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Protein kinases: mechanisms and downstream targets in inflammation mediated obesity and insulin resistance

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Abstract

Obesity induced low-grade inflammation (metaflammation) impairs insulin receptor signaling (IRS). This has been implicated in the development of insulin resistance. Insulin signaling in the target tissues is mediated by stress kinases such as p38 mitogen-activated protein kinase (MAPK), c-Jun NH2-terminal kinase (JNK), inhibitor of NF-kB kinase complex beta (IKKβ), AMP activated protein kinase (AMPK), protein kinase C (PKC), Rho associated coiled-coil containing protein kinase (ROCK) and RNA-activated protein kinase (PKR), etc. Most of these kinases phosphorylate several key regulators in glucose homeostasis. The phosphorylation of serine residues in the insulin receptor (IR) and IRS-1 molecule results in diminished enzymatic activity in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. This has been one of the key mechanisms observed in the tissues that are implicated in insulin resistance especially in Type II Diabetes Mellitus (T2-DM). Identifying the specific protein kinases involved in obesity induced chronic inflammation may help in developing the targeted drug therapies to minimize the insulin resistance. This review is focused on the protein kinases involved in the inflammatory cascade and molecular mechanisms and their downstream targets with special reference to obesity induced T2-DM.

Keywords

Obesity; Insulin resistance; Inflammation; Protein kinases

Introduction

Obesity has been implicated as a major risk factor for the development of metabolic syndrome. The incidence of obesity and metabolic syndrome are increasing rapidly in the developed as well as in developing countries [1]. It has been estimated that more than one third of adults and 17% of young population in the United States are obese. Obesity has been

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recognized as a major health problem in US [2]. Obesity-induced chronic inflammation is critical in the pathogenesis of insulin resistance, diabetes, and metabolic syndrome.

Inflammatory cell infiltration and activation of the pro-inflammatory cytokine network in hypertrophied and hyperplasic adipocytes in obesity along with hypoxia, oxidant stress, and endoplasmic reticulum (ER) stress leads to chronic inflammation [3]. Diseases such as rheumatoid arthritis, multiple sclerosis and type-2 diabetes (T2-DM) are known to be induced by low grade chronic inflammation [4]. Low grade chronic inflammation leads to impaired insulin receptor signaling and metabolic instability termed as “metaflammation” [5]. The intracellular signal transduction pathways are usually activated in response to the inflammation resulting in increased secretion of pro-inflammatory cytokines. Pro-inflammatory cytokines further mediate the cascade of events leading to insulin resistance [6].

Insulin signaling is essential for maintaining glucose homeostasis and the regulation of its metabolism in the liver, muscle and adipose tissues. Inflammation and pro-inflammatory cytokines affect the insulin signaling, thereby modulating glucose absorption [7]. Additionally, the inflammatory signal transduction cascade is known to function at both cellular and molecular level. Hence understanding the regulation of the inflammatory response and its effects on insulin signaling is essential towards developing novel therapeutic approaches to treat the inflammatory mediated metabolic conditions such as T2-DM. This review highlights the major signaling pathways associated with insulin resistance.

Immune cells and cytokines in obesity

The key immune cell population that regulates chronic inflammation and insulin resistance includes macrophages, T cells, dendritic cells (DCs), natural killer T cells (NKTs), B cells, neutrophils, and eosinophils. Apart from their role in mediating the immune response, cytokines secreted by these cells play a major role in obesity induced T2-DM [8]. Studies have shown that the number of tissue macrophages directly correlated with the adipose size in obesity [9]. The hypertrophied adipocyte induced inflammation increases circulating cytokines (TNF-α, IL-6 and IL-1β) and chemokines (MCP-1) [10] levels, ultimately leading macrophage recruitment. Further, macrophage recruitment can also be mediated by neutrophil elastase, a protease secreted by neutrophils plays a potential role in insulin resistance [11]. This contributes to the increased number of tissue macrophages and pro-inflammatory environment thereby playing a key and important role in insulin resistance [12].

The T-cell profile in adipose tissue also plays an important role in obesity and insulin resistance. Pathogenic $CD4^+$ and $CD8^+$ T cells have been implicated in obesity-associated inflammation. $CD4^+$ T cells in adipose tissue secretes IFN-γ and induces the polarization of macrophages towards the M1 classically activated phenotype [13]. Fabbrini et al., [14] demonstrated that there was an increase in Th17 and Th22 cells in adipose tissue of metabolically abnormal insulin-resistant obese subjects. Additionally, the obese state is also characterized by an increased accumulation of Th1 cells and a reduction of regulatory T cells. This changes may influence the cytokine level and their effect on inflammation and

insulin resistance [15]. Increased IL-6 and STAT3 levels from T cells subsets have been identified as key mediators in insulin resistance associated with obesity [16]. The pro-inflammatory cytokines from T cells can be synergistically augmented by B cells. DeFuria *et al.*, [17] reported that B cells increase the pro-inflammatory T-cell function in obesity/T2-DM through contact-dependent mechanisms; thereby mediating the obesity induced insulin resistance. NKT cells in adipose tissue were recently identified to be involved in the development of inflammation mediated by obesity, but the molecular mechanism behind the principle remains uncertain [18]. Adipose tissue DCs play a prominent role in obesity mediated chronic inflammation by increasing the production of pro-inflammatory Th17 cells [19].

Thus T cells contribute to obesity induced insulin resistance by increasing the pro-inflammatory M1 macrophages through pro-inflammatory cytokines. This increased pro-inflammatory cytokines and M1 macrophages further enhancing the inflammation. Thus inflammatory cells amplify the axes of inflammation and also contribute in the development of obesity induced insulin resistance.

Protein kinases and inflammatory signaling

The inflammatory signals perceived at cellular level are mediated by the corresponding cytokine and chemokine receptors and intracellular specific kinases. The pro-inflammatory cytokine induced activation of receptors such as IL-1, toll like receptor-4, TNF-α and IL-6 leads to stimulation of downstream protein kinases. These protein kinases further increase the release of additional mediators which increases inflammation [8] and potentially results in insulin resistance. Thus the status of insulin resistance is determined by the type of activated kinases and their downstream regulation [20, 21].

Apart from inflammatory cells, the abnormalities in the lipid metabolism and lipid metabolites do play a role in the pathophysiology of insulin resistance [22]. Inflammatory cells and higher levels of saturated fatty acids (SFA) and lipids, and ceramide (composed of sphingosine and a fatty acid) interlinks the inflammatory and protein kinase pathways involved in obesity induced insulin resistance. Stimulation with SFA, ceramides and liposaccharide (LPS) in pro-inflammatory M1 macrophages induces pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) through inflammatory protein kinase cascades [23]. Therefore, SFA and its derivative ceramide converge with inflammatory pathway IKKβ/NFκB, JNK1/AP1 and PKC signaling pathways to stimulate inflammation and thus can potentially induce insulin resistance [24].

Insulin sensitivity and resistance depends on the specific kinases such as AMP-activated protein kinase (AMPK), IκB kinase (IKK), protein kinase C (PKC) and mitogen-activated protein kinases (MAPKs) that acts on the insulin receptor substrate [8]. Similarly the role of Rho associated coiled-coil containing protein kinase (ROCK), [25, 26] and RNA-activated protein kinase (PKR) [27] in pathogenesis of insulin resistance has been documented. Insulin mediated activation of intrinsic tyrosine kinase in the insulin receptor (IR) leads to tyrosine phosphorylation of IRS1. This further activates its substrates phosphatidylinositol 3-kinase (PI3K) and Akt, leading to increased glycogen synthesis, glucose uptake and protein

synthesis [28]. AMPK is considered a positive regulator of insulin sensitivity. It is reported to be associated with increased GLUT4 translocation and subsequent glucose uptake [29]. Studies reported that dysregulation of AMPK as the central mechanism behind the insulin resistance mediated diabetes [30].

The mitogen-activated protein kinases (MAPKs) are well known for their role in inflammatory responses through phosphorylation of serine/threonine residues of target proteins [31]. The deficiency of MAPKs has been associated with reduced insulin sensitivity [32]. In insulin resistant conditions, serine kinases such as IB kinase (IKK) and JNK become activated by pro-inflammatory stimuli [33] and impair insulin sensitivity. Thus, understanding the interplay between inflammation, obesity and protein kinases, and identifying the specific protein kinases involved in phosphorylation may help in developing the targeted drug therapies to minimize the insulin resistance. This might play a key role in the prevention of diabetes.

AMP activated protein kinase (AMPK) and its upstream kinases

AMPK is a serine/threonine kinase composed of α, β and γ subunits [34]. The AMPKs are activated in the cytoplasm by upstream kinase mediated phosphorylation of Thr^{172} on the serine/threonine protein kinase domain at its N-terminus [35]. The upstream kinases of AMPKs includes liver kinase B1 (LKB1), the calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ), and the transforming growth factor-beta-activated kinase 1 (TAK1) [36–38]. The role of AMPK and its upstream kinases using *in-vitro* and *in-vivo* models are highlighted in Table 1.

Many studies have demonstrated the role of activated AMPK in regulating the glucose metabolism. Activated AMPK controls the glucose uptake by increasing expression and translocating GLUT4 or by phosphorylation of the 160 kDa Akt substrate [44] (Fig. 1). AMPK also contributes to glucose control by reducing gluconeogenesis through suppression of the expression of glycolytic genes encoding phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [45]. AMPK enhances insulin sensitivity either by directly regulating PI3K or by suppressing the negative feedback loop of IRS1 regulation via inhibition of mTOR/S6K [46]. Along with regulating insulin signaling, AMPK is an upstream kinase for metabolic enzymes such as acetyl-CoA carboxylase (ACC) [47] and HMG-CoA reductase [48]. It also regulates the fatty acid synthesis and plays a potential role in hepatic steatosis [49]. Glucagon mediated inhibitory protein kinase (PKA) increases the inhibitory phosphorylation of AMPK Ser^{173} and reduces the activating phosphorylation of AMPK Thr^{172} in the regulation of insulin signaling [50].

Expression of AMPK is essential for decreasing the pro-inflammatory stimuli (TNFα, IL-6 and IL-1) and increasing the production of the anti-inflammatory cytokine, IL-10 [51, 52]. Stimulation of inflammatory signals down regulates the AMPK activity [51]. The reduction in expression/activity of AMPK in inflammatory cells in obesity can potentially lead to development of inflammation induced diabetes [53]. The pro-inflammatory cytokine, TNFα, reduces the phosphorylation of AMPK Thr^{172} that was reported to suppress the activation of AMPK via protein phosphatase 2C (PP2C) [54]. Increased TNFα leads to PP2C mediated

inactivation of AMPK, which increases fatty acid levels and potentially insulin resistance. However, such reduction in AMPK activity was not noticed in TNF receptor knockdown mice which indicates that AMPK reduction is multifactorial and several other pro and anti-inflammatory mediators may play important role. A recent study shows that AMPK in macrophage promotes FA oxidation to reduce macrophage inflammation and insulin resistance [55].

AMPK regulates insulin homeostasis by reducing the phosphorylation of mTOR in the cytoplasm. Increased mTOR signaling has been implicated in the pathogenesis of obesity and the development of insulin resistance in metabolic syndrome [56] and modulation of mTOR signaling may suppress insulin resistance and T2-DM [57] The disruption of mTORC1 signaling in macrophages reduce inflammation and insulin resistance by inhibiting JNK/NFκB pathway activation in obesity [58]. The interplay between NF-κB signaling pathway and liver AMPK/mTOR/autophagy axis in relation to hepatic steatosis and insulin resistance has also been discussed [59]. The role of suppressing mTOR/SREBP-1 mediated lipogenesis in the liver and restoring insulin signaling in skeletal muscle has also been shown [60]. These studies suggest the role of mTOR in insulin resistance pathogenesis. Understanding the mTOR signaling may provide future therapeutics and interventions against obesity, insulin resistance, and diabetes.

Most of the anti-diabetic drugs reported to date target and activate AMPK indirectly by inhibiting mitochondrial Complex I. Side effects of the existing drugs could be eliminated by identifying anti-diabetic agents which could activate AMPK directly without affecting mitochondrial respiration [61]. Though activation of muscle and liver AMPK is advantageous [62] in terms of glucose uptake, its activation in pancreas inhibits glucose stimulated insulin secretion and β-cell function *in vivo* [42]. Treatment with Astragalus polysaccharide induces phosphorylation of AMPK Thr^{172} along with the upstream kinases CaMKKβ and LKB1 and results in increased glucose uptake [63]. Since inhibition of satellite cell-specific AMPKα1 is involved in the obesity induced muscle degeneration, preventing its inhibition and enhancing the activation of AMPK may be useful [64].

In summary, AMPK is a positive regulator of glucose uptake and its activity depends on its phosphorylation state and inflammatory molecules are the major contributor of AMPK inhibitory phosphorylation. Other than the inflammatory molecules, kinases mediating the synthesis of inflammatory cytokines are also considered as a source of AMPK inhibitory phosphorylation. Thus decrease in AMPK activation leads to activation of pro-inflammatory downstream signaling pathways like IKK, PKCs, MAPK (ERK, JNK, p38) and results in obesity induced insulin resistance.

IκB kinase (IKK)

The transcription factor NF-κB is an important mediator of the inflammatory response. Under normal conditions NF-κB remains inactive in the cytoplasm by interacting with IκB proteins. The subtypes of IκB include IκBα, IκBβ and IκBε and their phosphorylation is essential for activating NF-κB. Phosphorylated IκB undergoes ubiquitination mediated proteasomal degradation which allows for NF-κB translocation into the nucleus [65, 66].

IκB kinase (IKK) has been identified as the upstream kinase of IκB. To date, four different IKK namely IKKα, IKKβ, IKKε and TANK-binding kinase 1(TBK1) have been reported [67].

In addition to NF-κB activation, IKKs are also involved in the phosphorylation of other substrates [68], one such target being IRS1 [69]. IKKβ-mediated serine phosphorylation of IRS1 inhibits tyrosine phosphorylation of IRS1 and subsequent insulin signaling (Fig. 2). The emerging studies from the past decade have revealed the role of IKK mediated activation of NF-kB in obesity induced metabolic disorders especially diabetes. Chiang *et al.* [70] reported the role IKKβ in high-fat diet induced NF-kB activation subsequently increased IKKβ in liver, adipocytes and adipose tissue macrophages suggesting it as an attractive therapeutic target for obesity induced diabetes.

Studies with IKKβ knockdown mice revealed an increase in the level of adiponectin and hepatic insulin sensitivity [71]. Absence of IKKβ in hepatocytes provides local insulin responsiveness, whereas absence of IKKβ in myeloid cells provides protection towards the global insulin resistance in a mouse model [72]. Knocking out the interferon regulatory factors 3 (IRF3) results in diet induced hepatic insulin resistance and steatosis. Conversely over-expressed IRF3 interacts with IKKβ in the cytoplasm and preserves glucose and lipid homeostasis in liver [73]. The cytoplasmic interaction of IRF3 and the kinase domain of IKKβ represses the inhibitor of nuclear factor kappa B kinase beta subunit/nuclear factor kappa B (IKKβ/NF-κB) signaling and plays a regulatory role in insulin resistance. Kamon *et al.* [74] documented that inhibition of IKKβ ameliorates TNFα-mediated down-regulation of adiponectin secretion and stimulates insulin-stimulated Akt activity in adipocytes. This suggests that inhibitors of IKKβ reduce insulin resistance by targeting the TNFα signaling pathway. In normal conditions the IKKβ/NF-kB complex remains inactive in the hypothalamus, whereas in obese condition IKKβ/NF-kB gets activated and disrupts insulin/leptin signaling [75]. Recent report have shown that using SC-514 [76] and peptide-based inhibition [77] of IKK attenuated NF-κB activation with suppressed inflammation and reduced development of long-term diabetes inflammatory complications.

Though there are reports on IKKβ as a potent target of obesity induced insulin resistance, Rohl *et al.* [78] noticed that expression of muscular IKKβ is not essential for obesity induced insulin resistance. In addition to the existing, firmly established IKKα and IKKβ, Reilly et al., [67] explained the role of the additional kinase IKKε. Regulation of IKKε in the hypothalamus of obese mice has been recently analyzed and reported as the main inflammatory mediator in the hypothalamus. In both liver and fat of IKKε knockdown mice, the modulated gene was noticed as the target gene for IRFs regulation but its mechanism remained unclear [79]. Studies by Chiang et al., [70] revealed higher levels of IKKε in liver and adipose tissue of high-fat diet induced obese animal models. IKKε knockout (KO) mice are protected from the diet induced obesity and insulin resistance. Inhibition of IKKε with either chemical means on small interfering RNA suggested a reduction in the inhibitory phosphorylation of IRS1Ser^{307} and insulin resistance via IR/IRS-1/Akt and JAK2/STAT3 pathways [80].

These results and literature clearly suggest that IKK plays a tissue specific role and identification of the correct IKK isoform and its specific inhibitor with global effect can prevent obesity induced insulin resistance and subsequent development of metabolic syndrome. Furthermore, TNFα produced via IKK signaling inhibits AMPK Thr^{172} phosphorylation and increases the level of saturated fatty acid (SFA) [23]. Thus suppression of IKK in target tissues associated with obesity can be a potential therapeutic target for the treatment of insulin resistance in obesity.

Role of Protein kinase C in obesity and insulin resistance

Fatty acid metabolites are known to impair insulin signaling by tissue-specific activation of specific isoforms of protein kinase C (PKC) in the cytoplasm. Lipid dependent PKC activation has been associated with insulin resistance in type 2 diabetics [81]. To date, 12 isoforms of three major groups: conventional/Ca^{2+} and phospholipid dependent (α, βI, βII and γ), novel/Ca^{2+} independent and phospholipid dependent (δ, ε, η, θ and μ), and atypical/Ca^{2+} and phospholipid independent (ζ and ι/λ) have been reported [82].

In skeletal muscle, PKCα is the most abundant among the various isoforms of PKC and its activation is associated with high FFA content. Activation of PKCα mediates the phosphorylation of IRS1 (IRS1 Ser^{307}, IRS1Ser^{1101}) [28]. Similar to PKCα, PKC-θ was noticed to be activated by cytosolic diacylglycerol and induced by direct phosphorylation of IRS1Ser^{1101} [83]. The isoform PKCβ is the upstream kinase for Jun amino-terminal kinase, IKK mitogen/extracellular-regulated kinase. These isoforms promote the respective Ser^{307} and Ser^{612} phosphorylation of IRS1 [84]. Increased expression of PKCβ is associated with the increase in white adipose tissue mass and its knock-out results in reduction in triglyceride level and WAT mass [85].

Among the various isoforms of PKC, the skeletal muscle isoform was found to be enriched with the novel/Ca^{2+} independent and phospholipid dependent PKC-θ [86], while the PKCε was shown to be expressed in liver, adipocytes and 3T3 fibroblasts [87]. Kumashiro et al., [88] reported that activation of PKCε increased proportionally with hepatic diacylglycerol (DAG) content, which is a predictor of insulin resistance. Using antisense oligonucleotides against liver PKCε, it has been demonstrated that targeting PKCε with a specific inhibitor could be a novel therapeutic target for fat-induced hepatic insulin resistance and type 2 diabetes [87]. Although other PKCs are reported to induce insulin resistance by mediating inhibitory phosphorylation of IRS $Ser^{307/612/1101}$, PKCε mediates insulin resistance by directly targeting the expression of insulin receptor (IR) [28] (Fig. 3). However, some studies also reported the inverse correlation between IR expression and PKCε level in diabetic obese rats has also been reported [89].

The High-mobility group AT-hook 1 (HMGA1) protein has been recognized as a transcriptional regulator of IR gene expression and its defect decreases IR expression with an increase in type 2 diabetes mellitus susceptibility [90]. Under the phosphorylated state, the DNA binding ability of HMGA1 declines and thus the mechanism behind the phosphorylation/dephosphorylation of HMGA1 will determine the expression of IR [91]. Upon FA induced phosphorylation, PKCε translocates to the nuclear region and mediates

phosphorylation of HMGA1. The absence of a nuclear localization signal (NLS) makes it unclear as to how PKCε is mobilized to the nuclear region for HMGA1 phosphorylation. This could be explained by recent studies conducted by Dasgupta et al., [92] which reported that F-actin recognizes the phosphorylated form of PKCε and mobilizes it to the nuclear compartment.

Increased proliferation of pancreatic endocrine cells is an essential event to fulfil insulin demand. In stress-free conditions PKCδ displays a specific dual role; it mediates the inhibitory phosphorylation of $p21^{Cip1/WAF1}$ (cell cycle inhibitor) at Ser^{146} and promotes β-cell proliferation by nuclear extrusion and induced cytosolic accumulation of $p21^{Cip1/WAF1}$ [93]. Under FFA induced stress however, PKCδ promotes nuclear accumulation of pro-apoptotic factors [94]. Mingo-Sion et al., [95] reported PKCδ mediated inhibition of insulin receptor signaling in diabetic models via IRS-1 Ser312 phosphorylation. Genetic inhibition of PKCδ with RNA interference and pharmacological inhibition with rottlerin revealed a decline in $p21^{Cip1/WAF1}$ Ser^{146} phosphorylation increasing nuclear accumulation and subsequent apoptosis [96]. On the other hand, PKCδ WT transgenic mice overexpressing PKCδ increased cytosolic accumulation of the cell cycle inhibitory $p21^{Cip1/WAF1}$. Different splice variants of PKCδ involving in apoptosis (PKCδI) and pro-survival pathway (PKCδII and PKCδVIII) have also been reported [97, 98]. Patel et al. [99] demonstrated that TRA2B regulated splice variants PKCδI is essential for clonal expansion of pre-adipocytes and its expression decreases with terminal differentiation. The molecular switch which operates the dual role of PKCδ is unclear and has to be elucidated in order to facilitate favorable tuning of PKCδ activation. Further studies to identify the molecular mechanisms behind the alternative splicing will be helpful in the development of a novel therapeutic target for management of obesity induced insulin resistance.

To summarize, PKCs activation by ongoing inflammation results in IRS-1 Ser phosphorylation. This leads to decrease in binding of insulin with its receptor. Activated PKCs also enhance the production of pro-inflammatory mediators, thus increasing the inflammation contributing to obesity induced insulin resistance.

Mitogen activated protein kinase (MAPK) and insulin signaling

MAPKs have now been identified in a wide range of inflammatory disease states ranging from cancer to obesity induced diabetes. Broadly, MAPKs consist of seven families and are classified into two groups [100]. The first group, referred to as classical MAPKs, includes extracellular regulated kinase 1/2 (ERK1/2), p38 kinase, c-Jun N-terminal kinase (JNK) and ERK5. The second group, referred as atypical MAPKs, consists of ERK3, ERK4, ERK7 and Nemo-like kinase (NLK). Activation of inflammatory responsive MAPKs is mediated by protein kinase cascades containing a series of three or more upstream protein kinase; MAP3Ks activate MAPK MAP2Ks by dual phosphorylation on serine/threonine residues, MAP2Ks then activate MAPKs by dual phosphorylation on tyrosine and threonine residues. The phosphorylation in target amino acid varies with specific MAPKs. Though most of the MAPKs require dual phosphorylation of threonine and a tyrosine at T-X-Y motif, ERK3 and 4 as well as NLK require a single tyrosine as the phosphorylation site [101]. Activated MAPKs can either phosphorylate the subsequent protein kinase or non-protein kinases such

as transcriptional factors. Downstream of the activating stimuli, the kinase cascades may themselves be stimulated by combinations of small G proteins, MAP4Ks, scaffolds, or oligomerization of the MAP3K in a pathway [102]. Activation of upstream kinases of the MAPK cascade, MAPKs and the target proteins has been reviewed by Broom *et al*. [101]. The role of protein kinase Map4k4 has been further discussed in obesity-induced hyperinsulinemia and insulin resistance by promoting insulin secretion from β cells [103]. Involvement of the MAPK signaling pathways has been recently reported in mediating the beneficial effects of tartary buckwheat flavonoids on redox balance and insulin resistance in insulin-resistant HepG2 cells [104].

Role of ERKs in glucose metabolism and insulin resistance

The extracellular signal-regulated kinase (ERK) signaling pathway in the cytoplasm is activated by inflammatory signals. It activates downstream kinases and transcriptional factors, and mediates insulin resistance in target tissues (Fig. 4). Activation of ERK1/2 (p44/42 mitogen-activated protein kinases) has been characterized as a key event in insulin signaling and diabetes. ERK1/2 activation requires phosphorylation at Thr^{202} and Tyr^{204}. The activation cascade comprises of two upstream kinases, MEK1/2 and a upstream Raf kinase (ERK1/2 kinases kinases) [105, 106]. The Raf kinase is the only MAP3K recruited by receptor tyrosine kinases for activation of the ERK pathway. It plays a major role in glucose uptake and metabolism in liver and skeletal muscles, regulates insulin secretion as well as insulin production at the transcriptional and translational levels. The hormone mediated insulin secretion also increases ERK1/2 activity [107], thus playing an important role in insulin signaling and metabolism.

Elevated blood glucose levels lead to calcium entry and calcineurin induced ERK activation. The other nutrients which influence blood glucose concentration and hormones like glucagon-like peptide I (Glp1) are also involved in ERK activation [108]. The activation of ERK1/2 decreased the responsiveness of insulin gene promoter by elevated glucose levels and induce insulin resistance [109]. This inhibition of insulin gene promoter is induced by expression of CCAAT/enhancer binding protein beta (C/EBP-β). This protein binds to the insulin gene promoter in an ERK1/2-dependent manner and regulates insulin transcription in beta cells. In type II diabetes, ERK1/2 also inhibits insulin gene transcription by phosphorylating the factors that inhibit the insulin gene promoter. Insulin-activated ERK1/2 phosphorylates SREBP2 [110] and SREBP1a, thereby enhancing their transactivation potential and linking ERK1/2 signaling with key regulators of lipid metabolism. Phosphorylation of ERK1/2 is mediated by Raf, however it can be Raf independent [111]. Raf independent phosphorlylation is mediated by Bromodomain-containing protein 2 (Brd2). Brd2 is a nuclear serine/threonine kinase which also regulates the activation of ERK1/2 with special reference to adipogenesis. Although, the role of ERK and its activation in mediating the insulin resistance has been studied in detail, there is a need to further investigate the physiological significance of these modifications *in-vivo,* as well as the effect of inhibition of ERK on insulin resistance. Recently Liu *et al*., [112] reported, serum and glucocorticoid-regulated protein kinase 1 (SGK1) negatively regulate the activation of ERK1/2 and play a significant role in insulin signaling.

ERK-5 is also termed as big MAP kinase 1 (BMK1). The basic function of ERK5 relates to endothelial function and vascular integrity [113]. It has been documented that the expression of ERK5 in endothelial cells in heart, placenta, lung, kidney and skeletal muscle tissue remains dominant [114]. ERK5 could regulate the signaling either by phosphorylating further downstream kinases or by transcriptional activation of the target genes. Activation of ERK5 is similar to other MAPKs and is mediated by dual phosphorylation at Thr^{218}/Tyr^{220} via a specific upstream kinase MEK5 [115]. ERK5 has an opposing action to calcineurin on NFATc4-mediated gene transcription which changes the levels of adipokines expression. The deletion of ERK5 increased adiposity with dysregulation in adipokines secretion, leptin resistance, and impaired glucose handling [116]. It has been identified that above effect of ERK5 in diabetic nephropathy is mediated by TGFβ1 induced phosphorylation of MEK5 without involvement of the MAP3K Ras [117]. There are no extensive studies on ERK3, ERK4 and ERK7, hence there is a need to study their role in insulin resistance.

Taken together, ERK has a bimodal activity. ERK1/2 activation by obesity induced inflammation induces the production of free fatty acids and pro-inflammatory cytokines. This leads to increased inflammation and insulin resistance. Whereas, ERK5 activation alternates the nuclear translocation of AP-1 and decrease the production of pro-inflammatory cytokines.

Obesity, insulin resistance and JNK

Studies have suggested the role of cJun NH2-terminal kinase (JNK) in the mechanism of obesity-induced insulin resistance [118]. A high fat diet causes activation of the JNK1 signaling pathway leading to insulin resistance and obesity, and manifests a chronic low-grade inflammatory response. This results in activation of stress protein kinase pathways (including the cJun NH2-terminal kinase JNK1) in the cytoplasm and plays a critical role in the pathogenesis of obesity-induced insulin resistance [119] (Fig. 5). Three isoforms of JNKs include JNK1, JNK2 and JNK3. JNK1 and JNK2 are expressed ubiquitously, whereas JNK3 is expressed in a limited number of tissues, including brain and heart [20].

There are several potential mechanisms involved in JNK activation in obesity. High fat diet induces ER stress [120] and the unfolded protein response (UPR) pathway that leads to JNK1 activation [121]. Saturated fatty acids might act as ligands for toll-like receptors that activate the JNK pathway [122]. These fatty acids can also activate the JNK pathway by protein kinase C-mediated activation of the mixed-lineage protein kinase (MLK) group of MAP3Ks [123] and subsequent JNK activation is mediated by the JIP1 scaffold protein [124]. HFD-induced insulin resistance is associated with chronic low-grade inflammation and the expression of inflammatory cytokines such as tumor necrosis factor [125] and adipokines can cause JNK activation [20]. The relative contribution and mechanistic relationships between these above mentioned pathways remains to be determined.

In the experimental approach to confirm the role of JNK, it has been demonstrated that $Jnk1^{-/-}$ mice are protected against HFD-induced insulin resistance [126]. Specifically, feeding a HFD to mice with tissue-specific Jnk1-deficiency in insulin target tissues (e.g. fat and muscle) causes casual obesity, yet these mice exhibit elevated insulin sensitivity

compared with non-ablated mice [127]. In Jnk2$^{-/-}$ mice, though JNK2 is involved in metabolic regulation but its function could be masked by complete expression of JNK1 [128]. Similarly, a recent study revealed that though JNK deficiency in macrophages does not alter the obesity in HFD mice, but improves insulin sensitivity [129]. In spite of its role in the development of type 2 diabetes development, disruption of the Mapk9 gene encoding JNK2 resulted in protection against autoimmune destruction of β-cells by maintaining the Th1/Th2 balance [130]. In contrast to the well documented negative roles of JNKs (JNK1 and JNK2) in an obesity associated signaling pathway, JNK3 has been reported to be a mediator of anti-apoptotic activity in insulin-secreting cells [131] and a potential role of JNK3 as a candidate marker to assess islet quality prior to transplantation has been reported [132]. These results suggest that JNK2 is an important contributor for both T1-DM and T2-DM. Knockout and inhibition of JNK1 reduces HFD adiposity, improves glucose tolerance and insulin sensitivity suggesting the crucial role of JNK1 in development of insulin resistance.

P38 kinases and insulin resistance

The p38 is among the stress activated serine/threonine protein kinase MAPKs. Its family is composed of four members, α, β, γ and δ. Expression of the isoforms of p38 varies between tissues; the α and β isoforms are expressed ubiquitously, the γ isoform is specific to skeletal muscle and the expression of δ isoform was found in the testes, pancreas and small intestines [133]. The p38 MAPKs are activated by the MAP3K and MAP2K cascade (Fig. 6). They are subject to activation by one of the several upstream MAP3Ks such as TAK1, ASK1, LK3, MEKK1-4 and TAO1-3 [134]. The activation of p38 MAPKs results in lipotoxicity in adipocytes and stellate cells. Significantly increased phosphorylation of p38 MAPK has been found in adipose and bowel tissue [135]. The p38 MAPK plays important roles in regulating glucose metabolism in skeletal muscle and adipose tissue. Studies with cultured skeletal muscle cells revealed that p38 MAPK enhances insulin and exercise or AMPK-induced glucose uptake by activating GLUT4 [136, 137].

Insulin has been identified as a regulatory molecule for ERKs and p38 in adipogenesis, but the molecular mechanism has not been fully characterized. Recently, Liu *et al.* [138] reported the role of Steroid Receptor RNA Activator (SRA) in adipogenesis. Overexpression of SRA inhibits the phosphorylation of p38 MAPK and JNK and SRA knockdown results in reduced insulin receptor levels with decreased phosphorylation of IRS-1 and Akt resulting in the activation of p38 MAPK and JNK. Inhibition of p38MAPK has been discussed as a novel strategy to alleviate FFA induced hepatic insulin resistance in obesity-associated disorders [139]. Makeeva et al., [140] reported that nitric oxide-induced p38 activation promotes TAB1 independent activation of ERK in β-cell death resulting in decreased insulin secretion. MAPKs are important in the development of insulin resistance in skeletal muscle tissue, via a p38 MAPK-dependent mechanism.

These studies suggest the important roles of p38 MAPKs in obesity and insulin resistance. However, it has been reported that activation of p38 MAPK in the livers of obese mice reduces ER stress in obese and diabetic mice [141]. This positive effect of p38 MAPK is mediated by Thr^{48} and Ser^{61} phosphorylation of X-box binding protein 1 (XBP1s)

transcriptional factor and is associated with improvement in its nuclear migration. XBP1s is an important regulator of adiponectin multimerization and its over expression improves glucose tolerance and insulin sensitivity in both lean and obese (ob/ob) mice [142]. Since there are mixed results in relation to the role of MAPKs in obesity and insulin resistance, there is a need to further investigate their effects on cellular metabolism in obesity mediated type 2 diabetes and insulin resistance.

Rho kinase (ROCK)

Rho-kinase (ROCK) is a Serine/Threonine protein kinase with two isoforms, ROCK1 (ROCKβ) and ROCK2 (ROCKα) [143]. ROCK1 has been suggested as a novel regulator of glucose homeostasis and insulin sensitivity [25]. The depletion of ROCK1 in mice results in decreased insulin-stimulated PI3K associated IRS-1 signaling, and decreased phosphorylation of Akt, AS160, S6K, and S6 in the skeletal muscle, thereby causing systemic insulin resistance by impairing insulin signaling [25]. In muscle of obese diabetic human subjects, insulin stimulated ROCK1 was noticed to be decreased with up-regulation of its kinase inhibitor RhoE [144]. These studies suggest that decreased ROCK1 in the skeletal muscle is associated with insulin resistance. However, the role of ROCK1 in adipose tissue is suggested to be inducing insulin resistance. These have been reported in diet induced or genetically obese mice where increased ROCK1 mediated negative regulation of insulin signaling [26] The role of ROCK2 also seems to induce obesity mediated insulin resistance and cardiac dysfunction [145]. The role of ROCK in insulin homeostasis appear to be dependent on tissue specificity and isoform function [145]. Further studies are needed to determine the role of ROCK and to develop further potential target strategies to improve insulin resistance.

RNA-activated protein kinase (PKR)

Inflammatory pathways involved in obesity induced insulin resistance are also regulated by the activation of double-stranded RNA-dependent protein kinase (PKR), a pattern recognition receptor (PRR) [146]. PKR is a key constituent of the metaflammasome, activated by nutrients such as fatty acids and by ER stress [147]. The interaction between the PKR, and inflammatory kinases, such as IKK and JNK, IRS1 has also been reported [148]. The absence of PKR leads to decreased activation of JNK and IKKβ which are key kinases in the development of insulin resistance. This suggests that PKR is an important modulator of insulin signaling in obesity [27]. Activation of PKR has been associated with the inhibition of pancreatic β-cell proliferation through sumoylation-dependent stabilization of P53 in the development of T2-DM [146]. PKR also directly interacts with insulin receptor signaling components and inhibits insulin action [149]. These studies suggest the role of PKR activation in the pathogenesis of the insulin resistance, and inhibition of the PKR activation may be a potential therapeutic approach to improve insulin resistance. This has been reported in mice model with small molecules inhibitors of PKR [147].

Recent therapies targeting protein kinases to wrestle against obesity induced type2 diabetes

A wide range of therapeutic molecules targeting the phosphorylation of obesity-associated kinases has been reported to act against insulin resistance and type 2 diabetes mellitus (Table 2).

Atorvastatin, a powerful drug to decrease cholesterol level, has been identified to have anti-inflammatory potency, decreased macrophage infiltration and expression levels of TNF-alpha, IL-6, and GLUT4 in adipose tissue [165]. It is unclear whether these anti-inflammatory properties will have any long term effect on insulin resistance. Similarly, Glucagon-like peptide-1 has been developed against Type 2 diabetes and is known to induce active phosphorylation of AMPK Thr^{172}. This reduces body weight, serum FFA, and triglyceride levels [152, 166]. Unlike other compounds, Thienopyridone (Compound A-769662) is a direct activator of AMPK [167]. The main demerit of A-769662 is that it exhibits AMPK-independent effects and has poor oral absorption [168]. Recently C24 has been reported as a small molecule which was structurally optimized from PT1 to improve oral bioavailability [154]. Both PT1 and C24 caused an increase in AMPK activity and ACC phosphorylation [154, 169, 170]. Triterpenoids derived from bitter melon showed an anti-diabetic effect via CaMKKβ, a key upstream kinase responsible for the activation of AMPK [61]. In addition to IKK mediated NFκB activation, IKK is also reported to be involved in inhibitory phosphorylation of IRS1 [69], Furthermore, Kamon et al., [74] documented an increase in glucose tolerance by inhibiting IKKβ using IMD-0354. Salicylate was reported to impair FFA- induced insulin resistance by targeting IKK and preventing IKK induced serine phosphorylation of IRS-1 (Ser 307) and IRS-2 (Ser 233) [157]. Hypocrellin A, a fungal perylene quinonoid pigment from *Hypocrella bambuase* targets a specific PKC isoform other than PKC-θ and ameliorates insulin resistant induced abnormalities in glucose and lipid metabolism [86]. Two small molecules, which are inhibitors against PKC-λ/ι, have been developed by Sajan *et al.*, [159] and have been shown to prevent abdominal obesity, hepatosteatosis, hyper triglyceridemia and hypercholesterolemia in obese rodent models. Komers et al., [171] reported that p38 activity was noticed to be increased in renal cortices of diabetic rats. Similarly, a p38 MAPK inhibitor compound (PHA666859) was documented as a novel therapeutic molecule against diabetic retinopathy [172]. A recent report by Ozaki et al., [161] revealed improvement in insulin sensitivity and glucose tolerance in the diabetic mice treated with MEK inhibitor PD184352. Specific inhibition of TAK1 with administration of 5Z-7-oxozeaenol delayed the onset of autoimmune diabetes in spontaneous and cyclophosphamide-induced experimental diabetic mice [163]. Thus targeting the p38 MAPK with its specific inhibitor could be a novel strategy to protect against diabetic associated tissue damage. Bargut *et al.,* [164] have reported that fish-oil diet prevents adiposity and modulates white adipose tissue inflammation through activation of AMPK and inhibition of MAPK and RAS pathways. Metformin, a biguanide agent activates AMPK via inhibition of PKA dependent inhibitory phosphorylation of AMPK [50] and increase in LKB1 mediated AMPK activation [173]. These drugs and therapies render a hope to develop better therapies to counter the kinases involved in insulin resistance.

Conclusion

In conclusion, protein kinases play an important role in downstream signals perceived from the cell surface. The role of IKK, PKC, MAPK (ERK, JNK, p38) in inducing insulin resistance has been well established in several animal models. However, developing targeted therapies, either drugs or inhibitors, has not been very successful to date. Changes in the cell surface molecules that can further influence the cytoplasmic protein kinases can be reasonable as good therapy to prevent the development of resistance. Among the kinases reported, AMPK alone was noticed as positive regulator of insulin signaling. Hence, there is a need to study and research the role of other kinases in relation to insulin resistance, so that a potential novel therapy can be designed. It is now well established that kinase signaling is required for the metabolic events and kinase specific activation is associated with detrimental effects in obesity that contribute to Type 2 diabetes. Strategies to prevent and/or ameliorate obesity remain important in the overall treatment of insulin resistance and type 2 diabetes.

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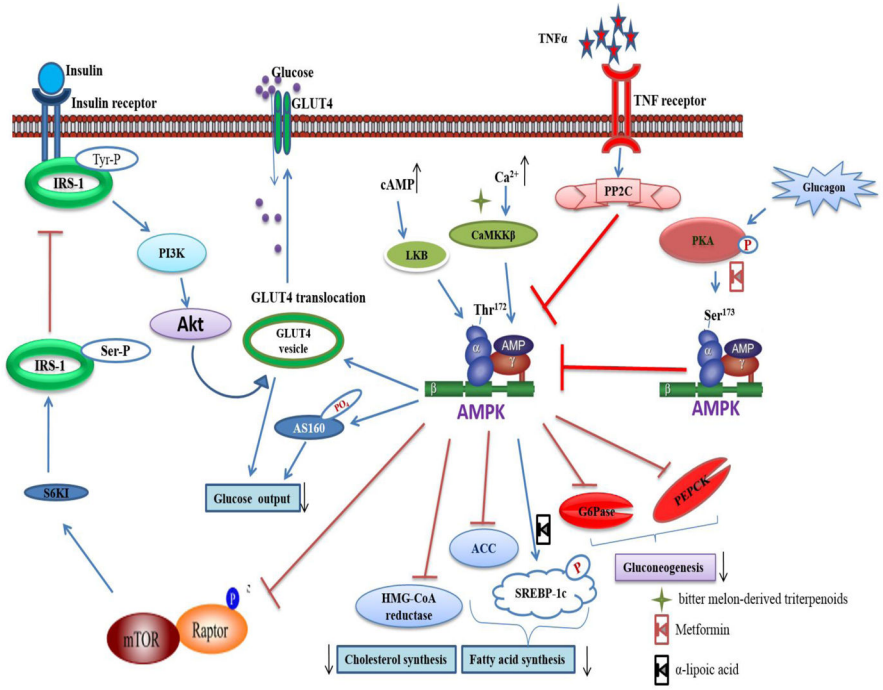

Fig. 1.

AMPK mediated regulation of glucose uptake. Under increase cAMP or Ca^{2+} content, AMPK gets activated by Thr^{172} phosphorylation via the upstream kinases LKB, CaMKKβ and TAK. The positive phosphorylation improves glucose uptake by either increasing the expression of GLUT-4 or by phosphorylation of Akt substrate of 160 kDa (AS160). AMPK promotes PI3K mediated GLUT-4 translocation indirectly by inhibiting the mTOR mediated inhibitory Ser phosphorylation of IRS-1. AMPK is the upstream kinase of factors important for gluconeogenesis, Fatty acid and cholesterol synthesis and down regulates their actions. The glucagon mediated PKA phosphorylation induces the inhibitory phosphorylation of AMPK Ser^{173} and reverses the whole positive effect of AMPK. Obesity induced low grade inflammatory signals such as TNFα are involved in PP2C mediated inhibition of AMPK Thr172 phosphorylation. The metformin has been recognized as PKA inhibitor and α-lipoic acid was noticed to inhibit SREBP activation. Whereas bitter melon-derived triterpenoids activates the CaMKKβ.

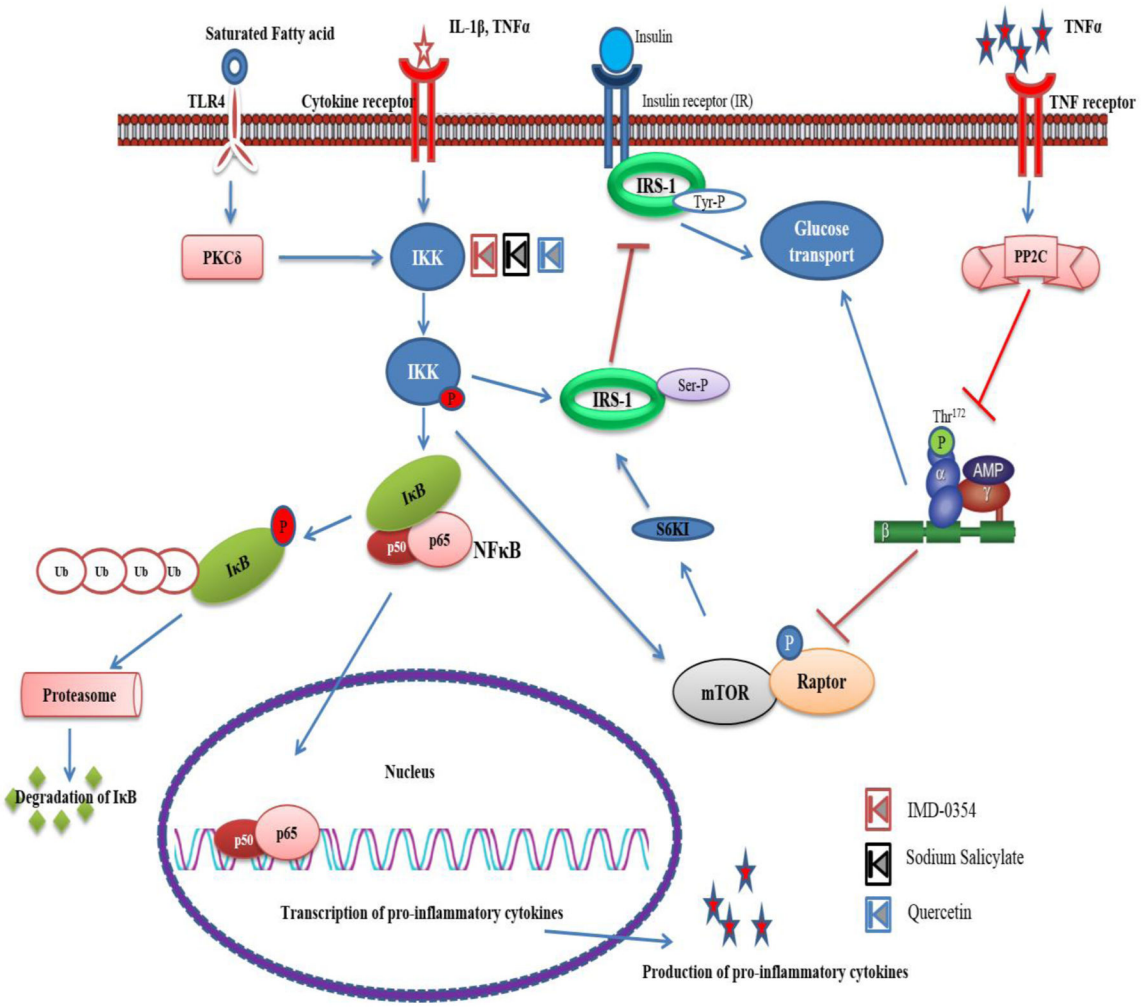

Fig. 2.

IKK mediated insulin resistance under the influence of inflammatory signal. The inflammatory signal perceived at the receptor activates IKK and leads to subsequent activation and nuclear translocation of NFκB. The NFκB mediated transcription of inflammatory cytokines mediates insulin resistance via inhibitory phosphorylation of AMPK. The compound IMD-0354, Sodium Salicylate and Quercetin inhibits the IKK.

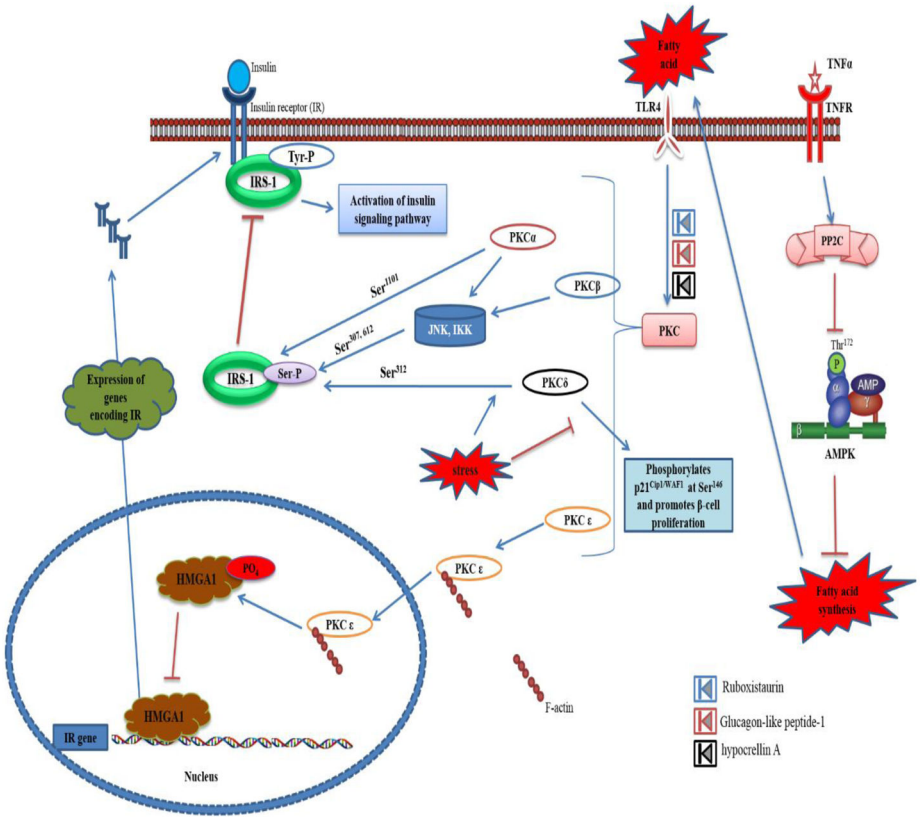

Fig. 3.

Role of PKC in insulin resistance. Upon activation by the fatty acids, PKC α,β,δ either directly involves in inhibitory phosphorylation of IRS or activates JNK or IKK for their subsequent Ser phosphorylation of IRS. Whereas the FA activated PKCε translocates to nucleus with the help of F-actin and suppresses the transcription of insulin receptor by phosphorylating the HMGA1. The activation of PKCs were inhibited by Ruboxistaurin, Glucagon-like peptide-1 and hypocrellin A.

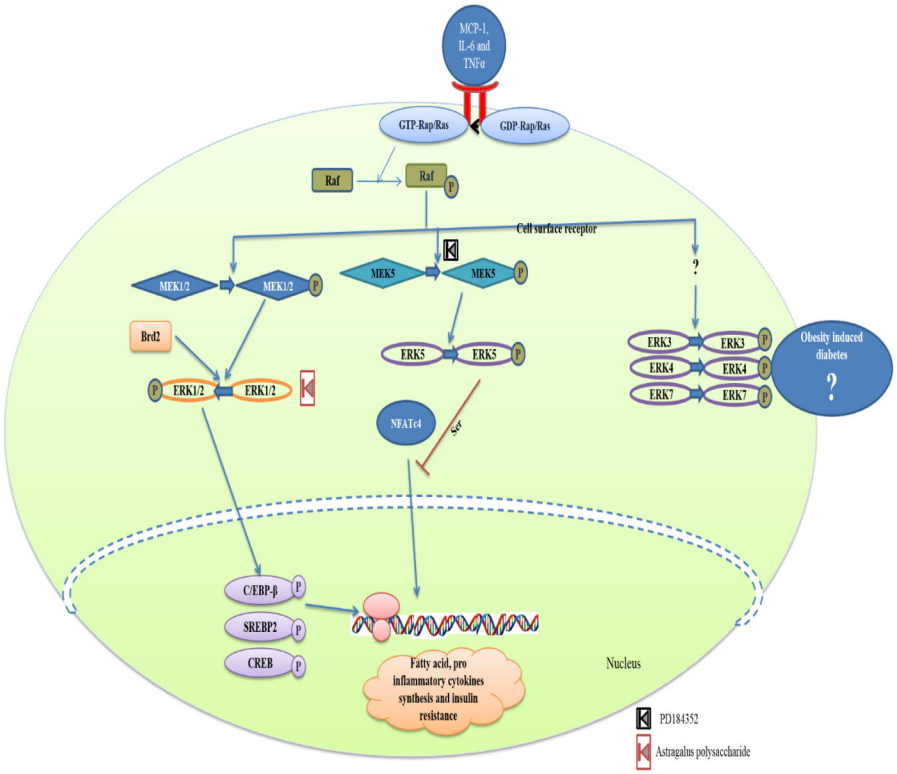

Fig. 4.

Role of ERK in obesity mediated diabetes. The inflammatory signals received as the result of obesity phosphorylates the Raf (MAP3K) via activation of G-protein coupled receptor. Activated MAP3K activates ERKs via activation of ERKs specific MAP2Ks (MEKs). The Brd involves in raf independent activation of ERKs. Activation of ERK1/2 phosphorylates its target transcriptional factors and mediates production of fatty acid, inflammatory cytokines and mediates development of Insulin resistance. ERK5 prevents the above insulin resistance by preventing the nuclear translocation of AP1 synergic partner NFATc4 via ser phosphorylation. The Astragalus polysaccharide was reported to inhibit the activity of ERK and the compound PD184352 inhibits the activity of MEK.

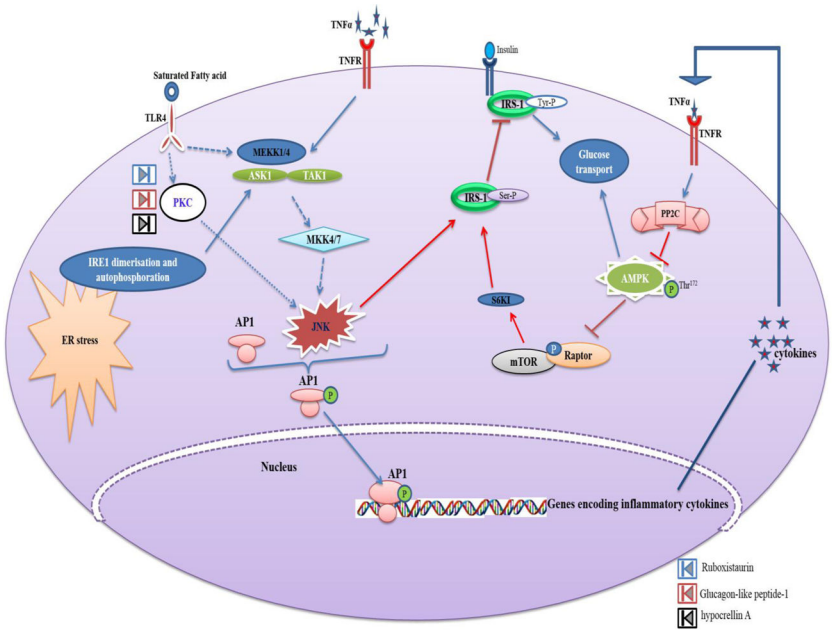

Fig. 5.

Activation of JNK in response obesity induced inflammatory signals and its role in insulin resistance. JNK gets activated by four routes; in first route the TNFα mediated activation of MAP3Ks (MEKK1/4, ASK1 and TAK-1) activates JNK via MAP2K (MKK4/7); in second route JNK activation occurs by Endoplasmic Reticulum (ER) stress mediated activation of MAP3K (ASK1); in third route, MAP3K (MEKK1/4) activated by TLR4 with perception of fatty acid signals activated the JNK; in fourth route TLR4 perceives the fatty acid signal and activates JNK independent of MAP3K via PKC. The activated JNK promotes nuclear translocation of AP1 transcription factor and mediates synthesis of inflammatory cytokines responsible for development of insulin resistance. In addition to induction of inflammatory cytokines mediated impairment in insulin signaling, JNK induces insulin resistance by direct inhibitory ser phosphorylation of IRS-1. The Ruboxistaurin, Glucagon-like peptide-1 and hypocrellin A inhibits the activity of PKC, thereby further activation of JNK will be attenuated.

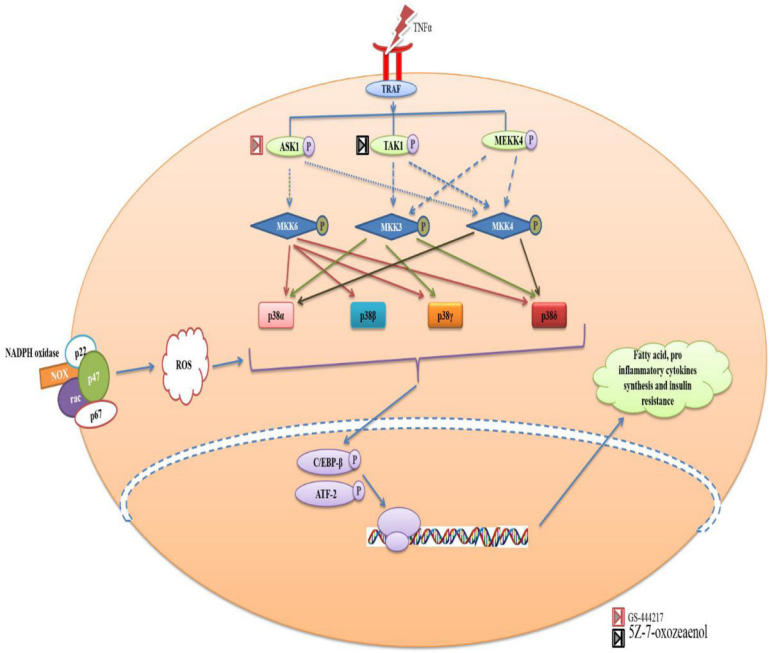

Fig. 6.

MAPK p38 activation in response to obesity induced inflammation. As a result of inflammatory stress mediated by TNFα, MAP3Ks (MKK6, TAK1 and MKK4) gets activated. Further the MAP3Ks activates the respective MAP2Ks. The phosphorylation of MAP2Ks activates the specific p38 MAPKs, which consequently activates the transcription factors of genes involved in synthesis of cytokines and fatty acid. Inspite of the inflammation mediated activations, p38 MAPKs are even activated by oxidative stress released by NADP oxidase. The compound GS-444217 inhibits the activity of ASK1 and the 5Z-7-oxozeaenol inhibits the activity of TAK1

Table 1

Functional validation of kinases in obesity associated complications

Sl. No	Research Model	Observation	Reference
1.	Over expression of AMPKα2 cDNA in mice	Decrease in blood glucose level with increase in hepatic lipid metabolism	[39]
2.	Over expression of AMPKα1 hyperlipidemic type 2 diabetic rats	Decrease in blood glucose concentration, plasma and hepatic triglyceride content.	[40]
3.	AMPK α1/2 siRNA transfected myotubes	Decreased Chlorogenic acid mediated glucose transport	[41]
4.	CaMKKβ Over expressed in CCL13 cells	Increased phosphoryation of AMPK	[37]
5.	Overexpression of AMPK in pancreatic islets of mice	Decreased β-cell function	[42]
6.	Genetic inhibition of PKC-ζ	Inhibited metformin mediated phosphorylation of AMPK-Thr^{172} and its upstream kinase	[43]

Table 2

Molecules reported with overcome obesity induces diabetes and their respective target protein kinases

Sl. No	Therapeutic molecule	Therapeutic effect	Target Kinase	Model	Reference
1.	Astragalus polysaccharide	Stimulates glucose uptake	AMPK	*In-vitro*	[63]
2.	5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR)	LKB1-independent AMPK phosphorylation	AMPK	*In-vitro*	[150]
3.	Conjugated linoleic acid	Stimulates glucose uptake	AMPK	*In-vitro*	[151]
4.	Metformin	Inhibits PKA dependent inhibitory phosphorylation of AMPK	AMPK	*In-vitro*	[50]
5.	Glucagon-like peptide-1	Decreases body weight, serum FFA, and triglyceride	AMPK	*In-vivo*	[152]
6.	Thienopyridone (Compound A-769662)	Improves glucose homeostasis	AMPK	*In-vivo*	[153]
7.	C24	reduce blood glucose and lipid levels and improved glucose tolerance	AMPK	*In-vivo*	[154]
8.	Resveratrol	Stimulates glucose uptake	AMPK	*In-vivo*	[155]
9.	α-lipoic acid	Reduces hepatic lipogenesis	AMPK	*In-vivo*	[156]
10.	Chlorogenic acid	Stimulates glucose transport in skeletal muscle	AMPK	*In-vivo*	[41]
11.	Bitter melon-derived triterpenoids	Improve glucose disposal	CaMK Kβ	*In-vivo*	[61]
12.	IMD-0354	Stimulates adiponectin levels and overcomes glucose intolerance	IKKβ	*In-vivo*	[74]
13.	Sodium Salicylate	Prevents FFA-induced hepatic insulin resistance	IKKβ	*In-vivo*	[157]
14.	Amlexanox	Increases thermogenesis and improves weight loss and insulin sensitivity	IKKε	*In-vivo*	[67]
15.	CAYMAN10576	Decreased adiposity, hypophagia and increased in energy and glucose metabolism	IKKε	*In-vivo*	[80]
16.	Ursolic acid	Restoration of insulin signaling	IKKβ	*In-vivo*	[158]
17.	Hypocrellin A	Improves glucose and lipid metabolism under insulin resistant and diabetic condition	PKC	*ex vivo*	[86]
18.	Ruboxistaurin	ameliorates insulin resistance	PKC	*In-vivo*	[158]
19.	Aurothiomalate	Prevents complications such as hyperglycemia, hyperinsulinemia, abdominal obesity, hepatosteatosis, hypertriglyceridemia, and hypercholesterolemia	PKC-λ/ι	*In-vivo*	[159]
20.	Quercetin	Prevents early diabetes tissue injury	IKK	*In-vivo*	[160]
21	PD184352	Improves insulin sensitivity and glucose tolerance	MEK	*In-vivo*	[161]
22	GS-444217	reduce renal inflammation under diabetic nephropathy condition	ASK1	*In-vivo*	[162]
23	5Z-7-oxozeaenol	Delays onset of autoimmune diabetes	TAK1	*In-vivo*	[163]
24	Fish oil	Activates AMPK, inhibits MAPK and RAS pathways	AMPK	*In-vivo*	[164]