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Review

FAM3 gene family: A promising therapeutical target for NAFLD and type 2 diabetes

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) and diabetes are severe public health issues worldwide. The Family with sequence similarity 3 (FAM3) gene family consists of four members designated as FAM3A, FAM3B, FAM3C and FAM3D, respectively. Recently, there had been increasing evidence that FAM3A, FAM3B and FAM3C are important regulators of glucose and lipid metabolism. FAM3A expression is reduced in the livers of diabetic rodents and NAFLD patients. Hepatic FAM3A restoration activates ATP-P2 receptor-Akt and AMPK pathways to attenuate steatosis and hyperglycemia in obese diabetic mice. FAM3C expression is also reduced in the liver under diabetic condition. FAM3C is a new hepatokine that activates HSF1-CaM-Akt pathway and represses mTOR-SREBP1-FAS pathway to suppress hepatic gluconeogenesis and lipogenesis. In contrast, hepatic expression of FAM3B, also called PANDER, is increased under obese state. FAM3B promotes hepatic lipogenesis and gluconeogenesis by repressing Akt and AMPK activities, and activating lipogenic pathway. Under obese state, the imbalance among hepatic FAM3A, FAM3B and FAM3C signaling networks plays important roles in the pathogenesis of NAFLD and type 2 diabetes. This review briefly discussed the latest research progress on the roles and mechanisms of FAM3A, FAM3B and FAM3C in the regulation of hepatic glucose and lipid metabolism.

Contents

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1. Introduction

In 2013, about 380 million people had diabetes worldwide, and this number would rise to about 590 million in 2035 [1]. In China, the prevalence of diabetes among adults is estimated to be as high as 10.9% to 11.6%, affecting more than 100 million people [2,3]. What is more serious is that the prevalence of prediabetes among Chinese adults is estimated to be 35.7% to 50.2%, affecting about 300 to 500 million people [2,3]. Type 2 diabetes is mainly characterized by pancreatic β cell dysfunction and peripheral insulin resistance, and accounts for more than 90% of diabetic patients. Non-alcoholic fatty liver disease (NAFLD) describes a pathophysiological process covering steatosis (simple fatty liver), non-alcoholic steatohepatitis (NASH) and cirrhosis, and it is a strong risk factor for many diseases including hyperlipidemia, insulin resistance and type 2 diabetes [4–8]. In the past decades, the prevalence of NAFLD had also become epidemic worldwide. It is estimated that the prevalence of NAFLD is 14% to 30% of the general population of western countries [9,10]. The prevalence of NAFLD among children is estimated to be 3% to 10%, and increases to 40% to 70% among obese children [11]. In diabetic patients, the prevalence of NAFLD has been estimated to be as high as 74% [12]. In China, the overall prevalence of NAFLD ranges from 20% to 42% depending on region [13–16]. Clearly, type 2 diabetes and NAFLD have become serious global public health issues.

In 2002, Zhu et al. identified four new genes [17] and classified them into one novel gene family termed Family with Sequence Similarity 3 (FAM3). These four genes are called FAM3A, FAM3B, FAM3C and FAM3D, respectively [17]. Because initial analysis revealed that FAM3B protein is highly expressed within pancreatic islets [17], FAM3B has also been referred to as Pancreatic Derived Factor (PANDER) in subsequent studies. In the past decade, intensive studies have revealed that some members of the FAM3 gene family play important roles in regulating glucose and lipid metabolism. This review briefly discussed the latest research on FAM3A, FAM3B (PANDER) and FAM3C. In particular, their roles in the regulation of hepatic glucose and lipid metabolism will be summarized and discussed.

2. FAM3A and Hepatic Glucose/Lipid Metabolism

According to the initial analysis conducted during the discovery of the FAM3 gene family, FAM3A mRNA is ubiquitously expressed among tissues of human and rodents [17]. Both human and mouse FAM3A proteins are composed of 230 amino acid residues and share high sequence similarity [17].

2.1. FAM3A Represses Hepatic Gluconeogenesis and Lipogenesis

FAM3A expression is decreased in human livers with steatosis when compared with healthy livers [18], suggesting that it may be involved in regulating hepatic glucose and lipid metabolism. To further determine the potential roles of FAM3A in glucose and lipid metabolism, its expression in the main metabolic tissues of obese diabetic mice was analyzed. Both FAM3A mRNA and protein are reduced in the liver and adipose tissue of obese mice [18]. Swimming exercise improved steatosis and hyperglycemia in db/db mice with a significant restoration of hepatic FAM3A expression. To directly address the role of FAM3A in hepatic glucose and lipid metabolism, it had been overexpressed in the livers of High-Fat diet (HFD) mice and db/db mice. Seven days after FAM3A overexpression, glucose intolerance, hyperglycemia and insulin resistance in diabetic mice were markedly improved [18]. Hepatic glucose production and steatosis were also attenuated by FAM3A overexpression in diabetic mice [18]. Gene profile analysis indicated that FAM3A overexpression inhibited the expression of key gluconeogenic and lipogenic genes including phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6pase) and fatty acid synthase (FAS), and increased the expression of genes involving lipid oxidation such as adiponectin receptor 1 (AdipoR1), uncoupling protein 2 (UCP2) and peroxisome proliferator-activated receptor-α (PPARα) [18]. FAM3A overexpression elevated phosphorylated Akt and adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK, pAMPK) levels in obese diabetic mouse livers. In contrast, hepatic FAM3A silencing caused hepatic lipid deposition and fasting hyperglycemia in normal C57BL/6 mice. FAM3A

silencing reduced pAkt and AdipoR1 levels and increased the expression of PEPCK and G6pase in normal mouse livers [18]. Akt is a crucial kinase controlling the expression of glucose and lipid metabolizing genes in various tissues by targeting forkhead box protein O1 (FOXO1), sterol regulatory element binding protein-1 (SREBP-1) and other transcription factors [19–25]. Although Akt activation promotes lipid synthesis via the activation of SREBP-1 pathway [19,26–28], there is increasing evidence that amelioration of steatosis by drugs or physical exercise is associated with increased Akt activity in the liver [29–31].

In cultured hepatocytes, FAM3A overexpression induced Akt activation in a PI3K-dependent, but insulin receptor-independent manner. FAM3A protein is predominantly present in mitochondrial but not cytosolic fraction. FAM3A overexpression promoted adenosine triphosphate (ATP) production in cultured hepatocytes and mouse livers. Released ATP has been reported to activate the PI3K/Akt signaling pathway via the ATP receptors (P2 receptors, which are divided in P2X and P2Y subtypes) in several other cell types [32–37]. We found that FAM3A elevated cytosolic free calcium levels via both P2X receptor-mediated influx of extracellular calcium and P2Y receptor-mediated release of calcium from internal calcium storage in cultured hepatocytes [18]. FAM3A-induced Akt activation was completely abolished by the inhibition of calmodulin (CaM) using chlorpromazine (CPZ). Hepatic FAM3A overexpression also suppressed gluconeogenic gene expression and ameliorated fasting hyperglycemia in type 1 diabetic mice [18]. However, because FAM3A-induced Akt activation is partially repressed by the P2 receptor inhibition but completely blocked by CaM inhibition, it is likely that other mechanism is also involved in FAM3A-mediated Akt phosphorylation in addition to P2 receptor signaling transduction. In pancreatic islet β cells, an increase in intracellular ATP levels can close ATP-sensitive potassium (K_{ATP}) channels to open L-type calcium channels, resulting in an influx of extracellular calcium [38–41]. Several lines of evidences have suggested that the opening of L-type calcium channels is also affected by K_{ATP} channel in hepatocytes [42–44]. We found that FAM3A-induced Akt activation was partially inhibited by the blockade of L-type calcium channels using nifedipine (unpublished data), suggesting that increased intracellular ATP levels may also contribute to FAM3A-induced increase in cytosolic calcium levels and Akt activation. Collectively, FAM3A activates Akt via the ATP-P2 receptor-Ca^{2+}-CaM-PI3K pathway independent of insulin receptor signaling transduction in hepatocytes [18].

In obese mouse livers, the expression of ATP synthase β subunit (ATPSβ) is reduced with a decrease in ATP content [45]. In support of the findings observed in FAM3A study [18], hepatic overexpression of ATPSβ increased liver ATP content, activated Akt, suppressed gluconeogenic gene expression, and ameliorated hyperglycemia and fatty liver in db/db mice [45]. In cultured hepatocytes, ATPSβ overexpression similarly activated the PI3K-Akt pathway via ATP-P2 receptor-CaM signaling transduction independent of insulin [45]. Importantly, FAM3A and ATPSβ overexpression promoted the nuclear exclusion of FOXO1 in P2 receptor-dependent manner in cultured hepatocytes [45,46]. Collectively, these findings strongly suggested that the ATP-P2 receptor signaling pathway plays important roles in the regulation of Akt and FOXO1 activities, and subsequent glucose and lipid metabolism in hepatocytes independent of insulin.

2.2. FAM3A Upregulates Hepatic AdipoR1 Expression

Adiponectin receptor 1 (AdipoR1) expression is reduced in the livers of obese diabetic rodents [47,48]. Hepatic AdipoR1 overexpression increased lipid oxidation and ameliorated hyperglycemia in db/db mice, whereas disruption of AdipoR1 repressed UCP2 expression and increased hepatic gluconeogenesis [49]. Moreover, activation of the adiponectin receptor signaling induces AMPK activation [50–52]. FAM3A overexpression upregulated AdipoR1 and UCP2 protein levels in diabetic mouse livers, whereas FAM3A silencing reduced AdipoR1 expression in normal mouse livers [18]. It is likely that upregulation of AdipoR1 contributes to FAM3A's beneficial effects on steatosis and hyperglycemia beyond Akt signaling transduction.

2.3. FAM3A and Hepatic AMPK Activation

AMPK represents an attractive target for the treatment of NAFLD and diabetes, and its activity is modulated by various factors such as adiponectin, metformin, and an increase in intracellular AMP/ATP ratio [50,53–55]. Metformin targets mitochondrial complex I to inhibit ATP synthesis [56,57], leading to an increase in AMP/ATP ratio and activation of AMPK [58,59]. Metformin also suppresses hepatic gluconeogenesis via AMPK-independent mechanism [56]. However, a decrease in ATP synthesis and cellular ATP content is associated with many diseases including heart failure, obesity, NAFLD and diabetes [45,60–64]. Because ATP is the key energy storage molecule and an important signaling molecule [18,34,45,65,66], enhancing ATP production by FAM3A or ATPSβ activation may have some unique advantages in the treatment of NAFLD and diabetes when compared with metformin. Thus far, the mechanism of FAM3A-induced AMPK activation in diabetic mouse livers remains uncharacterized. One possibility is that FAM3A activates AdipoR1 signaling transduction to induce AMPK phosphorylation [18,50,51].

2.4. FAM3A Improves Insulin Resistance

Lipid transfer from adipose tissue to the liver (ectopic fat deposition) plays an important role in the progression of fatty liver under insulin resistant conditions [67–69]. When insulin resistance occurs, lipolysis in adipose tissues increases, which results in an elevation in circulating free fatty acid (FFA) levels, leading to increased FFA uptake and excessive lipid deposition in the liver [70–72]. FAM3A overexpression improved global insulin resistance in both HFD-induced diabetic mice and db/db mice. Improvement of global insulin resistance by FAM3A should also contribute to its beneficial effects on steatosis.

2.5. FAM3A Is a Target Gene of PPARγ

Peroxisome proliferator-activated receptor (PPAR) superfamily members (PPARα, PPARδ and PPARγ) play crucial roles in

controlling glucose and lipid metabolism in various tissues [73,74]. PPARγ agonist treatment or PPARγ overexpression potently induced FAM3A expression in cultured human and mouse hepatocytes [75]. In contrast, activation of PPARα and PPARδ failed to induce FAM3A expression in cultured hepatocytes. A putative peroxisome proliferator response element (PPRE) is located at −1258/−1246 of the human *FAM3A* gene promoter. PPARγ but not PPARα and PPARδ directly binds to this PPRE-like motif in human *FAM3A* gene promoter and activates its transcriptional activity. Similarly, a PPRE-like motif is also found at −805/−791 of mouse *FAM3A* gene promoter. Activation of PPARγ, but not PPARα and PPARδ, stimulates the transcriptional activity of mouse *FAM3A* promoter [75]. These findings revealed that FAM3A is a new target gene of PPARγ. During the differentiation of 3T3L1 preadipocytes, the expression of both PPARγ and FAM3A expression are increased. FAM3A is further revealed to promote 3T3L1 preadipocyte differentiation via the activation of ATP-P2 receptor-Akt proliferative pathway [76]. Under obese condition, a decrease in FAM3A expression in adipocytes contributes to adipose dysfunction and type 2 diabetes [76]. Another report confirmed that FAM3A expression is increased during the adipogenesis of 3T3L1 cells [77]. However, it had also been reported that FAM3A overexpression inhibited 3T3L1 adipogenesis in the same study [77].

2.6. *NFE2/miR-423-5p Axis Represses FAM3A Signaling Transduction*

In the past decade, dysregulated miRNA profile in tissues and circulation had been shown to be associated with type 2 diabetes [78]. Circulating miR-423-5p level had been reported to be dysregulated in diabetic or obese patients [79–81].

Human and mouse FAM3A mRNAs are shown to be the direct targets of miR-423-5p, which share identical sequence between human and mouse. In the livers of obese mice and steatotic patients, miR-423-5p expression is increased with a decrease in FAM3A expression [82]. miR-423-5p overexpression repressed, whereas miR-423-5p inhibition activated FAM3A-ATP-Akt in cultured hepatocytes. In cultured hepatocytes, miR-423-5p inhibition repressed gluconeogenesis, whereas miR-423-5p overexpression promoted gluconeogenesis. Hepatic miR-423-5p inhibition activated FAM3A-ATP-Akt pathway and improved hyperglycemia, insulin resistance, gluconeogenesis and fatty liver in obese diabetic mice. In contrast, hepatic miR-423-5p overexpression repressed FAM3A-ATP-Akt pathway and promoted hyperglycemia with increased gluconeogenesis and lipid deposition in the liver of normal mice [82]. With the improvement of insulin resistance, hepatic miR-423-5p repression inhibits the lipolysis process of white adipose tissue and reduces serum FFA levels. miR-423-5p is further shown to be a direct target gene of transcriptor nuclear factor-erythroid 2 (NFE2), the expression of which is increased in the livers of obese mice and NAFLD patients. Hepatic NFE2 overexpression induced miR-423-5p expression to repress FAM3A-ATP-Akt pathway, promoting hyperglycemia and lipid deposition in the liver [82]. Overall, under obese condition, the activation of NFE2/miR-423-5p axis causes dysregulated glucose and lipid metabolism by repressing hepatic FAM3A signaling transduction.

2.7. *FAM3A and Other Diseases*

FAM3A protects liver against ischemia/reperfusion injury (IRI) by activating Akt survival pathway, and repressing inflammation and oxidative stress [46]. PPARγ agonist exerts beneficial effects on liver IRI by activating hepatic FAM3A signaling transduction [46]. FAM3A also stimulates the proliferation and migration of vascular smooth muscular cells (VSMCs) [83], and protects neuronal HT22 cells against apoptosis triggered by oxidative and endoplasmic reticulum (ER) stress [84,85].

To date, the mechanism of FAM3A-mediated enhancement in ATP production remains unclear. Moreover, further study is also needed to elucidate the mechanism(s) through which FAM3A activates AdipoR1 expression and AMPK in hepatocytes.

3. PANDER in the Pathogenesis of NAFLD and Type 2 Diabetes

Circulating PANDER is increased in patients with metabolic syndrome, and its level is correlated positively with metabolic syndrome components in a Chinese population. Notably, circulating PANDER level can predict the risk of type 2 diabetes in a Chinese population [86]. Another clinical report found that serum PANDER level is elevated in diabetic patients. Serum PANDER level is correlated negatively to pancreatic β cell function in diabetic patients [87]. These clinical studies revealed that an increase in circulating PANDER represents a common pathogenic process in the progression of metabolic syndrome and type 2 diabetes.

3.1. *Circulating PANDER Mediates the Crosstalk Between Pancreatic β Cell and Liver*

Extensive research had been performed to determine the biological functions of PANDER in pancreatic islets since its discovery in 2002. Recombinant PANDER treatment or PANDER gene overexpression induced the apoptosis of pancreatic α and β cells [88–91]. Glucose, free fatty acids and proinflammatory cytokines potently induce PANDER gene expression in pancreatic β cells [92–97]. In the islets of db/db mice, PANDER mRNA and protein expression are increased, which are reversed by rosiglitazone administration with the attenuation of hyperglycemia and insulin resistance [98]. These findings suggested that PANDER exerts deleterious effects on pancreatic β cells under insulin resistant or diabetic conditions. Paradoxically, PANDER-deficient mice exhibit impaired insulin secretion in response to glucose. Isolated PANDER-deficient mouse islets also exhibit blunted insulin secretion when challenged with glucose [99]. These observations suggested that PANDER has dual roles in regulating pancreatic β cell functions. PANDER facilitates insulin secretion in physiological condition, whereas abnormally increased PANDER expression exerts deleterious effects on pancreatic β cell functions under insulin resistant condition [98,100]. Similarly, low expression level of IL-1β is beneficial for the induction of insulin secretion and proliferation of pancreatic β cells in physiological condition [101]. However,

excessive expression and secretion of IL-1β exert deleterious effects on pancreatic β cells under insulin resistant or diabetic condition [101–103].

PANDER protein is co-secreted with insulin via a Ca^{2+} influx-dependent manner in pancreatic β cell lines and primary cultured mouse islets [104]. Moreover, islet α cells also secrete PANDER in response to arginine and insulin [105]. Circulating PANDER is increased in obese C57BL/6 mice [106]. PANDER is further shown to bind to the cell membrane of mouse liver cells and human HepG2 cells, inducing insulin resistance in hepatocytes [107]. One recent study reported that transgenic mice with PDX-1-driven specific overexpression of PANDER in pancreatic β cells develop fasting hyperglycemia and hepatic insulin resistance [108]. PANDER transgenic mice exhibit lower pAMPK level and increased lipid deposition in the liver than that in wild type mice [108]. Quantitative mass spectrometry-based proteomic analyses revealed that PANDER transgenic mouse liver displays activated liver X receptor (LXR) and lipogenic pathways when compared with wild type mouse livers. Several target genes of LXR including fatty acid synthase (FAS) and cholesterol 7α-hydroxylase (CYP7A1) are also upregulated in PANDER transgenic mouse livers [109,110]. Collectively, these in vitro and in vivo findings revealed that the liver is a direct target of islet-secreted PANDER. So far, the PANDER receptor has still not been identified.

3.2. *Liver-Derived PANDER in Glucose and Lipid Metabolism*

PANDER mRNA and protein expression are increased in the livers of HFD-fed mice and db/db mice. Hepatic PANDER overexpression induced global insulin resistance and steatosis in normal C57BL/6 mice. Gene profile analysis revealed that PANDER overexpression reduced pAkt, pAMPK and pFOXO1 protein levels but increases FOXO1, PPARγ, SREBP-1 and FAS protein levels in mouse livers [111]. In contrast, hepatic PANDER silencing improved global insulin resistance and steatosis in db/db mice. PANDER silencing increases pAkt, pAMPK and pFOXO1 expression levels, and reduces FOXO1, PPARγ, SREBP-1 and FAS expression levels in db/db mouse livers. Moreover, hepatic PANDER overexpression increases serum very low-density lipoprotein-triglyceride (VLDL-TG) levels in normal mice, whereas PANDER silencing reduced VLDL-TG levels in db/db mice [111]. Other reports also confirmed that hepatic PANDER overexpression induced glucose intolerance and fasting hyperglycemia in normal mice [112,113]. In support of these findings, hepatic glucose production is suppressed in PANDER-deficient mice [99].

In HepG2 cells, PANDER overexpression reduced pAkt and pFOXO1 levels in both absence and presence of insulin stimulation. PANDER overexpression promoted lipid deposition in cultured hepatocytes. In support, hepatic FOXO1 overexpression promoted lipid deposition in normal mouse livers [111]. Interestingly, FOXO1 overexpression induced PANDER expression in mouse livers and hepatocytes [111]. This suggests that the crossregulation between PANDER and FOXO1 finally results in excessive FOXO1 activity, which promotes lipogenesis and gluconeogenesis in the liver. In support, PANDER-deficient mice are resistant to HFD-induced fasting hyperglycemia [106]. PANDER-deficient mouse livers exhibited lower hepatic TG content than wild type mice. Moreover, PANDER-deficient mice also had higher hepatic pAkt and pAMPK levels than wild type mice [114]. Inhibition of PANDER expression has been proposed to mediate the beneficial effects of aerobic swimming training on steatosis in obese mice [115]. Moreover, Ratliff et al. cloned another PANDER gene fragment from liver cells, and elucidated that the PANDER promoter activity is potently activated by glucose in hepatocytes. Glucose-induced activation of the PANDER promoter activity was enhanced by carbohydrate-responsive element binding protein (ChREBP) [116]. This suggests that upregulation of PANDER is a novel mechanism for explaining the lipogenic and gluconeogenic effects of ChREBP [117]. However, one recent study reported that adenovirus-mediated overexpression of PANDER decreased TG content in normal mouse livers [118]. Although the reason for the discrepancy is not known, it is generally accepted that PANDER promotes gluconeogenesis and lipogenesis in the liver.

It is likely that insulin resistance, inhibition of AMPK activity and upregulation of PPARγ also contribute to PANDER-induced lipid deposition in the liver [111,112,114]. Wilson et al. reported a secretory form of PANDER in the medium of cultured hepatocytes with PANDER overexpression [112]. Conditioned medium collected from hepatocytes with PANDER expression induces gluconeogenic gene expression and promotes gluconeogenesis in cultured hepatocytes [112]. However, secretory PANDER isoform cannot be detected in mouse livers and cultured hepatocytes with or without PANDER overexpression in several other reports [111,113,118]. These findings suggested that liver-derived PANDER likely promotes lipogenesis and gluconeogenesis via the intracellular and extracellular mechanisms. Moreover, PANDER also impairs glucagon-like peptide 1 (GLP-1) production in intestinal L cells in vivo and in vitro, revealing a new mechanism of PANDER in the regulation of glucose and lipid metabolism [113]. Taken together, increased PANDER expression inhibits the activities of Akt and AMPK, leading to FOXO1 overactivation and promoting lipogenesis/gluconeogenesis in hepatocytes under obese or insulin resistant status.

Overall, the experimental evidences in animal and cell culture models support the clinical observations that circulating PANDER is a novel biomarker for NAFLD and type 2 diabetes.

3.3. *PANDER and Other Diseases*

PANDER regulates the survival of various cell types. siRNA-mediated silencing of PANDER induces apoptosis of HCT8, HCT116, A549, N9 and C2C12 cells [119]. PANDER promotes the invasion and metastasis of human colon cancer cells [120]. In Epstein-Barr virus (EBV)-positive human gastric tumors, methylation of the PANDER gene is increased with a decrease in PANDER mRNA expression [121]. PANDER also stimulates VSMC proliferation and migration by inhibiting miR-322-5p expression [122].

4. FAM3C in the Regulation of Hepatic Glucose and Lipid Metabolism

So far, the role of FAM3C, also called interleukin-like EMT inducer (ILEI), in the regulation of glucose and lipid metabolism remain unknown. FAM3C expression is reduced in the

liver but not other metabolic tissues of obese mice [123]. Hepatic FAM3C overexpression improved glucose intolerance, insulin resistance and steatosis in obese diabetic mice. In obese mouse livers, FAM3C overexpression increased Akt phosphorylation, and reduced the mRNA and protein levels of gluconeogenic and lipogenic genes [123]. Mechanistically, FAM3C promotes Akt activation in CaM-dependent but insulin independent manner. However, FAM3C-induced Akt phosphorylation is not dependent on influx of extracellular calcium and release of internal calcium. Collectively, FAM3C induced-activation of CaM-PI3K-Akt pathway is not dependent on calcium and insulin [123].

Three calmodulin genes, designated as CALM1, CALM2 and CALM3, respectively, exist in mammalians, and all of them encode one identical CaM protein [124]. Further in vitro and in vivo experiments revealed that FAM3C activates heat shock factor 1 (HSF1) to induce CALM1 transcription and elevate CaM protein to activate PI3K-Akt pathway, suppressing gluconeogenesis independent of calcium and insulin in hepatocytes [123]. Moreover, FAM3C also represses the mTOR-SREBP1-FAS lipogenic pathway in hepatocytes in vitro and in vivo, which partially explains the beneficial effects of FAM3C on steatosis [123].

In type 1 diabetic mouse livers, FAM3C-HSF1-CaM axis is repressed. FAM3C overexpression activated HSF1-CaM-Akt pathway to normalize the fasting blood glucose levels of type 1 diabetic mice to that of normal mice. Hepatic HSF1 overexpression also activated CaM-Akt pathway to ameliorate hyperglycemia of type 1 diabetic mice. What's more, FAM3C secretion is necessary for its impact on Akt activation and gluconeogenesis suppression in hepatocytes, revealing that FAM3C is a new hepatokine (Chen Z and Yang J, Oncotarget 2017, in press).

5. CaM in FAM3A and FAM3C Signaling

FAM3A and FAM3C activate CaM-Akt pathway via different mechanisms. FAM3A activates CaM-Akt pathway through Ca^{2+}-dependent mechanism, whereas FAM3C promotes the activation of CaM-Akt pathway in Ca^{2+}-independent manner. Together, these findings revealed that CaM signaling pathway plays important roles in suppressing hepatic gluconeogenesis and lipogenesis independent of insulin. As one of important protein with many functions [125], CaM activity is tightly regulated at transcriptional and functional levels.

CaM inhibitor CPZ is one antipsychotic drug that is primarily prescribed to treat psychotic disorders such as schizophrenia [126]. However, long-term administration of CPZ always triggers hyperglycemia in animals and human [127]. Several mechanisms including global inflammation, stimulation of hepatic glycogenolysis, inhibition of pancreatic insulin release and decrease of peripheral glucose uptake had been proposed to explain CPZ's hyperglycemic effects [127,128]. Moreover, CPZ also represses mitochondrial β-oxidation of long-chain fatty acids in hepatocytes [129]. Based on a series of our findings [18,45,82,123], it is very possible that long-term administration of CPZ also promotes hepatic gluconeogenesis and lipogenesis to cause dysregulated glucose and lipid metabolism. To address this issue will be useful for avoiding the hyperglycemic effects of CPZ kind of drugs.

6. Crosstalks Among FAM3 Gene Family Members in the Progression of NAFLD and Diabetes

Under obese status, an increase in PANDER expression is always associated with a decrease in FAM3A and FAM3C expression in the liver. Physical exercise significantly reduces PANDER expression with an increase in FAM3A expression in the livers of db/db mice [18,111]. It is likely that the imbalance among hepatic FAM3A, PANDER and FAM3C expression and signaling networks contributes to the progression of steatosis and type 2 diabetes. In particular, the imbalance between PANDER and FAM3A expression contributes much to the repression of Akt and AMPK activities in hepatocytes under obese condition. Then, an

Table 1 – Summarization and comparison of basic characteristics of FAM3A, FAM3B and FAM3C.

	FAM3A		FAM3B (PANDER)		FAM3C (ILEI)	
Chromosomal location	Human	Mouse	Human	Mouse	Human	Mouse
	Xq28	X; X A7.3	21q22.3	16 C4; 16 57.47 cM	7q31.31	6 A3.1; 6 9.24 cM
Amino acid residues	230		235		227	
Tissue distribution	Ubiquitous expression among mouse and human tissues		Highly expressed in pancreas of human and mouse; also ubiquitously expressed in other tissues		Ubiquitous expression among mouse and human tissues	
Alterations in diabetic state	Expression decreased in liver and adipose of obese diabetic mice, and in steatotic human liver		Expression increased in pancreatic islet and liver of diabetic rodents; circulating levels are increased in diabetic rodents and human		Expression decreased in liver of type 1 and type 2 diabetic mice	
Main mechanisms of action	Suppresses hepatic gluconeogenesis and lipogenesis via the activation of Akt/AMPK activities and upregulation of AdipoR1		Negatively regulates pancreatic β cell functions; promotes hepatic gluconeogenesis and lipogenesis via the inhibition of Akt/AMPK activities and activation of lipogenic pathway		Suppresses hepatic gluconeogenesis and lipogenesis via the activation of HSF1-CaM-Akt pathway and inhibition of mTOR-SREBP1-FAS pathway	

interesting question raised is how this imbalance happens. As discussed above, PANDER expression is activated by FFAs and FOXO1 [98,111], while FAM3A expression is induced by PPARγ activation, but repressed by high levels of FFAs and insulin, and miRNA [18,75,82]. FAM3C expression was repressed by FFAs [123]. So, one possibility is that FAM3A and FAM3C may repress PANDER expression by Akt-mediated inhibition of FOXO1 activity. On the other hand, PANDER may repress hepatic FAM3A and FAM3C expression by inducing FFAs accumulation in hepatocytes. Moreover, PANDER may also upregulate FAM3A expression via the activation of PPARγ in hepatocytes [111]. Elucidation of how and when the imbalance among hepatic FAM3A, PANDER and FAM3C expression and functions occur will shed light on better understanding the pathogenesis of steatosis and type 2 diabetes.

About 20–30% of patients with steatosis will progress to NASH [130]. So far, whether FAM3 gene family members are involved in the pathogenesis of NASH remains unknown. FAM3A expression is reduced in human livers with steatosis [18,82]. NASH is mainly characterized by hepatic inflammation and fibrosis beyond lipid deposition [7]. Given the roles of FAM3A in attenuating steatosis, inflammation, oxidative stress and ER stress [18,46,82,84,85], which are the risk factors for NASH [7], it may also represent a promising target for treating NASH. To determine the roles and mechanisms of FAM3 gene family members in NASH is of great importance.

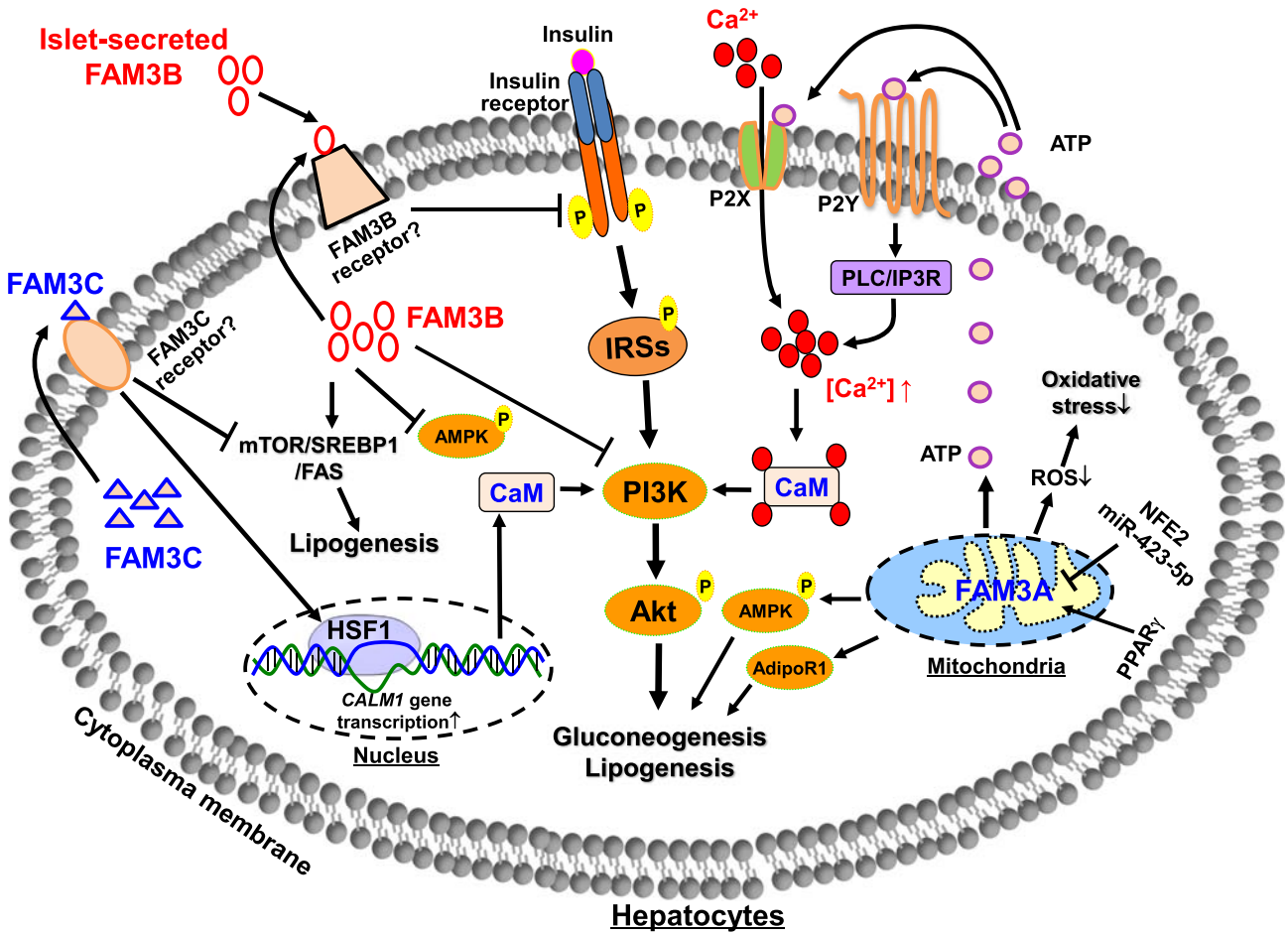

Fig. 1 – FAM3A, FAM3B (PANDER) and FAM3C are novel regulators of hepatic glucose and lipid metabolism. FAM3A activates CaM-Akt pathway through ATP-P2 receptor-mediated increase in cellular Ca^{2+} level. FAM3C activates HSF1 to induce CALM1 transcription, increasing CaM protein level to activate PI3K-Akt pathway independent of cellular Ca^{2+}. In contrast, islet- and liver-derived FAM3B (PANDER) inhibits Akt activation in hepatocytes. The receptors for FAM3B and FAM3C have still not been identified. IRSs, insulin receptor substrates; PI3K, phosphoinositide 3-kinase; AMPK, adenosine 5′-monophosphate-activated protein kinase; AdipR1, adiponectin receptor 1; mTOR, mammalian target of rapamycin; SREBP1, sterol regulatory element-binding protein 1; FAS, fatty acid synthase; CaM, calmodulin; HSF1, heat shock factor 1; ROS, reactive oxygen species; ATP, adenosine triphosphate; NFE2, nuclear factor erythroid-derived 2; P2X, P2X receptor subtypes; P2Y, P2Y receptor subtypes; PLC, phospholipase C; IP3R, inositol 1,4,5-trisphosphate receptor.

7. Modulation of FAM3A, PANDER and FAM3C Expression for Treating NAFLD and Diabetes

As discussed above, activating FAM3A/FAM3C or inhibiting PANDER could be potential strategy for treating NAFLD and type 2 diabetes. The expression changes in diabetic tissues, tissue distribution, main action mechanisms and other basic information of FAM3A, PANDER and FAM3C are summarized in Table 1. Based on the previous study, potential activators or inhibitors of various transcriptors could be used to modulate FAM3A, PANDER and FAM3C expression. FAM3A expression is activated by transcriptor PPARγ [75] but repressed by NFE2 in hepatocytes [82]. So, PPARγ agonists or NFE2 inhibitors could be potential molecules for inducing FAM3A expression in hepatocytes. Actually, PPARγ agonist rosiglitazone's beneficial effects on liver IRI are achieved through induction of FAM3A expression [46]. It is of great interest whether FAM3A is involved in the hypoglycemic effects of PPARγ agonists. PANDER expression is induced by transcriptors FOXO1 [111] and ChREBP-1 [116] in hepatocytes. Potential inhibitors of FOXO1 and ChREBP-1 could be used to inhibit PANDER expression in hepatocytes. In particularly, interrupting the crossregulation network between PANDER and FOXO1 will be important for reducing FOXO1 activity in hepatocytes [111]. Heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) can bind with the 3′-UTR of FAM3C mRNA to repress its translation [131]. Moreover, the stability of FAM3C protein is affected by ubiquitination factor E4A (UBE4A), a cognate ubiquitin ligase that interacts with FAM3C protein [132]. Inhibitors of hnRNP E1 and UBE4A may be used for inducing FAM3C expression. Overall, further intensive study on the regulatory mechanisms of FAM3 gene family members will provide more potential targets for modulating their expression to treat NAFLD and type 2 diabetes.

8. Summary and Perspective

FAM3A, PANDER and FAM3C are novel regulators of hepatic glucose and lipid metabolism via the modulation of gluconeogenic and lipogenic pathways (Fig. 1). The imbalance among hepatic FAM3A, PANDER and FAM3C signaling networks plays important roles in the development and progression of NAFLD and type 2 diabetes. Clearly, correcting this imbalance either by inhibiting PANDER expression and/or by activating FAM3A and FAM3C expression in the liver

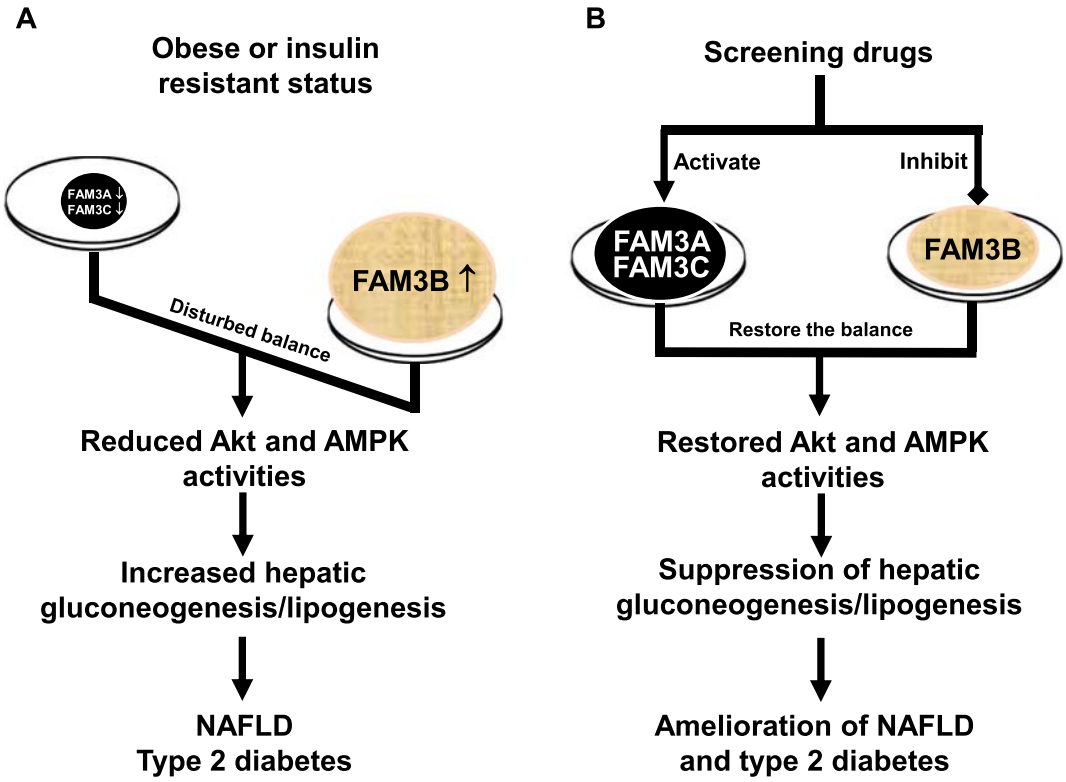

Fig. 2 – Correcting the imbalance in signaling networks among FAM3 gene family members is the potential strategy for treating NAFLD and type 2 diabetes. A) Under obese condition, upregulation of PANDER expression and/or repression of FAM3A and FAM3C expression will lead to the imbalance of these important Akt and AMPK activity regulators, contributing to the development of metabolic disorders including NAFLD and type 2 diabetes. B) Designing or screening drugs that either activate FAM3A and FAM3C expression, or inhibit PANDER expression is the potential strategy for the treatment of NAFLD and type 2 diabetes. NAFLD, non-alcoholic fatty liver disease.

represents a novel strategy for the treatment of NAFLD and type 2 diabetes (Fig. 2). In particular, given its roles in the activation of Akt and AMPK pathways, and inhibition of oxidative stress and inflammation in hepatocytes, activating FAM3A may hold great promise for the treatment of NAFLD and diabetes.

Author Contributions

X.Z., W.Y., J.W. and Y.M. wrote the manuscript. J.Y. and Y.G. revised and edited manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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