

• 综述 •

The Class IIa Deacetylase and Its Inhibitors: Possible Therapies for Insulin Resistance and Type 2 Diabetes

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Abstract Class II histone deacetylases (HDACs) include HDAC4, 5, 7 and 9, which are subject to phosphorylation, thereby regulating their nuclear and cytoplasmic translocation. They play a role in glycolipid metabolism in liver and adipose tissues, thus aggravating the degree of insulin resistance and the progression of diabetes. Inhibitors of class II HDACs have become a hot research topic. They aim to inhibit enzymatic activities, improve metabolism and insulin sensitivity.

Key words type 2 diabetes; insulin resistance; histone deacetylase (HDAC); inhibitor

II a 类组蛋白去乙酰化酶及抑制剂在胰岛素抵抗与糖尿病中的潜在作用

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摘要 II 类组蛋白去乙酰化酶(HDACs)包括 HDAC4、5、7 和 9,它们能够发生磷酸化作用和介导核质转位。HDACs 在肝、脂肪组织中的糖脂代谢中发挥作用,可加重胰岛素抵抗程度和糖尿病进展。II 类 HDACs 抑制剂目前已成为研究热点,旨在抑制酶活性,改善代谢和胰岛素敏感性。

关键词 2 型糖尿病; 胰岛素抵抗; 组蛋白去乙酰化酶; 抑制剂

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1 Introduction

Nucleosomes are the basic unit of chromosomes and consist of DNA and histones. Post-translational protein modifications (PTMs) are a common feature of proteins. Acetylation, a kind of PTM, could occur on particular residues of histones and other proteins. Histone deacetylases (HDACs) have deacetylation effects and are central to the modification of chromosomes and regulation of gene expression. Histone acetylation neutralizes the positive charge of the histone lysine residue, thus facilitating the dissociation of DNA and histone octamers and relaxing the nucleosomal structure, thereby activating transcription. Conversely, histone deacetylation leads to dense chromosomes, and gene transcription is inhibited.

To date, 18 human HDACs have been identified and can be divided into four categories: (1) Class I

HDACs include HDAC1, 2, 3 and 8. They are widely expressed and located in the nucleus and mainly regulate histone acetylation, cell survival and proliferation. (2) Class II HDACs including HDAC4, 5, 6, 7, 9, 10, that associated with the yeast protein HDA1, mainly locates in the cytoplasm, and shuttle between the cytoplasm and the nucleus^[1]. (3) Class

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III HDACs, also known as sirtuins, include SIRT1-SIRT7^[2] (4) Class IV HDACs; HDAC11, shuttles between the nucleus and cytoplasm^[3]. Class II HDACs are further subdivided into class IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10)^[4,5]. Studies have confirmed that Class II HDACs are closely related to metabolism.

2 Class II HDACs

2.1 Distribution

The expression of type IIa HDACs has certain tissue specificity^[6]. For example, HDAC4 and HDAC9 are rich in the muscle, heart and brain. HDAC4 is highly expressed in the brain and bones, and HDAC7 is rich in endothelial and thymus cells.

2.2 Structure

Class IIa HDACs, in addition to their C-terminal catalytic domains, also have an N-terminal domain that is subject to signal-dependent phosphorylation at highly conserved serine residues. The N-terminal domain contains the interacting binding site of the transcription factor myocyte enhancer factor 2 (MEF2) family members and 14-3-3 chaperone protein^[7]. Studies showed that other transcription factors such as Runx2, calmodulin-binding transcription activators, and serum response factors can also interact with class IIa HDACs^[8].

2.3 Phosphorylation

Phosphorylation of class IIa HDACs is very important and the N-terminal domains are reversibly phosphorylated to control their distribution in the nucleus and cytoplasm, and regulate transcriptional repression. Under basal conditions, class IIa HDACs are not phosphorylated and are predominantly located in the nucleus, and recruited to the target gene by interaction with transcription factors, thereby inhibiting transcription. Under specific signals, class IIa HDACs are phosphorylated and the interaction with transcription factors is abrogated, thus activating transcription of the target genes. In addition, class IIa HDACs may also be phosphorylated in the cytoplasm, leading to retention in the cytoplasm. Therefore, nuclear-cytoplasm translocation is closely related to the inhibition of the target genes. Recent studies identified that kinases and phosphatases were regulated during the nuclear-cytoplasm translocation of class IIa HDACs. Ca^{2+} /calmodulin-dependent protein kinases (CaMKs) are class IIa-type HDACs kinases. CaMK I and IV are capable of phosphorylating all members of class IIa HDACs. In the cellular model, CaMKs induced the translocation of class IIa HDACs from the nucleus to the cytoplasm, so that the target gene was repressed. HDACs are phosphorylated by protein kinase D, bind to the 14-3-3 protein, and shuttle from the nucleus to the cytoplasm^[9].

Although histone acetylation reduction is usually associated with gene silencing in the promoter region, evidence suggests that HDACs could also activate some genes. For example, in the yeast, HDAC Hos2 is essential for gene activation, and the absence of HDAC1 and 2 homologues Rpd3 results in inhibition of transcription at telomeres 7-9. HDACs are also associated with transcriptional activation of certain genes in higher eukaryotes^[10].

In recent years, more and more studies have confirmed that, HDACs also act on many cell substrates in addition to histones, and acetylation may be associated with phosphorylation.

2.4 The role of class IIa HDACs in type 2 diabetes and insulin resistance

Phenotypic analysis of class IIa HDAC transgenic and knockout mouse models has confirmed its key role in growth and differentiation^[4,5]. Zhou *et al* reported the representation and phenotypes of gene knockout mice for each class IIa HDAC family member. Mice lacking HDAC4 showed poor bone formation due to early bone calcification^[11] and mice lacking HDAC5/HDAC9 exhibited a further increase in cardiac hypertrophy in a stress response induced by human-induced aortic stenosis^[12]. Mice lacking HDAC7 manifested embryonic lethality on day 11^[13].

2.4.1 HDAC4/5/7 There is a link between the class IIa HDACs and energy and glucose metabolism. Mihaylova *et al*^[14] showed that HDAC4, 5, and 7 are expressed in the liver and that knockdown of HDAC4/5/7 led to a significant reduction in fasting blood glucose levels. In addition, high-fat diet (HFD)-fed mice showed a significant reduction in fasting blood sugar levels and improved glucose tolerance after knockout of class IIa HDACs that were phosphorylated by the activated protein kinase family members and located in the cytoplasm. HDAC is dephosphorylated and translocated into the nucleus in response to the fasting hormone glucagon and was recruited to the gluconeucine-6-phosphatase (G6PC) promoter and enhanced gluconeogenesis. HDAC4 and HDAC5 interacted with class I HDAC3, leading to deacetylation and activation of the FOXO family transcription factors, as well as their target gene expression. Elimination of class IIa HDACs in mouse hepatocytes led to inhibition of the FOXO target gene, lowered blood glucose levels, and increased glycogen storage. Knockdown of class IIa HDACs in mice with type 2 diabetes improved hyperglycemia, suggesting that inhibitors of I/II HDAC may be potential therapeutic targets for metabolic syndromes.

In a study using *Drosophila* as a model, Wang *et al*^[15] found that HDAC4 was phosphorylated by salt-inducible kinase 3 (SIK3) in the cytoplasm under feeding conditions. However, SIK3 inactivation during fasting

resulted in dephosphorylation and nuclear translocation of HDAC4, as well as FOXO deacetylation.

Glucose uptake is mainly dependent on glucose transporter 4 (GLUT4) that transfers extracellular glucose into cells through the cell membrane. The GLUT4 gene promoter contains two domains: MEF2 binding domain and domain I. When MEF2 binds to the MEF2 binding domain, GLUT4 enhancer factor binds to domain I, and transcription activity is at its highest^[16]. Studies have also shown that a decrease in MEF2 expression was associated with reduced expression of GLUT4^[17]. In particularly, class IIa HDACs, HDAC4 and 5 can inhibit the transcriptional activity of MEF2 and regulate GLUT4 expression through its deacetylase activity, tightening the chromatin structure so that the transcriptional complex cannot bind to DNA, thereby inhibiting transcription. The study also reported that overexpression of HDAC5 reduced GLUT4 expression in cardiac tissues. Phosphorylation of HDAC5 serine 259 and 498 by AMPK resulted in dissociation from the MEF2 domain.

2.4.2 HDAC9 Studies have shown increasing levels of HDAC9 expression in HFD-fed mice. Knockout of HDAC9 protected mice from hyperglycemia, high cholesterol, fatty liver disease, and improved glucose tolerance and insulin sensitivity caused by HFD feeding^[18]. In another study, the expression of class II HDACs, especially HDAC5 and HDAC9, were increased in the brains of C57BL/6 J mice fed a HFD for 6 months. In addition, these mice also showed increased fasting blood glucose levels and reduced insulin sensitivity compared with control mice.

It is noteworthy that during adipocyte differentiation, of the 11 HDACs family members (except class III), only HDAC9 mRNA levels were significantly decreased prior to the increase in fat gene expression. Overexpression of HDAC9 blocked differentiation of 3T3-L1 preadipocytes, suggesting that HDAC9 may be the key to initiating adipogenesis and may play a negative regulatory role in this process^[19].

It is well known that chronic hepatitis C virus (HCV) infection is associated with an increased risk of hyperglycemia and type 2 diabetes^[20]. A recent study examined the increase in HDAC9 activity in the liver of HCV-infected transgenic mice and patients, and showed that it was associated with an increase in FOXO1-mediated hepatic gluconeogenesis activity^[21]. The study also showed that HDAC9 may be linked with inhibition of liver gluconeogenesis and reducing blood sugar.

These studies all demonstrate the regulation of class IIa HDACs in glucose and energy metabolism. Changes in HDACs levels led to decreased insulin sensitivity and metabolic dysfunction.

3 Class IIa HDAC inhibitors

Class IIa HDAC inhibitors (HDACIs) increase histone acetylation levels and alter gene transcriptional activity^[22]. At present, most of the inhibitors capable of inhibiting the activity of class IIa HDACs are class I/II HDAC inhibitors, while the inhibitors that specifically inhibit the activity of class IIa HDACs are less.

HDACIs can be categorized according to the structure:

(i) Short-chain fatty acids: butyric acid, valeric acid, phenylbutyric acid and their salt compounds.

(ii) Hydroxamic acids: trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), α -carboxycinnamic acid bis-hydroxamide, and givinostat (ITF2357). TSA was the first natural histone deacetylase inhibitor discovered.

(iii) Cyclic tetrapeptides: Trapotoxin A, Apicidin, FK228, CHAP31.

(iv) Benzamides: Etinostat, MS-275, CI-994, and MGCD0103.

(v) Electrophilic ketones: trifluoromethyl ketone, α -ketoamides.

(vi) Trithiocarbonates: a new type of HDACI.

At present, HDACIs have received extensive attention as a treatment for various diseases. For example, sodium valproate (VPA) is used for extensive^[26] and focal epilepsy, and another pan-HDACI, sodium butyrate, is for the treatment of sickle cell anemia and beta-anemia^[27]. SAHA was approved by the FDA for the treatment of skin T cell leukemia. HDACIs are also a new type of targeted anticancer drugs^[28] that can target tumor growth, differentiation, and apoptosis, and have little effect on normal cells. Sodium butyrate has anti-depressant and anxiolytic effects, and can also be used to treat Alzheimer's disease^[29]. TSA can inhibit cardiac hypertrophy, reverse atrial fibrosis, and improve left ventricular function^[30].

HDACIs could also regulate glucose homeostasis, reduce pancreatic β -cell apoptosis and have important effects on insulin resistance and diabetes^[31]. HDACIs are expected to be new drugs for the prevention and treatment of diabetes and could improve and delay complications of diabetes^[24].

In vitro and *in vivo* studies have shown that HDACIs have protective effects on pancreatic β cells. Christensen *et al* found a relationship between histone hyperacetylation and β cells survival^[25]. Evidence suggests that HDACIs could regulate pancreatic development and protect from cytokine-induced β -cells death^[26].

An *in vitro* study by Larsen *et al* reported that SAHA and TSA, class I/II HDAC inhibitors were able

to partially block cytokine-induced β -cell cytotoxicity. However, these drugs could not restore glucose-stimulated insulin secretion^[27]. SAHA did not have protective effects against β -cell death in non-obese mice with type 1 diabetes mellitus, but when SAHA was used in combination with the dipeptidyl peptidase-IV inhibitor, MK-626, the number of β -cells increased. Lewis *et al* showed that the broad-spectrum HDACI, ITF2357, reduced blood glucose levels and improved islet function in mice with streptozotocin-induced type 1 diabetes mellitus. Some studies have shown that ITF2357 treatment leads to increased islet cell viability, insulin secretion, and reduced apoptosis^[28]. Specific class IIa HDACIs MC1568 could regulate pancreatic development^[29] and had a protective effect on cytokine-induced beta cell death^[26]. These studies demonstrate the beneficial effects of HDACIs on β -cell protection.

HDACIs affect insulin resistance in peripheral tissues, such as muscle, liver, and fat. In muscle, HDACIs increase the translocation of GLUT4, thereby increasing glucose uptake. In the livers of ob/ob mice, IRS-1 deacetylation was inhibited and the insulin signaling pathway was partially restored by TSA treatment.

VPA could stimulate insulin release from isolated islets *in vitro*, but hyperinsulinemia was observed in VPA-treated patients regardless of body weight. Pylvanen *et al* concluded that increasing levels of insulin concentration may be due to a reduction in hepatic insulin degradation^[30]. Therefore, further experiments are needed to elucidate the effect of HDACIs on human insulin resistance and insulin secretion.

In addition to the important roles in insulin sensitivity and glucose utilization, HDACIs have a certain therapeutic role in obesity and dyslipidemia. It was reported that MC1568, an inhibitor of type IIa HDACs, could reduce PPAR γ -induced 3T3-L1 adipogenesis, whereas the Class I HDACs-specific inhibitor, MS275, completely blocked adipocyte formation^[31].

4 Problems and prospects

There are still few studies on class IIa HDACs in glucose and lipid metabolism, and its specific role is not fully understood. Further clarification of the mechanisms of class IIa HDACs in metabolic regulation, in different physiological and pathological conditions is required. Finding specific class IIa HDACs inhibitors will provide new ideas for the prevention and treatment of metabolic diseases.

Disclosure of interest

The authors declare that they have no competing

interest.

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