

Role of Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4 signaling in Liver and Metabolic Diseases

Felix Ampadu¹, Vibhudutta Awasthi¹, Aditya D. Joshi^{1*}

¹Department of Pharmaceutical Sciences, University of Oklahoma Health Sciences
Center, Oklahoma City, Oklahoma 73117

Running Title: Role of MAP4K4 signaling in metabolic diseases

Corresponding Author: *Dr. Aditya D. Joshi, Department of Pharmaceutical Sciences, University of Oklahoma Health Sciences Center, 1110 N. Stonewall Ave., Oklahoma City, Oklahoma 73117, Tel: 405-271-6593 (Ext: 47471), E-mail: aditya-joshi@ouhsc.edu

Number of Pages: 29

Number of Tables: 0

Number of Figures: 3

Number of References: 47

Abstract word count: 123

Abbreviations: IHH, immortalized human hepatocytes; MAPK, mitogen activated protein kinase; MAP4K4, mitogen-activated protein kinase kinase kinase kinase 4; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease.

Recommended Section: Gastrointestinal, Hepatic, Pulmonary, and Renal

Abstract

MAP4K4 is a serine/threonine protein kinase belonging to the germinal center kinase (GCK) sub-group of sterile 20 protein (Ste20p) family of kinases. MAP4K4 has been involved in regulating multiple biological processes and a plethora of pathologies, including systemic inflammation, cardiovascular diseases, cancers, metabolic and hepatic diseases. Recently, multiple reports have indicated the upregulation of MAP4K4 expression and signaling in hyperglycemia and liver diseases. This review provides an overview of our current knowledge of MAP4K4 structure and expression, as well as its regulation and signaling, specifically in metabolic and hepatic diseases. Reviewing these promising studies will enrich our understanding of MAP4K4 signaling pathways and in the future, will help us design innovative therapeutic interventions against metabolic and liver diseases using MAP4K4 as a target.

Significance Statement

Although most studies on the involvement of MAP4K4 in human pathologies are related to cancers, only recently its role in liver and other metabolic diseases is beginning to unravel. This mini review discusses recent advancements in MAP4K4 biology within the context of metabolic dysfunction and comprehensively characterizes MAP4K4 as a clinically relevant therapeutic target against liver and metabolic diseases.

Introduction

In mammals, the mitogen activated protein kinase (MAPK) family belongs to the Ste-20-like family of serine/threonine kinases due to its shared homology with sterile 20 serine protein kinase (encoded by sterile 20 gene) in *Saccharomyces cerevisiae* (Johnson and Lapadat, 2002). MAPK family encompasses over 30 member proteins classified into p21-activated kinases (PAK-I) and germinal center kinase (GCK-I to VIII) sub-groups (Delpire, 2009). The MAPKs are capable of phosphorylating their own serine and threonine residues via auto-phosphorylation and can phosphorylate their substrates at serine and threonine sites, both leading to regulation of downstream signaling (Johnson and Lapadat, 2002). In the canonical pathway, MAP3K activates MAP2K, which in turn induces threonine/tyrosine phosphorylation and subsequently activates downstream MAPK members, including extracellular signal-regulated protein kinases (ERKs), p38 MAPK, and stress-activated protein kinases / c-Jun N-terminal kinases (SAPK / JNKs) (Johnson and Lapadat, 2002). The MAP3Ks can be regulated by small GTP-binding proteins as well as activated by mitogen activated protein kinase kinase kinase (MAP4K) family members (Chuang et al., 2016a).

MAP4K belongs to GCK sub-family and includes MAP4K1 (hematopoietic progenitor kinase, HPK1), MAP4K2 (GCK), MAP4K3 (germinal center kinase-related protein kinase, GLK), MAP4K4 (hepatocyte progenitor kinase-like / germinal center kinase-like kinase, HGK), MAP4K5 (kinase homologous to SPS1/STE20, KHS), MAP4K6 (misshapen like kinase 1, MINK), and MAP4K7 (TNF receptor-associated factor 2 (TRAF2) and NCK-Interacting protein kinase, TINK) (Chuang et al., 2016a). These MAP4K family members have been implicated in a wide range of pathophysiological

processes, including tissue homeostasis, viral infections, autoimmune diseases, malignancies, cardiotoxicity, and inflammatory and metabolic diseases (Singh et al., 2023). More specifically, mitogen-activated protein kinase kinase kinase 4 (MAP4K4) signaling has been explicitly involved in cancer progression and metastasis, systemic inflammation, atherosclerosis, type 2 diabetes mellitus, insulin resistance, and hepatic diseases (Singh et al., 2023). This mini review illustrates our current understanding of MAP4K4 signaling in the liver and metabolic diseases.

Structure, regulation, expression, and tissue distribution of MAP4K4

MAP4K4, also known as a HGK (hepatocyte progenitor kinase-like /Germinal Center Kinase-like Kinase - human ortholog) and NIK (Nck-interacting Kinase - mouse ortholog) (Delpire, 2009), is a serine/threonine protein kinase that serves as an upstream regulator of MAPK signaling (Singh et al., 2023). The *MAP4K4* gene is located on chromosome 2q11.2 in humans and comprises 33 exons (Wright et al., 2003). Human MAP4K4 protein contains ~ 1239 amino acids, and the molecular structure of MAP4K4 is well-conserved across species (Gao et al., 2016). Human MAP4K4 has an N-terminal kinase domain and a C-terminal hydrophobic leucine-rich citron-homology (CNH) domain (Wright et al., 2003). Whereas mouse MAP4K4 has two proline-rich motifs in the intermediate domains through which MAP4K4 interacts with the SH3 domain of NCK adapter protein (Becker et al., 2000). Alternative splicing of the *MAP4K4* transcript results in five functional isoforms that display similarity between kinase and CNH-domains, but the intermediate regions are distinct (Dan et al., 2001;

Cesana et al., 2023). It is noteworthy that both kinase and CNH domains are required for the complete activation of JNK activity by MAP4K4 (Dan et al., 2001).

MAP4K4 is widely expressed in humans and rodents, with notably elevated levels observed in the brain, adipose tissue, lymph nodes, and testes (Gao et al., 2016). Recent investigations have also demonstrated MAP4K4 expression in both hepatocytes as well as in hepatic non-parenchymal cells (Anand et al., 2022). Our own unpublished data show robust expression of MAP4K4 in immortalized hepatocytes as well as mouse and human liver tissue.

The precise mechanisms controlling the gene expression of *MAP4K4* remain unelucidated. However, evidence suggests that epigenetic alterations, such as DNA methylation, may influence its modulation (Wright et al., 2003). Increased methylation of CpG islands in the promoter region of *MAP4K4* gene has been observed in the T cells of individuals diagnosed with type 2 diabetes, and this epigenetic modification has been linked to changes in its mRNA expression levels (Chuang et al., 2016b). Moreover, the expression of *MAP4K4* mRNA increased following the activation of the tumor suppressor protein p53, suggesting that p53's regulation of MAP4K4 could have important therapeutic implications in cancer treatment (Gao et al., 2016). Ephrin receptors, which play a role in cell migration and tissue remodeling, also stimulate the activation of MAP4K4 (Xue et al., 2001). This was supported by a study in which whole-body or endothelial-specific MAP4K4 knockout mice displayed mortality during early embryogenesis and abnormalities in mesodermal differentiation and cell migration (Wright et al., 2003). Besides its involvement in embryonic development, MAP4K4 also

plays a role in cell migration, proliferation, survival, ion transport, and cytoskeletal adhesion (Bouzakri and Zierath, 2007; Gao et al., 2016; Golforoush et al., 2020).

MAP4K4 downstream effectors and signaling pathways

MAP4K4 plays a significant role in inflammation by regulating the production of cytokines and various immune cells (Chuang et al., 2016a; Singh et al., 2023). In this regard, the effects of MAP4K4 is intricately linked with the tumor necrosis factor alpha (TNF α)-induced JNK signaling pathway (Tripolitsioti et al., 2017b). MAP4K4 activation occurs in cells during exposure to TNF α stimulation, which leads to a targeted activation of JNK via a kinase cascade involving the mitogen-activated protein kinase kinase kinase 7 (MAP3K7/TAK1), mitogen-Activated Protein Kinase Kinase 4 (MAP2K4/MKK4), and mitogen-Activated Protein Kinase Kinase 7 (MAP2K7/MKK7). The activation of JNK is inhibited by the presence of dominant-negative mutants of MAP3K7/TAK1, MAP2K4/MKK4, and MAP2K7/MKK7, suggesting that JNK likely operates through the MAP3K7-MAP2K4-MAP2K7 kinase cascade (Yao et al., 1999). Additionally, the C-terminal citron-homology domain of MAP4K4 can interact with a small GTP-binding protein, Ras-related protein 2 (Rap2), but not with Ras-related protein 1 (Rap1 or Ras), to enhance JNK activation (Chuang et al., 2016a). It is noteworthy that MAP4K4 has a dual role as it not only mediates the signaling process but also facilitates the expression of TNF α (Yao et al., 1999; Tripolitsioti et al., 2017a). This means TNF α can increase the expression of MAP4K4 mRNA through the transcription factors c-Jun and activating transcription factor 2 (ATF2), and MAP4K4 can

facilitate the expression of TNF α in a positive feedback loop (Tesz et al., 2007; Tripolitsioti et al., 2017a).

The activation of the Nuclear factor- κ B (NF- κ B) pathway leads to the upregulation of genes associated with inflammation in various cells, such as macrophages, endothelial cells, and adipocytes (Liu et al., 2011). Interestingly, MAP4K4 can regulate NF- κ B signaling independent of the JNK pathway. Studies supporting the assertion above show that RNA interference-mediated suppression of MAP4K4 does not impact JNK, p38, ERK, or inhibitor of nuclear factor- κ B kinase (IKK) activation in macrophages following lipopolysaccharide (LPS) stimulation (Flach et al., 2015). Additionally, recent research has demonstrated that endothelial MAP4K4 contributes to increased endothelial permeability (Vania et al., 2020). Consequently, this can enhance the occurrence of vascular inflammation and the advancement of atherosclerosis by increasing the expression of NF- κ B target genes (Roth Flach et al., 2015). The silencing of MAP4K4 in endothelial cells has been demonstrated to decrease atherosclerotic lesions in mouse models by reducing the expression of cell surface adhesion molecules and suppressing the activation of NF- κ B, a crucial regulator of endothelial cell activation and atherosclerosis (Flach et al., 2015).

MAP4K4 is also involved in the regulation of the Notch signaling pathway (Liu et al., 2011). The Notch signaling pathway is evolutionarily preserved and is essential for determining cell fate, development, and the regulation of tissue stability. The pathway is initiated when ligands bind to notch receptors. This causes the receptor to be cleaved by proteases and releases the notch intracellular domain (NICD). NICD then translocates to the nucleus and interacts with transcription factors, such as C Promoter

Binding Factor 1 (CBF1) and Suppressor of Hairless (Lag-1) of the CSL family, to regulate target gene expression (Vanderbeck and Maillard, 2020). MAP4K4 is identified as a downstream effector of Notch signaling, regulating Notch-dependent gene expression. Chen et al showed that inhibition of MAP4K4 reduces Notch signaling and the expression of Notch target genes (Chen et al., 2023). Moreover, in a targeted genomic CRISPR-Cas9 screen, MAP4K4 was found to be essential for glioblastoma invasion, with its knockdown resulting in decreased expression of the Notch pathway genes (Prolo et al., 2019).

JAK/STAT are essential components of a signaling pathway involved in the regulation of key cellular processes, including immune response, proliferation, and differentiation (Bousoik et al., 2022). There is direct evidence of MAP4K4 interaction with Signal transducer and activator of transcription 3 (STAT3) in human embryonic kidney (HEK293T) cells (Wright et al., 2003). Moreover, the JAK/STAT pathway can crosstalk with the receptor tyrosine kinase (RTK)/Ras/MAPK pathway components regulated by MAP4K4 (Rawlings et al., 2004). JAK/STAT can indirectly promote Ras signaling by transcriptionally activating Suppressor Of Cytokine Signaling 3 (SOCS3), which binds to Ras-specific GTPase-activating proteins (RasGAP), a negative regulator of Ras signaling, leading to reduced activity of the Ras pathway (Rawlings et al., 2004).

MAP4K4 is also an upstream activator of Hippo/Yes-associated protein (YAP) pathway. The Hippo signaling pathway consists of a complex cascade of kinases that regulate cell fate, tissue homeostasis, and organ size (Zheng and Pan, 2019). Furthermore, it has been associated with a wide range of physiological and pathological phenomena, such as apoptosis, cancer, and immunity (Zheng and Pan, 2019). MAP4K4 has been

shown to function in tandem with Mammalian Ste20-like kinases 1/2 (MST1/2) to activate and phosphorylate large tumor suppressor kinases 1/2 (LATS1/2), which then phosphorylate the YAP and transcriptional co-activator with PDZ-binding motif (TAZ) in the Hippo signaling pathway (Meng et al., 2015; Zheng et al., 2015). Investigations involving MST1/2 knockout cells were unable to inhibit YAP/TAZ phosphorylation, suggesting that other kinases are also involved in LATS1/2 activation in the Hippo pathway (Meng et al., 2015). Recently, *in vitro* human kinome screening performed using LATS1 as a substrate revealed several MAP4K family members including MAP4K1/HPK1, MAP4K2/GCK, MAP4K3/GLK, MAP4K4/HGK, MAP4K6/MINK1, and TRAF2 and MAP4K7/ TNIK, as potent LATS1/2-activating kinases (Meng et al., 2015). This study suggested that MAP4Ks are components of the Hippo pathway that directly phosphorylate and activate LATS1/2 kinases. Moreover, MAP4Ks play a crucial role in regulating the physiological activities of LATS1/2 and YAP/TAZ in response to various upstream signals (Meng et al., 2015; Zheng et al., 2015).

Furthermore, the involvement of MAP4K4 in the AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways has been demonstrated in cultured adipocytes (Guntur et al., 2010; Virbasius and Czech, 2016). When AMPK is activated, it promotes catabolic processes - such as the absorption of glucose and the oxidation of fatty acids, while simultaneously inhibiting anabolic processes - including the synthesis of proteins and lipids (Guntur et al., 2010). The administration of a known activator of AMPK, oligomycin in MAP4K4-depleted cells resulted in a decreased AMPK phosphorylation. Conversely, adipocytes modified to overexpress MAP4K4 exhibited increased AMPK phosphorylation (Danai et al., 2013). This observation implies that

MAP4K4 functions as a positive regulator of AMPK signaling. Additionally, MAP4K4 serves as a suppressor of the mTOR signaling pathway, which plays a crucial role in regulating cellular development and metabolism. The aforementioned study also demonstrated that MAP4K4 plays a significant role in enhancing the activity of sterol-regulated element binding protein 1 (SREBP-1) and lipogenesis (Danai et al., 2013). This regulation occurs in an mTOR-dependent manner and is independent of JNK-signaling (Danai et al., 2013). SREBP-1 controls the activity of glucose transporter 4 (GLUT4), peroxisome proliferator-activated receptor (PPAR γ), and several genes involved in lipogenesis (Danai Laura et al., 2015). The expression of SREBP-1 is upregulated by the mechanistic target of rapamycin complex 1 (mTORC1) and downregulated by AMPK. Additionally, the reduction of MAP4K4 augments the phosphorylation of both mTOR and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) (Danai et al., 2013). The impact of depleting MAP4K4 on the activation of mTOR for the regulation of 4EBP1 seems to occur downstream of protein kinase B (AKT) in the insulin signaling pathway, since the phosphorylation of AKT remains unaffected following the depletion of MAP4K4 (Danai et al., 2013).

Role of MAP4K4 in metabolic diseases

A few studies have investigated the role of MAP4K4 in obesity, insulin resistance, and hyperglycemia (Danai Laura et al., 2015; Flach et al., 2016; Roth Flach et al., 2017). Preadipocytes cultured from abdominal subcutaneous adipose tissue biopsies indicated an increased expression of MAP4K4 in obese individuals (Isakson et al., 2009). Moreover, silencing MAP4K4 in 3T3-L1 adipocytes resulted in an increased expression

of CCAAT-enhancer-binding protein factors α and β (C/EBP α and β), PPAR γ , SREBP1 and GLUT4, leading to increased adipogenesis, and glucose uptake (Tang et al., 2006) (Fig. 1). Further investigation into the mechanisms behind MAP4K4's effects revealed that it suppresses adipocyte lipogenesis primarily by inhibiting SREBP1 through pathways that involve AMPK and mTOR but are independent of JNK signaling (Guntur et al., 2010; Danai et al., 2013; Si et al., 2020) (Fig. 1). In contrast to the *in vitro* data, the *in vivo* adipose-specific MAP4K4 knockout mouse model fed with a high-fat diet did not show adverse phenotypic changes in visceral and subcutaneous adipose tissues. Knocking out MAP4K4 in adipose did not alter systemic glucose tolerance or insulin response (Danai Laura et al., 2015) (Fig. 2). These findings suggest that while MAP4K4 plays a role in regulating adipocyte function and glucose metabolism at a cellular level, *in vivo* systemic effects involve additional factors and pathways. Therefore, further comprehensive research is necessary to elucidate the systemic effects of MAP4K4 signaling and its potential interactions with other metabolic processes.

In another elegant study, individuals harboring genetic variations of MAP4K4 (single nucleotide polymorphisms within *MAP4K4* gene) showed glucose intolerance, insulin resistance, and dysfunction of pancreatic β cells, along with higher levels of cytokines TNF α and IL-6 resulting from low-grade systemic inflammation that is commonly associated with type 2 diabetes (Sartorius et al., 2012; Chuang et al., 2016b; Li et al., 2016). Silencing MAP4K4 in rat primary β cells protected the pancreatic cells against TNF α -induced damage and insulin resistance (Bouzakri et al., 2009). Additionally, myotubes transfected with *MAP4K4* siRNA protected against TNF α -induced insulin resistance (Bouzakri and Zierath, 2007) (Fig. 1).

To examine *the in vivo* role of MAP4K4 in glucose metabolism, a tamoxifen-inducible global MAP4K4 knockout mouse model (iMAP4K4-KO) was generated (Danai Laura et al., 2015). High-fat diet (60 kcal% fat) fed iMAP4K4-KO mice displayed lower fasting glucose levels and less pancreatic cell growth despite having similar weight gain (Danai Laura et al., 2015) (Fig. 2). Insulin tolerance test performed on iMAP4K4-KO mice showed enhanced insulin responsiveness, emphasizing that systemic knockout of MAP4K4 protects against the high-fat diet-induced insulin resistance (Danai Laura et al., 2015). Notably, treatment with MAP4K4 inhibitor, PF-06260933 in ob/ob model of type II diabetes, as well as atherosclerosis-prone apolipoprotein E-deficient (ApoE^{-/-}) and low-density lipoprotein receptor-deficient (LDLR^{-/-}) models of atherosclerosis resulted in reduced plasma glucose levels which indicates that systemic pharmacological MAP4K4 inhibition can induce insulin sensitivity and manifest anti-diabetic effects (Ammirati et al., 2015) (Fig. 2). Interestingly, knocking out MAP4K4 from liver or adipose did not affect insulin sensitivity or glucose metabolism in mice fed with chow or high-fat diet (Danai Laura et al., 2015) (Fig. 2). However, skeletal muscle-specific MAP4K4 knockout mice generated by crossing MAP4K4-floxed mice with Myf5-cre mice showed protection against hyperglycemia and insulin resistance via AKT signaling. This strongly suggests that MAP4K4 expression and MAP4K4-regulated signaling pathways in Myf5-positive skeletal muscle cells significantly modulate insulin signaling (Danai Laura et al., 2015) (Fig. 2).

Significance of MAP4K4 signaling in liver diseases

A recent study identified a positive correlation between hepatic *MAP4K4* mRNA expression and the severity of the metabolic dysfunction-associated steatotic liver disease (previously known as non-alcoholic fatty liver disease) activity score in a cohort of 62 patients. In both human and rodent livers, *MAP4K4* is expressed in hepatic parenchyma (hepatocytes) and non-parenchymal cells (Kupffer and stellate cells). Moreover, immunocytochemistry and subsequent visualization using electron microscopy indicated that in oleate-treated immortalized human hepatocytes (IHH), both mitochondrial matrix and outer mitochondrial membrane have detectable levels of *MAP4K4* expression (Anand et al., 2022).

Knocking out *MAP4K4* using RNA interference in oleic acid-treated IHH showed less lipid accumulation due to reduced lipid uptake and synthesis, upregulated β -oxidation, and increased triglyceride efflux compared to hepatocytes transfected with a non-targeting siRNA control (Anand et al., 2022) (Fig. 1). The mRNA levels of *SREBP-1* and *PPAR γ* involved in lipid uptake and synthesis were significantly lower, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha α (*PGC1 α*) expression known for hepatic fatty acid oxidation and ketogenesis was higher in *MAP4K4* silenced IHHs, indicating that *MAP4K4* regulates lipotoxicity in human hepatocytes. Silencing *MAP4K4* also protected human hepatocytes against endoplasmic reticulum / oxidative stress-induced apoptosis as evidenced by lower levels of superoxide radicals, hydrogen peroxide, C/EBP Homologous Protein (CHOP), BAX, and caspase 3. Additionally, the expression of anti-apoptotic marker *BCL2* was upregulated in response to *MAP4K4* attenuation (Anand et al., 2022) (Fig. 1). Under basal conditions as well as after oleic acid treatment, *MAP4K4* silenced IHH showed lower phosphorylation of JNK and ERK,

whereas AKT-phosphorylation at serine 473 was elevated (Anand et al., 2022). Pharmacological inhibition of MAP4K4 using a bona fide inhibitor, PF-06260933, in oleate-treated IHH resulted in a significant reduction of lipid content and endoplasmic reticulum / oxidative stress (Fig. 1). On the contrary, IHHs overexpressing MAP4K4 exhibited increased lipid accumulation and endoplasmic reticulum / oxidative stress (Anand et al., 2022) (Fig. 1). Overall, these studies suggested the involvement of MAP4K4 signaling in metabolic dysfunction-associated steatotic liver disease and the inhibition of MAP4K4 protected hepatocytes against lipotoxicity (Fig. 1).

Interestingly, *in vivo* studies performed with tamoxifen inducible whole-body MAP4K4 knockout (iMAP4K4-KO) and hepatocyte-specific MAP4K4 knockout mice (Alb MAP4K4-KO) challenged with high-fat diet did not attenuate hepatic steatosis (Danai Laura et al., 2015) (Fig. 2). These confounding results are potentially attributed to the residual expression of hepatic MAP4K4 in iMAP4K4-KO mice and the presence of MAP4K4 in non-parenchymal cells of Alb MAP4K4-KO mice, which might exert compensatory effects. Nevertheless, these aspects of MAP4K4 inhibition need further investigation. Since MAP4K4 affects embryonic development, an inducible liver cell-specific MAP4K4 conditional knockout mouse models will need to be constructed to dissect the effects of hepatocyte and non-parenchymal cell-targeted MAP4K4 ablation in adult mice.

Apart from MAP4K4's involvement in metabolic dysfunction associated steatotic liver disease, a single study has indicated upregulation of MAP4K4 signaling (phosphorylation of MAP4K4 at serine 794) in chronic ethanol-induced liver injury model (Singh et al., 2022). As discussed above, MAP4K4 is an upstream activator of the Hippo

/ YAP pathway that is dysregulated in fatty liver disease of metabolic as well as alcohol origin (Ardestani et al., 2018; Bou Saleh et al., 2021; Nguyen-Lefebvre et al., 2021), implying that MAP4K4 inhibition will benefit in alcoholic hepatitis (Bou Saleh et al., 2021). However, the significance of MAP4K4 in alcoholic liver disease model needs to be further investigated.

Conclusions and Future Directions

Although most studies performed to date focused on the involvement of MAP4K4 in human pathologies related to cancers, its role in liver and other metabolic diseases has only recently begun to unravel. Initial studies using both *in vitro* and *in vivo* models have shown that mammalian MAP4K4 serves as a plausible master regulator for multiple signaling pathways involved in the development and progression of metabolic diseases. How the crosstalk among MAP4K4 and its downstream effectors, including JNK, NF- κ B, Notch, JAK/STAT, AMPK-mTOR, Hippo, and other pathways, plays out in metabolic diseases warrants further investigation (Fig. 3). An intriguing subject of inquiry would be to decipher the tissue- and cell- specific roles of MAP4K4 in the progression of liver disease. The complexity of its involvement further intensifies as MAP4K4 also affects glycemic levels and obesity. Besides genetic models, pharmacological manipulations of MAP4K4 will help decode its pivotal pathophysiological role. The future development of MAP4K4 modulators represent a promising direction for research, offering potentially new therapeutic avenues against liver and metabolic diseases. However, it is essential to approach this field with careful consideration, as understanding the impact of

MAP4K4 signaling on liver disease remains in its infancy, necessitating further comprehensive studies to elucidate its mechanisms and effects fully.

Data Availability Statement

The authors declare that this article does not contain any generated or analyzed datasets.

AUTHORSHIP CONTRIBUTIONS

Wrote or contributed to the writing of the manuscript: Ampadu, Awasthi, Joshi

References

Ammirati M, Bagley SW, Bhattacharya SK, Buckbinder L, Carlo AA, Conrad R, Cortes C, Dow RL, Dowling MS and El-Kattan A (2015) Discovery of an in vivo tool to establish proof-of-concept for MAP4K4-based antidiabetic treatment. *ACS medicinal chemistry letters* 6:1128-1133.

Anand S, Caputo M, Xia Y, Andersson E, Cansby E, Kumari S, Ståhlman M, Porosk R, Keuenhof K, Höög JL, Nair S, Marschall H-U, Blüher M and Mahlapuu M (2022) Inhibition of MAP4K4 Signaling Initiates Metabolic Reprogramming to Protect Hepatocytes From Lipotoxic Damage. *The Journal of Lipid Research*.

Ardestani A, Lypse B and Maedler K (2018) Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism. *Trends Endocrinol Metab* 29:492-509.

Becker E, Huynh-Do U, Holland S, Pawson T, Daniel TO and Skolnik EY (2000) Nck-interacting Ste20 kinase couples Eph receptors to c-Jun N-terminal kinase and integrin activation. *Molecular and cellular biology* 20:1537-1545.

Bou Saleh M, Louvet A, Ntandja-Wandji LC, Boleslawski E, Gnemmi V, Lassailly G, Truant S, Maggiotto F, Ningarhari M, Artru F, Anglo E, Sancho-Bru P, Corlu A, Argemi J, Dubois-Chevalier J, Dharancy S, Eeckhoutte J, Bataller R, Mathurin P and Dubuquoy L (2021) Loss of hepatocyte identity following aberrant YAP activation: A key mechanism in alcoholic hepatitis. *Journal of hepatology* 75:912-923.

Bousoik E, Mahdipoor P, Alhazza A and Aliabadi HM (2022) Combinational silencing of components involved in JAK/STAT signaling pathway. *European Journal of Pharmaceutical Sciences* 175:106233.

Bouzakri K, Ribaux P and Halban PA (2009) Silencing mitogen-activated protein 4 kinase 4 (MAP4K4) protects beta cells from tumor necrosis factor- α -induced decrease of IRS-2 and inhibition of glucose-stimulated insulin secretion. *Journal of Biological Chemistry* 284:27892-27898.

Bouzakri K and Zierath JR (2007) MAP4K4 gene silencing in human skeletal muscle prevents tumor necrosis factor- α -induced insulin resistance. *Journal of Biological chemistry* 282:7783-7789.

Cesana M, Vaccaro L, Larsen M, Kibæk M, Micale L, Riccardo S, Annunziata P, Colantuono C, Di Filippo L and De Brasi D (2023) Integrated exome and transcriptome analysis prioritizes MAP4K4 de novo frameshift variants in autism spectrum disorder as a novel disease–gene association. *Human Genetics* 142:343-350.

Chen K, Yuan X, Wang S, Zheng F, Fu Z, Shen Z, Cheng X, Wang Y, Tang S and Ni H (2023) MAP4K4 promotes ovarian cancer metastasis through diminishing ADAM10-dependent N-cadherin cleavage. *Oncogene* 42:1438-1452.

Chuang H-C, Wang X and Tan T-H (2016a) MAP4K family kinases in immunity and inflammation. *Advances in immunology* 129:277-314.

Chuang HC, Wang JS, Lee IT, Sheu WH and Tan TH (2016b) Epigenetic regulation of HGK/MAP4K4 in T cells of type 2 diabetes patients. *Oncotarget* 7:10976-10989.

Dan I, Watanabe NM and Kusumi A (2001) The Ste20 group kinases as regulators of MAP kinase cascades. *Trends in cell biology* 11:220-230.

Danai Laura V, Roth Flach Rachel J, Virbasius Joseph V, Garcia Menendez L, Jung Dae Y, Kim Jong H, Kim Jason K and Czech Michael P (2015) Inducible Deletion of Protein Kinase Map4k4 in Obese Mice Improves Insulin Sensitivity in Liver and Adipose Tissues. *Molecular and Cellular Biology* 35:2356-2365.

Danai LV, Guilherme A, Guntur KV, Straubhaar J, Nicoloso SM and Czech MP (2013) Map4k4 suppresses Srebp-1 and adipocyte lipogenesis independent of JNK signaling [S]. *Journal of lipid research* 54:2697-2707.

Delpire E (2009) The mammalian family of sterile 20p-like protein kinases. *Pflügers Archiv-European Journal of Physiology* 458:953-967.

Flach RJR, Danai LV, DiStefano MT, Kelly M, Menendez LG, Jurczyk A, Sharma RB, Jung DY, Kim JH and Kim JK (2016) Protein kinase mitogen-activated protein kinase kinase kinase 4 (MAP4K4) promotes obesity-induced hyperinsulinemia. *Journal of Biological Chemistry* 291:16221-16230.

Flach RJR, Skoura A, Matevossian A, Danai LV, Zheng W, Cortés C, Bhattacharya S, Aouadi M, Hagan N, Yawe JC, Vangala P, Menendez LG, Cooper MP, Fitzgibbons TP, Buckbinder L and Czech MP (2015) Endothelial Protein Kinase MAP4K4 Promotes Vascular Inflammation and Atherosclerosis. *Nature Communications*.

Gao X, Gao C, Liu G and Hu J (2016) MAP4K4: an emerging therapeutic target in cancer. *Cell & bioscience* 6:1-9.

Golforoush PA, Narasimhan P, Chaves-Guerrero PP, Lawrence E, Newton G, Yan R, Harding SE, Perrior T, Chapman KL and Schneider MD (2020) Selective protection of human cardiomyocytes from anthracycline cardiotoxicity by small molecule inhibitors of MAP4K4. *Scientific Reports* 10:12060.

Guntur KV, Guilherme A, Xue L, Chawla A and Czech MP (2010) Map4k4 negatively regulates peroxisome proliferator-activated receptor (PPAR) γ protein translation by suppressing the mammalian target of rapamycin (mTOR) signaling pathway in cultured adipocytes. *Journal of Biological Chemistry* 285:6595-6603.

Isakson P, Hammarstedt A, Gustafson B and Smith U (2009) Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor- α , and inflammation. *Diabetes* 58:1550-1557.

Johnson GL and Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 298:1911-1912.

Li T-T, Qiao H, Tong H-X, Zhuang T-W and Wang T-T (2016) Association of common genetic variants in mitogen-activated protein kinase kinase kinase 4 with type 2 diabetes mellitus in a Chinese Han population. *Chinese medical journal* 129:1179-1184.

Liu A-W, Cai J, Zhao X-L, Jiang T-H, He T-F, Fu H-Q, Zhu M-H and Zhang SH (2011) ShRNA-targeted MAP4K4 inhibits hepatocellular carcinoma growth. *Clinical Cancer Research* 17:710-720.

Meng Z, Moroishi T, Mottier-Pavie V, Plouffe SW, Hansen CG, Hong AW, Park HW, Mo J-S, Lu W and Lu S (2015) MAP4K family kinases act in parallel to MST1/2 to activate LATS1/2 in the Hippo pathway. *Nature communications* 6:8357.

Nguyen-Lefebvre AT, Selzner N, Wrana JL and Bhat M (2021) The hippo pathway: A master regulator of liver metabolism, regeneration, and disease. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 35:e21570.

Prolo LM, Li A, Owen SF, Parker JJ, Foshay K, Nitta RT, Morgens DW, Bolin S, Wilson CM and Vega L JC (2019) Targeted genomic CRISPR-Cas9 screen identifies MAP4K4 as essential for glioblastoma invasion. *Scientific reports* 9:14020.

Rawlings JS, Rosler KM and Harrison DA (2004) The JAK/STAT Signaling Pathway. *Journal of Cell Science*.

Roth Flach RJ, DiStefano MT, Danai LV, Senol-Cosar O, Yawe JC, Kelly M, Garcia Menendez L and Czech MP (2017) Map4k4 impairs energy metabolism in endothelial cells and promotes insulin resistance in obesity. *American Journal of Physiology-Endocrinology and Metabolism* 313:E303-E313.

Roth Flach RJ, Skoura A, Matevossian A, Danai LV, Zheng W, Cortes C, Bhattacharya SK, Aouadi M, Hagan N and Yawe JC (2015) Endothelial protein kinase MAP4K4 promotes vascular inflammation and atherosclerosis. *Nature communications* 6:8995.

Sartorius T, Staiger H, Ketterer C, Heni M, Machicao F, Guilherme A, Grallert H, Schulze MB, Boeing H and Stefan N (2012) Association of common genetic variants in the MAP4K4 locus with prediabetic traits in humans.

Si Z, Guan X, Teng X, Peng X, Wan Z, Li Q, Chen G, Tan J and Li J (2020) Identification of CYP46A1 as a new regulator of lipid metabolism through CRISPR-based whole-genome screening. *The FASEB Journal* 34:13776-13791.

Singh SK, Roy R, Kumar S, Srivastava P, Jha S, Rana B and Rana A (2023) Molecular Insights of MAP4K4 Signaling in Inflammatory and Malignant Diseases. *Cancers* 15:2272.

Singh V, Huang E, Pathak V, Willard BB, Allende DS and Nagy LE (2022) Phosphoproteomics identifies pathways underlying the role of receptor-interaction protein kinase 3 in alcohol-associated liver disease and uncovers apoptosis signal-regulating kinase 1 as a target. *Hepatology Communications* 6:2022-2041.

Tang X, Guilherme A, Chakladar A, Powelka AM, Konda S, Virbasius JV, Nicoloso SM, Straubhaar J and Czech MP (2006) An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPAR γ , adipogenesis, and insulin-responsive hexose transport. *Proceedings of the National Academy of Sciences* 103:2087-2092.

Tesz GJ, Guilherme A, Guntur KV, Hubbard AC, Tang X, Chawla A and Czech MP (2007) Tumor necrosis factor α (TNF α) stimulates Map4k4 expression through TNF α receptor 1 signaling to c-Jun and activating transcription factor 2. *Journal of Biological Chemistry* 282:19302-19312.

Tripolitsioti D, Grotzer M and Baumgartner M (2017a) The Ser/Thr kinase MAP4K4 controls pro-metastatic cell functions. *J Carcinog Mutagen* 8:2157-2518.

Tripolitsioti D, Grotzer M and Baumgartner M (2017b) The Ser/Thr kinase MAP4K4 controls pro-metastatic cell functions. *J Carcinog Mutagen* 8:2.

Vanderbeck A and Maillard I (2020) Notch Signaling at the Crossroads of Innate and Adaptive Immunity. *Journal of Leukocyte Biology*.

Vania V, Wang L, Tjakra M, Zhang T, Qiu J, Tan Y and Wang G (2020) The interplay of signaling pathway in endothelial cells—Matrix stiffness dependency with targeted-therapeutic drugs. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1866:165645.

Virbasius JV and Czech MP (2016) Map4k4 Signaling Nodes in Metabolic and Cardiovascular Diseases. *Trends in Endocrinology & Metabolism* 27:484-492.

Wright JH, Wang X, Manning G, LaMere BJ, Le P, Zhu S, Khattry D, Flanagan PM, Buckley SD and Whyte DB (2003) The STE20 kinase HGK is broadly expressed in human tumor cells and can modulate cellular transformation, invasion, and adhesion. *Molecular and cellular biology* 23:2068-2082.

Xue Y, Wang X, Li Z, Gotoh N, Chapman D and Skolnik EY (2001) Mesodermal patterning defect in mice lacking the Ste20 NCK interacting kinase (NIK). *Development* 128:1559-1572.

Yao Z, Zhou G, Wang XS, Brown A, Diener K, Gan H and Tan T-H (1999) A novel human STE20-related protein kinase, HGK, that specifically activates the c-Jun N-terminal kinase signaling pathway. *Journal of Biological Chemistry* 274:2118-2125.

Zheng Y and Pan D (2019) The Hippo signaling pathway in development and disease. *Developmental cell* 50:264-282.

Zheng Y, Wang W, Liu B, Deng H, Uster E and Pan D (2015) Identification of Happyhour/MAP4K as alternative Hpo/Mst-like kinases in the Hippo kinase cascade. *Developmental cell* 34:642-655.

FOOTNOTES

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases grant R01 DK122028 (to A.D.J.). The authors declare no conflict of interest.

Figure Legend

Fig. 1: Metabolic effects of MAP4K4 signaling in known *in vitro* models. The effects of MAP4K4 overexpression in adipocytes and hepatocytes are illustrated (solid arrows). Additionally, the impact of pharmacological inhibition (PF-06260933) and RNA silencing of MAP4K4 in adipocytes, hepatocytes, pancreatic β cells, and myotubes on key metabolic processes is shown (dashed arrows). Changes in gene/protein expression and metabolic parameters are represented as increase (\uparrow), decrease (\downarrow), and no change (\leftrightarrow).

Fig. 2: Significance of *in vivo* MAP4K4 signaling in metabolic dysfunction associated models. The effects of physiological MAP4K4 signaling on adipocytes, hepatocytes, and

skeletal muscles in high-fat diet administered C57BL6 (wild-type) model of MASLD is illustrated (solid arrows). Additionally, consequences of whole-body and tissue-specific (adipose, hepatocytes and skeletal muscles) MAP4K4 ablation in high-fat diet administered C57BL6 model of MASLD on glucose metabolism is shown (dashed arrows). Effects of pharmacological inhibition of MAP4K4 using PF-06260933 in obese diabetic (*ob/ob*) chow diet (CD) administered mice, apolipoprotein E-deficient (*ApoE*^{-/-}) knockout western diet (WD) administered mice, and low-density lipoprotein receptor (*LDLR*^{-/-}) knockout custom high cholesterol western diet (HC) administered mice on glucose metabolism is also depicted (dashed arrows). Changes in parameters are represented as increase (↑), decrease (↓), and no change (↔).

Fig. 3: Role of MAP4K4 signaling in liver diseases. Schematic diagram illustrating downstream MAP4K4 targets and plausible hepatic pathologies involved in response to altered MAP4K4 signaling.

Figure 1

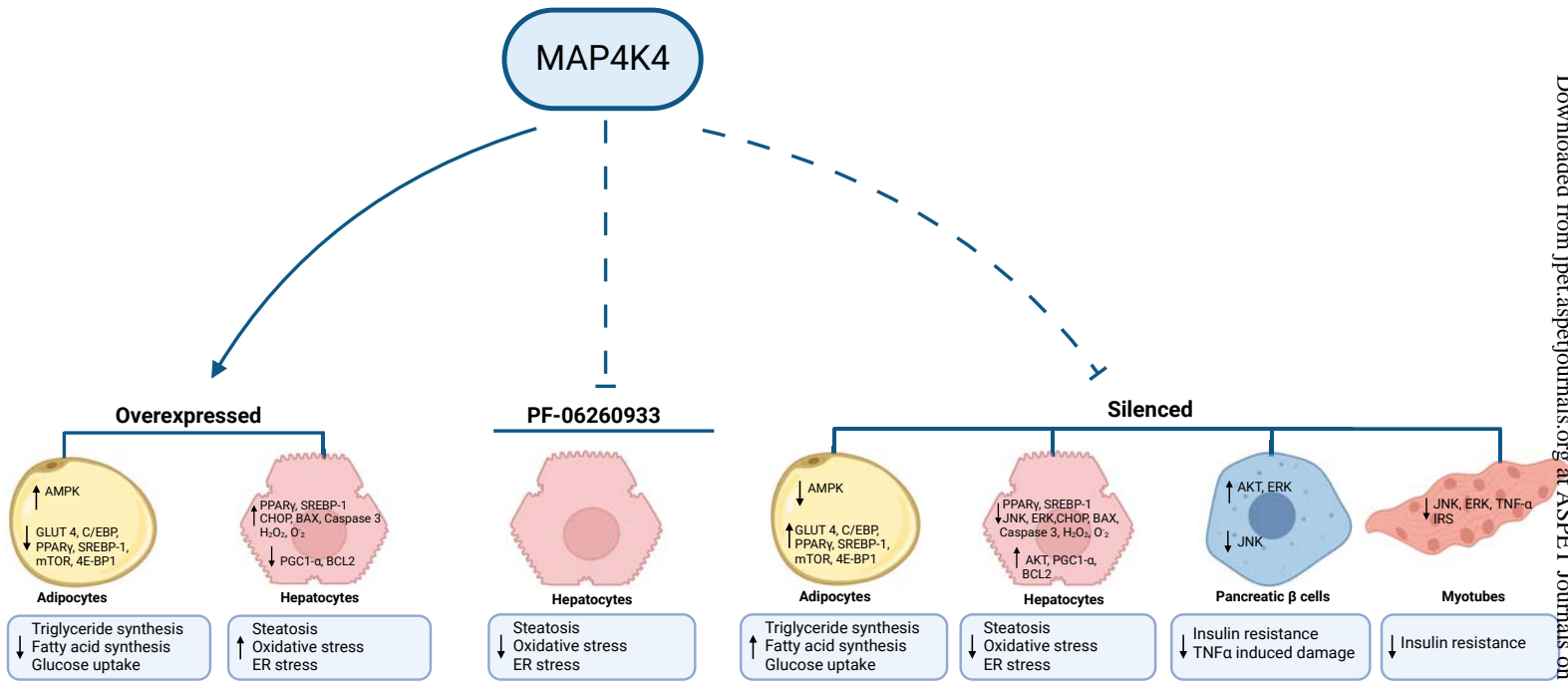


Figure 2

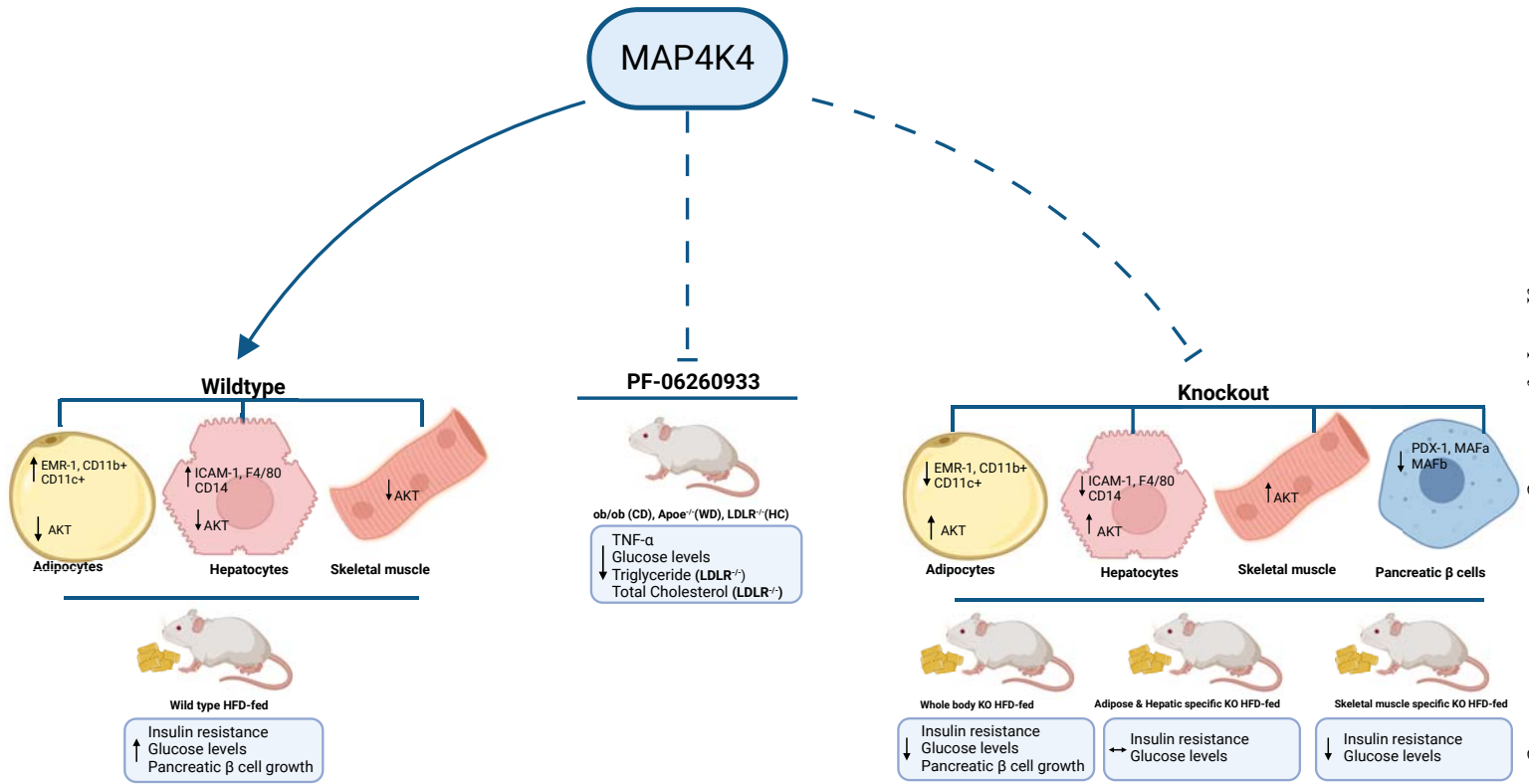


Figure 3

