Stress kinases in the modulation of metabolism and energy balance

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Abstract

Obesity is a new global pandemic, with growing incidence and prevalence. This disease is associated with increased risk of several pathologies, including diabetes, cardiovascular diseases, and cancer. The mechanisms underlying obesity-associated metabolic changes are the focus of efforts to identify new therapies. Stress-activated protein kinases (SAPK), including cJun N-terminal kinases (JNKs) and p38, are required for cellular responses to metabolic stress and therefore might contribute to the pathogenesis of obesity. Tissue-specific knockout models support a cell-type-specific role for JNK isoforms, in particular JNK1, highlighting its importance in cell homeostasis and organ crosstalk. However, more efforts are needed to elucidate the specific roles of other JNK isoforms and p38 family members in metabolism and obesity. This review provides an overview of the role of SAPKs in the regulation of metabolism.

Key Words

- ▶ JNK
- ▶ p38
- ▶ SAPK
- ▶ metabolism
- ▶ obesity
- ▶ stress
- ▶ adipose tissue
- ▶ brain and signal transduction

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Introduction

The totality of biochemical reactions that a cell requires to grow, survive and respond to external stimuli is known as metabolism. These processes are classified into two interdependent groups. Catabolism is the set of reactions that break down complex molecules to produce energy. This process produces NADH or ATP, which are required to synthesize macromolecules such as proteins, lipids and nucleic acids. This set of synthetic reactions is known as anabolism. Anabolism and catabolism play opposing roles in the maintenance of cell functions, but these processes are tightly coordinated in order to preserve cell homeostasis. Endocrine hormones, such as insulin, finely regulate the molecular pathways involved in the activation of anabolic and catabolic enzymes. Whenever this coordinated regulation is lost, anabolism and catabolism become unbalanced, giving rise to metabolic disorders.

Obesity, the most common metabolic disorder worldwide, develops when energy intake exceeds energy expenditure (Speakman 2010). Obesity is a major cause of morbidity and mortality, and is associated with increased risk of type 2 diabetes mellitus, heart disease, metabolic syndrome, hypertension, stroke, and certain forms of cancer (Haslam & James 2005). The design of new treatments requires knowledge of the diverse molecular mechanisms that contribute to obesity and its associated diseases.

MAPK signalling cascades transduce a variety of extracellular signals that regulate cellular responses (Sabio & Davis 2014). Three groups of MAPKs have been identified: the ERK, the p38 MAPKs, and the cJun N-terminal kinases (JNK). ERKs are mainly activated by mitogens and differentiation signals, JNK and p38 MAPK are activated by stress stimuli and are

Figure 1

Stress-activated protein kinase signaling pathway. Like all MAPK family members, the SAPKs, JNKs and p38s, are activated by a phosphorylation cascade. SAPKs are activated by stimuli such as cytokines and free fatty

acids, which are abundant in the obese state. Activation culminates with the phosphorylation of specific target proteins, inducing the appropriate cell response depending on the cell stimulus.

collectively named stress-activated protein kinases (SAPK) (Fig. 1) (Sabio & Davis 2014). The SAPK family is composed of the four isoforms of p38 (p38 alpha, beta, gamma and delta) and the three isoforms of JNK (JNK1, 2, and 3) (Paul *et al.* 1997). While the JNK pathway has been extensively studied and JNK1 has been implicated in the development of obesity and insulin resistance (Sabio & Davis 2010), the role of p38 MAPKs in this context has received less attention. Obesity results in a low-grade inflammation state (Neels & Olefsky 2006), with increased levels of proinflammatory cytokines such as tumour necrosis factor alpha (TNF α) and interleukin 6 (IL6) and of circulating free fatty acids (FFA), causing the activation of stress-induced mechanisms in the cell (Hotamisligil 2006).

cJun N-terminal kinases

Three genes encode the members of JNK family. While *Jnk1* and *Jnk2* are ubiquitously expressed, *Jnk3* is

specifically expressed in brain, testis and heart (Davis 2000, Chang & Karin 2001). Each JNK member can be expressed in several splice variants, which can be separated in two groups based on their size: the short forms of about 46 kDa (JNK1 α 1, JNK1 β 1, JNK2 α 1, JNK2 β 1, JNK3 α 1) and the long forms of about 54 kDa (JNK1 α 2, JNK1 β 2, JNK2 α 2, JNK2 β 2, JNK3 α 2) (Gupta *et al.* 1996, Yu *et al.* 2004). Many cells types express at least four JNK splice variants, with JNK1 variants predominating over JNK2 variants among the short forms and the reverse pattern observed for the long forms (Gupta *et al.* 1996, Yu *et al.* 2004). The function and the exact modulation of each of these splice variants is still unsolved.

JNKs are activated in response to a wide range of stimuli, such as cytokines (IL6, TNF α), environmental stresses (hypoxia, u.v. and ionizing radiation), toxins, drugs and metabolic changes, including obesity and hyperlipidaemia. JNKs are directly activated by two upstream MAP kinase kinase (MAP2K) enzymes, MKK4

and MKK7, through a dual phosphorylation on tyrosine and threonine in the conserved Thr-Pro-Tyr motif in their activation loop (Paul *et al.* 1997). The more than 50 proteins identified as JNK substrates include c-Jun, which once phosphorylated can dimerize with JunB, JunD or c-Fos to form the transcription factor activator protein-1 (AP-1) (Hotamisligil *et al.* 1996a); insulin receptor substrate 1 (IRS1) (Kim *et al.* 2004b); c-myc; p53, and numerous transcription factors (Davis 2000, Chang & Karin 2001, Bogoyevitch & Kobe 2006). Through the phosphorylation of their targets, JNKs induce a specific cell response to the specific stimulus, characterized by apoptosis, cell proliferation, and cell migration.

Despite its importance for brain function (Pirianov *et al.* 2007, Yoon *et al.* 2012), the JNK3 isoform as yet has no demonstrated role in metabolism. In contrast, a large body of literature attests to the key roles of JNK1 and JNK2 isoforms as regulators during obesity-induced inflammation and metabolic disorders (Tuncman *et al.* 2006,

Singh *et al.* 2009, Czaja 2010, Han *et al.* 2013). The JNK pathway is strongly activated in liver, adipose tissue and muscle during dietary and genetically-induced obesity (Hirosumi *et al.* 2002). Hirosumi *et al.* (2002) showed that mice with whole-body knockout of JNK1, but not JNK2, are protected against obesity and insulin. This finding correlates with a reduced total JNK activation in JNK1 knockout, whereas JNK2 knockout mice do not show this effect. Moreover, JNK1 knockout mice gain less weight (Hirosumi *et al.* 2002) and are resistant to liver steatosis induced by a diet deficient in methionine and choline (Schattenberg *et al.* 2006). Here, we provide an overview of the roles of JNK1 and JNK2 in metabolism and metabolic disorders (Fig. 2).

Liver

In mammals, the liver plays an essential role in metabolism, and is central to the maintenance of glucose

Figure 2

Organ-specific roles of JNK isoforms in metabolism. JNK proteins play a pivotal role in metabolism. Functions of JNK1 and JNK2 are organ- and cell-specific, and have been extensively studied in tissue-specific knockout mouse models.

and lipid homeostasis in the body. Hepatic insulin resistance and non-alcoholic fatty liver disease (NAFLD) are the most frequent liver consequences of the obese state. Using adenoviruses to overexpress or downregulate JNK, Nakatani *et al.* (2004) defined a central role for the hepatic JNK pathway in determining whole-body insulin sensitivity. These authors showed that hepatic overexpression of WT JNK induces insulin resistance in non-diabetic mice, whereas suppression of the JNK pathway in liver ameliorated insulin resistance in genetically obese mice (Nakatani *et al.* 2004). Moreover, studies in whole-body JNK1-knockout mice have shown that this kinase controls lipid accumulation and lipid uptake in liver (Imbernon *et al.* 2013). These findings indicate that the role of JNKs isoforms can differ between cell types. In fact, the phenotype of whole-body JNK1-knockout mice (Hirosumi *et al.* 2002) and adenovirus-treated mice (Nakatani *et al.* 2004) could be due to the ablation of JNK1 in parenchymal cells or extra hepatic tissues, highlighting the importance of cell and organ crosstalk. Hepatocyte-specific deletion of JNK1 provided further information about its role in liver steatosis and insulin resistance during obesity. Intriguingly, these mice displayed increased insulin resistance, glucose intolerance and hepatic steatosis, showing that JNK1 plays a protective role in hepatocytes (Sabio *et al.* 2009). In contrast, hepatocyte-specific simultaneous knockout of JNK1 and JNK2 induces systemic protection against high fat diet (HFD)-induced insulin resistance (Vernia *et al.* 2014). Vernia *et al.* demonstrated that JNK could indirectly inhibit the activity of the nuclear peroxisome proliferator-activated receptor alpha (PPAR α), thus preventing production of fibroblast growth factor 21 (FGF21). FGF21 plays a pivotal role in control ketogenesis, insulin sensitivity, glycaemia and obesity. In consequence, they found that lack of JNK1/2 in hepatocytes protects against obesity-induced diabetes (Vernia *et al.* 2014). JNK1 modulates liver insulin resistance and steatosis through two distinct molecular actions. On one hand, JNK1, upon activation by proinflammatory cytokines (e.g. TNF α) and circulating FFAs, phosphorylates the IRS1 on serine 307, preventing its interaction with the insulin receptor. This blocks insulin cellular signalling, inducing insulin resistance (Hotamisligil *et al.* 1996b, Aguirre *et al.* 2000). On the other hand, JNK1 is necessary in hepatocytes for the homeostatic maintenance of gluconeogenesis and lipogenesis and the prevention of hepatic insulin clearance due to the elevated expression of insulin receptor in this cell type (Sabio *et al.* 2009).

Adipose tissue

The most important organ for lipid storage is adipose tissue, the only tissue with high capacity to expand in a non-transformed state. However, adipocytes are not merely fat storage cells, in fact play a central role in metabolism and organismal homeostasis through the secretion of adipokines (Gavrilova *et al.* 2000). In the obese state, adipocyte enlargement induces molecular and cellular alterations that affect whole-body metabolic homeostasis. Increased lipolysis in adipocytes leads to increased hyperlipidaemia and lipid accumulation in peripheral organs, in particular muscle and liver. Moreover, adipocytes begin to produce a number of proinflammatory cytokines, such as IL6, TNF α , monocyte-chemoattractant protein-1 (MCP-1), inducible isoform nitric oxide synthase (iNOS), transforming growth factor-beta1 (TGF- β 1), and plasminogen activator inhibitor-1 (PAI-1) (Hotamisligil *et al.* 1993). Obese adipose tissue also recruits macrophages that contribute to the generation of a chronic inflammation state and insulin resistance (Weisberg *et al.* 2003, Xu *et al.* 2003). Adipocytes are thus target of stress stimuli during obesity, and, as mentioned previously, genetically- and dietary-induced obesity both activate JNK in adipose tissue (Hirosumi *et al.* 2002). The scaffold protein JNK interacting protein 1 (JIP1) has been shown to play a central role in JNK activation in adipose tissue (Jaeschke *et al.* 2004). JIP1^{-/-} mice are resistant to diet-induced obesity and show less severe insulin resistance. JNK cannot be activated in the adipose tissue and muscle of JIP1^{-/-} mice, preventing the acquisition of insulin resistance in those tissues. However, the livers of JIP1^{-/-} mice have no defect in JNK activation, which may account for the incomplete protection against insulin resistance in these animals. This demonstrates that adipose tissue protection from insulin resistance is cell specific and it is not due to a whole body protection from obesity (Jaeschke *et al.* 2004). Mice with adipocyte-specific JNK1-deficiency have been used to analyse the role of JNK1 in adipose tissue (Sabio *et al.* 2008). When fed a high-fat diet, these mice show a similar body-mass gain to their control counterparts. However, the adipocyte JNK1-knockout mice are protected against insulin resistance in adipose tissue. This phenotype could be due to the absence of JNK1-mediated inhibitory phosphorylation of IRS1. Surprisingly, knockout of JNK1 in adipose tissue also induces protection against insulin resistance in liver (Sabio *et al.* 2008). Adipose JNK1-knockout mice exhibit a strong reduction in adipose tissue production of IL6, reflected in decreased

levels of circulating IL6. This proinflammatory cytokine is known to induce hepatic insulin resistance (Klover *et al.* 2003, Kim *et al.* 2004a). In conclusion, JNK1 in adipose tissue regulates local insulin sensitivity through IRS1 phosphorylation and also controls adipocyte-IL6 production and adipocyte crosstalk with other organs. In the obese state, adipose JNK1 induces hepatic insulin resistance through IL6 production (Sabio *et al.* 2008).

Bone-marrow-derived cells

Obesity induces a low-grade chronic inflammation that could be partly responsible for the observed metabolic changes (Hotamisligil 2006, Neels & Olefsky 2006). Increasing food intake increases adipose tissue fat storage, which induces adipocyte enlargement, hypoxia, stress, cell death, and proinflammatory cytokine production. These changes have been proposed to induce immune-cell recruitment to adipose tissue and produce a chronic activation of the immune response (Ferrante 2013). However, how fat deposition in adipose tissue activates the immune system is not completely understood. The obese state also induces immune activation in organ-resident macrophages, in particular Kupffer cells in the liver, probably inducing insulin resistance (Lanthier *et al.* 2010). In order to assess the role of JNK in the immune system in obesity and obesity-induced disorders, several studies have examined mice lacking JNK proteins in myeloid or bone-marrow-derived specific knock out mice. Zhang *et al.* (2011) examined the effect of a high-fat diet on transgenic mice overexpressing dominant-negative JNK in adipose tissue and macrophages. These mice showed reduced weight gain, insulin resistance, glucose intolerance and hepatic steatosis. Moreover, these mice had smaller-than-normal adipocytes, reduced macrophage infiltration in adipose tissue, and less-severe whole-body inflammation (Zhang *et al.* 2011). The authors concluded that JNK isoforms are responsible for the crosstalk between adipose tissue and macrophage infiltration, and showed that abrogation of their expression in both cell types is necessary to obtain the same phenotype seen in JNK1 full body knock out mice (Zhang *et al.* 2011). However, these results do not fully elucidate the role of JNK proteins expressed in hematopoietic cells. Solinas *et al.* (2007) used adoptive transfer to demonstrate that JNK1 deletion in non-hematopoietic tissues protects against weight gain and, partly as a consequence, from insulin resistance. Moreover, they showed that hematopoietic JNK1 removal reduces obesity-induced inflammation and protects against HFD-induced insulin

resistance without affecting obesity (Solinas *et al.* 2007). However, another group reported that JNK1-deficient bone marrow transplantation was not sufficient to induce protection against insulin resistance and macrophage infiltration of adipose tissue in a WT recipient, whereas this protection was induced by the absence of JNK1 in parenchymal cells (Vallerie *et al.* 2008). Taken together, these results highlight the importance of understanding the role of JNK2 in the myeloid lineage. To address this question, the Davis laboratory generated a myeloid-cell-specific knockout mouse model for JNK1 and JNK2 (Han *et al.* 2013). When fed a HFD, these mice show the same obesity and hyperlipidaemia responses as WT controls, but are more insulin sensitive (Han *et al.* 2013). The myeloid JNK1/2-knockout mice also show less macrophage infiltration and decreased M1 polarization in adipose tissue. M1 polarization was also decreased in hepatic macrophages, accompanied by increased M2 polarization, highlighting an important role for JNK in M1 polarization. *In vitro* experiments further confirmed an intrinsic defect in M1 polarization (Han *et al.* 2013). Finally, a role has been described for JNK2 in T cells in type 1 diabetes (Jaeschke *et al.* 2005). Non-obese diabetic (NOD) mice lacking JNK2 exhibit a decrease in spontaneous diabetes and insulinitis, probably due to increased Th2 polarization of CD4+T cells (Jaeschke *et al.* 2005). The authors of this study reported that this T-cell polarization effect was not seen in JNK1^{-/-} NOD mice, indicating a specific role for JNK2 in the regulation of T-cell polarization (Jaeschke *et al.* 2005).

Skeletal muscle

Skeletal muscle is a very large organ that requires abundant available energy to carry out its functions. Myocytes therefore play a major role in insulin-induced glucose disposal and are sensitive to obesity-induced insulin resistance. Evidence suggests that circulating FFAs, whose levels are increased in obesity, might be responsible for skeletal muscle insulin resistance (Shulman 2000, Roden 2004). FFAs induce the phosphorylation cascade that activates JNKs, suggesting a possible a role for JNKs in skeletal muscle insulin resistance. Moreover, JNK1 is activated in muscle from HFD-fed mice (Hirosumi *et al.* 2002). Muscle-specific JNK1-knockout mice reveal a role for this isoform in the induction of insulin resistance in skeletal muscle fed a HFD (Sabio *et al.* 2010a). These mice are sensitive to HFD-induced obesity. However, they are protected against obesity-induced insulin resistance and show increased glucose uptake by muscle, probably as result of a reduction

in IRS1 serine phosphorylation. Surprisingly, adipose tissue and liver are not positively affected by JNK1 deficiency in skeletal muscle (Sabio *et al.* 2010a). Instead, these mice show increased hepatic steatosis and circulating levels of triglycerides, due to a reduction in lipoprotein lipase (*Lpl*) gene expression in muscle. Moreover, the augmented blood levels of triglycerides induce an increase in adipose tissue macrophage infiltration (Sabio *et al.* 2010a). Interestingly, inhibiting JNK in muscle by overexpressing heat shock protein 72 (HSP72) improves HFD-induced hyperglycaemia and hyperlipidaemia (Chung *et al.* 2008). Moreover, it prevents glucose intolerance and insulin resistance, and reduces HFD-induced weight gain (Chung *et al.* 2008). Taken together, these results indicate that lack of JNK1 expression in muscle modulates insulin sensitivity in a cell-autonomous manner but does not influence insulin sensitivity in other tissues (Sabio *et al.* 2010a). However, inhibiting total JNK activity in muscle can ameliorate the whole-body response to HFD (Chung *et al.* 2008). It would be interesting to study the effect of JNK2 deficiency in muscle to identify its possible role in tissue crosstalk.

Pancreatic β cells

Insulin is produced by β cells in the pancreatic islets of Langerhans. In a healthy state, β cells, which make up 65–80% of the cells in the islet, respond to increases in serum glucose by secreting insulin, which induces glucose uptake in peripheral organs. Chronic hyperglycaemia induces changes in β -cell gene expression that lead to cell hypertrophy and loss of differentiation (Jonas *et al.* 1999). Glucose-induced insulin gene expression is also inhibited by saturated fatty acids (which are increased in obesity) through the activation of JNK in β cells, resulting in the phosphorylation and inhibition of IRS1 and IRS2 (Solinas *et al.* 2006). Moreover, overexpression experiments have shown that activation of JNK by oxidative stress leads to β -cell failure (Kaneto *et al.* 2002, 2005). JNK-interacting protein 1, a scaffold protein that regulates JNK activity (Whitmarsh *et al.* 2001), has been identified as a potential regulator of type 2 diabetes in humans (Waeber *et al.* 2000) and has been shown to mediate β -cell apoptosis (Haefliger *et al.* 2003). Despite these results, experiments in animals lacking JIP1/JIP2 protein indicate that these proteins are not essential for β -cell viability (Standen *et al.* 2009). Activation of JNK in β cells by overexpression of constitutively active MKK7 results in impaired insulin production (Lanuza-Masdeu *et al.* 2013), a result consistent with previous work showing that JNK2^{-/-} β cells in

non-obese mice are protected against apoptosis induced by T cells in a model of autoimmune type 1 diabetes (Jaeschke *et al.* 2005). All these results indicate a specific role of JNK in β cells as a regulator of insulin production and secretion, and prolonged JNK activation, as in the obese state, can induce islet dysfunction.

CNS

In the CNS, the onset of hypothalamic resistance to leptin and insulin is a key event in the development of obesity and impaired glucose homeostasis and systemic immune responses (Belgardt & Bruning 2010, Tschop *et al.* 2010, Vogt & Bruning 2013). Both hormones act on hypothalamic neurons to decrease food intake and hepatic glucose production and to increase energy expenditure (Obici *et al.* 2002, Belgardt & Bruning 2010). Two neuronal populations, each characterized by the expression of specific neuropeptides, have potent effects on energy homeostasis (Contreras *et al.* 2015, Lopez & Tena-Sempere 2015). One population consists of the POMC and CART neurons, which provide a strong anorexigenic effect, decreasing food intake and body weight. In contrast, the second population of AgRP and NPY neurons has a potent orexigenic effect, increasing food intake. Since the first evidence demonstrating that brain-specific deletion of insulin receptor (InsR) was associated with diet-sensitive obesity (Bruning *et al.* 2000), many reports have tried to identify the neurons and mechanism by which this hormone affects the brain. In this context, the failure of conditional depletion of JNK1 in several peripheral insulin-sensitive tissues to recapitulate the phenotype of whole-body JNK1 knockout mice (Sabio *et al.* 2008, 2009, 2010a,b) suggests the possibility that JNK1 activation in the CNS might contribute to the maintenance of energy balance and glucose metabolism. JNK activation in hypothalamus during obesity has been linked to endoplasmic reticulum stress, inflammation, and hyperlipidaemia (Ozcan *et al.* 2004, Prada *et al.* 2005). In 2010, two groups independently generated two brain-specific conditional JNK1 knockout models (Belgardt *et al.* 2010, Sabio *et al.* 2010b) using the Nestin-cre system, which is used to ablate a gene of interest in neurons and astrocytes in the CNS (Tronche *et al.* 1999). Both studies showed that these mice are protected against HFD-induced obesity, insulin resistance and glucose intolerance at the central and peripheral level (Belgardt *et al.* 2010, Sabio *et al.* 2010b). Reduced body-weight gain correlated with increased energy expenditure and locomotor activity and a higher body temperature

(Belgardt *et al.* 2010, Sabio *et al.* 2010b). Moreover, the mice had depressed lipid accumulation in brown fat and elevated plasma levels of T₃ and T₄ thyroid hormones, which correlated with high levels of thyrotropin-releasing hormone (TRH) in hypothalamus. These data indicate that JNK1 in CNS controls body homeostasis by regulating the hypothalamic–pituitary–adrenal axis (Belgardt *et al.* 2010, Sabio *et al.* 2010b). Two newly generated mouse models are helping to further clarify the role of JNK1 in the CNS: deletion of JNK1 and JNK2 in pituitary gland and conditional overexpression of constitutively active JNK in AgRP neurons (Vernia *et al.* 2013, Tsaousidou *et al.* 2014). Vernia *et al.* found that lack of JNK1 and 2 in the anterior pituitary gland protects against weight gain during obesity and diabetes. Moreover, these mice have elevated energy expenditure and circulating levels of T₃, T₄ and thyroid stimulating hormone (TSH). The study shows that JNK1/2 regulate expression of *Dio2*, which encodes the enzyme responsible for T₄ to T₃ conversion in the pituitary gland. Impairment of this conversion reduces the negative feedback regulation of the expression of TSH, resulting in sustained production of TSH, T₃ and T₄ (Vernia *et al.* 2013). In the second model, mice expressing constitutively active JNK1 in AgRP neurons become obese when fed a HFD and are leptin resistant at the neuronal and systemic level (Tsaousidou *et al.* 2014). These results indicate that JNKs, in particular JNK1, play a pivotal role in central nervous control of obesity. Further efforts are needed to address the role of JNK3, a mainly CNS isoform, in metabolism and energy homeostasis.

p38 MAPK

The four p38 MAPK isoforms – α , β , γ , δ – are encoded by different genes and have different tissue expression patterns (Sabio & Davis 2014). The p38 α isoform is widely expressed, but is less abundant in the brain, where p38 β MAPK is the major isoform (Beardmore *et al.* 2005). The p38 δ isoform is highly expressed in a limited number of tissues, including neutrophils and endocrine glands (Sumara *et al.* 2009, Cuadrado & Nebreda 2010, Ittner *et al.* 2012, Gonzalez-Teran *et al.* 2013), while p38 γ kinase is expressed in all tissues, with high levels found in muscle (Beardmore *et al.* 2005).

All p38 isoforms are activated, in response to appropriate stimuli, by dual phosphorylation in the activation loop sequence Thr-Gly-Tyr. p38 MAPKs can be activated by MKK3, MKK4 or MKK6 in cell-free systems, but their activation in intact cells is primarily mediated by

MKK3 and MKK6 (Enslen *et al.* 2000, Brancho *et al.* 2003, Remy *et al.* 2010). However, most of these studies were performed in immortalized fibroblasts and how specific p38 MAPK isoforms are activated in response to different stimuli in other cells types is largely unknown. The function of p38 α has been studied in several physiological processes, and although no specific role for this kinase has been defined in metabolism, p38 α is known to phosphorylate several key proteins involved in glucose and lipid metabolism (Fig. 3).

Liver

Although the specific role of the different p38 isoforms in liver metabolism has not been studied, several p38 MAPK substrates have been implicated in metabolic processes. Here we summarize these substrates and current knowledge about the potential action of p38 isoforms in liver metabolism. Downstream targets of p38 include two important transcription factors involved in gluconeogenesis: transcriptional PPAR gamma coactivator-1 alpha (PGC1 α) and CREB. Upon phosphorylation, PGC1 α and CREB activities are enhanced, and they bind to sites in the promoter regions of the genes encoding phosphoenolpyruvate carboxykinase kinase (PEPCK) and glucose-6 phosphatase, activating transcription of these gluconeogenic genes (Deak *et al.* 1998, Puigserver *et al.* 2001, Cao *et al.* 2005). Experiments with pharmacological inhibitors and p38-targeting shRNA have suggested that p38 might modulate gluconeogenesis via this mechanism (Cao *et al.* 2005). PGC1 α also co-activates many other transcription factors, including PPAR α and FOXO1. Furthermore, p38 has been proposed to directly phosphorylate PPAR α , enhancing its activity (Juge-Aubry *et al.* 1999). p38 also phosphorylates CCAAT-enhancer-binding protein alpha (C/EBP α) (Qiao *et al.* 2006), a transcription factor that regulates glucose homeostasis (Yang *et al.* 2005). Phosphorylation on serine enhances C/EBP α transactivation activity and increases PEPCK gene expression (Qiao *et al.* 2006). A more recent study showed that p38 phosphorylates the spliced form of X-box binding protein 1 (Xbp1s), promoting its nuclear translocation, reducing endoplasmic reticulum stress, and improving glycaemia during obesity (Lee *et al.* 2011). All these studies point to a potentially important role of p38 kinases as regulators of glucose homeostasis in the liver. However, extensive research in conditional knockout and transgenic mouse models will be needed to define the function of each p38 isoform in liver homeostasis.

Figure 3

Roles of p38 isoforms in metabolism. p38 targets include several proteins with a prominent role in metabolism regulation, such as TNF α , C/EBP β , and PPAR α , suggesting a role for these SAPKs in metabolism and metabolic

disease. Further studies in tissue-specific knockout mouse models are needed to clarify the specific roles of each p38 isoform.

Adipose tissue

Adipose tissue formation depends on tight regulation of cell growth, proliferation and differentiation (Gehart *et al.* 2010). It has been proposed that p38 might modulate adipocyte differentiation through the phosphorylation of C/EBP β (Engelman *et al.* 1998), a key transcription factor for adipose tissue differentiation (Tanaka *et al.* 1997). *In vitro* experiments with p38 α/β inhibitors have shown that p38 phosphorylates and activates C/EBP β , enhancing PPAR γ transcription and promoting adipocyte differentiation. It also has been postulated that p38 α regulates brown adipose tissue differentiation by controlling uncoupling protein 1 (UCP1) expression levels through the phosphorylation of the transcription factors ATF2 and PGC1 α (Cao *et al.* 2004, Tseng *et al.* 2008). However, more experimental work is needed in knockout and conditionally deficient animals in order to define the role of p38 MAPK in adipose tissue *in vivo*.

Pancreatic β cells

Although it has been speculated that p38 α might promote β cell failure, a part from *in vitro* inhibitor studies, there are only a few supporting evidences (Gehart *et al.* 2010). A very elegant recent study showed that p38 δ controls insulin secretion through the phosphorylation and inhibition of polycystic kidney disease 1 protein (PKD1) (Sumara *et al.* 2009). PKD1 regulates insulin secretion and cell survival, and mice lacking p38 δ are protected against stress-induced β cell death and have elevated insulin secretion.

Bone-marrow-derived cells

The p38 MAPK family regulates TNF α levels, a key cytokine in the control of systemic inflammation. While p38 α regulates TNF α expression by controlling its transcription and mRNA stability (Campbell *et al.* 2004,

Kang *et al.* 2008), recent studies demonstrate key roles for p38 γ and p38 δ in the posttranscriptional regulation of TNF α biosynthesis, with myeloid-specific p38 γ/δ gene ablation triggering defects in TNF α production (Risco *et al.* 2012, Gonzalez-Teran *et al.* 2013). Translational elongation of nascent pro-TNF α protein is mediated by eukaryotic elongation factor 2 (eEF2) kinase (Gonzalez-Teran *et al.* 2013), which is inhibited by p38 γ/δ -mediated phosphorylation (Knebel *et al.* 2001). Neutrophils are an important cell type that triggers inflammation and monocyte migration (Nathan 2006) and that makes an important contribution to liver steatosis development and metabolic dysfunction (Talukdar *et al.* 2012, Bertola *et al.* 2013, Mansuy-Aubert *et al.* 2013). p38 γ/δ have also been shown to control their recruitment and mobility (Ittner *et al.* 2012, Gonzalez-Teran *et al.* 2013). However, the specific contribution of these kinases to chronic inflammation induced by metabolic disease has not been tested.

Conclusions

Several reports have demonstrated that SAPKs control several metabolic events and their excessive activation is associated with deleterious effects during obesity. Most of these studies have examined JNK family members, in particular the isoforms JNK1 and JNK2. Further research is needed to clarify the function of the different p38 isoforms during obesity and in metabolic regulation and to define the cell types in which these kinases perform their function. The functions of p38 γ/δ have not been studied extensively *in vivo*, and very little is known about their substrates and functions. Recent reports suggest that the four isoforms of p38 have widely differing functions (Sumara *et al.* 2009, Gehart *et al.* 2010, Sabio *et al.* 2010b, Ittner *et al.* 2012, Gonzalez-Teran *et al.* 2013, Sabio & Davis 2014). Studies in knockout and conditionally deficient mouse models for each p38 kinase are needed in order to define the role of these proteins in health and disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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