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The role of Pdcd4 in tumor suppression and protein translation

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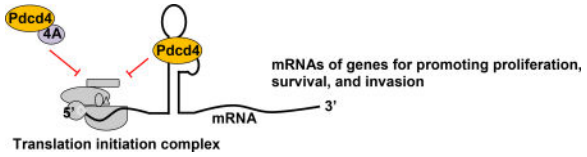
Abstract

Programmed cell death 4 (Pdcd4), a tumor suppressor, is frequently down-regulated in various types of cancer. Pdcd4 has been demonstrated to efficiently suppress tumor promotion, progression, and proliferation. The biochemical function of Pdcd4 is a protein translation inhibitor. Although the fact that Pdcd4 inhibits protein translation has been known for more than a decade, the mechanism by which Pdcd4 controls tumorigenesis through translational regulation of its target genes is still not fully understood. Recent studies show that Pdcd4 inhibits translation of stress-activated-protein kinase interacting protein 1 to suppress tumor invasion, depicting a picture of how Pdcd4 inhibits tumorigenesis through translational inhibition. Thus, understanding the mechanism of how Pdcd4 attenuates tumorigenesis by translational control should provide a new strategy for combating cancer.

Graphical Abstract



Programmed cell death 4 (Pdcd4) functions as a tumor suppressor and an inhibitor of protein translation. Pdcd4 is the binding partner of eIF4A. By binding with eIF4A, Pdcd4 inhibits eIF4A's helicase activity to attenuate translation of mRNAs with structured 5'UTR. In addition, Pdcd4 may directly bind with mRNA to interfere the translation process. The translational targets of Pdcd4 are involved in cell proliferation, survival, and invasion. Thus, understanding the mechanism by which Pdcd4 suppresses translation may provide a new strategy for cancer intervention.

Keywords

eIF4A; tumor promotion; tumor invasion; proliferation

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Conflict of interest statement

The authors have declared no conflict of interest.

Introduction

The cDNA of Programmed cell death 4 (Pdcd4) was initially cloned from mouse cells when Pdcd4 expression was up-regulated by treatment with dexamethasone (Shibahara et al. 1995). Subsequently, this gene was identified in different species including human, rat, and chicken (Azzoni et al. 1998, Goke et al. 2002, Schlichter et al. 2001); and the deduced amino acid sequences are highly conserved among these species. For example, the amino acid sequence of Pdcd4 from human and mouse shares about 92% identity and 96% homology. The expression of Pdcd4 is frequently down-regulated in numerous types of cancers including breast, colon, liver, lung, pancreas, and skin cancers. It has been demonstrated that Pdcd4 functions as a tumor suppressor, inhibiting tumor promotion, invasion/metastasis, and proliferation. The established biochemical function of Pdcd4 is that Pdcd4 inhibits eIF4A's helicase activity and thereby suppresses protein translation. Thus, suppression of tumorigenesis by Pdcd4 is believed to occur through translation inhibition of a set of genes involved in tumor cell proliferation, migration, invasion, and metastasis. In this review, we will discuss current understanding of the mechanisms by which Pdcd4 regulates tumorigenesis through translational control of its target gene expression.

Structural motifs of Pdcd4

Pdcd4 protein composed of 469 amino acids contains two so-called "MA-3" domains, two phosphorylation sites, two clusters of positively charged residues, and two nuclear export signals (NES) (Figure 1). Amino acid sequence alignment and protein secondary structure analysis indicate that the two MA-3 domains on Pdcd4 reside at residues 164 to 275 and 327 to 440. The MA-3 domain typically spans about 120 amino acid residues and contains 7 or 8 α-helices, which is also found in other proteins including translation initiation factor eIF4G, nonsense-mediated mRNA decay 2 protein, and the nuclear cap-binding protein CBP80 (Aravind and Koonin 2000). Crystal structure and mutation analyses demonstrate that Pdcd4 uses MA-3 domains to interact with eukaryotic initiation factor 4A (eIF4A) (Chang et al. 2009, Loh et al. 2009, Yang et al. 2004). In addition, Pdcd4 protein can be phosphorylated by Akt or p70S6K at Ser67, and phosphorylation at Ser67 is recognized by β transducin repeats-containing proteins (β-TRCP), an E3 ligase, resulting in proteasomal degradation of Pdcd4 (Dorrello et al. 2006, Palamarchuk et al. 2005). Palamarchuk *et al.* also suggested that phosphorylation of Pdcd4 at Ser67 and Ser457 leads to nuclear localization (Palamarchuk et al. 2005). Pdcd4 was also reported to bind with RNA through two clusters of positively charged amino acids (Bohm et al. 2003, Wedeken et al. 2010), and these two clusters are required for Pdcd4 to bind with *A-MYB* and *C-MYB* mRNAs and suppress their translation (Biyanee et al. 2015, Singh et al. 2011). Since Pdcd4 contains two NES signals, it is speculated to shuttle between nucleus and cytoplasm (Bohm et al. 2003). Pdcd4 suppresses protein translation in cytoplasm via binding with eIF4A, but the function of Pdcd4 in nucleus is unknown.

Regulation of Pdcd4 expression

Pdcd4 expression can be regulated at levels of epigenetics, transcription, post-transcription, and post-translation, which have been previously discussed (Yang et al. 2014). Here, we only

discuss two essential and well-known mechanisms: ubiquitin-mediated degradation and micro RNA-mediated degradation.

As aforementioned, Pdcd4 can be phosphorylated at Ser67 by p70S6K and Akt. Upon phosphorylation by p70S6K or Akt, Pdcd4 is recognized and bound by an ubiquitin E3 ligase, β-TRCP (Dorrello et al. 2006). The β-TRCP belongs to the F-box family, the substrate recognition component of Skp1-Cul1-F-box protein (SCF) ubiquitin ligase complex, which transfers ubiquitin molecules to target proteins for proteasomal degradation (Cardozo and Pagano 2004). Thus, after binding with β-TRCP, Pdcd4 is ubiquitinated and consequently degraded by proteasome. The other important mechanism to regulate Pdcd4 expression is through microRNA (miRNA). miRNA is a RNA molecule containing 20–25 nucleotides that binds with target RNA at 3' untranslated region (3'UTR) and negatively regulates gene expression. The 3'UTR of Pdcd4 contains a perfectly matched target sequence for miR-21 (Asangani et al. 2008, Lu et al. 2008). miRNA-21 binds with *pdcd4* mRNA to attenuate the translation of *pdcd4* mRNA. Bioinformatics analyses predict that more than 80 micro RNAs potentially target Pdcd4, suggesting an important role of microRNA in regulating Pdcd4 expression (Dweep et al. 2011).

Pdcd4 inhibits tumor promotion

Using RNA differential display assay, Pdcd4 was found to be highly expressed in JB6 transformation-resistant cells comparing to the JB6 transformation susceptible cells (Cmarik et al. 1999). The mouse epidermal JB6 cells have 3 genetic variants, namely transformation-resistant, transformation-susceptible, and transformed cells. Exposure of transformation-susceptible JB6 cells to tumor promoter such as 12-O-tetradecanoylphorbol-13-acetate (TPA) or tumor necrosis factor α (TNFα) results in neoplastic transformation. On the other hand, the transformation-resistant JB6 cells resist TPA- or TNFα-induced transformation. Thus, this cell culture system is an ideal tool for studying the biochemical events during tumor promotion. To demonstrate the inhibitory role of Pdcd4 on tumor promotion, expression of *PDCD4* antisense in transformation-resistant JB6 cells to lower the Pdcd4 protein level results in a gain of transformation-susceptible phenotype (Cmarik et al. 1999). In consistence with this finding, ectopic expression of *PDCD4* cDNA in transformation-susceptible JB6 cells render cells insensitive to TPA-induced neoplastic transformation (Yang et al. 2003b). In addition to these cultured cell studies, transgenic mice with over-expression of Pdcd4 in skin efficiently reduces 7,12-dimethylbenz(a)anthrance (DMBA)/TPA induced papilloma formation (Jansen et al. 2005), while knockout of Pdcd4 expression in mouse skin increases the number of papilloma after DMBA/TPA treatment (Schmid et al. 2008). Collectively, these results indicate that Pdcd4 inhibits tumor promotion.

Pdcd4 inhibits tumor invasion and metastasis

Beyond inhibiting tumor promotion, over-expression of *PDCD4* cDNA also suppresses tumor invasion or intravasation in many types of cancer cells including breast, colon, liver, gastric, and ovarian cancer (Gonzalez-Villasana et al. 2012, Leupold et al. 2007, Nieves-Alicea et al. 2009, Wei et al. 2012, Yang et al. 2006, Yu et al. 2014, Zhang et al. 2009). On

the other hand, lowering Pdcd4 expression by *PDCD4* siRNA or shRNA stimulates invasion in breast, colon, lung, and nasopharyngeal cancer cells (Santhanam et al. 2010, Wang et al. 2008, Wang et al. 2010, Yang et al. 2015, Zhen et al. 2017). In animal studies, knockdown of Pdcd4 in colon cancer cells promotes metastasis to lymph node and liver when these cells are injected into cecum of nude mice (Wang et al. 2013). Mice with Pdcd4 knockdown develops tumors with frequent metastasis to liver and kidney (Hilliard et al. 2006). Moreover, knockdown of Pdcd4 also causes a decrease in expression of several epithelial markers including E-cadherin, α-catenin, and γ-catenin as well as an increase in expression of mesenchymal markers like β-catenin, N-cadherin, and fibronectin in both cultured cells and mice (Wang et al. 2008, Wang et al. 2010, Wang et al. 2013), suggesting that Pdcd4 knockdown induces epithelial to mesenchymal transition (EMT). EMT is the process for cells to acquire a fibroblast-like phenotype, lose epithelial polarity, and boost migration to penetrate the surrounding extracellular matrix (Grunert et al. 2003), which is a key step for tumor cells to gain invasive and metastatic ability. All of the above evidence indicates that Pdcd4 functions as an invasion/metastasis inhibitor.

Pdcd4 inhibits cell proliferation

Immunohistochemical staining of human intraductal papillary mucinous neoplasm of pancreas tissues showed that Pdcd4 expression is inversely correlated with the level of proliferation marker Ki-67 (Hayashi et al. 2010), suggesting that Pdcd4 may regulate tumor cell proliferation. In line with the animal studies, over-expression of Pdcd4 suppresses proliferation in several cancer cell lines including breast carcinoma (Cuesta and Holz 2016), hepatocellular carcinoma (Zhang et al. 2009), nasopharyngeal carcinoma (Zhen et al. 2013), glioma (Chen et al. 2016), and mesenchymal stem cells (Liu et al. 2017). Conversely, knockdown of Pdcd4 stimulates proliferation by increasing cyclin D1 expression in colon carcinoma cells (Guo et al. 2011). Similarly, Pdcd4 null ($Pdcd4^{-/-}$) mouse embryonic fibroblast (MEF) cells appear to proliferate faster than wild-type ($Pdcd4^{+/+}$) MEF cells (Wang et al. 2017). These findings indicate that Pdcd4 suppresses cell proliferation. In contrast to the aforementioned results, Ullman and colleagues showed that Pdcd4 can be methylated at Arg110 and the methylated Pdcd4 interacts more robustly with eIF4A resulting in enhanced viability of cells during nutrient and growth factor depletion (Fay et al. 2014, Powers et al. 2011). It is believed that Pdcd4 binds with eIF4A to inhibit translation of mRNAs with structured 5’ untranslated region (5’UTR) (see below). Usually, the mRNAs of growth factors, growth promotion genes, and oncogenes carry structured 5’UTR (De Benedetti and Harris 1999). It is unknown how Pdcd4 improves cell viability during nutrient and growth factor depletion by binding with eIF4A. Thus, it is necessary to identify the translational targets of Pdcd4 under such conditions.

Pdcd4 is the binding partner of eIF4A

In the yeast two-hybrid assay, using Pdcd4 cDNA as bait to screen the mouse cDNA library, Pdcd4 was identified to interact with eIF4A and this interaction was further confirmed by glutathione S-transferase pull-down, immunoprecipitation, immunolocolization, and mammalian two-hybrid assays (Goke et al. 2002, Yang et al. 2003a, Zakowicz et al. 2005). The Pdcd4 crystal structure showed that each MA-3 domain consists of eight or nine α-

helices (Chang et al. 2009, Loh et al. 2009). The analyses of Pdcd4-eIF4A co-crystal structure further revealed that Pdcd4 uses these two MA-3 domains to interact with eIF4A (Chang et al. 2009, Loh et al. 2009), in which one Pdcd4 molecule is sandwiched between two molecules of eIF4A (Chang et al. 2009, Loh et al. 2009). Pdcd4 binds to eIF4A in an approximately vertical position with each MA3 domain contacting both N- and C-terminal domains of eIF4A (Chang et al. 2009, Loh et al. 2009). The interactions between Pdcd4 and eIF4A are ionic interactions or hydrogen bonds mediated by several conserved residues on both Pdcd4 and eIF4A. For example, Glu249 and Asp253 in one MA-3 domain of Pdcd4 form salt bridges with Arg161 and Arg110 in the first eIF4A molecule and the Asp414 and Asp418 of the other MA3 domain interact similarly with the Arg161 and Arg110 in the second eIF4A molecule. Consistent with these observations, mutation of Glu249 to Lys, Asp253 to Ala, Asp414 to Lys, or Asp418 to Ala in Pdcd4 forfeits the binding ability to eIF4A in the mammalian two-hybrid assay (Yang et al. 2004). In contrast to the crystal structure analyses, Waters *et al.* used NMR analyses to reveal that Pdcd4 and eIF4A forms Pdcd4-eIF4A complex in a 1:1 ratio in solution and only one MA3 domain of Pdcd4 interacts with one eIF4A molecule (Waters et al. 2011). In support of this notion, they found that only double mutation of Glu249 to Ala/Asp253 to Ala but Asp414 to Ala or Asp 418 to Ala abolish Pdcd4's binding ability to eIF4A in immunoprecipitation assays. It is possible that the interactions between Pdcd4 and eIF4A are different in crystal and solution conditions. However, it is unclear whether the discrepancy of the result of mutation analyses is due to the different experimental methods, i.e. mammalian two hybrid vs. immunoprecipitation.

Pdcd4 is an inhibitor of translation

By binding with eIF4A, an ATP dependent RNA helicase, Pdcd4 inhibits eIF4A's helicase activity (Yang et al. 2003a). The eIF4A is critical for unwinding structured mRNA, allowing translation initiation complex to move along the mRNA and locate the translation initiation codon during translation initiation. Thus, inhibition of eIF4A's helicase activity is expected to curb translation of mRNAs with structured 5'UTR more efficiently than those without. This model was supported by the finding that Pdcd4 suppresses translation of the luciferase with a synthetic stem-loop structure at 5'UTR greater than the one without it (Yang et al. 2004). In agreement with this finding, Wedeken *et al.,* reported that *P53* 5'UTR mRNA can form a stable secondary structure and Pdcd4 efficiently inhibits translation of luciferase fused with *P53* 5'UTR (Wedeken et al. 2011). Additionally, knockdown of Pdcd4 increases the distribution of *P53* mRNA to polysomal fractions in a polysomal fractionation assays, suggesting that p53 is a genuine target of Pdcd4 (Wedeken et al. 2011). Under stress conditions such as UV radiation, Pdcd4 expression is downregulated and thereby p53 translation increases in response to stress induced apoptosis (Figure 2). Recently, this model was further confirmed by identification of another Pdcd4 target, stress-activated-protein kinase interacting protein 1 (Sin1), whose mRNA also forms a stable secondary structure at 5'UTR (Wang et al. 2017). Pdcd4 inhibits translation of luciferase fused with *SIN1* 5'UTR (Sin1-5'UTR-Luc) in a concentration dependent manner but not the luciferase without *SIN1*-5'UTR. However, Pdcd4 mutant defective in eIF4A binding is not able to inhibit translation of Sin1-5'UTR-Luc. In agreement with above observations, inhibition of eIF4A

activity by chemical inhibitor, silvestrol, sufficiently inhibits Sin1-5'UTR-Luc translation and Sin1 expression, suggesting that eIF4A activity plays a role in Pdcd4 inhibited Sin1 translation. Sin1 is an exclusive component of mammalian target of rapamycin complex 2 (mTORC2). Inhibition of Sin1 translation by Pdcd4 results in repression of mTORC2 activity and thereby suppresses Akt activation to attenuate tumor proliferation and invasion (Figure 2). Taken together, these results reveal that Pdcd4 inhibits eIF4A activity to suppress translation of mRNAs with structured 5'UTR.

Apart from the eIF4A dependent mechanism, Pdcd4 may directly bind with mRNA to suppress its translation. It was reported that Pdcd4 inhibits mRNA translation of antiapoptotic proteins, Bcl-x_L and X chromosome-linked inhibitor of apoptosis (XIAP), through directly binding to the internal ribosome entry site element and blocking the formation of translation initiation complex (Liwak et al. 2012). In response to survival signaling, p70S6K2 phosphorylates Pdcd4 resulting in Pdcd4 degradation, which leads to enhanced translation of XIAP and Bcl-x_L (Liwak et al. 2012) (Figure 2). Moreover, Fehler *et al.* suggested that by association with poly(A) binding protein, Pdcd4 may bind with *C-MYB* mRNA and impede its translation elongation (Fehler et al. 2014). c-Myb is a transcription factor, whose target genes are involved in proliferation and survival (Ramsay and Gonda 2008) (Figure 2). Whether Pdcd4 binds with mRNA through a specific sequence, motif, or tertiary structure remains unknown, requiring further investigation.

Mechanisms of regulating tumorigenesis by Pdcd4

Over-expression of Pdcd4 in JB6 transformation-susceptible cells inhibits both basal and TPA-induced AP-1 transactivation (Yang et al. 2001). Transcription factor AP-1 is a homodimer or heterodimer of Jun and Fos proteins, which plays a crucial role in regulation of tumor promotion. For instance, repression of AP-1 transactivation by dominant negative c-Jun or AP-1 inhibitor, SR11302, leads to inhibition of TPA-induced neoplastic transformation in JB6 cells (Dong et al. 1994, Li et al. 1997). Several downstream targets of AP-1 dependent transcription are involved in cell proliferation, transformation, and invasion (Ozanne et al. 2000). Therefore, inhibition of AP-1 activation by Pdcd4 is a key event for suppression of tumorigenesis. Using transactivation assays, Pdcd4 was further shown to inactivate AP-1 transactivation through inhibition of c-Jun activation in mouse JB6 cells (Yang et al. 2003b). Furthermore, Bitomsty *et al.* discovered that Pdcd4 interferes activation and phosphorylation of c-Jun by suppression of Jun N-terminal kinase (JNK) activation in avian QT6 cells (Bitomsky et al. 2004). How does Pdcd4 inhibit JNK and c-Jun activation? Pdcd4 was found to regulate the expression of a JNK upstream kinase, mitogen-activated protein kinase kinase kinase kinase 1 (MAP4K1), to control the activation of JNK, c-Jun, and AP-1 in colon HT29 cells (Wang et al. 2012) (Figure 3). Mutation of the c-Myc binding site, located within the −536 bp of MAP4K1 promoter, dramatically reduces MAP4K1 promoter activity (Wang et al. 2012). In addition, lower c-Myc expression by *C-MYC* siRNA inhibits MAP4K1 expression (Wang et al. 2012). These findings indicate that c-Myc regulates MAP4K1 expression. Moreover, knockdown of Pdcd4 suppresses E-cadherin expression inducing activation of β-catenin dependent transcription and thereby stimulating expression of its downstream targets, c-Myc and urokinase-type plasminogen activator (u-PAR) (Wang et al. 2010). The uPAR, a 55–60 kDa glycosylated receptor, binds to its ligand

and leads to degradation of extracellular matrix components allowing tumor cells to penetrate basal membrane during invasion (Blasi 1993). Collectively, when Pdcd4 is knocked down, elevates c-Myc and u-PAR expression. Both contribute to the promotion of tumor invasion (Kariko et al. 1993) (Figure 3).

Loss of E-cadherin is a distinctive feature for EMT to gain the ability of migration and invasion. E-cadherin expression can be regulated by various mechanisms including repression by zinc-finger transcription factors, Snail (Wang et al. 2010). Snail, a transcription repressor, binds to E-boxes on the E-cadherin promoter and suppresses its transcription (Cano et al. 2000). Knockdown of Snail in the Pdcd4 knockdown cells restores the E-cadherin promoter activity, showing that Snail does contribute to the inhibition of E-cadherin expression by Pdcd4 knockdown. How does Pdcd4 regulate Snail expression? Recently, we found that knockdown of Akt abolishes Pdcd4 knockdown-induced Snail expression (Wang et al. 2017). As the consequence of Snail downregulation, E-cadherin expression increases in Akt knockdown cells. One potential pathway for Akt up-regulating Snail expression is through activation of NF-κB (Julien et al. 2007). Akt phosphorylates IKKβ resulting in phosphorylation of the NF-κB inhibitor, IκB, and the phosphorylated IκB is subsequently ubiquitinated and degraded leading to NF-κB activation. The other possible mechanism is that Akt phosphorylates glycogen synthase kinase 3 (GSK3) to inactivate it, preventing proteasomal degradation of Snail (Zhou et al. 2004). Further investigation is needed to determine through which pathway Akt regulates Snail expression.

Pdcd4 has been demonstrated to regulate Akt activation (Guo et al. 2011, Lankat-Buttgereit et al. 2008, Wei et al. 2012). Akt mediates numerous cellular functions including proliferation, invasion, and metastasis, and is frequently activated in many types of human cancers (Vivanco and Sawyers 2002). Akt activity is mainly regulated by 3-phosphoinositide-dependent kinase 1 (PDK1) and mTORC2. Phosphorylation of Thr308 by PDK1 increases Akt kinase activity, but its maximal activity requires phosphorylation of Ser473 by mTORC2 (Guertin and Sabatini 2007). Recent studies demonstrate that Pdcd4 inhibits mTORC2 activation via suppression of Sin1 translation to attenuate Akt activity (Wang et al. 2017). Inhibition of Sin1 translation by Pdcd4 sufficiently attenuates invasion in colon carcinoma (Wang et al. 2017), suggesting that Pdcd4 inhibits the mTORC2-Akt axis to suppress invasion (Figure 3). In addition, regulation of Akt activation by Pdcd4 also contributes, at least in part, to the promotion of cell proliferation by controlling cyclin D1 expression (Guo et al. 2011). Collectively, Pdcd4 suppresses Sin1 translation to attenuate the mTORC2 activity resulting in inhibition of Akt activation and expression of its downstream targets, cyclin D1 and Snail. It is known that Snail negatively regulates E-cadherin expression to suppress β-catenin and AP-1 dependent transcriptions.

As mentioned above, Pdcd4 may directly inhibit translation of anti-apoptotic proteins, XIAP and Bcl-x_L to promote apoptosis. In addition, Pdcd4 has been reported to increase apoptosis by up-regulating pro-apoptotic proteins. Up-regulation of Pdcd4 expression in the lung of K-Ras-null mice via aerosol delivery of *PDCD4* cDNA significantly increases the expression of pro-apoptotic proteins BAD and Apaf-1 (Jin et al. 2006). In the same study, the authors also found that up-regulation of Pdcd4 suppresses the expression of cell cycle regulatory molecule, cyclin-dependent kinase 4 (CDK4) (Jin et al. 2006). While down-regulation of

Pdcd4 by delivery of *PDCD4* shRNA into the lung of A/J mice enhances CDK4 expression (Hwang et al. 2010), indicating that Pdcd4 inhibits cell proliferation and induces apoptosis in mouse lung. In addition, it has shown that Pdcd4 inhibits invasion in breast cancer cells by enhancing the expression of tissue inhibitor of metalloproteinase 2 (TIMP2) (Gonzalez-Villasana et al. 2012, Nieves-Alicea et al. 2009). The TIMP2 is a natural inhibitor of the matrix metalloproteinases (MMPs), which suppresses cell migration and invasion by preventing penetration of extracellular matrix. Lankat-Buttgereit *et al.* found that Pdcd4 inhibits carbonic anhydrase type II (CAII) expression (Lankat-Buttgereit et al. 2004). CAII hydrates CO_2 to bicarbonate, which is an important substrate for synthesis of lipids, amino acids, and pyrimidine for fast growth tumor cells.

Conclusions and future directions

Pdcd4 is well positioned to be an integrator of inhibiting protein translation and suppressing tumorigenesis. It appears that Pdcd4 inhibits translation of a set of genes including Sin1, p53, c-Myb, Bcl-X_L, and XIAP to suppress tumorigenesis (Fehler et al. 2014, Liwak et al. 2012, Wang et al. 2017, Wedeken et al. 2011). Thus, identification of more Pdcd4 translational targets will allow us to understand in depth how Pdcd4 inhibits tumorigenesis. Pdcd4 is known to inhibit the activation of Akt, one of the most frequently activated kinases in numerous cancers, which regulates cell survival, proliferation, apoptosis, and invasion. In addition, recent studies indicates that Pdcd4 inhibits mTORC2 activation, which also plays a fundamental role in regulating tumor cell migration, invasion, and metastasis (Zhou and Huang 2011). Since Pdcd4 expression is frequently down-regulated in cancerous cells, restoration or stimulation of Pdcd4 expression is expected to inhibit the mTORC2-Akt axis, which should be a promising strategy for cancer prevention and treatment. Thus, compounds that efficiently elevate Pdcd4 expression should have great potential for cancer therapeutics.

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Abbreviations

CAII	carbonic anhydrase type II
5'UTR	5'untranslated region
eIF4A	eukaryotic initiation factor 4A
β-TRCP	β-transducin repeats-containing proteins

EMT	epithelial to mesenchymal transition
GSK3	Glycogen synthase kinase 3
JNK	Jun N-terminal kinase
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1
mTORC2	mammalian target of rapamycin complex 2
p70S6K	70 kDa ribosomal S6 kinase
Pdcd4	programmed cell death 4
Sin1	stress-activated-protein kinase interacting protein 1
TIMP2	tissue inhibitor of metalloproteinase 2
TPA	12-O-tetradecanoylphorbol-13-acetate
XIAP	X chromosome-linked inhibitor of apoptosisp

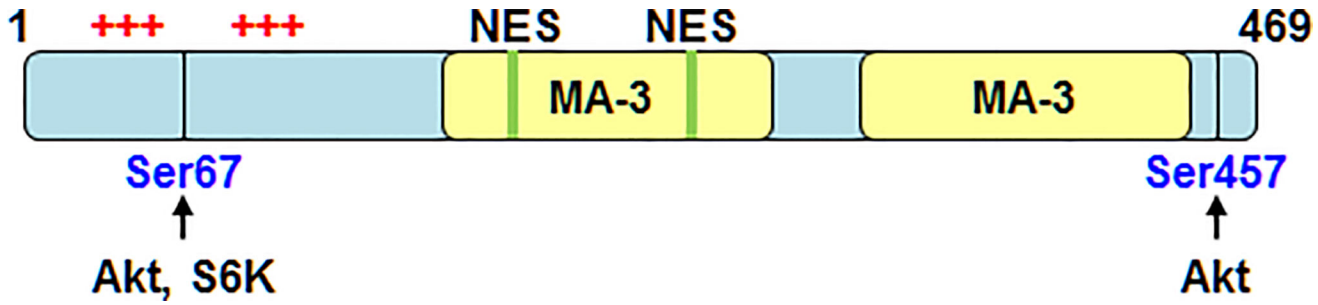

Figure 1.

Schematic diagram of the functional motifs and sites of Pdcd4. Arrows indicate the phosphorylation sites by Akt or S6K1. Green boxes designate the potential nuclear translocation signals (NES). The +++ indicates the cluster of positively charged residues. The MA-3 domains are in yellow.

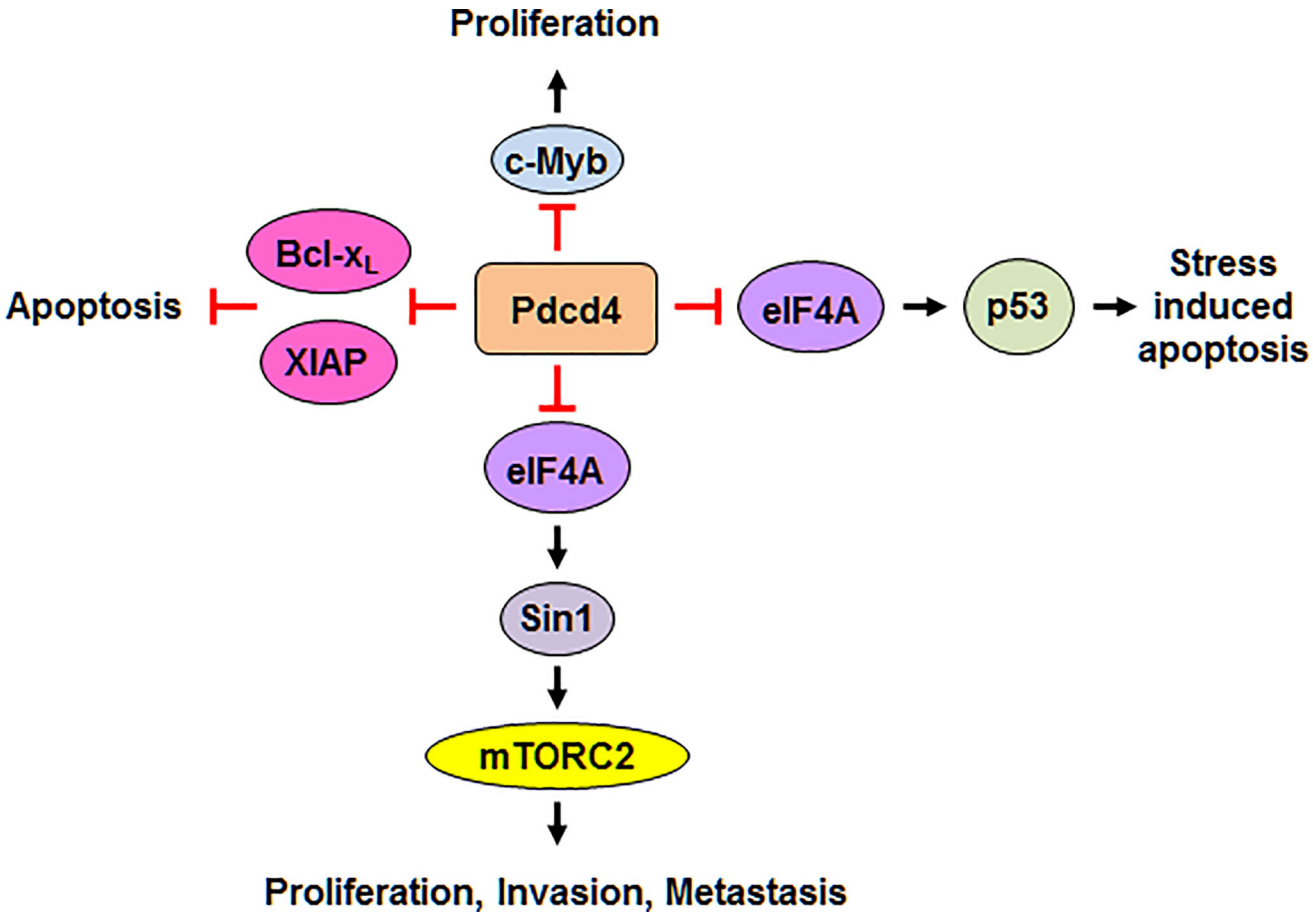

Figure 2.

Pdcd4 translational targets are involved in regulation of proliferation, apoptosis, invasion, and metastasis. Pdcd4 inhibits eIF4A activity to suppress translation of p53 and Sin1. Inhibition of p53 translation results in repression of stress induced apoptosis, while inhibition of Sin1 translation attenuates cell proliferation, invasion, and metastasis. Besides partners with eIF4A, Pdcd4 may directly bind with mRNAs of c-Myb, Bcl-xL, and XIAP to inhibit their translations, leading to suppression of proliferation and anti-apoptosis.

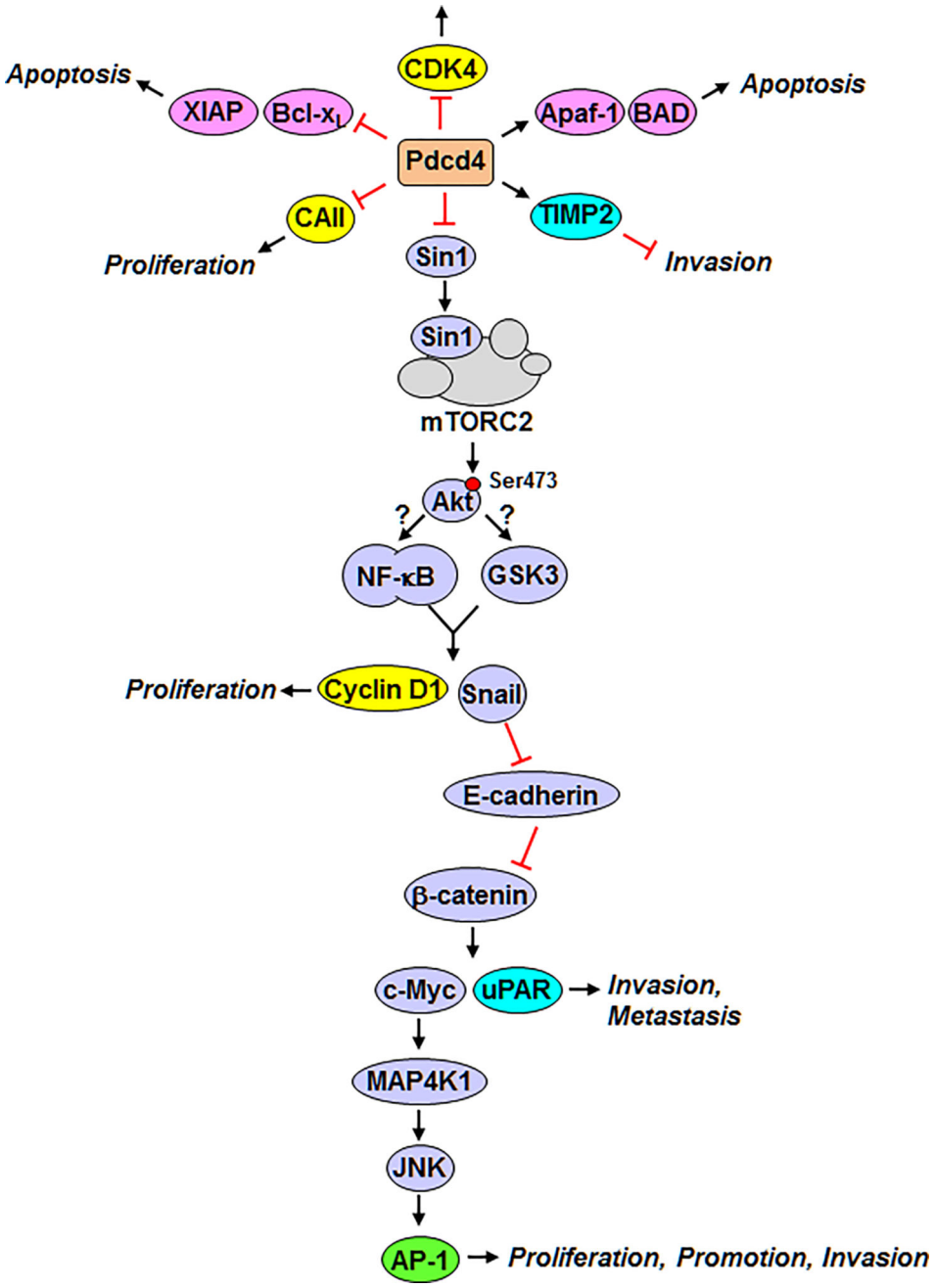

Figure 3.

Pdcd4 suppresses tumorigenesis through inhibition of multiple pathways. Pdcd4 inhibits Sin1 translation to inactivate mTORC2. Suppression of mTORC2 activity attenuates activation and phosphorylation at Ser473 of Akt resulting in decreased expression of cyclin D1 and Snail through inactivation of NF-κB or GSK3. Reduction of cyclin D1 expression leads to decreased cell proliferation. The transcription repressor Snail negatively regulates E-cadherin expression resulting in activation of β-catenin dependent transcription and increased c-Myc and u-PAR expression. u-PAR has been demonstrated to promote tumor cell invasion; c-Myc subsequently stimulates MAP4K1 expression and thereby activates

AP-1 dependent transcription to regulate tumor proliferation, promotion, and invasion. Inhibition of TIMP expression by Pdcd4 was also reported to suppress invasion. Pdcd4 may repress cell proliferation through suppression of anti-apoptotic XIAP and Bcl-x_L or stimulation of apoptotic Apaf-1 and BAD expression. Attenuation of cell proliferation by Pdcd4 also can be achieved by inhibition of CDK4 and CAII expressions. The color of yellow, blue, and pink indicates the proteins involved in proliferation, invasion, and apoptosis, respectively. The protein with multiple functions is in green.