Chapter 14
Splice Variants of MDM2 in Oncogenesis

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Abstract Many types of human cancers overexpress MDM2 protein. A common characteristic among these cancers is an associated increase in *mdm2* splice variants. Provided here is a comprehensive list, based on a literature review, of over 70 *mdm2* variants. These variants are grouped according to in-frame versus out-of-frame status and their potential (or ability) to be translated into isoform proteins. We describe the putative functions for these *mdm2* splice variant mRNAs, as well as the mechanistic drivers associated with increased *mdm2* transcription and splicing. The paradoxical signal transduction functions of the most commonly studied variants *mdm2-a,-b* and *-c* are addressed for their outcomes in the presence and absence of wild-type p53. These outcomes vary from tumor promotion to growth arrest. Finally, we present issues in the detection of endogenous MDM2 protein and how many of the antibodies commonly used to detect MDM2 do not present a full picture of the cellular representation of the isoform proteins. This review provides a focusing lens for individuals interested in learning about the complexities of *mdm2* mRNAs and their protein isoforms as well as the roles MDM2 isoforms may play in cancer progression.

Keywords MDM2 • Splicing

Introduction

Many human cancers over-express MDM2 and the *mdm2* gene locus produces a diverse array of *mdm2* splice variants [1, 2]. Alternative splicing is predominantly co-transcriptional [3] with approximately 6.3 alternatively spliced transcripts

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occurring per human gene [4]. The coordination of splicing with transcription highlights the importance of alternative splicing in signal transduction. Unlike the average human gene locus, the *mdm2* gene gives rise to more than the average 6.3 alternative spliced transcripts. Increased transcription and splicing of *mdm2 is* associated with increased tumorigenesis [1]. However, it is unclear exactly what biological functions the alternative spliced *mdm2* transcripts contribute to tumorigenesis. We reviewed the literature describing *mdm2* splice variants associated with oncogenesis and found that at least 72 have been described. It has not been determined how many of these splice variants express protein in cancer cells.

The association of high MDM2 expression with tumorigenic potential was identified in 1991 in the Donna George laboratory [5]. In 1996, the Lunec group detected increased expression of *mdm2* splice variants in multiple tumor types [6]. The alternative spliced transcripts were named *mdm2*-a, *mdm2*-b, *mdm2*-c, *mdm2*-d and *mdm2*-e in order to contrast them with the full-length version called *mdm2-fl* [6]. Since 1996 the list of *mdm2* alternative spliced variants related to oncogenesis has vastly increased (see Table 14.1). We will focus on the three most commonly found, and best studied, exon skipped transcripts that can be translated into protein [23].

Table 14.1 Known MDM2 splice variant transcripts

Group	Mdm2 variant	Transcript size	In frame	Amino acid number	Predicted protein size (kDa)	Apparent protein size (kDa)	Reference
A	Hdm365	365	Nuclear				[7]
	MYO-2	262	No				[8]
	MYO-3	269	No				[8]
	MYO-5	302	No				[8]
	MYO-6	313	No				[8]
	MYO-7	337	No				[8]
	MYO-10	371	No				[8]
	MYO-13	440	No				[8]
	MYO-14	485	No				[8]
	MYO-16	490	No				[8]
	MYO-17	514	No				[8]
	MYO-19	544	No				[8]
	MYO-21	575	No				[8]
	MYO-22	578	No				[8]
	MYO-27	724	No				[8]
	MYO-28	736	No				[8]
	MYO-29	763	No				[8]
	MYO-30	791	No				[8]
	MYO-31	893	No				[8]
	MYO-33	1,385	No				[8]
	Hdm2-1007	1,007	No				[9]
	Mdm2-FB26		No				[10]
	Mdm2-FB28		No				[10]

(continued)

Table 14.1 (continued)

Group	Mdm2 variant	Transcript size	In frame	Amino acid number	Predicted protein size (kDa)	Apparent protein size (kDa)	Reference
	Mdm2-FB30		No				[10]
	Mdm2-FB55		No				[10]
	Mdm2-Var1		No				[10, 11]
	Mdm2-219	219	No				[12, 13]
	Mdm2-254	254	No				[12]
	Mdm2-H	386	No				[14]
	Mdm2-LN229a	259	No				[15]
	Mdm2-LN229b	195	No				[15]
	Mdm2-LN18	234	No				[15]
	Mdm2-G116	201	No				[15]
	Mdm2-G150	211	No				[15]
B	MYO-1	252	Yes	84	9.6		[8]
	MYO-4	285	Yes	95	10.9		[8]
	MYO-8	351	Yes	117	13.4		[8]
	MYO-9	360	Yes	120	13.8		[8]
	MYO-11	405	Yes	135	15.5		[8]
	MYO-12	405	Yes	135	15.5		[8]
	MYO-18	486	Yes	162	55.8		[8]
	MYO-20	546	Yes	182	20.9		[8]
	MYO-23	621	Yes	207	23.7		[8]
	MYO-24	654	Yes	218	25		[8]
	MYO-25	660	Yes	220	25.2		[8]
	MYO-26	717	Yes	239	27.4		[8]
	MYO-32	1,095	Yes	365	41.9		[8]
	Hdm2-1338	1,338	Yes	446	50.7		[9]
	Hdm2-1200	1,200	Yes	400	45.5		[9]
	P2-Mdm2-10	1,338	Yes	446	50.7		[16]
	P2-Mdm2-C1	906	Yes	302	34.6		[16]
	Mdm2-N1_40	522	Yes	174	20		[17]
	Mdm2-KB2	732	Yes	243	27.9		[17]
	Mdm2-KB3	219	Yes	73	8.4		[17]
	Mdm2-JN1	207	Yes	69	7.9		[17]
	Mdm2-DS2	364	Yes				[17]
	Mdm2-DS3	294	Yes	98	11.2		[17]
	Mdm2-IS1	456	Yes	152	17.4		[17]
	Mdm2-PM2	393	Yes	131	15		[17]
	Mdm2-EU2	297	Yes	99	11.4		[17]
	Mdm2-281	281	Yes	94	10.8		[12]
	Mdm2-DelE	327	Yes	109	12.5		[13]
	Mdm2-DelF	303	Yes	101	11.6		[13]
	Mdm2-FB29	1,074	Yes	358	41.1		[10]
	Mdm2-FB25	750	Yes	250	28.7		[10]
	Mdm2-Var2		Yes				[11]

(continued)

Table 14.1 (continued)

Group	Mdm2 variant	Transcript size	In frame	Amino acid number	Predicted protein size (kDa)	Apparent protein size (kDa)	Reference
C	Mdm2-A (Alt2)	941	Yes	295	33.8	75	[6, 18–20]
	Mdm2-A1	798	Yes	270	22.6	55	[19, 21]
	Mdm2-B (Alt1)	707	Yes	217	24.9	48	[6, 12, 14, 17–20, 22]
	Mdm2-C (Alt3)	1,016	Yes	321	36.6	85	[6, 14, 18–20]
	Mdm2-D	449	Yes	132	15.1	30	[6, 19, 20]
	Mdm2-E	303	Yes	102	11.7	16	[6, 19, 20]
	Mdm2-F	1,391	Yes	468	53.7	85	[2, 14, 19]
	Mdm2-G	1,361	Yes	444	50.9	85	[2, 14, 21]
	Mdm2-FL	1,526	Yes	491	55	90	[6]

Table 14.2 Common MDM2 transcripts associated with cancers

Cancer type	MDM2-A/ALT2 (Exons 4–9)	MDM2-B/ALT1 (Exons 4–11)	MDM2-C/ALT3 (Exons 5–9)	Reference
Colorectal	No	Yes	No	[9]
Ovarian	Yes	Yes	Yes	[6]
Bladder	Yes	Yes	Yes	[6]
Leukemia	Yes	Yes	Yes	[6]
Breast carcinoma	Yes	Yes	Yes	[12, 13, 22, 24, 25]
Soft tissue sarcoma	Yes	Yes	Yes	[1, 10, 17]
Hodgkin's lymphoma	Yes	Yes	Yes	[22, 26]
Glioblastoma	Yes	Yes	Yes	[1, 15, 20]
Liposarcoma	Yes	Yes	Yes	[1]
Lung carcinoma	Yes	Yes	Yes	[1, 27]
Oral squamous carcinoma	No	Yes	Yes	[8]
Burkitt's lymphoma	Not tested	Not tested	Yes	[16]
Osteosarcoma	Not tested	Not tested	Yes	[16]
Pediatric rhabdomyosarcoma	Yes	Yes	Yes	[10]

The most commonly detected isoforms are *mdm2*-a, *mdm2*-b and *mdm2*-c. These three *mdm2* splice variants are found in human leukemia, soft tissue sarcoma, Hodgkin's lymphoma, glioblastoma, rhabdomyosarcoma, liposarcoma, and many different carcinomas including ovarian, breast, bladder, lung and oral squamous cell carcinoma (see Table 14.2). It is highly likely that the protein products MDM2-A, MDM2-B and MDM2-C contribute to the diversity of the human cancer proteome [23]. In addition to *mdm2* spliced variants potentiating cancer proteome diversity, alternative spliced *mdm2* products have also been associated with a small nuclear RNA processed form called *hdm365* [7]. This suggests that some human *mdm2* transcripts may possess RNA-based functions.

Transcripts of Human *mdm2* in Cancer

Two different promoters, P1 and P2, control transcription of the *mdm2* gene, giving rise to two different mRNA messages that encode MDM2-FL [28]. The *mdm2* gene has 12 exons and the P1 promoter drives transcription from upstream of exon 1 and coordinates the splicing out of exon 2. The P1 promoter is responsible for basal *mdm2* transcription and is controlled in part by NF-κB binding sites [29]. The P2 promoter drives transcription from upstream of exon 2 and is controlled by numerous transcription factors. Directly adjacent to the P2 dependent promoter are two binding sites for the transcription factor p53 and in response to stress p53 activates *mdm2* transcription [28, 30]. Other transcription factor binding sites adjacent to the P2 promoter include the Ets/Ap-1, E-box, RXR and Smad binding sites and GC boxes (reviewed in [31]). The Ras signaling pathway can stimulate *mdm2* transcription via the Ap-1/Ets sites [32]. TGF-β signaling stimulates transcription via Smad 2/3 transcription factors binding to the Smad binding sites and MYCN in neuroblastoma cells binds to the E-box near the P2 promoter [33, 34]. The GC boxes are the regions that bind the Sp1 transcription factor. A single nucleotide polymorphism in the GC box region, at position 309, that changes a T to G increases the affinity for Sp1 binding to drive *mdm2* transcription [35]. Patients who are homozygous G/G SNP 309 in the *mdm2* gene have increased susceptibility to multiple cancers [35, 36]. In addition, the tissue-specific RXRγ transcription factor and binding region in retinoblastoma cells can activate P2 dependent *mdm2* expression [37]. High levels of estrogen also activate *mdm2* by activating P2 promoter transcription [38, 39]. While promoter usage has not been shown to be a factor in the alternative splicing of *mdm2* transcripts, the robust signaling of oncogenes present in cancers often drives P2 *mdm2* oncogene mediated transcription [40, 41]. We hypothesize that this increased transcription from the P2 promoter changes the *mdm2* splice variant isoform ratio.

In 2002, Bartel, Taubert and Harris summarized the existence of over 40 different human tumor associated *mdm2* splice variants [2]. At that time the list of distinctive *mdm2* mRNAs was as follows: *mdm2*-fl, *mdm2*-a, *mdm2*-b, *mdm2*-c, *mdm2*-d, *mdm2*-e, *mdm2*-a1, *mdm2*-kb2, *mdm2*-kb3, *mdm2*-jn1, *mdm2*-ds2, *mdm2*-ds3, *mdm2*-is1, *mdm2*-gk1, *mdm2*-pm2, *mdm2*-eu2, *mdm2*-bl, *mdm2*-n, *mdm2*-fb25, *mdm2*-fb26, *mdm2*-fb28, *mdm2*-fb29, *mdm2*-fb30, *mdm2*-fb55, *mdm2*-281 bp, *mdm2*-219 bp, *mdm2*-254 bp, *mdm2*-f, *mdm2*-g, *mdm2*-h, *mdm2*-ln229a, *mdm2-ln229b*, *mdm2*-ln18, *mdm2*-g116, *mdm2*-g150, *mdm2*-var2, *mdm2*-var1, *mdm2-delF*, *mdm2*-delE, and *mdm2*-fb60 (see Table 14.1). These transcripts are found in human cancers, but until recently, no corresponding endogenous protein products had been detected. Many of the *mdm2* splice variant transcripts produced by exon skipping lack the coding region for the p53 interacting domain [2]. The most common variants associated with many different types of human cancers (and missing the p53 interacting domain) are: *mdm2*-a (lacking exons 4–9), *mdm2*-b (lacking exons 4–11), and *mdm2*-c (lacking exons 5–9) (see Table 14.2). All three of these have the potential to endogenously encode oncogenic proteins (see Table 14.3) (reviewed in [23]).

Table 14.3 Biological activity of MDM2-A, MDM2-B, and MDM2-C

Biological/ biochemical activity	MDM2-A	MDM2-B	MDM2-C	References
Increases tumor formation in mice	Yes	Yes	Not Tested	[42, 43]
Alters tumor spectrum in transgenic mice	Yes	Yes	Not Tested	[42, 44]
Overexpression is incompatible with normal mouse development, or viability after birth, in a wt p53 background	Yes	Yes	Not Tested	[42, 43]
Transformation of NIH 3 T3 cells	Yes	Yes	Yes	[6]
Overexpression inhibits proliferation of cells with wt p53	Yes	Yes	No	[26, 43, 45]
Increases proliferation of some cell lines with wt p53	No	Yes	Yes	[26, 42, 45]
Overexpression increases proliferation of cells lacking wt p53	Yes	Yes	Yes	[42, 44, 45]
Upregulates p21, Cyclin D1 and Cyclin E	Yes	Yes	Not Tested	[26, 43]
Inhibits apoptotic signaling by upregulating p65 RelA	Not Tested	Yes	Not Tested	[42]
Binds Mdm2-FL	Yes	Yes	Yes	[24, 43, 45, 46]

Alternatively spliced *mdm2* transcripts in human cancer continue to be detected. The list has increased in number beyond the previously identified 40 (see Table 14.1). The most recent additions to the list of *mdm2* splice variants come from a study of oral squamous cell carcinoma (OSCC). This study shows that *mdm2* splice variants associate with increased likelihood to form OSCC [8] as *mdm2* splice variants are detected in 89 % of oral squamous cell carcinoma [40]. Four splice variants in OSCC are the previously identified *mdm2*-b, *mdm2*-c, *mdm2*-pm2 and *mdm2*-eu2 (with *mdm2*-b found most often). Interestingly, 26 *mdm2* OSCC variants are novel isoforms. Those found to be in-frame range in size from 252 bp to 1,095 bp, and were named: MYO-1, MYO-4, MYO-8, MYO-9, MYO-11, MYO-12, MYO-18, MYO-20, MYO-23, MYO-24, MYO-25 and MYO-32. What is consistent for the in-frame oral cancer variants is that they retain the MDM2 ring-finger binding domain. A significant number of OSCC *mdm2* transcripts are out-of-frame. These range in size from 262 bp to 1,385 bp and were named: MYO-2, MYO-3, MYO-5, MYO-6, MYO-7, MYO-10, MYO-13, MYO-14, MYO-17, MYO-19, MYO-21, MYO-27, MYO-28, MYO-29, MYO-30, MYO-31 and MYO-33.

At least 72 *mdm2* alternative spliced transcripts have been identified in human cancers. This number of 72 includes those 40 compiled in 2002 [2], the OSCC transcripts [8], two novel transcripts that we documented that are driven from the

P2 promoter (P2*mdm2*-10 and P2*mdm2*-C1 [16]) and the RNA-based functional form, *hdm365* [7]. It is likely that the tally of 72 variable *mdm2* transcripts is an underestimate because they continue to be identified. Moreover, while many *mdm2* alternatively spliced transcripts have been detected, the identification of endogenous MDM2 splice variant polypeptides is still lacking. This is partially due to a lack of specific antibodies to detect them. However, the fact that many of the alternative and aberrantly spliced *mdm2* messages are not competent to encode protein suggests that some *mdm2* splice variants might function as regulatory RNA molecules.

The ENCODE project consortium guidelines for functional elements of the genome demonstrates that only a small percentage of the genome (2.9 %) covers areas of protein-coding exons. Furthermore, 62 % of the genome represents RNA molecules with only 5.5 % accounted for in protein-annotated regions [4]. Therefore, the majority of the functional RNA molecules encoded by the human genome represent non-coding regions and for *mdm2* transcripts may indicate a major RNA-based function.

Out-of-Frame Versus In-Frame *mdm2* Transcripts

There are over 70 known splice variant transcripts and they represent alternatively and aberrantly spliced mRNAs (see Table 14.1). Alternatively spliced *mdm2* transcripts are those that result due to exon-exon splicing and give rise, more often than not, to in-frame transcripts with the potential to produce protein [2]. Aberrantly spliced transcripts represent those that result due to the use of cryptic internal splice sites within the *mdm2* exon or intron sequences [2]. Aberrant *mdm2* splicing produces transcripts that are mainly out-of-frame and these do not have the potential to generate protein.

Of the known *mdm2* transcripts, approximately 46 % do not encode protein and all but one of these is spliced out-of-frame to the full-length *mdm2* transcript (Table 14.1, group A). Group A represents this subset of numerous *mdm2* transcripts generated in human cells. One major product in this group is the *hdm365* transcript (in Table 14.1) that potentially has an RNA-based function [7]. This transcript is initiated from the P2 promoter of *mdm2* and retains exons 2, 3, 4 and 5 [7]. The *hdm365* transcript resides in the nucleus and is located at sites of *mdm2* transcription [7]. This localization suggests a role for this *mdm2* transcript in splicing or regulation of the *mdm2* mRNA message.

The *mdm2* transcripts that are assumed, but not proven, to encode protein make up approximately 41.7 % of the identified *mdm2* transcripts (Table 14.1, group B). Group B represents both alternatively and aberrantly spliced *mdm2* transcripts. It is not clear if these transcripts form protein in the cell, as the tools to properly identify each potential MDM2 protein isoform need to be developed.

The final category of *mdm2* transcripts accounts for 12.5 % of the known *mdm2* transcripts. They have been confirmed by in vitro translation assays to encode MDM2 protein isoforms (Table 14.1, group C). Interestingly, only MDM2-FL,

MDM2-A, MDM2-B, MDM2-C, MDM2-D and MDM2-E have been shown to have a biological function in vitro or in vivo [6, 18, 24, 26, 42–44]. Furthermore, none of these MDM2 protein isoforms except for the full-length MDM2 (MDM2-FL) have been detected as expressed endogenously in cancer cells. Although there are high levels of *mdm2* transcripts found in cancers, the level of transcripts do not correlate with high MDM2 protein levels [12, 17]. The reason for this may be due to the absence of proper antibody epitope recognition since antibodies detect some, but not all, MDM2 isoforms within the background of MDM2-FL.

Full-length MDM2, translated from exons 3–12, possesses both oncogenic and tumor suppressive properties [31]. Translation of the MDM2 protein begins in exon 3 and P2-derived transcripts are more efficiently translated than P1-derived transcripts [30]. Some oncogenic properties of MDM2 come from the ability of the protein to interact with the tumor suppressor p53 and target it for proteasome-mediated degradation [47]. However, MDM2 also interacts with the p53 mRNA and increases the translation of p53 protein [48]. This apparent paradox for MDM2 function is increased in complexity by the fact that some *mdm2* splice variants have the capacity to encode polypeptides that lack portions of the p53 interacting domain [2, 31]. Therefore, some of this paradoxical behavior may be explained by determining the functions of specific MDM2 splice variant isoforms.

Mechanisms That Drive Alternative Splicing of *mdm2* Transcripts in Cancer

It is common to find a loss of splicing fidelity in cancer cells [49]. The mechanisms responsible for changes in splicing in cancer continue to emerge. Evidence attributes some of these changes to variations in *cis*-regulatory elements, sequences within the RNA which effect splice-site usage and recognition [50]. Many Serine/Arginine rich (SR) and heterogeneous ribonucleoprotein (hnRNP) splicing factor proteins are up-regulated in cancers and these *trans*- acting splicing factors can increase splicing events [49, 51, 52]. The oncogene c-MYC drives upregulation of specific splicing factors including polypyrimidine-tract binding protein (PTB) and hnRNP A1 and A2 (reviewed in [51–53]). With oncogenes driving alternative splicing, it is not surprising that alternatively spliced transcripts of *mdm2* are found in many different cancers (see Tables 14.1 and 14.2). The *mdm2* splice variants *mdm2-a, mdm2*-b and *mdm2*-c result from exon skipping. This exon skipping occurs because some *mdm2* introns have a defective polypyrimidine tract, a *cis*-regulatory element important for splicing factor binding and 3′ splice site recognition [21]. The splice variants *mdm2*-d and *mdm2*–e on the other hand result from an aberrant splicing mechanism that does not use the normal exon-intron boundaries [21]. Interestingly, some known aberrantly spliced *mdm2* transcripts have a common splicing pattern due to sequences of high homology in the *mdm2* transcript that serve as cryptic splice donor and acceptor sites for splicing factor binding [2].

Alternative splicing of *mdm2* transcripts and transcription from the P2 promoter are also driven by genotoxic stress conditions such as cisplatin or ultraviolet radiation [22, 25]. Some splice variants produced under genotoxic stress conditions, like *mdm2*-b, are seen at high frequency in cancers [40]. A conserved *cis*-regulatory element in intron 11 of the *mdm2* gene promotes this stress-induced regulation of *mdm2* splicing [40]. Stress-induced splicing, in particular that seen with cisplatin treatment, induces co-transcriptional *mdm2* exon skipping through disruption of the EWS-YB1 interaction [41]. EWS is a protein that interacts with the RBP7 subunit of RNA pol II and YB1 interacts with the spliceosome [54, 55]. The stress-induced cotranscriptional exon skipping of *mdm2* produces *mdm2* variants missing the p53 interaction domains. Therefore, exon skipping may help to promote a more robust p53 response by inhibiting the production of MDM2 that interacts with p53 [41].

The Biological Functions of Ectopically Expressed MDM2-A, MDM2-B, and MDM2-C

The biological outcomes of ectopically expressed MDM2-A, MDM2-B, and MDM2-C range from growth activation to growth inhibition under different circumstances (see Table 14.3). The variable outcomes are associated with the presence or absence of wild-type p53 protein. For example, if wild-type p53 is expressed then MDM2-A transgenic homozygous mouse pups die of unknown causes shortly after birth [43]. The only mice that survive with MDM2-A are hemizygous [43]. However, in a *p53*-null background homozygous mice survive and the expression of MDM2-A alters the tumor spectrum of transgenic *p53*-null mice toward increased T-cell lymphomagenesis [44]. Additionally, *p53* heterozygous mice crossed with MDM2-A expressing transgenic mice develop aggressive mammary tumors [44]. Furthermore, the expression of MDM2-A in *p53*-null mouse embryo fibroblasts (MEFs) promotes cell transformation [44]. The exogenous expression of MDM2-A in wild-type MEFs inhibits cell growth. This inhibition of cell growth correlates with an increase in p53 transcriptional activity and high p21 protein levels [43]. Similarly, in the immortalized primary BJ fibroblast cell line ectopic expression of MDM2-A up-regulates p21 along with Cyclin D1 and Cyclin E [26]. Exogenously expressed MDM2-A interacts with endogenous MDM2-FL and activates wild-type p53 activity thus explaining some of the differences seen in a *p53*-null background [43].

Similar to MDM2-A, exogenous expression of MDM2-B also has differential outcomes in the presence or absence of wild-type p53. The exogenous expression of MDM2-B in transgenic mice is not compatible with normal development [42]. Only when MDM2-B is expressed under a promoter with limited tissue expression are mice able to survive. The transfection of the *mdm2*-b into NIH/3T3 cells increases cell proliferation and transformation capabilities [42]. Interestingly, the expression of MDM2-B in NIH/3T3 cells interferes with the induction of apoptosis without

affecting p53 stability or activity and is linked to an increase of p65 RelA protein levels [42]. Surviving MDM2-B transgenic mice with tissue specific expression have increased tumorigenesis that correlates with this increase in p65 protein levels [42]. MDM2-B expression also increases cell proliferation in p53-null, ARF-null and Rb-null MEFs, therefore indicating a p53-independent mechanism of action [42]. However, other studies show exogenously expressed MDM2-B interacts with MDM2-FL protein localizing MDM2-FL to the cytoplasm in numerous cell lines to allow wild-type p53 protein to be activated [24, 46]. Ectopic expression of MDM2-B also up-regulates p21 expression in immortalized BJ fibroblasts correlating with an inhibition of cell proliferation [26].

Our laboratory works on the MDM2-C splice variant. Unpublished studies from our laboratory were presented at the 2011 MDM2 Workshop in New York City and were recently published [45]. We have designed a specific antibody toward MDM2-C to detect the endogenous MDM2-C protein isoform and we have explored the biological functions of MDM2-C. Exogenous expression of MDM2-C in the presence or absence of p53 in H1299 lung carcinoma cells showed increased colony formation as compared to MDM2-FL or vector control [45]. Therefore, like MDM2-A and MDM2-B, MDM2-C shows a p53-independent transformation function. Furthermore, the transfection of *mdm2*-c in the presence or absence of *p53* into H1299 cells increased colony formation, indicated by transforming ability. The co-transfection of *mdm2*-c and *p53* into H1299 cells did not significantly decrease p53 transcriptional activity or change p53 protein levels and MDM2-C was also able to interact with MDM2-FL [45]. Therefore, unlike MDM2-A and MDM2-B, MDM2-C does not increase the activity of wild-type p53. An in vivo mouse model has yet to be carried out for MDM2-C. Until this is done, we will not know the full biological functions of MDM2-C.

Detection of Endogenous MDM2

There are a number of MDM2 specific antibodies that detect the endogenous MDM2 protein in cancer cells and cancer tissues (reviewed in [23]). These MDM2 antibodies recognize epitopes of multiple MDM2 domains including the amino terminus, the central region, and carboxyl terminus of the protein. However, the MDM2 antibodies utilized to determine MDM2 protein levels in cancers are often to the central region. Therefore, they are not appropriate to detect the majority of MDM2 splice variant isoforms. This is especially true since the main antibody used in immunohistochemistry of cancer tissues for MDM2 protein expression is IF2 (Ab-1). The epitope of recognition for the IF2 antibody lies within amino acids 26–169, which represents the p53-binding domain of the MDM2 protein and spans exons 4 and 5 [19, 56]. Therefore, using the IF2 antibody (or any other antibody to a region deleted by a splicing event) will not show a true representation of the levels of MDM2 protein present in the cancer tissue.

Work to examine the expression of endogenous MDM2 splice variant protein isoforms is being carried out in our laboratory. We generated a rabbit polyclonal antibody to the MDM2-C isoform. The MDM2-C rabbit polyclonal antibody specifically detects MDM2-C, and not MDM2-FL, expressed by an in vitro translation system [45]. This MDM2-C specific antibody also detects endogenously expressed MDM2-C in cancer cell lines and cancer tissues. To our knowledge, we are the first group to generate an antibody specific for an MDM2 spliced variant protein isoform. The *mdm2*-c transcript is the third most common *mdm2* transcript found in cancer cells and tissues [1]. Therefore, detection of the MDM2-C protein isoform may provide a new cancer biomarker. MDM2 endogenous expression undoubtedly results from a mixture of *mdm2* transcripts such as *mdm2*-a, *mdm2*-b, and *mdm2*-c. The proteins expressed from these transcripts are all potential cancer biomarkers. It is important that these biomarkers be detected with the proper MDM2 antibodies that are specific for various isoforms. The use of antibodies to MDM2 in the clinic have led to the conclusion that cancers with high levels of spliced variant transcripts have less MDM2 protein [57]. In actuality, not detecting MDM2 protein in breast cancers with *mdm2* splice variant transcripts is a false negative [57]. Future research needs to make use of MDM2 splice-variant specific antibodies, or antibodies to either the extreme amino or carboxyl terminus of MDM2, in order to evaluate the true nature of MDM2 protein expression in cancer.

Summary

The diverse array of *mdm2* splice variants in human cancers suggests they have functional significance and can serve as cancer biomarkers. To date, MDM2 protein biomarker studies have been carried out with antibodies that give false negative results for the accumulation of MDM2 isoforms lacking central regions of the polypeptide. Future MDM2 biomarker studies must be carried out with consideration given to detecting multiple isoforms. In order to detect multiple MDM2 isoforms, antibody reagents must recognize either the amino or carboxyl terminus of MDM2 because as shown in Table 14.1 most *mdm2* splice variants retain these regions. Alternatively, future MDM2 biomarker studies could make use of mixtures of antibodies with specificity to the MDM2 amino and carboxyl termini as well as the specific amino acid splice junction residues for focused splice variants. Recommendations for future MDM2 biomarker studies should combine new methods for the detection of *mdm2* splice variant RNA messages along with the detection of multiple MDM2 isoform proteins. This is because the MDM2 polypeptides and RNA sequences may cooperate in the transformation process. The oncogenic MDM2 pathway is a central node in cancer progression that may make use of many isoforms of the MDM2 protein and *mdm2* RNA and future research should center on this exciting oncogenic hub.

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