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FOXO transcription factors in non-alcoholic fatty liver disease☆

X. Charlie Dong

Department of Biochemistry and Molecular Biology, Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN, USA

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a chronic progressive liver disorder that begins with simple hepatic steatosis and progresses to non-alcoholic steatohepatitis, fibrosis, cirrhosis, and even liver cancer. As the global prevalence of NAFLD rises, it is increasingly important that we understand its pathogenesis and develop effective therapies for this chronic disease. Forkhead box O (FOXO) transcription factors are key downstream regulators in the insulin/insulin-like growth factor 1 (IGF1) signaling pathway, and have been implicated in a range of cellular functions including the regulation of glucose, triglyceride, and cholesterol homeostasis. The role of FOXOs in the modulation of immune response and inflammation is complex, with reports of both pro- and anti-inflammatory effects. FOXOs are reported to protect against hepatic fibrosis by inhibiting proliferation and transdifferentiation of hepatic stellate cells. Mice that are deficient in hepatic FOXOs are more susceptible to non-alcoholic steatohepatitis than wild-type controls. In summary, FOXOs play a critical role in maintaining metabolic and cellular homeostasis in the liver, and dysregulation of FOXOs may be involved in NAFLD development.

Keywords

Forkhead box O (FOXO); Non-alcoholic fatty liver disease (NAFLD); Insulin-like growth factor 1 (IGF1); Steatosis; Inflammation; Fibrosis

1. Introduction

Forkhead box O (FOXO) transcription factors belong to the O subfamily of the forkhead box protein family.[1] There is a single *FOXO* gene in *Caenorhabditis elegans* (*DAF-16*) and *Drosophila* (*dFOXO*), and four *FOXO* genes (*FOXO1/3/4/6*) in mammals. FOXO proteins are highly conserved, especially the forkhead box and transactivation domains, and RAC-alpha serine/threonine-protein kinase (AKT) conserves three major phosphorylation sites (Fig. 1). Mammals and other animals, such as *Caenorhabditis elegans* and *Drosophila*, share similar insulin/insulin-like growth factor (IGF) 1 signaling cascades (Fig. 2). Insulin/IGF1

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Conflict of interest

The author declares that he has no conflict of interest.

activate insulin receptor/IGF1 receptor, which subsequently activate insulin receptor substrates through tyrosine phosphorylation. The activated insulin receptor substrates stimulate phosphoinositide 3-kinase, which converts Phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] to phosphatidylinositol-3,4,5-trisphosphate [$PI(3,4,5)P_3$]. This stimulates 3-phosphoinositide-dependent protein kinase 1 and mechanistic target of rapamycin complex 2, which activate AKT at Thr308 and Ser473, respectively.[2–4] FOXOs are the immediate downstream effectors of AKT (Fig. 3).

FOXO transcriptional activity can be regulated by various post-translational modifications, though is predominantly regulated by phosphorylation and acetylation.[5] AKT kinases play a critical role in FOXO inactivation by phosphorylating a few conserved serine/threonine sites of each FOXO (FOXO1-Thr24/Ser256/Ser319, FOXO3-Thr32/Ser253/Ser315, FOXO4-Thr32/Ser197/Ser262, FOXO6-Thr26/Ser184).[6] In addition to AKT, there are a number of other kinases that can phosphorylate FOXOs, including adenosine monophosphate (AMP)-activated protein kinase (AMPK), c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase, mammalian sterile 20-like kinase 1, and protein kinase R-like endoplasmic reticulum kinase.[7] In addition to phosphorylation, FOXOs can be acetylated by p300/ cyclic AMP response element-binding protein (CBP) acetyltransferases and deacetylated by sirtuin (SIRT) 1 and histone deacetylase 3.[8–17]

FOXOs have pleiotropic functions in animal systems, with effects on cell survival, anti-oxidative stress, autophagy, and metabolism (Fig. 4). In this short review, I will summarize our current understanding of liver FOXOs and their role in NAFLD development.

2. FOXOs in glucose and lipid metabolism

The interplay between FOXO transcription factors and insulin and nutrient signaling pathways means that FOXOs play an important role in both glucose and lipid metabolism (Fig. 3).[18–40] The role of FOXOs in the regulation of genes that are critically involved in glucose, triglyceride, and cholesterol metabolism is summarized below.

2.1. FOXOs in hepatic glucose metabolism

FOXOs have been shown to play a critical role in hepatic glucose homeostasis. Knockout of either *FoxO1* alone or *FoxO1/3/4* altogether specifically in mouse liver leads to lower blood glucose levels under both fasting and non-fasting conditions.[21,25,26,35,36,40] *FoxO6* whole body knockout mice also exhibit lower levels of fasting and non-fasting blood glucose.[18] In response to starvation, FOXOs transcriptionally activate the hepatic gluconeogenic program by inducing a number of genes including phosphoenolpyruvate carboxykinase 1, glucose-6-phosphatase catalytic subunit, and pyruvate dehydrogenase kinase 4.[24,26,35,36,38,40,41] Meanwhile, FOXOs also inhibit glycolysis, likely through suppression of glucokinase and pyruvate kinase gene expression (Fig. 4).[24,26,35,36,38,41] By doing so, FOXOs help maintain normal blood glucose levels during starvation. However, under insulin resistant or diabetic conditions, with the tight control of insulin signaling lacking, FOXOs continuously activate hepatic gluconeogenesis and thereby promote hyperglycemia.[26,41]

2.2. FOXOs in hepatic triglyceride metabolism

FOXOs play a critical role in triglyceride homeostasis by regulating *de novo* lipogenesis, fatty acid oxidation, import of free fatty acids from the blood circulation, and export of triglyceride-rich very low density lipoproteins to the blood circulation (Fig. 4). In the regulation of de novo lipogenesis, FOXOs suppress the lipogenic master regulator sterol regulatory element binding protein (SREBP) 1 at the transcriptional level. As a result, a number of genes involved in fatty acid biosynthesis are also modulated by FOXOs, including acetyl-CoA carboxylase alpha, fatty acid synthase, adenosine triphosphate citrate lyase, malic enzyme 1, mitochondrial glycerol-3-phosphate acyltransferase, and stearoyl-CoA desaturase 1.[20,21,23,29,31,36–38] Moreover, FOXOs activate lipolysis and fatty acid oxidation genes including adipose triacylglycerol lipase, hormone-sensitive lipase, lipoprotein lipase, and carnitine palmitoyltransferase 1.[21,31,37,38,42] Interestingly, FOXO1 also suppresses expression of the G0/G1 switch-2 gene that encodes an inhibitor of adipose triacylglycerol lipase.[37] FOXO1 has been shown to upregulate fatty acid transporters such as Leukocyte differentiation antigen CD36.[43] In addition, FOXOs promote lipid droplet breakdown through activation of lipophagy, an autophagy process that degrades lipid droplets for energy production. A number of autophagy-related genes including autophagy related 5 (*ATG5*), *ATG12, ATG14*, beclin 1, phosphatidylinositol 3-kinase catalytic subunit type 3 (*PIK3C3*), and sestrin 3 are regulated by FOXOs. A role of autophagy in the promotion of lipid metabolism in the liver has been suggested by numerous studies;[34,44–49] however, the underlying mechanism remains largely unclear.

2.3. FOXOs in hepatic cholesterol metabolism

FOXOs also regulate a number of genes involved in cholesterol biosynthesis and metabolism (Fig. 4). *SREBP-2*, the master regulator of cholesterol biosynthesis, is a direct target of FOXOs, especially FOXO3.[32] Hepatic *FoxO1/3/4* triple knockouts show increased expression of the *SREBP-2* gene.[32] As expected, a number of SREBP-2 target genes including 3-hydroxy-3-methylglutaryl-CoA reductase and 3-hydroxy-3-methylglutaryl-CoA synthase 1 are also suppressed by FOXOs.[21,23,32,36,38] In addition to cholesterol biosynthesis, FOXO1 regulates cholesterol conversion to bile acids by modulating bile acid biosynthetic genes including cytochrome P450 family 7 subfamily A polypeptide 1 (*CYP7A1*), *CYP7B1*, and *CYP8B1*, although there are inconsistent findings with regard to the role of FOXO1 in the *CYP7A1* gene regulation. FOXO1 also upregulates the genes encoding biliary cholesterol transporters—ATP binding cassette subfamily G member 5 and member 8.[50–56]

In addition, FOXOs regulate low-density lipoprotein (LDL)-cholesterol homeostasis. Normally, LDL-cholesterol is degraded through a LDL receptor (LDLR)-mediated clearance process; however, when the level of proprotein convertase subtilisin/kexin type 9 (PCSK9) is elevated, the interaction between PCSK9 and LDLR leads to the degradation of LDLR and causes an increase in LDL-cholesterol.[57] Interestingly, the *PCSK9* gene is suppressed by FOXO3 and SIRT 6. When FOXO3 or SIRT 6 is deficient in the liver, circulating LDL-cholesterol levels are elevated.[58]

3. FOXOs in non-alcoholic steatohepatitis

As FOXOs play a critical role in glucose and lipid homeostasis, it is not surprising that dysregulation of hepatic FOXOs may lead to metabolic disorders. Studies of *FoxO* gene knockouts and overexpression in mice have provided strong evidence regarding the role of FOXOs in hepatic steatosis. On a regular diet, deletion of *FoxO1/3* or *FoxO1/3/4* genes in mouse liver leads to mild or moderate hepatic steatosis, respectively.[29,31,36] Overexpression of a constitutively active *FOXO1* transgene reduces hepatic triglyceride content.[37,38] When challenged by high-fat diets, *FoxO1/3/4* liver-specific knockout mice develop very severe hepatic steatosis, especially on a high-fat plus cholesterol diet.[29]

FOXOs have been shown to modulate inflammation through regulation of a number of genes including interleukin 1 beta, toll-like receptor 4, C-C motif chemokine ligand 2, C-C motif chemokine receptor 2, and adhesion G protein-coupled receptor E1 (also named *EMR1 or F4/80*) (Fig. 4). Overexpression of constitutively active *FOXO1* mutant in macrophages mediated by a LysM-Cre induces the expression of the C-C motif chemokine receptor 2 gene and increases the number of proinflammatory M1-type macrophages in mouse adipose tissue[59] (though whether similar changes occur in hepatic macrophages or Kupffer cells is unclear). Mice that are deficient in *FoxO1/3/4* specifically in hepatocytes are susceptible to high-fat plus cholesterol diet-induced inflammation and liver injury.[29] It has been reported that FOXO1 expression and activity is elevated in patients with steatohepatitis.[60] More studies are needed to clarify the role of FOXOs in human non-alcoholic steatohepatitis.

4. FOXOs in fibrosis

Human NAFLD is a progressive liver disease that begins with simple steatosis, transitions to hepatic inflammation, and later develops fibrosis as extracellular matrix proteins such as collagen gradually accumulate in the liver. Hepatic stellate cells (HSCs) are believed to play a crucial role in the development of liver fibrosis.[61] FOXO1 has been shown to inhibit proliferation and transdifferentiation of HSCs, partly through the regulation of cyclin-dependent kinase inhibitor 1B and superoxide dismutase 2.[62] After a bile duct ligation, $FoxO1^{+/-}$ mice are more predisposed to hepatic fibrosis than wild-type mice.[62] Using the immortalized human HSC cell line LX-2, it has been shown that FOXO1 and FOXO3 are also involved in the tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of HSCs.[63] In addition to their effect on HSCs, FOXO1/3/4 in hepatocytes play a protective role in diet-induced liver fibrosis. When hepatic *FoxO1/3/4* genes are deleted in mice, expression of fibrogenic genes including type I collagen alpha 1 and tissue inhibitor of metalloproteinase 1 is greatly elevated after the knockout mice are challenged with either a high-fat or high-fat plus cholesterol diet.[29]

5. Conclusions

As FOXOs have been implicated in longevity in different organisms,[5,64–67] their salutary functions in the liver, including maintaining glucose, triglyceride, and cholesterol homeostasis, and modulating inflammation and fibrosis, may contribute to the prolonged lifespan and protection against NAFLD (Fig. 5). Importantly, FOXO activity needs to be

controlled according to dynamic environmental cues, as over- or under-activation may lead to undesirable consequences. For example, under insulin resistant conditions, FOXOs are constitutively active, resulting in elevated hepatic glucose output and M1-type macrophage activation.[21,30,40,59,60,68–71] Additional studies are needed to fully understand the role of FOXOs in normal hepatic function and NAFLD development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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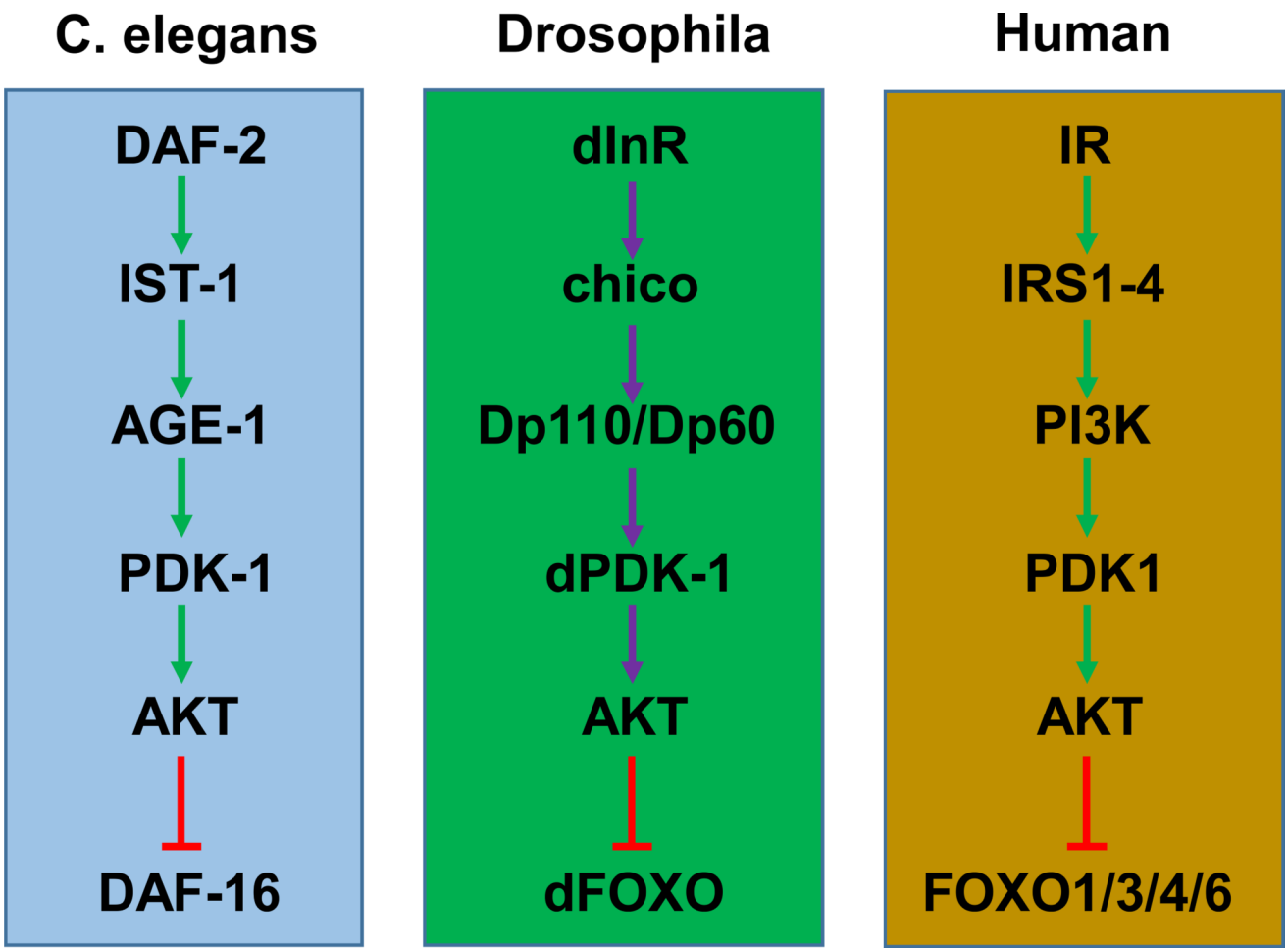

Figure 1. The insulin/insulin-like signaling pathways are evolutionally conserved

The FOXO transcription factors are regulated by the insulin/insulin-like signaling pathways that are well conserved in *C. elegans*, *Drosophila*, and mammals. Upon stimulation by insulin or insulin-like growth factors (IGFs), the insulin/IGFs receptors are activated, and subsequently the signaling cascade of IRS→PI3K→PDK1→AKT is activated as well. As a result, FOXOs are phosphorylated and inhibited by AKT.

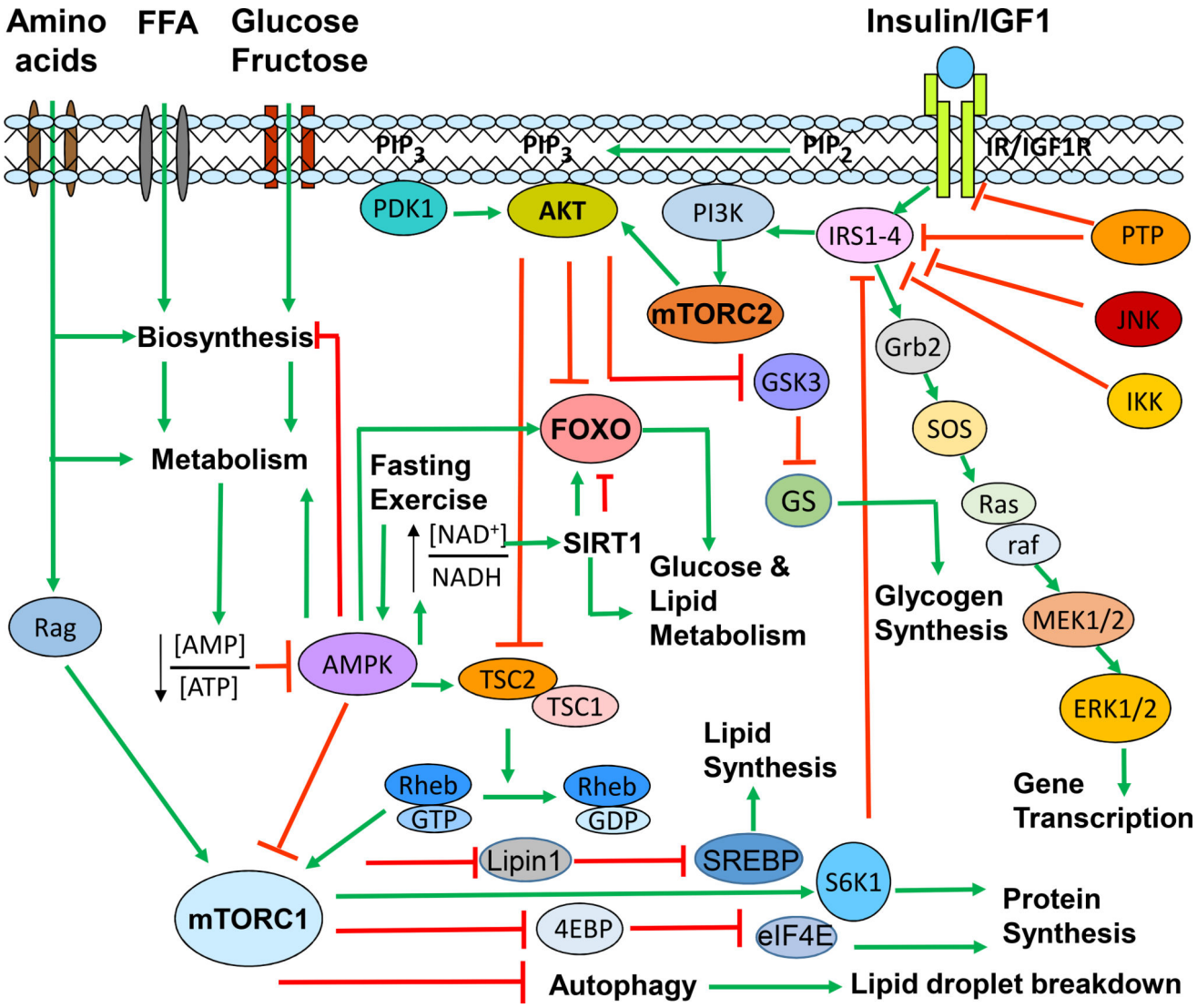

Figure 2. Insulin signaling and nutrient sensing pathways in hepatocytes

Major signaling cascades in the insulin and amino acid signaling pathways are outlined in this simplified diagram. Insulin and nutrient signaling is normally integrated to maintain metabolic homeostasis. Insulin plays a critical role in glucose, lipid, and protein metabolism. Upon insulin stimulation, the insulin signaling cascade (IR→IRS→PI3K→PDK1/mTORC2→AKT) is activated. As a major kinase in the downstream of the insulin signaling, AKT controls hepatic glucose and lipid homeostasis. AKT activates glycogen synthesis by inhibiting GSK3 through phosphorylation. Meanwhile, AKT also inhibits the FOXO transcriptional activity for hepatic gluconeogenesis through phosphorylation and nuclear exclusion of FOXO. AKT also promotes lipid and protein synthesis through activation of mTORC1. In addition to insulin, amino acids also activate mTORC1 to promote protein synthesis and inhibit autophagy. mTORC1 stimulates lipogenesis through activation of SREBPs. FOXO is also modulated via deacetylation by SIRT1, an NAD^+-dependent deacetylase. The energy sensor AMPK regulates metabolic homeostasis through activation of FOXO and inhibition of mTORC1.

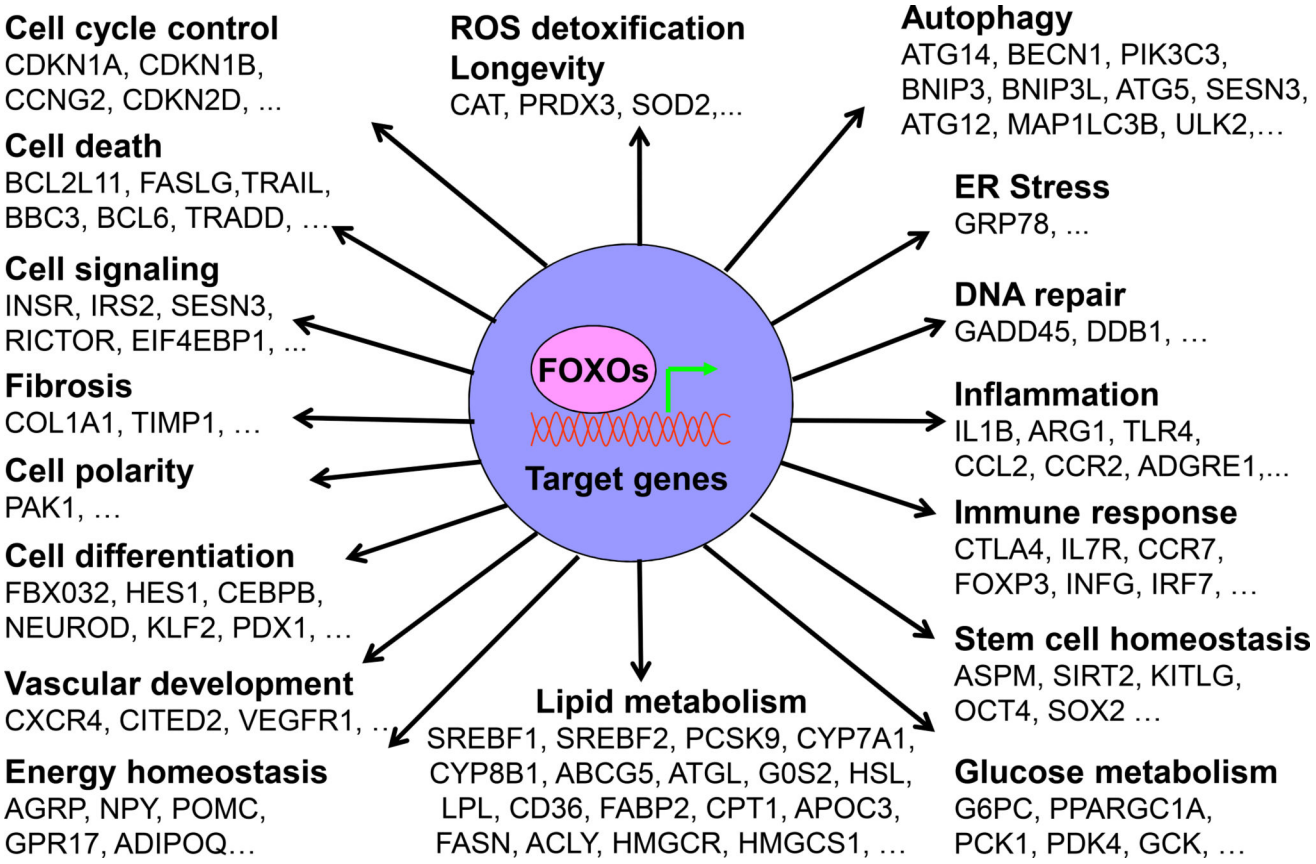

Figure 3. FOXOs have pleiotropic functions

Major FOXO functions are highlighted here to indicate the involvement of FOXOs in multiple cellular processes including cell cycle control, cell differentiation, glucose and lipid metabolism, energy homeostasis, autophagy, ROS detoxification, ER stress, DNA repair, and immune response. Numerous genes have been identified as FOXO targets. Owing to the limited space, only a small number of the FOXO-regulated genes for each biological process are listed here.

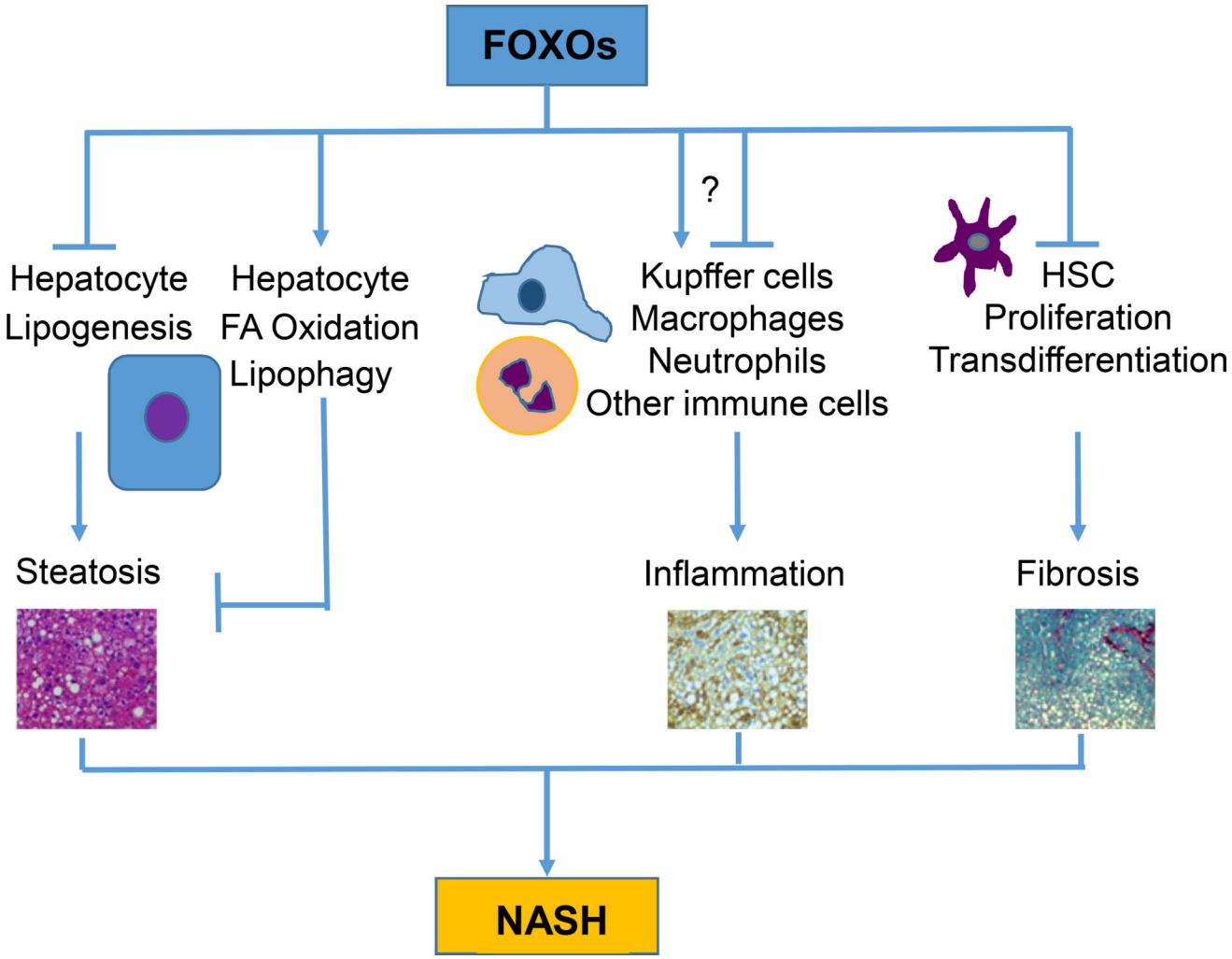

Figure 4. A working model depicting the involvement of FOXOs in the pathogenesis of NASH

This is a very simplistic view of FOXOs in the development of NASH from the perspective of three major cell types in the liver – hepatocytes, Kupffer cells, and hepatic stellate cells (HSCs). Crosstalks between these and other cell types are not illustrated here. In hepatocytes, FOXOs suppress the development of steatosis by promoting lipophagy and fatty acid oxidation and inhibiting triglyceride and cholesterol biosynthesis. In immune cells including Kupffer cells and circulated macrophages, the role of FOXOs is not very clear as both pro- and anti-inflammation activities of FOXOs have been reported in the literature. Additional studies are needed to clarify the role of FOXOs in hepatic immune cells. In hepatic stellate cells (HSCs), FOXO1 has been shown to suppress HSC proliferation and transdifferentiation, thus inhibiting hepatic fibrosis.