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# Aberrant splicing of the DMP1-ARF-MDM2-p53 pathway in cancer

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## Abstract

Alternative splicing of mRNA precursors is a ubiquitous mechanism for generating numerous transcripts with different activities from one genomic locus in mammalian cells. The gene products from a single locus can thus have similar, dominant-negative, or even opposing functions. Aberrant alternative splicing has been found in cancer to express proteins that promote cell growth, local invasion, and metastasis. This review will focus on the aberrant splicing of tumor suppressor/oncogenes that belong to the DMP1-ARF-MDM2-p53 pathway. Our recent study shows that the *DMP1* locus generates both tumor-suppressive *DMP1α* (p53-dependent) and oncogenic *DMP1β* (p53-independent) splice variants, and the *DMP1β/α* ratio increases with neoplastic transformation of breast epithelial cells. This process is associated with high DMP1β protein expression and shorter survival of breast cancer (BC) patients. Accumulating pieces of evidence show that *ARF* is frequently inactivated by aberrant splicing in human cancers, demonstrating its involvement in human malignancies. Splice variants from the *MDM2* locus promote cell growth in culture and accelerate tumorigenesis *in vivo*. Human cancers expressing these splice variants are associated with advanced stage/metastasis, and thus have negative clinical impacts. Although they lack most of the p53-binding domain, their activities are mostly dependent on p53 since they bind to wild type MDM2. The *p53* locus produces splice isoforms that have either favorable (β/γ at the C-terminus) or negative impact (Δ40, Δ133 at the N-terminus) on patients' survival. Since the oncogenic alternative splicing products from these loci are expressed only in cancer cells, they may eventually become targets for molecular therapies.

### Keywords

splicing; oncogene; tumor suppressor gene; cancer; prognosis; DMP1 (DMTF1); ARF; MDM2; p53

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**Author Contributions**

Both authors contributed to all parts of the work.

**Conflicts of Interest**

The authors declare no conflicts of interest related to this work.

## Introduction

The alternative splicing (AS), differential selection of splice sites present within a pre-mRNA, leads to a production of multiple mRNAs from a single gene with divergent functions. A frequent outcome of AS is the production of proteins with a similar, dominant-negative, or even opposing functions as represented by overexpression of oncogenic ΔN isoforms from the *p63* and *p73* loci (1), and many genes in the pro-apoptotic pathway give also rise to anti-apoptotic isoforms by AS (2). This mechanism gives opportunities for cancer cells to produce proteins that accelerate cell growth, inhibit apoptosis, and promote metastasis.

Several splicing factors, RNA-binding proteins regulating AS have been identified as proto-oncogenes and are overexpressed in human cancers (2). Multiple cancer-associated genes such as *Cyclin D1, PKM, Bcl-x, CD44, hnRNP, p63*, and *p73* are alternatively spliced in tumors compared to adjacent normal (2–6). The activities of these tumor-associated splice isoforms vary from regulation of novel biological processes to dominant-negative regulation of the isoforms expressed in normal tissues (1–6).

The tumor suppressor p53 is activated upon cellular stresses such as DNA damage, oncogene expression, or hypoxia, which induces gene signature involved in DNA repair, cell cycle arrest, apoptosis, or autophagy (7, 8). Of note, the *TP53* locus generates multiple splicing variants with different tumor suppressive functions. The primary regulator of p53 protein stability is MDM2 that inhibits transcriptional activity, nuclear localization, and protein stability (9–11). Similarly, the *MDM2* locus give rise to multiple splicing isoforms with different functions (12). Their biological activities, the mode of overexpression, and clinical impacts of these splice variants are discussed.

$p19^{Arf}$ ($p14^{ARF}$ in humans) directly binds to Mdm2 (HDM2), sequesters Mdm2 to the nucleous and neutralize its activity, and thereby activates p53 (13, 14). The Myb-like transcription factor DMP1 (cyclin D-binding Myb-like Protein 1; DMTF1) governs the activity of the ARF-p53 pathway by binding to the *ARF* promoter and through physical interaction with p53 (15–20). The hDMP1-ARF-MDM2-p53 pathway thus provides cell autonomous tumor surveillance that force early stage cancer cells to undergo senescence and/or apoptosis to prevent the development of cancer (13–18). We recently analyzed 110 pairs of normal and cancer tissues from BC for loss of heterozygosity (LOH) of h*DMP1, INK4a/ARF, p53* and gene amplification of *Hdm2*, and found that LOH of the h*DMP1* locus was found in 42% of breast carcinomas while those of *INK4a/ARF* and *p53* were found in 19% and 34%, respectively. LOH for h*DMP1* was found in mutually exclusive fashion with that of *INK4a/ARF* and *p53*, and was associated with low Ki67 index and diploid karyotype, and longer relapse-free survival (20). We also found overexpression of the splicing variant DMP1β in human BC samples and conducted pathological and transgenic mouse studies focusing on DMP1β (21). The genomic structures for the *DMP1, INK4a/ARF, MDM2*, and *p53* loci, the roles of splice variants from these loci in carcinogenesis are discussed in this review.

## DMP1 (DMTF1)

The *Dmp1* gene (*Dmp1α*) was isolated in a yeast two-hybrid screen of a murine T cell library with cyclin D2 bait (22). D-type cyclins physically bind to the DNA-binding domain of Dmp1 and interferes with its ability to bind to DNA and cause cell cycle arrest (23). A later study has shown that Dmp1 (Dmp1α) is a critical transcriptional activator for *Arf* (18). The gene product, $p19^{Arf}$ (or $p14^{ARF}$) stabilizes nucleoplasmic p53 by binding to Mdm2, sequesters it in the nucleolus, and directly inhibits the ubiquitin ligase activity of Mdm2 (13, 14). *DMP1* is a tumor suppressor deleted in ~35% of human non-small-cell lung carcinomas and 42% of BC (20, 24, 25). Mitogenic signals from oncogenic Ras (26) and HER2/neu (27) have been shown to activate the *Dmp1* promoter, while physiological mitogens (28) as well as genotoxic stimuli mediated by NF-κB (29) cause repression. *Eμ-Myc, K-ras*$^{LA}$, *HER2/neu* or *cyclin D1*-driven tumor development was significantly accelerated in both $Dmp1^{+/-}$ and $Dmp1^{-/-}$ mice with no significant differences in the survival between the two cohorts, suggesting that *Dmp1* is haplo-insufficient tumor suppressor (24, 27, 30–32). In *ARF*-null cells, DMP1α directly binds to p53 and inhibits the ubiquitination and nuclear export for p53 (19), providing the secondary mechanism for DMP1α to prevent tumor development in *ARF*-deficient cells (17).

The *hDMP1* locus consists of 18 exons that encode three different splice variants (α, β, γ) with antagonizing biological activities (Fig. 1A; 33). The full-length isoform was designated as *DMP1α* while two other transcripts were named as *DMP1β* and *DMP1γ* (Fig. 1B). The DMP1β and DMP1γ proteins lack most of the DNA-binding and the whole C-terminal transactivation domains present in DMP1α, and therefore, are unable to transactivate $p14^{ARF}$ or other DMP1α-target genes (Fig. 1C; 21, 33). DMP1β was found to block differentiation and stimulate proliferation during PMA-induced differentiation U937 cells to macrophages while DMP1γ had little effect (33, 34). Hence, it was predicted that the DMP1β/γ isoforms have different functions than those of DMP1α for tumor suppression and myeloid differentiation. Since DMP1 is a critical mediator of BC (mammary tumor) suppression in humans and mice (20, 27, 35), we studied the role of the DMP1β/γ splice variants in mammary oncogenesis.

To study whether the *DMP1* mRNA is alternatively spliced in human BC, total RNAs were isolated from both tumors and adjacent neighbor tissue of 46 BC patients, and qRT-PCR was conducted for *DMP1* (21). The *DMP1β/α* isoform mRNA ratio was higher in 15 of 45 BC samples (~33%) than their neighbor pathologically normal tissues and was associated with poor relapse free survival of patients ($p = 0.0287$, $\chi^2 = 4.7859$; Fig. 1D). We them performed immunohistochemistry (IHC) with paraffin-embedded tumor tissues from 63 BC patients using DMP1β-specific antibody RAB. The data indicate that 35 of 63 (56%) BC specimens were highly stained with the RAB antibody relatively to the surrounding breast epithelial tissues (21). The patients with high DMP1β staining in the tumors relapsed earlier than the patients with low DMP1β ($p = 0.0050$, $\chi^2=7.8653$; 21). There was no correlation between DMP1β protein expression and LOH of the *DMP1* locus, suggesting that these two events occurred independent of each other. Taken together, our data indicate that the DMP1β protein is frequently overexpressed in BCs, which has negative impact on the BC patients' survival.

To examine the DMP1β function *in vivo*, we established *MMTV-DMP1β*$_{V5His}$ transgenic mouse lines (21). The transgenic females (42% multi-parous) developed mammary tumors with a mean latency of 16 months. Thus the onset of mammary tumors in *MMTV-DMP1β*-transgenic females was earlier than that of *MMTV-cyclin D1/D3/E, c-rel*, but later than *MMTV-neu* mice (21, 36). IHC demonstrated intense staining of the proliferation markers, Ki67 and cyclin D1 in *DMP1β*-transgenic mammary glands (21). Since *MMTV-Dmp1α* mice never developed mammary tumors, but rather inhibited tumor development in *MMTV-neu* mice (35), DMP1β has a distinct role from DMP1α *in vivo* (21).

In summary, the h*DMP1* locus generates both oncogenic (*DMP1β*) and tumor-suppressive (*DMP1α*) transcripts. It should be noted that the activity of DMP1β is independent of p53 in a BC cell line (21) although it has the ability to antagonize the activity of DMP1α in ARF induction, which was shown in DNA-binding assays and in cells by the close proximity of DMP1α/β in the nucleus (37). Since mammary tumors from *MMTV-DMP1β* mice show high expression of Ki67 and cyclin D1, it is likely that DMP1β is in the upstream of signaling cascades involving these molecules. Identification of DMP1β-binding proteins by mass spectrometric analyses and/or GeneChip microarrays will be essential to reveal the signaling pathways governed by DMP1β.

## INK4a/ARF

The *INK4a/ARF* locus on human chromosome 9p21 encodes a member of the INK4 family cyclin-dependent kinase inhibitors p16$^{INK4a}$ that regulate progression through the G1 phase of the cell cycle through RB (38) and an independent transcript named as an alternative reading frame gene named *ARF*, the product of which that antagonizes the activities of MDM2 in p53 inhibition (13, 14; Fig. 2A). Inactivation of the *ARF/INK4a* locus occurs by gene deletion, point mutation, and/or promoter hypermethylation, which have been reported in nearly half of human cancers (39). Since p16$^{INK4a}$ and ARF do not share any amino acid homology and regulate different pathways, *ARF* is not considered to be a splice variant for *INK4a*. However, both p16$^{INKAa}$ and ARF are tumor suppressors as demonstrated by gene knockout studies, acting in a non-redundant manner to contribute to tumor suppression since tumor development is accelerated in animals that lack both genes (13, 14, 38).

In addition to *INK4a* and *ARF*, the *INK4a/ARF* locus encodes the third transcript named *p12* in the human pancreas through the use of an alternative splice donor site within intron 1 (40). The transcript produces a 12kD protein composed of *INK4a* exon 1α and a novel exon (dark colored box at the C-terminus; (Fig. 2B). Although overexpression of p12 in human pancreatic cancer cells led to cell arrest at the G1 phase, it did not have any CDK4-inhibitory activity (40). Conversely, p12 exhibited a transactivating activity equivalent to 12% of wild type (WT) p53, the property of which were not found in 16$^{INK4a}$. Poi et al. examined the genetic status of *p12* in 40 pancreatic cancer specimens and found that *p12* alteration was found in of 70% of tumors (41). These results support that p12 is a tumor suppressive protein different from p16$^{INK4a}$ or 14$^{ARF}$, and its genetic inactivation is associated with pancreatic carcinogenesis.

Lin et al. reported the 4th transcript from the *INK4a/ARF* locus termed *p16γ*, in a primary T-cell acute lymphoblastic leukemia (T-ALL) patient sample and a neuroblastoma cell line (42; Fig. 2B). p16γ is identical to $p16^{INK4a}$ except that it contains an in-frame insertion of 197-bp between exons 2 and 3 (Fig. 2B). *p16γ* expression is detected in the majority of $p16^{INK4a}$-expressing primary ALL samples and other tumors, but was barely detectable in normal tissues. Functional analysis of p16γ revealed that it interacts with CDK4 and inhibits its kinase activity indicting that it is a novel regulator of the RB pathway (42).

Aberrant splicing has also been reported in the *INK4a/ARF* locus. Prowse et al. reported a splice acceptor site mutation for $p16^{INK4a}/p14^{ARF}$ within a BC, melanoma, neurofibroma-prone family. This splice site mutation affected both $p16^{INK4a}$ and $p14^{ARF}$, and appeared to result in transcripts that lack exon 2 (Fig. 2; 43). Other studies have also shown that the *ARF* transcript is lost as frequent as *INK4a* in human cancers by aberrant splicing (44, 45), consistent with the results of *ARF*-specific knockout mouse studies (13, 14). Together, both $p16^{INK4a}$ and $p14^{ARF}$ would be functionally inactive in human cancers with splice mutation.

## MDM2

The mouse and human *MDM2* gene spans 22–32kb of genomic DNA consisting of 12 exons (46, 47). Exons 2 to 11 contain the coding sequence of *MDM2*, whereas exons 1a and 1b are untranslated regions that regulate the rate of translation. The genomic structure for the human *MDM2* (*HDM2*) promoter region is shown in Fig. 3A. The locus has two independent promoters: P1 is located at 5' of exon 1a, P2 is between exons 1a and 1b. The p53 (asterisks; 48), AP1/ETS, and Smad (inverted triangles) - binding sequences are found within the first intron (10). Intron 1 also has retinoic acid receptor-binding sites (RXR; diamond), and the GC box containing *SNP309* associated with increased cancer susceptibility (49). HDM2 has been shown to be overexpressed in human soft tissue sarcomas, osteosarcomas, esophageal carcinomas, breast/bladder/ovarian carcinomas, etc. by gene amplification, promoter activation, or other unknown mechanisms with poor prognosis (10, 20, 50, 51).

The diversity in *MDM2* transcripts is caused by differential usage of the two promoters and alternative pre-mRNA splicing. More than 40 different *Mdm2* and *MDM2* alternative and aberrant transcripts have been identified in tumors and normal tissues (12, 51–53). In addition to the full-length (FL) 90-kDa protein, several truncated *HDM2* isoform products of 85, 76, and 57 kDa have been described in human breast carcinomas (54). The domain structures of the human full-length MDM2 (MDM2-FL) and its splice variants and their correlation with exons are shown in Fig. 3B (10, 55). Notably, the *MDM2* splice variants lack most of the amino acid sequences for p53-binding; the nuclear localization/export sequences (NLS/NES), but retain zinc finger and ring finger domains (Fig. 3B). Some of them (MDM2-B, MDM2-KB2, MDM-FB25) lack the acidic domain required for ubiquitinated MDM2 and p53 to be degraded by cytoplasmic proteasomes. *MDM2-A* is a common splice variant that is frequently detected in many tumors (56). The most abundant splice isoform *MDM2-B* has been described in various tumor types as well as normal tissues whereas many of the other variants have only been detected in one particular tumor type

(51). The mRNA increases in response to genotoxic stress, suggesting a substantial role for these transcripts (51).

In soft tissue sarcomas, *MDM2-A, MDM2-B*, and *MDM2-KB2* (Fig. 3B) are expressed. In rhabdomyosarcoma (RMS), six alternatively spliced transcripts were observed including *MDM2-A, MDM2-B, MDM2-C, MDM2-A1, MDM2-FB25*, and *MDM2-FB29* (Fig. 3B; 12). The isoforms lacking most of the p53-binding domain may function similar to MDM2-B, which can bind MDM2-FL and inhibit its ability to bind p53, and therefore limit its ability to target p53 for degradation (57).

## Functions of *MDM2* Isoforms

Early studies suggested that many *MDM2* splice variants display a dominant-negative effect on WT MDM2 (MDM2-FL). Indeed, upon binding of MDM2 splice variants with an intact COOH-terminal RING finger domain to MDM2-FL, the p53 protein becomes stabilized, resulting in a growth-inhibitory phenotype (51, 57). However, many Mdm2 splice isoforms lack most of the p53-binding domain (Fig. 3B) and accelerate tumorigenesis *in vivo*. Volk *et al.* reported that MDM2-A expression enhances p53 activity and decreases transformation *in vitro*, but it cannot confer tumor protection (56). Indeed, MDM2-A enhanced transformation of *p53*-null cells and changed the tumor spectra in both *Arf*- and *p53*-null mice. Steinman *et al.* demonstrated that *MDM2-B* promotes tumorigenesis in a transgenic mouse model (58). Fridman *et al.* showed that the murine equivalents of the human *MDM2-B*, *-D*, and *-E* splice variants significantly accelerated lymphomagenesis in an *Eμ-Myc* transgenic mice (59). Okoro *et al.* reported that the MDM2-C protein was highly expressed in human cancers and functioned as a p53-independent growth activator (60). These data provide evidence that most *MDM2* isoforms can contribute to tumor development *in vivo*. The outstanding difference in the activity of *MDM2* splice variants *in vivo* (accelerated tumorigenesis) and cultured cells (inhibition of cell growth) can be explained by the possibility that the *MDM2* splice variants act as oncogenes, and their forced expression trigger growth arrest in primary cells while they promote cell proliferation in cancer cells where the Arf-p53 pathway is inactivated.

## Prognostic values for aberrant *MDM2* splice variants

Yu et al. (52) studied 69 colorectal cancer specimens for *MDM2* mRNA and *p53* mutations. While 90% of colorectal cancer expressed the *MDM2-FL*, approximately half of them showed expression of some *MDM2* splice variants. Although there was no correlation between the expression of *MDM2* splicing variants and *p53* mutation(s), expression of *MDM2* splice isoforms was associated with advanced tumor stage and distant metastasis in WT *p53* cases (52).

Jacob et al. (61) studied *MDM2* splice variants in RMS. They found strong association of *MDM2-ALT1* (equal to *MDM2-B* in Fig. 3B; 62) with high-risk metastatic RMS. The *MDM2-ALT1* expression directly contributed to metastatic behavior and promoted the invasion of RMS cells through a matrigel-coated membrane. The *MDM2-ALT1* has become the first known molecular marker for high-grade metastatic disease in the most common RMS subtypes.

## TP53

Although p53 was originally cloned as an oncogene, the WT protein is a transcription factor that causes tumor suppression by mediating cellular functions including, senescence, apoptosis, DNA repair, and autophagy (7, 8, 63). The p53 protein is largely regulated by a negative feedback loop with MDM2, an E3 ubiquitin-protein ligase that mediates proteasomal degradation and nuclear export of p53 (9–11). Mutations in *p53* that disrupt the transactivating domains often contribute to tumorigenesis with gain-of-functions that contribute to all stages of tumor initiation and development (64). The human *p53* locus, *TP53* contains 11 exons (Fig. 4A), which encode major functional domains including the two transactivation domains (TAD1/2, 65), proline-rich domain (PRD), DNA-binding domain (DBD), nuclear import signal (L), tetramerization domain (4DE), and C-terminal regulatory domain (CTD; Fig. 4B).

The *TP53* genomic locus has two independent promoters, P1 and P2 (Fig. 4A). P1 starts at 5' of exon 1 and generates full-length (FL) p53 (p53α) and Δ40p53 while the P2 located in intron 4 generates N-terminal truncated variants, Δ133 and Δ160p53 (Fig. 4B). Theoretically, 16 isoforms are generated from four different N-terminal isoforms (FLp53, Δ40, Δ133, and Δ160p53) with four different C-terminal splice variants (α, β, γ, and δ generated by alternative splicing, Fig. 4B; 66). p53 is regulated at both transcriptional and post-translational levels (7–9). The *p53* promoter P1 has putative binding sites for transcriptional factors: YY-1, NF-κB, NF-1, AP-1, and basic helix-loop-helix family proteins (67). CpG site methylation for the P1 promoter was detected in some cases of BC (67).

The Δ40p53 isoform lacks TAD1 but retains TAD2, allowing for minimal transactivation of most p53 target genes (Fig. 4B; 68). However, Δ40p53 impairs growth suppression by p53 through oligomerizing with FLp53. In *p53*-null cells, Δ40p53 expression alone was insufficient to mimic the transcriptional activity of a p53-response element reporter, and co-transfection of Δ40p53 with FLp53 decreased total p53 transcriptional activity in a dose-dependent manner. However, low Δ40p53/FLp53 expression ratio increased p53 transcriptional activity over FLp53 alone due to Δ40p53 protection of FLp53 from Mdm2-mediated degradation (69). Thus Δ40p53 has differential effects on FL53 dependent on its level of expression: high Δ40p53 blocks FLp53 activity while low Δ40p53 stimulates it. The second largest ΔNp53 isoform, Δ133p53 lacks both TAD1/2 and PRD, and inhibits FLp53 activity (Fig. 4B, 70). Δ133p53α does not bind to p53-response elements, but inhibits FLp53 from binding to its target genes. Δ133p53 is expressed in a p53-dependent manner since P2 contains p53-resposive elements, raising the possibility that this isoform participates in a negative feedback loop to delicately modulate the FLp53 activity (70).

Bourdon et al. (68) cloned two *p53* isoforms using mRNA from normal human colon (Fig. 4A, see *α/β/γ*). They found that the short bands corresponded to three different splices of intron 9 of the human *p53* mRNA initiated in intron 4 (i.e. Δ*133p53α/β/γ*). *p53β* encodes a truncated p53 protein terminating with 10 additional amino acids (DQTSFQKENC), while the *p53γ* transcript produces a truncated p53 protein terminating with 15 additional amino acids (MLLDLRWCYFLINSS, Fig. 4B). Both splice variants are expected to encode the

p53 protein without the oligomerization domain due to stop codons in exon i9 (Fig. 4A). Other p53 variants (p53δ and p53ε) were reported by Hofstetter et al. in ovarian cancers (71). Future functional studies will be needed to define their roles in normal and neoplastic tissues.

Both p53β and γ proteins are found in the nucleus since they retain the NLS signal. Transfection of the *p53β* gene in the absence of WT *p53* had no effect on $p21^{CIP1}$ or *BAX* promoter activation. However, *p53β* co-expression with WT *p53* enhanced the p53 activity on the *BAX* promoter, but not on the $p21^{Cip1}$ promoter, suggesting promoter-specificity for this mechanism (68). Graupner et al. reported that p53β/γ proteins did not oligomerize to bind to DNA nor did they bind to WT p53 or MDM2 despite an efficient nuclear localization (72). These isoforms might indirectly modulate the WT p53 function by competing for its negative regulators preventing WT p53 from degradation. Fujita et al. reported that high p53β and low Δ133p53 were associated with replicative senescence in normal human fibroblasts (73). p53β cooperated with FLp53 to accelerate cellular senescence. The senescence-associated pattern of p53 isoform expression (i.e. high p53β and low Δ133p53) was observed in colon adenomas with senescent phenotypes. Thus high Δ133p53 and low p53β isoform expression in colon cancer may signal an escape mechanism from the senescence barrier during the progression from adenoma to carcinoma (73).

## Expression of p53 variants in cancer and their prognostic values

Germline mutations affecting *TP53* splicing have been reported in a number of families with Li-Fraumeni syndromes, suggesting that both coding and non-coding regions have to be examined in this hereditary disorder (74–76). BC is the most common malignancy in women; however, somatic *p53* mutation is not very common (18–25%; 77) in BC, suggesting that p53 may be inactivated by different mechanisms. Bourdon et al. (68) analyzed C-terminal *p53* splice variants in 30 BC patients where 5 of them had *p53* mutations (17%). In contrast to normal breast tissue where all three C-terminal splice variants are detected, Δ133p53α was found in 24 of 30 (80%), raising the possibility of inactivation of the p53 pathway by aberrant *p53* splicing rather than point mutation since Δ133p53α inhibits FLp53 from binding to its target genes.

Thompson et al. examined the clinical relevance of C-terminal *p53* splice variants in 248 patients with BC (78). *p53* mutation was identified in 26% cases, associated with shorter survival, histological grade 3, and estrogen receptor (ER)-negativity. Notably, the patient groups with a mutant *p53* cancer expressing p53β/γ isoforms had survival curves comparable to those with tumor(s) having WT *p53* (78). Similarly, p53β/γ expression was associated with ER-negative cancers with improved survival similar to ER-positive cancers (78). Thus the p53β/γ isoforms improve the survival of BC patients with *p53* mutation(s) or lacking ER.

Avery-Kiejd et al. analyzed relative mRNA expression of *p53* isoforms in BC (79). Δ40p53 was significantly upregulated in tumor tissue compared to the normal breast, and was significantly associated with an aggressive, triple-negative subtype (79). Conversely, p53β expression was negatively associated with tumor size and longer disease-free survival; thus

high p53β levels were protective, particularly in patients with a *p53* mutation, consistent with the report from Thompson et al (78).

Mutation of the *p53* tumor suppressor gene occurs very frequently (~96%) in high-grade serous ovarian cancer. Hofstetter et al. (80) examined the relationship between the expression of two p53 isoforms (Δ133p53 and Δ40p53) and prognosis in patients with serous ovarian cancer. They found that Δ133p53 constitutes an independent prognostic marker for improved recurrence-free and overall survival in patients with mutant *p53* suggesting that Δ133p53 might suppress the actions of mutant p53 (80). Novel therapeutic approaches could be built upon these findings since Δ133p53 blocks the activity of mutant p53 that often have gain-of-functions associated with aggressive disease and resistance to therapies.

Further details of *TP53* splice variants are discussed in references #81 – #83.

## Conclusive Remarks and Future Directions

Alternative splicing is a mechanism to increase the functional diversity of a gene from a single genomic locus. Both mutations in cis-acting splicing elements and changes in the expression/activity of splicing factors affect the splicing of numerous oncogenes and tumor suppressor genes. Signaling pathways governed by splice variants of DMP1-ARF-MDM2-p53 are summarized in Fig. 5. The activities of splice variants from this pathway converge on p53 except for *DMP1β* and novel transcripts from the *INK4a/ARF* locus - *p12* and *p16γ*. Since DMP1β induces cyclin D1 and Ki67 *in vivo*, the activity of this splice variant may be dependent on RB rather than p53. Conversely, splice variants from the *MDM2* locus are mostly dependent on p53 although they do not have the p53-binding domain, possibly through their physical interaction with WT MDM2. Both ΔNp53 and p53β/γ variants show their activities through modulation of WT p53.

Although early studies stressed the relative importance of $p16^{INK4a}$ than ARF in human cancers due to high frequency mutations of the former, recent studies have shown that the *ARF* transcript is lost as frequent as *INK4a* in human cancers by aberrant splicing. Inactivating point mutations for *INK4a* and aberrant splicing for the *INK4a/ARF* locus are considered to be independent since simultaneous inactivation for these have not been reported. In case of *p53* splice variants, the biological consequences and prognostic values of p53 isoforms are dependent on the status of *p53* since mutant p53 often has gain-of-functions that are advantage.

The underlying mechanisms causing alternative splicings have not yet been studied except for several publications on *MDM2* and *p53* (84–87), which is an important avenue of research in the future. Since the oncogenic alternative splicing products from these loci are expressed only in cancer cells, they may eventually become targets for molecular therapies.

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## Figure Legends

88. Mallakin A, Sugiyama T, Kai F, et al. The Arf-inducing transcription factor Dmp1 encodes transcriptional activator of amphiregulin, thrombospondin-1, JunB and Egr1. Int J Cancer. 2010; 126:1403–1416. [PubMed: 19816943]

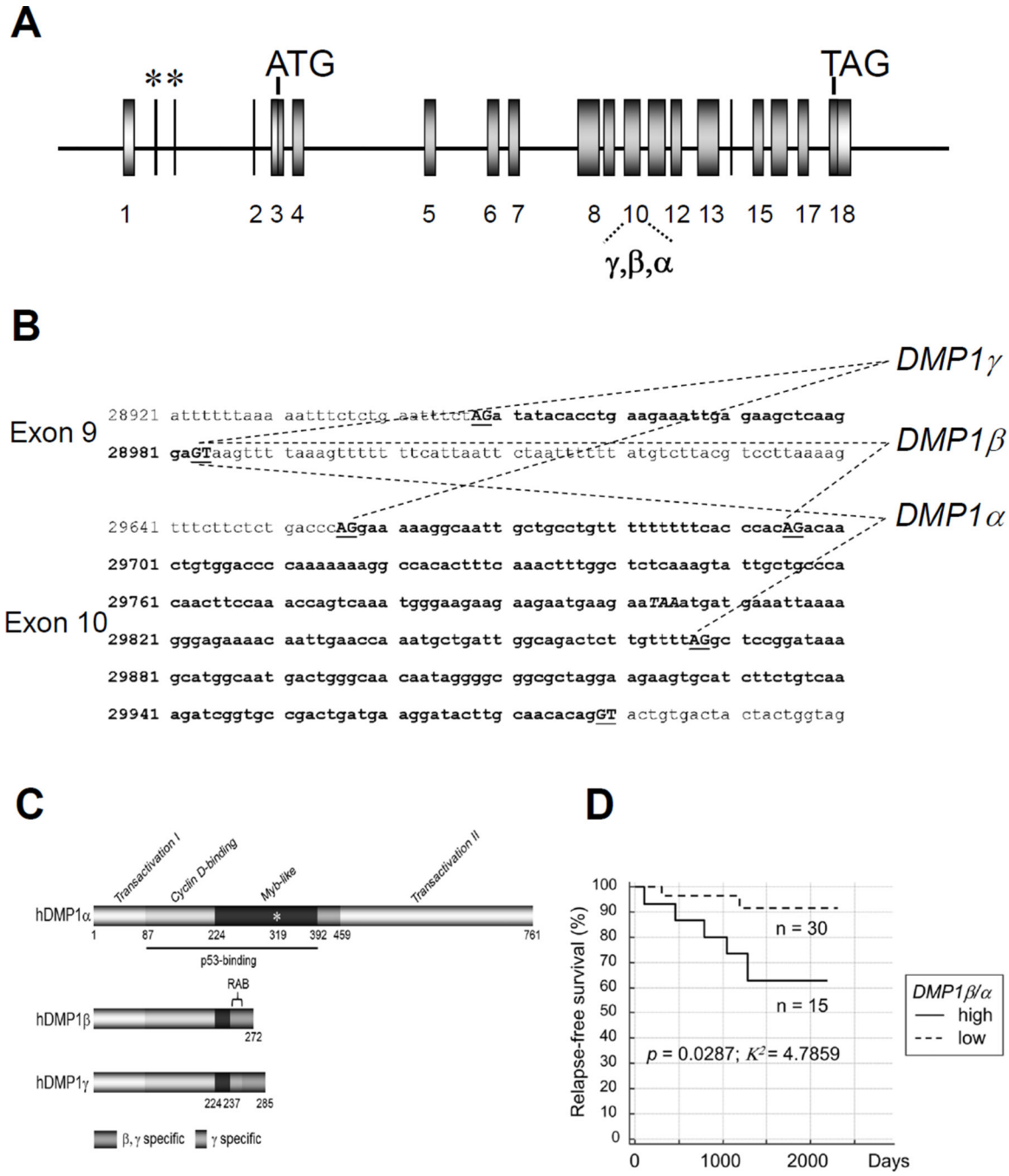


**Figure 1. The structure of the *hDMP1* locus and generation of the three splice variants**

**A.** The structure of the human *DMP1* locus on chromosome 7q21. DMP1 is a cyclin D-binding Myb-like Protein 1 that prevents tumor development by transactivating the *Arf* promoter as well as directly activating p53 through physical interaction (15–20, 88). Non-coding regions are colored white while coding regions are colored gray, respectively. The *DMP1* gene consists of 18 exons, of which 15 (exons 3–18) are encoding proteins. There are two very short exons between exon 1 and 2 (asterisks), which may regulate the rate of translation for *DMP1* mRNAs.

**B.** The nucleotide sequence of the *hDMP1* genomic locus, exon 9, intron 9, and exon 10. The exons are shown in bold and the introns are shown in normal. Splice donor (GT) and acceptor (AG) sites are underlined. The *hDMP1γ* mRNA is spliced from exon 9 to exon 10*γ*, the *hDMP1β* mRNA is spliced from exon 9 to exon 10*β* while the *hDMP1α* mRNA is spliced from exon 9 to exon 10α. The common stop codon for *DMP1β* and *γ* (TAA) has been shown in italic capital.

**C.** The DMP1α protein consists of 761 amino acids in mice and 760 amino acids in humans. Since there is a TAA stop codon in exon 10*β*, the *hDMP1β* and *γ* mRNAs generate truncated proteins with lower molecular weights, lacking most of the Myb-like repeats. The DMP1β/γ-specific region was used for immunization to raise an antibody to DMP1β, named RAB.

**D.** High *DMP1β/α* mRNA ratio (solid line, n = 15) is associated with shorter relapse-free survival in BC than the control group (discontinuous line, n = 30). $p = 0.0287$, $\chi^2 = 4.7859$.

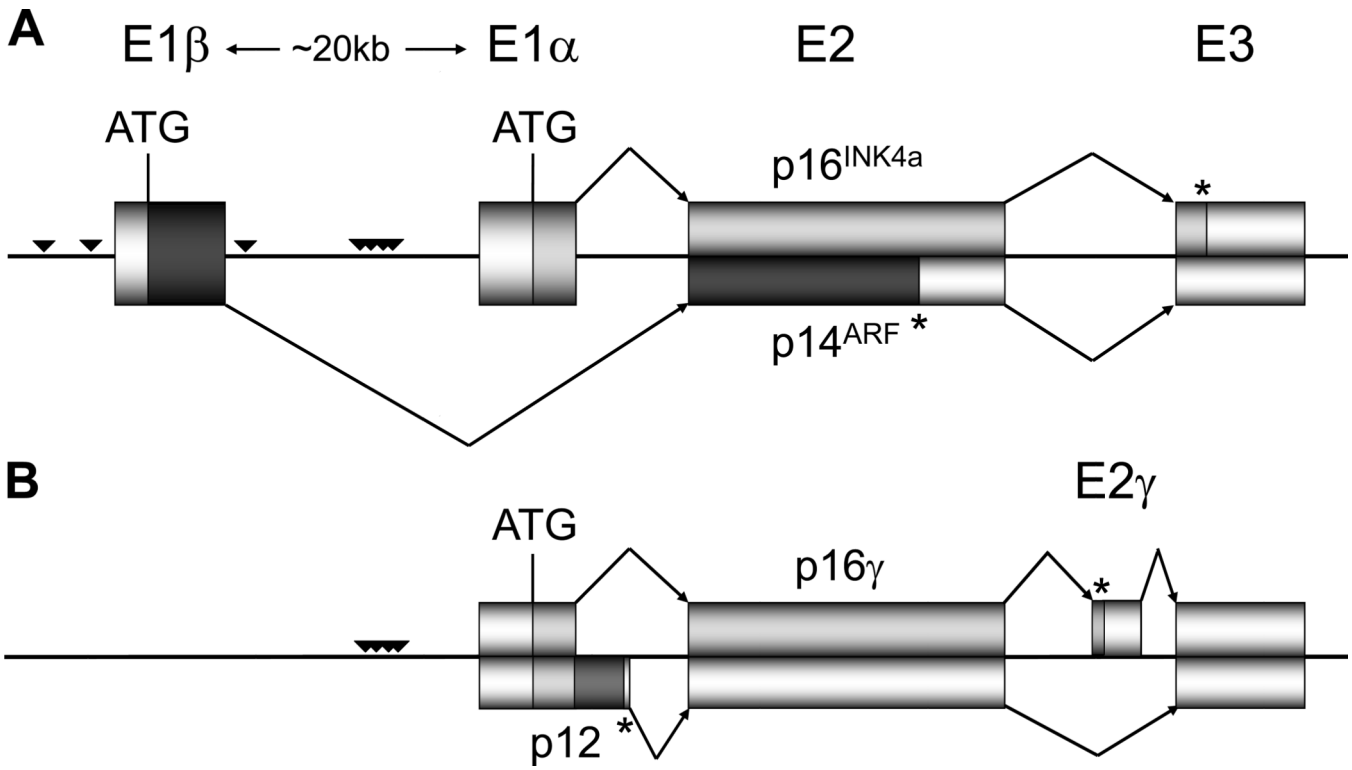


**Figure 2. The genomic structure of the human *INK4a-ARF* locus**

**A.** The *INK4a* mRNA starts from exon 1α, which is spliced into common exons 2 and 3. The *ARF* (alternate reading frame) mRNA starts from exon 1β, spliced into common exons 2 and 3. Only exon 1β and exon 2 are used to generate the ARF protein. The reverse triangles show DMP1α-binding consensus sequences. A stop codon is indicated by an asterisk (*).

**B.** The human *INK4a-ARF* locus generates two other transcripts, namely p16γ and p12 related to *INK4a*. The *p16γ* mRNA starts from exon 1α, exon 2, followed by a new exon 2γ, and exon 3, generating a different 16 kDa protein. A stop codon is indicated by an asterisk (*). The *p12* mRNA starts from exon 1α but has an insertion at the end of the exon, which will then be spliced into exons 2 and 3. This produces a shorter form of the protein that consists of exon 1α and the unique region in exon 1α, followed by a stop codon.

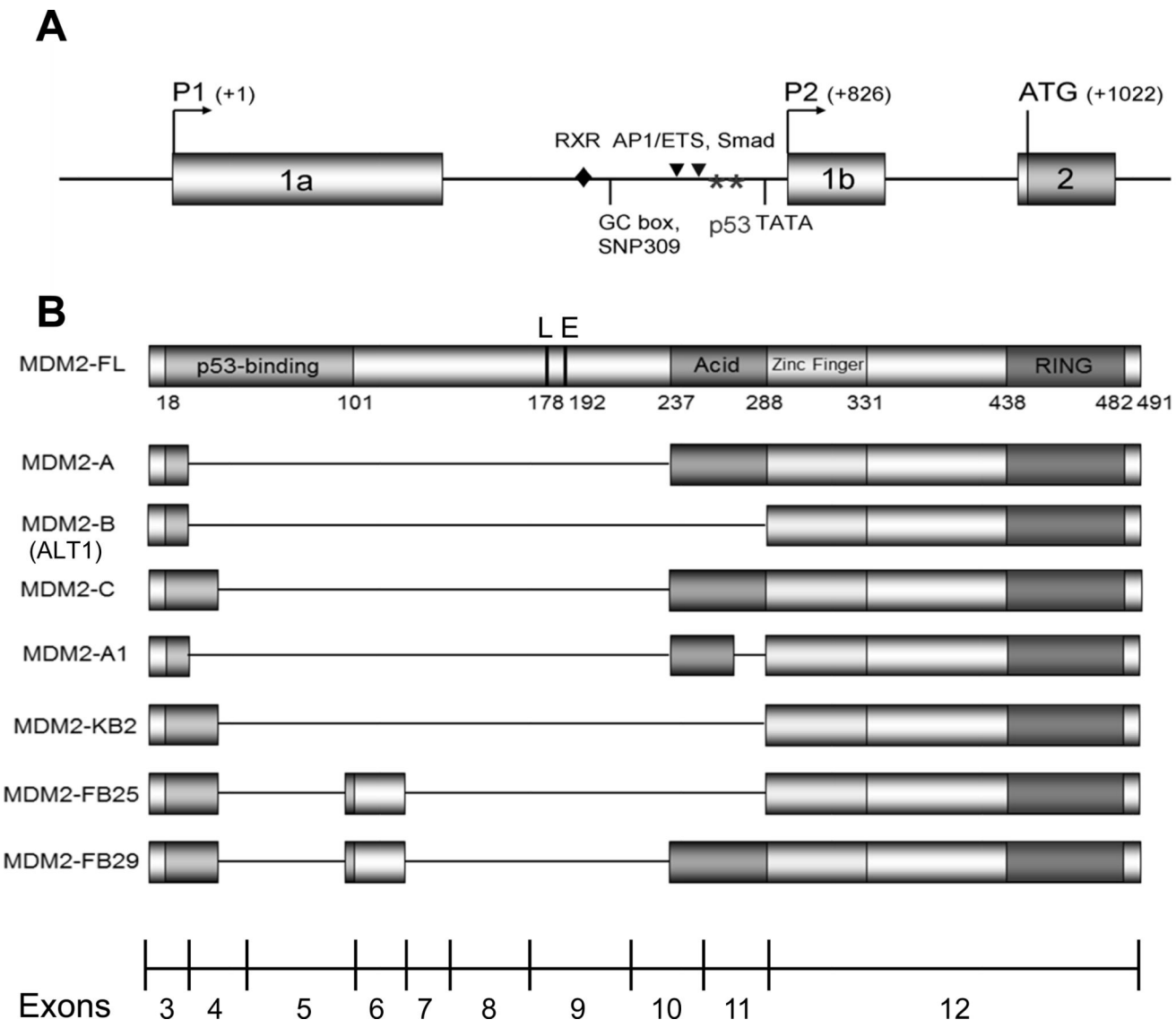


**Figure 3. The promoter and protein structure of the human *MDM2* splicing variants**

**A.** Genomic structure for the human *MDM2* promoter region. The locus has two independent promoters before exons1a (P1) and 1b (P2). The TATA box is between p53-binding sites and exon 1b. ATG is in the exon 2. The p53 (asterisks), AP1/ETS, and Smad-consensus sequences (inverted triangles) are in the intron 1. Intron 1 also has retinoic acid receptor-binding sites (RXR), the GC box containing SNP309.

**B.** Schematic representation of the full-length (MDM2-FL) and alternative spliced human *MDM2* mRNAs (12, 53) in human soft tissue sarcoma samples. Most *MDM2* spliced forms lack the p53-binding domain, nuclear localization (NL), and nuclear export (NE) signals. The bottom line shows the genomic structure for *Mdm2* (mouse). The exon 3 corresponds to exon 2 in Fig. 3A.

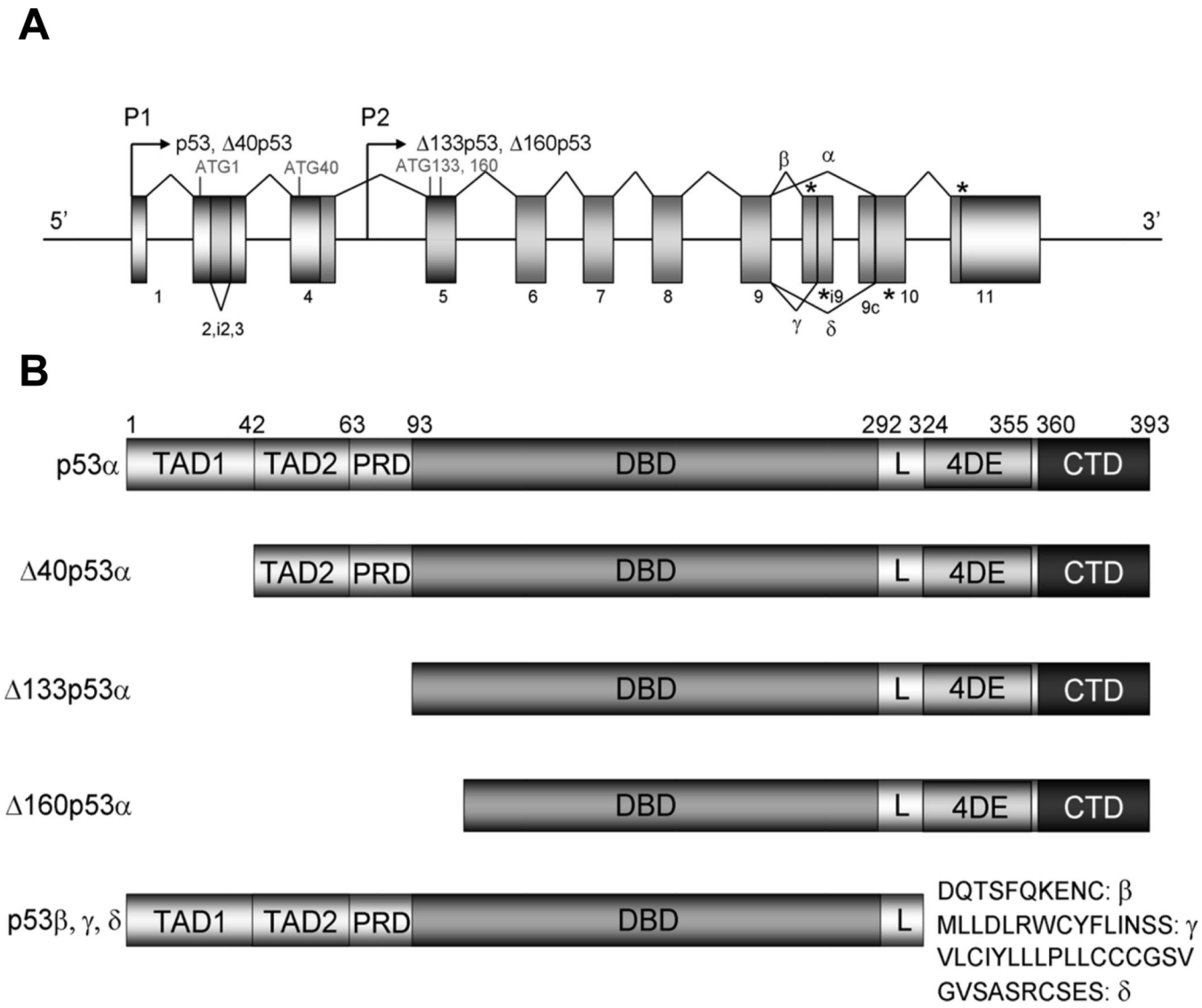


**Figure 4. The genomic and protein structures of the full-length p53 and its splice variants**

**A.** The genomic structure for the human *p53* locus (68, 71). Non-coding regions appear in white while coding regions are shown in gray, respectively. The stop codon is indicated by an asterisk (*). The *p53* locus has two independent promoters: P1 generates the full length p53 (p53α) and Δ40p53 while P2 generates Δ133p53 and Δp160p53. Δ133p53α plays an active role in tumor initiation and progression. Δ160p53 is a novel N-terminal *p53* isoform encoded by the Δ133p53 transcript. These two isoforms are differentially expressed in normal human tissues (83). Moreover, the locus generates *p53β, γ, δ* transcripts by alternative splicing at the 3' end. Theoretically there are 16 splice variants expressed in cells. **B.** The protein structures for full-length p53 (p53α) and its splice variants. Human p53 consists of 393 amino acids with 6 proposed domains. They are TAD1: transactivation domain 1; TAD2: transactivation domain 2; PRD: proline-rich domain; DBD: DNA-binding domain; L: nuclear import signal; 4DE: tetramerization domain; and CTD: C-terminal regulatory domain. MDM2 binds to TAD1. Δ40p53 lacks TAD1, but has TAD2 and PRD.

p53β/γ/δ lack the tetramerization and C-terminal regulatory domains, which are replaced by unique amino acid sequences.

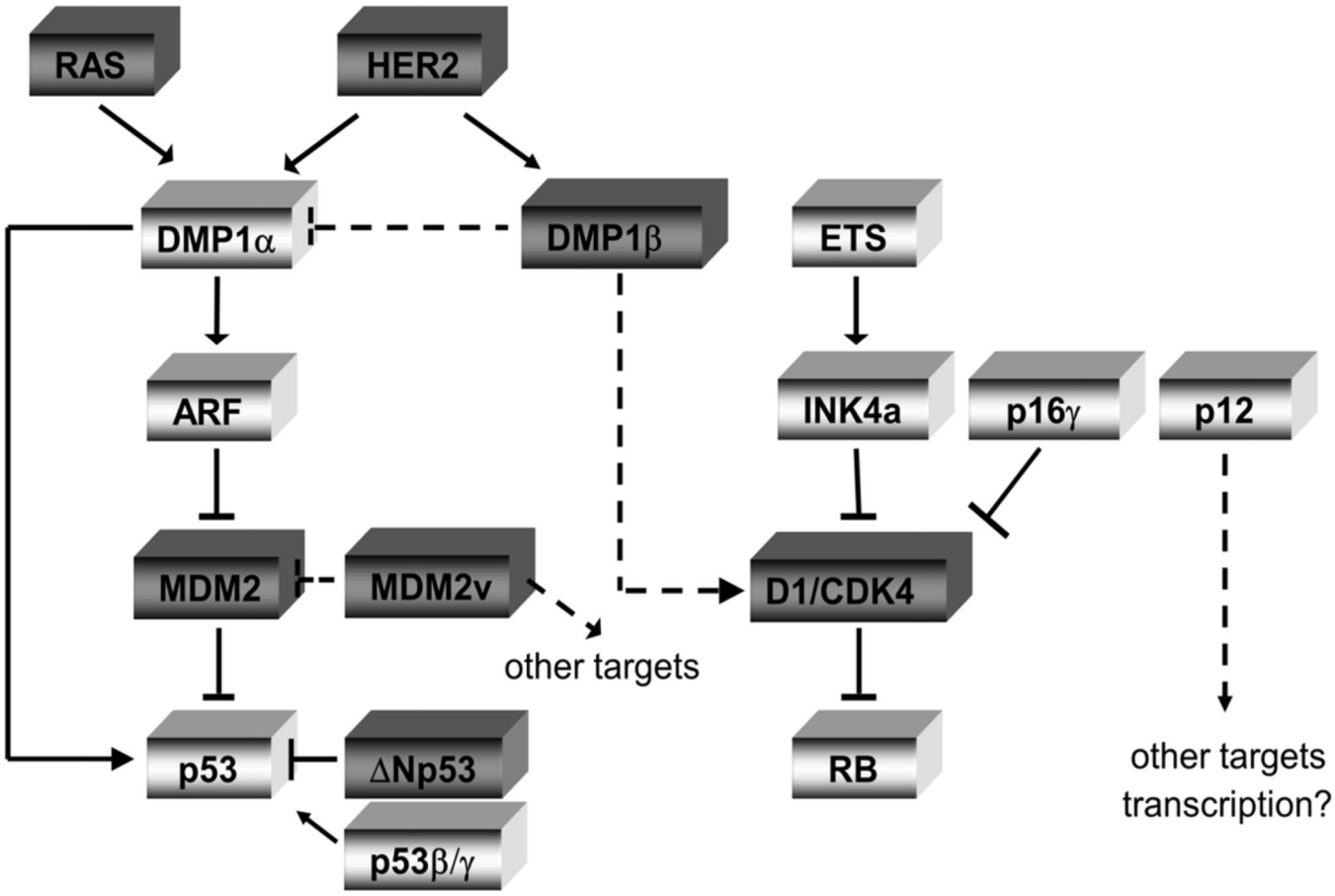


**Figure 5. Signaling pathways involved in aberrant splicing of the DMP1-ARF-MDM2-p53 pathway**

The *DMP1* gene is unique in that it produces both oncogenic (DMP1β) and tumor-suppressive proteins from the single genomic locus. DMP1α causes cell cycle arrest in Arf-p53-dependent fashion while DMP1β stimulates G1-S progression independent of p53 (21). Since both Ki67 and cyclin D1 proteins are upregulated in *MMTV-DMP1β* mice, DMP1β is considered to be in the upstream of the cyclin D1/CDK-RB pathway, but the molecular details for its action need future research. The activity of 16γ is dependent on the RB pathway while p12 does not have the CDK4-inhibitory activity but causes gene transactivation/repression, and thus might have other targets than RB (40–42). Splice variants from the *MDM2* locus act through MDM2, and thus are mostly dependent on p53 regardless of the fact that they lack the p53-binding domain. Both ARF and MDM2 have other targets than p53. Published studies indicate that p53 variants (both N- and C-terminal isoforms) are dependent on p53 for their biological activities.