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INVITED REVIEW

Proline dehydrogenase in cancer: apoptosis, autophagy, nutrient dependency and cancer therapy

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Abstract

L-proline catabolism is emerging as a key pathway that is critical to cellular metabolism, growth, survival, and death. Proline dehydrogenase (PRODH) enzyme, which catalyzes the first step of proline catabolism, has diverse functional roles in regulating many pathophysiological processes, including apoptosis, autophagy, cell senescence, and cancer metastasis. Notably, accumulated evidence demonstrated that PRODH plays complex role in many types of cancers. In this review, we briefly introduce the function of PRODH, then its expression in different types of cancer. We next discuss the regulation of PRODH in cancer, the downstream pathways of PRODH and the therapies that are under investigation. Finally, we propose novel insights for future perspectives on the modulation of PRODH.

Keywords PRODH · L-proline · Apoptosis · Autophagy · p53 · Cancer

Abbreviations

PRODH	Proline dehydrogenase
POX	Proline oxidase
EAAs	Essential amino acids
NEAAs	Non-essential amino acids
TNBC	Triple-negative breast cancer
P5C	Pyrroline-5-carboxylate
ETC	Electron transport chain
TCA	Tricarboxylic acid cycle
PYCRS	P5C reductase
PIG6	P53-induced gene-6
PPARγ	Peroxisome proliferator activated receptor gamma
TZDs	Thiazolidinediones
oxLDL	Oxidized low-density lipoprotein
TME	Tumor microenvironment
CSC	Cancer stem cell
EMT	Epithelial–mesenchymal transition
NSCLC	Non-small cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
PCa	Prostate cancer
BRCA	Breast invasive carcinoma
LUAD	Lung adenocarcinoma
MMBC	Multifocal/multicentric breast cancer
UFBC	Unifocal breast cancer
NFAT	Nuclear factor of activated T cells

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TRAIL	Tumor necrosis factor-related apoptosis inducing ligand
NAC	N-acetyl-L-cysteine
L-THFA	L-tetrahydro-2-furoic acid
AICAR	5-Aminoimidazole-4-carboxamide ribonucleoside
NF-κB	Nuclear factor κB

Introduction

Cancer cells trigger metabolic reprogramming during their initiation and progression in response to the tumor microenvironment stimuli by directly or indirectly activating aberrant growth and survival signals (Pavlova and Thompson 2016; Agnihotri and Zadeh 2016; He et al. 2016; Chen et al. 2019; Yu et al. 2020; Y et al. 2019; He et al. 2019). The high rate of aerobic glycolysis and glutamine utilization are the two most significant features of cancer cell metabolic reprogramming (Byun et al. 2020; Bernfeld and Foster 2019; Lunt and Vander Heiden 2011; Chen et al. 2014). In addition to glutamine, other amino acids (e.g., serine, glycine, alanine, proline, etc.) are consumed in cancer cells for the generation of nucleotides, reactive oxygen, proteins, and oncometabolites (Muhammad et al. 2020). Based on dietary necessity, amino acids can be divided into essential amino acids (EAAs) and non-essential amino acids (NEAAs) (Choi and Coloff 2019). Several recent reports have uncovered the important role of NEAAs in the pathology of cancer (Coloff et al. 2016). Strategies that target NEAAs metabolism are still in clinical trials and therapy. For example, the allosteric inhibitor of glutaminase, compound BPTES (bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide) that blocks glutamine utilization has been reported to play an anti-tumor role in a variety of cancers (Rajeshkumar et al. 2017; Yuneva et al. 2012; Le et al. 2012; Gross et al. 2014; Xiang et al. 2015). Other inhibitors of glutaminase, like CB-839 and compound 968, that block glutaminase activity inhibit the growth of transformed/cancer cells (Gross et al. 2014). Except for blocking glutamine utilization, limiting cellular cysteine could induce a unique cell death program known as ferroptosis and result in an anti-tumor effect(Mao et al. 2018). Inhibitors of the transporter xCT, like sorafenib and sulfasalazine, that block the transport of cystine, an oxidized dimer form of NEAA cysteine, have been already approved by the U.S. Food and Drug Administration (FDA) for tumor treatment (Koppula et al. 2020; Lei et al. 2020; Lo et al. 2008).

As one of the NEAAs, proline is the sole proteinogenic secondary amino acid that endows several functions not possible with other amino acids (Liu et al. 2020). Hence, proline is essential for collagen synthesis to support collagen physical stability (Shoulders and Raines 2009). Proline can also act as a 'helix breaker', as it contributes to the 3D structure of proteins by introducing a kink to disrupt the α-helix conformation (Cordes et al. 2002; Williams and Deber 1991; Burke et al. 2020). It is well established that proline plays a critical role in molecular recognition, protein stability, signaling transduction and cell redox reactions (Phang et al. 2010). Because proline has α-amino nitrogen within a pyrrolidine ring, proline has its own enzyme family that is distinct from most amino acids.

Proline dehydrogenase (PRODH), also known as proline oxidase (POX), is a mitochondrial inner-membrane protein that catalyzes proline to produce pyrroline-5-carboxylate (P5C) in the first step of proline catabolism. In this reaction, generated electrons transferred to the mitochondrial electron transport chain (ETC) for ATP or ROS generation, which finally mediates downstream signal pathways and biological processes (Fig. 1). For instance, PRODH generates ATP to promote tumor cell survival in some nutrient stress like hypoxia and glucose depletion (Liu and Phang 2012). In other cases, PRODH also induces apoptosis and autophagy in cancer cells through ROS generation, and functions as a tumor suppressor (Liu et al. 2006, 2009). As the complex and integral role of PRODH in cancer, PRODH has sparked great attention in the proline metabolism research field. Interestingly, recent reports suggested that PRODH

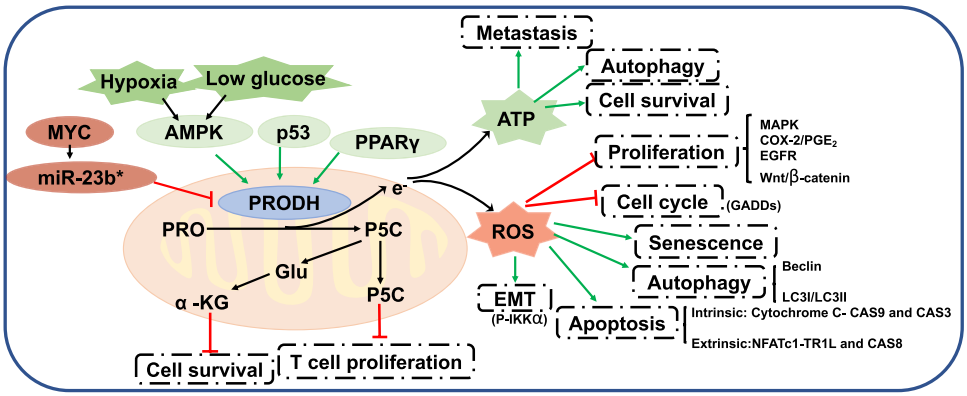

Fig. 1 Potential roles of PRODH in cancer cells through different mechanisms. PRODH has been demonstrated regulated by miR-23b*, AMPK, p53 and PPARγ. Inducible PRODH results in ATP/ROS generation via catalyzing L-proline to P5C. Then, the increasing ROS and ATP contribute to a broad range of cell actions that depend on microenvironment

may play a promote tumor or anti-tumor role depending on the environment and cell types.

A series of new discoveries have reported noval effects and mechanism of PRODH and proline catabolism in cancer. Hence, we will conclude the previous studies and related discoveries during the last few years. The principal focus of this review is the specific relationship between proline catabolism and cancer progression. We will discuss the regulation of PRODH in cancer, the downstream pathways of PRODH, and therapies that are under investigation. Finally, we will explore the possible potential mechanism of PRODH function in inhibiting cancer or promoting cancer in some circumstances.

Proline metabolism in cancer

PRODH is identified as one of the p53-induced genes related to apoptosis, which binds to mitochondrial inner membranes and catalyzes the oxidation of proline to P5C. P5C is an intermediate in the metabolic interconversions between the tricarboxylic acid (TCA) cycle and urea cycle (Adams 1970; Phang 2019). P5C converts to glutamic-γ-semialdehyde (GSA) spontaneously and carries out two dehydrogenation reactions for α-KG generation, a critical intermediate of the TCA cycle. In the other reaction pathway, P5C converts to ornithine catalyzed by ornithine aminotransferase and participates in the urea cycle (Phang 2019). The reverse conversion of P5C to proline is proline biosynthesis that contains three isoform P5C reductases (PYCRS), which also play important roles in cancer progression. Existing studies have identified the protumor role of PYCR1 in different cancers, including melanoma (De Ingeniis et al. 2012; Ye et al. 2018), renal cell carcinoma (Weijin et al. 2019), breast cancer (Loayza-Puch et al. 2016; Ding et al. 2017), hepatocellular carcinoma (Zhuang et al. 2019) and colorectal cancer (Yan et al. 2019; Burke et al. 2020). Several reviews conclude the unequivocal role of PYCR1 and proline biosynthesis in cancers based on different mechanisms (Burke et al. 2020; Phang 2019). However, PRODH seems to play complex roles which depend on cancer types and microenvironment. Hence, this review will concentrate on PRODH and proline catabolism in cancer.

PRODH is the enzyme in the first step of proline catabolism, which donates electrons to the electron transport chain for ROS or ATP production contributing to a series of biology reaction including signaling transduction, oxidation reaction, immune-inflammatory reaction, etc. Previous studies have established a series of observations focus on PRODH-mediate proline metabolism on several cancers (Phang 2019; Liu et al. 2008, 2005, 2010). Recent reports have discovered novel aspects of PRODH in different cancer types (Burke et al. 2020; Cappelletti et al. 2018; Fang et al. 2019); it is necessary to sum up the roles and mechanisms related to PRODH.

Regulatory mechanisms of PRODH

Transcriptional regulation of PRODH by transcriptional factors

To ensure appropriate functioning of PRODH in cancer cell regulation, the expression and activity of PRODH are subjected to a variety of pathways regulation. *PRODH* was first identified as a p53-induced gene-6 (PIG6) in a model for apoptosis(Polyak et al. 1997), and this discovery opened a new direction with a surge of reports related to the proline metabolism in subsequent studies. Since this discovery, the p53 response elements (REs) in the promoter and introns of *PRODH* were identified (Maxwell and Kochevar 2008; Raimondi et al. 2013; Liu et al. 2020). Interestingly, one of the p53 Res, located in *PRODH* introns, is efficiently transactivated by p53 members, p63 and p73 (Raimondi et al. 2013). The transcriptional activation of *PRODH* by p53 also has been demonstrated in other independent investigations (Nagano et al. 2016; Donald et al. 2001). Mutant p53 has been shown to reduce mRNA expression of PRODH in renal cancer (Maxwell and Rivera 2003). Similarly, mutant p53 reduced PRODH expression in colon cancer cell lines compared with wild-type p53 (Liu et al. 2020). However, p53 may not be the only factor that transactivates PRODH expression; other factors may also result in the variation of expression and activity of PRODH. The characteristics of PRODH finally showed in cancer cells mainly depended on the roles of predominant factors which may counteract the role of others. This transformation might account for discrepancies of PRODH in different cancer types.

PRODH expression can also be promoted by peroxisome proliferator activated receptor gamma (PPARγ), a ligand-dependent transcription factor that belongs to the nuclear hormone receptor superfamily (Pandhare et al. 2006). PPARγ can play a role in controlling the expression of networks of genes related to inflammation, lipid metabolism, adipogenesis, and metabolic homeostasis through forming obligate heterodimers with retinoid X receptor (RXR) and binding to specific response elements in the promoter regions of target genes (Ahmadian et al. 2013). *PRODH* promoter was found to be activated by PPARγ ligand troglitazone in colon cancer cells with both PPARγ-dependent and independent mechanisms (Pandhare et al. 2006). The oxidized low-density lipoprotein (oxLDL) is a potential factor that increased cancer risk(Tian et al. 2019). oxLDL could markedly increase PRODH expression based on one of its components, 7-ketocholesterol, through PPARγ in several cancer cells(Zabirnyk et al. 2010).

Posttranscriptional regulation and post-translational modification of PRODH

Apart from transcriptional regulation of *PRODH* as discussed above, PRODH expression can also be regulated at mRNA levels by microRNAs. MicroRNAs were first discovered in 1993 (Lee et al. 1993), and many studies have identified microRNAs roles in cancer biology for patients' prognosis, disease classification, and clinical treatment trials (Rupaimoole and Slack 2017; Bertoli et al. 2015). MicroRNAs negatively regulate mRNA level through binding to complementary sequences in the 3′ untranslated region (UTR) of target mRNAs (Krol et al. 2010; Hayes et al. 2014). PRODH expression was found to be suppressed by miR-23b* in renal cancer (Liu et al. 2010). Moreover, oncogene Myc suppresses PRODH expression indirectly through miR-23b*-mediated pathway (Liu et al. 2012b). However, there are no more studies related to posttranscriptional regulation of PRODH in cancer. Considering the multi-functions of microRNAs in regulating cancer biology, further investigations about searching for potential microRNA target to PRODH is worthy.

To date, accumulating evidence demonstrate that *PRODH* is specifically regulated by several transcriptional factors like tp53 and PPARγ. There are few studies focus on PRODH related post-translational modification, including ubiquitination, phosphorylation, acetylation, methylation, etc. An epigenome-wide gene–age interaction analysis reveals that the elderly LUAD patients have better survival with lower methylation of PRODH (Chen et al. 2020). However, no more related studies and experiments reports the regulation of PRODH by post-translational modification. The underlying mechanisms of its enzyme activity and protein stability may be the key diver cause complex roles of PRODH on specific cancer. The post-translational regulation has been proved to contribute to tumor metabolism regulation, immunological tumor microenvironment (TME) modulation and cancer stem cell (CSC) stemness maintenance (Telerman and Amson 2009; Deng et al. 2020). Hence, the post-translational modification of PRODH, which mediates the "quantity" and "quality" of PRODH, needs further investigations.

PRODH expression and cancer

Since *PRODH* was first identified as a p53-induced gene-6 (PIG6) in 1997(Polyak et al. 1997), there have been several reports demonstrating its down expression in various cancers and multiple effects on cancer cell cycle arrest, cell senescence, apoptosis, and autophagy. Subsequently, some findings have reported that PRODH is up-regulated in some challenging circumstances and contributes to cancer progression through influencing tumor growth, EMT, metastasis, and T cell infiltration (Table1).

Table 1 The role of PRODH in cancer

Tumor types	Functions	Characteristics
Breast cancer	Pro-tumor	Supports 3D growth and metastasis (Elia et al. 2017), generates ATP and as a drug target in MCF10A cell (Elia et al. 2017)
	Anti-tumor	PRODH was correlated with better prognoses in BRCA patients (Wang et al. 2020). Induced PRODH increases anti- apoptotic autopghagy in TNBC cells (Fang et al. 2019). PRODH promotes apoptosis in MCF7 cells (Zareba et al. 2017)
Prostate Cancer (PCa)	Pro-tumor	The up-regulation of PRODH inhibits T cell proliferation and functions (Yan et al. 2018). The expression of PRODH is up- regulated in PCa tissues and higher in the advanced tumors (Yan et al. 2018). PRODH increased tumor growth in animal model (Yan et al. 2018)
Pancreas cancer	Pro-tumor	PRODH is overe-expressed in PDAC tissues (Olivares et al. 2017). PRODH supports cell survival and proliferation in glucose and glutamine-restricted conditions, promotes PDAC growth, and supports TCA metabolism (Olivares et al. 2017)
NSCLC	Pro-tumor	PRODH is up-regulated in NSCLC tumor tissues (Liu et al. 2020). Low methylation of PRODH plays a protective effect for LUAD patients survival(Chen et al. 2020)
Renal cancer	Anti-tumor	The expression of PRODH is reduced both in renal cancer cell lines and renal carcinoma tissue samples (Liu et al. 2010; Maxwell and Rivera 2003). PRODH induces apoptosis (Maxwell and Rivera 2003)
Tongue Squamous Cell Carcinoma	Anti-tumor	Propolis promote PRODH-dependenat apoptosis in CAL-27 cells. (Celińska-Janowicz et al. 2018)
Colorectal cancers	Anti-tumor	PRODH induces apoptosis in DLD-1 cell lines (Liu et al. 2006, 2005). PRODH induces G2 cell cycle arrest to inhibit cell growth and inhibites tumor development in a mouse xenograft model (Liu et al. 2009). PRODH is down-regulated (Liu et al. 2009)
Rectum cancer		PRODH is down-regulated (Liu et al. 2009)
Stomach cancer		PRODH is down-regulated (Liu et al. 2009)
Liver cancer		PRODH is down-regulated (Liu et al. 2009)

PRODH expression is markedly down-regulated compared to the corresponding normal tissues in renal and digestive tract tumors, including colorectal, rectum, stomach, and liver (Liu et al. 2009). Indeed, subsequent reports confirmed the ability of PRODH to trigger apoptosis through both intrinsic and extrinsic apoptotic pathway, and demonstrated that PRODH functions as a tumor suppressor in a variety of cancer cell type such as colorectal, renal, and tongue squamous cell cancer (Maxwell and Rivera 2003; Liu et al. 2005, 2009; Celińska-Janowicz et al. 2018). Several reports have proved this characteristic of PRODH independently (Hu et al. 2007; Liu et al. 2008; Toloczko-Iwaniuk et al. 2020). Intriguingly, PRODH activates apoptosis through multiple mechanisms, most of which are mediated by ROS production. By this mitochondrial (intrinsic) apoptotic pathway, cytochrome c is released from mitochondria into cytosol and results in the activation of caspase-9 and caspase-3. What is more, PRODH was shown to activate the extrinsic apoptotic pathway via increasing the expression of NFATc1, a transcription factor, and promoting its localization to the nucleus, which results in the induction of the death receptor TRAIL (extrinsic) and activation of caspase-8 (Liu et al. 2006). In addition to inducing apoptosis, PRODH also plays a role in arresting DLD-1 cells in G_2 phase via upregulating the expression of GADDs, a gene that affects growth arrest and DNA damage (Liu et al. 2009). However, the potential mechanism of PRODH upregulating GADDs to block cell cycle was not carried out for further research.

Autophagy, a multistep lysosomal-mediated pathway that eliminates damaged organelles and invading pathogens to support nutrient recycling, is intimately linked to a cell's live/die decision(Amaravadi et al. 2019; Zhang et al. 2019). Follow-up experiments showed that PRODH was involved in autophagy and promoted cells survival when cells counteract nutrient deprivation or hypoxia. PRODH has been identified as an important regulator in oxLDL-mediated prosurvival autophagy through the generation of superoxide and subsequent up-regulation of beclin-1(Zabirnyk et al. 2010). PRODH also acts as an energy source for providing ATP under nutrient stress conditions. The expression of PRODH is up-regulated in various cancer cells including colon, breast, prostate, melanoma, lung, and ovarian under hypoxia tumor microenvironment which is mainly dependent on AMPK activation (Liu et al. 2012a). Similarly, glucose deprivation increased PRODH expression and promoted PRODH catalytic activity for maintenance of ATP levels that is AMPK dependent (Pandhare et al. 2009).

Although PRODH is down-expressed in renal and digestive tumor samples, PRODH is up-regulated in non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma (PDAC), prostate cancer (PCa), and some breast invasive carcinoma (BRCA) subtypes (Table 1). This up-regulation of PRODH in tumor tissues promotes cell growth, survival or metastasis through another mechanism. PRODH has been reported to induce IKKα activity through generation of ROS and results in the up-regulation of related inflammatory genes (Liu et al. 2020). PRODH has also been shown to promote NSCLC cell growth, migration and invasion (Liu et al. 2020). An epigenome-wide gene-age interaction analysis has revealed the reversed role of PRODH on survival in different age stage of lung cancer patients (Chen et al. 2020). Young patients with high methylation of *PRODH* was the best survival group, and low methylation of *PRODH* takes as an increased protective effect on LUAD patients survival with increased age (Chen et al. 2020). Interestingly, significant correlation was observed between the specific CpG probe and expression of PRODH in LUAD patients ($r = -0.23$, $p = 3.38*10^{-5}$). This significant heterogeneity of *PRODH* methylation effect on different age stage may contribute to providing new evidence for researching the switch roles of PRODH in context and searching for new specific biomarkers and therapeutic strategies for improving prediction accuracy and treatment efficacy.

PRODH has also been shown to contribute to promoting breast cancer by supporting growth and metastasis of breast cancer cells (Fang et al. 2019). PRODH activity, dependent on P5C recycling via PYCR1, was shown to support spheroidal growth by sustaining ATP production. What is more, PRODH is significantly higher expressed in metastases compared to primary breast cancers in patients. Strikingly, the number of metastases was significantly reduced once PRODH activity was inhibited by L-THFA, whereas the weight of primary breast tumors remained unchanged. These data indicated that PRODH seems to be more specifically important in metastatic growth compared to primary growth (Elia et al. 2017). In accordance, a differential analysis mentioned PRODH as one of the significantly up-regulated genes in the multifocal/multicentric breast cancer (MMBC) patients between invasive MMBC and unifocal breast cancer (UFBC). However, this study did not present any actual data for PRODH expression (Lang et al. 2018). These data indicate that PRODH activity seems to be important in supporting metastasis in this specific site or organ (Fang et al. 2019).

A role for PRODH in promoting prostate cancer has also been reported (Yan et al. 2018), which mainly focuses its role on inhibiting T cell infiltration. High levels of P5C, a metabolite converted from proline by PRODH, have shown to be released by prostate cancer cells and result in T cells signaling suppression by increasing ROS but decreasing cytokines and ATP production. Moreover, these aforementioned phenotypes were reversed with PRODH knockdown. Similarly, the up-regulation of PRODH increased tumor growth in animal model and decreased $CD3^+$, $CD4^+$ and $CD8^+$ T cells infiltration. The expression of PRODH was up-regulated in human prostate cancer tissues compared

with its corresponding non-neoplastic tissue. Further, the expression of PRODH was higher in the advanced tumors among the different stages of PCa. Collectively, this study provides a novel perspective of PRODH on impairing immune cell functions through promoting the release of P5C from prostate cancer and finally creating a microenvironment that improves cancer cell survival. In the other hand, this study provides a new standpoint of PRODH for tumor immunotherapy.

Given that PRODH is closely related to the pathological processes in multiple types of cancers by serving as an anti-tumor or protumor member, mechanistic insights into how PRODH converts its dual role on specific context and cancer types could be valuable for its translational implications in clinical settings, such as the development of precision medicine on gene-oriented treatment.

The role of PRODH and L-proline catabolism in cancer

The physiological activity of PRODH is mainly involved in regulating redox statues, inflammatory reaction, intercellular signaling and cell death fate. As aforementioned, PRODH is up-regulated in some challenging circumstances, such as hypoxia or glucose restriction, or some specific type of cancer; whereas, PRODH is down-regulated in several tumors and plays an anti-tumor role through different signaling pathway. In this part, we will summarize the above specific functions of PRODH and the related mechanism in cancer.

PRODH functions in tumor process through ROS-mediated mechanism

ROS is identified as a group of molecular oxygen in different patterns, which are formed by a series of reduction–oxidation reactions and the electron transport chain (Sabharwal and Schumacker 2014). H_2O_2 and O_2^- are the most key terms of ROS generated by the ETC and various enzymes, including NADPH oxidases, pyruvate dehydrogenase, acyl-CoA dehydrogenase, proline oxidase and et al. (Prasad et al. 2017; Srinivas et al. 2019). With the variation of ROS intracellular concentration stimulated by various stressors or metabolic enzymes, ROS seems to play in a beneficial or deleterious role by various mechanisms(Sies and Jones 2020). The low concentration of ROS is associated with some cellular responses like proliferation, migration and differentiation. The high concentration ROS exposure leads to inflammation, metastasis, growth arrest and cell death (Sies and Jones 2020). Because mitochondria are the major source of ROS (Sies and Jones 2020; Sabharwal and Schumacker 2014), PRODH, an enzyme that located in mitochondrial inner membrane, which donates an electron to the ETC for generating ROS via oxidizing proline to P5C, may contribute to triggering redox signaling under normal condition or the initiation of cancer under pathophysiological conditions.

As mentioned above, multiple experiments independently revealed that PRODH activates apoptosis through ROS generation both in intrinsic and extrinsic pathways in a variety of cancer types (Fig. 1) (Celińska-Janowicz et al. 2018; Liu et al. 2005, 2006; Maxwell and Rivera 2003). Previous works have demonstrated that PRODH activates apoptosis through both p53-dependent and p53-independent pathways (Rivera and Maxwell 2005; Maxwell and Davis 2000). Although PRODH was not up-regulated in DECV, a derivative cell line of ECV-304 cell that is resistant to p53-mediated apoptosis, apoptosis was induced in both cell lines for P5C production, which indicates that PRODH is capable of inducing apoptosis in a p53-independent pathway (Maxwell and Davis 2000). Interestingly, this study revealed the contribution of P5C but not ROS in inducing apoptosis. The potential role and mechanism of P5C for apoptosis still need further elucidation.

Other reports demonstrated the role of ROS for serving as an intracellular second messenger in signaling cascades and regulating gene expression through stimulating signal transduction and protein phosphorylation (Chio and Tuveson 2017). PRODH activates nuclear factor of activated T cells (NFAT), an indicator of activated calcineurin, through ROS production, and induces cytochrome c release from the mitochondria into the cytoplasm which finally results in apoptosis (Rivera and Maxwell 2005). Another study based on this finding has discovered that PRODH also stimulates the expression of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) mediated by NFAT. Additionally, PRODH was involved in blocking MAPK signaling through reducing the phosphorylation of ERK, JNK and p38 (Liu et al. 2006). MnSOD, an antioxidant enzyme that defense against oxidative stress, reverses this reduction of MAPK signaling and inhibits PRODH-induced apoptosis (Liu et al. 2006). However, the detailed mechanism by which PRODH blocks the phosphorylation and the interaction of these pathways that mediates PRODH-induced apoptosis still need further investigation.

In addition to NFAT and TRAIL, COX-2/PGE_2, EGFR and β-catenin signaling have shown to play important roles in PRODH-induced apoptosis. COX-2/PGE_2 pathway contributes to metastatic spread, tumor development and maintenance (Greenhough et al. 2009; Echizen et al. 2018; Luo and Zhang 2017). PRODH suppresses COX-2/PGE_2, EGFR and β-catenin/APC activities, and this suppression was reversed by MnSOD, indicating that ROS/superoxides generated by PRODH was involved in this process (Liu et al. 2008). Similarly, phosphorylation of EGFR and COX-2 was reduced with PRODH addition. Furthermore, this study suggested that celecoxib, a COX-2 inhibitor, could increase the

expression of PRODH and induce apoptosis in oral squamous cell carcinoma (Toloczko-Iwaniuk et al. 2020).

Accumulated evidence suggest that ROS contributes to the induction and maintenance of cellular senescence through diverse pathways including mitochondrial DNA damage, signaling pathways and induction of microRNAs (Davalli et al. 2016). PRODH has also been identified as a senescence-specific gene induced by low dose of etoposide treatment (Nagano et al. 2016). The following study explored PRODH functions in senescence process; they revealed that PRODH promotes senescence and DNA damage via ROS production. And this promotion effect could be impaired by N-acetyl-L-cysteine (NAC), a potent ROS scavenger, which indicates that ROS may be involved in PRODH-mediated senescence. Moreover, etoposide-induced senescence and ROS production were suppressed by L-tetrahydro-2-furoic acid (L-THFA), an inhibitor for PRODH enzymic activity, which indicates that PRODH induces senescence through its enzymic activity (Nagano et al. 2017).

Autophagy (Nazio et al. 2019) is a process that promotes cell survival in response to multiple stimuli like viral infection, nutrient deprivation and genotoxic stress. ROS has been demonstrated to be involved in sustaining autophagy for its signal transduction role (Filomeni et al. 2015). Contrary to the anti-tumor roles described above, PRODH also serves as a survivor in some stress circumstance through ROS. For instance, PRODH is induced by AMPK pathway to initiate protective autophagy under hypoxia via generating ROS. However, PRODH switches its way from generating ROS to ATP under low glucose for cell energy to promote cell survival (Liu and Phang 2012). The potential mechanism of this switch needs more further investigation.

In addition, increased ROS with PRODH promotes phosphorylation of IKKα, which results in the up-regulation of several inflammatory genes in NSCLC cells (Liu et al. 2020). As previously described, ROS production by PRODH is intracellular and affects phosphorylation level of MAPK and COX-2. These findings indicate PRODH-mediated ROS may function as a signaling molecule that contributes to activating multiple critical elements of pathway. PRODH-mediated ROS generation whether inhibits or supports tumor progression depends on certain circumstance and cancer types; therefore, PRODH takes a complicated and flexible role in cancer.

PRODH functions in the tumor process through ATP-mediated mechanism

Proline catabolism via PRODH switches to support ATP production in several condition. Under nutrient stress conditions, especially with glucose deprivation, proline functions as a stress substrate accompanied with increased PRODH enzyme activity for ATP production to maintain cellular energy levels (Pandhare et al. 2009). Glucose depletion induced phosphorylation of AMPK, which indirectly induced PRODH activity through inhibiting mTOR (Pandhare et al. 2009). Consistently, PRODH activity was induced in a time- and dose-dependent tendency with a synthetic AMPK activator—5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), addition (Pandhare et al. 2009). In addition to response to nutrient stress conditions, PRODH contributes to ATP production for supporting energy during spheroidal growth (Elia et al. 2017). Consistently, inhibition of complex III of ETC by antimycin A impaired spheroidal growth, which indicates the critical role of ATP in sustaining spheroidal growth (Elia et al. 2017).

However, additional related reports focus on PRODH-ATP axis functions and triggering mechanisms on cancer progression still need to be further studied.

PRODH regulates tumor process through pyrroline-5-carboxylate generation

P5C is conversed from proline in the first step of proline catabolism, and can be converted into glutamate catalyzed by the P5CDH enzyme (Yan et al. 2018). Glutamate-derived α-Ketoglutarate (α-KG) is a key intermediate of the TCA cycle. Hence, PRODH may take regulatory functions for tumor processes by contributing to P5C-glutamate-α-KG generation. In yeast and plants, P5C seems to cause ROS accumulation, stress response, and cell death (Zhu 2002; Zareba and Palka 2016; Borsani et al. 2005). However, P5C regulates these tumor processes directly or indirectly through multiple mechanisms. P5C plays a harmful role on T cells by inhibiting proliferation and function via inducing SHP1 expression in prostate cancer cells (Yan et al. 2018). Interestingly, P5C recycling to proline via PYCRs sustains PRODH activity during spheroidal growth, which contributes to energy production and metastasis formation in breast cancer cells (Elia et al. 2017). Some studies also found that PRODH generated α-KG by P5C conversion and increased α-KG led to HIF-1 signaling suppression (Verma 2006; Koivunen et al. 2007). Moreover, α-KG decreased the expression of HIF1α and Wnt/β-catenin target genes significantly through enhancing α-KG-mediated degradation of HIF1α in colon cancer (Wen et al. 2019). On the other hand, α-KG has been shown to directly bind to IKKβ and nuclear factor κB (NF-κB) signaling, which results in the increasing uptake of glucose and tumor cell survival by upregulating GLUT1. This finding reveals a critical role of α-KG-mediated signal pathway in brain tumor, and also provides a potential interpretation for the dual role of PRODH in some context (Wang et al. 2019). α-KG is also a cofactor of the KDM5, which can actively remove lysine trimethylation of H3K4me3. Hence, α-KG could enhance KDM5 activity for H3K4 demethylation. Given that α-KG plays an important role on histone

modifications, the study that connects proline catabolism to histone modifications may be a novel area for investigation (Su et al. 2021).

Therapeutic strategies targeting PRODH in cancer

So far, various anticancer chemicals and molecules approach to activate or inhibit PRODH activity have been explored, including succinate, MnSOD et al.(Table 2). It is challenging to decide appropriate treatments targeting PRODH. Thus, summarizing the role and mechanism of PRODH and the potential therapeutic drug may provide alternatives to step out dilemma.

It is conceivable that PRODH could be indirectly regulated by compounds targeting upstream pathways. One study showed that etoposide activates p53 and promotes p53-mediated induction of PRODH, causing apoptosis and cell senescence (Maxwell and Davis 2000). Additionally, TZDs treatment markedly increased PRODH activity and protein through promoting the binding of PPARγ to the PRODH promoter, leading to PRODH-mediated ROS generation and apoptosis (Pandhare et al. 2006; Kim et al. 2007).

Moreover, compounds targeting PRODH activity directly have shown anticancer pharmacological properties. L-lactate is a reversible competitive inhibitor of PRODH in several bacterial (Kowaloff et al. 1977). L-THFA, a compound reported to inhibit PRODH activity in several studies, has also been shown to reduce spheroidal growth. Moreover, L-THFA treatment on mice significantly inhibits metastasis formation via impairing PRODH activity without any obvious adverse effects in normal cells and organ functions (Elia et al. 2017). Interestingly, inhibition of PRODH activity by L-THFA impairs cell migration and invasion formation in non-small lung cancer cells (Liu et al. 2020). L-THFA has also been reported to be a competitive inhibitor of PRODH in bacterial (Lee et al. 2003) and other mammalian cells. These data suggest that PRODH may function as a promising drug target in specific cancer types.

Other molecules targeting PRODH downstream signal have also been reported. N-acetyl cysteine, a ROS scavenger that is widely used as a pharmacological antioxidant (Ezeriņa et al. 2018), reduced ROS level generated by PRODH (Yan et al. 2018; Hancock et al. 2016), blocking apoptosis or reversing T cell cytokines secretion (Yan et al. 2018). MnSOD has also been found to inhibit PRODH-mediated apoptosis through reducing the release of cytochrome c (Liu et al. 2005).

As PRODH plays a complex and important role in tumor process, the detailed therapeutic schedules targeting PRODH are subjected to future investigation.

Conclusions and future perspectives

Recent discoveries have solidified the importance of PRODH and proline catabolism in cancer process. PRODH promotes apoptosis and tumor suppression in several cancer cells such as renal and colorectal cancer cells. The expression of PRODH is often down-regulated in these tumors, limiting PRODH-mediated apoptosis and anti-tumor roles. However, PRODH also functions as an oncogenic protein to support tumor cells survival, growth and metastasis in other contexts. The expression of PRODH are up-regulated in these cancer cells like PCa and PDAC. Hence, searching

Table 2 The role of PRODH activator/inhibitor treatment

Treatment	Functions	Modes	Mechanism
Etoposide	Activator	Increases expression	Activates PRODH in p53-dependent and induces apoptosis/senescence (Maxwell and Davis 2000; Rivera and Maxwell 2005)
TZDs	Activator	Increases expression	Activates PRODH through PPARγ signaling (Pandhare et al. 2006)
AICAR	Activator	Increases activity	A synthetic AMPK activator, increases PRODH activity through AMPK-mediated signal (Pandhare et al. 2009)
Celecoxib	Activator	Increases expression	A COX-2 inhibitor, increases PRODH and PPARγ expression (Toloczko-Iwaniuk et al. 2020)
L-THFA	Inhibitor	Inhibits PRODH activity	A competitive inhibitor of PRODH enzymic activity, reduces metastatic spread in breast cancer (Elia et al. 2017), and impairs cell migration and invasion in NSCLC (Liu et al. 2020)
Lactate	Inhibitor	Inhibits PRODH activity	A competitive inhibitor of PRODH, forms the complex with PRODH to inhibit PRODH activity (Zhang et al. 2004; Kowaloff et al. 1977)
NAC	Inhibitor	Inhibits PRODH- dependent ROS	An antioxidant, inhibits ROS generation by PRODH (Hancock et al. 2016)
Succinate	Inhibitor	Inhibits PRODH-dependent ROS	An uncompetitive inhibitor of PRODH/POX activity, inhibits ROS generation by PRODH (Hancock et al. 2016)
MnSOD	Inhibitor	Reduces the release of cytochrome c	An antioxidant, reduces the release of cytochrome *c* from mitochondria into cytosol by PRODH (Liu et al. 2005)

for clinical pharmacological compounds that target the proline pathway is meaningful for tumor-targeted therapy in different types of tumor.

Although pioneering studies of PRODH have advanced our understanding of its fundamental functions and roles in pathological process, studies of post-translational modifications of PRODH are still in blank area. Hence, further studies should focus on investigating post-translational modifications that controls PRODH expression and biological functions in pathophysiological conditions.

In summary, PRODH is involved in the pathogenesis of various cancers. The upstream regulators, downstream signal pathway, biological function, and potential targeted drugs of PRODH are required for prospective therapeutic approach in the future.

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Declarations

Conflict of interest None.

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