The function of miRNAs and their potential as therapeutic targets in burn-induced insulin resistance (Review)

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Abstract. Burns are common accidental injuries. The main clinical manifestations of severe burn injury are insulin resistance and high metabolism. Insulin resistance results in hyperglycemia, which may lead to skeletal muscle wasting and suspended wound healing. It also elevates the risk of infection and sepsis. Studies have indicated that insulin receptor (IR) and insulin receptor substrate 1 (IRS1) are essential factors involved in the regulation of blood glucose levels. Moreover, the suppression of the IR/IRS1 signaling pathway results in insulin resistance. Recent studies have also indicated that miRNAs, which are small non-coding RNAs consisting of 20-23 nucleotides, target the 3'-untranslated region (3'-UTR) of *IRS1* mRNA and attenuate protein translation. miRNAs also play an important role in the development of type II diabetes (T2D) and obesity-induced insulin resistance. In the present review, we discuss the involvement of miRNAs in burn-induced insulin resistance through the targeting of the IR/IRS1 signaling pathway. We also discuss the possibility of miRNAs a novel therapeutic target in insulin resistance in burn patients.

Contents

1. Introduction
2. Insulin resistance is a direct consequence of burn injury
3. Involvement of the insulin signaling pathway in glucose metabolism
4. Suppression of the insulin signaling pathway by burn injuries
5. Involvement of miRNAs in the regulation of insulin resistance
6. Potential role of miRNAs in clinical therapy
7. Conclusions

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1. Introduction

Mature miRNAs are small non-coding RNAs that consist of 19-23 nucleotides. The first miRNAs were characterized in *C. elegans* in 1993 (1). Subsequently, an increasing number of miRNAs were identified in plants and animals. To date, over 1,000 miRNAs have been identified in humans, which regulate approximately 60% of mammalian gene expression (2,3). miRNA genes are transcribed as primary miRNAs by RNA polymerase II (4). Following transcription, the precursor miRNA (stem-loop with approximately 80 nucleotides) is generated by Drosha (5). Subsequently, the precursor miRNA is exported from the nucleus to the cytoplasm and digested into a mature miRNA by Dicer, an RNA polymerase III enzyme (6). The main function of miRNAs is to regulate gene expression at the translational level. miRNAs can bind to the 3'-untranslated region (3'-UTR) of a target mRNA and suppress its translation (1). Recent studies have indicated that miRNAs play an essential role in a variety of diseases, including cancer (7), type I (8) and type II diabetes (T2D) (9), autoimmunity disease (10), and cardiovascular diseases (11). Thus, miRNAs have a potential effect on clinical diagnosis, prognosis and therapy.

Burn injury is a complex trauma which is caused by factors such as heat, electricity, chemicals and radiation (12). Inflammation is one of the host responses to injury, and following burn injury, the levels of inflammatory mediators, such as tumor necrosis factor α (TNF-α) (13), transformation growth factor β (TGF-β) (14), interleukin (IL)-2 and IL-6 (15), are markedly increased. Burn injury also leads to cardiovascular damage and enhances vascular permeability, which results in the loss of body fluid (16). Clinical studies have demonstrated that burn injury may induce insulin resistance and affect glucose and fat metabolism (17,18). Insulin resistance contributes to the attenuation of wound healing, which enhances the risk of infection (19). As previously demonstrated, a topical insulin injection can accelerate wound healing in diabetes through the activation of the Akt and Erk signaling pathways (20).

Previous studies have indicated that miRNAs play a critical role in regulating insulin resistance induced by mitochondrial dysfunction or diabetes through the inhibition of insulin receptor substrate 1 (IRS1) protein translation (21,22). The suppression of the insulin receptor (IR)/IRS1 signaling pathway is the key mechanism responsible for burn-induced

insulin resistance; therefore, miRNAs may also be implicated in burn-induced insulin resistance. The identification of the specific miRNAs which are involved in burn-induced insulin resistance may lead to the development of novel therapeutic targets for clinical therapy.

2. Insulin resistance is a direct consequence of burn injury

A previous study using a rat model demonstrated that no difference in insulin secretion was detected between the sham and burn groups; however, the sensitivity of insulin was significantly suppressed in the burn group (23). Insulin resistance and hyperglycemia are crucial risk factors for increased mortality in patients with severe burn injuries (24). In another study on burned children, it was demonstrated that insulin resistance can last up to 3 years (25). Intensive insulin therapy is an efficient manner to control the blood glucose of severely burned patients, as it can decrease the risk of infection and sepsis, improve hepatic and rental function, and suppress acute inflammation (24).

3. Involvement of the insulin signaling pathway in glucose metabolism

Glucose is one of the most important energy sources for the human organism. It is usually stored in the liver and muscle cells in the form of glycogen (26). After eating, elevated blood glucose is converted into glycogen (27). During the conversion, blood glucose is firstly transported into cells by the plasma membrane protein glucose transporter (GLUT), and its four isoforms, GLUT1, GLUT2, GLUT3 and GLUT4, have been well-characterized (28). GLUT4 is primarily expressed in muscle and fat cells. The insulin signaling pathway plays an important role in regulating its translocation (Fig. 1). Insulin binds to the IR and induces the autophosphorylation of the receptor at tyrosine residues (29). Following autophosphorylation, the receptor further recruits the IRS and promotes its phosphorylation at tyrosine residues (30). Phosphorylated IRS subsequently binds to the regulatory subunit, p85, of the phosphoinositide-3 kinase (PI3K) and activates its catalytic subunit p110, which is responsible for stimulating the phosphoinositide-dependent kinase (PDK) (31). As the upstream kinase of Akt, activated PDK promotes the phosphorylation of Akt at Thr308 and Ser473 (32), and phosphorylated Akt mediates the translocation of GLUT from the cytoplasm to the membrane (33) (Fig. 1). Apart from its role in GLUT translocation, Akt has also been implicated in regulating glycogen synthesis. Glycogen synthase (GS) is a key enzyme involved in converting glucose into glycogen, and there are two isoforms in mammals, the muscle isoform (34) and the liver isoform (35). Both isoforms are inactivated due to phosphorylation at the NH2- or COOH-terminal residues mediated by glycogen synthesis kinase 3 (GSK3) (36). Insulin dephosphorylates and restores the function of GS through Akt- or protein kinase A-mediated phoshorylation and the inactivation of GSK3 (37,38). In skeletal muscle, insulin enhances glycogen synthesis in the absence of GSK3 phosphorylation (39). Glucose-6 phosphate induces glycogen synthesis through the activation of GS in a cyclic AMP-stimulated protein kinase-dependent manner (40).

4. Suppression of the insulin signaling pathway by burn injuries

After a burn injury is sustained, the activation of the insulin signaling pathway is significantly suppressed, and blood glucose levels are markedly increased (41). The results from experiments carried out in our, as well as other laboratories have indicated that the levels of lipopolysaccharides (LPS), TNF-α and interleukins are increased following burn injury (13,15). These factors are involved in the regulation of the phosphorylation and degradation of IRS1 which, in turn, results in insulin resistance (Fig. 2). LPS, the stimulator of inducible nitric oxide synthase (iNOS), plays an essential role in inducing hyperglycemia and insulin resistance, which can be restored by the iNOS inhibitor (42,43). iNOS also enhances the ubiquitination and degradation of IRS1 (44). The deficiency of iNOS attenuates the burn-induced skeletal muscle insulin resistance (45). Glucose uptake is decreased following exposure to TNF-α (46). TNF-α inhibits the autophosphorylation of IR and its substrate IRS1 tyrosine phosphorylation (46). It can also suppress the function of IRS1 by triggering phosphorylation at Ser307 which induces the degradation of IRS1 (47-49). IL-6 has been shown to induce insulin resistance in HepG2 cells (50) and 3T3-L1 adipocytes (51), which can promote IRS1 degradation by upregulating the expression of the suppressor of cytokine signaling 3 (SOCS3) (52). IL-1β induces adipocyte insulin resistance through the downregulation of IRS1 (53). Due to the burn-induced IRS1 degradation, the interaction between IRS1 and PI3K is repressed, and insulin stimulates PKB/Akt activation, which is impaired after burn injury (54). Subsequently, the phosphorylation of GSK3β at Ser9 mediated by activated Akt is decreased; the enhanced activity of GSK3β has been detected in the skeletal muscle of rats following burn injury (55). Thus, the phosphorylation of GS mediated by GSK3 is augmented, and the conversion of glucose into glycogen is significantly attenuated (Fig. 2). Taken together, these data indicate that the TNF-α-, LPS- or IL-induced IRS1 protein degradation is involved in promoting burn-induced insulin resistance and hyperglycemia.

5. Involvement of miRNAs in the regulation of insulin resistance

As small non-coding RNAs, miRNAs play a pivotal role in post-transcriptional regulation. miRNAs can bind to and promote the deadenylation and degradation of target mRNAs (56,57). Translational repression is another important function of miRNAs. It can directly bind to the 3'-UTR of target mRNAs and inhibit the translational initiation (58). Studies have demonstrated that miRNAs are involved in the regulation of multiple insulin resistance-induced diseases (Fig. 3). In T2D, miR-144 has been shown to promote insulin resistance by directly targeting *IRS1* mRNA (22). The suppression of IRS1 mediated by miR-126 has also been shown to result in mitochondrial dysfunction and insulin resistance (21). The sitmulation of Akt activation by insulin is critical for glycometabolism, and the obesity-induced miR-143 overexpression has been shown to lead to hyperglycemia by inactivating the Akt signaling pathway (59). A study using let-7 family transgenic mice demonstrated that let-7 overexpression
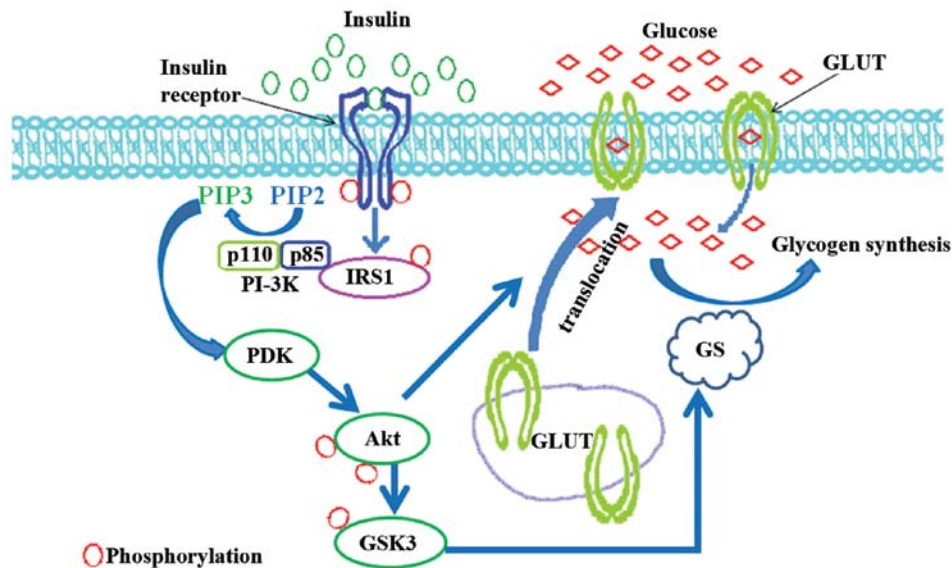
Figure 1. Schematic diagram of the inolvement of hte insulin signaling pathway in the regulation of glucose transport and glycogen synthesis. Insulin binds to its transmembrane receptor, insulin receptor (IR), and promotes its autophosphorylation at tyrosine residues (p-IR). Activated p-IR recruits IR substrate (IRS) and enhances its activation by mediating its phosphorylation (p-IRS). p-IRS subsequently binds to p85, the regulatory subunit of phosphoinositide-3 kinase (PI3K), and elevates the activation of its catalytic subunit p110, which subsequently activates phosphoinositide-dependent kinase (PDK). As the upstream kinase of Akt, PDK promotes the phosphorylation of Akt (p-Akt) at Thr308 and Ser473. Activated Akt regulates glucose metabolism in two pathways. One is promoting glucose transporter GLUT translocation from the cytoplasm to the membrane, which mediates glucose uptake; another one is repressing the function of glycogen synthesis kinase 3 (GSK3) by enhancing its phosphorylation at Ser9, and then enhancing the activation of GS and promoting glycogen synthesis.
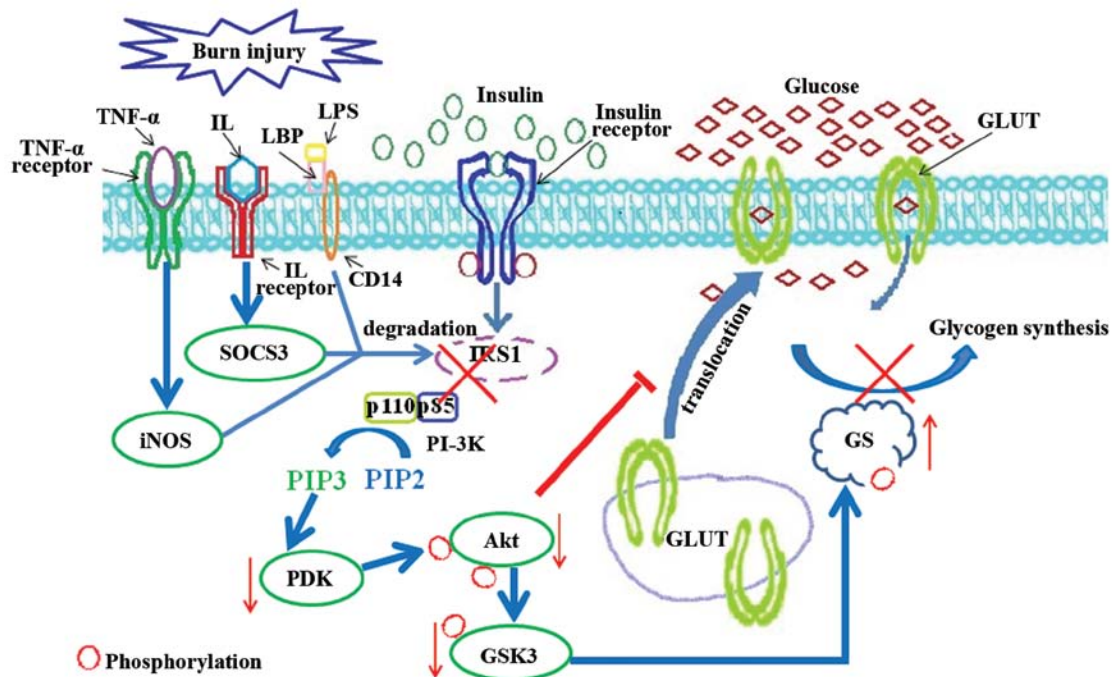

Figure 2. Schematic diagram of burn-induced insulin resistance through the suppression of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway mediated by insulin receptor substrate 1 (IRS1) degradation. After burn injury, the blood concentration of tumor necrosis factor (TNF)-α, interleukin (IL) and lipopolysacchride (LPS) is significantly increased. TNF-α binds to its receptor and induces inducible nitric oxide synthase (iNOS) production. ILs, IL-6 in particular, bind to corresponding receptors and promote the expression of suppressor of cytokine signaling-3 (SOCS3). LPS binds to CD14 with the help of the LPS binding protein (LBP) and activates the intracellular signaling pathway. All the cytokines induce IRS1 protein degradation, which suppresses the PI3K/Akt signaling pathway, and subsequently suppresses glucose transporter (GLUT) translocation and glycogen synthesis, which results in burn-induced insulin resistance and hyperglycemia.

may contribute to the development of T2D (60). Protein tyrosine phosphatase 1B (PTP1B) impairs the insulin signaling pathway through the dephosphorylation of IR at tyrosine residues. The 3'-UTR of *PTP1B* mRNA is the target of miR-122,
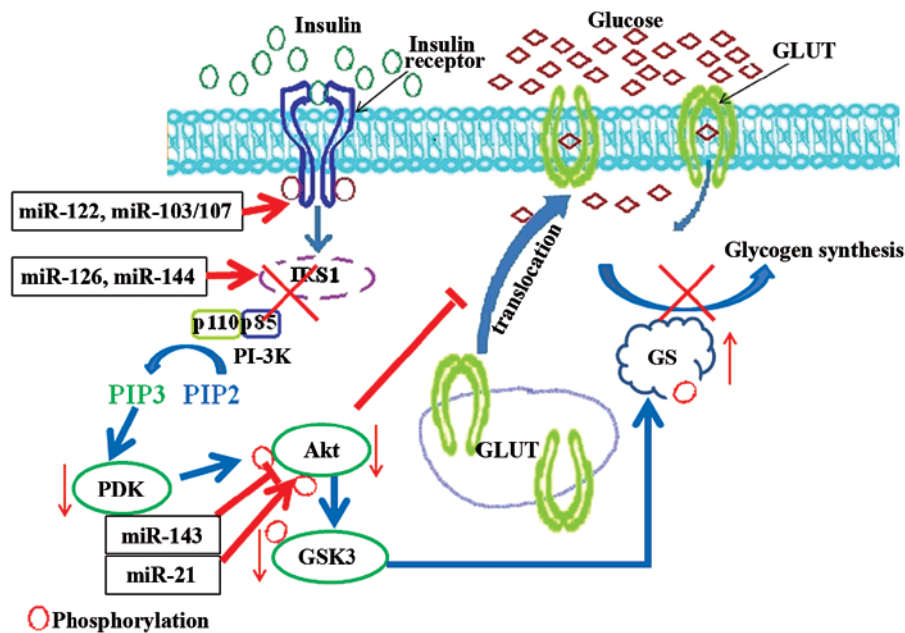
Figure 3. The miRNAs involved in regulating the insulin signaling pathway and glucose metabolism. miRNAs play an important role in the regulation of insulin signaling. Protein tyrosine phosphatase 1B (PTP1B) is the phosphatase of the insulin receptor (IR), and miR-122 inhibits the production of PTP1B and promotes the activation of the insulin signaling pathway. miR-103/107 also elevates the caveolin-mediated IR activation and enhances insulin sensitivity. miR-126 and miR-144 directly bind to the 3'-untranslated region (3'-UTR) of insulin receptor substrate 1 (IRS1) mRNA and suppress its translation, which abrogates the connection with phosphoinositide-3 kinase (PI-3K) and induces insulin resistance. In obesity-induced insulin resistance, upregulated miR-143 impairs the insulin-induced Akt activation. In adipocytes, The repressed expression of miR-21, which directly targets the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), the key phosphotase of Akt, results in Akt inactivation and impairs insulin-mediated glucose metabolism.

and decreased miR-122 expression has been shown to result in hepatic insulin resistance (61). In mouse models of obesity, miR-103/107 is upregulated. The blockage of miR-103/107 has been shown to promote insulin sensitivity by elevating caveolin-1-mediated IR activation (62). Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), the direct target of miR-21 (63), is the key phosphotase of Akt which can negatively regulate the Akt signaling pathway. In insulin-resistant adipocytes, the suppressed expression of miR-21 and impaired Akt signaling pathway has been observed (64). The transport of glucose is also regulated by miRNAs, and the transmembrane protein GLUTs play an essential role in glucose transport. Elevated miR-133 levels have been shown to reduce the insulin-stimulated glucose uptake by downregulating GLUT4 expression (65). In cardiomyocytes, miR-223 has been shown to promote GLUT4 expression and increase glucose uptake (66). Insulin resistance is the intrinsic complication of polycystic ovary syndrome (PCOS), and overexpressed miR-93 in patients with PCOS binds to the 3'-UTR of *GLUT4* mRNA and reduces its protein translation (67). Microarray analysis has further indicated that the expression of several miRNAs is altered following burn injury. In comparison with normal skin tissue, a total of 32 upregluated and 34 downregulated miRNAs were identified in the skin tissue of patients who sustained burn injuries (68). The expression levels of miR-144 in the skin tissue of the burned patients, which can directly target the 3'-UTR of *IRS1* mRNA (22), were 16-fold higher than those in normal skin tissue (68). This suggests that miRNAs, such as miR-144, play an essential role in promoting burn-induced insulin resistance by suppressing the activation of the IR/IRS signaling pathway.

6. Potential role of miRNAs in clinical therapy

Although miRNAs were only discovered 20 years ago, their molecular mechanisms of action involving the repression of target gene expression have been elucidated. Moreover, evidence indicates that miRNAs are associated with the development of a number of human diseases. The process from the time of discovery of an miRNA to the development of clinical therapeutic drug targets is rapidly approaching. miR-34a has been shown to suppress the development of prostate and lung cancer (69,70), and the use of a miR-34a mimic is currently in the developmental stage for cancer therapy (www. iptonline. com, The Therapeutic Potential of microRNAs). Another miRNA, miR-208, which is involved in promoting chronic heart failure (71), is also undergoing investigation in preclinical trials (www. iptonline.com, The Therapeutic Potential of microRNAs). Its antagonist is expected to be used in the therapy of heart disease. miR-122 has been shown to be associated with hepatitis C virus infection (72), and its antagonist, miravirsen, has undergone phase II clinical trials for the therapy of patients with hepatitis C virus (73). Miravirsen may be the first miRNA-related drug for clinical therapy. Taken together, the mimics or antagonists of miRNAs are expected to be widely used in clinical therapy.

7. Conclusions

In the response to burn injury, the expression of several miRNAs, including insulin resistance-associated miRNAs, is altered. This change may play a pivotal role in mediating burn-induced insulin resistance, which results in hyperglycemia and reduces

wound healing. Further research focusing on the involvement of miRNAs in the regulation of burn-induced insulin resistance may lead to the development of novel therapeutic targets for the treatment of burn injuries.

Acknowledgements

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