KeAi
ADVANCING RESEARCH
EVOLVING SCIENCE

Review article

Progress in the biological function of alpha-enolase

CrossMark

Hong Ji [1], Jianfa Wang [1], Jingru Guo, Yue Li, Shuai Lian, Wenjin Guo, Huanmin Yang[*], Fanzhi Kong, Li Zhen, Li Guo, Yanzhi Liu

College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, China

A B S T R A C T

Alpha-enolase (ENO1), also known as 2-phospho-D-glycerate hydrolase, is a metalloenzyme that catalyzes the conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. It is a multifunctional glycolytic enzyme involved in cellular stress, bacterial and fungal infections, auto-antigen activities, the occurrence and metastasis of cancer, parasitic infections, and the growth, development and reproduction of organisms. This article mainly reviews the basic characteristics and biological functions of ENO1.

© 2016, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Lohman and Mayerho discovered enolase in muscle extracts when studying the conversion of 3-phosphoglyceric acid into pyruvic acid in 1934, Subsequent studies have shown that three types of enolase isoenzymes exist in mammals: α-enolase (ENO1) is present in almost all mature tissues; β-enolase (ENO3) exists primarily in muscle tissues; and γ-enolase (ENO2) occurs mainly in nervous and neuroendocrine tissues. All enolases are composed of two identical subunits. The molecular weights of enolases range from 82 to 100 ku. In humans and other mammals, 3 independent genetic loci (α, β and γ) encode the 3 enolase isozymes. However, another enolase that is different from ENO1, ENO2 and ENO3 has recently been discovered in human and mouse sperm. This newly discovered enolase, termed enolase 4 (ENO4), is related to sperm motility and male reproduction (Nakamura et al., 2013). The present article mainly reviews the basic characteristics and biological functions of ENO1.

2. Basic characteristics of ENO1

Alpha-enolase, also known as 2-phospho-D-glycerate hydrolase, is a metalloenzyme that catalyzes the conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. Petrak et al. (2008) statistically analyzed the frequency of the appearance of various terms related to human, rat and mouse proteins in the journal "Proteomics" (volumes from 4 to 6, from 2004 to 2006) and calculated the frequency of each term present in the database. It was found that ENO1 was a protein with an extremely high term frequency. Pancholi (2001) compared the amino acid sequences of ENO1 derived from 39 species. They found that although the amino acid sequences of ENO1 are different between species, ENO1 appears to be highly conserved. Alpha-enolase exhibits an overall amino acid sequence homology across species from 40% to 90% (Pancholi, 2001). Alpha-enolase proteins derived from various species are all composed of two structural domains: a smaller N-terminus and a larger C-terminus. The N-terminus shows a $\beta 3\alpha 4$ topology, while the C-terminus shows an $h\beta\beta\alpha\alpha$ (βa) 6 topology.

For most enolases, fluoride acts as an inhibitor, while Mg^{2+} is the most important metal activator. In yeast systems, metal cations and fluoride bind to enolase at the active center of the enzyme, forming a complex. The complex blocks the binding of substrates to the enzyme in yeast systems, thereby exerting an inhibitory effect.

* Corresponding author.
E-mail address: yanghuanmin@aliyun.com.cn (H. Yang).
[1] These authors contributed equally to this work.

Studies have also found that Mg^{2+} is an activator of *Leuconostoc mesenteroides* 512FMCM. In addition, Mn^{2+} and Zn^{2+} exhibit the same effect (Lee et al., 2006). Manganous ions shows a strong activating effect on the enolases of *Candida albicans* and yeast. However, the activating effect of Mn^{2+} on carp muscle enolase is rather weak. Compared with Mn^{2+}, Zn^{2+} exerts a much stronger activating effect on carp enolase but a weaker effect on yeast enolases and *C. albicans* enolases. The above discoveries indicate that distinct activators and inhibitors exist for different animal enolases.

Alpha-enolase is abundantly expressed in most cells. Alpha-enolase is abundant in the cytoplasm and is also present at the cell surface and in nuclei (Pancholi and Fischetti, 1998). Because of the conservative nature of glycolytic enzymes (including ENO1) across millions of years, this class of enzymes is generally considered rather "dull". Glycolytic enzymes have even been labeled "void of sophisticated regulatory functions" because only minor changes in the concentrations of the enzymes occur in the presence of external stimuli and the enzymes only play a catalytic role in certain reactions. However, unlike the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene, *ENO1* is not a housekeeping gene. The expression of *ENO1* changes with the occurrence of pathological changes in organisms and over the course of cell growth. Current studies show that, in addition to their glycolytic activities, the glycolytic enzymes play important roles in several biological and pathophysiological processes. Specifically, studies demonstrate that ENO1 is closely related to cancer, systemic fungal disease, odontopathy and autoimmune diseases.

3. Biological functions of ENO1

3.1. Alpha-enolase participates in stress responses

A number of studies have shown that ENO1 plays an important role in the development of stress responses. Proteomic studies on the mechanisms underlying the development of cellular stress responses have shown that ENO1 is one of the proteins that are expressed differentially before and after stress exposure. Data have shown that cells often express specific proteins (such as heat shock proteins [HSPs]) and glucose-regulated proteins (including ENO1) to adapt to high temperatures and glucose deprivation (Young and Elliott, 1989). Under conditions of chronic hypoxic stress, the expression levels of 5 cellular stress-related proteins (with molecular weights of 34, 36, 39, 47 and 57 ku) are significantly altered in mammalian endothelial cells. Moreover, the changes in protein expression levels are time and oxygen concentration dependent (Graven et al., 1993). It has been confirmed that the 47-ku protein expressed by endothelial cells is ENO1. It is speculated that ENO1 promotes anaerobic metabolism upon hypoxic stress, thereby exerting a protective effect on cells.

Studies in microorganisms have found that the expression of several HSPs is induced in microbes of the genus *Saccharomyces* upon exposure to high temperature stress. One HSP, HSP48, has been identified as ENO1. Studies have shown that during the cellular response to hypoxic stress, ENO1 acts as a stress protein and activates the expression of hypoxia-inducible factor-1 (HIF-1) (Aaronson et al., 1995), indicating that ENO1 may enhance the protective effect on cells through promoting anaerobic metabolism. The above results indicate that HSP48 is the expression product of the *ENO1* gene and is related to temperature tolerance and growth regulation in microorganisms. Iida and Yahara (1985) found that the expression of the *ENO1* gene is regulated by the heat shock resistance (*HSR1*) gene and that mutations in HSR1 render microorganisms extremely tolerant to high temperatures. These findings suggest that ENO1 is closely related to heat stress (Iida and Yahara, 1985). Although ENO1 is also an HSP, the stability of ENO1 varies

with the thermal environment. It has been found that the ENO1 proteins of yeasts and streptococcal strains isolated from rats exhibit high thermal stability. In contrast, the thermal stability of ENO1 from carp muscles, rabbit muscles and bovine brain is rather poor (Kustrzeba-Wójcicka and Golczak, 2002).

Some researchers have found that extracellular signal-regulated kinase 1/2 (ERK1/2) achieves its functions in cardiomyocyte contraction and survival by regulating ENO1. This finding indicates that ENO1 enhances the contractile force of impaired cardiomyocytes in hypoxic conditions. Under the stimulatory condition of ischemic hypoxia, ENO1 restores cellular ATP levels and prevents the death of cardiomyocytes. Stress generated upon cardiomyocyte injury activates ENO1 in the cytoplasm. Activated ENO1 is transported to the cytoskeleton and contractile filaments to stabilize these structures via its molecular chaperone activity (Mizukami et al., 2004). In summary, ENO1 may play critical roles in a variety of stress responses. Such effects of ENO1 are essential for the survival of cells.

3.2. ENO1 and bacteria

Alpha-enolase exists as a cell surface protein in a variety of prokaryotic and eukaryotic organisms. In addition, ENO1 is capable of binding to plasminogen. Therefore, it is likely that ENO1 plays important roles in the development and progression of disease through regulation of the extracellular and intravascular fibrinolytic systems. Alpha-enolase is also related to many types of bacterial infections; research in this area has mainly focused on streptococcal and *Diplococcus pneumoniae* infections.

A study conducted by Pancholi and Fischetti (1998) showed that as a potent plasminogen-binding protein on the bacterial surface, ENO1 is related to the pathogenic effects of mucosal pathogen group A streptococci and *Diplococcus pneumonia*. In addition, it has been found that enolase on the surface of mucosal pathogen group A streptococci possesses a stronger capacity for binding to plasminogen in comparison with other surface proteins of streptococci (Berge and Sjobring, 1993).

In studying *D. pneumoniae*, researchers discovered that ENO1 is the only enolase present in this species. Lenz et al. (2003) found that ENO1 present on the surface of *Streptococcus pneumoniae* comes from inside the bacterial cell and is then secreted to the cell surface. However, some researchers believe that *S. pneumoniae* ENO1 is acquired from other cells that have undergone apoptosis or other forms of cell death (Adrian et al., 2004). In addition, ENO1 located on the surface of *D. pneumoniae* adheres to plasminogen, which is abundant in the human body and enhances the activity of plasmin, a critical step in *D. pneumoniae* infection (Bergmann et al., 2005). Alpha-enolase of *D. pneumoniae* promotes the plasmin-mediated degradation of the reconstituted basement membrane and simultaneously induces the formation of neutrophil extracellular traps (NETs) (Mori et al., 2012). However, these effects of ENO1 are not related to interleukin 8 (IL-8) and lipopolysaccharide (LPS). The above effects of ENO1 not only increase the activity of bacteria in human blood but also enhance the antigen recognition capability of neutrophils in the innate immune response. Kolberg et al. (2006) conducted a study on *S. pneumoniae* and showed that the binding of ENO1 to plasmin is sufficient to cause pneumonia, despite the low expression level of ENO1 on the surface of *Streptococcus pneumonia*.

Staphylococcal enolase is a laminin binding protein, suggesting that the enolase exerts its functions through pathways involving the binding of enolase to extracellular matrix proteins (such as fibronectin on the surface of gram-positive bacteria) (Pancholi and Fischetti, 1998). However, it is puzzling that despite the structural similarity between streptococcal surface ENO1 and staphylococcal

ENO1, streptococcal ENO1 does not bind to laminin. As a staphylococcal surface protein, the pathogenesis of ENO1 remains unclear.

However, studies have revealed that ENO1 is also present on the surface of *L. mesenteroides* 512FMCM, a non-pathogenic bacterium (Lee et al., 2006). Plasmin exerts its functions through binding to ENO1 located on the surface of *D. pneumoniae*. Alpha-enolase of *L. mesenteroides* lacks plasminogen-binding sites and is incapable of binding to plasmin (Kolberg et al., 2006). Further in-depth studies must be conducted to determine the role of surface ENO1 in *L. mesenteroides*.

3.3. ENO1 and fungi

Alpha-enolase is present in a large number of fungal species. There are two enolase genes in yeast, and enolase has been detected in cancer patients experiencing *Candida* infections. Studies have also demonstrated that all tissues infected with *C. albicans* contain high concentrations of plasminogen. Alpha-enolase of *C. albicans* is a binding protein for plasma plasminogen and plasma fibrin. The binding of ENO1 to plasminogen and fibrin plays a critical role in *Candida* infection-induced host cell damage (Jong et al., 2003). Evidence indicates that *C. albicans* surface ENO1 serves as a receptor for plasma plasminogen, thus establishing a connection between fungal infection and target protein hydrolysis.

However, sequence analysis of enolase genes failed to detect the presence of a C-terminal lysine. Therefore, it cannot be determined whether the invasiveness of the fungus is due to the plasma plasminogen-binding activity of enolase. Using a model of *Candida* infection, Sundstrom and Aliaga (1992) found that enolase is an immunogenic antigen. Therefore, it is believed that enolase may serve as a marker for invasive candidiasis. Unlike gram-positive bacteria, *Candida* enolase is located in the cell wall, suggesting that enolase may be involved in cell wall synthesis. Angiolella et al. (1996) showed that ENO1 of *C. albicans* is associated with glucan in the inner cell wall, indicating that ENO1 may serve as an antigen during systemic *Candida* infection.

A study conducted by De Backer et al. (2001) and Lo et al. (2005) revealed that knockout of the *ENO1* gene reduced the growth rate, drug sensitivity, mycelium formation and virulence of *C. albicans*. Ko et al. (2013) also knocked out the *ENO1* gene of *C. albicans* and found that the formation of germination tubes is reduced in *C. albicans* carrying a mutated *ENO1* gene. This finding indicates that ENO1 plays an important role in germination tube formation. Alpha-enolase gene mutations may reduce the virulence of *C. albicans* and render *C. albicans* more sensitive to certain drugs. Mason et al. (1993) showed that *C. albicans* grows slowly in yeast growth medium after knockout of the *ENO1* gene, as the yeast growth medium provides a sufficient energy supply. However, the growth of the mutants is completely suppressed if glucose is present in the yeast culture medium. This finding suggests that the *C. albicans* mutant is incapable of infecting rats. Because the blood of rats contains glucose, it is difficult for *C. albicans* to proliferate. In addition, studies have shown that ENO1 is a novel surface virulence factor of *Trichomonas vaginalis* (Mundodi et al., 2008).

The above results indicate that ENO1 plays a key role in the mechanism underlying the occurrence of fungal infections. However, the specific roles of ENO1 remain inconclusive and require further in-depth study.

3.4. ENO1 and cancer

There have been remarkable scientific achievements in assessing the relationship between ENO1 and cancer and the role of ENO1 in cancer. In mammals, the *ENO1* gene encodes a 37-ku protein, c-myc promoter binding protein 1 (MBP-1). As MBP-1 is a general transcriptional repressor, it exhibits an inhibitory effect on certain types of cancer. Researchers originally discovered MBP-1 in human cervical cancer. It has been found that MBP-1 binds to the sequence just 5′ of the TATA box in the P2 promoter of the human proto-oncogene c-myc and prevents the formation of the transcription complex, thereby achieving a transcription-inhibiting effect (Chang et al., 2006). Exogenous MBP-1 inhibits cell growth and accelerates the apoptosis and necrosis of breast cancer cells (Ghosh et al., 2006), neuroblastoma cells (Merkulova et al., 1997) and non-small cell lung carcinoma cells (Ghosh et al., 2006; Keller et al., 2000) by inhibiting *c-myc* gene transcription or by binding to molecular chaperones, which is consistent with the extremely low expression level of MBP-1 in tumor cells. Recent *in vitro* studies have shown that the physiological level of MBP-1 is regulated by glucose and that changes in MBP-1 lead to altered cell proliferation levels (Ghosh et al., 2006; Ejeskär et al., 2005; Sedoris et al., 2007). The above results indicate that ENO1 exerts an inhibitory effect on certain types of cancer.

Alpha-enolase overexpression has been observed in many tumor cells. Comparative proteomic studies of certain cancer cells and normal tissues have shown that significant differences exist in ENO1 expression levels. The results suggest that the up-regulation of the expression levels of ENO1 and other glycolytic enzymes may be related to aerobic glycolysis in cancer cells and the development of malignant tumors. *In vitro* transfection of ENO1 into neuroblastoma induces apoptosis in a large number of cells (Ejeskär et al., 2005). However, transfection of *ENO1* mRNA into K562 cells (a human erythroleukemia cell line) fails to produce a significant impact on the cells. The findings of such studies indicate that *ENO1* mRNA exhibits no generalized toxicity and exerts its effect through different pathways. Experimental results also show that *ENO1* mRNA fails to activate any death or growth inhibitory pathways in K562 cells. However, work on stomach cancer cells has shown that ENO1 is the center of a protein–protein interaction network composed of 74 stomach cancer-associated proteins, and silencing of the *ENO1* gene results in growth inhibition of stomach cancer cells and cell cycle arrest (Yan et al., 2011). In lung cancer tissue, the *ENO1* expression level is increased, while the *MBP-1* expression level is decreased (Altenberg and Greulich, 2004). These results suggest that ENO1 and MBP-1 are regulated by different pathways in tumor cells. In summary, because different cells were used in these studies and defects in certain cellular pathways might exist, researchers have obtained different results regarding the role of ENO1 in the occurrence of cancers (Ejeskär et al., 2005).

Recently, a great deal of evidence has shown that ENO1 is related to the occurrence and metastasis of malignant tumors. Studies have found that ENO1 activity is high in migrating breast cancer cells (Tu et al., 2010). Alpha-enolase is also expressed in pancreatic cancer cells and liver cancer cells (Takikita et al., 2009). Altenberg and Greulich (2004) conducted a study using gene chips and expressed sequence tags (ESTs). The results support the existence of a correlation between ENO1 and the occurrence of tumors and revealed that an increased ENO1 expression level on cell surface promotes the invasion of small cell lung cancer and head and neck cancer. Fu et al. (2015) showed that ENO1 enhances the glycolysis, growth, migration and invasion of small-cell lung cancer cells through a focal adhesion kinase (FAK)-mediated phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Recently, it has been found that the ENO1 expression level is higher in estrogen receptor-positive (ER+) breast cancer compared with estrogen receptor-negative (ER−) breast cancer. Chu et al. (2011) believe that patients with high ENO1 expression levels are more likely to exhibit a worse prognosis. This conclusion was confirmed in a study on canine breast cancer. Merkulova et al. (1997) demonstrated that enolase and other glycolytic enzymes are associated with troponin-

related functions in muscles. Such a connection may induce the production of adenosine triphosphate (ATP), which is required for muscle contraction (Keller et al., 2000). In tumor cells, ENO1 and other glycolytic enzymes may be closely associated with the cytoskeleton, which may be closely related to the migration of tumor cells.

However, most non-small cell lung cancer (NSCLC) patients showing down regulated ENO1 expression exhibit a poor prognosis (Chang et al., 2003). A study conducted by Cheng et al. (2011) demonstrated that the expression level of ENO1 is elevated in the nasopharyngeal mucosa of patients with nasopharyngeal carcinoma (NPC). However, ENO1 expression is not related to cervical lymph node metastasis and post-radiotherapy recurrence and metastasis in NPC patients. These two experimental findings are inconsistent with the results of many previous studies regarding the correlation between cancer cell migration and ENO1 expression. The specific reasons require further investigation. In addition, it has recently been shown that ENO1 expression is upregulated on the surface of mouse tumor cells expressing a mimic of the p53 isoform Δ133p53α in response to plasminogen activation. The results indicate that the p53 subunit induces inflammatory responses by increasing ENO1 expression levels on the surface of tumor cells (Sawhney et al., 2015).

Although the existence of an N-terminal active center is a unique characteristic of ENO1, ENO1 and MBP-1 are derived from a single transcript and share a common C-terminus (myc promoter binding region). Therefore, both MBP-1 and ENO1 may serve as tumor promoters. Studies have shown that downregulation of ENO1 and MBP-1 expression reduces the invasive capacity of follicular thyroid carcinoma cells (Trojanowicz et al., 2009). This result is consistent with the notion presented in the literature that "ENO1 may serve as a metabolic enhancer for certain tumor cells because an increase in ENO1 expression levels promotes ATP production". However, inconsistent experimental results have also been reported. For example, researchers overexpressed the *ENO1* gene in neurocytoma and observed a decreased cell growth rate and induction of apoptosis. The results indicate that ENO1 possesses tumor-suppressing effects and plays a key role in the metastasis of neurocytoma (Sedoris et al., 2007). Studies have produced different findings regarding the role of ENO1 in the occurrence and metastasis of cancers, which may be related to the types of cells studied and might also be closely related to ENO1 expression levels.

3.5. ENO1 may act as an autoantigen

Alpha-enolase has been discovered on the surface of a number of cell types (including the monocytic cell line U937, T cells, B cells, peripheral blood mononuclear cells and human brain cancer cells), indicating that ENO1 may act as an autoantigen. Additionally, anti-ENO1 antibodies have been detected in many human diseases, including Hashimoto's encephalopathy, retinopathy, rheumatic arthritis, systemic sclerosis, autoimmune ovarian failure, relapsing polychondritis and lung cancer. Alpha-enolase autoantibodies were recently found in the serum of patients with lupus nephritis and autoimmune retinopathy (Guillou et al., 2013; Coupland et al., 2014). Therefore, anti-ENO1 antibodies have been recommended as specific diagnostic indicators for certain diseases.

A study conducted by Sato et al. (2000) showed that anti-ENO1 antibodies were detectable in the serum from 6.9% of patients to 13.8% of patients with various types of lung cancer. The results indicate that ENO1 may serve as an immunizing antigen in patients with lung cancer. It has also been shown that anti-ENO1 antibodies are present in from 25% of patients to 66% of patients with rheumatoid arthritis (Mosca et al., 2006). Alpha-enolase expression

levels are increased on the surface of monocytes and macrophages in patients with rheumatoid arthritis. In contrast, ENO1 expression levels on the surface of mononuclear macrophages are not enhanced in patients with osteoarthritis. Evidence has suggested that ENO1 autoantibodies stimulate the production of inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 alpha/beta (IL-1α/β) and interferon gamma (IFN-γ) in patients with rheumatoid arthritis, resulting in increased serum concentrations of inflammatory factors (Bae et al., 2012). In a study attempting to identify the target antigens that are recognizable by anti-fibroblast antibodies in patients with systemic sclerosis, ENO1 was recognized as an antigen and was considered to be related to interstitial pneumonia (Terrier et al., 2010).

At present, ENO1 has been identified as an antigen in patients with inflammatory bowel disease. Anti-ENO1 antibodies are also present in these patients. The serum of 49% of patients with ulcerative colitis and 50% of patients with Crohn's disease (which are two types of inflammatory bowel disease) contains anti-ENO1 antibodies. The expression level of *ENO1* mRNA is increased in the colonic mucosa of patients with inflammatory bowel disease, and anti-ENO1 antibodies are also present in the serum of such patients. The process of producing anti-ENO1 antibodies may be as follows: ENO1 is released from cells after apoptosis or necrosis. Alpha-enolase then undergoes the process of antigen presentation and is taken up by antibody-producing cells in tissues. As an HSP, ENO1 promotes the process of cellular uptake.

A study conducted by López-Alemany et al. (2003) showed that anti-ENO1 antibodies reduce cell surface-mediated prothrombin activation by from 84 to 90%, thereby decreasing the dissolution of fibrin. The concentrations of fibrinogen and fibrin are increased, which promotes the production of proinflammatory factors and chemokines and induces inflammatory responses. Accordingly, high concentrations of anti-ENO1 antibodies are detected during inflammation. In human streptococcal pharyngitis-induced acute rheumatic fever, anti-streptococcal ENO1 antibodies bind to ENO1 on the surface of hematopoietic cells, and the ENO1 expression level on the surface of leukocytes is increased simultaneously. The assessment of patient serum samples has demonstrated that ENO1 is a cross-reacting antigen and plays an important role in streptococcal infection-induced autoimmune disease (Fontán et al., 2000).

Because of its antigenic nature, ENO1 is often used in vaccine-related research. Researchers believe that ENO1 may serve as a potential marker for pneumococcal vaccines. However, when Adrian et al. analyzed anti-ENO1 antibodies in the serum of patients infected with *S. pneumoniae* and uninfected individuals (Adrian et al., 2004), it was found that there were no significant differences in the titer, transport and infectivity of the antibodies, which may be directly related to the low expression level of ENO1 on the cell surface. Kolberg et al. (2006) also believe that ENO1 is not an ideal candidate marker for vaccines due to its low expression level on the surface of *S. pneumoniae*.

3.6. ENO1 and gene transcription

Alpha-enolase may act as a nuclear DNA-binding protein and regulate the transcription of related genes. Studies have shown that ENO1 is present in the nuclei of a number of cell types. For example, both the cytoplasm and the nucleus of endothelial cells and HeLa cells contain ENO1. Schirle et al. (2003) found that ENO1 is located in the nucleus of Burkitt lymphoma BL60 cells and human embryonic kidney 293 (HEK293) cells. A study conducted by Merkulova et al. (2000) revealed that ENO1 is translocated from the cytoplasm to the region surrounding the nucleus during the process of muscle regeneration. Wang et al. (2005) demonstrated that the nuclear DNA-binding protein ENO1 is only expressed in the

zona fasciculate, and not in the zona reticularis, of the adrenal cortex.

In many organisms, mitochondrial and nuclear DNA encode small RNAs. A double lipid bilayer membrane protects the mitochondrial matrix, where the replication, transcription and translation of mitochondrial DNA are conducted. The above processes are carried out via mitochondrial targeting of ENO1. In *Saccharomyces cerevisiae*, ENO1 acts as an important molecular chaperone in the mitochondrial localization of transfer RNAs (tRNA). The pathways involved are extremely complex and may include the recognition of tRNA by ENO1, binding of ENO1 to tRNA, and transport of the tRNA-ENO1 complex to the mitochondria. On the mitochondrial surface, ENO1 facilitates the binding of tRNA to transporter proteins and the entry of mitochondrial aminoacyl-tRNA synthetase into mitochondria. It has been speculated that the binding of ENO1 to tRNA results in structural changes in the tRNA. Therefore, researchers believe that ENO1 may act as a molecular chaperone and exert a regulatory effect on gene transcription (Entelis et al., 2006).

3.7. Other functions of ENO1

A relationship may exist between ENO1 and parasitic infection in the host. Sporulation of *Eimeria tenella* occurs in an aerobic environment, whereas excystation occurs in an anaerobic environment. After excystation, the sporozoites invade host cells. Therefore, sporozoites must adapt to the oxygen-free environment. At this stage, glycolysis produces ATP and, eventually, lactic acid. The production of lactic acid via glycolysis is the most important metabolic pathway of *Plasmodium* parasites in blood (Labbé et al., 2006). The characteristics and localization of the enolase of *Plasmodium falciparum* have been studied, and it has been found that ENO1 on the surface of *Plasmodium* merozoites possesses immunogenicity. In addition, anti-ENO1 antibodies are detectable in serum. Growth inhibition of *Plasmodium* parasites cultured *in vitro* does not appear to be caused by ENO1-induced inhibition of glycolytic activity (Labbé et al., 2006), as the inhibitory effect cannot be directly blocked by certain non-glycolytic pathways. It has been hypothesized that growth inhibition is induced by the surface protein-related functions of *Plasmodium* ENO1 or by indirect effects of the binding of anti-ENO1 antibodies to *Plasmodium* surface proteins.

Studies have shown that certain glycolytic enzymes, including enolase and lactate dehydrogenase (LDH), may provide evidence of the origins of species of the phylum Apicomplexa and serve as targets in diseases caused by apicomplexan parasites (Dzierszinski et al., 1999). The expression of enolase appears to be parasitic phase-specific, which varies as the niche environment and the metabolic requirements of the parasites change (Coppin et al., 2003). The expression level of the stable ENO1 enzyme is relatively high during the bradyzoite stage, whereas the expression level of the less stable ENO2 becomes higher during the tachyzoite stage. Toxoplasma ENO1 and ENO2 display different subcellular localizations and exhibit additional functions other than glycolysis (Ferguson et al., 2002).

Alpha-enolase from turtles and lampreys (tau-crystallin) exhibits a molecular weight of 48 ku and exists as dimers. In the crystalline lens of mammals and chickens, the activity of enolase is lower in cells that have grown for long periods of time in comparison with newly produced epithelial cells. It has been demonstrated that the translation of ENO1 is regulated over time or as the organism ages, which is consistent with the notion that the expression level of ENO1 in the crystalline lens does not increase gradually during embryonic development in ducks (Wistow et al., 1988). Alpha-enolase expression levels were examined in rabbits during epithelial regeneration after the debridement of corneal edges. The results showed that there was a significant difference in the ratio of ENO1 to total protein levels between uninjured and injured rabbit corneas. However, the role of ENO1 in eye development needs to be further investigated.

Alpha-enolase is also closely related to muscle growth and development in animals. Studies have demonstrated the existence of a significant difference in ENO1 expression levels in muscle samples between 2-week-old and 12-week-old Rugao chickens. The results indicate that ENO1 is closely related to the growth and development of chicken muscles. In young chickens, *ENO1* and *ENO2* are essential genes for muscle growth and development. In addition, *ENO1* expression levels gradually decrease while *ENO2* expression levels gradually increase in chicken muscles during the hatching process (Chang et al., 2011).

It was recently discovered that ENO1 and ENO4 are present in the sperm of humans, mice and other animals. In ENO4 mutant mice, sperm motility is reduced, and ATP production is decreased, indicating that ENO4 is related to sperm activity and male reproduction. Enolase 4 may be an important glycolytic enzyme in sperm. Alpha-enolase is also present in the rat testis and sperm. However, whether ENO1 plays a role in the glycometabolism of sperm has yet to be determined.

4. Conclusions

Over the past several decades, research on ENO1 has gradually extended, and new biological functions of ENO1 are being discovered. Specifically, ENO1 has been found to be involved in cellular stress, bacterial and fungal infections, autoantigen activities, the occurrence and metastasis of cancer, parasite infections, and the growth, development and reproduction of organisms. These discoveries are conducive to achieving a detailed understanding of the functions of ENO1. However, the mechanisms by which ENO1 affects certain cellular functions are not entirely clear. Future in-depth exploration in the field is expected to further elucidate the specific functions and mechanisms underlying the occurrence of ENO1, which may ultimately result in ENO1 becoming a therapeutic target and diagnostic marker for a variety of diseases and promote the advancement and development of clinical medicine.

References

Aaronson RM, Graven KK, Tucci M, Mcdonald RJ, Farber HW. Non-neuronal enolase is an endothelial hypoxic stress protein. J Biol Chem 1995;270:27752–7.

Adrian PV, Bogaert D, Oprins M, Rapola S, Lahdenkari M, Kilpi T, et al. Development of antibodies against pneumococcal proteins alpha-enolase, immunoglobulin A1 protease, streptococcal lipoprotein rotamase A, and putative proteinase maturation protein A in relation to pneumococcal carriage and otitis media. Vaccine 2004;22:2737–42.

Altenberg B, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics 2004;84:1014–20.

Angiolella L, Facchin M, Stringaro A, Maras B, Simonetti N, Cassone A. Identification of a glucan-associated enolase as a main cell wall protein of *Candida albicans* and an indirect target of lipopeptide antimycotics. J Infect Dis 1996;173:684–90.

Bae S, Kim H, Lee N, Won C, Kim HR, Hwang YI, et al. α-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. J Immunol 2012;189:365–72.

Berge A, Sjobring U. A novel plasminogen-binding protein from *Streptococcus pyogenes*. J Biol Chem 1993;268(34):25417–24.

Bergmann S, Rohde M, Preissner KT, Hammerschmidt S. The nine residue plasminogen-binding motif of the pneumococcal enolase is the major cofactor of plasmin-mediated degradation of extracellular matrix, dissolution of fibrin and transmigration. J Thromb Haemost 2005;94(2):304—11.

Cheng C, Long X, Li X, Xie M, Guo M. The expressions of enolase in the nasopharyngeal cancer tissue. J Clin Otorhinolaryngol Head Neck Surg 2011;25:554—6.

Chang GB, Zhen T, Huang ZY. Analysis on differentially expressed genes of muscle tissues in Rugao chicken at the ages of 2 and 12 weeks by microarray. Thai J Vet Med 2011;41(3):299—307.

Chang GC, Liu KJ, Hsieh CL, Hu TS, Charoenfuprasert S, Liu HK, et al. Identification of alpha-enolase as an autoantigen in lung cancer: its overexpression is associated with clinical outcomes. Clin Cancer Res 2006;12(19):5746—54.

Chang YS, Wu W, Walsh G, Hong WK, Mao L. Enolase-α is frequently down-regulated in non-small cell lung cancer and predicts aggressive biological behavior. Clin Cancer Res 2003;9:3641—4.

Chu PY, Hsu NC, Liao AT, Shih NY, Hou MF, Liu CH. Overexpression of α-enolase correlates with poor survival in canine mammary carcinoma. BMC Vet Res 2011;7:62.

Coppin A, Dzierszinski F, Legrand S, Mortuaire M, Ferguson D, Tomavo S. Developmentally regulated biosynthesis of carbohydrate and storage polysaccharide during differentiation and tissue cyst formation in Toxoplasma gondii. Biochimie 2003;85:353—61.

Coupland SG, Bursztyn LL, Belrose J, Fraser JA, Proulx AA. Role of α-enolase autoantibodies related to rod-bipolar cell function in non-paraneoplastic autoimmune retinopathy. Graef Arch Clin Exp 2014;55:331.

De Backer MD, Nelissen B, Logghe M, Viaene J, Loonen I, Vandoninck S, et al. An antisense-based functional genomics approach for identification of genes critical for growth of Candida albicans. Nat Biotechnol 2001;19(3):235—41.

Dzierszinski F, Popescu O, Toursel C, Slomianny C, Yahiaoui B, Tomavo S. The protozoan parasite toxoplasma gondii expresses two functional plant-like glycolytic enzymes. Implications for evolutionary origin of apicomplexans. J Biol Chem 1999;274:24888—95.

Ejeskär K, Krona C, Carén H, Zaibak F, Li L, Martinsson T, et al. Introduction of in vitro transcribed ENO1 mRNA into neuroblastoma cells induces cell death. BMC Cancer 2005;5:161.

Entelis N, Brandina I, Kamenski P, Krasheninnikov IA, Martin RP, Tarassov I. A glycolytic enzyme, enolase, is recruited as a cofactor of tRNA targeting toward mitochondria in Saccharomyces cerevisiae. Gene Dev 2006;20:1609—20.

Ferguson DJP, Parmley SF, Tomavo S. Evidence for nuclear localisation of two stage-specific isoenzymes of enolase in Toxoplasma gondii correlates with active parasite replication. Int J Parasitol 2002;32:1399—410.

Fontán PA, Pancholi V, Nociari MM, Fischetti VA. Antibodies to streptococcal surface enolase react with human α-enolase: implications in poststreptococcal sequelae. J Infect Dis 2000;182:1712—21.

Fu QF, Liu Y, Fan Y, Hua SN, Qu HY, Dong SW, et al. Alpha-enolase promotes cell glycolysis, growth, migration, and invasion in non-small cell lung cancer through FAK-mediated PI3K/AKT pathway. J Hematol Oncol 2015;8:22.

Ghosh AK, Steele R, Ryerse J, Ray RB. Tumor-suppressive effects of MBP-1 in non-small cell lung cancer cells. Cancer Res 2006;66:11907—12.

Graven KK, Zimmerman LH, Dickson EW, Weinhouse GL, Farber HW. Endothelial cell hypoxia associated proteins are cell and stress specific. J Cell Physiol 1993;157:544—54.

Guillou C, Avenel G, Derambure C, Verdet M, Golinski ML, Hiron M, et al. Potential in vitro immunomodulatory effects of the recombinant human alpha-enolase on peripheral blood mononuclear cells (PBMCs) from healthy donors. Ann Rheum Dis 2013;72:2627.

Iida H, Yahara I. Yeast heat-shock protein of Mr 48,000 is an isoprotein of enolase. Nature 1985;315:688—90.

Jong AY, Chen SH, Stins MF, Kim KS, Tuan TL, Huang SH. Binding of Candida albicans enolase to plasmin(ogen) results in enhanced invasion of human brain microvascular endothelial cells. J Med Microbiol 2003;52(8):615—22.

Keller A, Demeurie J, Merkulova T, Géraud G, Cywiner-Golenzer C, Lucas M, et al. Fibre-type distribution and subcellular localisation of alpha and beta enolase in mouse striated muscle. Biol Cell 2000;92:527—35.

Ko HC, Hsiao TY, Chen CT, Yang YL. Candida albicans ENO1 null mutants exhibit altered drug susceptibility, hyphal formation, and virulence. J Microbiol 2013;51:345—51.

Kolberg J, Aase A, Bergmann S, Herstad TK, Rødal G, Frank R, et al. Streptococcus pneumoniae enolase is important for plasminogen binding despite low abundance of enolase protein on the bacterial cell surface. Microbiology 2006;152:1307—17.

Kustrzeba-Wójcicka I, Golczak M. Enolase from Candida albicans — purification and characterization. Comp Biochem Physiol B, Biochem Mol Biol 2002;126:109—20.

Labbé M, Péroval M, Bourdieu C, Girard-Misguich F, Péry P. Eimeria tenella enolase and pyruvate kinase: a likely role in glycolysis and in others functions. Int J Parasitol 2006;36:1443—52.

Lee JH, Kang HK, Moon YH, Cho DL, Kim D, Choe JY, et al. Cloning, expression and characterization of an extracellular enolase from Leuconostoc mesenteroides. FEMS Microbiol Lett 2006;259:240—8.

Lenz LL, Mohammadi S, Geissler A, Portnoy DA. SecA2-dependent secretion of autolytic enzymes promotes Listeria monocytogenes pathogenesis. Proc Natl Acad Sci U S A 2003;100:12432—7.

Lo HJ, Wang JS, Lin CY, Chen CG, Hsiao TY, Hsu CT, et al. Efg1 involved in drug resistance by regulating the expression of ERG3 in Candida albicans. Antimicrob Agents Chemother 2005;49:1213—5.

López-Alemany R, Longstaff C, Hawley S, Mirshahi M, Fabregas P, Jardi M, et al. Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-enolase. Am J Hematol 2003;72(4):234—42.

Mason AB, Buckley HR, Gorman JA. Molecular cloning and characterization of the Candida albicans enolase gene. J Bacteriol 1993;175(9):2632—9.

Merkulova T, Dehaupas M, Nevers MC, Creminon C, Alameddine H, Keller A. Differential modulation of alpha, beta and gamma enolase isoforms in regenerating mouse skeletal muscle. Eur J Biochem 2000;267:3735—43.

Merkulova T, Lucas M, Jabet C, Lamande N, Rouzeau J, Gros F, et al. Biochemical characterization of the mouse muscle-specific enolase: developmental changes in electrophoretic variants and selective binding to other proteins. Biochem J 1997;323:791—800.

Mizukami Y, Iwamatsu A, Aki T, Kimura M, Nakamura K, Nao T, et al. ERK1/2 regulates intracellular ATP levels through α-enolase expression in cardiomyocytes exposed to ischemic hypoxia and reoxygenation. J Biol Chem 2004;279(48):50120—31.

Mori Y, Yamaguchi M, Terao Y, Hamada S, Ooshima T, Kawabata S. α-Enolase of streptococcus pneumoniae induces formation of neutrophil extracellular traps. J Biol Chem 2012;287:10472—81.

Mosca M, Chimenti D, Pratesi F, Baldini C, Anzilotti C, Bombardieri S, et al. Prevalence and clinico-serological correlations of anti-alpha enolase, anti-C1q, and anti-dsDNA antibodies in patients with systemic lupus erythematosus. Rheumatology 2006;33:695—7.

Mundodi V, Kucknoor AS, Alderete JF. Immunogenic and plasminogen-binding surface-associated-enolase of Trichomonas vaginalis. Infect Immun 2008;76:523—31.

Nakamura N, Dai Q, Williams J, Goulding EH, Willis WD, Brown PR, et al. Disruption of a spermatogenic cell-specific mouse enolase 4(ENO4) gene causes sperm structural defects and male infertility. Biol Reprod 2013;88:90.

Pancholi V, Fischetti VA. α-Enolase, a novel strong plasmin(ogen) binding protein of the surface of pathogenic streptococci. J Biol Chem 1998;273:14503—15.

Pancholi V. Multifunctional alpha-enolase: its role in diseases. CMLS-Cell Mol Life Sci 2001;58:902—20.

Petrak J, Ivanek R, Toman O, Cmejla R, Cmejlova J, Vyoral D, et al. A hit parade of repeatedly identified differentially expressed proteins. Proteomics 2008;8:1744—9.

Sedoris KC, Thomas SD, Miller DM. C-myc promoter binding protein regulates the cellular response to an altered glucose concentration. Biochemistry 2007;46:8659—68.

Sato N, Nabeta Y, Kondo H, Sahara H, Hirohashi Y, Kashiwagi K, et al. Human CD8 and CD4 T cell epitopes of epithelial cancer antigens. Cancer Chemother Pharmacol 2000;45:S86—90.

Sawhney S, Hood K, Shaw A, Braithwaite AW, Stubbs R, Hung NA, et al. Alpha-enolase is upregulated on the cell surface and responds to plasminogen activation in mice expressing a Δ133p53α mimic. PLoS One 2015;10:e0116270.

Schirle M, Heurtier MA, Kuster B. Profiling core proteomes of human cell lines by one-dimensional PAGE and liquid chromatography—tandem mass spectrometry. Mol Cell Proteomics 2003;2(12):1297—305.

Sundstrom P, Aliaga GR. Molecular cloning of cDNA and analysis of protein secondary structure of Candida albicans enolase, an abundant, immunodominant glycolytic enzyme. J Bacteriol 1992;174:6789—99.

Takikita M, Altekruse S, Lynch CF, Goodman MT, Hernandez BY, Green M, et al. Associations between selected biomarkers and prognosis in a population-based pancreatic cancer tissue microarray. Cancer Res 2009;69:2950—5.

Terrier B, Tamby MC, Camoin L, Guilpain P, Bérézné A, Tamas N, et al. Antifibroblast antibodies from systemic sclerosis patients bind to α-enolase and are associated with interstitial lung disease. Ann Rheum Dis 2010;69:428—33.

Trojanowicz B, Winkler A, Hammje K, Chen Z, Sekulla C, Glanz D, et al. Retinoic acid-mediated down-regulation of ENO1/MBP-1 gene products caused decreased invasiveness of the follicular thyroid carcinoma cell lines. J Mol Endocrinol 2009;42:249—60.

Tu SH, Chang CC, Chen CS, Tam KW, Wang YJ, Lee CH, et al. Increased expression of enolase alpha in human breast cancer confers tamoxifen resistance in human breast cancer cells. Breast Cancer Res Treat 2010;121:539—53.

Wang W, Wang L, Endoh A, Hummelke G, Hawks CL, Hornsby PJ. Identification of α-enolase as a nuclear DNA-binding protein in the zona fasciculata but not the zona reticularis of the human adrenal cortex. J Endocrinol 2005;184:85—94.

Wistow GJ, Lietman T, Williams LA, Stapel SO, De Jong WW, Horwitz J, et al. Tau-crystallin/alpha-enolase: one gene encodes both an enzyme and a lens structural protein. J Cell Biol 1988;107:2729—36.

Yan GR, Xu SH, Tan ZL, Yin XF, He QY. Proteomics characterization of gastrokine 1-induced growth inhibition of gastric cancer cells. Proteomics 2011;11:3657—64.

Young RA, Elliott TJ. Stress proteins, infection, and immune surveillance. Cell 1989;59:5—8.