PPM1D in Solid and Hematologic Malignancies: Friend *and* Foe?

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ABSTRACT

In the face of constant genomic insults, the DNA damage response (DDR) is initiated to preserve genome integrity; its disruption is a classic hallmark of cancer. Protein phosphatase Mg^{2+}/Mn^{2+}–dependent 1D (PPM1D) is a central negative regulator of the DDR that is mutated or amplified in many solid cancers. PPM1D overexpression is associated with increased proliferative and metastatic behavior in multiple solid tumor types and patients with *PPM1D*-mutated malignancies have poorer prognoses. Recent findings have sparked an interest in the role of *PPM1D* in hematologic malignancies. Acquired somatic mutations may provide hematopoietic stem cells with a competitive advantage, leading to a substantial proportion of mutant progeny in the peripheral blood, an age-associated phenomenon termed "clonal hematopoiesis" (CH). Recent large-scale genomic studies have identified *PPM1D* to be among the most frequently mutated genes found in individuals with CH. While *PPM1D* mutations are particularly enriched in patients with therapy-related myeloid neoplasms, their role in driving leukemic transformation remains uncertain. Here, we examine the mechanisms through which *PPM1D* overexpression or mutation may drive malignancy by suppression of DNA repair, cell-cycle arrest, and apoptosis. We also discuss the divergent roles of PPM1D in the oncogenesis of solid versus hematologic cancers with a view to clinical implications and new therapeutic avenues.

Introduction

Our cells constantly acquire somatic mutations from endogenous and environmental sources and must rely on the DNA damage response (DDR) to preserve genomic integrity. The DDR is a complex network of cellular pathways that function to sense DNA damage, signal the presence of damage, and mediate DNA repair. These signaling networks bring about various cellular outcomes including cell-cycle arrest, DNA repair, and apoptosis when the damage is too extensive for repair. The tumor suppressor, p53, plays a central role in activating the DDR and via regulation of multiple nodes of the DDR signaling cascade (1, 2). Early studies identified genes transcriptionally regulated by p53, one of which was called *WIP1* (wild-type p53-induced protein), later known as *PPM1D* (protein phosphatase Mg^{2+}/Mn^{2+}–dependent 1D) or *PP2Cδ* (3). Following DNA repair, p53 induces the expression of PPM1D which in turn acts as a negative regulator of the DDR to restore cellular homeostasis (4–6).

Over the last 20 years, mutations and amplifications in *PPM1D* have been identified in several cancer-associated clinical contexts implicating it as a proto-oncogene (7–12). *PPM1D*-overexpressing solid cancers exhibit advanced tumor stage, increased metastatic potential, and poorer prognosis (8, 13–16). In the blood, mutations in *PPM1D* are often found in individuals with clonal hematopoiesis (CH; ref. 17), a premalignant expansion of mutant hematopoietic stem cells. *PPM1D* mutations in the blood are enriched in individuals who were treated with chemotherapy for solid tumors, suggesting mutation of *PPM1D* offers a selective advantage (12, 18). However, whether these mutations promote hematologic malignancies is still unclear.

In this review, we will highlight the DDR pathways modulated by PPM1D and reflect on the degree to which the oncogenic properties of *PPM1D* mutations can be accounted for by its role in regulating p53. We will discuss the contexts and mechanisms in which *PPM1D*-mutant cells gain dominance over wild-type cells and the relevance of this so-called "clonal emergence" for cancer development. We will highlight remaining questions in the field about the conflicting clinical implications and divergent roles of PPM1D in solid and hematologic cancers.

PPM1D Amplifications and Mutations

The *PPM1D* gene consists of six exons on chromosome 17q23 in humans (19). The three domains of the PPM1D protein include the N-terminus, the phosphatase domain, and the C-terminus. The phosphatase domain of PPM1D is evolutionarily conserved with that of the other members in the protein phosphatase 2C (PP2C) family of Ser/Thr phosphatases (3). Genomic aberrations of *PPM1D* can present as amplifications of chromosome 17q as seen in ovarian and breast cancer (**Fig. 1A**; refs. 4, 7, 20–22). This results in increased expression of wild-type PPM1D that is correlated with the gene dosage and copy-number variation which can range from 4 to 27 (23). It is also important to note that several other cancer-associated genes are also located on chromosome 17q including *BRCA1, ERBB2, NF1, RAD51C, BRIP1*, and *BIRC5* (24). Therefore, overexpression of *PPM1D* in 17q amplifications may act cooperatively with the increased expression of other oncogenes to promote tumorigenesis.

With the advent of next-generation sequencing, mutations in *PPM1D* were first reported in 2013 (25). Strikingly, almost all mutations are nonsense or frameshift mutations spanning across the terminal exon of the *PPM1D* gene with no clear hotspot (**Fig. 1B**). Importantly, exon 6 mutations are all located downstream of the catalytic domain, and studies have demonstrated that truncation of the protein has minimal effect on the phosphatase activity of PPM1D.

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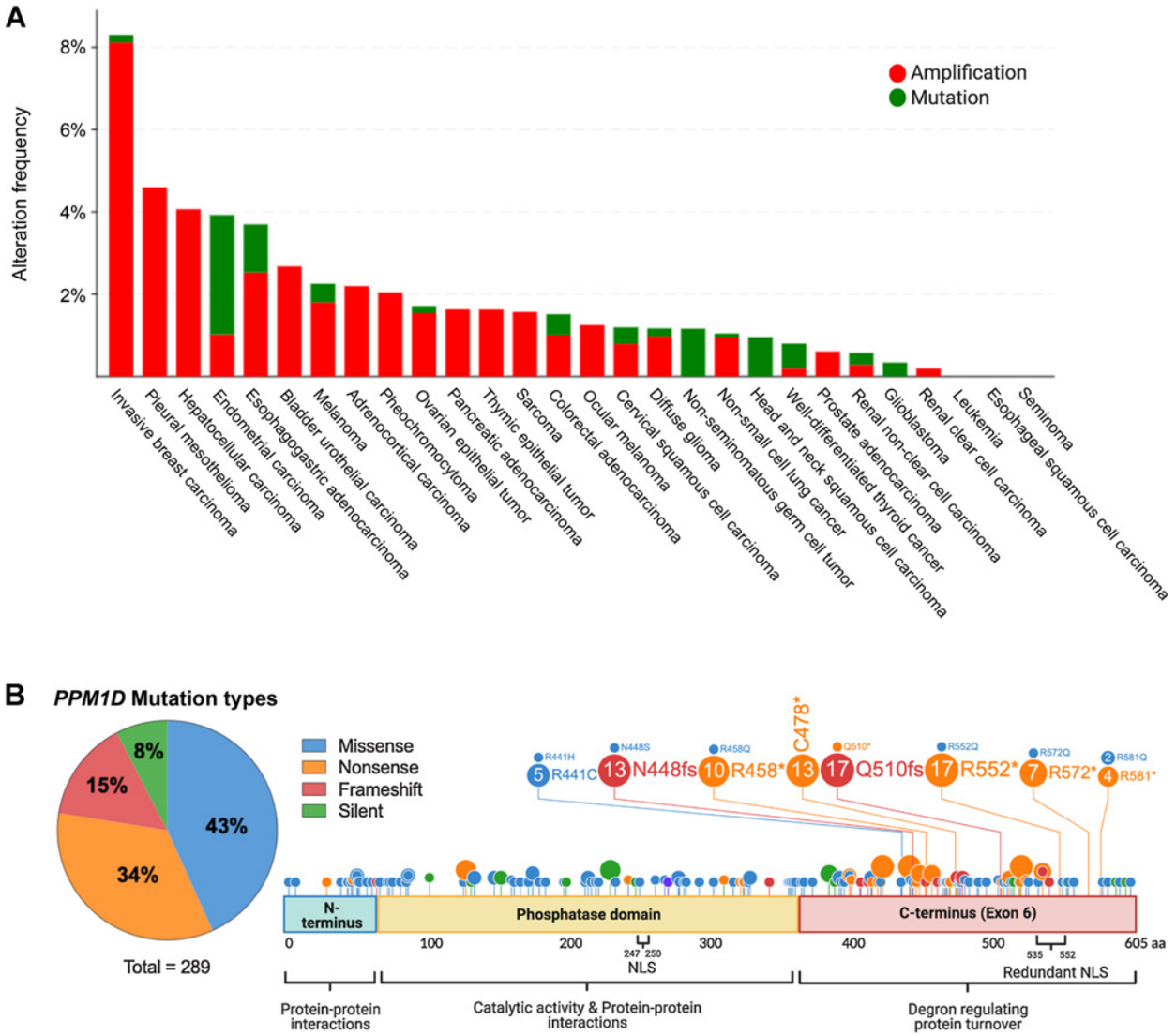

Figure 1.

PPM1D truncating mutations in solid cancers. **A,** Histogram showing the prevalence of *PPM1D* amplifications and C-terminal truncating mutations by cancer type, as indicated on the *x*-axis. The percentage of cases with *PPM1D* genomic alterations is indicated on the *y*-axis. The data were obtained from Pan-Cancer studies available in the cBioPortal database (10,967 total samples), which was then filtered to show only cancer types with more than 50 cases. **B,** Lollipop plot showing the location of the truncating mutations in the context of the domains of the *PPM1D* gene. A total of 289 *PPM1D* mutations were identified across 43 histology types from the COSMIC database. A pie chart of the mutation types is included with missense and nonsense mutations being the most common. The phosphatase domain and exon 6 of PPM1D are shown. Several mutation hotspots are noted.

Instead, these mutations result in the loss of a C-terminal degradation motif leading to the stabilization and accumulation of the mutant protein (12, 18, 26). Truncated PPM1D protein can accumulate in the cell up to 16 times the level of full-length PPM1D even in the absence of stressors where wild-type PPM1D levels would be low (12). This finding suggests that *PPM1D* truncating mutations could mimic the effect of *PPM1D* amplifications, as both alterations increase levels of PPM1D protein in the cell. However, it remains an open question as to whether the truncation variant has neomorphic effects or different interaction partners than full-length PPM1D. Two common cancer cells lines, HCT116 (colon cancer) and U2OS (osteosarcoma), both harbor heterozygous *PPM1D* truncating mutations (25). We have curated a list of additional cell lines with *PPM1D* truncating mutations and amplifications (**Table 1**) using the Cell Model Passports database from the Sanger Institute (27). In the next section, we will discuss the overall consequences of increased PPM1D activity on the DDR, cell cycle, and apoptosis and how dysregulation of these pathways can promote the formation of solid versus hematologic malignancies.

PPM1D Is a Negative Regulator of the DDR

The DDR is integral to maintaining genome integrity by coordinating the arrest of normal cellular functions and cell cycling to recruit

Table 1. List of cell lines with *PPM1D* truncating mutations, amplifications, and copy-number gains curated from the Cell Model Passports database.

Cell line	Cancer type	Nucleotide change	Amino acid change	Classification	*TP53* status
SUP-T1	TALL	c.1528_1529insA	p.N512fs*16	Frameshift	1
MN-60	BALL	p.N512fs*2	c.1529delA	Frameshift	0
HCC1569	BRCA	c.1344delT	p.L450fs*1	Frameshift	1
PA-1	OV	c.1370delC	p.A457fs*8	Frameshift	0
PA-1	OV	p.A457fs*8	c.1370delC	Frameshift	0
U2OS	OS	c.1372C>T	p.R458*	Nonsense	0
BB58-HNC	HNC	p.N477fs*6	c.1427delA	Frameshift	0
CCK-81	CRC	p.N512fs*2	c.1529delA	Frameshift	1
CL-34	CRC	p.R572*	c.1714C>T	Nonsense	1
CW-2	CRC	p.K336fs*3	c.1003delA	Frameshift	0
HCM-SANG-520-C18	CRC	p.N512fs*2	c.1529delA	Frameshift	1
HCT-116	CRC	c.1344delT	p.L450fs*1	Frameshift	0
SNU-175	CRC	p.N512fs*16	c.1528_1529insA	Frameshift	0
HEC-108	EC	p.L546fs*1	c.1632delC	Frameshift	1
HEC-6	EC	p.N512fs*16	c.1528_1529insA	Frameshift	1
NCI-H3122	NSCLC	p.N512fs*16	c.1528_1529insA	Frameshift	1

Cell line	Cancer type	Copy number	Cell line	Cancer type	Copy number
ZR-75-30	BRCA	40	NCI-H650	NSCLC	5
MCF7	BRCA	30	LN-229	GBM	5
HCC2218	BRCA	23	NCI-H2081	SCLC	5
BT-474	BRCA	23	KATOIII	GC	5
MDA-MB-361	BRCA	9	CFPAC-1	PC	5
SK-MEL-5	MEL	7	NCI-H64	SCLC	5
HCC1428	BRCA	7	NCI-H740	SCLC	5
UACC-893	BRCA	7	SK-MEL-3	MEL	5
NCI-H508	CRC	7	DAN-G	PC	5
CAKI-1	RCC	6	SK-MES-1	SqCLC	5
NCI-H28	MS	6	SW780	BC	5
U-2-OS	OS	6	SK-N-DZ	NB	5
NCI-H1993	NSCLC	6	NCI-H2009	NSCLC	4
PANC-02-03	PaC	6	MDA-MB-453	BRCA	4
SK-MEL-1	MEL	6	MHH-ES-1	EW	3
PC-3	PrC	5	NCI-H2405	NSCLC	3
MDA-MB-330	BRCA	5	MOLT-4	TALL	3

Abbreviations: BALL, B-cell acute myeloid leukemia; BC, bladder cancer; BRCA, breast cancer; CRC, colorectal; EC, endometrial carcinoma; GBM, glioblastoma; GC, gastric cancer; MEL, melanoma; MS, mesothelioma; NB, neuroblastoma; OS, osteosarcoma; OV, ovarian carcinoma; PaC, pancreatic cancer; PrC, prostate cancer; RCC, renal cell carcinoma; SCLC, small cell lung cancer; TALL, T-cell acute lymphoblastic leukemia.

downstream effectors that repair damaged DNA. As a homeostatic regulator of the DDR, PPM1D is activated in response to exogenous (i.e., radiation, chemicals, or chemotherapy) and endogenous (i.e., reactive oxygen species or DNA replication errors) stimuli (28). When activated, PPM1D attenuates the stress response through dephosphorylation of p53 (5), DNA damage sensors (ATM, ATR; ref. 29), cell-cycle checkpoint proteins (CHK1, CHK2, p21; refs. 30, 31), apoptotic proteins (BAX, DAXX; ref. 32), among others. Through this coordinated network of events, the DDR is inactivated, and the cell resumes normal cell cycling and homeostasis. Defects in the DDR lead to genomic instability and allow for the accumulation of driver aberrations that promote neoplastic growth. It is important to understand the role of PPM1D in modulating DNA repair to contextualize how its overexpression can lead to a blunted DDR (33–37) to promote malignant transformation. There are three DNA damage repair pathways that PPM1D is known to regulate: double-stranded break (DSB) repair, nucleotide excision repair (NER), and base excision repair (BER). An in-depth graphical summary of dephosphorylation sites is provided in **Fig. 2**.

DSB repair

DSBs result from exposure to ionizing radiation, chemicals (i.e., bleomycin and specific chemotherapeutic agents), and endogenous replication stress. DSB repair begins when ataxia telangiectasia mutated (ATM) undergoes autophosphorylation and orchestrates DSB repair by recruiting downstream effectors. Importantly, p-ATM phosphorylates H2AX at Ser139, which then becomes referred to as γ-H2AX. γ-H2AX is a highly specific and sensitive molecular marker for the initiation of the DDR, as it serves as a docking site for the recruitment of DNA repair proteins to DSB sites (33). As part of a negative feedback loop, PPM1D suppresses the activation of the ATM-dependent signaling cascade through dephosphorylation (29). PPM1D also directly dephosphorylates γ-H2AX, which further inhibits the recruitment of DNA repair factors after damage is successfully repaired. Notably, premature dephosphorylation of γ-H2AX by PPM1D can lead to failure in the recruitment of DNA repair proteins and delayed DNA repair (37).

Independent of γ-H2AX, the MRE11-RAD50-NBS1 (MRN) complex also plays a crucial role as an initial sensor of and responder to

Table 2. Summary of *PPM1D* genetic amplification and mutations in solid cancers.

Cancer type	*PPM1D* status	%	Prognosis (if available) and characteristics	Ref.
Ovarian	Amplification	10%	Silencing of *PPM1D in vitro* led to reduced cell survival.	(22)
Breast	Amplification	16%	Attenuation of apoptosis *in vitro*. Cooperated with *RAS* to transform primary MEFs.	(7)
	Amplification	11%	Associated with poor prognosis.	(4)
	Amplification	6%	More prevalent in $HER2^{+}$ breast cancers (19%) No association between *PPM1D* gene amplification or overexpression with disease-free, metastasis-free, or overall survival.	(20)
Neuroblastoma	Amplification	28% (9/32)	High expression of *PPM1D* correlated with significantly worse survival outcomes.	(54)
Medulloblastoma	Amplification and overexpression	64%	Increased *PPM1D* expression associated with metastasis and decreased survival. Associated with *CXCR4* and *GRK5* upregulation.	(51, 55, 83)
Pancreatic adenocarcinoma	Amplification	51% (86/169)	43% had metastatic disease at follow-up and harbored at least one mutation in *MDM2*, *MDM4*, or *WIP1.*	(23)
	Overexpression	55%	*PPM1D* expression positively correlated with tumor grade; promotes cell migration and invasion *in vitro* and tumor growth *in vivo.*	(94)
Colorectal	Overexpression	68% (252/368)	*PPM1D* expression significantly increased in tumors with nodal and distant metastasis and advanced TNM stages.	(14)
Papillary thyroid	Overexpression	63% (56/89)	*PPM1D* expression positively correlated with tumor size and lymph node metastasis.	(84)
Prostate cancer	Overexpression	56.4% (132/234)	*PPM1D* expression positively correlated with Gleason score, T-stage, lymph node status, and shorter biochemical recurrence-free survival, and decreased overall survival.	(15)
Salivary carcinoma	Overexpression	100% (82/82)	Correlated with malignant disease and poor prognosis.	(57)
Non–small cell lung cancer	Overexpression	69% (52/75)	Positively correlated with clinical stage, lymph node metastasis, and pathologic differentiation.	(16)
Nasopharyngeal carcinoma	Overexpression	Unclear	Positively correlated with advanced clinical stage, lymph node metastasis, response to ionizing radiation; poor 5-year survival.	(58)
Renal cell carcinoma	Overexpression	68% (53/78)	Positively correlated with T stages, lymph node metastasis, clinical stages and tumor differentiation, with poor overall survival.	(60)
Esophageal squamous cell carcinoma	Overexpression	69% (70/101)	Poor prognosis, lymph node metastasis, inferior 5-year survival.	(53)
Osteosarcoma	Overexpression	51% (23/45)	Higher levels of PPM1D detected in patients with distant metastasis and unfavorable prognosis.	(59)
Glioma	Truncating mutation	23% (3/13) 18%	*PPM1D* mutations mutually exclusive with *TP53* mutations but always found in conjunction with *NF1* mutations and frequently with *H3F3A*.	(8,46)

DNA damage by modulating the activity of ATM at DSBs (38). In addition to its role in the DDR, the MRN complex also mediates cell-cycle checkpoints and telomere maintenance. Yamaguchi and colleagues have shown that NBS1 is dephosphorylated by PPM1D at Ser343 *in vitro* (39). While it remains unclear how NBS1-S343 dephosphorylation may affect the DDR-specific role of the MRN complex, S343-mutant variants of NBS have defective CHK2 activation and inappropriate cell-cycle progression following genotoxic stress (40). Therefore, constitutive downregulation of NBS1 at S343 by PPM1D overexpression may promote tumorigenesis due to increased cell cycling and mutagenesis.

There is some evidence that PPM1D regulates certain aspects of non-homologous end joining (NHEJ) and homologous recombination (HR), the two major pathways that mediate DSB repair. In the context of NHEJ, PPM1D has been shown to dephosphorylate LSD1 resulting in impaired recruitment of 53BP1 to DSBs after bleomycin exposure (35). On the contrary, PPM1D was recently shown to promote HR by forming a stable complex with BRCA1-BARD1, which is critical for the timely recruitment of these substrates to DSBs (41). In summary, PPM1D appears to inhibit NHEJ while promoting HR. However, given the complexity of events in DSB repair, the net effect of PPM1D mutations on HR and NHEJ activity remains to be explored.

NER

NER is the key pathway involved in the repair of bulky, helix-distorting DNA damage including UV-induced genomic lesions. Following UV damage, p53 is phosphorylated by ATR and induces expression of XPC, p48XPE, and GADD45 to facilitate NER (42). Because p53 enhances NER, suppression of p53 activity by PPM1D should inhibit expression of these NER effectors. However, overexpression of PPM1D has been shown to inhibit NER activity in both p53-proficient and p53-deficient cell lines. Inhibition of NER activity was not observed with overexpression of a phosphatase-dead form of PPM1D, suggesting that the catalytic activity of PPM1D plays a direct role in regulating NER. Furthermore, mouse embryonic fibroblasts (MEF) derived from *Ppm1d*-deficient mice exhibited the opposite phenotype, with faster genome-wide resolution of damage after UV exposure (34). PPM1D was found to regulate NER via dephosphorylation of XPC and XPA. This activity

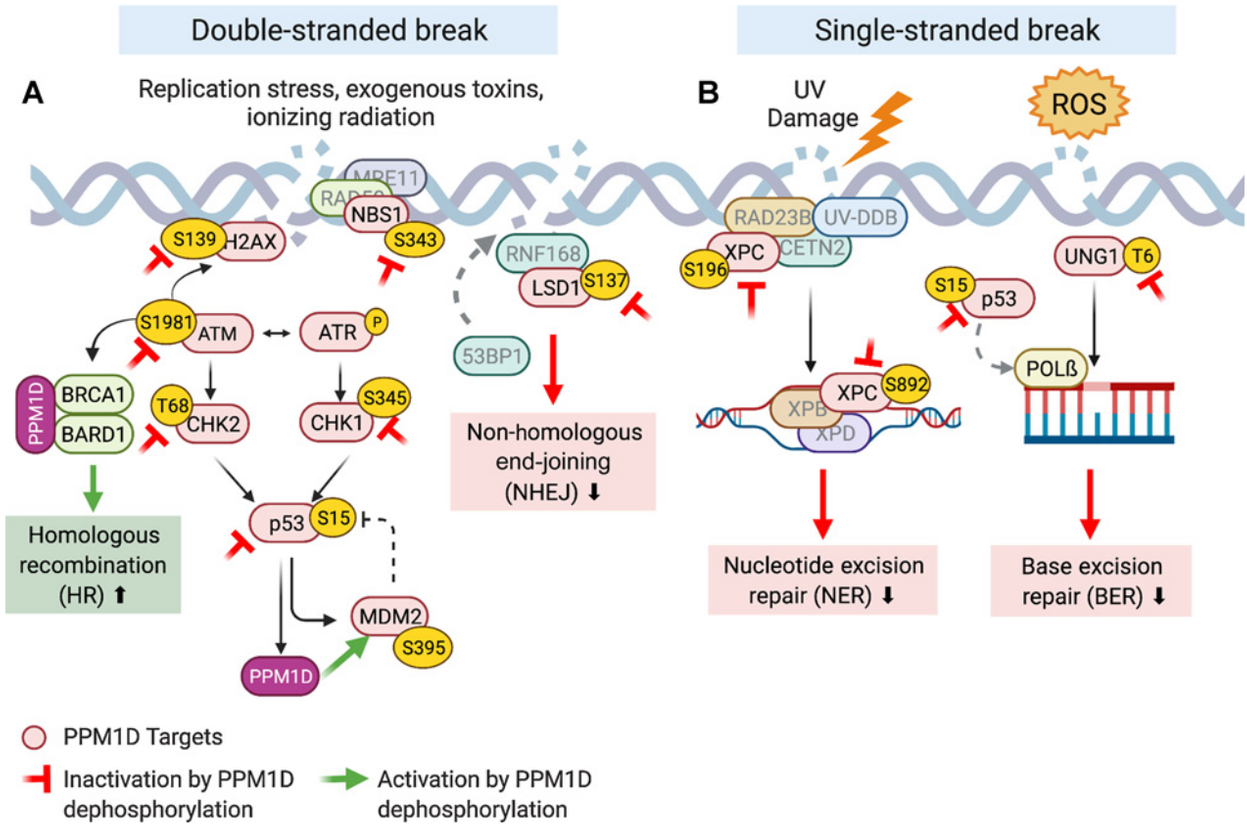

Figure 2.

The role of PPM1D in DNA repair. The red proteins are known PPM1D targets. The red inhibitory symbol denotes the inactivation of that protein upon PPM1D dephosphorylation. The green arrow represents activation of the protein upon dephosphorylation. **A,** PPM1D inhibits key players involved in DSB repair resulting in decreased NHEJ and increased HR. **B,** PPM1D inhibits several BER and NER proteins leading to decreased single-stranded break repair.

appeared to be specific to PPM1D, as members of other classes of phosphatases failed to dephosphorylate XPA at the same sites (34).

BER

BER is responsible for repairing non–helix-distorting base lesions that typically result from deamination, oxidation, or alkylation of nuclear and mitochondrial DNA. BER is initiated by DNA glycosylases, which recognize, and excise mismatched or damaged bases. P53 has been shown to promote BER through the regulation of several BER glycosylases as well as DNA polymerase ß, the main polymerase involved in short-patch BER (2). *Ppm1d*$^{-/-}$ MEFs were shown to have 6-fold higher BER activity compared with wild-type MEFs, independent of *Tp53* status. These results suggest that PPM1D suppresses BER activity. Indeed, it was later discovered that PPM1D dephosphorylates UNG2, a key uracil-DNA glycosylase that excises uracil bases from DNA, to shut down BER and return the cell to homeostasis (36). However, BER can be initiated by other DNA glycosylases, each with a substrate specificity for a particular type of damaged DNA base. Therefore, while PPM1D may decrease BER in response to UV-mediated damage, it remains unknown whether it decreases the repair of other types of damaged bases.

Consequences of mutant PPM1D in the DDR

Overall, PPM1D inhibits several DNA repair pathways involved in DSB repair, NER, and BER. In contrast, PPM1D seems to promote HR a distinct DNA repair mode. This finding is further bolstered by studies demonstrating that inhibition of PPM1D sensitizes cancer cells to PARP1 inhibition, a key player in mediating DNA repair and HR (41, 43). One caveat of the DNA repair studies described above is that many were performed by comparing WT cells with *PPM1D* knockout models. While one could infer that PPM1D overexpression models behave in an opposite manner, whether this is the case remains an open question. Similarly, as mentioned previously, while PPM1D truncation variants found in cancer have preserved phosphatase activity, it remains unclear whether it interacts with the same targets as wild-type PPM1D. Furthermore, the genomic consequences of the combined suppression of DSB repair, NER, and BER activity that is presumed to occur with excess PPM1D is still unknown. One possibility is excess PPM1D simply leads to delayed DNA repair that occurs with normal fidelity. Another possibility is that fidelity of DNA repair is compromised, leading to accumulation of mutations in the genome. This may increase the risk of a "second hit" mutation activating an oncogene or inhibiting a tumor suppressor. If such is the case, we may expect *PPM1D*-mutant cells to harbor a unique mutation signature representing a combination of distinct signatures reflective of the corresponding defective DNA repair pathways. Addressing these critical unanswered questions will shed light on the mechanism and potency of *PPM1D* mutations and amplifications in driving cancer.

Relationship between PPM1D and TP53

The tumor suppressor p53 has long been known to be the "guardian of the genome" and transcriptionally regulates hundreds of downstream effectors to promote cell-cycle arrest (*CDKN1A* and *GADD45A*), DDR (*XPC, DDB2*, etc.), and cell fate pathways including apoptosis (*PUMA, BAX*) and senescence. Mutations in *p53* lead to the dysregulation of these critical cellular pathways and allow the neoplastic transformation of cells into cancer (1). In normal cells, p53 becomes activated and turns on these genome-protective pathways. A major role of PPM1D is to attenuate this activation. P53 in fact transcriptionally activates the expression of PPM1D, which then dephosphorylates p53 at Ser15, which is a critical posttranslational modification required to stimulate transactivation of p53-responsive promoters (44); thus, PPM1D dephosphorylation of p53 directly deescalates the DDR. PPM1D also indirectly inhibits p53 by dephosphorylating upstream activating kinases such as ATM, ATR, CHEK1, and CHEK2. In addition, PPM1D dephosphorylates MDM2, an E3 ubiquitin ligase, which in turn tags p53 for proteasomal degradation (6). Together, all these actions serve to turn down the DDR broadly, and p53 specifically.

Given the suppressive role of PPM1D on p53 activity, *PPM1D* amplifications and mutations are thought to mimic partial loss of *TP53*. Nevertheless, p53 undergoes more than 300 different posttranslational modifications (PTMs) including ubiquitination, acetylation, and phosphorylation, which instigate programs independent of PPM1D (45). Therefore, while PPM1D directly inhibits p53-Ser15–dependent roles, other p53-initiated programs that are dependent on ubiquitination or acetylation may not be affected. Thus, to understand the impact of *PPM1D* alterations, it is important to determine the phenotypic, as well as mechanistic relationship to *TP53* alterations. A recent study found that germline overexpression of human *PPM1D* in mice could induce tumors that were phenotypically similar to those developed in mouse models with *TP53* mutations (24). Here, they exposed *PPM1D* mice to sublethal whole-body irradiation. Interestingly, they observed that the tumor spectrum was more comparable with that of *TP53* loss-of-function mouse models rather than *TP53* knockout mice. These findings support the hypothesis that PPM1D overexpression leads to only partial impairment of p53.

As *PPM1D* and *TP53* mutations both act through similar signaling pathways, we would expect functional redundancy to having both a knockdown of p53 and an upregulation of PPM1D within the same cell. Indeed, early studies showed that *PPM1D* genetic alterations and *TP53* mutations appear to be mutually exclusive in solid cancers. For example, *PPM1D* amplifications were almost exclusively found in *TP53* wild-type tumors in one breast cancer study (21), and *PPM1D* truncating mutations and *TP53* inactivating mutations were mutually exclusive in brainstem gliomas (8, 46). These reports suggest that, in certain contexts, mutations in both genes confer minimal additional advantage over mutations in either gene alone. Similarly, a recent analysis of 10,225 patients from The Cancer Genome Atlas database revealed that *PPM1D* is amplified significantly more often in *TP53* wild-type than *TP53*-mutant tumors (47). Yet, other studies have observed *PPM1D* amplifications and mutations to cooccur with *TP53* mutations in some tumor samples (20, 48). This conflict suggests that PPM1D may confer additional advantages to cancer cells through p53-independent mechanisms, including through mTOR signaling pathways, DNA repair pathways (NER and BER), and NFκB signaling pathway, among others (34, 36, 49).

In the blood, true comutations are more difficult to identify, as bulk sample sequencing does not distinguish between mutations in separate subclones versus within the same cell. *PPM1D* mutations have been reported to be comutated with *TP53* more frequently than expected by chance alone in therapy-related myelodysplastic syndrome (t-MDS; ref. 11), and mutations in both genes are enriched after exposure to chemotherapy (12). Yet, single-cell genome sequencing studies recently revealed that *PPM1D* and *TP53* mutations were typically present in separate clones in the blood (50). This finding is consistent with the hypothesis of functional redundancy. If PPM1D acts through similar pathways as p53, one would expect *PPM1D*- and *TP53*-mutant cells to have similar mutational profiles and prognosis. However, in t-MDS, the presence of *TP53* mutations was strongly associated with complex chromosomal abnormalities, whereas the presence of *PPM1D* mutations without concurrent *TP53* mutations was associated with lower frequencies of complex karyotypes at frequencies comparable with *TP53* and *PPM1D* wild-type cases. It is possible that partial suppression of p53 activity by PPM1D results in less genome instability than complete loss-of-function *TP53* mutations. In addition, complex karyotype is only one form of genetic alteration, and *PPM1D* mutants could have additional alterations or mutational signatures distinct from *TP53* mutants. Comparing the mutation burden and signatures between *PPM1D* and *TP53* mutants through whole-genome sequencing studies would shed light on the degree of functional overlap between the two genes.

Finally, *TP53* mutations are associated with a poorer overall prognosis than *PPM1D* mutations in t-MDS and are also much more prevalent in *de novo* leukemias (11). These findings suggest that loss of *TP53* is a more potent oncogenic driver than excess PPM1D, at least in the blood. As both mutations are enriched following exposure to chemotherapy and are highly prevalent in therapy-related acute myeloid leukemia (t-AML) and t-MDS, clonal expansion of *PPM1D* mutants may be preferable. In fact, an expanded *PPM1D* clone with high fitness may help suppress the rise of more potent oncogenic clones in the bone marrow and blood. Perhaps, in this way, *PPM1D* mutants can be viewed as a "friend" compared with *TP53*-mutant clones which have higher potential for malignant transformation. It would be interesting to experimentally compete *TP53*- and *PPM1D*-mutant clones head-to-head in the blood under varying conditions of stress. If *PPM1D* mutants do have a competitive advantage in certain stress conditions, we could infer that these cells function through p53-independent mechanisms. In contrast, if PPM1D acts predominantly through p53, one would expect *PPM1D* mutants to have similar, if not lower, competitiveness compared with *TP53* mutants. Overall, these studies may help to illuminate why *PPM1D* mutations are more enriched in t-AML, but not in other types of *de novo* hematologic and solid malignancies compared with *TP53* mutations.

PPM1D in Solid Cancers

PPM1D amplifications were first found in human cancers in the early 2000s, shortly after the gene was discovered. Initial studies used microarray-based comparative genomic hybridization to identify amplification of chromosome region 17q23 harboring the *PPM1D* locus in 11%–16% of primary breast cancer samples (4, 7, 21). Importantly, *PPM1D* amplifications were found to be associated with poorer survival and more aggressive disease in breast cancer patients (4, 21). These early findings established *PPM1D* as a potential oncogene in cancer research and were soon followed by numerous studies that identified either *PPM1D* genomic amplifications or increased *PPM1D* gene expression in a wide variety of other solid tumors including neuroblastoma (1, 36) medulloblastoma (51, 52), esophageal

squamous cell carcinoma (53), and more (summarized in **Table 1**). Several recurring features and characteristics are notable across *PPM1D*-overexpressing cancer types. For example, *PPM1D* overexpression is associated with significantly decreased overall, recurrence-free, or 5-year survival (4, 54–57), lymph node metastases as well as distant metastases, and advanced tumor stage across several different solid cancer types (14–16, 23, 52, 55, 58–60). Broadly, these findings suggest that *PPM1D* overexpression can serve as a valuable prognostic factor for risk stratification of patients with solid cancer.

Like *PPM1D* amplifications, *PPM1D* truncating mutations have also been identified across solid cancers (8, 25, 48, 61). *PPM1D* mutations have been established as oncogenic drivers in *de novo* diffuse midline glioma formation and are required for *in vivo* gliomagenesis (62). Sequencing tumor tissue from larger patient cohorts will illuminate whether, like *PPM1D* amplifications, patients with tumors harboring truncating *PPM1D* mutations have worse survival outcomes and metastatic potential than those without. In other solid cancer types, *PPM1D* mutations seem to play more of a supporting oncogenic role. In a colorectal cancer study, mice with truncated *Ppm1d* ($Ppm1d^{T/+}$) were crossed with mice harboring an inactivating mutation in the tumor suppressor *Apc* (Apc^{min}). Double mutant mice had significantly increased colonic polyps, accelerated tumor formation, and greater tumor penetrance compared with Apc^{min} mice alone. However, no intestinal polyps were found in $Ppm1d^{T/+}$ mice, suggesting that *Ppm1d* mutations alone are not potent enough to drive tumor initiation. Furthermore, organoids derived from $Ppm1d^{T/+}/Apc^{min}$ mice were resistant to 5-fluorouracil (5-FU) treatment and sensitivity was restored after pharmacologic inhibition of PPM1D (63). Overall, *PPM1D*-mutant cancer cells appear to have a similar chemoresistance phenotype as *PPM1D*-overexpressing cancer cells, and PPM1D inhibitors can potentially work synergistically with traditional chemotherapeutic agents in both instances.

PPM1D Mutations in the Blood—A Top Hit in CH

In 2014, multiple landmark studies described the phenomenon of CH, where large clones derived from single hematopoietic stem cells (HSC) were found to comprise a significant proportion of peripheral blood in individuals with no history of hematologic diseases. These expanded cell populations often harbor somatic mutations in one of several recurrently mutated genes. In some studies, *PPM1D* was found to be one of the most mutated genes in individuals with CH (17, 64). CH has since been found to be associated with an increased risk of hematologic malignancies (17), cardiovascular disease (65), and increased all-cause mortality (66). Around the same time as the discovery of CH, a series of large-cohort studies reported an enrichment of *PPM1D* truncating mutations in the peripheral blood of patients with solid cancer compared with control patients without cancer. Because *PPM1D* mutations were previously associated with various solid tumors, and the blood samples were thought to represent "germline" variants, the studies speculated that *PPM1D* germline mutations could be a risk factor or biomarker for development of these solid cancers (9, 25). However, additional studies that included analysis of matched blood and lung tumor samples revealed a discordance; *PPM1D* mutations were detected in the blood but not in the tumor (10). These findings suggested that *PPM1D* mutations had a hematopoietic origin and reflected somatic, not germline, mutations of *PPM1D* followed by clonal expansion of mutant hematopoietic cells.

Indeed, the observed frequency of *PPM1D* mutations in the blood of these patients with solid cancer (0.2%–1.5%) was similar to that reported in the general population (0.12%; ref. 17). Furthermore, a breast cancer cohort study noted that the presence of *PPM1D* truncating variants in the blood was positively correlated with age (67), which is consistent with age-related CH. However, the enrichment of *PPM1D* mutations in the blood in association with patients having solid tumors suggested that other variables besides age may contribute. Indeed, it was noted that all patients with ovarian cancer reported to exhibit *PPM1D* mutations had previously received chemotherapy treatment (68). Subsequent large-scale sequencing studies in patients who had been treated for solid tumors (MSK-IMPACT) validated the significant association between somatic *PPM1D* mutations in the blood and prior chemotherapy exposure (69). Together, these studies pointed to the concept of therapy-related CH and the view that *PPM1D* mutations can occur in aging-related CH, but that they are highly enriched after exposure to chemotherapy due to clear positive selection for mutant clones in the blood.

PPM1D-related CH not only has implications for leukemia predisposition but has also been shown to be associated with worse outcomes after autologous stem cell transplantation (66), promote heart failure (70), and alter immune cell function (71) in murine models. Recently, *Ppm1d* overexpression in murine immune cells was found to alter the degree of immunosuppression within the tumor microenvironment to increase tumor progression. This finding would suggest that *PPM1D* CH could impact disease progression and outcomes for patients that have PPM1D wild-type solid tumors. Pharmacologic inhibition of PPM1D could reprogram *Ppm1d*-mutant neutrophils toward a higher antitumor potential by promoting tumor infiltration (71). These findings expand on the clinical relevance of *PPM1D* CH and highlight the potential therapeutic benefits of PPM1D inhibitors.

PPM1D Mutations in the Blood—A Bystander in AML?

From the perspective of hematologic malignancies, *PPM1D* mutations appear in specific subsets of disease. Notably, *PPM1D* mutations are significantly more common in t-AML/t-MDS compared with primary AML and MDS (1112). In-depth analysis revealed that *PPM1D* mutations are significantly associated with prior exposure to specific genotoxic agents, including platinum therapy, topoisomerase I and II inhibitors, and radiotherapy (69, 72). Comparable with its frequency in CH, *PPM1D* is the eighth most mutated gene in myeloproliferative neoplasms (MPN). One study found *PPM1D* mutations in 19% of patients with MPN, of whom 10 carried the *PPM1D* mutation only after treatment with hydroxyurea. Generally, *PPM1D* mutations were found to be acquired later in disease based on mutational tree mapping (73).

Regarding the prognostic impact of these mutations, we found no significant difference in overall survival between *PPM1D*-mutated and non–*PPM1D*-mutated cases of t-AML/t-MDS, respectively (12). Similarly, while *PPM1D* mutations were associated with a significant HR of death of 1.64 ($P_{adjusted} = 0.002$), further stratification revealed that *PPM1D* mutations were not associated with adverse prognoses in patients without coexisting *TP53* mutations (11). Even in *de novo* AML and MDS, *PPM1D* mutations do not appear to be associated with worse overall outcomes. Consistent with earlier studies, Al Hinai and colleagues identified *PPM1D* truncating mutations in 0.6% of newly diagnosed AML, with sizable clones [variant allele frequency

(VAF) ~45%] in three of the cases. As this cohort was followed into clinical remission after chemotherapy treatment, the frequency of *PPM1D* mutations increased to 4% cases. Yet, the presence *PPM1D* mutations in clinical remission do not appear to predict AML relapse (74). Another study of MDS patients with deletion of chromosome 5q identified *PPM1D* truncating mutations in 5.6% cases and *TP53* monoallelic mutations in 15% cases, but observed that the rate of disease progression and lenalidomide resistance was independent of mutation status in either gene. However, lenalidomide resistance was associated with the acquisition and expansion of novel *PPM1D* and *TP53* mutations (75). The findings from both studies suggest that treatment may induce the acquisition of new mutations and confer a selective fitness advantage to hematopoietic clones harboring *PPM1D* mutations. However, the contribution of these mutant clones to disease progression is unclear. Several cases of *PPM1D* truncating mutations have been identified in pediatric therapy–related myeloid neoplasms, although it remains unknown whether these *PPM1D* mutations were present pretreatment (76). These observations lead to two models on the origin and evolution of these mutations: (i) cytotoxic therapies directly induce the mutations, which then clonally expand; or (ii) the mutations were initially present at low VAFs, which may be undetectable depending on depth of sequencing, but preferentially survive and repopulate the hematopoietic compartment after exposure to cytotoxic therapy. Recent studies utilizing whole-genome sequencing of single cell–derived hematopoietic colonies have suggested that *PPM1D* CH-associated mutations can occur early in life, and even *in utero* (77). In addition, the detection of the same somatic mutations with deep sequencing before and after cytotoxic exposure in multiple cases appears to support the latter model (78–80), but the models are not necessary mutually exclusive.

The Role of PPM1D in Oncogenesis

PPM1D has emerged as an oncogenic candidate due to its inhibitory effects on multiple tumor suppressors and DDR regulators. Several studies have experimentally demonstrated that *Ppm1d* overexpression accelerates transformation of MEFs in cooperation with other classic oncogenes such as *Hras, Neu1*, and *Myc*, compared with either *Ppm1d* overexpression or activated oncogenes alone (7, 81, 82). Conversely, deletion of *Ppm1d* yielded the opposite phenotype, with suppression of oncogene-driven transformation of MEFs. Loss of *Ppm1d* impairs carcinogenesis in two mammary tumor models (82) and *Ppm1d* knockout mice have lower lifetime incidences of cancer (9–11, 13, 51). Therefore, *PPM1D* overexpression and *PPM1D* deletion represent opposite ends of the oncogenic spectrum in cancer: overexpression confers a pro-oncogenic effect, whereas deletion confers an anti-oncogenic effect, supporting a significant contributing role of PPM1D in oncogenesis.

The oncogenic effects of PPM1D do not necessarily require genetic modification via gene duplication or activating mutations. Numerous solid cancer cell lines and patient samples have been shown to upregulate *PPM1D* at the mRNA level without copy-number gains (14, 15, 52, 83, 84). This is also true in several leukemia cell lines and primary human AML samples (13). However, *PPM1D* expression in AML seemed to vary according to cytogenetic and molecular status, owing to the heterogeneity and complexity of leukemia development (85). Nevertheless, these findings indicate that upregulation of *PPM1D* supports survival and disease progression in both solid and hematologic malignancies. While mechanisms of PPM1D-mediated oncogenesis based on cancer type has been reviewed recently by others (86), in this section, we will explore the overarching mechanisms in which PPM1D overexpression promotes tumorigenesis. These mechanisms converge on decreased cell-cycle arrest, resistance to apoptosis, and increased metastatic potential.

Decreased cell-cycle arrest

One of the key mechanisms by which *PPM1D*-overexpressing cells can gain proliferative capacity is through the loss of cycle-cell arrest. Upon sensing DNA damage, the DDR is activated to stop normal cellular functions to allow the resolution of damaged DNA prior to DNA replication. This process is critical to maintaining genome integrity by preventing the propagation of harmful genetic lesions. *PPM1D*-overexpressing cells dysregulate cell-cycle checkpoints by persistent dephosphorylation and inactivation of several cell-cycle regulators including ATM (29), CHK1 (5), CHK2 (30), and p53 (ref. 5; **Fig. 3A**).

One of the downstream targets of ATM is the cyclin-dependent kinase (CDK) inhibitor, $p27^{Kip1}$, which binds to CDK2 and inhibits G_1 progression into S-phase. PPM1D not only impairs this cell-cycle checkpoint through ATM dephosphorylation, but it also dephosphorylates $p27^{Kip1}$ at S140 (87). In addition, CHK1 and CHK2 both phosphorylate p53, leading to upregulation of another CDK inhibitor, $p21^{Cip1}$ (88). Truncated PPM1D was found to suppress the expression of *Cdkn1a* (the gene encoding p21) after ionizing radiation exposure in the mouse colon (63) due to the inhibition of p53. PPM1D also dephosphorylates p38 MAPK which leads to decreased expression of the CDK inhibitor, p16, in human patients with breast cancer (82, 89) and non–small cell lung cancer (90).

Several studies in various *PPM1D*-mutated solid cancer cell lines also exhibited impaired cell-cycle arrest *in vitro* (14, 15, 58, 84). U2OS (osteosarcoma) and HCT116 (colorectal) cell lines harboring endogenous *PPM1D* mutations have impaired G_1 cell-cycle arrest after ionizing radiation (IR) (25). Similarly, *PPM1D*-mutated retinal pigmented epithelial cell lines and primary mouse neuronal stem cells (mNSC) also failed to arrest at the G_1 and G_2 checkpoint after IR and continued to proliferate under genotoxic stress (62, 63). Phosphoproteomic studies done in *PPM1D*-mutant mNSCs and patient-derived *PPM1D*-mutant glioma cell lines showed that proteins related to cell cycling and DNA damage were differentially dephosphorylated in the *PPM1D*-mutated samples (62). In hematologic malignancies, *PPM1D*-mutant leukemia cells also exhibit increased cell-cycle progression to G_2–M and proliferative advantages following exposure to cytarabine (18). Normal HSCs must persist throughout one's lifetime to reconstitute the hematopoietic system. Therefore, cell-cycle regulation is critical to maintaining normal HSC function over time. It is currently unknown how *PPM1D* mutations may affect HSC cell cycling, activation, and quiescence. However, given the dormant nature of HSCs, this could potentially give us clues as to why *PPM1D* mutations are not as prevalent in hematologic malignancies.

Several studies have shown that inhibition of *PPM1D* by RNA interference leads to reduced cell proliferation and colony formation ability in thyroid, colorectal, and lung cancer cell lines (84, 90–92). This loss of proliferation was accompanied by increased G_0–G_1 cell-cycle arrest and accumulation of cells in the sub-G_1 phase. In addition, there was a significant downregulation of cyclin B1 in both lung and colorectal cell lines, suggesting another possible mechanism by which *PPM1D* mutant cells escape cell-cycle arrest (91, 92). In papillary thyroid cancer cell lines, siRNA knockdown of *PPM1D* decreased proliferation with a concurrent increase in phospho-p38 MAPK and p53. Interestingly, chemical inhibition of p38 restored the proliferative and colony-forming abilities of *PPM1D* knockdown cells, indicating that suppression of the p38 MAPK pathway is a mechanism by which

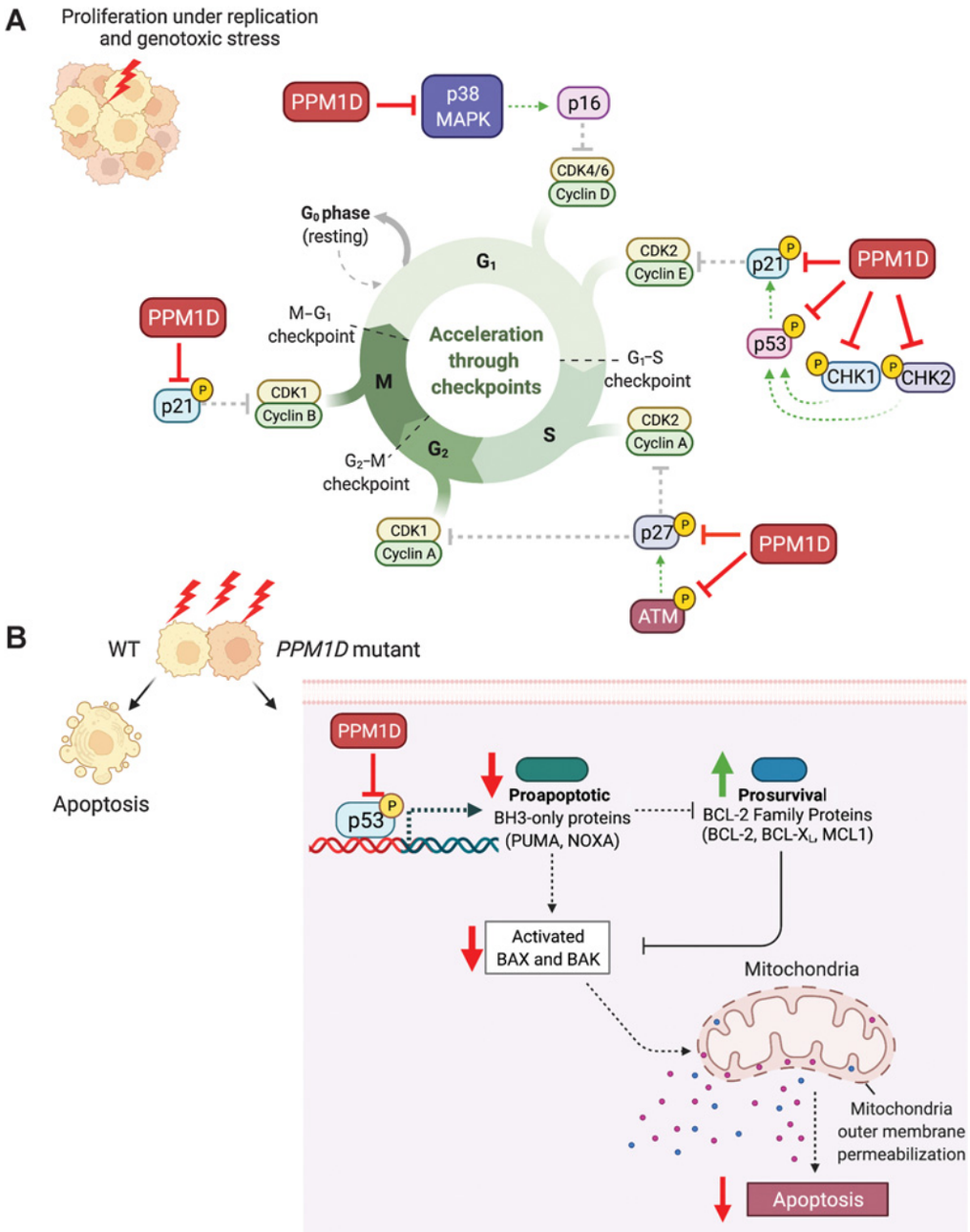

Figure 3.

PPM1D-mediated suppression of cell-cycle arrest and apoptosis. **A,** PPM1D inhibits key cell-cycle regulators including p53, CHK, CHK2, p21, p27, and p38 leading to loss of cell-cycle arrest. **B,** PPM1D inhibits p53 leading to loss of proapoptotic factors resulting in suppression of apoptosis.

PPM1D overexpression promotes proliferation. Overall, *PPM1D* overexpression can affect multiple pathways that result in abnormal cellular proliferation under external stress that can lead to the accumulation and propagation of genetic mutations.

Resistance to apoptosis

Resistance to apoptosis is another key feature of *PPM1D*-overexpressing cells. P53 induces apoptosis by transcriptional activation of the proapoptotic BH3-only family members *PUMA* and *NOXA*. These proteins control cell death by inhibiting the prosurvival BCL2 family proteins, resulting in the derepression of the cell death effectors, BAX and BAK. Activation of BAX and BAK lead to mitochondrial outer membrane permeabilization leading to caspase activation and subsequent apoptosis (**Fig. 3B**; ref. 93). Activation of apoptosis during the DDR allows the elimination of cells with unrepaired DNA lesions. Therefore, *PPM1D* overexpression prevents p53-mediated induction of these proapoptotic factors to allow cells to escape apoptosis. In AML, pancreatic, ovarian, and papillary thyroid cancer cell lines, PPM1D

was also found to suppress apoptosis through dephosphorylation of p38 MAPK, which cross-talks with the p53 pathway (13, 84, 94, 95). Furthermore, *PPM1D* knockdown increased apoptosis, which was reversed by inhibition of p38 phosphorylation (94). This finding once again demonstrates how *PPM1D*-overexpressing cells act through the p38 MAPK pathway to escape both apoptosis and cell-cycle arrest.

The resistance of *PPM1D*-overexpressing cells to apoptosis also extends to stress conditions, such as exposure to chemotherapy and IR. In medulloblastoma cells, *PPM1D* overexpression inhibited p53-mediated apoptosis and cell-cycle arrest following etoposide treatment (52). We and others have demonstrated that *PPM1D*-mutant HSCs undergo less apoptosis compared with wild-type cells after chemotherapeutic insults and ionizing radiation (12, 96). Strikingly, this difference in apoptosis is compounded with multiple rounds of chemotherapy, such that a small proportion of *PPM1D*-mutant HSCs can significantly outcompete wild-type cells following multiple treatments. In *Ppm1d*-truncating mutant mouse models, mutant thymocytes were found to not only have impaired DDR and cell-cycle arrest, but also decreased apoptosis in response to IR. The net effects of these impairments allowed the propagation of cells with improperly repaired lesions and promoted the formation of IR-induced lymphoma (96).

Conversely, PPM1D inhibition has been shown to promote chemosensitivity in colon cancer cells through increased apoptosis following exposure to oxaliplatin, 5-FU, and adriamycin (97). In MCF-7 breast cancer cells, downregulation of PPM1D was also able to sensitize cells to doxorubicin-induced apoptosis through p53 activation of Bax (98). Whether *PPM1D* amplifications or mutations are enriched in the subset of patients who are refractory to cancer treatment or have disease recurrence remains an open question of great clinical interest. Addressing this gap may highlight the need to develop clinically effective PPM1D inhibitors to resensitize *PPM1D*-overexpressing tumors to chemotherapy.

Increased metastatic potential

Finally, *PPM1D*-overexpressing solid cancers have an increased tendency to metastasize to lymph nodes and distant sites and there are several proposed mechanisms by which PPM1D promotes this invasive behavior and migration. Buss and colleagues observed that *PPM1D* expression is significantly increased in metastatic medulloblastoma, and that cells with high *PPM1D* expression have increased levels of CXCR4, a cell surface chemokine receptor that is associated with metastatic behavior. Stimulation of medulloblastoma cells with the CXCR4-ligand, SDF, activated PI-3K signaling and promoted growth and invasion in a p53-dependent manner. In contrast, knocking-out *PPM1D* decreased cell surface accumulation of CXCR4 and inhibited migration and invasion (55).

In pancreatic cancer, PPM1D was shown to promote cell migration and invasion through the Wnt/β-catenin pathway via downregulation of the tumor suppressor ASPP2 (94). Studies in other solid cancer cell lines have also shown that PPM1D expression is positively correlated with the expression of matrix metallopeptidase 2 and 9 (MMP-2 and MMP-9), enzymes that degrade extracellular matrix, plakophilin-2 (PKP-2), a positive regulator of endothelial growth factor receptor signaling, and VEGF-C, a potent angiogenic factor that mediates metastasis to lymph nodes. Consistent with these findings, knockdown of *PPM1D* reduced MMP-9, PKP2, and VEGF-C expression and inhibited invasion and migration. On the contrary, overexpression of *MMP-9*, *PKP2*, or *VEGF-C* in a *PPM1D* knockdown background restored the invasive and migratory phenotype. These studies point to MMP-9, PKP2, and VEGF-C as likely downstream targets of PPM1D activity that are yet to be explored (57–59, 99).

Although the hematopoietic system does not fall under the same physical restraints as solid cancers, the effects of *PPM1D*-mutant cells on the bone marrow microenvironment has yet to be elucidated. Studies have shown that *Ppm1d*-mutant macrophages exhibit a more proinflammatory profile (70). On the other hand, PPM1D has been shown to negatively regulate NFkB and TGFß signaling (100, 101). It would be of interest to study how *Ppm1d*-mutant progenitors in the bone marrow might modulate the activation and differentiation of neighboring HSCs to either promote or suppress growth of other malignant clones.

PPM1D in Solid versus Hematologic Malignancies

The role of *PPM1D*-activating mutations as a supporting oncogene in solid cancer is bolstered by compelling clinical evidence demonstrating that increased *PPM1D* mRNA expression is not only present in a significant subset of tumors but is also associated with more aggressive disease and worse survival outcomes. In contrast to solid malignancies, *PPM1D* mutations appear to play a distinct, more passive role in the hematologic realm. In cases where the frequency of *PPM1D* mutations is lower than the leukemia blast percentage, PPM1D likely plays the role of a passive bystander that is positively selected for following exposure to therapy and is clonally distinct from the driver clone (**Fig. 4**). Another possibility is that *PPM1D*-mutant clones could act as active bystanders, where they indirectly promote disease progression through alterations of the microenvironment or cell competition dynamics in the bone marrow, although this remains an open question. Future work is needed to clarify the precise role of *PPM1D* in hematologic malignancies, particularly the discrepancy between its prevalence in clonal expansion and in *de novo* malignancy.

Given that PPM1D affects many DDR and stress response pathways, it is possible that *PPM1D* mutants have increased suppression of these pathways leading to genomic instability and accumulation of DNA damage over time. Whether *PPM1D* mutants have an increased overall mutation burden at baseline and following exposure to genotoxic stresses is under active investigation. One plausible hypothesis is that *PPM1D* mutations potentiate the acquisition of additional mutations, which then work cooperatively with PPM1D to accelerate the initiation or progression of cancer. Indeed, *PPM1D* had been found to broadly comutate with multiple other

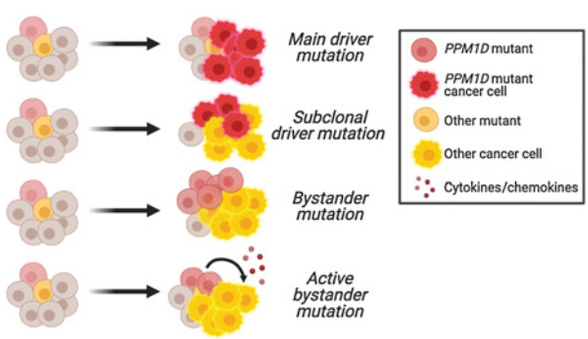

Figure 4.
Potential roles of *PPM1D* mutations in the blood. Schematic showing the potential roles of *PPM1D* mutations as driver, subclonal, bystander, or active bystander mutations in leukemogenesis. The precise role is unknown.

genes in solid tumors, t-AML and t-MDS (12). Systematic study of the effect of different cooperating mutations on the rate of transformation in different cancer contexts will enable us to better understand the potency of *PPM1D* amplification and mutations as an oncogenic driver in hematologic malignancies.

The frequent appearance of *PPM1D* mutations and amplifications in cancer also suggests an underlying competitive advantage that may precede the manifestation of disease. Broadly, there are two possibilities for this fitness advantage: an intrinsic advantage independent of stressors and/or an advantage that is dependent on the presence of external stressors. We have discussed the intrinsic cellular advantages in the previous sections in which *PPM1D* overexpression can drive suppression of cell-cycle arrest and apoptosis. In contrast to solid tumors, where *PPM1D* overexpression promotes fitness in cooperation with other oncogenes, external stressors appear to play a more major role in the expansion of *PPM1D*-mutant clones in the hematologic setting (**Fig. 5**). Selection of *PPM1D*-mutant clones under cytotoxic therapies in the hematopoietic setting raises an interesting question of whether *PPM1D*-mutant cells are similarly selected for in solid tumors following exposure to chemotherapy. While solid tumors (i.e., ovarian cancer) refractory to cisplatin treatment may be enriched for *PPM1D* mutations, this has not yet been explored. In support of this possibility, premalignant *PPM1D* clonal expansion has recently been observed in nonhematopoietic tissues, including the esophageal lining. One study identified clones harboring *PPM1D* somatic mutations in 13% of normal esophageal epithelial samples (102). In several elderly individuals, the mutated clones were found to have expanded and replaced the majority of normal epithelium. Of note, the authors observed that heavy alcohol consumption and tobacco use, both of which are known mutagenic agents, substantially accelerate clonal expansion in the esophagus. Interestingly, the aldehyde metabolites from alcohol have been shown to cause DNA DSBs and chromosomal rearrangements, reminiscent of genotoxic therapy (103). Therefore, environmental exposures may also play a significant role in the expansion of premalignant *PPM1D*-mutant clones in normal, nonhematopoietic tissues. Altogether, these findings suggest that various environmental exposures associated with cellular or genotoxic stress can affect *PPM1D* clonal dynamics and transformation into malignancy.

Concluding Remarks

Through the suppression of DNA repair, cell cycle, and apoptosis, *PPM1D* overexpression can drive uncontrolled cellular growth to promote malignancy. Further studies on PPM1D will lead to a more complete understanding of the mechanisms through which PPM1D promotes oncogenesis, particularly in the context of its divergent role in solid and liquid malignancies. It remains an open question as to what the exact significance of *PPM1D* genomic aberrations is in normal tissues and premalignant states. Could PPM1D act as a "friend" by competing with and suppressing the growth of other, more "oncogenic" clones such as *TP53* mutants? At what point does it turn from being a "friend" into a "foe"? Studies have demonstrated that *PPM1D* is more tumorigenic when it cooperates with other oncogenes (63). Inclusion of *PPM1D* in sequencing panels for normal and malignant tumor and blood samples will increase our knowledge of cooperating mutations and expand our understanding of the clinical implications of *PPM1D* overexpression.

PPM1D is an attractive therapeutic target given its prevalence in many cancers and its oncogenic potential. Yet, we are still lacking a clinically effective small molecular inhibitor. However, our understanding of PPM1D in the DDR lends new strategies for cancer therapies. Several studies have demonstrated that inhibition of PPM1D *in vitro* can modulate the sensitivity of cancer cells to other DDR-targeted therapies including PARP inhibition (41) and MDM2 antagonists (85, 104). Further investigations could yield additional druggable targets that

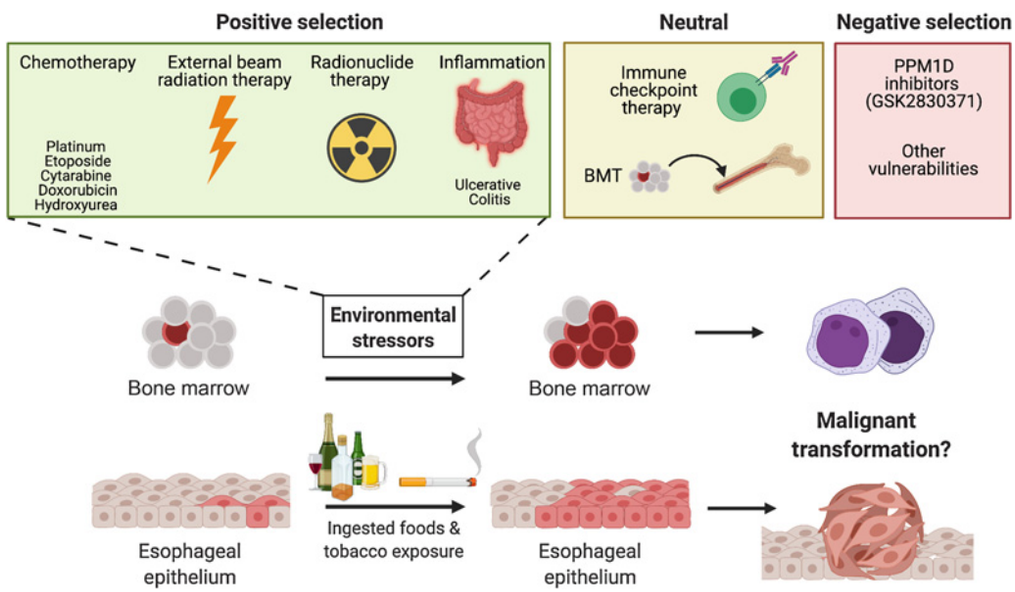

Figure 5.

Environmental conditions that promote the selection of *PPM1D* mutants in the blood and esophageal lining. Schematic representation of how premalignant clonal expansion of *PPM1D* mutants in the blood and esophagus is shaped by multiple environmental stressors. These stressors can have a positive, neutral, negative effect on *PPM1D*-mutant clonal dynamics.

either sensitize or confer synthetic lethality to cells bearing *PPM1D* mutations, an avenue that remains to be explored and would contribute greatly towards PPM1D-specific therapeutic development.

Authors' Disclosures

No disclosures were reported.

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