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Biological Activities of Receptor-interacting Protein 140 in Adipocytes and Metabolic Diseases

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Abstract

Receptor-interacting protein 140 (RIP140) is best known for its functional role as a wide-spectrum transcriptional co-regulator. It is highly expressed in metabolic tissues including mature adipocyte. In the past decade, molecular biological and biochemical studies revealed extensive and sequential post-translational modifications (PTMs) of RIP140. Some of these PTMs affect RIP140's sub-cellular distribution and biological activities that contribute to the development and progression of metabolic diseases. The biological activity of RIP140 that translocates to the cytoplasm in adipocytes is to regulate glucose uptake, adiponectin secretion and lipolysis. Accumulation of RIP140 in the cytoplasm promotes adipocyte dysfunctions, and provides a biomarker of early stages of metabolic diseases. Administering compounds that reduce cytoplasmic accumulation of RIP140 in high fat diet-fed animals can ameliorate metabolic dysfunctions, manifested in improving insulin sensitivity and adiponectin secretion, and reducing incidences of hepatic steatosis. This review summarizes studies demonstrating RIP140's PTMs and biological activities in the cytoplasm of adipocyte, signaling pathways stimulating these PTMs, and a proof-of-concept that targeting cytoplasmic RIP140 can be an effective strategy in managing metabolic diseases.

Keywords

RIP140; Post translational modification (PTM); Adipocyte; Type II diabetes mellitus (T2DM); Endothelin-1 (ET-1); Ambresentan; GLUT4; Adiponectin; Nuclear translocation; Biomarker; Therapeutics

INTRODUCTION

Adipocyte Dysfunctions and Metabolic Disorders

Metabolic syndrome, also known as syndrome X, presents one of the most challenging health issues in the modern society. Patients with metabolic syndrome tend to have multiple metabolic and inflammatory disorders, including type II diabetes mellitus (T2DM), atherosclerosis, hypertension, cardiomyopathy, rheumatoid arthritis and Alzheimer's diseases [1–3]. The main features of metabolic syndrome include the decrease in circulating

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CONFLICT OF INTEREST

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HDL and the increase in circulating triglyceride (TG), low density lipoprotein (LDL) and fasting blood glucose (4). Epidemiological and etiological analyses reveal that obesity is the major trigger for metabolic syndrome. Mechanistically, it is known that the enlarged and dysfunctional adipose tissues play a critical role in aggravating metabolic disorders [1, 5].

Adipose tissues, initially considered as inert tissues that merely store energy as TG, consist of two major types, white (WAT) and brown (BAT) adipose tissues, that have distinct functions in modulating whole body metabolism and energy homeostasis. BAT has higher mitochondrial activities and expresses marginally detectable levels of RIP140. Upon exposure to a low temperature, BAT utilizes TG to produce heat and maintain body temperature. Conversely, WAT stores energy after food intake and expresses much higher levels of RIP140. The stored energy is usually utilized during fasting, through TG hydrolysis [1, 6], to release glycerol and free fatty acids. Released fatty acids provide energy for other tissues and can produce ketone body in the liver. In addition to storing and utilizing lipid, there are also important metabolic processes occurred in adipocytes. For instance, insulin can promote glucose transporter 4 (GLUT4) trafficking to facilitate glucose uptake in adipocytes. In fact, insulin-stimulated glucose uptake in adipocytes is vital to the maintenance of systemic insulin sensitivity [7]. To this end, a defect in GLUT4 trafficking or loss of GLUT4 expression in adipocytes can also induce systemic insulin resistance in muscle and liver, which results in diabetes [1, 7]. Additionally, adipocytes, mainly white adipocytes, can secrete various cytokines such as adiponectin, resistin, and leptin to modulate multiple physiological processes including systemic insulin sensitivity, glucose and lipid metabolism, inflammatory potential, and animal behavior [1, 8–10]. Adiponectin can elevate systemic insulin sensitivity, improve endothelial function, and produce anti-inflammatory effects - all are protective against the development of metabolic and cardiovascular diseases (e.g., cardiac hypertrophy, ischemic heart disease and heart failure) [9, 11, 12]. Leptin can down regulate appetite, exacerbate inflammatory response, modulate bone mass, and affect puberty [13, 14].

Dysfunctions in adipocyte could be detrimental because of the potential to damage a wide spectrum of physiological processes. High caloric diet-induced adipocyte dysfunctions include reduced glucose uptake, increased release of free fatty acids, and dramatic changes in their secreted adipokine profiles [1]. In obese individuals and patients of T2DM, adiponectin secretion is decreased but leptin secretion is increased despite the increase in their adipose tissue mass, which further aggravates disease progression [1]. Disturbing adipocyte lipid storage and glucose uptake can elevate systemic insulin resistance and elicit lipotoxicity in liver and muscle [1, 15, 16]. Deregulating lipolysis can dampen cardiac function by increasing aberrant lipid deposition in cardiomyocytes. The accumulation of lipid in heart eventually causes lipotoxic cardiomyopathy, one metabolic disorder with a high mortality [5, 16, 17]. All these studies show that maintaining normal functions of adipocytes is vital to the homeostatic control of metabolism, behavior, and cardiovascular functions. An interesting theory suggests that obesity, which usually is associated with enlarged adipocytes, may reflect the adaptation of the system to reduce lipotoxicity and can be protective because it may reduce over-nutrition induced metabolic burden [16, 18]. However, after prolonged morbid obesity, adipocytes reduce the ability to store TG and

uptake glucose, and alter their secreted adipokine profiles, thereby contributing to the development of metabolic diseases.

The Physiological Role of RIP140

Receptor-interacting protein 140 (RIP140), also known as receptor interacting protein 1 (NRIP1) was initially identified as a coregulatory protein of estrogen receptor α (ER α) and orphan nuclear receptor TR2 [19–21]. Subsequently, RIP140 was found to interact with a wide spectrum of transcription factors including all the nuclear receptors tested. In most of these studies, RIP140 was shown to exert a corepressive activity for target gene transcription, primarily by recruiting repressive co-regulatory molecules such as HDAC3, CtBP and CDK8 [22–24]. In contrast, several studies showed that RIP140 could also act as a transcription coactivator, most notably for NF- κ B's activation of proinflammatory cytokine production in macrophages [25, 26]. RIP140 expression was detected mainly in ovary, heart and metabolic tissues such as adipose, muscle, and liver. In adipocytes, RIP140 was found to repress the expression of UCP1 and GLUT4 to reduce mitochondria activity and glucose uptake, respectively [27, 28], and to increase the expression of key lipogenic enzymes to promote lipogenesis [27, 29]. The physiological role for RIP140 in ovulation and metabolism was demonstrated in studies using RIP140-null mice. These whole body RIP140 knockout mice were defected in ovulation and resistant to high-fat diet (HFD)-induced T2DM and associated metabolic disorders [27, 30–32]. Interestingly, RIP140 appeared to also regulate gluconeogenesis and lipid storage in hepatocytes, could be involved in the metabolic response during starvation and in cancer cachexia [33], and might affect skeletal muscle and cardiac muscle metabolism to modulate muscle strength and cardiac functions [2, 34]. All these studies suggest that RIP140 is involved in diet-induced diabetes and metabolic diseases through its nuclear activity in adipocytes, liver and muscle.

A systemic mass-spectrometry investigation revealed surprisingly complex post translational modifications (PTMs) of RIP140. The PTMs of RIP140 include phosphorylation, acetylation, methylation, PLP conjugation, ubiquitination, and sumoylation [31, 35–43], which can modulate its gene regulatory activity, protein stability, cellular distribution, and/or interacting partners (Fig. 1 and Table 1) [31, 44]. These PTMs can be categorized into two principal pathways, one is to stimulate RIP140 nuclear export and cytoplasmic activities and the second is to enhance RIP140 nuclear retention and its gene regulatory activities (Fig. 2). Conceivably, signaling pathways that elicit these PTMs of RIP140 can modulate normal adipocyte functions. This review summarizes those PTMs related to RIP140's cytoplasmic distribution and activities in adipocytes, as well as signaling pathways triggering these PTMs. The pathophysiological manifestation of these PTMs is reviewed with a particular focus on the development of metabolic diseases.

Cellular Signaling to Promote Nuclear Export of RIP140

There are various forms of PTMs that could occur on RIP140. Among these, a cascade of sequential PTMs is specifically responsible for RIP140's nuclear export, or cytoplasmic accumulation, in adipocytes. This pathway is initiated from nuclear PKC ϵ -mediated phosphorylation on Ser-102 and Ser-1003 of RIP140 in the nuclei, which triggers 14-3-3-dependent recruitment of protein arginine methyl transferase 1 (PRMT1) that methylates

Arg240, Arg650, and Arg948 of RIP140 [19, 20]. After arginine methylation, the modified RIP140 is then exported to the cytoplasm by associating with exportin 1. It appears that, along 3T3-L1 adipocyte differentiation processes, RIP140 expression is gradually elevated and increasingly exported to the cytoplasm, in consistence with the increase in its serine phosphorylation followed by subsequent arginine methylation [40]. The functional relevance of these enzymes was demonstrated by silencing or inhibiting PKC ϵ or PRMT1, which reduced the cytoplasmic RIP140 level by enhancing its nuclear retention, resulting in increased lipid accumulation in adipocytes.

In mice, HFD feeding could increase nuclear PKC ϵ activity, which elevates the cytoplasmic RIP140 level in epididymal adipose tissue [45]. This may be due to accumulated intracellular DAG that stimulates PKC ϵ [46], the principal trigger for RIP140's nuclear export pathway. This is supported by the finding that a DAG kinase inhibitor (that blocks DAG catabolism to elevate DAG level) could enhance RIP140 accumulation in the cytoplasm [26]. In addition to intrinsic signals like intracellular lipid accumulation, circulating endothelin-1 (ET-1) can also provide an extrinsic signal to activate PKC ϵ in adipocytes (Fig. 2) [26, 47]. ET-1, the most potent vasoconstrictor secreted by endothelial cells, regulates important biological processes such as wound healing and regulation of blood vessel tone. ET-1 is the major therapeutic target in managing pulmonary hypertension and pulmonary fibrosis [48–50]. Clinically, obese and T2DM patients have higher levels of circulating ET-1. Several studies have suggested that elevated circulating ET-1 might contribute to adipocyte dysfunctions [50–52]. In agreement with these suggestions, we have demonstrated that ET-1 indeed stimulates the accumulation of RIP140 in the cytoplasm of adipocytes [47]. It appears that after binding to ET_A receptor on adipocytes, ET-1 activates PLC β to produce DAG, which activates nuclear PKC ϵ [47]. Accordingly, targeting either PLC β or PKC ϵ by inhibitors or siRNAs can effectively diminish ET-1-induced accumulation of RIP140 in the cytoplasm of adipocytes. Importantly, we have demonstrated that a clinically approved ET_A antagonist, ambrisentan, effectively blocks the accumulation of cytoplasmic RIP140 in primary epididymal adipose tissues in HFD-fed mice and abrogates the progression of metabolic syndromes in these animals (see the following section). Altogether, these studies demonstrated two major pathways that can stimulate RIP140's nuclear export: intrinsic and extrinsic pathways. The intrinsic pathway is initiated by the accumulation of intracellular lipid content, especially the DAG level, whereas the extrinsic pathway is elicited by circulating ET-1. Both pathways utilize nuclear PKC ϵ to promote the accumulation of RIP140 in the cytoplasm, supporting PKC ϵ -PRMT1 as the principal pathway to modulate RIP140's nuclear export *in vitro* and *in vivo*. In addition to ET-1, several inflammatory cytokines such as RANTES and CCL5 can also activate PLC β [53, 54]. Therefore, inflammatory cytokines may also stimulate nuclear export of RIP140 to affect adipocyte functions.

Functions of Cytoplasmic RIP140 in Adipocytes

In studies using ultracentrifugation to separate subcellular fractions of adipocytes, RIP140 was detected in fractions of nucleus, cytosol, endoplasmic reticulum and lipid droplet, but not in mitochondria. This result was supported in immunochemical analyses of adipocytes co-labeled with markers of specific organelles [26]. The pattern of sub-cellular distribution

of RIP140 in adipocytes suggests that RIP140 may interact with a wide spectrum of proteins to exert distinct functions in different subcellular locations (Fig. 3). By examining RIP140-interacting proteins identified from a yeast two hybrid screening, we have determined an important functional role for RIP140 in adipocytes, mediated by its interacting with an Akt substrate AS160 [45]. AS160, a GTPase-activating protein (GAP), can dampen vesicle transport, docking and tethering by inactivating different Rab GTPases. AS160's activity can be blocked by Akt-mediated phosphorylation [7, 45, 55], which is negated by its interacting with RIP140 (see following). In adipocytes, GLUT4 transporter is carried in specialized vesicles (GLUT4 vesicles) whose trafficking to plasma membrane can be inhibited by AS160 in resting cells. Upon insulin stimulation, Akt is activated to phosphorylate AS160 whose activity (inactivating Rab GTPases) is then inhibited, which leads to a robust increase in GLUT4 vesicle trafficking [7, 55]. Relevant to this pathway, cytoplasmic RIP140 competes with Akt for AS160 binding, rendering hypo-phosphorylation of AS160 even after insulin stimulation, which maintains AS160 activity to impede GLUT4 vesicle trafficking and glucose uptake [45]. In agreement with this result, RIP140-null adipocytes rescued with a mutated RIP140 that could not be exported to the cytoplasm have increased glucose uptake, as compared to RIP140-null adipocytes rescued with the wild-type RIP140. Since a short-term HFD readily stimulates RIP140's nuclear export in adipocytes, the accumulation of RIP140 in the cytoplasm may in fact indicate an early, pre-diabetic condition that impedes adipocytes' normal functions.

Another function of RIP140 is to decrease adiponectin secretion. Adiponectin is stored in trafficking adiponectin vesicles around the trans-Golgi networks (TGNs); trafficking of these vesicles is also stimulated by insulin [11]. Cytoplasmic RIP140 also retards adiponectin secretion from adipocytes [56]. Interestingly, this inhibition is not caused by ER retention or changes in disulfide bond formation for adiponectin oligomers. Rather, cytoplasmic RIP140 inhibits adiponectin secretion by maintaining AS160 activity that also modulates the traffic of adiponectin vesicles. Therefore, by interacting with AS160, cytoplasmic RIP140 can reduce adiponectin secretion and GLUT4 vesicle trafficking. However, the specific Rabs regulating adiponectin vesicles remain to be determined [55].

The third cytoplasmic function of RIP140 in adipocytes is exerted through its association with lipid droplets (LDs) *via* direct interaction with perilipin A [26]. Perilipin A, a structural protein of lipid droplets, can regulate the formation of LDs. By interacting with perilipin A, RIP140 promotes lipolysis through recruiting hormone-sensitive lipase (HSL) to LDs and releasing the comparative gene identification-58 (CGI-58), an activator of adipose triglyceride lipase (ATGL), to activate ATGL. These dynamic changes in molecular interactions result in increased substrate availability for HSL and the activation of ATGL. As such, nuclear RIP140 acts to promote lipogenesis in differentiating adipocytes; but in fully differentiated adipocytes, RIP140 leaves the nucleus, rendering reduced lipogenesis [38–40]. Furthermore, the accumulation of RIP140 in the cytoplasm would promote lipolysis to further reduce the lipid contents. RIP140 located to the cytoplasm also impedes glucose uptake, which would decrease the available carbon source for lipogenesis and further contribute to diminished lipid accumulation. Therefore, the net outcome of exporting RIP140 from the nucleus to the cytoplasm in adipocytes is to reduce their overall lipid load,

which could be important for their survival and in maintaining normal functions. It would be interesting to determine whether cytoplasmic RIP140 may modulate adipocytes' survival.

Cytoplasmic RIP140 as A New Therapeutic Target in Managing Metabolic Diseases

HFD feeding stimulates RIP140's accumulation in the cytoplasm of adipocytes through both intrinsic (intracellular lipid content) and extrinsic (circulating ET-1) stimulations; the cytoplasmic RIP140 could dampen glucose uptake and adiponectin secretion and increase lipolysis. As such, the accumulation of RIP140 in the cytoplasm of adipocytes may serve as a biomarker of early stages of metabolic disease. Accordingly, reducing cytoplasmic RIP140 in adipocytes might be beneficial. To provide a proof-of-concept, we have shown that a clinically approved drug that blocks ET-1 signaling, ET_A antagonist ambrisentan, can substantially reduce HFD-induced cytoplasmic accumulation of RIP140 in their epididymal adipose tissues [47]. Further, primary adipocytes from ambrisentan-treated mice are more efficient in glucose uptake, and these animals have higher concentrations of circulating adiponectin, fewer inflammatory signs (such as crown like structures) in their epididymal adipose tissues, better glucose tolerance, and fewer incidences of liver steatosis, as compared to the placebo control (saline) or the control treatment (hydralazine) [47]. This study demonstrates the benefit of blocking nuclear export of RIP140 to prevent and manage diet-induced diabetes and other metabolic disorders. However, it remains to be determined whether ET_A agonists would enhance lipid accumulation by retaining nuclear RIP140. A better understanding of the control of nuclear export of RIP140 could be helpful to the design of therapeutic drugs that may modify the disease course of metabolic syndromes related to adipocyte dysfunctions and uncontrolled lipogenesis. Novel drugs may be developed to harness the benefits of increasing lipid loads with reduced detrimental effects such as lipolysis and insulin resistance in adipocytes.

CONCLUSION

RIP140 plays important roles in metabolism and can contribute to the development of metabolic diseases [25, 26, 28, 30, 31, 45]. Recent studies have further revealed surprisingly complicated PTMs of RIP140, as well as complex signaling pathways leading to these PTMs. Of particular significance is the wide spectrum of biological consequences elicited by these PTMs. To this end, accumulation of RIP140 in adipocyte cytoplasm can be detrimental because of its effects on multiple processes aggravating the severity of metabolic diseases, such as reducing glucose uptake and adiponectin secretion and increasing lipolysis. Since PTMs of RIP140 in adipocytes are triggered by alterations in the pathophysiological and/or nutritional status, drugs targeting any step in the signaling cascades triggering these PTMs could be useful in managing metabolic diseases. However, it is unclear whether RIP140 can be exported to the cytoplasm in other types of cells such as macrophages that also express high levels of RIP140. It is also unclear whether RIP140 could affect cytoplasmic processes in cell types other than adipocytes. Understanding signal specificity in a particular biological context involving RIP140 is vital to the development of therapeutics that could have a greater therapeutic index. It has been suggested that RIP140 antagonizes another metabolic regulator PGC-1; it will be interesting to investigate whether the activity of RIP140 in the

cytoplasm can be regulated by PGC-1 and if RIP140 may cooperate with PGC-1 to modulate the metabolic status of adipocytes.

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Fig. (1).
Schematic illustration of post-translational modifications (PTMs) of RIP140. The boxes indicate the full length RIP140 with repressive domains 1 to 4 (RDs 1–4).

Fig. (2).

Sequential phosphorylation then methylation promotes nuclear export of RIP140. Activated nuclear PKCε phosphorylates RIP140 on Ser-102 and Ser-1003. Phosphorylation on RIP140 then facilitates its recruiting PRMT1 through the action of 14-3-3. The associated PRMT1 triggers arginine methylation on Arg-240, Arg-650, and Arg-948. The arginine-methylated RIP140 recruits exportin-1, leading to nuclear export of RIP140.

Fig. (3).

HFD-induced accumulation of RIP140 in the cytoplasm and the biological activities of cytoplasmic RIP140 in adipocytes. HFD-feeding elevates circulating ET-1 and DAG levels. Increase in ET-1 and intracellular DAG activates PKC ϵ , initiating the signaling pathway to stimulate nuclear export of RIP140. In the cytoplasm, RIP140 can interact with AS160 to block the traffic of GLUT4 and adiponectin vesicles. Cytoplasmic RIP140 can also interact with perilipin on the surface of lipid droplets to increase lipolysis. Ambrisentan blocks ET-1 signal to reduce RIP140's accumulation in the cytoplasm, thereby lessening the severity of HFD-induced metabolic diseases.

Table 1

Post-translational Modifications of RIP140

Modification	Residues	Enzyme	Function
Phosphorylation	S102, S1003	PKC ϵ	Nuclear export
	T202, T207	Erk2	Transrepression
	S104, S358, S380, S488	?	?
	S519, S531, S543, S672		
	Y364, Y418, Y436	Sky	Ubiquitination
Methylation	R240, R650, R948 P	PRMT1	Nuclear export
	K591, K653, K757	?	Transrepression
Acetylation	K158, K287	P300	Transrepression
	K111, K311, K482, K529	?	?
	K 687, K932		
PLP conjugation	K613	?	Transrepression
Sumoylation	K757, K1157	?	Transrepression