splice variants were aggressive and displayed a similar pathology to lymphomas expressing full-length *MDM2*. These data provide evidence that at least some *MDM2* isoforms can contribute to tumor development in an *in vivo* mouse model. However, in contrast to a transforming function, *MDM2-B* is expressed in both normal and malignant mammary tissues (16).

Another aspect to be considered regarding a potential transforming function of the *MDM2* isoforms is the amount and/or the translation efficiency of a given mRNA as well as the ratio of two or more splice variants. Because it is unknown under what conditions p53-independent functions of full-length *MDM2* may be active, it is impossible to predict the function of each splice variant in all the model systems evaluated. In many cases, *MDM2* isoforms may not be functional and, therefore, would not affect the cell negatively. However, many of the proteins synthesized from alternatively and/or aberrantly spliced mRNAs can play either a transforming role or a "normal" physiological role dependent on the cell type. In addition, recent data from Bartl *et al.* (38) suggest a new RNA-based function of *MDM2*. The authors describe a 365-bp,

Table 1. Sequence of the Anti-MDM2 Oligonucleotides

Oligonucleotide	Sequence ^a	Target Sequence in Exon	Characteristic	References
Kondo	GACATGTTGGTATTGCACAT	Exon 3	Phosphorothioate oligonucleotide	(39–41)
HDMAS5	GATCACTCCCACCTTCAAGG	Exon 7	Phosphorothioate oligonucleotide	(42, 43)
Anti-MDM2-MBO	<u>UGACACCTGTTCTCACUCAC</u>	Exon 7	Mixed backbone oligonucleotide	(44–46, 48, 51, 55–57)

^aUnderlined sequences, 2'-O-methyl-RNA linkages between nucleotides.

alternatively spliced transcript that is composed of the first five *MDM2* exons and is highly stress inducible. It appears to be the major processing product of the *MDM2* mRNA both in normal and in cancer cells (38). Therefore, it seems likely that even transcript variants that are not translated fulfill a function as small non-mRNAs. Future studies are required to clearly define the functions of the many *MDM2* transcript variants and how their expression is controlled.

Down-Regulation of the *MDM2* Messenger RNA by AS-ODNs

The use of AS-ODNs targeted to different *MDM2* mRNAs may be a useful approach to evaluate the functions of alternatively spliced isoforms. In addition, *MDM2* antisense designed to down-regulate splice variant and/or full-length *MDM2* mRNAs may be a useful anticancer therapeutic approach (Fig. 1). However, many of the *MDM2*-AS-ODNs reported to date were designed to target sequences not present within the majority of splice variants and therefore would only down-regulate full-length *MDM2* expression. Here, we summarize current progress on the development of *MDM2*-AS-ODNs as a potential anticancer therapy.

The first study to use *MDM2*-AS-ODNs (Table 1) to down-regulate *MDM2* expression was published by Kondo *et al.* (39). It was shown that glioblastoma U87-MG cells were more susceptible to cisplatin-induced apoptosis when the cells were cotreated with cisplatin and *MDM2*-AS-ODNs that targeted the first 20 nucleotides of the open reading frame. A decrease of the *MDM2* protein level could be observed, although p53 protein levels remained constant. These findings (39) suggest an important role for *MDM2* in the development of resistance to cisplatin. Teoh *et al.* (40) studied the effect of the same oligonucleotide in a series of multiple myeloma cell lines. Treatment with *MDM2*-AS-ODNs resulted in decreased DNA synthesis and cell viability as well as a G₁ cell cycle arrest associated with pRb-E2F1 binding. Furthermore, apoptosis was induced in the AS-ODN-treated cells (40). In another study using the same *MDM2*-AS-ODNs described by Kondo *et al.* (39), we observed decreased *MDM2* protein expression 24 h after antisense treatment and an 80% decrease in the colony-forming ability of *MDM2*-AS-ODN-treated undifferentiated sarcoma cells (US8/93) compared with control cells that received a scrambled control oligonucleotide (41). Although *MDM2* splice variants were not evaluated during these studies, the *MDM2*-AS-ODN used had the potential to down-regulate many of the common splice forms

Table 2. Summary of Published *in Vivo* Applications of Anti-MDM2 Oligonucleotides

Tumor Type	Cell Line	Animal Model	p53 Gene Status	Oligonucleotide, Amount, Schedule	Drugs	Effects of Cotreatment	References
Prostate cancer	DU-145, PC-3	Mice	ND ^a	Anti-MDM2-MBO, 25 mg/kg/day, 4 × 5 days/wk	Irinotecan, paclitaxel, rituxan	Synergistic effect (+ irinotecan) slightly increased activity (+ paclitaxel, rituxan)	(56)
Colon cancer	LS174T	Mice	Wild-type	Anti-MDM2-MBO, 20 mg/kg/day, 5 days/wk	10-Hydroxy-camphothecin, 5-fluorouracil	Synergistically or additive therapeutic effects	(46, 57)
Glioblastoma multiforme	DLD-1 U87-MG	Mice	Mutant Wild-type	Anti-MDM2-MBO, 25 mg/kg/day, 5 days/wk	Irinotecan, paclitaxel	Inhibition of tumor growth, 39- and 63-fold activity of irinotecan and paclitaxel	(48)
Rhabdomyosarcoma	RD	Rat	Mutant	Anti-MDM2-MBO, 100 µg continuously over 1 wk	–	Significantly reduced tumor growth, decreased mutant p53 levels	(51)
Breast cancer	MCF-7	Mice	Wild-type	Anti-MDM2-MBO, 25 mg/kg/day, 3 × 5 days/wk	Irinotecan, paclitaxel, 5-fluorouracil	Synergistically or additive therapeutic effects	(47)
Colon cancer	MDA-MB-468 GEO	Mice	Mutant ND	Anti-MDM2-MBO, 10 mg/kg/day, 2 × 5 days/wk	Cisplatin, topotecan	Potential of effects of cisplatin, topotecan	(45)
Osteosarcoma	SJSA	Mice	ND	Anti-MDM2-MBO, 25 mg/kg/day	10-Hydroxy-camphothecin, Adriamycin	Synergistic effect of cotreatment	(44)
Choriocarcinoma	JAR	Mice	ND	Anti-MDM2-MBO, 25 mg/kg/day	10-Hydroxy-camphothecin, Adriamycin	Synergistic effect of cotreatment	(44)

^aND, not determined

(Fig. 2) in addition to full-length MDM2. However, because splice variant expression was not analyzed during these studies, it is not possible to evaluate how potential changes in splice variant expression may have influenced the observed results.

Chen *et al.* (42) investigated the effects of improved phosphorothioate MDM2-AS-ODNs in JAR, SJSA, and MCF-7 cells. The MDM2 protein level was reduced up to 5-fold by using an oligonucleotide termed HDMAS5-ODN (Table 1), resulting in activation of p53 (42). This oligonucleotide was directed against a sequence within exon 7 of the MDM2 mRNA, a region that is omitted in many splice variants. Similar results were observed by Sato *et al.* (43) in osteosarcoma U2-OS cells treated with HDMAS5-ODN in combination with DNA-damaging drugs such as mitomycin C and cisplatin. The drug-mediated cell killing was even more pronounced when the expression of both MDM2 and p21 was blocked by antisense transfection (43). Encouraging *in vitro* results of MDM2-AS-ODN treatment led to the development of xenograft models of different tumors. These xenograft models include osteosarcoma SJSA cells and choriocarcinoma JAR cells (44), colon cancer GEO cells (45), LS174T and DLD-1 cells (46), breast cancer MCF-7 and MDA-MB-468 cells (47), several glioblastoma multiforme cell lines (48), and prostate cancer cell lines (49). In these studies, the human tumor cells were injected into the inguinal area, grown to xenografts, and subsequently treated with either an anti-MDM2 mixed backbone oligonucleotide (anti-MDM2-MBO; Table 1; 44) alone or in combination with chemotherapeutic drugs such as irinotecan, paclitaxel, 5-fluorouracil, or radiation (Table 2; 47–51). MDM2-MBO is another oligonucleotide targeted to exon 7 that is deleted in most splice variants (Fig. 2). In most studies, the MDM2-AS-ODNs were given by i.p. injection at 5 consecutive days. Compared with untreated animals, treatment of the respective xenografts with MDM2-AS-ODNs led to significant antitumor activity in terms of slowed tumor growth and prolonged survival that was attributed to inhibition of MDM2 expression. In cell lines with wild-type p53 such as MCF-7 (47) and U87-MG (48), these antitumor effects were accompanied by elevated levels and increased activity of wild-type p53, whereas in cell lines with mutant p53 such as MDA-MB-468 (47) and T98G (48), the p53 protein levels remained unaffected. In contrast, treatment of an orthotopic xenograft model of rhabdomyosarcoma RD cells (p53^{mt/-}) in the peritoneum of nude rats with MDM2-AS-ODNs resulted in decreased levels of both MDM2 and mutant p53 (51). In general, synergistic effects were observed when cells or xenografts were treated with a combination of MDM2-AS-ODNs and the respective drug or radiation compared with the treatment with either of these agents alone (47–50). However, combination of irinotecan and a MDM2 mismatch control oligonucleotide had the similar effect as the combination of irinotecan and MDM2-AS-ODNs (47–49). The authors speculated that treatment with oligonucleotides, in general, increases the uptake of the active metabolite of irinotecan, SN-38 (46).

Conclusions

It has been shown that MDM2 not only acts as an oncogene but also displays growth-inhibitory functions. However, it is currently unclear how these different functions are controlled or

influenced by expression of alternatively spliced isoforms of MDM2. The use of AS-ODNs to inhibit the expression of genes of the p53-MDM2 pathway has the potential to become an exciting new cancer therapy (52, 53). MDM2-AS-ODNs specifically inhibit MDM2 expression, and they employ their antitumor activity via different mechanisms, regardless of p53 status. This is particularly noteworthy given the high mutation frequency of the p53 gene in human cancer (54). The activity of conventional drugs or radiation can be synergistically enhanced if given in combination with MDM2-AS-ODNs, and the effects appear to be independent of p53 gene status or the different MDM2 isoforms that might be expressed. In light of the current findings, effective cancer therapy may involve inhibiting MDM2 expression, in addition to blocking binding of MDM2 to p53, to inhibit all the growth-promoting activities of MDM2. However, whether the AS-ODNs influence expression of any MDM2 splice variants is currently unknown. As specific splice variants have now been shown to play a role in tumorigenesis, knowing whether they are down-regulated together with full-length MDM2 will be important for interpretation of future data.

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References

- Fakharzadeh, S. S., Trusko, S. P., and George, D. L. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J.*, 10: 1565–1569, 1991.
- Dubs-Poterszman, M. C., Tocque, B., and Wasylyk, B. MDM2 transformation in the absence of p53 and abrogation of the p107 G₁ cell-cycle arrest. *Oncogene*, 11: 2445–2449, 1995.
- Jones, S. N., Hancock, A. R., Vogel, H., Donehower, L. A., and Bradley, A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc. Natl. Acad. Sci. USA*, 95: 15608–15612, 1998.
- Brown, D. R., Thomas, C. A., and Deb, S. P. The human oncoprotein MDM2 arrests the cell cycle: elimination of its cell-cycle-inhibitory function induces tumorigenesis. *EMBO J.*, 17: 2513–2525, 1998.
- Folberg-Blum, A., Sapir, A., Shilo, B., and Oren, M. Overexpression of mouse Mdm2 induces developmental phenotypes in *Drosophila*. *Oncogene*, 21: 2413–2417, 2002.
- Leng, X., Connell-Crowley, L., Goodrich, D., and Harper, J. W. S-phase entry upon ectopic expression of G₁ cyclin-dependent kinases in the absence of retinoblastoma protein phosphorylation. *Curr. Biol.*, 7: 709–712, 1997.
- Martin, K., Trouche, D., Hagemeyer, C., Sorensen, T. S., La Thangue, N. B., and Kouzarides, T. Stimulation of E2F1/DP1 transcriptional activity by MDM2 oncoprotein. *Nature*, 375: 691–694, 1995.
- Xiao, Z. X., Chen, J., Levine, A. J., Modjtahedi, N., Xing, J., Sellers, W. R., and Livingston, D. M. Interaction between the retinoblastoma protein and the oncoprotein MDM2. *Nature*, 375: 694–698, 1995.
- Pinkas, J., Naber, S. P., Butel, J. S., Medina, D., and Jerry, D. J. Expression of MDM2 during mammary tumorigenesis. *Int. J. Cancer*, 81: 292–298, 1999.
- Bueso-Ramos, C. E., Manshour, T., Haidar, M. A., Yang, Y., McCown, P., Ordonez, N., Glassman, A., Sneige, N., and Albitar, M. Abnormal expression of MDM-2 in breast carcinomas. *Breast Cancer Res. Treat.*, 37: 179–188, 1996.
- Bartel, F., Taubert, H., and Harris, L. C. Alternative and aberrant splicing of MDM2 mRNA in human cancer. *Cancer Cell*, 2: 9–15, 2002.
- Sigalas, I., Calvert, A. H., Anderson, J. J., Neal, D. E., and Lunec, J. Alternatively spliced *mdm2* transcripts with loss of p53 binding domain sequences: transforming ability and frequent detection in human cancer. *Nat. Med.*, 2: 912–917, 1996.
- Matsumoto, R., Tada, M., Nozaki, M., Zhang, C. L., Sawamura, Y., and Abe, H. Short alternative splice transcripts of the *mdm2* oncogene correlate to malignancy in human astrocytic neoplasms. *Cancer Res.*, 58: 609–613, 1998.
- Kraus, A., Neff, F., Behn, M., Schuermann, M., Muenkel, K., and Schlegel, J. Expression of alternatively spliced *mdm2* transcripts correlates with stabilized wild-type p53 protein in human glioblastoma cells. *Int. J. Cancer*, 80: 930–934, 1999.

15. Hori, M., Shimazaki, J., Inagawa, S., Itabashi, M., and Hori, M. Alternatively spliced MDM2 transcripts in human breast cancer in relation to tumor necrosis and lymph node involvement. *Pathol. Int.*, *50*: 786–792, 2000.
16. Lukas, J., Gao, D. Q., Keshmeshian, M., Wen, W. H., Tsao-Wei, D., Rosenberg, S., and Press, M. F. Alternative and aberrant messenger RNA splicing of the *mdm2* oncogene in invasive breast cancer. *Cancer Res.*, *61*: 3212–3219, 2001.
17. Bartel, F., Meye, A., Wurl, P., Kappler, M., Bache, M., Lautenschlager, C., Grunbaum, U., Schmidt, H., and Taubert, H. Amplification of the MDM2 gene, but not expression of splice variants of MDM2 mRNA, is associated with prognosis in soft tissue sarcoma. *Int. J. Cancer*, *95*: 168–175, 2001.
18. Bartel, F., Taylor, A. C., Taubert, H., and Harris, L. C. Novel *mdm2* splice variants identified in pediatric rhabdomyosarcoma tumors and cell lines. *Oncol. Res.*, *12*: 451–457, 2002.
19. Tamborini, E., Della, T. G., Lavarino, C., Azzarelli, A., Carpinelli, P., Pierotti, M. A., and Pilotti, S. Analysis of the molecular species generated by MDM2 gene amplification in liposarcomas. *Int. J. Cancer*, *92*: 790–796, 2001.
20. Evdokiou, A., Atkins, G. J., Bouralexis, S., Hay, S., Raggatt, L. J., Cowled, P. A., Graves, S. E., Clayer, M., and Findlay, D. M. Expression of alternatively-spliced MDM2 transcripts in giant cell tumors of bone. *Int. J. Oncol.*, *19*: 625–632, 2001.
21. Garcia, J. F., Villuendas, R., Sanchez-Beato, M., Sanchez-Aguilera, A., Sanchez, L., Prieto, L., and Piris, M. A. Nucleolar p14(ARF) overexpression in Reed-Sternberg cells in Hodgkin's lymphoma: absence of p14(ARF)/Hdm2 complex is associated with expression of alternatively spliced Hdm2 transcripts. *Am. J. Pathol.*, *160*: 569–578, 2002.
22. Hong, D. H. and Li, T. Complex expression pattern of RPGR reveals a role for purine-rich exonic splicing enhancers. *Invest. Ophthalmol. Vis. Sci.*, *43*: 3373–3382, 2002.
23. Bartel, F., Pinkert, D., Fiedler, W., Kappler, M., Wurl, P., Schmidt, H., and Taubert, H. The Expression of alternatively and aberrantly spliced transcripts of the MDM2-mRNA is not tumor-specific. *Int. J. Oncol.*, *24*: 143–151, 2004.
24. Celotto, A. M. and Graveley, B. R. Alternative splicing of the *Drosophila* Dscam pre-mRNA is both temporally and spatially regulated. *Genetics*, *159*: 599–608, 2001.
25. Cooper, T. A. and Mattox, W. The regulation of splice-site selection, and its role in human disease. *Am. J. Hum. Genet.*, *61*: 259–266, 1997.
26. Blencowe, B. J. Exonic splicing enhancers: mechanism of action, diversity and role in human genetic diseases. *Trends Biochem. Sci.*, *25*: 106–110, 2000.
27. Jensen, K. B., Dredge, B. K., Stefani, G., Zhong, R., Buckanovich, R. J., Okano, H. J., Yang, Y. Y., and Darnell, R. B. Nova-1 regulates neuron-specific alternative splicing and is essential for neuronal viability.
28. Schlott, T., Reimer, S., Jahns, A., Ohlenbusch, A., Ruschenburg, I., Nagel, H., and Droese, M. Point mutations and nucleotide insertions in the MDM2 zinc finger structure of human tumors. *J. Pathol.*, *182*: 54–61, 1997.
29. Mariatos, G., Gorgoulis, V. G., Kotsinas, A., Zacharatos, P., Kokotas, S., Yannoukakos, D., and Kittas, C. Absence of mutations in the functional domains of the human MDM2 oncogene in non-small cell lung carcinomas. *Mutat. Res.*, *456*: 59–63, 2000.
30. Silva, J., Silva, J. M., Dominguez, G., Garcia, J. M., Rodriguez, O., Garcia-Andrade, C., Cuevas, J., Provencio, M., Espana, P., and Bonilla, F. Absence of point mutations at codon 17 of the *mdm2* gene (serine 17) in human primary tumors. *Mutat. Res.*, *449*: 41–45, 2000.
31. Taubert, H., Kappler, M., Meye, A., Bartel, F., Schlott, T., Lautenschlager, C., Bache, M., Schmidt, H., and Wurl, P. A MboII polymorphism in exon 11 of the human MDM2 gene occurring in normal blood donors and in soft tissue sarcoma patients: an indication for an increased cancer susceptibility? *Mutat. Res.*, *456*: 39–44, 2000.
32. Rallapalli, R., Strachan, G., Cho, B., Mercer, W. E., and Hall, D. J. A novel MDMX transcript expressed in a variety of transformed cell lines encodes a truncated protein with potent p53 repressive activity. *J. Biol. Chem.*, *274*: 8299–8308, 1999.
33. Rallapalli, R., Strachan, G., Tuan, R. S., and Hall, D. J. Identification of a domain within MDMX-S that is responsible for its high affinity interaction with p53 and high-level expression in mammalian cells. *J. Cell. Biochem.*, *89*: 563–575, 2003.
34. Evans, S. C., Viswanathan, M., Grier, J. D., Narayana, M., El Naggar, A. K., and Lozano, G. An alternatively spliced HDM2 product increases p53 activity by inhibiting HDM2. *Oncogene*, *20*: 4041–4049, 2001.
35. Dang, J., Kuo, M. L., Eischen, C. M., Stepanova, L., Sherr, C. J., and Roussel, M. F. The RING domain of Mdm2 can inhibit cell proliferation. *Cancer Res.*, *62*: 1222–1230, 2002.
36. Steinman, H. A., Gosselin, J., and Jones, S. N. Analysis of tumorigenesis in MDM2 transgenic mice. *Proc. Am. Assoc. Cancer Res.*, *43*: 1064, 2002.
37. Fridman, J. S., Hernandez, E., Hemann, M. T., de Stanchina, E., Cordon-Cardo, C., and Lowe, S. W. Tumor promotion by Mdm2 splice variants unable to bind p53. *Cancer Res.*, *63*: 5703–5706, 2003.
38. Bartl, S., Ban, J., Weninger, H., Jug, G., and Kovar, H. A small nuclear RNA, hdm365, is the major processing product of the human *mdm2* gene. *Nucleic Acids Res.*, *31*: 1136–1147, 2003.
39. Kondo, S., Barnett, G. H., Hara, H., Morimura, T., and Takeuchi, J. MDM2 protein confers the resistance of a human glioblastoma cell line to cisplatin-induced apoptosis. *Oncogene*, *10*: 2001–2006, 1995.
40. Teoh, G., Chen, L., Urashima, M., Tai, Y. T., Celi, L. A., Chen, D., Chauhan, D., Ogata, A., Finberg, R. W., Webb, I. J., Kufe, D. W., and Anderson, K. C. Adenovirus vector-based purging of multiple myeloma cells. *Blood*, *92*: 4591–4601, 1998.
41. Meye, A., Wurl, P., Bache, M., Bartel, F., Grunbaum, U., Mansa-ard, J., Schmidt, H., and Taubert, H. Colony formation of soft tissue sarcoma cells is inhibited by lipid-mediated antisense oligodeoxynucleotides targeting the human *mdm2* oncogene. *Cancer Lett.*, *149*: 181–188, 2000.
42. Chen, L., Agrawal, S., Zhou, W., Zhang, R., and Chen, J. Synergistic activation of p53 by inhibition of MDM2 expression and DNA damage. *Proc. Natl. Acad. Sci. USA*, *95*: 195–200, 1998.
43. Sato, N., Mizumoto, K., Maehara, N., Kusumoto, M., Nishio, S., Urashima, T., Ogawa, T., and Tanaka, M. Enhancement of drug-induced apoptosis by antisense oligodeoxynucleotides targeted against Mdm2 and p21WAF1/CIP1. *Anticancer Res.*, *20*: 837–842, 2000.
44. Wang, H., Zeng, X., Oliver, P., Le, L. P., Chen, J., Chen, L., Zhou, W., Agrawal, S., and Zhang, R. MDM2 oncogene as a target for cancer therapy: an antisense approach. *Int. J. Oncol.*, *15*: 653–660, 1999.
45. Tortora, G., Caputo, R., Damiano, V., Bianco, R., Chen, J., Agrawal, S., Bianco, A. R., and Ciardiello, F. A novel MDM2 anti-sense oligonucleotide has anti-tumor activity and potentiates cytotoxic drugs acting by different mechanisms in human colon cancer. *Int. J. Cancer*, *88*: 804–809, 2000.
46. Wang, H., Wang, S., Nan, L., Yu, D., Agrawal, S., and Zhang, R. Antisense anti-MDM2 mixed-backbone oligonucleotides enhance therapeutic efficacy of topoisomerase I inhibitor irinotecan in nude mice bearing human cancer xenografts: *in vivo* activity and mechanisms. *Int. J. Oncol.*, *20*: 745–752, 2002.
47. Wang, H., Nan, L., Yu, D., Agrawal, S., and Zhang, R. Antisense anti-MDM2 oligonucleotides as a novel therapeutic approach to human breast cancer: *in vitro* and *in vivo* activities and mechanisms. *Clin. Cancer Res.*, *7*: 3613–3624, 2001.
48. Prasad, G., Wang, H., Agrawal, S., and Zhang, R. Antisense anti-MDM2 oligonucleotides as a novel approach to the treatment of glioblastoma multiforme. *Anticancer Res.*, *22*: 107–116, 2002.
49. Wang, H., Yu, D., Agrawal, S., and Zhang, R. Experimental therapy of human prostate cancer by inhibiting MDM2 expression with novel mixed-backbone antisense oligonucleotides: *in vitro* and *in vivo* activities and mechanisms. *Prostate*, *54*: 194–205, 2003.
50. Grunbaum, U., Meye, A., Bache, M., Bartel, F., Wurl, P., Schmidt, H., Dunst, J., and Taubert, H. Transfection with *mdm2*-antisense or wtp53 results in radiosensitization and an increased apoptosis of a soft tissue sarcoma cell line. *Anticancer Res.*, *21*: 2065–2071, 2001.
51. Wurl, P., Bartel, F., Meye, A., Kappler, M., Bache, M., Schmidt, H., Schonfelder, M., and Taubert, H. Growth reduction of a xenotransplanted human soft tissue sarcoma by MDM2 antisense therapy via implanted osmotic minipumps. *Int. J. Oncol.*, *20*: 1087–1093, 2002.
52. Wang, H., Prasad, G., Buolamwini, J. K., and Zhang, R. Antisense anticancer oligonucleotide therapeutics. *Curr. Cancer Drug Targets*, *1*: 177–196, 2001.
53. Zhang and Wang, H. MDM2 oncogene as a novel target for human cancer therapy. *Curr. Pharm. Des.*, *6*: 393–416, 2000.
54. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53 mutations in human cancers. *Science*, *253*: 49–53, 1991.
55. Chen, L., Lu, W., Agrawal, S., Zhou, W., Zhang, R., and Chen, J. Ubiquitous induction of p53 in tumor cells by antisense inhibition of MDM2 expression. *Mol. Med.*, *5*: 21–34, 1999.
56. Li, M., Luo, J., Brooks, C. L., and Gu, W. Acetylation of p53 inhibits its ubiquitination by Mdm2. *J. Biol. Chem.*, *277*: 50607–50611, 2002.
57. Wang, H., Nan, L., Yu, D., Lindsey, J. R., Agrawal, S., and Zhang, R. Anti-tumor efficacy of a novel antisense anti-MDM2 mixed-backbone oligonucleotide in human colon cancer models: p53-dependent and p53-independent mechanisms. *Mol. Med.*, *8*: 185–199, 2002.
58. Bartel, F. and Meye, A. Bedeutung des Onkogens *mdm2* für die Entwicklung neuer Therapiestrategien. In: F-W. Rath and M. Schonfelder (eds.), Diagnostik und Therapie der Weichteilsarkome: Stand und Perspektiven. 168–177. Leipzig: Karger Verlag, 2001.
59. Schlott, T., Thasler, W., Gorzel, C., Pahernik, S., Brinck, U., Eiffert, H., and Droese, M. Detection of MDM2 alterations in cultured human hepatocytes treated with 17 β -estradiol or 17 α -ethinylestradiol. *Anticancer Res.*, *22*: 1545–1552, 2002.

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