Enolase 1: A paradigm of metabolic enzyme moonlighting in tumorigenesis (Review)

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Abstract. Enolase 1 (ENO1) plays a pivotal role in tumor development, recognized as a multifunctional oncogene across diverse cancers. Initially known for its central role in glycolysis, where it catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate, the influence of ENO1 extends far beyond. Recent studies have unveiled its additional roles in promoting tumor progression through plasminogen receptor activity, nucleic acid binding activity and signaling functions. The function of ENO1 is intricately regulated by a wide array of post-translational modifications, such as phosphorylation, ubiquitination, acetylation, methylation, succinylation and glycosylation. These modifications fine-tune its enzymatic activity, stability and subcellular localization, thereby affecting tumor behavior. ENO1 holds significant diagnostic and prognostic value, with its expression levels closely linked to tumor malignancy and patient survival outcomes. In preclinical models, multiple therapeutic approaches targeting ENO1 have demonstrated tumor progression-inhibiting effects. Consequently, drug development efforts centered on ENO1 are gaining momentum, with anticancer agents targeting this protein showing promising potential. As ENO1 emerges as a novel therapeutic target in oncology, the present review summarizes the latest research progress on ENO1 in the field of cancer.

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Abbreviations: ENO1, enolase 1; PLG, plasminogen; MBP-1, c-myc promoter-binding protein 1; HIF-1α, hypoxia-inducible factor 1-alpha; c-Myc, myc proto-oncogene protein; YAP1, Yes-associated protein 1; PD-L1, programmed death-ligand 1; PTM, post-translational modification; m^6A, N6-methyladenosine; m^6C, 5-methylcytosine; NSUN2, NOP2/Sun RNA methyltransferase 2; YBX1, Y-box binding protein 1; CCDC65, coiled-coil domain containing 65; WTAP, Wilms tumor 1-associating protein; GPS2, G protein pathway suppressor 2; RACK1, receptor for activated C kinase 1; PRMT6, protein arginine methyltransferase 6; SUMO, small ubiquitin-like modifier; O-GlcNAc, o-linked β-n-acetylglucosamine; PRMT5, protein arginine methyltransferase 5; KAT2A, lysine acetyltransferase 2a; USP21, ubiquitin specific peptidase 21; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FBXW7, f-box/WD repeat-containing protein 7; PEP, phosphoenolpyruvate; ROS, reactive oxygen species; AKT, protein kinase B; PI3K, phosphoinositide 3-kinase; mTOR, mechanistic target of rapamycin; AMPK, AMP-activated protein kinase; ERK, extracellular signal-regulated kinase; EMT, epithelial-mesenchymal transition; TME, tumor microenvironment; MAPK, mitogen-activated protein kinase; CPT1A, carnitine palmitoyltransferase 1a; PTMs, post-translational modifications; HRE, hypoxia response element

Key words: enolase 1, glycolysis, tumorigenesis, post-translational modifications, moonlighting protein

Contents

1. Introduction

Elevated levels of ENO1 have been observed in various types of cancer and are associated with a poor prognosis (1). For example, elevated ENO1 expression has been noted in colorectal cancer (CRC) (2), hepatocellular carcinoma (HCC) (3), gastric cancer (4), breast cancer (5), glioma (6),

non-Hodgkin lymphoma (7,8), bladder cancer (9) and head and neck cancers (10). Overexpression of ENO1 is typically linked to enhanced tumor proliferation, invasion and metastatic potential (2,4,11-13). Notably, therapies targeting enolases have shown significant efficacy, with several enolase inhibitors demonstrating the ability to eliminate tumors (14).

Metabolic reprogramming in tumor cells, particularly the 'Warburg effect', is a central focus of cancer research (15). This process not only supplies bioenergy and biosynthetic precursors for rapidly dividing tumor cells but also creates an immunosuppressive microenvironment that fosters tumor progression (15-18). ENO1, a key catalyst in glycolysis that converts 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP), was traditionally considered to function solely in metabolic pathways. However, emerging evidence suggests that the oncogenic role of ENO1 extends far beyond its classical glycolytic activity. As a multifunctional protein, ENO1 promotes extracellular matrix degradation and tumor metastasis through its plasminogen (PLG) receptor activity (19,20). It also regulates the translation and stability of critical messenger RNAs, such as Yes-associated protein 1 (YAP1), as a nucleic acid-binding protein (21). Furthermore, ENO1 acts as a signaling scaffold protein, activating key oncogenic pathways like phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), while inhibiting AMP-activated protein kinase (AMPK)/mechanistic target of rapamycin (mTOR) (22). The diverse and precisely regulated functions of ENO1 are largely attributed to its extensive post-translational modifications (PTMs), including phosphorylation, ubiquitination, acetylation, methylation and succinylation (23-27). These modifications serve as a complex molecular code that dynamically governs ENO1's enzymatic activity, protein stability, subcellular localization and 'functional switching' across various biological processes. Moreover, ENO1 plays a pivotal role in mediating chemotherapy resistance and shaping the immunosuppressive tumor microenvironment (TME), highlighting its immense potential as a therapeutic target (9, 28). Currently, various intervention strategies targeting ENO1, including small-molecule inhibitors, natural product derivatives and therapeutic vaccines, have exhibited significant antitumor effects in preclinical models (29,30). The present review aimed to systematically summarize the multifaceted oncogenic functions of ENO1 in tumors and elucidate the multilevel regulatory network that controls its expression and activity. Additionally, it discussed the latest therapeutic strategies targeting ENO1 and their prospects for clinical translation, while identifying key scientific questions for future exploration in this field.

2. ENO1: A multifunctional protein

ENO1 was first identified as a pivotal enzyme in the glycolytic pathway, catalyzing the conversion of 2-PG to PEP, a crucial step in glycolysis. Elevated ENO1 expression supports the 'Warburg effect', meeting the high metabolic demands of rapidly proliferating tumors (31,32). Traditionally, glycolytic enzymes were considered functionally specialized and lacking regulatory signaling capabilities, with stable expression levels. However, unlike GAPDH, studies have revealed that ENO1 is not merely a housekeeping gene but a multifunctional protein. It promotes tumor progression through multiple mechanisms, including its glycolytic function and various additional activities, with its expression closely linked to malignant phenotypes, such as abnormal proliferation, invasion, drug resistance and immune evasion in tumor cells. Notably, its functions and involvement in pathophysiological processes are largely determined by its subcellular localization (6,12,33).

Structural basis of ENO1. The structural diversity of ENO1 underpins its functional diversity. The human ENO1 gene spans over 18 kb and contains 12 exons (34). Its promoter lacks canonical TATA and CAAT boxes but is GC-rich and contains potential SP1 binding sites (34), along with a hypoxia response element (HRE) that mediates transcriptional activation by hypoxia-inducible factor 1 (HIF-1) (35). An upstream inverted Alu sequence may act as a transcriptional repressor (36). Through alternative splicing regulated by the AKT/protein kinase R-like endoplasmic reticulum kinase/eukaryotic initiation factor 2 α pathway, the ENO1 gene also produces c-myc promoter-binding protein 1 (MBP-1), a shorter protein variant with distinct functions (37). The crystal structure of human ENO1 has been resolved at 2.2 Å, revealing its classic dimeric form (38). Each monomer consists of 434 amino acids (~48 kDa) and comprises two domains: an N-terminal domain (residues 1-138) with a β-fold and three α-helices and a larger C-terminal domain (residues 139-432) folding into an α/β barrel. Mammals possess three tissue-specific enolase isoforms (ENO1, ENO2 and ENO3) encoded by distinct genes, with ENO1 widely distributed across tissues, ENO2 confined to neuron-associated tissues and ENO3 predominantly found in muscle tissue. While enolases are generally fluoride-sensitive and Mg^{2+}-dependent, ENO1 possesses distinct surface properties that underlie its unique moonlighting functions, including PLG receptor and nucleic acid-binding activities (38).

Catalytic activity of ENO1. ENO1's classic enzymatic function is the catalytic conversion of 2-PGA to PEP. ENO1 operates as a homodimer and lacks catalytic activity as a monomer (39). Its catalytic residues are highly conserved across eukaryotes, with the active site located in a cleft between the N-terminal and C-terminal domains, containing both the substrate-binding pocket and the metal ion-binding site. Catalytic activity requires a divalent metal ion (Mg^{2+} or Mn^{2+}) to stabilize the substrate conformation and neutralize the negative charge of the phosphate group, thereby lowering the reaction energy barrier (40-43). The active site typically accommodates two metal ions: a high-affinity conformational ion and a low-affinity catalytic ion (44), with the latter binding only upon substrate (or analog) engagement (45). The dehydration reaction catalyzed by ENO1 occurs in three steps: First, the C-terminal domain of ENO1 binds and activates the substrate, 2-PGA (39). At this stage, the domain closes, creating a hydrophobic environment that encapsulates 2-PGA. This shields the substrate from water molecules, preventing interference with the dehydration process. The closure of the domain is triggered by Mg^{2+} binding (40,45,46). The second step involves dehydration and proton transfer. A highly conserved lysine residue, likely Lys345, acts as a base, abstracting a proton from the C2 position of 2-PGA. This leads to the formation of an unstable carbocation intermediate, which rapidly isomerizes into an enol pyruvate intermediate. This enol

pyruvate intermediate is a pivotal transient state in the catalytic cycle (40,47,48). Almost simultaneously, an acidic residue, likely glutamate 211, donates a proton to the C3 hydroxyl group of the enol intermediate, thereby completing the dehydration process (48,49). Subsequently, a double bond forms upon carbon rearrangement, yielding PEP, the hallmark biochemical reaction of enolases. Substrates positioned at the ENO1 active site can interact with two metal ions and the electrostatic interactions between these ions are crucial for propelling the reaction forward (40). Lys396 and Ser41 play a role in stabilizing the negative charge of the transition state and binding Mg^{2+}, thereby facilitating reaction progression (50). After the product is released, ENO1 rapidly reverts to its initial conformation, preparing to accept the next substrate molecule (20).

ENO1's PLG-activating activity. ENO1 functions as a cell-surface PLG receptor, its earliest identified non-glycolytic role (19,51). This activity, independent of its enzymatic function (38), involves specific surface lysine residues and a putative binding motif (FFRSGKY, residues 250-256) that interacts with PLG (Kd ~1.9 μM) (52). This characteristic is shared with numerous PLG receptors (53) and its binding mode to PLG is likely dominated by polar interactions arising from complementary surface conformations (38,54). By concentrating PLG and facilitating its activation by urokinase plasminogen activator (uPA) or tissue plasminogenactivator (tPA), ENO1 enhances localized plasmin generation, promoting extracellular matrix degradation and tumor cell invasion/metastasis (52). Lys345 plays a vital role in capturing the R-proton from PLG, with Glu211 becoming protonated and forming a hydrogen bond with the α-hydroxy group of PLG (40). Additionally, Lys345, Glu211 and the metal cation of ENO1 may participate in PLG activation (55).

ENO1: A nucleic acid-binding protein. ENO1 has been found to directly bind to RNA, participating in the regulation of RNA metabolism and function. It binds to the cytosine-uracil-guanine-rich element in YAP1 mRNA, promoting YAP1 translation (56). ENO1 also binds to the 3' untranslated regions (3'UTRs) of Klf2 and FUS mRNA, thereby stabilizing them to inhibit pyroptosis (57). Conversely, ENO1 facilitates the degradation of IRP1 mRNA to suppress ferroptosis (33). Furthermore, ENO1 functions as a DNA-binding protein, inhibiting tumorigenicity by binding to the c-Myc promoter, depending on the binding activity of residues 97-237. This DNA-binding domain is retained in the C-terminal region shared with its splice variant MBP-1, explaining MBP-1's antitumor activity (58).

3. ENO1 enzyme activity: Primarily regulated by PTMs

The regulation of ENO1 enzyme activity involves allosteric control, reversible covalent modifications and adjustments in enzyme abundance. In practice, ENO1 function is determined by both its concentration, regulated at the mRNA transcription and protein stability levels (Fig. 1) and its specific activity (the rate at which a unit concentration of enzyme catalyzes a specific reaction), which is dynamically adjusted mainly through diverse PTMs (Fig. 2).

Regulation of ENO1 mRNA and translation. ENO1 mRNA expression is finely regulated through multiple epigenetic mechanisms, including post-transcriptional modifications such as 5-methylcytosine (m^5C) and N^6-methyladenosine (m^6A). The m^5C modification, catalyzed by methyltransferase NOP2/Sun RNA methyltransferase 2 (NSUN2) and recognized by reader protein Y-box binding protein 1 (YBX1), is crucial (59). Knockout of NSUN2 in CRC cells markedly reduces ENO1 mRNA and protein levels, thereby altering ENO1-dependent glucose metabolism pathways (59). This modification also influences ENO1 expression via the transcription factor c-Myc (60), while coiled-coil domain containing 65 (CCDC65) suppresses transcription by impairing c-Myc binding to the ENO1 promoter (61). Notably, the accumulation of lactate catalyzed by ENO1 induces lactylation at histone H3K18, activating NSUN2 transcription and forming a positive feedback loop for m^5C modification (59). Beyond m^5C modification, ENO1 mRNA undergoes m^6A methylation at adenine position 359. The m^6A modification promotes its binding to the m^6A-reading protein YTH N6-methyladenosine RNA binding protein F1, thereby enhancing ENO1 translation efficiency (62). This process is regulated by the RNA binding motif protein 15/methyltransferase-like 3 complex (63) and Wilms tumor 1-associating protein (WTAP) (64), which further enhance ENO1 mRNA m^6A methylation to promote tumor glycolytic activity (65). Additionally, lysine methyltransferase 5A catalyzes monomethylation of lysine 20 on histone H4 (H4K20me1), which binds to the ENO1 promoter and inhibits its transcriptional activity (66).

Hypoxia-induced ENO1 expression. The presence of HREs in the ENO1 promoter enables its transcriptional upregulation under hypoxia, making ENO1 a key player in cellular hypoxic responses (35,67,68). Under hypoxic conditions, HIF-1α stabilizes and translocates to the nucleus, binding to ENO1's HREs to activate transcription (69,70). Hypoxia preferentially induces full-length ENO1 over its alternative splice variant MBP-1, attenuating MBP-1-mediated repression of the c-myc promoter and leading to c-myc upregulation (71). The regulation of ENO1 expression under hypoxia primarily relies on the HIF-1α signaling pathway, with positive regulators such as Mucin 1 and histone H2A histone family member X (72) stabilizing HIF-1α to promote its recruitment to the ENO1 promoter (73,74). Conversely, G protein pathway suppressor 2 binds to receptor for activated C kinase 1 (RACK1), stabilizing the HIF-1α-RACK1 complex, which triggers HIF-1α polyubiquitination and degradation, ultimately inhibiting ENO1 transcription (75). By contrast, SIX homeobox 1 drives glycolytic gene expression independently of HIF-1α by recruiting histone acetyltransferases histone acetyltransferase binding to ORC1 and nuclear receptor coactivator 3 to the ENO1 promoter. These enzymes catalyze acetylation modifications of histone H4K5 and H3K4 (H4K5ac and H3K4ac), respectively, thereby activating ENO1 expression (76). These studies not only elucidate the multi-level regulatory network governing ENO1 under hypoxic conditions but also underscore its pivotal role in tumor metabolic reprogramming as a key strategy for tumor cells to adapt to hypoxic microenvironments.

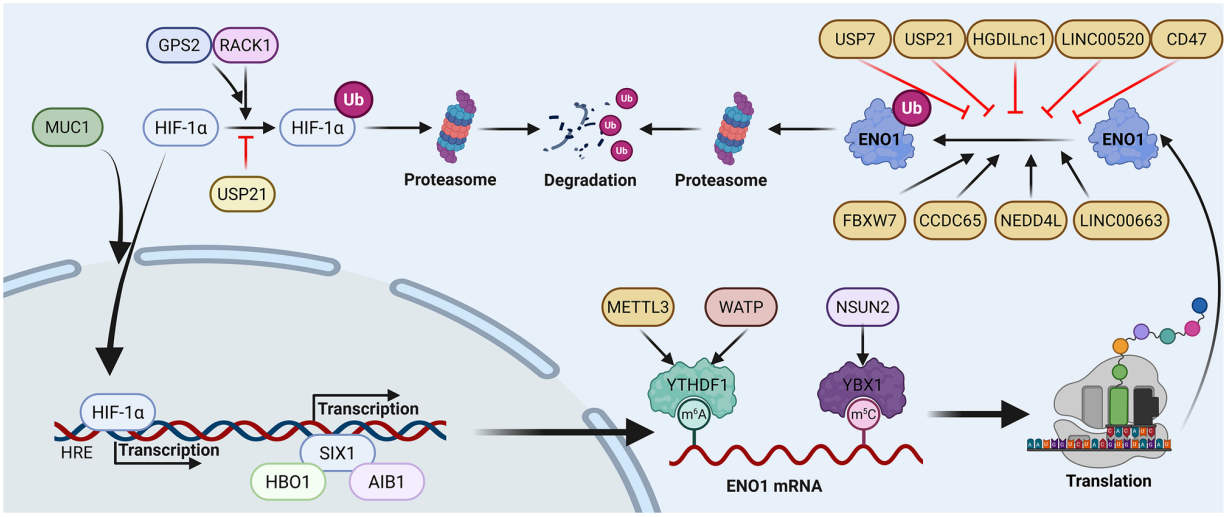

Figure 1. From transcription to post-translational modification, ENO1 expression is influenced by multiple pathways. HIF-1α regulates the transcription of ENO1 mRNA through the HRE region, while the stability of mature mRNA is controlled by m^5C and m^6A modifications. The stability of the ENO1 protein is regulated by numerous factors, ultimately leading to its degradation via ubiquitination. ENO1, enolase 1; HIF-1α, hypoxia-inducible factor 1-alpha; HRE, hypoxia response element; c-Myc, myc proto-oncogene protein; m^5A, N6-methyladenosine; GPS2, G protein pathway suppressor 2; RACK1, receptor for activated C kinase 1; MUC1, Mucin 1; USP21, ubiquitin specific peptidase 21; METTL3, methyltransferase-like 3; WTAP, Wilms tumor 1-associating protein; YTHDF1, YTH N6-methyladenosine RNA Binding Protein F1; CCDC65, coiled-coil domain containing 65; FBXW7, F-box and WD repeat domain containing 7; YBX1, Y-box binding protein 1; NDD4L, NEDD4 like E3 ubiquitin protein ligase.

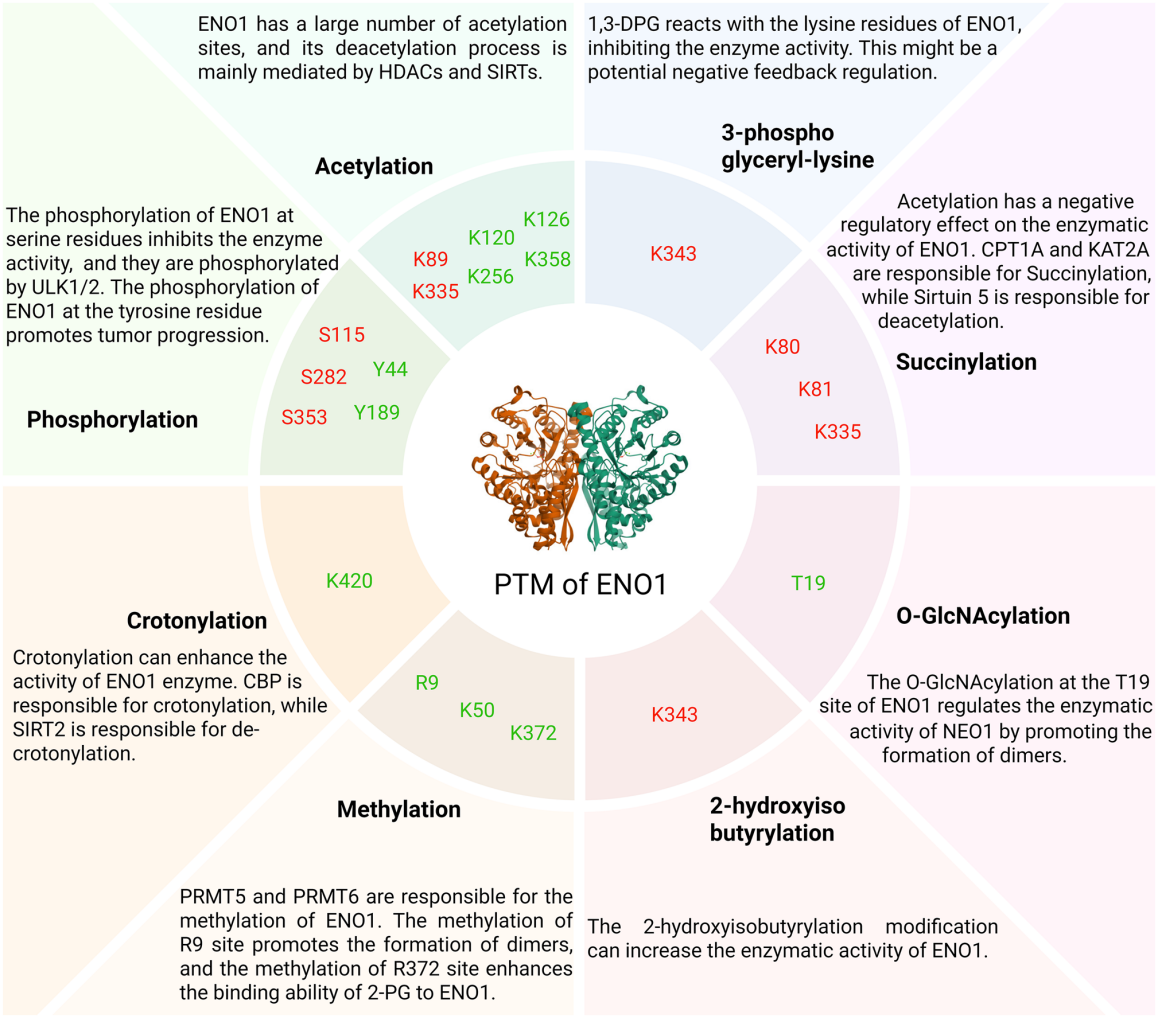

Figure 2. PTMs of ENO1 are highly diverse. The modified sites that have been studied are shown in the image. Red font indicates modifications at this site that inhibit enzyme activity, while green font denotes modifications that enhance enzyme activity or carcinogenic activity. ULK, Unc-51 like autophagy activating kinase; PRMT, protein arginine methyltransferase; CPT1A, carnitine palmitoyltransferase 1a; KAT2A, lysine acetyltransferase 2a.

Regulation of ENO1 stability by ubiquitination. Ubiquitination is a key post-translational modification process that regulates protein stability and degradation (77). Although ubiquitination is a protein modification process, its regulation of the ENO1 protein ultimately achieves changes in enzyme concentration by affecting its stability. The E3 ubiquitin ligase f-box/WD repeat-containing protein 7 (FBXW7) directly facilitates ENO1 ubiquitination and degradation (61,78), a process finely modulated by upstream regulators: CCDC65 (61) recruits FBXW7 to amplify ENO1 ubiquitination, while the long non-coding RNA LINC00520 binds to ENO1, competitively inhibiting FBXW7-mediated ubiquitination and thus stabilizing ENO1 (79). Deubiquitination also plays a pivotal role in ENO1 stability regulation. Ubiquitin specific peptidase 21 (USP21) influences ENO1 through multiple pathways: On the one hand, USP21 directly deubiquitinates ENO1 to enhance its stability; on the other hand, USP21 indirectly upregulates HIF-1α expression by deubiquitinating and stabilizing the heat shock protein (HSP)90, thereby promoting ENO1 transcription (80). Additionally, EMC2 recruits ubiquitin specific peptidase 7, which directly deubiquitinates ENO1 to stabilize it (24).

Beyond the classic ubiquitin-proteasome system, other factors also target ENO1 for degradation regulation. The E3 ligase neural precursor cell expressed developmentally downregulated 4-like has been shown to specifically target ENO1, increasing its ubiquitination levels to promote degradation (81,82). ENO1 stability is negatively regulated by LINC00663, which enhances the E6AP-mediated ubiquitin-proteasome pathway (83). Conversely, the long non-coding RNA HGDILnc1 (84) and CD47 (85) interact with ENO1 to protect it from proteasomal degradation. Furthermore, SUMOylation, the covalent attachment of a small ubiquitin-like modifier (SUMO) to target proteins (86), is involved in ENO1 modification. Specifically, the K202 and K343 sites of ENO1 have been demonstrated to undergo SUMO2 and SUMO3 modification, indicating that SUMOylation may play a crucial role in regulating ENO1 stability and cellular metabolic functions (84). Collectively, these multilayered regulatory mechanisms highlight the precise control of ENO1 protein turnover in cancer cells.

Phosphorylation of ENO1. Phosphorylation is one of the most common PTMs of ENO1, usually exerting a negative regulatory effect on its activity by adding phosphate groups to specific serine, threonine, or tyrosine residues (87). Under conditions of amino acid and growth factor deprivation, Unc-51 like autophagy activating kinase1/2 directly phosphorylate the Ser115 and Ser282 residues of ENO1. This phosphorylation reduces ENO1's enzymatic activity and redirects increased carbon flux to the pentose phosphate pathway to generate nicotinamide adenine dinucleotide phosphate (reduced coenzyme II). This process helps maintain cellular energy and redox homeostasis at both cellular and organismal levels, protecting cells from reactive oxygen species (ROS)-induced death (88). Additionally, when exposed to the oxazole compound KB2764, ENO1 can be phosphorylated at Ser353 by PKM, enhancing mitochondrial function and reducing cellular reliance on glycolysis (89). High-throughput studies have identified numerous phosphorylation sites on ENO1, including multiple serine residues (e.g., S27, S37, S40, S161, S170, S179, S254, S272, S198, S254, S263, S272, S291 and S419) (88-94) and three tyrosine residues (Y25, Y44 and Y287) (93,95). However, the specific biological functions of most of these sites, especially those located at dimer interfaces, remain to be fully elucidated.

Acetylation of ENO1. Acetylation typically occurs on lysine residues, altering protein charge and conformation by adding an acetyl group, which in turn affects protein function (87,96). This modification is widespread in glycolysis and the tricarboxylic acid cycle, with traces found on catalytic residues of ~2/3 of key enzymes (23). Moreover, acetylation modifications of different glycolytic enzymes show interdependent effects, suggesting the formation of regulatory networks within bioenergetic pathways (97). Deacetylation of ENO1 is mainly mediated by members of the histone deacetylase (HDAC) family. For instance, histone deacetylase 11 (HDAC11) inhibits ENO1's glycolytic activity in tumor cells by deacetylating lysine 335 (K335) (98). Other studies indicate that HDAC1 and HDAC7 also regulate ENO1's acetylation status by controlling acetylation at residues K120, K126 and K256, thereby enhancing enzyme activity (99,100). Furthermore, the deacetylase sirtuin 4 targets the K358 site of ENO1. Its deacetylation action increases ENO1's affinity for the substrate 2-PG, thereby boosting catalytic activity. Notably, this enhanced enzymatic activity is accompanied by weakened ENO1 binding to RNA, representing a functional switch partly mediated by acetylation (101). Notably, ENO1 can bind ~2,000 transcript sites. Although the specific functions of these RNA molecules are not fully understood, they directly inhibit ENO1's enzymatic activity. Experiments show that exogenously introduced RNA ligands reduce ENO1-dependent metabolite production while promoting serine biosynthesis. This RNA-binding capacity is regulated by acetylation at the K89 site, primarily controlled by the deacetylase SIRT2. This finding reveals a novel mechanism by which acetylation modifies metabolic enzyme activity through nucleosomally regulated pathways (102). In a large-scale acetylation study of central metabolic enzymes, K339, K390 and K402 of ENO1 were identified as acetylation sites. Although the direct effect of these modifications on enzyme activity was not validated in that study, structural analysis revealed that acetylated lysine residues occupy similar spatial positions and volumes within the active pocket as native substrates. Thus, it is hypothesized that the presence of one or more acetyl groups may hinder PEP binding, thereby inhibiting ENO1's catalytic activity (23).

Succinylation of ENO1. Succinylation, which involves the covalent attachment of a succinyl group to lysine residues, is a crucial PTM regulating ENO1 (103). Proteomic studies have identified ENO1 as one of the most heavily succinylated proteins, with key modification sites including K80, K81 and K335. Succinylation at these sites markedly inhibits ENO1's glycolytic activity (104,105). Notably, the K335 site, located near the substrate-binding pocket, may directly impede 2-PG binding or alter the conformation of the catalytic center. Carnitine palmitoyltransferase 1a (CPT1A) mediates succinylation at these sites through its succinyl-CoA transferase (LSTase) function, rather than its conventional carnitine

palmitoyltransferase function. Under glutamine-depleted conditions, CPT1A exhibits enhanced LSTase activity, thereby promoting ENO1 succinylation (105). Additionally, lysine acetyltransferase 2a (KAT2A) has also been reported to act as a succinyltransferase for ENO1, while the deacylase Sirtuin 5 mediates its removal (26,104,106). Although the precise effect of KAT2A-mediated succinylation on enzymatic kinetics requires further investigation, this modification is associated with pro-tumor phenotypes, promoting cell proliferation and migration while inhibiting apoptosis.

Methylation of ENO1. ENO1 methylation primarily occurs on arginine residues and is catalyzed by specific methyltransferases, playing a critical role in regulating its function. Protein arginine methyltransferase 5 (PRMT5) mediates symmetric dimethylation at R9 and R50. Methylation at R9, located at the dimer interface, is essential for stable dimer formation and full enzymatic activity (25). The R50 modification is associated with enhanced tumor cell metabolism and invasiveness (107). Protein arginine methyltransferase 6 (PRMT6) also methylates ENO1 at two key sites: Methylation at R9 similarly promotes dimerization, while methylation at R372 appears to enhance substrate (2-PG) binding affinity. Therefore, arginine methylation by PRMT5 and PRMT6 fine-tunes ENO1 activity, stability and oncogenic function through distinct molecular mechanisms (108).

O-linked β-n-acetylglucosamine (O-GlcNAcylation) of ENO1. O-GlcNAcylation is a dynamic monosaccharide modification of intracellular proteins (109,110) that can influence protein folding, stability, secretion and cell surface localization (111). O-GlcNAcylation at threonine 19 (T19) is a key regulatory modification of ENO1, markedly enhancing its glycolytic function. Mechanistically, this modification promotes the formation of enzymatically active dimers. Evidence shows that a T19A mutation severely impairs dimerization, resulting in a mutant protein with a higher Km for 2-PGA and a catalytic efficiency reduced by 80% compared to the wild type. Thus, T19 O-GlcNAcylation acts as a positive regulator of ENO1 by stabilizing its active dimeric form (112).

Other modifications of ENO1. Several additional PTMs further diversify the functional regulation of ENO1. Lysine 2-hydroxyisobutyrylation at K228 and K281 enhances its activity (113) and the deacylase CobB can reverse this modification at the conserved K343 site (114). The glycolytic intermediate 1,3-bisphosphoglycerate can spontaneously form 3-phosphoglyceryl-lysine (pgK) at K343, inhibiting ENO1 activity and constituting a direct metabolic feedback loop (115). This reaction occurs spontaneously, exploiting the electrophilic nature of 1,3-bisphosphoglycerate without requiring enzymatic catalysis (116). ENO1 is also modified by interferon-stimulated gene 15, although the functional consequences of this ISGylation event, first reported in 2005, remain undefined (117). Lysine crotonylation, predominantly at K420 and regulated by CBP/SIRT2, is elevated in tumors and enhances both enzymatic and oncogenic functions (118,119). Citrullination, the conversion of arginine residues to citrulline catalyzed by protein arginine deiminases (PADs) (120), has been identified at least 18 citrullination sites in ENO1. Although its effect on ENO1 activity is unclear, it serves as a diagnostic marker in rheumatoid arthritis (121).

4. ENO1: Mediator of multiple pro-tumor signaling

In tumorigenesis and progression, ENO1 not only fuels abnormal metabolic reorganization in tumor cells but also extensively contributes to malignant phenotypes such as proliferation, invasion, metastasis and drug resistance by regulating multiple signaling pathways. It activates the PI3K/AKT pathway to promote survival signaling, suppresses the AMPK/mTOR pathway to enhance anabolic metabolism, forms a positive feedback loop with the extracellular signal-regulated kinase (ERK) signaling pathway and regulates resistance mechanisms against various chemotherapeutic agents, including gemcitabine, cisplatin and 5-fluorouracil. Thus, ENO1 serves as a central hub within tumor signaling networks (Fig. 3).

ENO1: Tumorigenesis promotion through PI3K signaling. In tumors, the PI3K/AKT pathway is often constitutively activated due to genetic or epigenetic alterations (122). This pathway drives tumor cells toward aerobic glycolysis rather than mitochondrial oxidation, providing metabolic advantages and promoting malignant phenotypes (123-126). ENO1 facilitates the phosphorylation of FAK at Tyr397, leading to increased levels of phosphorylated (p-)PI3K (Tyr458) and p-AKT (Ser473), thereby activating the PI3K/AKT signaling pathway (127). Additionally, ENO1 can be phosphorylated at Y44, directly promoting the activation of PI3K and AKT at the aforementioned sites (128). ENO1 also activates TGF-β1 through PLG recruitment and plasmin (PL) generation, thereby activating the PI3K/AKT pathway (129). These mechanisms collectively support ENO1's role in enhancing this signaling pathway. Theoretically, any pathway regulating ENO1 protein levels and stability could influence its downstream signaling pathways. Reports confirm that circRPN2 and CCDC65 inhibit PI3K/AKT signaling by binding to ENO1 and accelerating its degradation (130,131). Conversely, fibroblast growth factor receptor-like 1, WW domain-binding protein 2, family with sequence similarity 126 member A and transient receptor potential cation channel subfamily C member 5 opposite strand positively regulate ENO1 function and downstream signaling by directly binding to the ENO1 protein, thereby promoting tumor proliferation and chemotherapy resistance (123,132-134).

ENO1-mediated modulation of AMPK/mTOR signaling. As a key glycolytic enzyme, ENO1 drives high glycolytic flux and ATP production in cancer cells, suppressing AMPK activation and subsequently enhancing mTOR signaling (135). Mechanistically, ENO1 inhibits AMPK phosphorylation at Thr172 while promoting phosphorylation of mTOR at Ser2447 and Akt at T308/S473, facilitating oncogenic growth and metastasis in cancers such as CRC (29,30,136-138). Notably, ENO1 can also activate AMPKα1 under certain conditions, contributing to cell proliferation and apoptosis resistance through AKT/GSK3β phosphorylation (136). Moreover, ENO1 can act through the PI3K/Akt/mTOR signaling, a pathway independent of AMPK, highlighting its multifaceted role

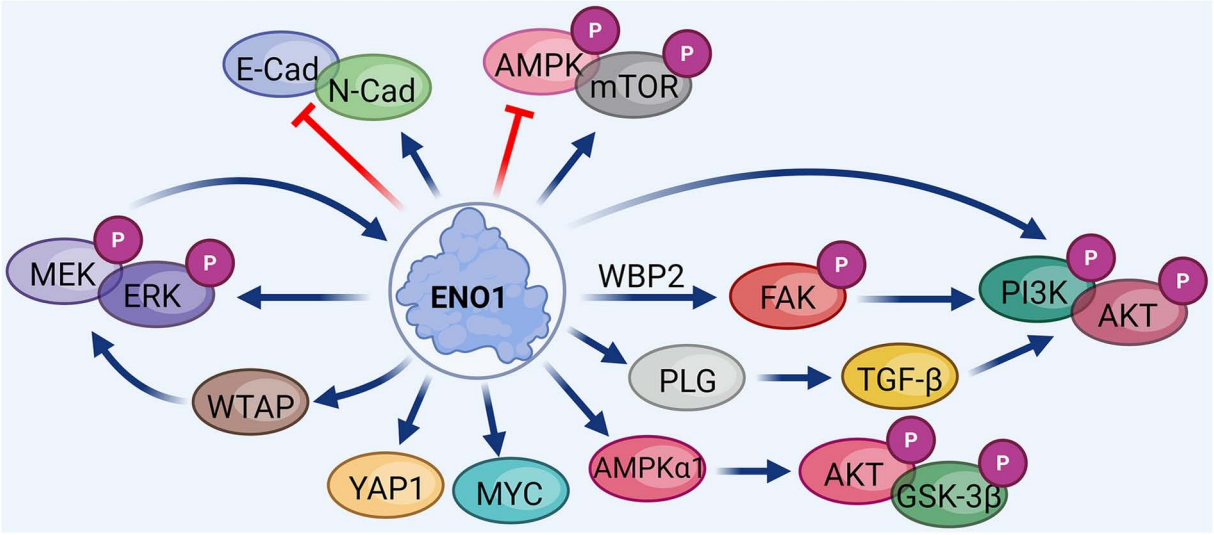

Figure 3. ENO1 occupies a central hub position in tumor signaling networks. It activates the PI3K/AKT pathway to promote survival signaling, suppresses the AMPK/mTOR pathway to enhance anabolic processes and forms a positive feedback loop with the ERK signaling pathway. ENO1, enolase 1; MEK, mitogen-activated extracellular signal-regulated kinase; ERK, extracellular signal-regulated kinase; AMPK, AMP-activated protein kinase; WTAP, Wilms tumor 1-associating protein; YAP1, Yes-associated protein 1; PLG, plasminogen; FAK, focal adhesion kinase; GSK, glycogen synthase kinase.

in metabolic signaling (139,140). Silencing ENO1 induces autophagy-dependent ferroptosis in breast cancer cells, closely linked to AMPK/mTOR signaling, as mTOR is a well-known autophagy inducer (141). Emerging evidence suggests that the AMPK/mTOR pathway may reciprocally regulate ENO1 expression and activity through transcriptional or post-translational mechanisms, indicating bidirectional crosstalk between ENO1 and this central metabolic hub (142). Further studies are needed to fully elucidate the complex interplay between ENO1 and AMPK/mTOR signaling in tumor metabolic rewiring.

Positive feedback between ENO1 and ERK signaling. The ERK signaling pathway, a critical member of the mitogen-activated protein kinase (MAPK) signaling pathway, plays a vital role in cellular processes such as proliferation, differentiation, migration and apoptosis (32). Substantial evidence indicates that ENO1 forms a positive feedback loop with the ERK signaling pathway. In CRC, high ENO1 expression promotes ERK phosphorylation, enhancing glycolysis and tumor growth (85). ENO1 may regulate ERK partly through its PLG-activating function, as its knockdown disrupts integrin-mediated cell-matrix adhesion, a process linked to ERK activation (12). Activated ERK1/2 signaling phosphorylates WTAP and enhances its stability, promoting m^6A modification of ENO1 mRNA, thereby increasing its stability and translation efficiency (64) and supporting tumor cell proliferation and survival (143). This positive feedback loop sustains the tumor's proliferative signal. Targeting ENO1 (ENOblock) and targeting ERK (SCH772984) both demonstrate antitumor effects, particularly in ENO1/ERK-hyperactive tumor subtypes, indicating that the ERK signaling pathway and ENO1 jointly drive tumor progression through multidimensional bidirectional interactions.

ENO1-mediated multiple drug resistance signaling. ENO1 overexpression drives resistance to gemcitabine in cancers such as prostate, pancreatic and cholangiocarcinoma through multiple interconnected mechanisms. A central pathway involves the post-transcriptional upregulation of YAP1. ENO1 binds to C-U-G-rich elements in YAP1 mRNA, enhancing its translation. Elevated YAP1 activates the Hippo signaling pathway and promotes protective autophagy, thus shielding tumor cells from GTP-binding protein overexpressed in skeletal muscle-induced apoptosis (21). The oncogenic effects of YAP1 are further amplified through the YAP1/phospholipase C beta 1/15-hydroxyprostaglandin dehydrogenase axis, stimulating arachidonic acid metabolism and leading to prostaglandin E2 accumulation, a key driver of ENO1-mediated progression that can be pharmacologically inhibited by aspirin (56). Additionally, ENO1 contributes to GEM resistance by modulating cellular redox balance, reducing intracellular ROS via glycolysis-enhanced 'Warburg' metabolism (144). ROS can reciprocally regulate YAP1 stability through CBP-mediated phosphorylation at Ser127, forming a regulatory circuit that sustains the resistant phenotype (145). Targeting this resistance pathway, modulation of ENO1 protein degradation has been shown to reverse gemcitabine resistance in preclinical models (80).

ENO1 enhances resistance to platinum-based DNA-targeted drugs in multiple tumor types. The formation of ENO1 dimers markedly contributes to lactate levels, thereby mediating cisplatin resistance (79,108). Lactic acid accumulation promotes DNA homologous recombination repair, a mechanism that is crucial for resistance to DNA-targeting drugs, including cisplatin, temozolomide and doxorubicin (146,147). Lactate further activates pro-survival signaling pathways such as Wnt and PI3K/Akt, contributing to cisplatin tolerance (132,148,149). These observations highlight ENO1-mediated glycolysis as a central metabolic determinant of broad chemoresistance. Increased glycolytic activity driven by ENO1 also underlies resistance to nucleoside analogs such as gemcitabine, often associated with MYC pathway activation and upregulation of ribonucleotide reductase M1 (RRM1) (150). Separately, in CRC, ENO1 directly induces epithelial-mesenchymal

transition (EMT) and confers resistance to 5-fluorouracil (5-FU) (151). Inhibition of ENO1 at Thr205 elevates CDH1 expression, reverses EMT and restores chemosensitivity (148). This strategy is particularly effective in TP53-mutated CRC; combined targeting of the JAK2-STAT3-UCHL3-ENO1 axis with pacritinib synergizes strongly with 5-FU, achieving >90% tumor reduction in preclinical models (152).

ENO1 also contributes to therapy resistance through additional pathways. In prostate cancer, cell surface-localized ENO1 interacts with extracellular matrix protein 1, inducing its phosphorylation at Y189. This modification facilitates the recruitment of adaptor proteins growth factor receptor-bound protein 2 and son of sevenless homolog 1, leading to downstream MAPK signaling activation and promoting resistance to hormonal therapies such as enzalutamide (153). Moreover, ENO1 plays a key role in castration-resistant prostate cancer. The histone demethylase lysine-specific demethylase 4B cooperates with c-Myc to bind the c-Myc response element within the ENO1 promoter, driving ENO1 transcription and positioning it as a promising therapeutic target in castration-resistant prostate cancer (154). These findings illustrate the diverse mechanisms through which ENO1 fosters drug resistance. Targeting ENO1 or its associated signaling networks represents a compelling strategy for overcoming treatment resistance and improving therapeutic outcomes.

5. ENO1-regulated tumor immune microenvironment

Overexpression of ENO1 is strongly linked to the upregulation of immune checkpoint molecules and resistance to immunotherapy, suppressing antitumor immune responses across diverse types of cancer through multiple mechanisms. It drives T cell exhaustion and interacts with key immune molecules, ultimately fostering an immunosuppressive microenvironment.

ENO1-mediated immunosuppressive microenvironment. ENO1 is crucial in shaping an immunosuppressive TME, mainly by inducing T cell dysfunction and exhaustion (155). Its overexpression is associated with increased expression of inhibitory immune checkpoints such as programmed cell death protein 1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and T-cell immunoreceptor with Ig and ITIM domains and contributes to resistance against anti-programmed death-ligand 1 (PD-L1) therapy (156). The mechanisms of ENO1-mediated immunosuppression differ among types of cancer. In pancreatic cancer (PC), ENO1 knockout reduces regulatory T cells (Tregs) and boosts IFN-γ and TNF-α production, transforming immunologically 'cold' tumors into 'hot' ones (157). It can also directly upregulate PD-L1 to hinder $CD8^+$ T cell infiltration (158). In intrahepatic cholangiocarcinoma, ENO1 activates AKT signaling to elevate fibrinogen-like protein 1, which binds to lymphocyte-activation gene 3 on T cells and suppresses their function (159). In esophageal squamous cell carcinoma, PLCE1-driven ENO1 upregulation enhances aerobic glycolysis and lactate production, impairing $CD8^+$ T cell activation and cytokine secretion (160). Moreover, hydrogen sulfide (H_2S)-induced disulfide modification at ENO1 Cys119 inhibits Treg activation. Reducing H_2S levels through a sulfur-restricted diet or endogenous depletion synergizes with anti-PD-L1/CTLA-4 therapy in CRC (161). In glioblastoma, ENO1 promotes M2-type microglial polarization (162), while in multiple myeloma, it dampens plasmacytoid dendritic cell (pDC) activity. This effect can be reversed by ENO1 inhibitors, which restore pDC-mediated activation of $CD8^+$ T and NK cells (163). Collectively, these findings underscore ENO1 as a master regulator of immune evasion and support its dual targeting for direct antitumor effects and immunotherapy enhancement.

Interaction of ENO1 with immune-related molecules. ENO1 participates in immune regulation by interacting with various immune-related molecules through direct and indirect mechanisms, with context-dependent effects across types of cancer. In CRC, O-GlcNAcylation of ENO1 at Ser249 disrupts its interaction with PD-L1, reducing PD-L1's association with the E3 ligase STIP1 homology and U-box containing protein 1. This inhibits PD-L1's ubiquitin-mediated degradation, increases its stability and ultimately facilitates immune evasion by suppressing T-cell activity (112). Conversely, in lung cancer, ENO1 interacts with PD-L1 and promotes its degradation via the ubiquitin-proteasome pathway, enhancing T cell-mediated antitumor immunity (27). In breast cancer, ENO1 contributes to an immunosuppressive microenvironment by directly binding to and stabilizing SPP1 mRNA, leading to elevated SPP1 expression. Subsequently, SPP1 activates the ITGB1 pathway, impairing $CD8^+$ T cell function and promoting tumor-associated macrophage polarization, thereby reducing the efficacy of anti-PD-L1 therapy (164).

6. Therapeutic approaches targeting ENO1

As a multifunctional oncogene, ENO1 has driven the development of various targeted therapeutic strategies. Research on ENO1 inhibitors has evolved from early natural products to a range of novel compounds identified through high-throughput virtual screening and rational drug design. Additionally, the exploration of therapeutic vaccines has broadened the therapeutic options for ENO1-related cancers (Fig. 4).

Research progress on ENO1 inhibitors. As early as the 1980s, numerous ENO1 inhibitors were discovered. Most of these inhibitors either directly target ENO1 to reduce its enzymatic activity or mimic metabolic intermediates to inhibit binding (165). Fluoride, identified as an ENO1 inhibitor during this period, markedly inhibits ENO activity in *Streptococcus mutans* at concentrations between 50 and 300 μM (Table I). It acts as a competitive inhibitor of enolase (166). Another ENO1 inhibitor discovered around the same period was phosphonoacetohydroxamate (PhAH) (165), which was not thoroughly evaluated until 2012. PhAH efficiently inhibits human enolases, showing similar inhibitory effects on both ENO1 and ENO2 and effectively suppresses glioblastoma proliferation and progression (30,167). 2-Fluoro-2-phosphonoacetohydroxamate (FPAH), a fluorinated derivative of the known inhibitor PhAH, has a lower pKa value for its phosphate group due to fluorination, bringing it closer to that of the natural substrate PEP. However, upon binding to ENO1, the fluorine atom of FPAH forms a tight non-covalent interaction with Gln164, forcing the nearby

Table I. Molecular drugs targeting enolase 1.

Name	Ki value	Mechanism	(Refs.)
(3-hydroxy-2-nitropropyl) phosphonate	6.4 nM (high pH, saturated Mg^{2+})	Aci-carboxylate form of simulated carbon negative ion intermediate	(165)
(Nitroethyl)phosphonate	1.2 μM (high pH, saturated Mg^{2+})	Competitive inhibitor, simulated carbon negative ion intermediate	(165)
Phosphonoacetohydroxamate	15 pM (saturated Mg^{2+}, ionized form)	Simulates carbon negative ion intermediates, directly coordinating with Mg^{2+}	(31,165)
Phosphonopyruvate oxime	75 μM (pH 8-9.3, ionized form)	Possible due to oxime group interference with the structure	(165)
(Phosphonoethyl)nitrolate	~14 nM (pH 9.0, 1 mM Mg^{2+})	Simulated carbon negative ion intermediate	(165)
Tartronate semialdehyde phosphate	500 nM (pH 8, containing glycerol)	Substrate analog	(165)
Fluoride	50-300 μM	Competitive binding at the active site	(166)
2-Fluoro-2-phosphonoacetohydroxamate	1.4 μM	Substrate analog	(167)
AP-III-a4	576 nM	Non-substrate analog, inhibitor	(168)
ENO1 DNA vaccine	Unknown	DNA vaccine	(179)
pHCT74-conjugated liposome	Unknown	Drug-carrying vehicle	(191)
SF2312	10-50 nM	Inhibitor, binding to Glu166 and His370	(30)
HEX/POMHEX	ENO1: 232 nM ENO2: 64 nM	Inhibitor, binding to R373	(171)
HuL227	Unknown	Monoclonal antibody	(172)
Ciwujianoside E	~18.1 μM	Inhibitor, between dimer interfaces	(129)

Ki, inhibition constant; ENO1, enolase 1.

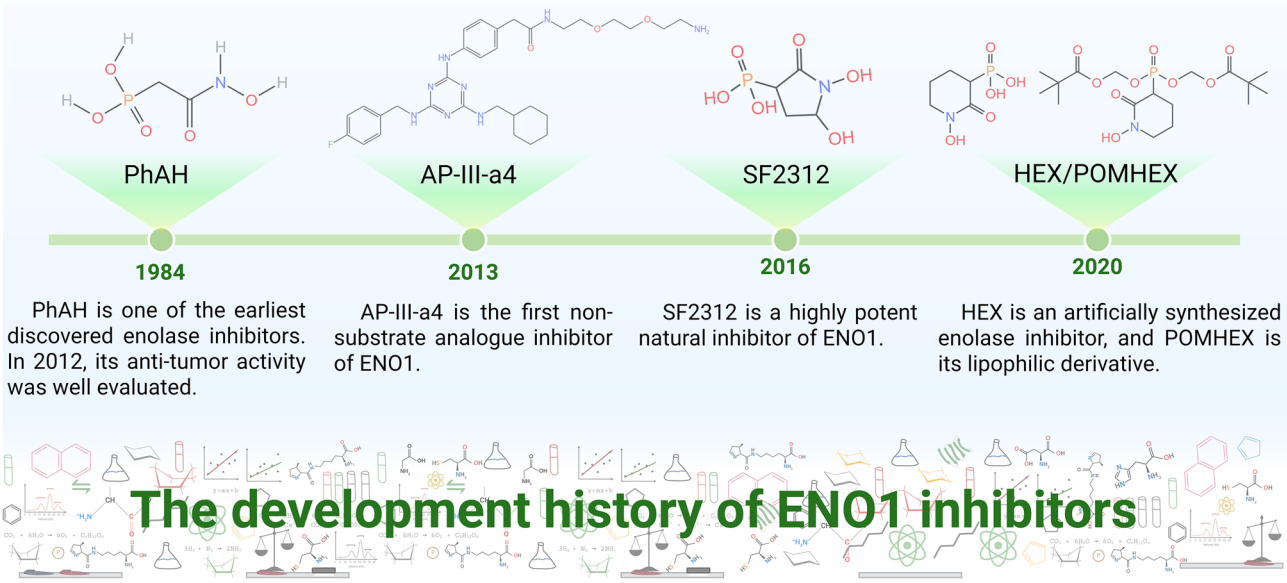

Figure 4. Key compounds in the development of ENO1 inhibitors. ENO1, enolase 1.

His156 to flip outward, resulting in a relatively weak binding affinity (167). AP-III-a4 is the first non-substrate analog inhibitor targeting ENO1 and also functions as a non-enzymatic site inhibitor (30,168). It inhibits tumor survival and migration, enhances the sensitivity of gastric cancer to cisplatin and increases the sensitivity of breast cancer to radiotherapy (169). In multiple myeloma, AP-III-a4 restores T cell and NK cell-mediated tumor killing by activating pDCs and synergistically enhances T cell-mediated cancer cell lysis with anti-PD-L1 antibodies (163). Additionally, AP-III-a4

induces the translocation of enolase into the cell nucleus, where it acts as a transcriptional repressor (168,170). HEX, a synthetic enolase inhibitor, selectively kills glioblastoma cells at low nanomolar concentrations, eradicates intracranial orthotopic tumors in mice and is well-tolerated. The carbonyl and hydroxylamine groups of HEX form chelates with magnesium ions (Mg^{2+}), while the anionic phosphonate forms a salt bridge with the R373 residue, thereby inhibiting ENO enzyme activity. Notably, HEX exhibits higher selectivity toward ENO2, with Ki values of 232 and 64 nM for ENO1 and ENO2, respectively. This makes HEX particularly suitable for tumors lacking ENO1, enabling maximal inhibition of tumor cell glycolysis while minimizing effects on normal cells (171). Due to its highly polar phosphoric acid structure, HEX may have impaired plasma membrane penetration. Consequently, the lipophilic POMHEX was developed, which undergoes enzymatic hydrolysis inside cells to release the active HEX, markedly improving its pharmacokinetic properties. HuL227 is a multi-target monoclonal antibody that binds to ENO1 on the tumor surface. It blocks ENO1's function as a PLG receptor, reducing plasmin activation, inhibiting cancer cell degradation of the extracellular matrix and impeding tumor invasion and metastasis. Simultaneously, HuL227 inhibits VEGF-A-induced PLG activation via ENO1 and markedly reduces vascular endothelial cell luminal formation. Additionally, HuL227 suppresses the migration and chemotaxis of androgen-independent prostate cancer cells (PC-3, DU145) induced by inflammatory mediators (TNFα, CCL2, TGFβ) (172). In recent years, Lung *et al* (173) screened 22 million chemical structures from the ZINC database that comply with Lipsky's five rules and identified compounds that bind to ENO1 through virtual screening. ZINC1304634, ZINC16124623, ZINC1702762 and ZINC72415103 are four ENO1 inhibitors identified through comprehensive computer-aided screening. These inhibitors are classified as non-mutagenic, non-carcinogenic and suitable for oral administration, indicating development potential (174).

Natural products and derivatives targeting ENO1. Several natural products and their derivatives have demonstrated promising ENO1-targeting activities through distinct mechanisms. The phosphonate antibiotic SF2312 binds to the ENO1 active site by coordinating two Mg^{2+} ions via its phosphate and carbonyl groups, while its 5-hydroxyl group forms hydrogen bonds with the catalytic residues Glu166 and His370. This interaction locks ENO1 in a closed conformation, blocking substrate entry and halting the catalytic cycle (29). Ciwujianoside E, a natural product, specifically inhibits the interaction between ENO1 and PLG, thereby preventing plasmin generation and TGF-β1 activation. In both *in vitro* and *in vivo* studies on Burkitt's lymphoma, Ciwujianoside E exhibited potent antitumor effects, suppressing cell proliferation and invasion (129). Subsequent studies have revealed that ginsenosides and their derivatives also exhibit inhibitory effects on ENO1. Rh2E2 (175) and 20 (S)-Rh2E2 (176), two synthetic ginsenoside derivatives, inhibit tumor growth and metastasis. Rh2E2 exhibits no toxic reactions at the maximum oral dose of 5.000 mg/kg. It specifically downregulates tumor glycolysis, fatty acid β-oxidation and the tricarboxylic acid cycle, thereby inhibiting ATP production and targeting tumor cell metabolism. 20 (S)-Rh2E2 reduces ENO1 protein levels, suppressing lactate and ATP production in lung cancer cells without affecting normal cells.

Therapeutic vaccines. Tumor therapeutic vaccines are a form of immunotherapy that activates the patient's immune system to recognize and attack cancer cells. Their core principle involves presenting tumor-specific antigens expressed by the tumor to the immune system (177,178). The earliest ENO1-targeted tumor therapeutic vaccine was a DNA vaccine. This ENO1 DNA vaccine induced both antibody and cellular immune responses, extending the average survival time of PC mice by 138 days. Vaccinated mice showed a significant increase in serum levels of anti-ENO1 immunoglobulin G. This antibody binds to cancer cell surfaces, inducing complement-dependent cell-mediated cytotoxicity. The ENO1 DNA vaccine reduced the number of myeloid-derived suppressor cells (MDSCs) and Tregs while enhancing multiple responses of T helper cells (179). Combining this vaccine with a PI3Kγ inhibitor produced synergistic effects (180). By inhibiting PI3Kγ to target MDSCs, this approach increased $CD8^+$ T cell and M1 macrophage infiltration in tumors while reducing Treg cells. Specific IgG and IFNγ targeting ENO1 also increased in the mouse circulation. In the meantime, pretreating PC mice with gemcitabine before inoculation with the ENO1 DNA vaccine unleashed the antitumor activity of CD4+ T cells, resulting in superior tumor suppression (181). These findings highlight the potential of ENO1-directed vaccines, especially when combined with immunomodulatory or chemotherapeutic agents, to overcome immunosuppression and enhance antitumor immunity.

Designing antigens targeting post-translational modification epitopes represents a novel strategy for activating tumor immune responses (182). Citrullinated ENO1 peptides, such as ENO1 11-25cit (15) in melanoma and ENO1 241-260cit (253) in HLA-DR4 transgenic mice, elicit potent Th1 responses and demonstrate antitumor activity not observed with their unmodified counterparts (183). Building on this, a refined citrullinated ENO1 (citENO1) vaccine peptide was shown to enhance $CD8^+$ T cell activation, inhibit tumor growth and synergize with PD-1 blockade (184). Another vaccine design incorporating the tumor-associated antigen mENO1 (Ag85B-ENO1 46-82) effectively boosted $CD8^+$ T cell infiltration and cytokine production (IFN-γ, TNF-α), promoted M1-like macrophage polarization and suppressed tumor progression in a lung cancer model (185). Collectively, these advances underscore the potential of ENO1-directed therapeutic vaccines to reprogram the immunosuppressive TME and enhance antitumor immunity.

7. Diagnostic and prognostic value of ENO1 in cancer

ENO1 is notably overexpressed in various types of cancer, such as HCC, PC, CRC and breast cancer. Its expression levels are closely associated with tumor stage, invasiveness and a poor prognosis. The diagnostic accuracy is substantially enhanced when ENO1 is used alone or in combination with traditional biomarkers such as CA19-9 and CEA.

Diagnostic value of ENO1. The diagnostic value of ENO1 mainly stems from its combined analysis with established biomarkers, which markedly improves diagnostic sensitivity

and specificity. In lung cancer diagnosis, co-analyzing ENO1 with CEA, SCC, NSE and CYFRA21-1 effectively boosts detection sensitivity (186). In gastric cancer, elevated serum anti-ENO1 autoantibody titers (AUC=0.656) have diagnostic utility and their combination with CEA levels can further inform patient prognosis (187). For PC diagnosis, ENO1 alone shows a sensitivity of 75.8% and a specificity of 88.2%. When combined with CA19-9, diagnostic sensitivity reaches 94.5% with an AUC of 0.935, outperforming any single currently available biomarker (188). This approach is particularly valuable in Lewis-negative patients with normal CA19-9 levels. Moreover, in oral submucosal fibrosis with atypical hyperplasia, ENO1 expression levels can predict the malignant progression of precancerous lesions (189). In summary, ENO1 holds substantial diagnostic value.

Prognostic value of ENO1 in multiple cancers. ENO1 expression is markedly prognostic across various types of cancer. In HCC, elevated ENO1 mRNA and protein levels in tumor tissues are linked to poorer overall survival and disease-free survival (3,190). Similarly, in CRC, ENO1 overexpression is strongly associated with advanced clinicopathological features, including deeper tumor invasion, lymph node metastasis, perineural invasion and a higher TNM stage, serving as an indicator of an unfavorable prognosis (2,138). Elevated ENO1 protein levels are detectable in the plasma of PC patients, with its expression markedly associated with lymph node metastasis, clinical staging and a poor prognosis (1,188). In triple-negative breast cancer, ENO1 overexpression is markedly associated with high-grade tumors and a poor prognosis (5). Additionally, combined detection of CD47 and ENO1 provides a reliable prognostic biomarker for CRC patients (85). Collectively, these findings establish ENO1 as a valuable prognostic marker in multiple types of cancer.

8. Conclusions and outlook

ENO1, as a multifunctional glycolytic enzyme, has roles that extend far beyond its traditional function in energy metabolism. The present review systematically clarified the multifaceted functions of ENO1 in tumorigenesis and progression, covering its diverse side roles as a metabolic enzyme, PLG receptor, nucleic acid-binding protein and signaling scaffold protein. The execution of these functions heavily relies on its extensive PTMs, which dynamically regulate its enzymatic activity, stability, subcellular localization and functional transitions. ENO1 promotes tumor proliferation, invasion, metastasis and drug resistance through mechanisms such as activating the PI3K/AKT pathway, inhibiting the AMPK/mTOR pathway and forming positive feedback loops with ERK. Furthermore, the regulatory role of ENO1 in the tumor immune microenvironment is gradually coming to light. Therapeutically, small-molecule inhibitors, natural product derivatives and therapeutic vaccines targeting ENO1 have demonstrated significant antitumor potential in preclinical models, especially when combined with immune checkpoint inhibitors or chemotherapy, displaying favorable synergistic effects. Regarding diagnosis and prognosis, ENO1 is highly expressed in multiple types of cancer and is associated with tumor malignancy and poor patient outcomes, making it a clinically valuable biomarker, either independently or in combination with others.

Despite significant progress in understanding ENO1's role in tumors, numerous unknowns remain to be explored. First, although the present review summarized the extensive PTM sites on ENO1, whether crosstalk exists between different modifications is still unknown. Elucidating how PTM combinations dynamically regulate ENO1's functional transitions and subcellular localization is of great value, particularly its response mechanisms within the dynamically changing TME. ENO1 plays critical functions in normal cells and physiological states, yet its functional regulation regarding tissue or tumor type specificity remains poorly characterized. Simultaneously, clarifying its unique mechanisms across diverse cancer contexts could facilitate the development of more targeted therapeutic strategies. Existing ENO1 inhibitors mainly target enzymatic activity, with limited approaches available for its non-enzymatic functions. Future approaches may involve designing multifunctional inhibitors or combination therapies that simultaneously block its metabolic and signaling scaffold functions. As a novel topic, the specific mechanisms by which ENO1 regulates immune cell function within the TME require further elucidation. Investigating the metabolic-immune crosstalk it mediates could offer new targets for overcoming immunotherapy resistance. Future interdisciplinary, multi-level investigations will advance ENO1 from mechanistic research to clinical application, ultimately driving breakthroughs in cancer treatment.

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Authors' contributions

XN completed the initial manuscript draft. MZ was responsible for translation and language editing. KZ handled the graphical visualizations. CW, JG and WF were responsible for literature collection, organization and screening. LZ, TJ and GZ provided financial support and ultimately reviewed

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Competing interests

The authors declare that they have no competing interests.

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The authors utilized DeepL, an AI-powered translation website, to assist in completing this article. While the website provided support during the translation process, the author bears full responsibility for the final content. Retrieved from: https://www.deepl.com/zh/translator.

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