

Lactate Dehydrogenase A as a Target of Cancer Therapy

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Abstract

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Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of pyruvate to lactate and on the contrary lactate to pyruvate. LDH is a tetramer consisting of two types of subunits M (LDHA) and H (LDHB). LDHA catalyzes the conversion of pyruvate to lactate, while LDHB converts lactate to pyruvate. LDHA increases its expression in cancer cells and is associated with cancer aggressiveness. Lactate that continues to increase gives the effect of an acidic microenvironment so that there is sufficient availability of oxygen for metabolism, which increases anaerobic glycolysis and decreases oxidative phosphorylation. Inhibition of LDHA can provide hope in cancer therapy because many studies have shown that inhibition of LDHA reduces cancer growth. This review will describe several cancer treatment approaches using LDH as a cancer therapy target until now.

Introduction

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of pyruvate to lactate and on the contrary lactate to pyruvate. Pyruvate which is converted to lactate indicates the occurrence of anaerobic glycolysis due to reduced oxygen availability in cells.¹ LDH is a tetramer consisting of two types of subunits M (LDHA) and H (LDHB). The H type is more prominent in the heart, while the M type is more prominent in skeletal muscle and in the liver. These subunits combine to form five types of tetramers, namely H₄, H₃M₁, H₂M₂, H₁M₃ and M₄. LDHA has a higher affinity for pyruvate and a high V_{max} for pyruvate reduction than LDHB. LDHA catalyzes the conversion of pyruvate to lactate, while LDHB converts lactate to pyruvate.^{1,2}

LDHA increases its expression in cancer cells and is associated with cancer aggressiveness. The increase in LDHA is due to the high oxygen consumption due to the very fast proliferation rate of these cells so that the cells experience hypoxia.³

Hypoxic conditions allow the action of LDHA to convert pyruvate to lactate. The increase in lactate gives an acidic microenvironment, so that even though there is sufficient oxygen, the metabolism that occurs is increased anaerobic glycolysis and decreased oxidative phosphorylation. This phenomenon is called the Warburg phenomenon.^{2,4}

High levels of lactate production are an indicator of metastasis and a poor prognosis in cancer patients. Lactate has now become a marker for tumor malignancy.⁴ While the increase in LDH enzymes in cancer patients can be the main target in cancer therapy.⁵ Inhibition of LDHA can provide hope in cancer therapy because many studies have shown that LDHA inhibition reduces cancer growth. This paper will describe several cancer treatment approaches using LDH as a cancer therapy target.

Structure, Isozyme and Function of LDH

The LDH enzyme (LDH, EC 1.1.1.27) has a tetrameric form with a molecular

weight of 144 kDa containing four subunits. Each monomer is formed by a polypeptide chain consisting of 334 amino acids.⁶ Each subunit has a different active center.⁷ The LDH enzyme is a dehydrogenase enzyme that has isozymes. Isozymes are enzymes that have different structures but catalyze the same reactions.⁸ Differences in the structure of isozymes from one another are caused by differences in amino acid sequences, modification of covalent bonds and conformational changes in three-dimensional structures.⁹

The LDH enzyme consists of five isozymes composed of two types of H subunits (LDHB) and M subunits (LDHA). These subunits combine to form four types of tetramers namely LDH1 (H₄); LDH2 (M₁H₃); LDH3 (M₂H₂); LDH4 (M₃H₁) and LDH5 (M₄).^{6,9} Sub-units B and A are encoded by two different genes, namely for sub-unit B by the LDH-H gene located on chromosome 12p12.2-p12.1, while for sub-unit A is coded by the LDH-M gene located on chromosome 11p15.4. The difference between subunits A and B can be seen in the LDH-M and LDH-H sequences.^{6,9}

These five isozymes have distribution in several types of organs such as the heart, liver, brain skeleton, kidneys and red blood cells. H₄ (LDH1) and H₃M (LDH2) isozymes are mainly present in the heart but to a lesser extent in the liver, skeleton and kidneys. The M₄ (LDH5) isozyme predominates in the liver and skeletal muscle, but to a lesser extent in heart muscle, brain and red blood cells.^{1,4} This difference in distribution has physiological significance. Organs whose metabolism is aerobic are found to contain a lot of LDH isozymes rich in subunits H is shown by the heart. On the other hand, organs capable of anaerobic metabolism contain isozymes rich in slow-moving M subunits such as H₃M (LDH4) and M₄ (LDH5) isozymes.⁴

The H subunit has a higher affinity for lactate than for pyruvate. This compound will be immediately converted by the LDH isozyme into pyruvic acid which can then be converted into acetyl-CoA to enter the Krebs cycle which takes place aerobically. Most tissues have the H₂M₂ (LDH3) isozyme. Under normal circumstances, the LDH present in the blood are LDH2, LDH1,

LDH3, LDH4 and LDH5. Normally, red blood cells contain more LDH2 than LDH1.⁸ The H subunit is more acidic because it has a residue. There are fewer basic amino acids that have a negative charge compared to the M subunit which has basic amino acids. Therefore, this second property can be utilized in electrophoretic separation. LDH1 migrates faster than LDH5.^{2,8}

Lactate dehydrogenase (LDH) is an enzyme that plays an important role in glycolysis. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate while simultaneously oxidizing NADH in the final step of anaerobic glycolysis. LDH enzyme can catalyze the reversible reaction of pyruvate to lactate. The LDH enzyme can be detected by its ability to catalyze the reduction of pyruvate in the presence of NADH or to catalyze the oxidation of lactate in the presence of NAD⁺.⁹ The structure of LDH can see in this figure 1 below.

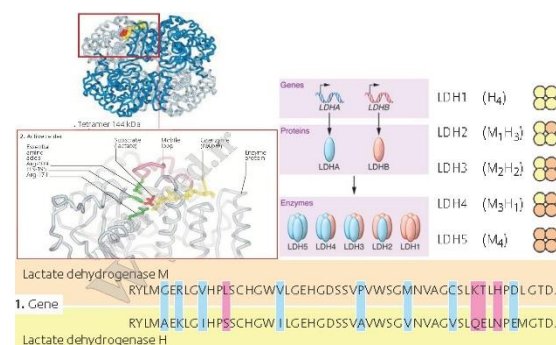


Figure 1. Structure, isozymes and gene of Lactate Dehydrogenase.⁸

Metabolism of LDH in Cancer Cells

Cancer is a disease that results in unrestrained cell proliferation and propagation, which invades and destroys other tissues and eventually kills the organism due to its spread to other areas of the body.² Exogenous factors such as chemicals, radiation, viruses and endogenous factors such as immunodeficiency and genetic alterations can lead to the development of cancer.

These changes are basically the result of mutations that benefit oncogene function and eliminate the function of cancer suppressor genes.¹¹ Cancer formation is a multilevel stage with four to seven steps that occur until the change of normal cells into malignant cells.⁴ Cell proliferation is an increase in the number of cells as a result of cell growth and development. Uncontrolled cell growth and development is a hallmark of cancer. Failure of regulation results in phenotypic changes in cancer.

Cancer cells perform a different metabolism than normal cells. In 1920 Warburg discovered that cancer cells metabolize glucose differently in the presence of oxygen. Glucose is converted to pyruvate, but the pyruvate produced is not used for oxidative phosphorylation because pyruvate is converted to lactate. This phenomenon initiates metabolic changes in cancer cells. The changes that occur provide an advantage for cancer cells to survive and carry out cell proliferation and advanced development.^{12,9} Metabolic changes create conditions for an acidic extracellular environment. At the start of tumor cell expansion, the cells will take oxygen around them so that the cells experience hypoxia and initiate the activation of the transcription factor Hypoxia-inducible transcriptional 1- α (HIF1- α).⁹

Gene Expression of HIF is a solution to overcome cells in hypoxic conditions and reduce dependence on aerobic respiration, as well as oxidative phosphorylation and prioritize anaerobic glycolysis. Therefore, cancer cells experience increased expression of glycolytic enzymes, glucose transport and inhibition of mitochondrial respiration. HIF also stimulates angiogenesis by regulation of several factors including vascular endothelial growth factor.^{5,9} HIF-1 α induces LDH protein with M-Subunit. LDH-M is also called LDH5 and is encoded by the LDHA gene whereas LDH-H is encoded by LDHB (not regulated by HIF). Both LDH-M and LDH-H are expressed under normal conditions, but in cancer cells LDH5 has increased expression.^{10,2}

Other metabolic changes make proto-oncogenes able to become oncogenes by

mutations that result in changes in protein structure and increase in protein concentration. This is due to the loss of protein regulation and stability which makes its existence and activity in the cell longer. Several signals are given to activate oncogenes.⁹ Ras signals are received when mutations occur and promote glycolysis and then the AKT kinase affects the signal to insulin to take up glucose which is used for regulation of cancer cells. The transcription factor Myc is also upregulated for various metabolisms. Loss of p53 protein prevents expression of the gene encoding SCO2 to synthesize cytochrome C which functions in mitochondrial respiration. The p53 protein also regulates metabolic regulation that depends on the transcription factor NF- Γ b.^{6,9}

The regulation of glucose metabolism involves various transcription factors. Myc and HIF-1 α can regulate LDH expression at the transcription and translation stages. Myc and HIF-1 α will attach to the hypoxia response element (HRE) 5'-CACGTC-3' and 5'-GACGTGCG-3' (respectively) which are the sites of the LDHA promoter. HIF-1 α will be activated by phosphatidylinositol 3 kinase (P13K) which initiates glycolytic enzymes including LDHA.^{6,12}

ErbB2(Her2/neu) is an oncogene that is highly expressed in breast cancer and is indicated for a poor prognosis. If it is related to LDHA expression, its activation is through P13K which can activate HIF-1 α and regulate LDHA gene regulation. In addition, heat shock factor 1 (HSF1) also plays a role in the expression of the LDHA gene by attaching directly to the LDHA promoter region.^{12,13} Another factor is pyruvate kinase isozyme M2 (PKM2) epidermal growth factor. PKM2 is a transcription coactivator that interacts with HIF-1 α . PKM2 induction is carried out by prolyl hydroxylase 2 which promotes HIF-1 α and attaches to HRE thereby exerting p300 coactivators, histone acetylation, and converting oxidative phosphorylation to glycolysis. PKM2 is also a β -catenin coactivator to induce Myc expression.^{9,12} The regulation of signaling between LDH, Myc and HIF1 in metabolism cancer can shown at figure 2.

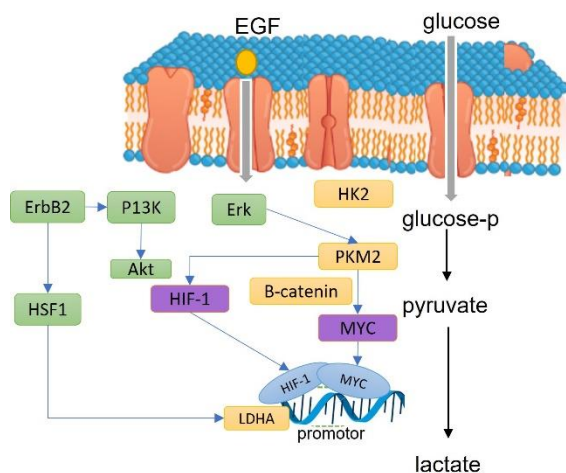


Figure 2. Regulatory Relationship between LDH-A, Myc and HIF-1 α in glucose metabolism.^{2,6,10,12}

Lactate Dehydrogenase as a Target Cancer Therapy

Based on explanation above, LDH has an important role in metabolism of cancer cells. Which is catalyzing the reaction of pyruvate into lactate and producing NAD⁺ which is needed to maintain the continuity of the glycolysis process so that allows cancer cells to develop and metastasize. Therefore the current direction of development is more emphasis on finding LDH enzyme inhibitors. Genetic studies show that LDH may be a good strategy for regulating metabolism in cancer cells. As a result of the development of several researchers, there are several ways to use LDH as a cancer therapy, as will be described below.

- *Inhibitory compounds*

Until now, researchers have developed assays and methods to identify LDH inhibitors, especially LDHA. The selected compounds need to have chemical similarities and if used do not cause other reaction disturbances and of course still maintain the stability of LDHA. The use of inhibitor compounds was evaluated through the level of lactate production,

oxygen consumption and ¹³C NMR spectroscopy. In addition, changes in metabolism, cell proliferation and apoptosis also need to be seen. The compound used as an LDHA inhibitor is 3-((3-carbamoyl-7(3,5-dimethylisoxazol-4-yl)-6-methoxyquinolin-4-yl) amino) benzoic acid as NADH-competitive. The results obtained are inhibitory compounds that have the potential to inhibit as little as 2-3 nM and at LDHB 10-80 times their inhibition. Rapid rates of lactate production can be inhibited in liver and breast cancer cell lines. The most consistent LDHA inhibition was when cells were subjected to hypoxic conditions and LDHB expression was still low. In addition, there was an increase in oxygen consumption in hepatocellular carcinoma cells at a dose of 3 microM, because higher concentrations would inhibit mitochondria.¹⁴

In addition, there are other compounds that can inhibit LDH development, namely 1-(phenylseleno)-4-(Trifluoromethyl) Benzene (15) and galloflavin (5) which suppresses tumor growth through apoptotic cell death interfering by inhibiting LDHA by increasing the potency of LDHB thereby interfering with cell proliferation.^{15,16}

- *Inhibition of Shuttle Lactate*

Lactate is a product produced from the pyruvate breakdown reaction by the LDH enzyme. The lactate produced can be a precursor for the formation of glucose in a lack of oxygen. In cancer cells, lactate is produced higher than normal cell production, therefore lactate will be removed from the cell. Lactate is released from the cell through a transporter, namely Monocarboxylate Transporter (MCT). The MCT mechanism occurs in simport, namely when lactate is released, protons will enter, this happens to maintain cell balance. It has been reported that the transporters that act on cancer cells are MCT 1 and MCT 4. Uptake of lactate by tumor cells occurs through the MCT 1 transporter which has a high affinity for lactate. Meanwhile, lactate is released via the MCT 4 transporter which has a low affinity for lactate¹⁷. Inhibition of MCT1 can provide a choice for tumor cells to use glucose, thereby reducing the

amount of glucose thereby providing hypoxic conditions to tumor cells and causing tumor cells to die. Inhibition of MCT4 can provide a direct potential for tumor cells to experience hypoxia so that tumor cells can die due to the accumulation of lactic acid in the cells.¹⁷

- *Inhibition RNAi*

RNAi is an interference RNA that is used to inhibit the occurrence of a gene expression. The LDH inhibition approach using RNAi aims to prevent LDH from being produced because it inhibits transcription and allows the degradation of the LDH gene. It has been reported that inhibition of LDHA mRNA with RNAi induced cell death in cancer cell lines with wild-type p53, mutant and without p53 and indicated that the endogenous LDHA formed impairs cancer cell defense. From the results of this study it was found that p53 can regulate NAD⁺ and reduce NADH. P53-dependent as an inhibitory molecule in cancer cell lines that helps the silencing of LDHA by RNAi. P53 inhibits by decreasing the activity of NAD⁺-dependent deacetylation of sirtuin 1 (SIRT1) and increasing the acetylation of p53 in cancer cells. Both of these occur due to the increased activity of NADPH-dependent oxidoreductase NQO1. Therefore, the combination of inhibiting the use of RNAi and adding p53 status to cancer cells can be used as therapy.^{6,18}

- *Inhibition with LDH Acetylation*

Acetylation is the process of adding an acetyl group. Acetylation of LDH means adding an acetyl group to LDH. LDH acetylation has been reported to play an important role in pancreatic tumors. Through spectrophotometric analysis it was suspected that there were eight acetylation sites, and the results of further studies found that acetylation occurred at lysine 5 (K5) which inhibited LDHA catalytic activity. Lysosomes.¹⁹

- *Inhibition of tyrosine phosphorylation*

Tyrosine phosphorylation is the process of attaching phosphate to tyrosine residues. It has been reported that the receptor tyrosine kinase oncogene FGFR1 directly phosphorylates LDHA. Phosphorylation at Y10 and Y83 gave activated LDHA by prolonging the binding of LDH to NADH. In addition, Y10 phosphorylation in LDHA is associated with the activation of the tyrosine kinase oncogene. Y10 saves cells by reducing cell proliferation and ATP when cells are hypoxic.²⁰

Several tyrosine kinase inhibitors have been found and are effective as antitumor agents, namely imatinib mesylate (STI571; Gleevec), gefitinib (Iressa), erlotinib (OSI-1774; Tarceva), lapatinib (GW-572016), canertinib (CI-1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), sunitinib (SU11248), and leflunomide (SU101). Inhibition of tyrosine kinase can interfere with signaling pathways and thereby interfere with the development of malignant cells. The ability of new tyrosine kinase inhibitors also continues to develop to solve cancer problems.²⁰

Conclusion

LDHA plays an important role in the growth of cancer cells. By inhibiting LDH from the gene to protein level, it can be used as a target for cancer therapy. Inhibition of LDH expression and suppression of lactic acid will affect cancer cell proliferation and growth thereby interfering with cancer development.

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