Aldolase - From Biochemistry to Laboratory Medicine

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Aldolase (ALD) (fructose-1,6-bisphosphate aldolase) is a 160 kDa, enzyme which catalyzes the conversion of fructose 1-6-biphosphate in glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the glycolytic metabolic pathway. There are also experimental data suggesting that nuclear ALD isoenzyme A might play an important role in cell proliferation. At the present time, the most useful serum markers of muscle injury following intense, prolonged exercise are: creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase myoglobin and troponin. Although serum ALD is not usually measured yet, it may be used together with CK to evaluate the status of muscle adaptation to training. Recent studies offered ALD a new perspective, as a future valuable biomarker in monitoring the evolution of muscle crush injuries, in order to prevent silent, but progressiv muscle fibers necrosis after injury. It has also been shown that ALD was an independent clinical prognostic marker in many other human cancers, being involved in some well-known signaling pathways.

Keywords: aldolase, crush syndrome, muscle injuries

Aldolase (fructose-1,6-bisphosphate aldolase) is a 160 kDa, enzyme which catalyzes the conversion of fructose 1-6-biphosphate in glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the glycolytic metabolic pathway [1].

Aldolase has two intracellular locations: the cytoplasm and the cell nucleus, where the is located in the heterochromatin region [2].

ALD has three isozymes (A, B, and C), encoded by three different genes. These three genes are differently expressed during the stages of cellular development and differentiation:

-ALD A - the muscle type - binds to the cytoskeleton actin-containing filaments in a tissuespecific binding manner.

-ALD B - the liver type - is expressed in hepatocytes and, also, has interactions with the cytoskeleton

-ALD C is expressed mainly in brain and other nervous tissue [1].

ALD - the fourth glycolitic enzyme

ALD converts a six-carbon molecule (fructose 1,6-bisphosphate - Fru1,6BP) in two three-carbon molecules: G3P and DHAP.

Glycolitic intermediates are very important for cancer cells metabolism. NADH generated by glycolysis, is reoxidized to NAD+ in the reducing reaction of DHAP to G3P [3, 4]. The resulted reducing equivalent (NADH) will be used for quinone reducing reaction in the inner mitochondrial membrane. NAD+ regeneration will, in turn, trigger a high glycolytic flux. G3P is also a starting source for the de novo synthesis of glycerolipids (triglycerides - TG). TG – fatty acid cycling needs a continuously supply of G3P from the glycolitic pathway [3, 4]. In this way is increased the glucose carbons entry into mitochondria, as pyruvate, and the enhanceing of Krebs cycle and ATP synthesis. Thus, this reaction also increases the entry of glycose carbons into mitochondria in the form of pyruvate, resulting in enhanced citric acid (tricarboxylic acid) cycle activity and ATP production [3, 4].

Without being one of the three key glycolitic enzymes, ALD, according to Marcondes studies, plays an important role of an intern glycolitic regulator [5].

Muscle-type 6-phosphofructo-1-kinase and ALD associate conferring catalytic advantages for both enzymes (Marcondes MC, 2011). In presence of ALD, 6-Phosphofructo-1-kinase (PFK) can not be modulated by its allosteric activators, ADP and Fru 2,6-BP, but can still be inhibited by its negative allosteric modulators, citrate and lactate. Dimers of these two sequential glycolitic enzymes associate forming heterotetramers [5]. The metabolic role of this association is ALD affinity for its substrate and activity enhancement. Marcondes' results suggest that the PFK – ALD association represents a catalytic advantage for both enzymes and may play an interesting role in channeling the glucose metabolism towards glicolitic oxidation [5].

ALD -a nuclear regulator factor

Glycolysis is a vital metabolic pathway. Its evolutionary importance is illustrated by the fact that it is active in all organisms groups, on Earth [2, 6]. Glycolytic enzymes capacity to interact with high energetic molecules and metabolic intermediates offers them the important role of signal sensors and integrators, as players in the cell fate regulation [2, 6] . As previously showed, ALD a glycolytic and gluconeogenetic enzyme localized both in cytoplasm and nuclei of cells. However, the role of ALD presence in nuclei is still unclear [2, 6]. Piotr Mamczur studies revealed that the ALD A nuclear localization is correlated with the proliferative activity of human squamous cell lung cancer cells (hSCC) and with Ki-67 (a key marker of proliferation) expression [2, 6].

Piotr Mamczur has showed that bloking cell cycle entry in S phase and inhibiting transcription caused the removal of ALD A from nuclei, suggesting that nuclear ALD A might play an important role in cells proliferation [2, 6]. In nonproliferative cells the intracellular distribution of this enzyme seems strongly related to the cellular oxidative status and the Akt - p38 mediated signals. Akt - p38 activity is controled by hormonal signaling and is related to the metabolic state of the cell and its environment. Thus, ALD A - related signaling pathway might be part of a complex

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molecular mechanism which combines informations regarding energetic status, glucose availability and stress, and ellaborates signals in order to control and the rate of cells proliferation and/or growth [2, 6].

ALD A - as future biomarker in laboratory medicine

Detection of tissue-specific proteins in serum is a valuable method to identify and quantify tissue damage (examples include the association of troponin I and CK-MB with myocardium) [1].

Serum aldolase activity should be used for diagnosing and monitoring disease activity [7, 8]. In addition, of oxidative stress biomarkers (like total antioxidant capacity, glutathione peroxidase and 8-hydroxydesoxyguanosine (8 - OHdG) [9] and inflammation biomarkers (like interleukine 6) [10] evaluation may be indicated for a better assessment of the pathological process.

Exper imental part

Muscle damage biomarker

The most frequent causes of muscle damage are:
-strenuous physical exercise
-electrical injury
-direct hits
-surgical trauma
-crush injuries (the crushing syndrome) [1].

ALD A and strenuous physical exercise

Muscle tissue can be damaged during intense, prolonged exercise as a consequence of mechanical and metabolic causes.

Skeletal muscle enzymes and proteins serum levels represent valuable markers of muscle tissue functional status and integrity. These markers could vary widely in different pathological and physiological conditions.

In present, the most useful serum markers of muscle injury following intense, prolonged exercise are: creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase myoglobin and troponin. Although serum ALD is not usually measured yet, it may be used together with CK to evaluate the status of muscle adaptation to training.

According to Brancaccio studies ALD A and carbonic anhydrase CAIII should be added to the previous list [1]. In addition, apoptosis in muscle cells is a consequence of strenuous exercises and may be triggered by an intensive oxidative stress [11, 12]. Therefore, evaluation of both, oxidative stress markers (like glutathione peroxidase and 8-hydroxydesoxyguanosine (8-OHdG) [9] and the antioxidant capacity should be used to establish the damage level of muscle cells.

ALD A and crush syndrome

In crush syndrome muscle tissue injurie is characterised by sarcomeric degeneration from Z-disk fragmentation [1]. Normally, muscle cell architecture allow to minimizes the stress targeted on the plasma membrane by channeling the forces from extracellular matrix to the cytoskeleton through the dystrophin-glycoprotein complex [1]. In crush injuries, membrane failure results in loss of cytoplasmic components (like enzymes) and allows the influx of extracellular ions. The increase in serum levels of such enzymes should be a valuable index of cellular necrosis or tissue damage following muscle crush injuries [1].

In the case of a crush injurie the skeletal muscles healing process follows a constant order, without significant changes. Three steps have been identified in this healing process: *destruction, repair and remodeling* [13]. The last two of steps (repair and remodeling) are closely interrelated [13].

Step 1: is represented by destruction and is characterized by tearing and subsequent myofibrils necrosis, hematoma formation and inflammatory cells proliferation [13]. Consequently, evaluation of some serum inflammation biomarkers, as interleukines [1], should be performed in parallel with the muscle enzymes measurements.

Step 2: include the repair (with necrotic tissue phagocytosis) and remodeling (myofibrils regeneration and concomitant connective scar tissue formation) processes, together with blood vessels formation and neural growth [13]. One process that is vital for regenerating the injured muscle is the vascularization. Vascular supply restoration represents the first sign of muscle regeneration [13].

Step 3: include the remodeling and maturation of the regenerated myofibrils, followed by scar tissue contraction and, finaly, recovery of functional capacity of the muscle tissue [13].

Myofibrils are fusiform and very long, consequently there is an imminent risk of necrosis starting in the crush injury site extending along the whole length of the fiber. However, a specific structure, called the contraction band, consisting of condensed cytoskeletal material, will act in such cases as a *firewall system* [14].

In present, serum CK is considered the most sensitive indicator of muscle crush injuries evaluation [15, 16].

However, there are several individual clinical case reports describing patients with fasciitis [17, 18] who had normal CK levels, but high serum ALD levels. Moreover, Nozaki and Pestronk recently reported the clinical features of some patients with selectively elevated ALD serum levels [19]. They have found that these patients presented a constellation of symptoms including: muscle weakness, myalgias, and muscle biopsies illustrating perimysial and perifascicular pathology [19]. In their study, Nozaki and Pestronk emphasized the diagnostic utility of serum ALD measurements in all patients with muscle complaints but normal serum CK [19]. The molecular mechanism by which ALD might be released from muscle cells without a parallel serum CK elevation has not been yet explained.

In order to find an explanation to these intriguing clinical data, Casciola-Rosen and his collaborators analyzed the gene and protein expression levels of ALD and CK, during in vitro muscle cell differentiation [15, 16]. Their experimental results showed that ALD is expressed at higher levels compared to CK, during in vitro *muscle regeneration* [15, 16]. They have also identifyed a phase during muscle cell differentiation in which cells express exclusively ALD A [15, 16].

In this regard, although CK and ALD A are frequently present, together, in the serum of autoimmune myopathies patients, however, a substantial percent of these patients have elevated serum ALD A levels and normal serum CK levels [15, 16].

Recent findings illustrated that early regenerating muscle cells may be an important target of the immune response in autoimmune myopathies. Muscle cell differentiation recapitulates embryonic development, with consequently expression of gene cassettes ment to guide the cells towards mature phenotype [20]. Casciola-Rosen et al. in vitro data demonstrated clearly that ALD A is expressed at highest levels in myoblasts and immature muscle cells, whereas CK expression is absent in the former, but becomes more and more significant with the differentiation progress [16]. Casciola and collaborators also confirmed these findings in vivo, using a model of mouse muscle injury and repair [16]. Immunoblotting experimental data of Casciola-Rosen' team revealed that ALD A is present in undamaged mouse muscle, but is expressed at 4 –fold higher levels in the damaged muscle, 4 days after injury. At 4 days after the injury the repairing muscle tissue consists almost exclusively of regenerating

fibers. In contrast, the highest CK levels are associated with undamaged muscle tissue, which include mature and differentiated fibers [16].

All these experimental data of Casciola-Rosen and collaborators [16] offer aldolase a new perspective, as a future, valuable biomarker in monitoring the evolution of muscle crush injuries, in order to prevent silent, but progressiv muscle fibers necrosis after injury.

Results and discussions

ALD A and surgical trauma

Surgical approaches are well known causes of damage to surrounding muscle tissue [21]. The muscle could be partially torn, stretched or even transected, resulting in muscle damage [21]. In their study, Bergin and his collaborators indicated a starting point for a more precise evaluation of this kind of muscle injury-biochemical markers like LDH, aspartate aminotransferase, myoglobin, troponin and, also, ALD and carbonic anhydrase III (CAIII) [21].

ALD A and cancer

ALD A is one of the three aldolase isozymes (A, B, and C), encoded by three different genes [22]. These aldolases are differentially expressed during development. ALD A is highly expressed during the embryo development [23] and in regenerating muscle fibers [16].

It has been showed that ALD A is implicated in various cellular functions and biological process like: muscle tissue maintenance, regulation of cell shape and mobility, actin filament organization and muscle contraction, and ATP biosynthesis [22].

Recent studies revealed, also, that ALD A has been highly expressed in many types of malignant cells ,including human lung squamous, renal cell and hepatocellular carcinomas [22].

Squamous cell carcinoma (SCC) represents the second most common type of lung cancer accounting for about 30% of all lung cancers [22]. Diagnosed early, lung SCC (LSCC) is well curable by surgical excision. However, after resection, many of LSCC diagnosed patients present a high rate of recurrence for metastasis and resistance to the existing chemotherapeutic agents. Therefore, in order to reduce mortality of LSCC, it is necessary to identify and characterize a constelation of molecular markers for early diagnosis and screening of recurrence and metastasis [22].

Sha Du and collaborators compared the protein profiles of clinical metastatic, non-metastastic LSCC tissues and adjacent normal lung tissues. They have identified a number of differentially expressed proteins with biological functions such as: carbohydrate metabolism, cell signaling regulation, , molecular chaperones, and protein synthesis. Among these proteins, they have found very interesting the ALD A isoenzyme, as an important enzyme of glycolysis, being known that a typical feature of tumor cells is the highly active glycolysis associated to apoptosis inhibition [22]. As Warburg first stated, cancer cells need to activate glycolysis in order to sustain proliferation, despite oxygen presence, because glycolysis ensure most of the molecular elements required for massive cell proliferation [22].

Sha Du and collaborators data revealed that depletion of ALD A results in an upregulation of epithelial markers and a down regulation of mesenchymal markers, suggesting that ALDO A is essential for mesenchymal morphology (characteristic of migrating cells) maintainence. Accordingly, their results revealed that high expression of ALD A was significantly relevant to a high degree of metastasis and low degree of pathologic staging. In other words, high expression of ALD A was related to a low survival rate and poor prognosis [22]. These findings suggest that ALD A should be regarded as a future potential marker for LSCC metastasis, prognosis prediction and as a target for clinical treatment of LSCC.

Recent studies have proven that ALD was an independent clinical prognostic marker in many other human cancers, being involved in some well-known signaling pathways [22, 23].

Conclusions

Overall, it is clear that ALD should not be regarded as an ordinary glycolytic enzyme anymore. More and more experimental data revealed new important roles of ALD in different cell molecular processes.

References

1. BRANCACCIO, P., LIPPI, G., MAFFULLI, N., Clin. Chem. Lab. Med., **48**, no. 6, 2010, p. 757–767.
2. MAMCZUR, P., DZUGAJ, A., Acta Biochim. Pol., **55**, 2008, p. 799–805.
3. LOCASALE, J.W., CANTLEY, L.C., Cell Metab., **14**, 2011, p. 443– 451.
4. ESTRELLA, V., CHEN, T., LLOYD, M., WOJTKOWIAK, J., CORNNELL, H.H., IBRAHIM-HASHIM, A., BAILEY, K., BALAGURUNATHAN, Y., ROTHBERG, J.M., SLOANE, B.F., JOHNSON, J., GATENBY, R.A., GILLIES, R.J., Cancer Res., **73**, 2013, p. 1524–1535.
5. MARCONDES, M.C., SOLA-PENNA, M., TORRES, R.S.G., ZANCAN, P., lubmb Life, **63**, no. 6, 2011, p. 435-445.
6. MAMCZUR, P., GAMIAN, A., KOLODZIEJ, J., DZIEGIEL, P., RAKUS, D., Biochim. Biophys. Acta, **1833**, 2013, p. 2812–2822.
7. CARNEIRO, S., ALVIM, G., RESENDE, P., SOUSA, M.A.J., CUZZI, T., RAMOS-E-SILVA, M., Skinmed: Dermatology for the Clinician, 6, no. 1, 2007, p. 46–47.
8. ONO, H., SUZUKI, N., MIZUNO, H., TATEYAMA, M., AOKI, M., ITOYAMA, Y., Rinsho Shinkeigaku, **49**, 2009, p. 119-122.
9. STANESCU, I., TOTAN, A., RUS, F., MIRICESCU, D., MOCANU, B., CALENIC, B., GREABU, M., Rev .Chim. (Bucharest), **68**, no. 6, p. 1201-1204.
10. LUPESCU, D., GREABU, M., TOTAN, A.,NAGEA, M., POPESCU, G.I., LUPESCU, O., DIMITRIU, A.L., STOIAN, I., GRINTESCU, I.M., CORNECI, D., Rev. Chim. (Bucharest), **69,** no. 10, 2018, p. 2762-2767.
11. KOCTURK, S., KAYATEKIN, B.M., RESMI, H., ACIKGOZ, O., KAYNAK, C.,OZER F., Eur. J. Appl. Physiol., **102**, 2008, p. 515–524.
12. LIU, J., YEO, H.C., OVERVIK-DOUKI, E., HAGEN, T., DONIGER, S.J., CHU, D.W., BROOKS, G.A., AMES, B.N., J. Appl. Physiol., **89**, 2000, p. 21–28.
13. FERNANDES, T.L., PEDRINELLI, A., HERNANDEZ, A.J., Rev. Bras. Ortop., **46**, no. 3, 2011, p. 247-255.
14. HURME, T., KALIMO, H., LEHTO, M., JARVINEN, M., Med Sci Sports Exerc., **23**, no. 7, 1991, p. 801-810.
15. CASCIOLA-ROSEN, L., HALL, J.C., MAMMEN, A.L., CHRISTOPHER-STINE, L., ROSEN, A., Clin. Exp. Rheumatol., **30**, no. 4, 2012, p. 548–553.
16. CASCIOLA-ROSEN, L., NAGARAJU, K., PLOTZ, P., WANG, K., LEVINE, S., GABRIELSON, E., CORSE, A., ROSEN, A., J Exp Med., **201**, 2005, p. 591–601.
17. FUJIMOTO, M., SATO, S., IHN, H., KIKUCHI, K., YAMADA, N., TAKEHARA, K., J. Rheumatol., **22**, 1995, p. 563–565.
18. QUINTERO-DEL-RIO, A.I., PUNARO, M., PASCUAL, V., J Clin Rheumatol., **8**, 2002, p. 99–103.
19. NOZAKI, K., PESTRONK, A., J. Neurol. Neurosurg. Psychiatry, **80**, 2009, p. 904–908.
20. ZHAO, P., HOFFMAN, E.P., Dev Dyn.2004, **229**, p. 380–392.
21. BERGIN, P.F., DOPPELT, J.D., KEPHART, C.J., BENKE, M.T., GRAETER, J.H., HOLMES, A.S., HALEEM-SMITH, H., TUAN, R.S., UNGER, A.S., J. Bone Joint Surg. Am., 93, no. 15, 2011, p. 1392-1398.
22. SHA, D., ZHUZHU, G., HAO, L., SONG, Y., WANG, L., GONG, L., LIU, L., XIAOYU, Q., HOU, Z., SHAO, S., Plos One, **9**, no. 1, 2014, p. 1-7.
23. CHANG, Y., YANG, Y., TIEN, C.P., YANG, C.J., HSIAO, M., Trends in Endocrinology & Metabolism, **29**, no. 8, 2018, p. 549-559.

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