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Dmp1 and tumor suppression

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Abstract

Dmp1 (cyclin *D* binding *m*yb-like *p*rotein *1*; also called Dmtf1) is a transcription factor that was isolated in a yeast two-hybrid screen through its binding property to cyclin D2. Although it was initially predicted to be involved in the cyclin D-Rb pathway, overexpression of Dmp1 in primary cells induces cell cycle arrest in an Arf, p53-dependent fashion. Dmp1 is a unique *Arf* regulator, the promoter of which is activated by oncogenic Ras-Raf signaling. *Dmp1* expression is repressed by physiological mitogenic stimuli as well as by overexpressed E2F proteins; thus, it is a novel marker of cells that have exited from the cell cycle. Spontaneous and oncogene-induced tumor formation is accelerated in both $Dmp1^{+/-}$ and $Dmp1^{-/-}$ mice; the $Dmp1^{+/-}$ tumors often retain and express the wild-type allele; thus, Dmp1 is haplo-insufficient for tumor suppression. Tumors from $Dmp1^{+/-}$ and $Dmp1^{-/-}$ mice often retain wild-type Arf and p53, suggesting that Dmp1 is a physiological regulator of the Arf-p53 pathway. The human DMP1 (*h*DMP1) gene is located on chromosome 7q21, the locus of which is often deleted in myeloid leukemia and also in some types of solid tumors. Post-translational modification of Dmp1 and its role in human malignancy remain to be investigated.

Keywords

Dmp1; cyclin D; Arf; p53; Ras; haplo-insufficiency

Discovery of Dmp1

D-type cyclins (D1, D2 and D3) are induced in the context of a delayed early response to growth factor stimulation, and their synthesis and assembly with their catalytic partners, cyclin-dependent kinase 4 (Cdk4) and Cdk6, depend upon the presence of mitogens (Sherr, 2000; Sherr and Robers, 2004; Giacinti and Giordano, 2006). Cyclin D-Cdk holoenzymes play two established roles in facilitating progression through the G_1 phase of the cell division cycle:(1) they catalyse the phosphorylation of the retinoblastoma protein (pRb), and (2) during G_1 progression, accumulating cyclin DCdk holoenzymes recruit Cdk inhibitors, such as $p27^{Kip1}$ and $p21^{Cip1}$, into higher order complexes, thereby neutralizing their effects on other Cdks and facilitating the activation of cyclin E-Cdk2 later in the G_1 phase (Sherr, 2000; Giacinti and Giordano, 2006). However, this established concept did not exclude the possibility of D-type cyclins of interacting with other proteins. Using a yeast two-hybrid interactive screen, Hirai and Sherr isolated a novel cyclin *D* binding *my*b-like *p*rotein *1* (designated as Dmp1; also called Dmtf1, cyclin *D* binding *my*b-like *t*ranscription *f*actor *1*), which binds specifically to the nonamer DNA consensus sequences CCCG(G/T)ATGT to activate transcription. Although Dmp1 is structurally related to the myb family proteins (c-myb, A-myb and B-myb), a subset of these Dmp1 recognition sequences contains a GGA trinucleotide core, which additionally

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functions as Ets-responsive elements. Dmp1 binds to all of the D-type cyclins directly *in vitro* and when co-expressed in insect Sf9 cells. Dmp1 can be phosphorylated by cyclin D-dependent kinases in Sf9 cells, suggesting that its transcriptional activity might be regulated through cyclin D/Cdks holoenzymes (Hirai and Sherr, 1996). Hirai and Sherr's very first report raised the possibility that cyclin D/Cdks might regulate gene expression in an Rb-independent manner, suggesting that D-cyclins might link other genetic programs to the cell cycle progression (Hirai and Sherr, 1996).

Dmp1 arrests cell cycle progression in rodent fibroblasts, the activity of which is antagonized by D-type cyclins in Cdk-independent fashion

Dmp1 contains a central DNA binding domain that includes three imperfect Myb-like repeats flanked by two acidic transactivation domains at the amino- and carboxyl-termini (Figure 1; Inoue and Sherr, 1998). Although Dmp1 does not belong to any gene family, it is structurally related to Myb proteins in the DNA-binding domain and transcription termination factor-1 in the transactivation domain (Evers and Grummt, 1995). So far, the negative regulatory domain in the c-Myb protein has not been identified in Dmp1 (Oh and Reddy, 1999). The Dmp1 protein migrates at 120–130 kDa, although the expected molecular weight is 85 kDa (Inoue and Sherr, 1998). This is considered to be due to extensive post-translational modification of the protein, possibly due to phosphorylation, at least in Sf9 cells (Hirai and Sherr, 1996). One preliminary report suggests the possible involvement of ERK in this process (Cheng M and Sherr CJ, personal communication). D-type cyclins associate with a region of the Dmp1 DNA-binding domain immediately adjacent to the Myb-like repeats to form heteromeric complexes that do not detectably interact with Cdk4 or with DNA (Figure 1). The segment of D-type cyclins required for its interaction with Dmp1 was mapped outside the ‘cyclin box’, which contains the residues predicted to contact Cdk4. Interestingly, the estrogen receptor and the steroid receptor co-activator bind with cyclin D1 outside the cyclin box, suggesting that the carboxyl-terminal half of cyclin D1 is important for its interaction with DNA-binding proteins (Bernards, 1999;Zwijsen *et al.*, 1997). Coexpression of any of three D-type cyclins (D1, D2 or D3) with Dmp1 in mammalian cells canceled its ability to activate gene expression, which was independent of Cdks (Inoue and Sherr, 1998). Over-expression of Dmp1 into mouse fibroblasts inhibits their entry into S phase. Cell cycle arrest depended upon the ability of Dmp1 to bind to DNA and to transactivate gene expression and was specifically antagonized by co-expression of D-type cyclins, including a D1 point mutant (D1KE) that does not bind to Cdk4 (Inoue and Sherr, 1998). From these early studies, it was predicted that Dmp1 induces genes that interfere with S phase entry and that D-type cyclins can override Dmp1-mediated growth arrest in a Cdk-independent manner. A study from another group showed that cyclin D1 and D2 specifically inhibited transcription through the v-Myb DNA-binding domain, but not the c-Myb DNA-binding domain (Ganter *et al.*, 1998). Analysis of a cyclin D1 mutant and a dominant-negative Cdk4 mutant suggested that this repression was independent of Cdks. Interestingly, the cyclin D-interacting domain was mapped within the v-Myb DNA-binding domain (Ganter *et al.*, 1998). Cyclin D1 also inhibits the activity of B-Myb, the promoter of which is a direct target for E2Fs (Horstmann *et al.*, 2000).

Although the physiological roles for cyclin D as a Cdk-independent repressor of transcriptional activation by Myb proteins remain to be determined, it was recently reported that the cyclin D1-associated kinase activity is largely dispensable for the development of retina and breast, but is essential for HER2/neu-induced breast tumorigenesis (Landis *et al.*, 2006). Thus, cyclin D1 has some physiological activity in normal tissue development that is independent of Cdk activation.

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Direct binding and activation of the *Arf* promoter by Dmp1

Through the extensive search for Dmp1-consensus sequences on naturally occurring promoters, it was found that the human *CD13/Aminopeptidase N* and the murine and human *Arf* promoters have high-affinity Dmp1-binding sequences (Inoue *et al*., 1998a, 1999; for INK4a/ARF reviews, see Kim and Sharpless, 2006; Sherr 2000, 2001, 2006). Dmp1 directly binds to a unique consensus site (5′-CCCGGATGC-3′) on the murine *Arf* promoter to activate its gene expression (Inoue *et al*., 1999). Dmp1-mediated *Arf* promoter activation was dependent on the consensus sequence, since the mutant reporter was not activated by Dmp1. Other Ets family proteins (Ets1, Ets2, Elf1 and Fli1) did not activate the *Arf* promoter by themselves, although the Dmp1/Ets site showed high affinity binding to the recombinant Ets protein. When Dmp1:ER virus-infected cells were stimulated with 4-HT, they increased both Arf mRNA and protein, and thereby induced Arf-, p53-dependent cell cycle arrest within 48 h (Inoue *et al.*, 1999). Although Dmp1 and E2F-1 bind to different sites on the *Arf* promoter and act additively in a transactivation assays, Dmp1 induces cell cycle arrest but does not provoke programmed cell death. The data suggest that Dmp1 activates *Arf* transcription but does not stimulate other collateral pathways, such as Apaf-1 or caspases that are essential for E2F1-mediated apoptosis. Therefore, apart from its established role in protecting cells from potentially oncogenic signals, $p19^{Arf}$ can be induced in response to anti-proliferative stimuli that do not obligatorily lead to cell death (Inoue *et al.*, 1999).

$p19^{Arf}$ activity is compromised but not eliminated in *Dmp1*-null cells

To study the role of Dmp1 *in vivo*, mice that lack *Dmp1* by disrupting exons that encode the Myb-like repeats were created (Inoue *et al.*, 2000). *Dmp1*-null animals are 20–30% smaller than their wild-type littermates at birth. Male $Dmp1^{-/-}$ mice remained smaller even in adults, however, female knockout mice eventually became indistinguishable from their $Dmp1^{+/+}$ or $Dmp1^{+/-}$ littermates. *Dmp1*-null mice have other miscellaneous phenotypes, such as generalized seizures, seminal vesicle dilatation caused by urologic syndromes, and poor mammary gland development in females (Inoue *et al.*, 2000). The growth of *Dmp1*-null MEFs (murine embryonic fibroblasts) is progressively retarded as cells are passaged in culture, however, unlike normal cells, $p19^{Arf}$ and p53 levels remain relatively low and the MEFs continued to grow slowly without reaching replicative senescence. The rate of $p16^{Ink4a}$ induction in *Dmp1*-null cells remained largely identical with those in $Dmp1^{+/+}$ and $Dmp1^{+/-}$ cells. The levels of Dmp1 dramatically increased from passage 2 to passage 3 in both $Dmp1^{+/+}$ and $Dmp1^{+/-}$ cells, and the accumulation of *Dmp1* preceded that of $p19^{Arf}$ (Inoue *et al.*, 2000). The data suggested that both Dmp1 and $p19^{Arf}$ are induced in response to stress signaling caused by non-physiological cell culture conditions. When wild-type MEFs were cultured beyond the period of replicative senescence, immortalized cell lines that had either a mutant p53 (~80%) or deleted *Arf* locus (~20%) were obtained (Inoue *et al.*, 2000). However, $Dmp1^{-/-}$ cells readily gave rise to established cell lines that retained wild-type *Arf* and functional p53 without overexpression of Mdm2, suggesting that the activity of the Arf-Mdm2-p53 pathway is strikingly impaired in $Dmp1^{-/-}$ cells (Inoue *et al.*, 2000).

Ras-mediated signaling pathways are critical for the mitogen-dependent induction of cyclin D1 and its assembly with Cdk4 (Cheng *et al.*, 1998). Overexpression of activated Ras initiates DNA synthesis independent of growth factor stimulation. Paradoxically, continued overexpression of oncogenic Ras and its various effectors elicit irreversible cell cycle arrest by upregulating the levels of $p16^{Ink4a}$, $p19^{Arf}$ and p53 (Lin *et al.*, 1998; Palmero *et al.*, 1998; Serrano *et al.*, 1997; for review, McMahon and Woods, 2001). Early passage *Dmp1*-null cells, like MEFs from either *Arf*-null or *p53*-null mice were transformed by oncogenic Ha-Ras alone, thus bypassing the effects of immortalizing oncogenes. These data suggest that loss of *Dmp1*

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compromises the Arf-, p53-dependent senescence response that suppresses oncogenic transformation. These activities are consistent with the role of Dmp1 as a tumor suppressor.

MEFs were not only the tissue that caused hyper-proliferation in culture. Splenic pre-B lymphocytes or T-lymphocytes isolated from *Dmp1*-null mice showed much higher proliferative capacity than $Dmp1^{+/+}$ cells when stimulated with appropriate mitogens (Inoue *et al*., 2000; Inoue K and Sherr CJ, unpublished data). Interestingly, the T-cell phenotype was even stronger in *Dmp1*-null T-cells than in *Arf*-null cells, suggesting that Dmp1 must have different target genes than $p19^{Arf}$ for T-cell proliferation (Inoue K *et al.*, unpublished data).

Both *Dmp1*-null and heterozygous mice are prone to tumor development; haploid-insufficiency of Dmp1 in tumor suppression

Consistent with the data obtained in MEF studies, *Dmp1*-null mice spontaneously developed lethal tumors in their second year of life with a mean latency of 83 weeks (Inoue *et al*., 2001). The most frequently encountered tumors were pulmonary adenomas/adenocarcinomas (42%). Vascular tumors, including hemangiomas and hemangiosarcomas (24%), hepatocellular adenomas/adenocarcinomas (18%) and B-cell lymphomas (15%) were also relatively common (Inoue *et al.*, 2001). The time of appearance and variety of tumors observed in *Dmp1*-null mice bore no obvious relationship to those in *Arf*-null or *p53*-null mice, which exhibit a different spectrum (Donehower *et al.*, 1992; Kamijo *et al*., 1999; Inoue *et al*., 2001). Treating neonatal *Dmp1*-null mice with dimethylbenzanthracene (DMBA) or ionizing radiation accelerated tumorigenesis, and many such animals developed multiple tumors of more than one histological type (Inoue *et al.*, 2000, 2001). In addition to lung and skin carcinomas, DMBA-treated $Dmp1^{-/-}$ and $Dmp1^{+/-}$ mice often developed ovarian tumors, T-cell leukemia/lymphomas, hepatocellular carcinomas and melanomas (Inoue *et al*., 2001). Since these tumors were not found in the control $Dmp1^{+/+}$ mice that received the same treatment, *Dmp1*-inactivation apparently contributed to the change of tumor spectra. In humans, epithelial tumors such as epidermoid carcinomas and adenocarcinomas are much more common than sarcomas or lymphomas, and carcinomas are almost exclusively found after 40 years of age. Given that *Dmp1*-null mice developed epithelial tumors in their second year of life, they might be useful as an animal model of human carcinogenesis.

E*μ-Myc* transgenic mice develop Burkitt-type B-cell tumors with a mean latency of about 6 months. When crossed onto a $Dmp1^{+/-}$ or $Dmp1^{-/-}$ background, lymphomas induced by the E*μ-Myc* transgene were greatly accelerated (mean latency, 12 weeks) with no differences between cohorts lacking one or two *Dmp1* alleles (Inoue *et al.*, 2001). Intriguingly, the latency in the $Dmp1^{+/-}$ or $Dmp1^{-/-}$ strains mimicked that of $Arf^{+/-}$, E*μ-Myc* transgenic mice (Eischen *et al.*, 1999), consistent with the idea that *Dmp1* loss lowers $p19^{Arf}$ expression (Inoue *et al.*, 1999, 2000). Tumors from *Dmp1* heterozygotes retained and expressed the wild-type *Dmp1* allele, and most contained detectable Dmp1 protein (Inoue *et al.*, 2001). Direct nucleotide sequencing of *Dmp1* reverse transcription–polymerase chain reaction (RT–PCR) products from five such tumors identified no mutations in the DNA-binding domain. The results provide strong evidence that Dmp1 is haplo-insufficient for tumor suppression (Inoue *et al.*, 2001; reviewed by Brooksbank, 2001; Quon and Berns, 2001). The combined frequencies of *p53* mutation and *Arf* deletion in the $Dmp1^{-/-}$ and $Dmp1^{+/-}$ cohorts were ~10 versus ~50% in $Dmp1^{+/+}$ littermates (Figure 2). These results provided strong genetic evidence that loss of even a single *Dmp1* allele alleviates the selection for *p53* mutation and *Arf* loss that otherwise occurs during E*μ-Myc*-induced lymphoma generation, indicating that Dmp1 is a physiological regulator of the Arf-p53 pathway *in vivo*.

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Regulation of the *Dmp1* promoter by oncogenic Ras

As transfected Dmp1 protein has a relatively long half-life (~12 h), it was speculated that transcriptional control played important roles in its regulation. In cultured primary cells, the *Dmp1* promoter was efficiently activated by oncogenic Ha-Ras^{V12}, but not by overexpressed c-Myc or E2F-1 (Sreeramaneni *et al.*, 2005). The *Dmp1* promoter activation by Ras^{V12} depended on Raf-MEK-ERK signaling because the double mutant Ras^{V12S35} activated the promoter and because U0126 completely blocked the promoter activation (Sreeramaneni *et al.*, 2005). Induction of $p19^{Arf}$ and $p21^{Cip1}$ by oncogenic Raf was compromised in *Dmp1*-null cells, which were resistant to Raf-mediated premature senescence (Sreeramaneni *et al.*, 2005). This indicated that Dmp1 is a critical target for oncogenic Raf-induced premature senescence. A Ras^{V12}-responsive element was mapped to the 5′ leader sequence of the murine *Dmp1* promoter, where endogenous fos and jun family proteins bind. The *Dmp1* promoter activation by Ras^{V12} was strikingly impaired in *c-jun* as well as in *junB* knock-down cells, suggesting the critical role of jun proteins in the *Dmp1* promoter activation.

Importantly, a Ras^{V12}-responsive element was mapped to the unique Dmp1/Ets site on the *Arf* promoter, where endogenous Dmp1 proteins bind upon oncogenic Raf activation (Sreeramaneni *et al.*, 2005). Therefore, activation of the *Arf* promoter by Ras/Raf signaling is mediated by Dmp1, and this is why *Dmp1*-null primary cells are highly susceptible to Ras-induced transformation. Although oncogenic Ras activates the E2F transcription factors, E2Fs do not play important roles in *Arf* induction by Ras (Palmero *et al.*, 2002; Rowland *et al.*, 2002). Collectively, these three reports indicate the presence of a novel jun-Dmp1 pathway that directly links oncogenic Ras-Raf signaling and $p19^{Arf}$, independent of the classical cyclin D1/Cdk4-Rb-E2F pathway (Figure 3).

One interesting piece of data is the response of the *Arf* promoter to D-cyclins. Overexpressed D-type cyclins antagonize Dmp1 transcriptional activity in a Cdk-independent fashion when tested with artificial promoter-reporter plasmids containing concatamerized Dmp1 consensus binding sequences or with some natural promoters, such as those derived from the *CD13/Aminopeptidase N* gene (Inoue and Sherr, 1998; Inoue *et al.*, 1998a). However, the results were reversed for the *Arf* promoter, where D-type cyclins cooperated to enhance the activity of Dmp1 in a Cdk4-dependent manner. The *Arf* promoter contains both Dmp1- and E2F-binding sites, enabling Ras^{V12}-induced cyclin D1 to assemble with Cdk4, promote the release of E2Fs from Rb, and thereby collaborate with Dmp1 in activating *Arf* gene expression (Inoue *et al.*, 1999). On the other hand, the *CD13/Aminopeptidase N* promoter, which lacks E2F-consensus sequences, can be experimentally suppressed by D-type cyclins, which, when overexpressed, can interfere with Dmp1 binding to DNA. The Dmp1/Ets-consensus sequences found within these two promoters is completely identical (CCCGGATGC) (Inoue *et al.*, 1998a,1999), consistent with the hypothesis that sequences flanking the Dmp1-binding site determine the responsiveness of the promoter to D-type cyclins. It is important to emphasize that interference of Dmp1 activity by D-type cyclins has not been demonstrated in situations where D-type cyclins accumulate to physiological levels. Indeed, the level of cyclin D1 achieved after Ha-Ras^{V12} expression was ten times lower than that generated by the cyclin D1 expression vector itself (Sreeramaneni R and Inoue K, unpublished data).

Regulation of Dmp1 by E2Fs and its expression in specific differentiated, non-proliferating cells

Although the *Dmp1* promoter is activated by oncogenic Ras signaling, it was repressed when the cells entered the S to G2/M phase of the cell cycle when both Dmp1 and Arf expressions were downregulated (Mallakin *et al.*, 2006). Subsets of E2Fs were specifically bound to the *Dmp1* promoter upon mitogenic signaling, and E2Fs 1–4 inhibited the *Dmp1* promoter in a

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reporter assay. It has been reported that E2F-DB mutant binds to endogenous E2F sequences and blocks their repressor as well as activator activity (Rowland *et al.*, 2002). The *Dmp1* mRNA was not downregulated by serum in E2F-DB(+) cells, suggesting that the *Dmp1* promoter repression by serum is E2F-dependent. It was reported that endogenous activating E2Fs, E2F1 and E2F3a were recruited to the *Arf* promoter in response to hyperproliferative oncogenic signaling, indicating that distinct subsets of E2F proteins contribute to the normal repression and oncogenic activation of *Arf* (Aslanian *et al.*, 2004). Surprisingly, all of the E2F1, E2F2 and E2F3a proteins were repressors on the *Dmp1* promoter, especially the former two. Thus, E2F1 has differential effects on the *Dmp1* promoter (repression) and the *Arf* promoter (activation) when overexpressed in rodent fibroblasts (Inoue *et al.*, 1999; Mallakin *et al.*, 2006). The E2F-mediated regulation of the *Dmp1* promoter is conserved in humans since the *hDMP1* promoter has a typical E2F site (5′-TTTCGCGC) and is efficiently repressed by E2Fs (Inoue K *et al.*, unpublished data). It is important to note that the *Dmp1* promoter is not the only promoter repressed by 'activating' E2Fs; repression of the human *telomerase* promoter as well as the tumor suppressor *ARHI* promoter by E2F1 have also been reported (Crowe *et al.*, 2001; Lu *et al.*, 2006). Although the detailed mechanism of E2F-mediated repression of the *Dmp1* promoter remains to be determined, one preliminary experiment suggests that the repression is Rb-independent since the E2F1 Y411C mutant that does not interact with Rb (Rowland *et al.*, 2002) inhibited the *Dmp1* promoter as well (Mallakin *et al.*, 2006).

Immunohistochemical staining was conducted to identify the pattern of Dmp1 expression in normal murine tissues compared with the proliferation marker Ki67, to search for Dmp1-expressing cells *in vivo* (Mallakin *et al.*, 2006). In thymus, the nuclei of mature T lymphocytes in the medulla were strongly positive for Dmp1, whereas Ki67 was detected only in the cortex. In intestine, Dmp1 was detected in the nuclei of superficial layers of the villi, whereas Ki67-positive cells were confined to the lower one-third of the crypt. Double staining for Dmp1 and Ki67 revealed that these two proteins were expressed in a mutually exclusive fashion in nearly all the tissues examined. This pattern of expression of Dmp1 is in contrast to that of c-myb protein in normal murine tissues, which is expressed specifically in actively proliferating cells in the testis and the thymus (Oh and Reddy, 1999). Interestingly, c-Myb and Dmp1 collaborate to activate the *CD13/Aminopep-tidase N* promoter (Inoue *et al.*, 1998a). Thus, c-myb and Dmp1 seem to play complementary roles in regulating expression of genes involved in cell differentiation.

The human DMP1 gene and cancer

In striking contrast to the accumulating information on murine Dmp1, very little is known about the involvement of *human* DMP1 (*h*DMP1) in human cancer. *h*DMP1 has very high structural homology with its murine counterpart (760 amino acids, 96% similarity and 95% identity with murine Dmp1 at protein level). The *h*DMP1 gene is located on human chromosome 7q21, a locus often deleted in human malignancies (Bieche *et al.*, 1992; Kerr *et al.*, 1996; Bodner *et al.*, 1999; Trovato *et al.*, 2004). One copy of the genes at the *hDMP1* locus was reportedly deleted in all the tumor cells with chromosome 7q abnormalities regardless of the detailed karyotype, suggesting that one allele loss of *hDMP1* could contribute to 7q-malignancies that are refractory to conventional chemotherapy (Bodner *et al.*, 1999). It has been reported that the *hDMP1* locus encodes at least three splicing variants, that is *h*DMP1α, β and γ (Tschan *et al.*, 2003). The full-length *h*DMP1α corresponds to murine Dmp1, which positively regulates the $p19^{Arf}$-p53 pathway (Tschan *et al.*, 2003). Therefore, *h*DMP1α is considered to have tumor-suppressor activity. The β- and γ-splicing variants do not bind to DNA, but they can make heterodimers with *h*DMP1α. Therefore, *h*DMP1β and γ proteins are dominant negative forms for *h*DMP1α when they are over-expressed (Tschan *et al.*, 2003). They are specifically expressed in immature hematopoietic cells. U937 cells that constitutively express *h*DMP1β isoform showed reduced cell surface expression of *CD13/Aminopeptidase*

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N and continued to proliferate even after phorbol 12-myristate 13-acetate treatment (Tschan *et al.*, 2003). Therefore, it is highly possible that splicing abnormalities that result in the overexpression of *β/γ* isoforms of *h*DMP1 contribute to human leukemogenesis. Although the endogenous products of *h*DMP1*β* and *γ* have not been reported, these proteins may bind to D-type cyclins, since they retain the amino-terminal DNA-binding domain required for cyclin D interaction (Inoue and Sherr, 1998; Tschan *et al.*, 2003).

Recently, it was reported that *hDMP1* is a potential target for Wilms tumor gene *WT1* as well as for *miR-15a* (Kiriakidou *et al.*, 2004; Elmaagacli *et al.*, 2005). Although the *WT1* gene has been isolated as a tumor suppressor of Wilms tumor, it is aberrantly over-expressed in human leukemic cells, especially in acute myelocytic leukemia and in blastic crisis of chronic myelocytic leukemia (Inoue *et al.*, 1994, 1997). Antisense oligonucleotides to *WT1* inhibit the growth of human leukemic cells and overexpression of *WT1* interferes with myeloid differentiation programs (Inoue *et al.*, 1998b; Yamagami *et al.*, 1996). It was reported that siRNA treatment of the *WT1* gene in a K562 cell line upregulated *hDMP1* (~3-fold) when the cells stopped proliferating and underwent apoptosis (Elmaagacli *et al.*, 2005). The *miR-15a* gene is located on human chromosome 13q14.3, which is often deleted in patients with B-cell chronic lymphocytic leukemia and is involved in apoptosis (Calin and Croce, 2006; Cimmino *et al.*, 2005). A combined computational-experimental approach predicted that *hDMP1* could be a potential target for *miR-15a* (Kiriakidou *et al.*, 2004). Although these experimental results were correlative, it is intriguing to speculate about a role for *h*DMP1 as a tumor suppressor in human leukemic cells.

Dmp1, on the cusp between oncogene and tumor suppressor signaling – future prospects

Accumulating evidence suggests that Dmp1 is a regulator of the Arf-p53 pathway, although the gene had been isolated through its binding to cyclin D2. The role of Dmp1 in Ink4-cyclin D/Cdk-Rb signaling remains to be determined. Dmp1 is a transcription factor located at the junction of oncogene-tumor suppressor gene signaling; thus, it is highly possible that the gene is inactivated in a significant percentage of human cancers. The molecular mechanisms of haploid insufficiency may be determined by the identification of novel signaling pathways regulated by Dmp1. The mechanism of post-translational modification and identification of Dmp1-binding partners is an undiscovered area, which might yield further information about how this mysterious transcription factor is regulated at protein levels.

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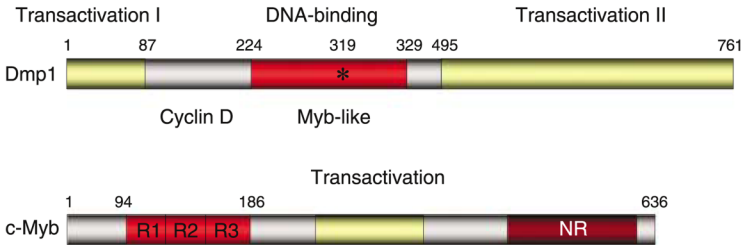

Figure 1.

The structure of the Dmp1 (Dmtf1) transcription factor. Both murine and human Dmp1 has three tandem myb-like repeats with two transactivation domains. Mutation of the lysine residue into glutamic acid abolishes its DNA binding. The cyclin Dinteraction domain has been mapped to the amino-terminal segment of the DNA-binding domain. The negative regulatory domain (NR) that is found in c-Myb has not been identified in Dmp1.

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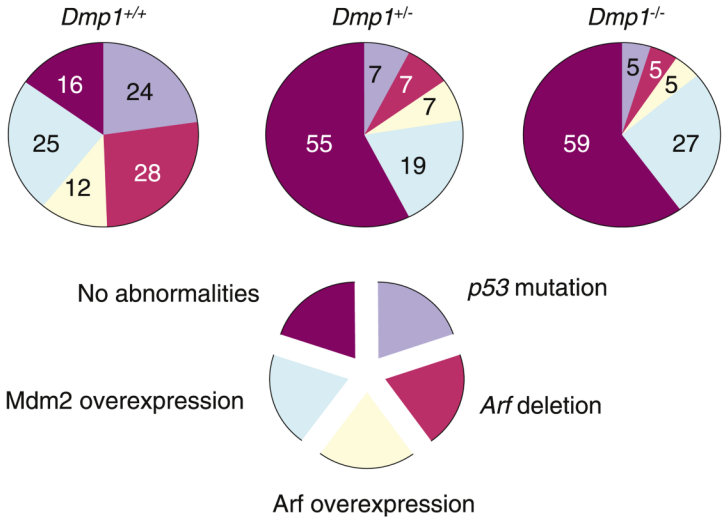

Figure 2.

Disruption of the Arf-Mdm2-p53 pathway in E*μ-Myc* lymphomas. In *Dmp1*$^{+/-}$ and *Dmp1*$^{-/-}$ E*μ-Myc* lymphomas, there is a striking reduction in the frequencies of *p53* mutations and *Arf* deletions. This suggests that Dmp1 is a regulator of the Arf-p53 pathway *in vivo*.

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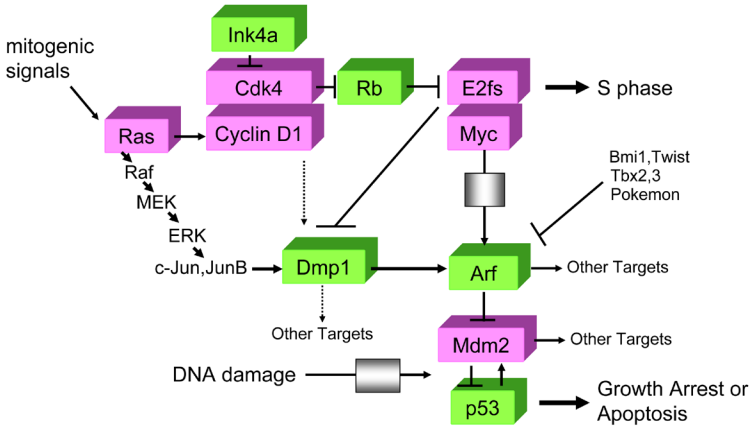

Figure 3.

Dmp1 links the Rb and p53 pathways. The novel 'Jun-Dmp1' pathway links oncogenic Ras-Raf signaling and the Arf-p53 pathway. This pathway is independent of the classical cyclin D1/Cdk4-Rb-E2F signaling. E2Fs directly bind to the *Dmp1* promoter and causes repression; thus they have differential effects on the *Dmp1* and on the *Arf* promoter. Overexpression of D-cyclins inhibits the activity of Dmp1 in a Cdk-independent fashion; however, on the *Arf* promoter, Dmp1 and cyclin D1 act synergistically, and this activity is dependent on Cdks. Dmp1 might have other targets than *Arf*, especially in T lymphocytes.