Review

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Structural and functional regulation of lactate dehydrogenase-A in cancer

Mark R Woodford[1,2,3], Victor Z Chen[1,3], Sarah J Backe[1,2,3], Gennady Bratslavsky[1,2,3] & Mehdi Mollapour*[,1,2,3]

[1]Department of Urology, SUNY Upstate Medical University, Syracuse, NY 13210, USA
[2]Department of Biochemistry & Molecular Biology, SUNY Upstate Medical University, Syracuse, NY 13210, USA
[3]Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY 13210, USA
*Author for correspondence: mollapom@upstate.edu

Dysregulated metabolism is one of the hallmarks of cancer. Under normal physiological conditions, ATP is primarily generated by oxidative phosphorylation. Cancers commonly undergo a dramatic shift toward glycolysis, despite the presence of oxygen. This phenomenon is known as the Warburg effect, and requires the activity of lactate dehydrogenase-A (LDHA). LDHA converts pyruvate to lactate in the final step of glycolysis and is often upregulated in cancer. LDHA inhibitors present a promising therapeutic option, as LDHA blockade leads to apoptosis in cancer cells. Despite this, existing LDHA inhibitors have shown limited clinical efficacy. Here, we review recent progress in LDHA structure, function and regulation as well as strategies to target this critical enzyme.

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The high-energy molecule ATP is essential to fuel biological processes in all living cells. ATP is generated most efficiently by glucose catabolism followed by oxidative phosphorylation. In brief, glucose is metabolized to pyruvate in the cytosol and subsequently converted to acetyl-CoA in the mitochondria. Acetyl-CoA then enters the tricarboxylic acid cycle, initiating the electron transport machinery and resultant ATP production. This pathway maximally generates 36 ATP per molecule of glucose, accounting for approximately 95% of cellular ATP [1]. The remaining 5% is the result of dedicated oxygen-independent glycolysis, a less efficient pathway yielding two ATP per molecule of glucose and concluding with the reduction of pyruvate to lactate [1]. This terminal reaction, catalyzed by LDH, dictates the relative usage of the two metabolic modalities [2].

LDHs are responsible for the NADH-dependent interconversion of pyruvate and lactate [3]. The four highly similar isozymes of LDH are encoded by four separate genes. *LDHA*, *LDHB* and *LDHD* are ubiquitous, while *LDHC* is testis specific [4]. LDHA is involved in ATP production under stress-induced hypoxia in muscle, and as such is often referred to as LDH-M [5]. *LDHB* is the sole isoform expressed in cardiomyocytes, and indicative of its role in the heart, is often denoted as LDH-H [5]. The significance of *LDHD* is poorly understood, however, it is the only variant able to utilize the D-isomer of lactate [4,6].

Despite highly similar structure and function, differential tissue expression suggests distinct cellular roles for these isozymes.

Lactate dehydrogenase

Structure & activity

The importance of LDH has long been appreciated, with research into its activity dating back more than 80 years. In fact, the nucleotide-binding motif known as the Rossmann fold was first identified in LDH in 1970, only later to be recognized as a ubiquitous structural feature across the proteome [7–11]. The substrate binding domain is the only other major structural feature in LDH, and together these features account for greater than 90% of the protein [8]. The remaining 20 amino acids at the N-terminus form an unstructured region that interacts with the C-terminus of a neighboring monomer and is critical for oligomeric assembly of LDH (Figure 1) [8].

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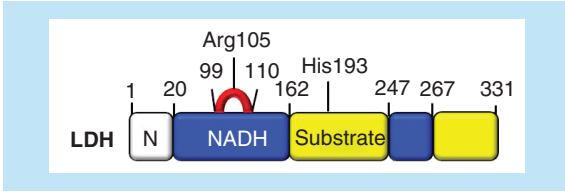

Figure 1. Schematic representation of LDHA protein. Blue corresponds to the unstructured N-terminal dimerization region, orange is the nucleotide-binding Rossmann fold, red is the catalytic loop and green forms the substrate-binding domain.

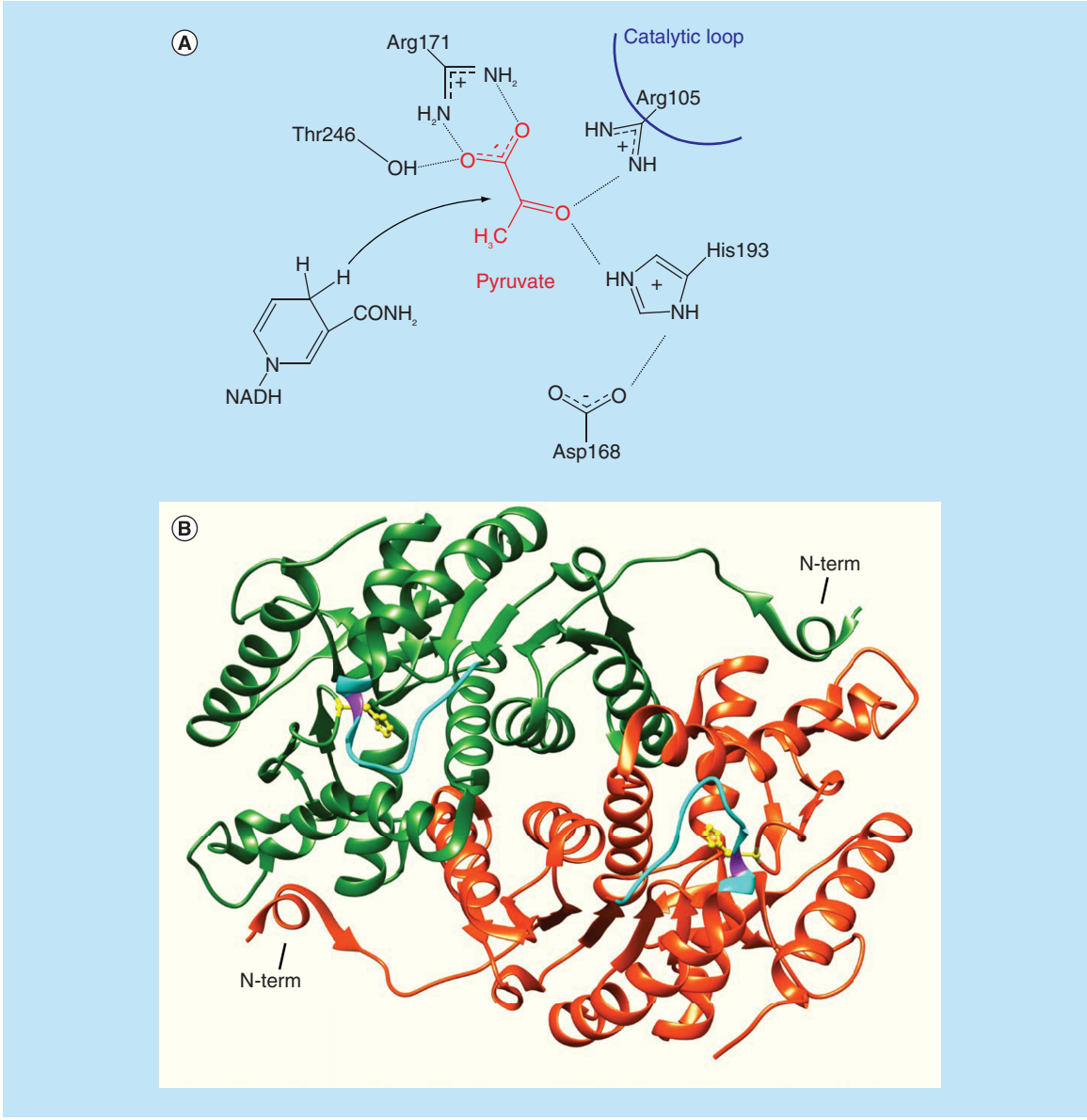

Figure 2. Structural architecture of the LDHA. (A) Catalytic site interactions of LDHA with pyruvate. Direct hydride transfer from NADH to pyruvate is stabilized by charged interactions in the binding pocket. His193 acts as a proton donor to form lactate. **(B)** Oligomer formation is mediated by the unstructured N-terminus, which interacts with the C-terminus of the adjacent monomer. The mobile loop region is colored in cyan, with Arg105 in magenta and His193 in yellow.

The ability of LDH to bind the nucleotide cofactor NADH is essential to allow substrate entry into the remodeled active site [12,13]. NADH interacts primarily with four residues (numbering based on LDHA), Asp168, Arg171, Thr246 and the catalytic His193 (Figure 2A [14–16]). Arg171 and Thr246 along with Ala236 provide substrate

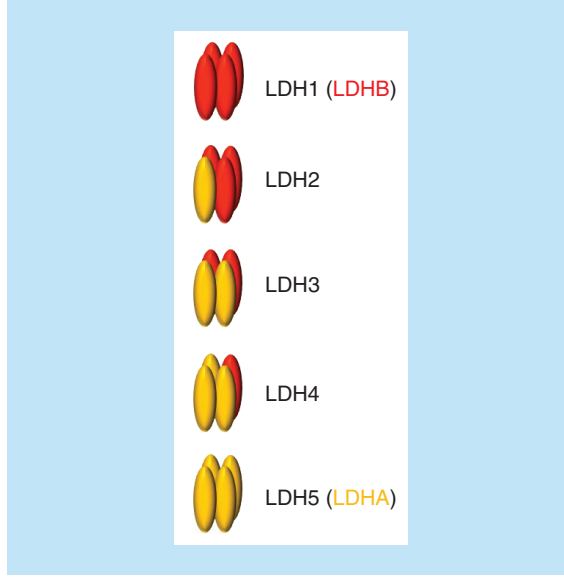

Figure 3. LDH tetramer configurations. LDHA (cyan) can homotetramerize to form LDH5, or interact with LDHB (red) to form heterocomplexes LDH4–LDH2. LDH1 consists of four monomers of LDHB.

coordination in the adjacent substrate-binding pocket [12]. This is followed by closure of a flexible loop formed by residues 99–110 and desolvation of the active site, committing LDH to catalysis [17]. This loop is stabilized by several contacts formed with nucleotide and substrate [18] and a critical hydrogen bond is formed between Arg105 and the substrate carbonyl [19,20]. This bond is essential, as mutation of Arg105 completely abrogates LDH activity [21]. The protonated His193 acts as a proton donor, initiating a reaction that terminates in the oxidation of NADH to NAD^+ and the release of NAD^+ and lactate [22,23]. The lactate is then excreted as waste, which can have a profound effect on the pH of the extracellular environment [24]. Interestingly, lactate can also be taken up through monocarboxylate transporter 1 and utilized as a metabolic fuel, though the mechanism remains under investigation [25,26].

LDHA–LDHB

LDH exists as an obligate dimer (Figure 2B), with each monomer hosting its own active site [27]. Upon cofactor and substrate binding or increasing protein concentration, LDH assembles into a highly active tetramer [28–33]. In cells, the tetramer can be composed of any combination of A and B isoforms, which share 75% sequence identity (Figure 3) [34]. Tetramer formation is regulated both by the abundance of substrate as well as post-translational modifications [35–37], which will be discussed in more detail later. LDHA exhibits a preference for pyruvate, whereas LDHB prefers lactate, suggesting a cellular role for LDH regulation based on both enzyme oligomer composition and substrate prevalence.

Warburg effect

From its initial characterization as a substrate-regulated enzyme, LDH activity has been inextricably linked to the abundance of intracellular pyruvate resulting from glucose catabolism [32]. Indeed, there is a clear connection between the metabolic requirements of various cell types and their LDH activity [38–40]. At steady state, several cell types preferentially utilize glycolysis, including the skeletal muscle during exercise, muscle stem cells during self-renewal and lymphocytes, which experience an increase in glycolytic flux during activation. In the first case, hypoxia induces the shift to glycolysis due to the increased demand for NAD^+ [41]. In muscle stem cells, glycolysis is activated during tissue repair, in an LDHA-dependent process [42]. This is antagonized by AMPKα1, demonstrating a role for the nutrient-sensing kinases AMPK and mTOR in the regulation of glycolysis [42]. Notably, the activation of lymphocytes occurs in highly oxygenated blood, so glycolysis occurs despite the capacity for oxidative phosphorylation [43]. This phenomenon is known as the Warburg effect.

The Warburg effect was first described by Otto Warburg in the 1920s, and is most commonly associated with cancer. After initial speculation that this phenotype resulted from compromised mitochondrial function, it has since been demonstrated that mitochondria retain activity [44]. More recently, focus has been shifted to deregulation of glycolytic pathway enzymes to explain this metabolic reprogramming [45].

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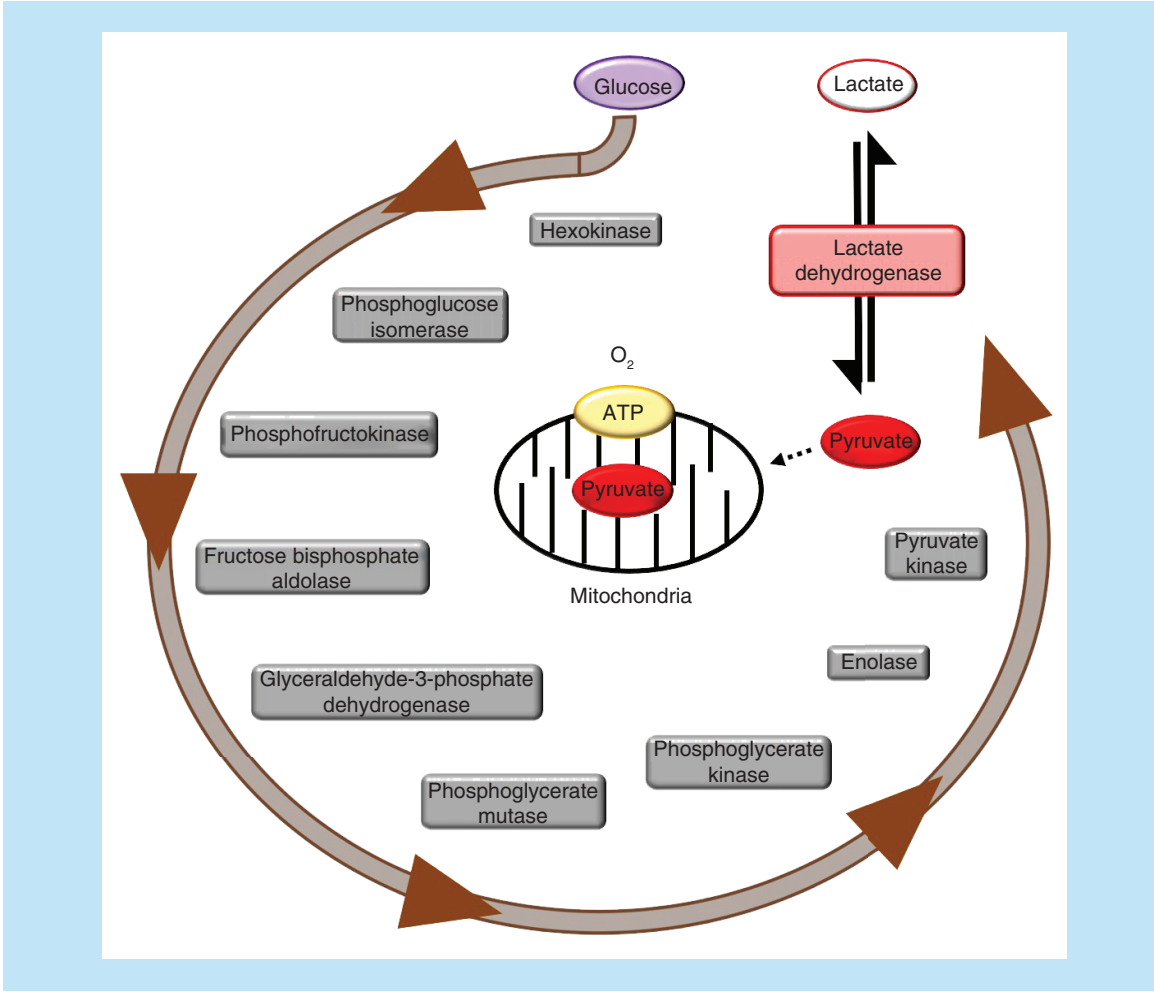

Figure 4. Biochemical steps involved in glycolysis. Glucose is metabolized to pyruvate in the cytosol, which can be subsequently utilized by mitochondrial oxidative phosphorylation or converted to lactate by LDHA.

Glycolysis provides a relatively small contribution to the intracellular ATP pool at steady state, indicative of a highly regulated process. The large number of enzymes involved in glycolysis have contributed to competing theories attempting to explain the Warburg effect. These include the increased uptake of glucose, altered isoform expression and hyperactivity of glycolytic enzymes. GLUT1 and GLUT3 are glucose transporters that are activated in response to AMPK, which is itself activated under ATP depletion [46]. GLUT1 and another glycolytic enzyme phosphoglucose isomerase are also upregulated in response to hypoxia [47], a common condition in solid tumors [48]. The first step of glycolysis, the phosphorylation of glucose to glucose-6-phosphate, is catalyzed by HK. HK-II (the most active isoform) becomes more abundant upon cellular transformation, which is also a consequence of HIF-1α induction [49]. However, inhibitors of GLUT1 and HK evaluated in preclinical and Phase I clinical trials have been found largely ineffective [50]. Pyruvate kinase works immediately upstream of LDHA, catalyzing the conversion of phosphoenolpyruvate to pyruvate. This reaction is coupled to the generation of ATP, and can be carried out by any of the four isozymes of pyruvate kinase. Pyruvate kinase isoform M2 (PKM2) is typically expressed during early development, however, it is also preferentially expressed in cancer [51]. Replacement of the PKM1 isozyme with PKM2 in cancers signifies a dedifferentiation to a more stem-like phenotype and is a predictor of poor outcome in cancer [51]. The dysregulation of these numerous proteins, however, fails to address the perseverance of glycolysis in the presence of competent oxidative phosphorylation machinery, as they all exist 'upstream' of the mitochondrial electron transport chain (Figure 4).

Taken together, the crucial role for LDH in maintaining redox homeostasis under hypoxic and anabolic conditions suggests a critical role in the hypertrophic growth of cancers.

Functional regulation of LDH

Much work has been done in the last 40 years to understand signaling inputs dictating LDH activity. The fruit of these efforts includes glycolytic and LDH-specific regulation at every level, and an array of existing and potential targets for inhibition in cancer.

Epigenetic effect

DNA methylation is an epigenetic modification often found to regulate gene expression by affecting binding of transcriptional proteins [52]. Indeed, methylation of promoter elements has been reported to govern differential expression of various LDH isozymes. *LDHC* is downregulated through methylation at nine sites in a 230 bp CpG island, and is hypomethylated in LDHC-expressing tissues at steady state [53]. A similar mechanism where loss of DNA methylation promotes expression has been shown for *LDHA* in R51 retinoblastoma cells [54]. Interestingly, methylation of a similar CpG-rich region in *LDHB* completely abrogates expression, leaving LDHA as the only LDH present in evaluated gastric and pancreatic cancer cell lines [55]. This finding presents promoter methylation as one potential explanation for the differential reliance on LDHA and LDHB observed in cancers. These data demonstrate that epigenetic regulation of LDH is a potential determinant of isozyme availability and regulation in cancer.

Transcriptional regulation

In addition to being hyperactivated in many cancers, expression of *LDHA* is also increased, implying transcriptional regulation of *LDHA* is an important suppressive mechanism. Transcription factors are proteins that play a critical role in the regulation of gene expression by binding to DNA and modulating transcription of target genes [56]. Transcriptional dysregulation is a common finding in cancers and supports the hyperproliferative capacity of cancer cells [57]. Interestingly, transcription factors such as HIF and c-Myc that have an established link to oncogenesis have also been found to regulate LDHA expression [58].

In the presence of oxygen, prolyl hydroxylation of HIF allows its recognition, ubiquitination and degradation by the tumor suppressor E3-ubiquitin ligase von-Hippel Lindau (VHL) [59]. When VHL is lost, HIF accumulates in cells. This promotes an oncogenic phenotype in part by inducing expression of LDHA and remodeling metabolism [60,61]. In HeLa and Hep3B cells, HIF1α binds to the transcriptional enhancer and promoter of LDHA and activates transcription, promoting a hypoxia-like transcriptional program [60,62]. A similar mechanism for LDHA regulation by both HIF1 and HIF2 has been observed in pancreatic cancer [60]. It has been well-established that hyperactivation of mTOR is abundant in cancers and is intimately involved in the regulation of glycolysis [63]. The HIF transcriptional program is induced by mTOR activation [64] (as well as HER2, described later [65]), providing a probable mechanism for the glycolytic dependence on mTOR in breast cancer. Notably, mTOR inhibition by rapamycin reverses the glycolytic reprogramming, which is magnified by coinhibition of LDHA with the substrate-competitive inhibitor oxamate [66].

As the 'guardian of the genome', p53 directs DNA repair through its essential role as a transcription factor [67]. As such, mutations in p53 can be found in approximately one-third of all cancers [68,69]. Perhaps surprisingly, p53 is also able to suppress the transcription of LDHA, along with the migration and invasion of cancer cells caused by LDHA hyperactivity [70]. This ascribes yet another tumor suppressive function to p53 with respect to the regulation of cellular metabolic transformation.

Deregulation of the oncogenic transcription factor c-Myc is a common finding in cancers [71]. The *LDHA* promoter is bound by c-Myc and transcriptionally activated [71]. Interestingly, knockdown of *LDHA* reduced c-Myc-mediated anchorage-independent growth [71]. These data suggest LDHA expression and activity is the functional outcome of c-Myc activity, and is critical in cancer initiation, growth and proliferation [72]. In agreement, it has been demonstrated that the c-Myc inhibitor JQ1 acts to inhibit ovarian cancer growth by indirectly reducing the activity of LDHA [73].

Pancreatic cancer has an exceedingly poor prognosis, as the 5-year survival rate is only 5% [74]. The transcription factor forkhead box protein M1 (FOXM1) has been demonstrated to be overexpressed in pancreatic cancer and contribute to oncogenesis [75,76], as well as induce glucose metabolism [77]. FOXM1 binds to the promoter of LDHA and another glycolytic enzyme phosphoglycerate kinase 1 and activates their transcription [74]. Increased expression of FOXM1 and LDHA was positively correlated in pancreatic cancer patient samples, which also correlated with clinical grade and stage [74].

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The receptor tyrosine kinase ErbB2 (HER2) is a known oncogene whose deregulation is most closely associated with poor prognosis in breast cancer [78]. ErbB2 expression has been shown to increase lactate production, however, the mechanism has only recently been uncovered [79,80]. Zhao *et al.* demonstrated ErbB2 upregulates expression of the transcription factor heat shock factor-1 (HSF1), which translocates to the nucleus and induces LDHA, but not LDHB [80]. Targeting the ErbB2/HSF1 axis may provide a specifically exploitable avenue to inhibit LDHA in breast cancer.

Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor reported to have tumor-suppressor activity in several cancers [81]. KLF4 expression is negatively correlated with LDHA expression, and this relationship is positively associated with tumor grade and stage [82]. Pancreatic cancer cell lines demonstrating low KLF4 (and thus high LDHA) demonstrated increased aerobic glycolysis, which could be rescued by exogenous expression of KLF4. KLF4 was shown to bind to two elements in the promoter of LDHA and negatively regulate its transcription [82], providing another mechanism for suppression of glycolysis in cells.

14-3-3 is a family of ubiquitously expressed adaptor proteins, with apparently opposing effects on LDHA and the metabolic recalibration of cancer [83]. These proteins are generally found to interact with amphipathic surfaces containing a phospho-Ser/Thr and are thus implicated in regulation of protein function [84]. 14-3-3σ is unique among family members for its noted tumor-suppressive ability as well as its structural distinction [85]. This isoform is reported to stabilize p53 [86], and is frequently lost in cancer due to transcriptional repression or increased degradation [87,88]. Indeed, loss of 14-3-3σ increased glucose uptake in HCT116 colorectal cancer cells, and induced the c-Myc-responsive transcriptional program, including LDHA [83]. 14-3-3ζ, however, increases cAMP response element-binding protein-mediated transcription of LDHA in human mammary epithelial cells [89]. This was shown to be a direct consequence of RAF/MEK/ERK pathway activation, and inhibiting MEK/ERK attenuated LDHA expression and tumor growth in these cells [89]. Subsequent research has shown that 14-3-3ζ is able to directly bind to phosphorylated LDHA and protect it from ubiquitination and degradation [90]. Interestingly, this interaction is regulated by the ubiquitin-binding protein CUEDC2, which stabilizes 14-3-3ζ [90]. Taken together, these data suggest that upregulation of LDHA expression is part of a transcriptional program responsible for promoting and/or sustaining oncogenesis. Additionally, the targeting of specific transcription factors with small-molecule inhibitors may provide anticancer effects as a consequence of the downregulation of LDHA.

Post-transcriptional regulation

miRNAs are short, noncoding RNAs (18–25 bases) that bind to the 3′-untranslated region (3′-UTR) [87] of target mRNA and regulate expression by modulating translation or inducing mRNA degradation. Since their discovery in 1993, miRNAs have been implicated in the regulation of many disease processes, including glycolysis, and are dysregulated across cancers. A detailed review has covered miRNA-mediated regulation of glycolytic enzymes as well as signaling pathways that feed into glycolysis, such as mTOR, c-Myc and HIF [91]. However, more recent research has identified roles for several miRNAs in the direct regulation of *LDHA*.

Bladder cancer is one of many malignancies that demonstrate upregulated LDHA expression. A binding site for miR-200c has been identified within the 3′-UTR of *LDHA*, suggesting a role for miR-200c in the regulation of LDHA expression and potentially glycolysis [92]. Previously, miR-200c was shown to play a role in epithelial-to-mesenchymal transition and curiously, high expression of miR-200c is associated with poor prognosis in several cancers [93]. Indeed, miR-200c is downregulated in bladder cancer, and restoring its levels decreases LDHA and suppresses glycolytic activity, and consequently the proliferation and invasion of cultured bladder cancer cell lines [92].

Though the term 'tumor-suppressor' generally refers to proteins, miR-449a is reported to have the characteristics of a tumor suppressor [94]. Expression and activity of LDHA was found to be downregulated in response to miR-449a expression in non-small-cell lung cancer cell lines [95]. This decrease in LDHA sensitized lung cancer cells to ionizing radiation, suggesting LDHA-mediated cell proliferation confers a survival advantage lost upon LDHA inhibition [95].

Six additional miRNAs capable of binding the 3′-UTR of *LDHA* have also been identified by target-prediction (miR34a/c, miR-369-3p, miR-374a, miR-4524a/b) [96]. *LDHA* is downregulated by these miRNAs and their expression is negatively correlated with that of LDHA in colorectal cancers. Cells re-expressing these miRNAs also demonstrated decreased glycolytic activity and colorectal cancer xenograft growth, consistent with modulation of LDHA [96]. Interestingly, a single point mutation was found in the 3′-UTR of *LDHA* in HCT116 colon and BxPC3 pancreatic cancer cells as well as 4/30 colorectal cancer tissue samples. This mutation (rs18407893)

abrogates binding of miR-374a [96], providing an intriguing explanation for the loss of control of LDHA in certain cancers.

Downregulation or loss of miR-34a is a common feature of many tumor types [97], and is further compounded by the revelation that miR-34a is responsive to the tumor-suppressor p53 [98]. In fact, it has been posited that miR-34a is involved in maintenance of the p53 transcriptome, as they influence overlapping pathways such as DNA repair and cell cycle progression [98]. Specifically, LDHA is a common target of both miR-34a and p53, with both factors negatively regulating LDHA expression [70,99]. Taken together, these findings show loss of p53, miR-34a, or both dramatically induce *LDHA* expression and promote the Warburg phenotype.

In a broader context, several miRNAs (including miR-92-1) have been reported to target and suppress the tumor-suppressor VHL [100,101]. This stabilizes HIF-1α and induces *LDHA* transcription [100,101], as discussed earlier. These miRNAs are demonstrated to be overexpressed in cancer [100], suggesting additional indirect mechanisms of *LDHA* overexpression and regulation of metabolic transformation.

The regulation of LDHA appears to occur both at the transcriptional and post-transcriptional levels. It then follows that regulation of the miRNAs and transcription factors themselves will indirectly govern *LDHA* expression, but the complicated feedback mechanisms have yet to be fully deciphered.

Post-translational modifications

Modulating protein–protein interaction is a key mechanism in the regulation of signal transduction. This is often accomplished by post-translational modification, including phosphorylation, ubiquitination and acetylation of individual amino acid residues. Consequently, these modifications alter the properties of the protein, commonly ligand binding, subcellular localization and affinity for its binding partners [102]. Several post-translational modifications have been reported for LDHA, causing changes in its oligomeric state, stability and catalytic activity.

One commonly modified residue in proteins is lysine (Lys), which is subject to acetylation, ubiquitination and SUMOylation. Acetylation at Lys5 induces degradation of LDHA, as ac-LDHA is a substrate for chaperone-mediated autophagy via HSC70 [103]. Furthermore, independent of degradation, this acetylation decreases LDHA catalytic activity. The histone deacetylase SIRT-2 reverses this modification and restores LDHA activity [103]. LDHA harboring an acetylation-mimetic mutation at this position reduced growth, proliferation and migration of cultured pancreatic cancer cells and mouse xenografts. Taken together, acetylation at Lys5 provides two-fold redundancy in the suppression of LDHA [103].

Alternatively, tyrosine phosphorylation of LDHA is reported to increase its NADH binding and enzymatic activity as well as promote tetramer formation. The first report of LDHA tyrosine phosphorylation demonstrated the direct action of v-Src on LDHA-Tyr239 [104,105] with this phosphorylation inducing nuclear translocation of LDHA where it associates with chromatin [106]. This interaction promotes a damage response transcriptional program by increasing histone acetylation as a result of lactate-mediated histone deacetylase inhibition [107,108]. Subsequent work has shown FGF receptor 1 (FGFR1) directly phosphorylates Tyr10 and Tyr83 in LDHA and induces phosphorylation of Tyr172 and Tyr239 [35]. Tyr83 is in close proximity to the nucleotide-binding domain of LDHA, and its substitution to Phe abrogates NADH binding [35]. Tyr10 is located in the unstructured N-terminus of LDHA, but not LDHB, and is reported to be involved in oligomeric assembly [12]. Indeed, phosphorylation of this residue increases tetramer formation, while the tetrameric fraction of LDHA-Tyr10Phe phospho-null point mutant is unchanged in the presence of FGFR1 [35]. Interestingly, this residue is also subject to phosphorylation by ABL, JAK2, FLT3, HER2 and Src kinases, suggesting a common mechanism for LDHA activation in cancers with unique-signaling deregulations [35,37]. Tyr10 was also found to be preferentially phosphorylated in cancer cell lines, and expression of LDHA-Tyr10Phe decreased cancer cell proliferation as compared with wild-type LDHA [35,109]. Functionally, expression of LDHA-Tyr10Phe in mouse xenografts of H1299 lung cancer cells significantly reduced tumor burden, consistent with the observation that LDHA activity is critical to tumor proliferation [110]. Additionally, FGFR1 or 2 expression in prostate cancer both phosphorylated and enhanced LDHA, while suppressing LDHB by inducing promoter methylation, a consequence of downregulated TET DNA demethylases [36]. Taken together, tyrosine phosphorylation of LDHA increases its enzymatic activity by enhancing tetramerization and increasing cofactor association.

Extrinsic modulation of LDH

A large body of research suggests that LDHA is largely responsible for supporting the Warburg phenotype and promoting cancer progression, likely due to its preference for pyruvate aligning with the intracellular abun-

dance [58,79,111–115]. However, despite the noted absence of LDHB in some cancers [55], and the reported finding that LDHB deletion can increase tumorigenicity in cancer [36], there does appear to be a role for LDHB in the maintenance and progression of malignancies [116,117]. Perhaps this phenomenon is a consequence of reuptake and metabolism of secreted so-called 'waste' lactate, the preferred substrate of LDHB [118,119]. In fact, conflicting reports imply cotargeting LDHA and LDHB is necessary to affect the proliferation of a subset of tumors [120,121]. In contrast, it has been found that the activity of LDHA, but not LDHB, is correlated with aggressiveness of tumor growth [110,122]. Knockdown of *LDHA* attenuates tumor growth *in vitro* [113] and in xenograft models of mouse mammary tumors and hereditary leiomyomatosis and renal cell cancer [79,123]. Furthermore, high expression of LDHA is correlated with poor survival in cancers [124,125]. Inhibition of LDHA has been demonstrated to both induce a return to oxidative phosphorylation (metabolic flexibility) [126] as well as apoptosis [35], a result of the unchecked production of mitochondrial reactive oxygen species. Taken together, it appears that the majority of cancers rely primarily on LDHA.

Small-molecule inhibitors of LDHA

The detailed knowledge of LDHA structure has led to the discovery and development of several small-molecule inhibitors competitive with nucleotide and/or substrate (Figure 5) [127–131]. Despite the existence of several promising preclinical inhibitors of LDH, there have been no clinical trials initiated based on these lead compounds. Inhibitors have shown limited *in vivo* efficacy, and because LDHA and LDHB are very structurally similar, often target both isoforms of LDH [132]. Interestingly, the observed differences in catalytic activity between LDH isozymes are thought to be the result of altered surface electrostatic interactions. In fact, the pK_a of the critical active site His193 increases from 7.3 in LDHA (likely protonated at physiologic pH) to 8.3 in LDHB (nonprotonated), representing a potentially exploitable distinction for rational design of LDHA-specific inhibitors [12].

The earliest described inhibitor is the substrate-analog oxamate [3]. This compound has been demonstrated to induce apoptosis or senescence through the inhibition of LDH in non-small-cell lung cancer [133], pancreatic cancer [134] and hepatocellular carcinoma [135], but is a low-affinity, nonselective inhibitor [126]. Another substrate-competitive inhibitor class, the 2-amino-5-aryl-pyrazines, demonstrate a modest preference for LDHA over LDHB, but have yet to be evaluated in a cell-based assay [136]. A more thoroughly characterized inhibitor, 1-(phenylseleno)-4-(trifluoromethyl) benzene, demonstrated the ability to specifically inhibit cell viability in a panel of cancer cell lines (IC_{50}: 45–84 μM) [137], and is currently under development. Two other substrate-competitive LDHA inhibitors have been gleaned and optimized from high-throughput screening, compound 9 and compound 24c, which have been found to be efficacious in prostate and lung cancers (compound 9) and osteosarcoma (compound 24c) [138,139].

Early nucleotide-competitive inhibitors such as gossypol and its derivatives are nonselective and are plagued by off-target toxicity [140–143]. Arguably the most successful inhibitor to date, FX11 has shown anticancer activity in lymphoma [110], pediatric osteosarcoma [58] and prostate cancer [114]. Despite these preliminary results, off-target interactions have scuttled the continued development of this inhibitor scaffold [50,127]. Quinoline-3-sulfonamides have shown some preclinical utility and are highly selective for LDHA, though their pharmacokinetic properties render them unsuitable for *in vivo* dosing [117].

Inhibitors simultaneously competitive with both substrate and cofactor have also been developed. GNE-140 inhibits LDHA in this manner in pancreatic cancer cells, however, continuous dosing is required to induce apoptosis [126]. Acquired resistance to GNE-140 is abolished by the AMPK inhibitor phenformin, suggesting metabolic flexibility and a return to oxidative phosphorylation in this system [126]. This phenomenon was similarly reported under oxamate inhibition [144]. Encouragingly, NHI-1 and NHI-2 demonstrate moderate selectivity for LDHA over LDHB and have antiproliferative effects in pancreatic cancer [145–148]. In 2017, a class of pyrazole-based inhibitors were characterized, yielding the noteworthy compound 63, capable of inhibiting LDHA in cells at nanomolar concentrations. Further optimizations are ongoing to prepare compound 63 for *in vivo* studies (Table 1) [132]. Interestingly, inhibitory peptides that block the tetramerization of LDHA have recently been developed. These peptides have not yet been tested in cultured cells, though specific disruption of the LDHA tetramer is likely to be both a feasible (due to the divergent sequences of the N-termini of LDHA and LDHB [5]) and successful therapeutic strategy, as discussed earlier [149].

Development of an LDHA-specific inhibitor is a clinical necessity, as LDHB is the dominant isoform in heart muscle and its inhibition could prove problematic, as cardiotoxicity dooms many promising candidate drugs [129,150].

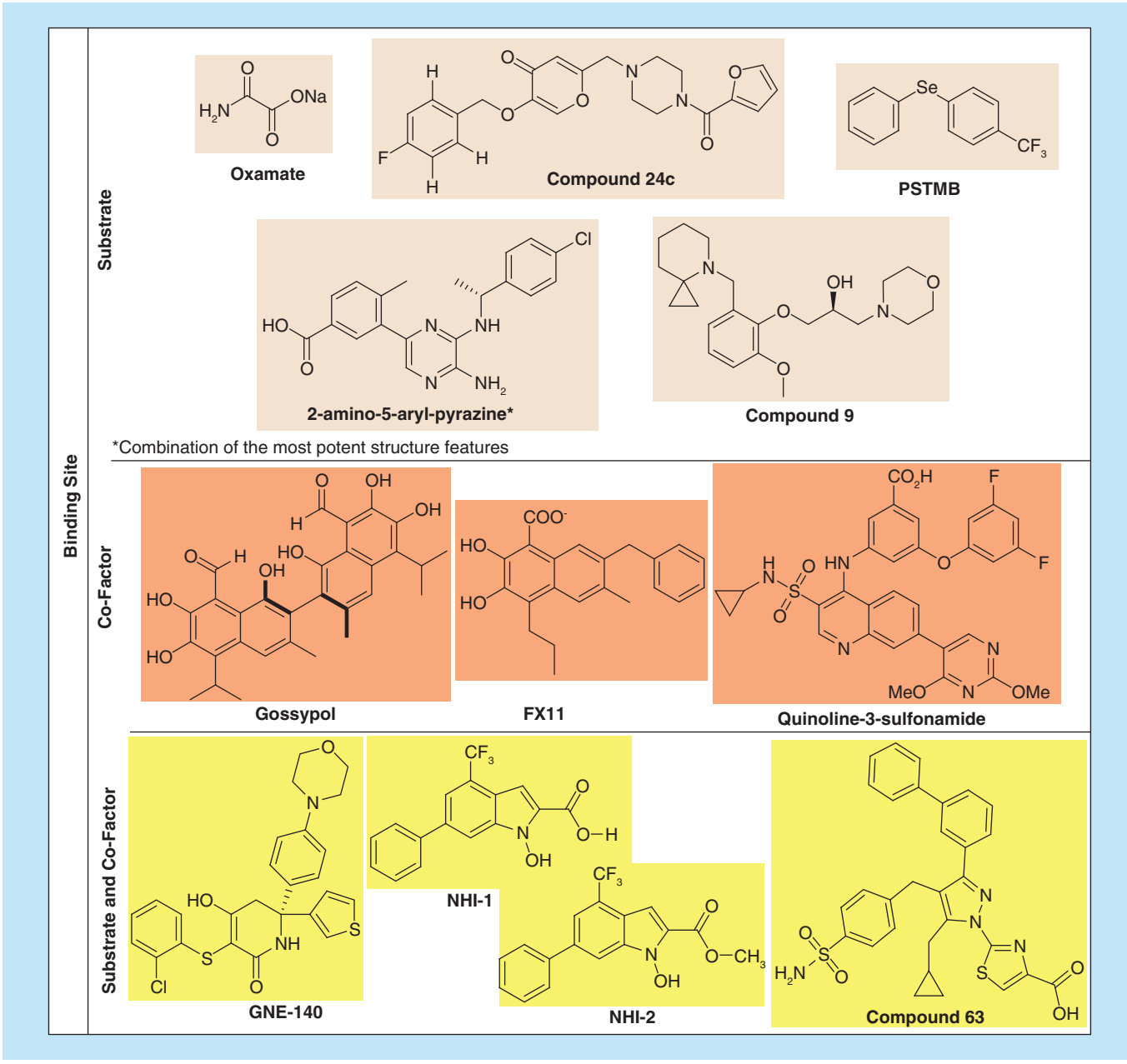

Figure 5. Structures of LDHA inhibitors. LDHA inhibitors divided according to mode of inhibition. Top: substrate-competitive; Middle: cofactor-competitive; Bottom: inhibitors that compete by occupying all or part of both binding sites.
PSTMB: 1-(phenylseleno)-4-(trifluoromethyl) benzene.

In the absence of an efficacious, well-tolerated LDHA-specific inhibitor, a detailed understanding of signals into LDH is required to develop targets for coinhibition strategies.

Conclusion

The vast scope of regulatory strategies employed in the suppression of glycolysis, and specifically LDHA, illuminate the critical importance of metabolic control. At steady state, repression of LDHA promotes oxidative phosphorylation, maintaining metabolic homeostasis. The accumulation of insults in cancer gradually shifts cells to a reliance on glycolysis, and ultimately unrestrained LDHA activity. This progressive deregulation supports a hypertrophic, oncogenic signaling profile (Figure 6). Inhibition of LDHA is detrimental to cancer cell growth and proliferation, therefore it stands to reason that regaining control of LDHA activity is of paramount importance.

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Table 1. Summary of LDHA inhibitor characteristics.

Inhibitor	Preclinical models	Lactate production	ECAR	Viabil.	ROS	Migrat.	Apop.	Continued development	Ref.
Oxamate	NSCLC, prostate cancer	↓	–	↓	↑	–	↑	N	[133–135]
2-amino-5-aryl-pyrazine	*In vitro*	↓	–	–	–	–	–	Y	[136]
Compound 24c	Prostate cancer	–	–	–	–	–	↑	Y	[138]
Compound 9	Osteosarcoma	↓	↓	↓	↑	–	–	Y	[139]
PSTMB	Colon cancer	–	–	↓	↑	–	↑	Y	[137]
Gossypol	Pulmonary fibrosis	↓	↓	↓	–	–	–	N	[143]
FX11	Prostate cancer, lymphoma, osteosarcoma	–	–	↓	–	↓	↑	N	[58,110,114]
Quinoline-3-sulfonamide	Hepatocellular carcinoma	↓	↓	↓	–	–	–	Y	[117]
GNE-140	Prostate cancer, colon adenocarcinoma	↓	↓	↓	–	–	–	N	[120,126]
NHI-1/2	PDAC	–	–	↓	–	↓	↑	Y	[144]
Compound 63	Prostate cancer, sarcoma	–	↓	↓	–	–	–	Y	[132]

Characterization of identified LDHA inhibitors, including cellular validations and development status. Downward-pointing arrows denote a decrease in the assay readout, upward-pointing arrows denote an increase.
ECAR: Extracellular acidification rate; NSCLC: Non-small-cell lung cancer; PDAC: Pancreatic ductal adenocarcinoma; PSTMB: 1-(phenylseleno)-4-(trifluoromethyl) benzene; ROS: Reactive oxidative species.

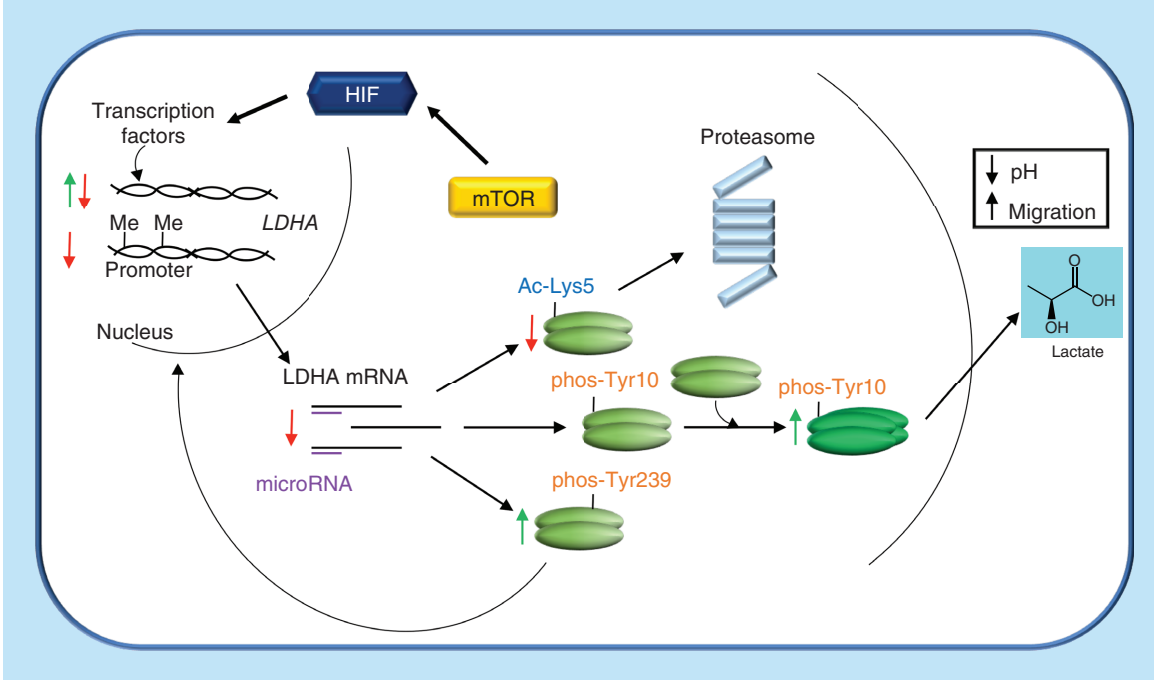

Figure 6. Molecular mechanisms of LDHA regulation. Transcription of *LDHA* in the nucleus is influenced by methylation and transcription factor binding. LDHA translation is affected by miRNAs. Post-translational regulation further governs LDHA protein activity. Hyperactive LDHA generates lactate, which is involved in cancer progression. The effect of various cellular regulatory mechanisms on LDHA is represented by green arrows (increased) or red arrows (decreased).
HIF: Hypoxia-inducible factor.

Future perspective

With the adoption of large-scale proteomics and genome-wide association studies, cellular signaling networks have become ever more entangled. As evidenced by this review, numerous overlapping signaling inputs contribute to the regulation of one well-understood enzymatic process. Accordingly, the search for new therapeutic targets

should begin with a comprehensive understanding of the regulation of these fundamental metabolic cycles. LDHA is regulated during transcription, post-transcriptional processing, and post-translational modification. The responsible proteins, as well as downstream effectors (such as those induced by lactate accumulation) should be examined as secondary therapeutic targets to short-circuit pro-survival signals in adaptable diseases such as cancer.

Executive summary

Background

- Cellular metabolism is an adaptable process responsible for generating the energy currency ATP. The two primary metabolic activities are cytosolic glycolysis and mitochondrial oxidative phosphorylation. Lactate dehydrogenase (LDH) catalyzes the NADH-dependent interconversion of pyruvate and lactate in the terminal step of glycolysis.

Lactate dehydrogenase

- LDHA and LDHB are the two primary isozymes of LDH. LDHA and LDHB can form mixed dimers and tetramers, with subunit composition influencing activity. LDHA favors lactate production, while LDHB primarily generates pyruvate.

Warburg effect

- Despite the presence of oxygen and functional mitochondria, cancers commonly undergo a metabolic shift from oxidative phosphorylation to glycolysis known as the Warburg effect. In the absence of oxidative phosphorylation, LDHA activity is required to fuel glycolysis through the generation of NAD^+. LDHA is thus essential to maintain the Warburg effect.

Functional regulation of LDH

- Transcriptional, post-transcriptional and post-translational regulation of LDHA modulate its expression and activity. Reduction of LDHA in Warburg-shifted cancer cells induces apoptosis, validating LDHA as a therapeutic target. Despite this, small-molecule inhibitors of LDHA have had limited clinical success. Understanding the effect of cellular regulatory strategies of LDHA expression and activity provides a potential avenue to more efficacious inhibition of LDHA.

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