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MINIREVIEWS

Galectin-3 and IL-33/ST2 axis roles and interplay in diet-induced steatohepatitis

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Abstract

Immune reactivity and chronic low-grade inflammation (metaflammation) play an important role in the pathogenesis of obesity-associated metabolic disorders, including type 2 diabetes and nonalcoholic fatty liver disease (NAFLD), a spectrum of diseases that include liver steatosis, nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Increased adiposity and insulin resistance contribute to the progression from hepatic steatosis to NASH and fibrosis through the development of proinflammatory and profibrotic processes in the liver, including increased hepatic infiltration of innate and adaptive immune cells, altered balance of cytokines and chemokines, increased reactive oxygen species generation and hepatocellular death. Experimental models of dietary-induced NAFLD/NASH in mice on different genetic backgrounds or knockout mice with different immune reactivity are used for elucidating the pathogenesis of NASH and liver fibrosis. Galectin-3 (Gal-3), a unique chimera-type β-galactoside-binding protein of the galectin family has a regulatory role in immunometabolism and fibrogenesis. Mice deficient in Gal-3 develop pronounced adiposity, hyperglycemia and hepatic steatosis, as well as attenuated liver inflammation and fibrosis when fed an obesogenic high-fat diet. Interleukin (IL)-33, a member of the IL-1 cytokine family, mediates its effects through the ST receptor, which is present on immune and non-immune cells and participates in immunometabolic and fibrotic disorders. Recent evidence, including our own data, suggests a protective role for the IL-33/IL-33R (ST2) signaling pathway in obesity, adipose tissue inflammation and atherosclerosis, but a profibrotic role in NASH development. The link between Gal-3 and soluble ST2 in myocardial fibrosis and heart failure progression has been demonstrated and we have recently shown that Gal-3 and the IL-33/ST2 pathway interact and both have a profibrotic role in diet-induced NASH. This review discusses the current evidence on

the roles of Gal-3 and the IL-33/ST2 pathway and their interplay in obesity-associated hepatic inflammation and fibrogenesis that may be of interest in the development of therapeutic interventions to prevent and/or reverse obesity-associated hepatic inflammation and fibrosis.

Key words: Galectin-3; Liver fibrosis; Interleukin-33; ST2; Nonalcoholic steatohepatitis

Core tip: Obesity-associated chronic low-grade inflammation (metaflammation) plays an important role in the pathogenesis of nonalcoholic steatohepatitis (NASH). Galectin-3 (Gal-3), a β-galactoside-binding protein, plays a regulatory role in metaflammation and tissue fibrosis. The Interleukin (IL)-33/ST2 pathway has a protective role in obesity and adipose tissue inflammation and promotes liver fibrosis. The characteristics of dietary-induced NASH differ in mice on different genetic backgrounds and Gal-3 and ST2 (IL-33R) knockout mice. In this report, we review current evidence on the roles of Gal-3 and the IL-33/ST2 pathway and their interplay in obesity-associated hepatic inflammation and fibrogenesis that may be of interest in the development of therapeutic interventions.

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INTRODUCTION

Metabolism and immunity are closely connected and in the conditions of chronic overnutrition and low energy expenditure during obesity immune-mediated metabolic control are exerted in metabolic tissues, including adipose tissue and the liver. Metabolism and immunity share regulatory components that are yet incompletely understood[1]. Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), a severe form of NAFLD that can progress to liver fibrosis and cirrhosis, are regarded as a hepatic manifestation of metabolic syndrome[2]. The etiopathogenesis of NASH remains poorly understood, but existing data indicate that it is far more prevalent in obese individuals[3]. Obesity is characterized by increased adipose tissue mass, but also ectopic lipid accumulation and chronic low-grade inflammation in metabolic tissues[4]. The adipose tissue of obese individuals contains infiltrating immune cells and increased local generation of proinflammatory cytokines, as a result of the activation of stress kinases including c-Jun N-terminal protein kinases (JNK), IκB kinase (IKK) and protein kinase PKR, which in turn interferes with insulin signaling and triggers insulin resistance in adipose tissue, liver and skeletal muscle[5,6]. Therefore, obese individuals are at increased risk for developing both type 2 diabetes and liver diseases.

The diverse immune cell populations in the liver together with the inflammatory potential of nonhematopoetic hepatic cells have a central role in obesity-associated steatohepatitis and fibrosis[7]. Evidence suggests that cytokines are important mediators of diet-induced hepatic steatohepatitis, and the balance between proinflammatory Th1 and anti-inflammatory Th2 cytokines are changed[8]. Animal models of NAFLD/NASH are used for studies of pathogenesis of obesity-associated liver pathology including dietary models of experimental mice on various backgrounds with different susceptibility for the development of high-fat diet (HFD)-induced obesity and related metabolic disorders.

Galectin-3 (Gal-3), an evolutionarily conserved lectin that is produced by various cell types including immune cells and adipocytes, participates in immunometabolism[9-11]. Gal-3 has pro- and anti-inflammatory roles depending on the pathophysiological condition and tissue type. Gal-3 has the ability to bind to and dispose of advanced-glycation end-products and lipoxidation end-products[11,12]. Gal-3 is upregulated in adipose tissue in obesity and evidence including our own data demonstrates increased adiposity, hyperglycemia and enhanced inflammation in adipose tissues and pancreatic islets in Gal-3 knockout mice[13], which also develop accelerated and more severe pathology in experimental models of atherosclerosis and metabolically-induced kidney damage[14,15]. Interleukin (IL)-33, a member of the IL-1 superfamily of cytokines that is expressed by epithelial, endothelial and innate immune cells, functions as a traditional cytokine produced from living cells and as a nuclear factor regulating gene transcription. IL-33 can function as an "alarmin" - released following cell necrosis to alert the immune system to tissue damage or stress- and exerts its biological effects through binding to the its receptor, ST2[16] that is present on immune and nonimmune cells[17]. The IL-33/ST2 pathway is involved in the regulation of inflammation and remodeling during obesity and NASH[18]. Administered IL-33 enhanced accumulation of Th2 cells and promoted polarization of M2 type macrophages in adipose tissue, and reduced adiposity and glucose levels in obese diabetic (*ob/ob*) mice[17]. Recent data give evidence that IL-33 promoted expansion of $ST2^+$ T regulatory cells in adipose tissue during obesity and attenuated adipose tissue inflammation and insulin resistance[18]. IL-33 seems to have a hepatoprotective role in Concanavalin A-induced hepatitis and in ischemia-reperfusion injury[19,20]. Thus, the evidence that IL-33/ST2 signaling is an important regulatory pathway in

immunometabolic diseases are accumulating, but the role of this axis in obesity-associated liver pathology is scarce.

Gal-3 and IL-33 are two unrelated molecules, but recent studies point to the link between Gal-3 and soluble ST2 (sST2) in the pathophysiology of adverse myocardial remodeling and heart failure[21] and both molecules are regarded as prognostic markers in patients with acute myocardial infarction and acute or chronic heart failure[22,23]. Gal-3 promotes myocardial fibrosis, whereas myocardial fibrosis and hypertrophy are prevented through interaction between IL-33 and ST2[24]. The interaction between Gal-3 and the IL-33/ST2 pathway in liver fibrosis has not been studied, and reported data indicate that both pathways have a profibrotic role in obesity-associated hepatic fibrosis. Thus, Gal-3 and the IL-33/ST2 pathway may be potential therapeutic targets for treating or preventing liver pathology associated with obesity.

Here, we review the roles of Gal-3 and the IL-33/ST2 axis in the pathogenesis of liver metabolic disorders related to obesity and their interplay in diet-induced fibrotic NASH. These findings stem from our research of the effects of the genetic deletion of Gal-3 and ST2 in mice on relevant translational models of susceptibility to obesity-associated diseases.

PATHOGENESIS OF NASH

NAFLD is regarded as a hepatic manifestation of metabolic syndrome, and has increasing incidence worldwide that is in line with the increased prevalence of obesity and type 2 diabetes. NAFLD is a spectrum of liver diseases that encompasses simple steatosis, NASH and cirrhosis, and it is strongly associated with obesity and metabolic syndrome[25]. Visceral adipose tissue (VAT) from lean individuals and animals contain predominantly M2 type (alternatively activated) tissue resident macrophages that attenuate inflammation by secreting IL-10, whereas the VAT of obese individuals characterize the shift towards the M1 (classically activated) macrophages which secrete proinflammatory cytokines, such as TNF-α, that promote insulin resistance[26]. NASH and the consequential liver fibrosis represent major health problems without effective therapy[25,26].

Increased lipolysis in adipose tissue and increased plasma free fatty acids together with metaflammation are believed to promote lipid accumulation in hepatocytes leading to liver steatosis[27,28]. The pathogenesis of fibrotic NASH is described in a "two-hit hypothesis", wherein insulin resistance acts as "first hit" that leads to liver steatosis which renders hepatocytes more susceptible to a "second hit" which could involve inflammatory and oxidative stress mediators, lipotoxic fatty acids, cholesterol and ceramides[29]. In addition to this concept of the two-hit model, it is now appreciated that a more complex multiple parallel hits model is more likely responsible for the disease progression[30]. Current evidence suggest that lipotoxicity and oxidative stress represent the major mechanisms underlying hepatocyte dysfunction which initiates a cascade of steatonecrosis and inflammation. Additionally, deposition of fibrous tissue is the natural consequence of hepatocyte injury that is mediated by chronic inflammation[30,31]. NASH is characterized by intrahepatic accumulation of innate and adaptive immune cells through action of chemokines and cytokines that may also promote activation of hepatic stellate cells (HSCs) and their differentiation into myofibroblasts, the key players in the pathogenesis of liver fibrosis[32-34]. Intrahepatic immune cells and hepatic cells sustain chronic inflammation and by action of TGF-β and the IL-33/IL-13 pathway induce transdifferentiation of HSCs into myofibroblastic cells responsible for excessive extracellular matrix (ECM) deposition, mostly in the form of collagen[34-36] as illustrated in Figure 1.

MOUSE MODELS OF NASH

Experimental models that mimic the human NASH are needed to study causes and pathogenesis of this disease and to serve as adequate models for testing novel therapeutic strategies for this disease. Various options of animal models of NAFLD/NASH are used and are divided into genetic, dietary and combination models. Animals with naturally occurring mutations that inactivate key genes (*e.g.,* leptin gene in the ob/ob mice), animals with specific genetic manipulations, and animals subject to environmental and dietary variations are used for this purpose. Mice with different genetic backgrounds, knockout and transgenic mice, have been used in studies of obesity-related metabolic diseases[37,38]. The reported genotype-dependent differences in the susceptibility to developing fibrotic NASH could be associated with differential immune and inflammatory responses to metabolic danger molecules in these mice.

We have used C57Bl/6 and BALB/c mice, which are strains commonly used in studies on immunoregulation and HFD-induced metabolic disorders. The C57Bl/6 mice and BALB/c mice are regarded as prototypic Th1- and Th2-type mouse strains, respectively. Recent evidence indicates that the balance between M1/M2 macrophages and Th1/Th2 lymphocytes is of critical importance for the outcome of obesity-related metabolic disorders. The constitutive and HFD-induced differences in the distribution of immune cells in metabolic tissues exist in C57Bl/6 and BALB/c mice. We have recently reported inherent and HFD-induced differences in immunometabolic phenotype in these two mouse strains[39]. In response to HFD, the C57Bl/6 mice exhibited greater weight gain, higher glycemia, increased adiposity and higher prevalence of proinflammatory innate and adaptive immune cells in

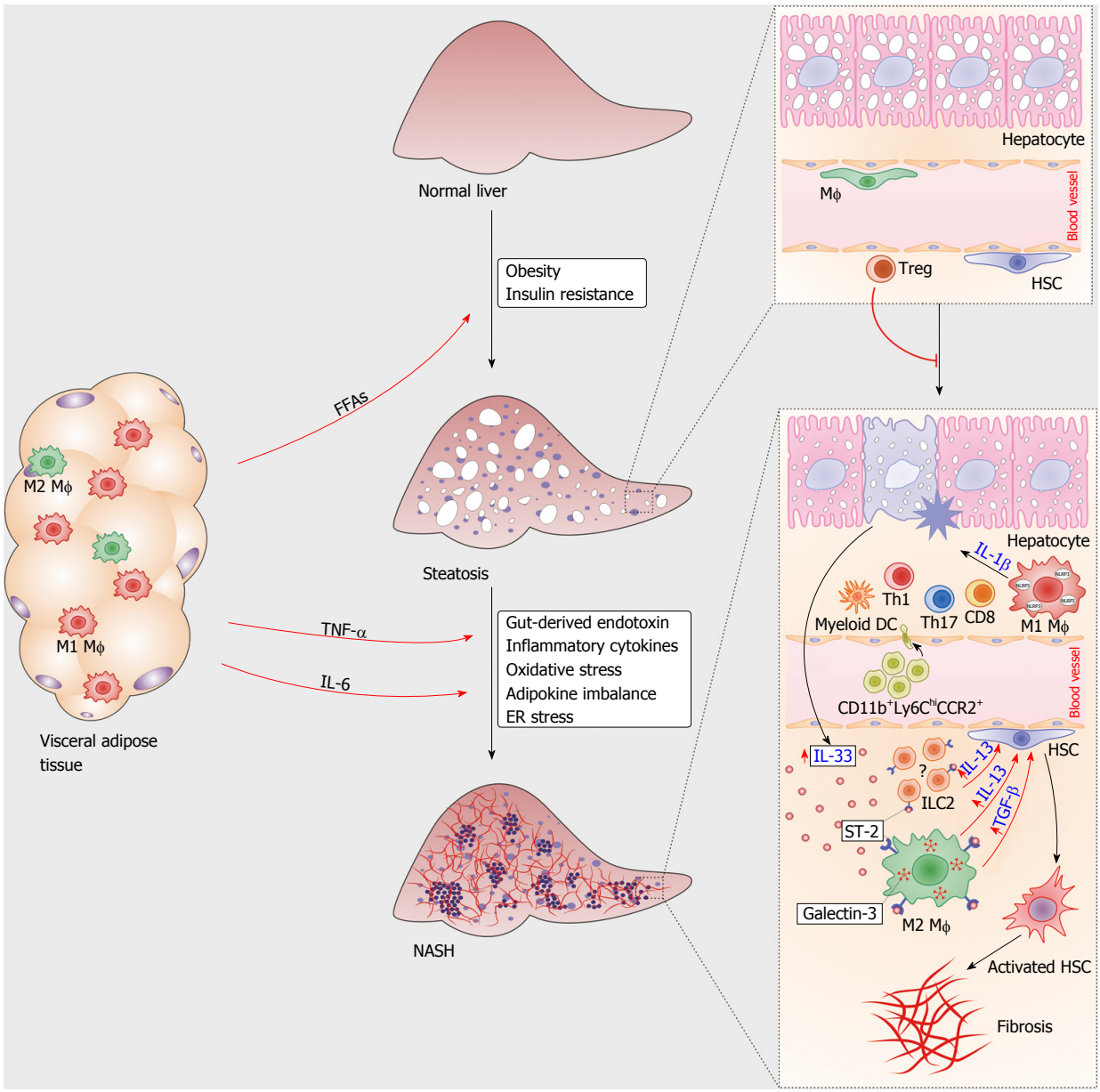

Figure 1 Immune cells in the pathogenesis of nonalcoholic steatohepatitis. During obesity, proinflammatory M1 macrophages and Th1-type lymphocytes infiltrated in the visceral adipose tissue mediate metaflammation that triggers insulin resistance. Increased amounts of free fatty acids (FFAs) released from adipose tissue accumulate in hepatocytes, causing liver steatosis. Liver regulatory T cells suppress metabolic inflammation. Multiple signals from visceral adipose tissue and gut polarize liver resident macrophages towards M1 type, promote chemotaxis of immune cells and hepatocyte injury. Damaged hepatocytes release IL-33, which promotes release of profibrogenic IL-13 and TGF-β from IL-33R (ST2)-positive macrophages. Liver resident innate lymphoid cells type 2 (ILC2s) might also respond to IL-33 by producing IL-13. Profibrogenic cytokines activate quiescent hepatic stellate cells, which transform to myofibroblasts, the key cells involved in the development of liver fibrosis.

VAT than the BALB/c mice. Th1-type mice on an HFD regimen were more susceptible to the development of visceral adiposity, liver inflammation and fibrosis, while the Th2-type mice were more susceptible to liver steatosis, which was associated with differential immune cell composition in adipose tissue and liver in this mouse strain[39]. In comparison to BALB/c mice, more numerous myeloid dendritic cells (DCs), proinflammatory macrophages and $CD11b^{+}Ly6C^{high}$ monocytes and $CD8^{+}$ T lymphocytes were found in livers of HFD-fed C57Bl/6 mice, together with higher levels of hepatic levels of IL-6, TNF-α and IFN-γ. C57Bl/6 and BALB/c mice differentially regulate the expression of genes related to lipid metabolism in liver in response to high-fat feeding, as HFD-induced marked liver steatosis and upregulated the hepatic LXRα and PPARγ genes in BALB/c mice. C57Bl/6 mice fed HFD developed liver fibrosis and had increased hepatic procollagen and TGF-β mRNA expression, and IL-33, IL-13 and TGF-β protein levels in liver homogenates, while diet-matched BALB/c mice had scarce collagen deposition in liver and lower levels of hepatic profibrogenic cytokines[39]. The representative images of HFD-induced liver pathology in C57Bl/6 and

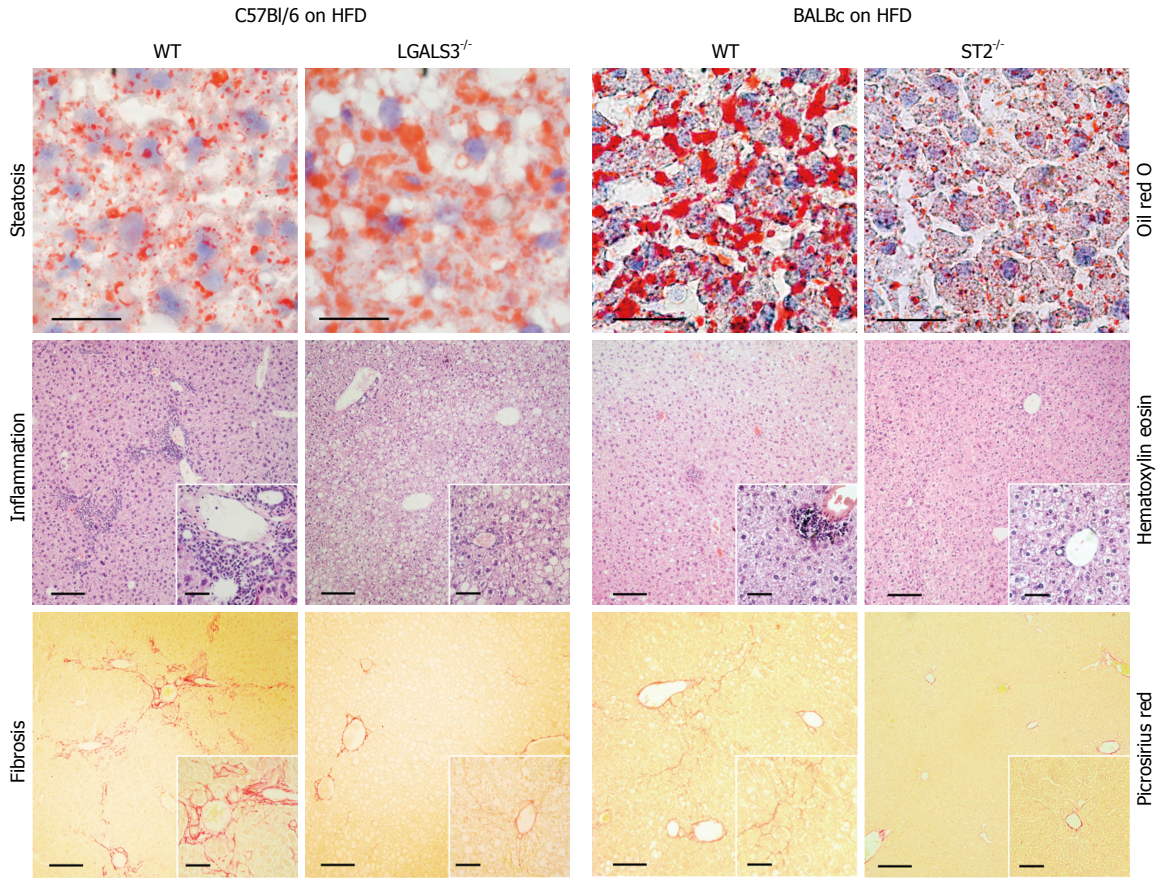

Figure 2 Galectin-3 and IL-33/ST2 axis in diet-induced steatohepatitis. Increased liver steatosis, but attenuated inflammation and fibrosis, in Galectin-3 knockout mice fed high-fat diet compared to diet matched C57Bl/6 wild-type mice (left panel). Decreased liver steatosis, inflammation and fibrosis in ST2 knockout mice fed high-fat diet compared to diet matched BALB/c wild-type mice.

BALB/c mice are shown in Figure 2. Strain-dependent differences in metabolic variables and composition of immune cells in metabolic tissues need to be considered in designing metabolic studies, particularly in studying obesity-associated fibrotic NASH.

GAL-3 IN HFD-INDUCED STEATOHEPATITIS

Galectins are evolutionarily conserved proteins that bind glycans, the carbohydrate structures broadly distributed on mammalian cells which can be altered by various pathological stimuli. There are 15 members of the galectin family which, *via* the carbohydrate-recognition domain (CRD), bind the cell surface β-galactosides and multiple terminal N-acetyllactosamine (LacNAc) sequences and cause intracellular signaling events involved in the regulation of various biological responses. The "galectin signalosome" has a role in many physiological and pathological conditions, and better understanding of its functions could lead to the development of novel therapeutic agents such as recombinant galectin proteins or specific galectin inhibitors.

Gal-3 has a unique "chimera-type" structure, having both lectin-like and CRDs, and can be present on the cell membrane, in the cytoplasm and the nucleus, and in extracellular spaces, including the systemic circulation[9]. Gal-3 recognizes endogenous glycans and modulates intracellular signaling pathways upon cell activation[40], proliferation[41,42] and apoptosis[43]. Gal-3 exerts important cell-cell and cell-ECM pro-adhesive roles[44,45], while also acting as a scavenger molecule for glucose and lipid adducts and in binding of microbial products, including endotoxin[46,47]. Gal-3 is expressed in innate and adaptive immunity cells and its production is altered in a variety of pathophysiological conditions, including autoimmune and inflammatory diseases, cancers and fibrotic disorders[13,48-50]. Gal-3 may have pro- and anti-inflammatory roles depending on the nature of the pathophysiological process, the type of tissue and the cellular localization.

Recent findings suggest an important regulatory role for Gal-3 in metabolic disorders, including obesity[13,51,52], diabetes[13,51], atherosclerosis[14], lipid-induced glomerular injury[15] and hepatic steatosis/inflammation[53]. The evidence so far, including our own data, indicates that gal-3 plays an important role in the regulation of adiposity, glucose metabolism, steatohepatitis and liver fibrosis in mice[13,53]. Obesity, *via* gain of ectopic fat and inducing metaflammation,

Table 1 Changes in mRNA levels of lipid metabolism and fibrosis-related genes in livers of LGALS3$^{-/-}$ and ST2$^{-/-}$ mice during high-fat diet-induced nonalcoholic steatohepatitis

Target gene	LGALS3$^{-/-}$ mice	ST2$^{-/-}$ mice
Abca-1	Upregulated	Unchanged
Cd36	Upregulated	Downregulated
LXR-α	Unchanged	Downregulated
PPARγ	Upregulated	Downregulated
Coll-α1	Downregulated	Downregulated
α-SMA	Downregulated	Unchanged
IL-33	Downregulated	Downregulated
IL-13	Downregulated	Downregulated
TGF-β	Unchanged	Unchanged

Abca-1: ATP-binding cassette transporter; LXR-α: Liver X receptor-alpha; PPARγ: Peroxisome proliferator-activated receptor gamma; Coll-α1: Procollagen alpha 1; α-SMA: Alpha-smooth muscle actin; IL-33: Interleukin-33; IL-13: Interleukin-13; TGF-β: Transforming growth factor-beta.

promotes insulin resistance, β cell failure and hepatic steatosis, thus representing the major risk factor for the development of type 2 diabetes and NAFLD.

The data regarding the role of Gal-3 in the pathogenesis of NAFLD are contrasting. Nomoto *et al*[54] have reported that Gal-3-deficient mice spontaneously develop steatosis at 6 mo of age. In addition, using the murine model of choline deficient, L-amino acid-defined diet - induced NASH that same group reported that ablation of Gal-3 led to a more pronounced steatosis and liver injury that could be related to and distinctive from the Gal-3 knockout mice on the CD1 background used in these studies[55]. In contrast, in a study by Iacobini *et al*[56], Gal-3 knockout mice were resistant to the development of steatosis and fibrotic NASH when fed an atherogenic diet. The authors demonstrated that proatherogenic HFD accelerated renal and aortic lesions, but attenuated NASH in Gal-3 knockout mice, which was accompanied by less fat deposition in liver and decreased oxidative stress. They also demonstrated that AGE/ALE levels and RAGE expression were decreased in the liver in spite of their increased circulating levels and that Gal-3 expressed on liver sinusoidal cells and endothelial cells has a major role in the uptake of these glucose and lipid adducts[56]. Gal-3 binds AGE/ALE *via* receptor-mediated endocytosis[57] and these harmful metabolic products are subsequently degraded by detoxifying enzymes[58].

NAFLD is strongly associated with obesity and meta-flammation, but the complex molecular mechanisms mediating development of liver steatosis and its progression to steatohepatitis and liver fibrosis are incompletely defined. The role of Gal-3 in the regulation of obesity-associated NASH has not been investigated. Therefore, we subjected wild-type (WT) and Gal-3 knockout mice on the C57Bl/6 background to obesogenic HFD (60% kcal from fat) for 24 wk and performed metabolic, histological, immunophenotypical and gene expression analyses in metabolic tissues[53]. We have demonstrated that the Gal-3-deficient mice fed HFD exhibit accelerated obesity and excess adiposity, hyperglycemia, insulin resistance, dyslipidemia and inflammatory changes in VAT and pancreatic islets. We have shown that obesity-associated hepatic lipid accumulation was uncoupled from the fibroinflammatory response in the liver in Gal-3 knockout mice in this experimental model[53]. In comparison to the WT mice, the HFD-fed Gal-3 knockout mice developed more pronounced liver steatosis which was accompanied by upregulation of hepatic FAS, PPAR-γ, Abca-1 and Cd36 mRNA expression, the lipogenic genes involved in fatty acid uptake and lipid synthesis[53] (Table 1). However, in obese Gal-3 knockout mice, liver injury, inflammation and fibrosis, and hepatic procollagen α1 and α-SMA mRNA expression were markedly lower compared to the WT mice on the same diet regimen, findings that were similar to the data reported by Iacobini *et al*[56]. The more pronounced hepatic fibro-inflammatory response induced by the obesogenic diet in the WT mice was associated with more numerous myeloid DCs and M1 macrophages (F4/80^{+}CD11c^{+}) infiltrated into the livers and higher hepatic F4/80, CD11c, NLRP3 inflammasome and IL-1β mRNA expression[53]. In contrast to the Gal-3 knockout mice, the WT mice on the obesogenic diet had increased percentages of CCR2^{+} proinflammatory monocytes (CD11b^{+}Ly6C^{hi}) in blood, bone marrow and liver and higher hepatic expression of CCL2[53]. HFD-fed Gal-3 knockout mice exhibited higher endotoxemia, but the hepatic TLR4 and CD14 NADPH-oxidase enzymes' mRNA expression was lower in comparison to the diet-matched WT mice. The obesity-driven greater steatosis was uncoupled with attenuated NASH in Gal-3-deficient mice, thus Gal-3 is involved in the progression of obesogenic diet-induced steatohepatitis in mice[53].

Furthermore, WT mice on HFD exhibited pronounced liver fibrosis accompanied by markedly higher hepatic expression of procollagen α1, IL-33 and IL-13 mRNA compared to Gal-3 knockout mice on the same diet regimen, while hepatic TGF-β mRNA expression was similar[53]. Gal-3 has an important profibrotic role and our data are consistent with the observation that Gal-3 disruption attenuated ECM production both *in vitro* in HSC cultures and *in vivo* in the model of carbon tetrachloride (CCl_4)-induced cirrhosis[59]. In this model, the disruption of the Gal-3 gene blocked TGF-β-mediated myofibroblast activation and procollagen expression, thus markedly attenuating CCl_4-induced liver fibrosis in mice.

Host-derived galectins may contribute to amplifying or attenuating of antimicrobial immune responses. Since Gal-3 directly interacts with the microflora and a variety of pathogenic bacteria, the contradictory results obtained when examining the role of Gal-3 in inflammation using Gal-3 knockout mice may at least in part be the consequence of different microbial populations and/or the involvement of

specific commensals in the disease pathogenesis under different experimental conditions[60]. Given the important involvement of the microflora in a variety of pathologies, including those of metabolic origin, a better understanding of the cross-talk between Gal-3 and commensal bacteria is necessary to clarify these issues.

Future studies will help to elucidate the role of Gal-3 in other metabolic tissues in the course of diet-induced obesity or aging. In particular, clarifying the mechanisms for the protective role of Gal-3 in pancreatic islets in the course of obesity would be of great importance. Obesity, diabetes, NASH and heart failure, and other diseases associated with inflammation in humans, are conditions that warrant better understanding of the role of Gal-3, especially in the light of current development of pharmacological inhibitors of Gal-3 for treatment of cancer and fibrosis.

IL-33/ST2 AXIS IN HFD-INDUCED STEATOHEPATITIS

The hallmarks of diet-induced steatohepatitis are the presence of liver steatosis, chronic inflammation in the liver and, under some circumstances, progression to liver fibrosis. Accumulated lipids in hepatocytes may promote the inflammatory response characterized by the increased infiltration of myeloid and lymphoid cells within the liver, activation of resident Kupffer cells and the secretion of pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6. Moreover, the most recent finding shows that IL-1 signaling promotes hepatic lipogenesis[61]. The role of other members of the IL-1 superfamily, including the IL-1 receptor antagonist, IL-18 and IL-33, together with IL-1 in obesity-associated liver pathology are incompletely defined.

IL-33 is a pleiotropic cytokine that binds to its plasma membrane receptor complex, comprising ST2 and the IL-1R accessory protein, and generally promotes Th2-type immune responses[62]. IL-33 exerts protective metabolic effects in obesity and atherosclerosis[17,63]. However, IL-33 promotes liver fibrosis through the activation and expansion of liver-resident innate lymphoid cells, which produce profibrotic IL-13[64]. The role of the IL-33/ST2 axis in obesity-associated liver pathology is not elucidated. We investigated the role of IL-33/ST2 signaling in the development of hepatic steatosis, inflammation and fibrosis using ST2-deficient mice on the BALB/c background which were placed on a long-term obesogenic HFD or high-fat high-fructose diet. The HFD-fed ST2-deficient mice exhibited increased weight gain and visceral adiposity compared with diet-matched WT mice. However, ST2 deletion markedly reduced hepatic steatosis, liver inflammation and fibrosis which was associated with lower expression of genes related to lipid metabolism in the liver. Innate immune cells, including $CD68^+$ macrophages and $CD11c^+$ DCs, were less numerous in HFD-fed ST2-knockout mice compared to WT controls. The HFD-fed ST2-knockout mice had less collagen deposition in the livers and lower numbers of profibrotic $CD11b^+Ly6C^{low}$ monocytes and Th17 cells in the liver, lower hepatic procollagen-α1, IL-33 and IL-13 mRNA expression, and lower serum levels of IL-33 and IL-13 compared with the diet-matched WT mice. Our findings suggest that the IL-33/ST2 axis has a complex role in obesity-associated metabolic disorders, as this pathway has a protective role in HFD-induced adiposity, but enhances liver steatosis, inflammation and fibrosis in NASH (Table 1). A very recent study[65] suggests that IL-33 treatment in HFD or methionine-choline-deficient diet (MCD)-fed C57Bl/6 mice attenuated hepatic steatosis, but aggravated hepatic fibrosis in a ST2-dependent manner. The reported effects of IL-33 on liver fibrosis are consistent with our data and the differential result regarding liver steatosis may be due to the genetic background of mice (*i.e.* BALB/c mice used in our experiments). Similar to our findings, the authors reported that the progression of NASH was associated with the increased mRNA expression level of IL-33 and ST2 in liver[65].

The significance of sST2 as a biomarker of liver fibrosis has been studied. The reported data demonstrate that the serum levels of sST2 were higher in patients with liver cirrhosis and hepatocellular carcinoma. These data suggest an important role of sST2 in the pathogenesis of hepatocellular carcinoma and liver cirrhosis as a possible marker of systemic inflammation. Further research is needed to evaluate the potential of sST2 as a prognostic marker in patients with liver fibrosis[66].

A recent study showed strong correlation between serum sST2 levels and fibrosis stages in patients with hepatitis B infection. It is suggested that serum levels of sST2 levels may be a reliable biomarker for evaluating the response to therapy for liver diseases causing fibrosis, including NAFLD[67].

GAL-3 AND IL-33/ST2 AXIS INTERACTION IN HUMAN PATHOLOGY

Gal-3 and the IL-33/ST2 axis are involved in myocardial remodeling. Gal-3 and sST2 are approved prognostic biomarkers that are involved in myocardial fibrosis and inflammation. Soluble Gal-3 is released by activated cardiac macrophages and stimulates proliferation of myofibroblasts and procollagen deposition. Higher concentrations of plasma Gal-3 are associated with myocardial remodeling, along with an increased risk of incident heart failure and mortality[68]. ST2 exists in two forms, a transmembrane receptor (ST2L) as well as a soluble decoy receptor (sST2). Through interaction between IL-33 and ST2L, myocardial fibrosis and hypertrophy are prevented. The sST2 acts as a decoy receptor that neutralizes IL-33,

so that the cardioprotective role of the IL-33/ST2L signaling pathway is lost, resulting in cardiomyocyte hypertrophy, apoptosis and fibrosis. Therefore, serum levels of sST2 are strongly predictive of adverse outcomes in patients with acute myocardial infarction or heart failure and significantly predict left ventricle remodeling. Thus, both sST2 and Gal-3 are reflective of fibrosis and cardiac remodeling, key events in heart failure[69].

Gal-3 and sST2 are promising biomarkers with additive diagnostic and prognostic value in the management of heart failure. Increased levels of Gal-3 indicate on-going fibrosis and reflect higher risk of heart failure, its severity and poor prognosis. The levels of sST2 are associated with the remodeling of left ventricle with significant prognostic value in heart failure. The head-to-head comparison of these two biomarkers in a large cohort of patients with a long-term follow-up revealed that sST2 is more important addition to established risk factors, so incorporation of the measurement of serum levels of sST2 into clinical practice is recommended[69]. Recently, serial testing for sST2 was shown to increase the prognostic information related to prediction of left ventricular remodeling and worsening of heart failure[70].

GAL-3 AND IL-33/ST2 AXIS INTERACTION IN NASH

In contrast to the opposite roles of Gal-3 and the IL-33/ST2 axis in myocardial remodeling, both Gal-3 and IL-33 exert profibrotic effects in liver fibrosis. Recently, evidence has demonstrated that Gal-3 is an important mediator in liver fibrotic models and that Gal-3 inhibitors protect against fibrotic disorders[71,72]. Tissue fibrogenesis is a complex process and newer data has pointed to the important cross-talk between cells of the immune system and tissue myofibroblasts in the evolution of liver fibrosis. Gal-3 is a molecule which can exert potent effects on multiple cell types, including myofibroblasts, and by altering the function of innate immune cells, such as macrophages[73]. In the CCl_4-induced model of liver fibrosis, Gal-3 ablation blocked HSC activation and collagen expression, as TGF-β failed to transactivate Gal-3-deficient HSCs, in contrast to the WT HSCs, suggesting that Gal-3 is required for TGF-β-mediated myofibroblast activation and ECM production. Gal-3 deletion reduced retention of TGF-β receptors at the cell surface and reduced phosphorylation and nuclear translocation of β-catenin, but had no effect on Smad2/3 phosphorylation[59]. Gal-3 represents a molecule that links macrophages, fibroblasts and the profibrotic response. Gal-3 mediates IL-4-induced alternative macrophage activation[74] and IL-4/IL-13 activated macrophages upregulate profibrotic genes and enhance fibrosis. Most recent data demonstrate a profibrotic role of IL-33 in a liver fibrosis model through ST2-dependent production of IL-13 by innate lymphoid cells (ILCs) that activate HSCs[64]. In our studies, we have addressed the issue of a possible regulatory role of Gal-3 in the newly described IL-33/ST2/IL-13 profibrotic pathway. Our recent data show that, in contrast to the Gal-3 knockout mice, HFD-fed WT C57Bl/6 mice had a higher number of hepatocytes that strongly expressed IL-33 and hepatic IL-13-expressing $CD11b^+$ myeloid cells, increased hepatic levels of IL-33 and IL-13 and increased mRNA expression of IL-33, ST2 and IL-13 in liver[53]. Moreover, IL-33 failed to induce ST2 upregulation and IL-13 production by Gal-3-deficient peritoneal macrophages *in vitro.* Similarly, exogenous IL-33 enhanced liver fibrosis in HFD-fed mice in both genotypes, albeit to a significantly lower extent in the Gal-3 knockout mice. This was associated with less numerous hepatic IL-13-expressing $CD11b^+$ cells. Whether Gal-3 is directly involved in the regulation of TLR4 and IL-13 mRNA expression remains to be elucidated. We and others[53,56] have shown that HFD increased TLR4 mRNA expression in livers of C57Bl/6 mice, which was markedly reduced in Gal-3-deficient mice[53]. A recent study also demonstrated that Gal-3 can be actively released by activated microglial cells and can bind directly to TLR4, thereby amplifying the typical TLR4-dependent proinflammatory response, including caspase-mediated inflammation[75].

IL-13 is produced by T lymphocytes, macrophages/DCs, mast cells and basophils and is induced by IL-33. The molecular mechanisms involved in Gal-3-dependent regulation of IL-13 mRNA expression are not known. We have shown that lower level of IL-13 mRNA in livers of HFD-fed Gal-3 knockout mice could be related to decreased hepatic IL-33 levels in these animals and the inability of Gal-3-deficient macrophages to respond to IL-33, which resulted in reduced percentages of $CD11b^+$ myeloid cells that expressed ST2 and IL-13 in livers[53].

The interaction of Gal-3 and the IL-33/ST2 pathway in diet-induced steatohepatitis is shown in Figure 3. These results provide evidence of a novel role for Gal-3 in regulating IL-33-dependent HFD-induced fibrotic NASH[53]. Thus, Gal-3 plays an important regulatory role in the newly described profibrotic IL-33/ST2/IL-13 pathway.

Further studies are necessary to clarify functions of intra and extracellular Gal-3 in liver fibrosis and in-depth research of the role of Gal-3 in IL-33 signaling is needed. A better understanding of the mechanisms regulating tissue fibrosis and targeted strategies to inhibit Gal-3 in the liver provide the rationale for the development of new therapeutic approaches for

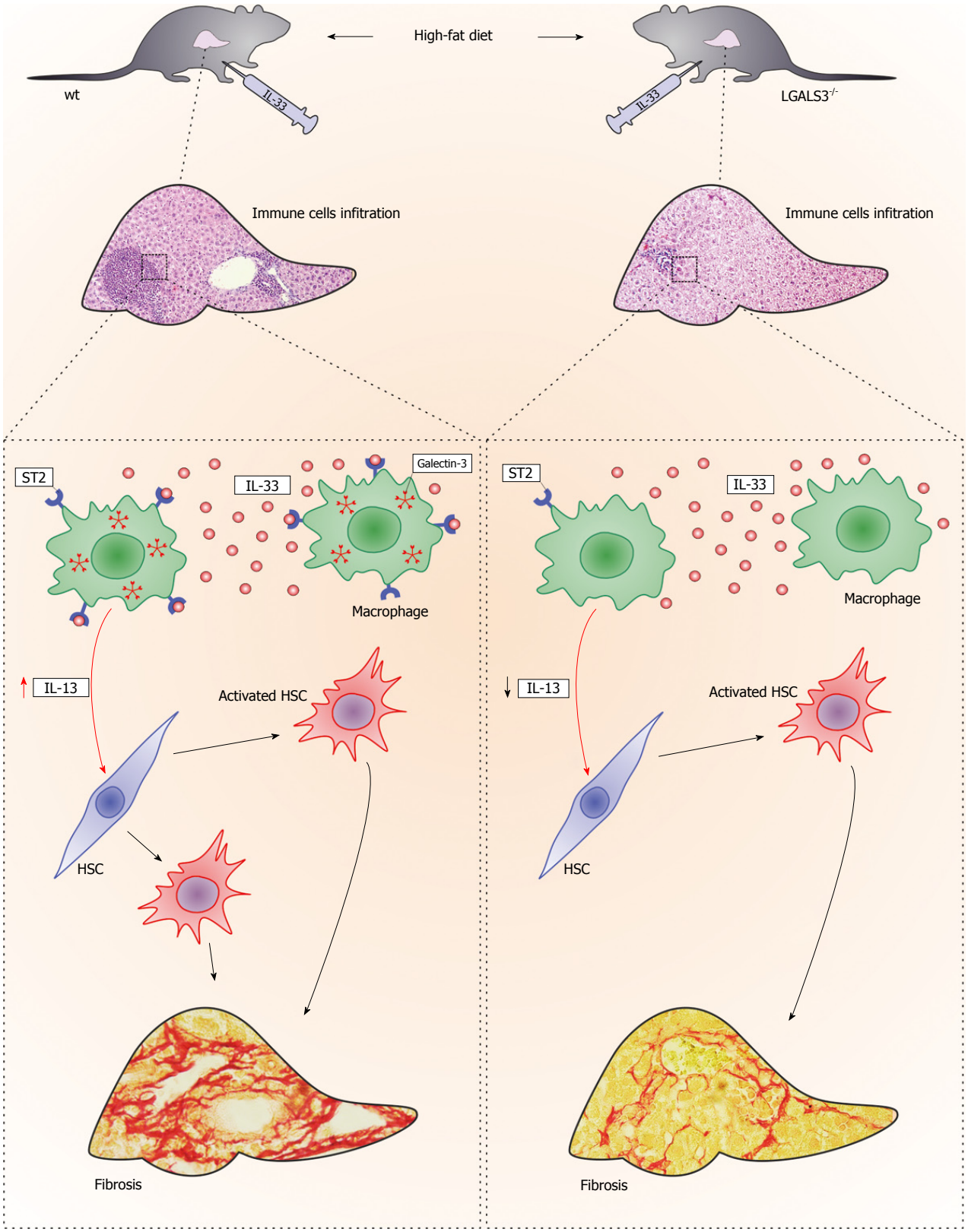

Figure 3 Galectin-3 and IL-33/ST2 axis interaction in diet-induced steatohepatitis. Administration of IL-33 *in vivo* enhanced high-fat diet-induced liver fibrosis in both genotypes of mice, although to a markedly lower extent in the galectin-3 knockout mice, which was accompanied by less numerous ST2-positive myeloid cells that express IL-13. Galectin-3 plays an important regulatory role in the newly described profibrotic IL-33/ST2/IL-13 pathway in hepatic fibrosis.

patients with liver fibrosis.

REFERENCES

1 **Osborn O**, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 2012; **18**: 363-374 [PMID: 22395709 DOI: 10.1038/nm.2627]

2 **Alam S**, Mustafa G, Alam M, Ahmad N. Insulin resistance in development and progression of nonalcoholic fatty liver disease. *World J Gastrointest Pathophysiol* 2016; **7**: 211-217 [PMID: 27190693 DOI: 10.4291/wjgp.v7.i2.211]

3 **Chang Y**, Jung HS, Cho J, Zhang Y, Yun KE, Lazo M, Pastor-Barriuso R, Ahn J, Kim CW, Rampal S, Cainzos-Achirica M, Zhao D, Chung EC, Shin H, Guallar E, Ryu S. Metabolically Healthy Obesity and the Development of Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* 2016; **111**: 1133-1140 [PMID: 27185080 DOI: 10.1038/ajg.2016.178]

4 **Chen L**, Chen R, Wang H, Liang F. Mechanisms Linking Inflammation to Insulin Resistance. *Int J Endocrinol* 2015; **2015**: 508409 [PMID: 26136779 DOI: 10.1155/2015/508409]

5 **Boura-Halfon S**, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. *Am J Physiol Endocrinol Metab* 2009; **296**: E581-E591 [PMID: 18728222 DOI: 10.1152/ajpendo.90437.2008]

6 **Emanuela F**, Grazia M, Marco de R, Maria Paola L, Giorgio F, Marco B. Inflammation as a Link between Obesity and Metabolic Syndrome. *J Nutr Metab* 2012; **2012**: 476380 [PMID: 22523672 DOI: 10.1155/2012/476380]

7 **Povero D**, Feldstein AE. Novel Molecular Mechanisms in the Development of Non-Alcoholic Steatohepatitis. *Diabetes Metab J* 2016; **40**: 1-11 [PMID: 26912150 DOI: 10.4093/dmj.2016.40.1.1]

8 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]

9 **Dumic J**, Dabelic S, Flögel M. Galectin-3: an open-ended story. *Biochim Biophys Acta* 2006; **1760**: 616-635 [PMID: 16478649 DOI: 10.1016/j.bbagen.2005.12.020]

10 **Pejnovic NN**, Pantic JM, Jovanovic IP, Radosavljevic GD, Djukic ALj, Arsenijevic NN, Lukic ML. Galectin-3 is a regulator of metaflammation in adipose tissue and pancreatic islets. *Adipocyte* 2013; **2**: 266-271 [PMID: 24052904 DOI: 10.4161/adip.24881]

11 **Pugliese G**, Iacobini C, Pesce CM, Menini S. Galectin-3: an emerging all-out player in metabolic disorders and their complications. *Glycobiology* 2015; **25**: 136-150 [PMID: 25303959 DOI: 10.1093/glycob/cwu111]

12 **Pugliese G**. Do advanced glycation end products contribute to the development of long-term diabetic complications? *Nutr Metab Cardiovasc Dis* 2008; **18**: 457-460 [PMID: 18674679 DOI: 10.1016/j.numecd.2008.06.006]

13 **Pejnovic NN**, Pantic JM, Jovanovic IP, Radosavljevic GD, Milovanovic MZ, Nikolic IG, Zdravkovic NS, Djukic AL, Arsenijevic NN, Lukic ML. Galectin-3 deficiency accelerates high-fat diet-induced obesity and amplifies inflammation in adipose tissue and pancreatic islets. *Diabetes* 2013; **62**: 1932-1944 [PMID: 23349493 DOI: 10.2337/db12-0222]

14 **Iacobini C**, Menini S, Ricci C, Scipioni A, Sansoni V, Cordone S, Taurino M, Serino M, Marano G, Federici M, Pricci F, Pugliese G. Accelerated lipid-induced atherogenesis in galectin-3-deficient mice: role of lipoxidation via receptor-mediated mechanisms. *Arterioscler Thromb Vasc Biol* 2009; **29**: 831-836 [PMID: 19359660 DOI: 10.1161/atvbaha.109.186791]

15 **Iacobini C**, Amadio L, Oddi G, Ricci C, Barsotti P, Missori S, Sorcini M, Di Mario U, Pricci F, Pugliese G. Role of galectin-3 in diabetic nephropathy. *J Am Soc Nephrol* 2003; **14**: S264-S270 [PMID: 12874444]

16 **Martin NT**, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* 2016; **17**: 122-131 [PMID: 26784265 DOI: 10.1038/ni.3370]

17 **Miller AM**, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, Xu D, Sattar N, McInnes IB, Liew FY. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res* 2010; **107**: 650-658 [PMID: 20634488 DOI: 10.1161/circresaha.110.218867]

18 **Han JM**, Wu D, Denroche HC, Yao Y, Verchere CB, Levings MK. IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. *J Immunol* 2015; **194**: 4777-4783 [PMID: 25870243 DOI: 10.4049/jimmunol.1500020]

19 **Volarevic V**, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N, Lukic ML. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol* 2012; **56**: 26-33 [PMID: 21703183 DOI: 10.1016/j.jhep.2011.03.022]

20 **Sakai N**, Van Sweringen HL, Quillin RC, Schuster R, Blanchard J, Burns JM, Tevar AD, Edwards MJ, Lentsch AB. Interleukin-33 is hepatoprotective during liver ischemia/reperfusion in mice. *Hepatology* 2012; **56**: 1468-1478 [PMID: 22782692 DOI: 10.1002/hep.25768]

21 **Shah RV**, Januzzi JL. Soluble ST2 and galectin-3 in heart failure. *Clin Lab Med* 2014; **34**: 87-97, vi-vii [PMID: 24507789 DOI: 10.1016/j.cll.2013.11.009]

22 **Ho JE**, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012; **60**: 1249-1256 [PMID: 22939561 DOI: 10.1016/j.jacc.2012.04.053]

23 **Shimpo M**, Morrow DA, Weinberg EO, Sabatine MS, Murphy SA, Antman EM, Lee RT. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation* 2004; **109**: 2186-2190 [PMID: 15117853 DOI: 10.1161/01.cir.0000127958.21003.5a]

24 **Sanada S**, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007; **117**: 1538-1549 [PMID: 17492053 DOI: 10.1172/jci30634]

25 **Machado MV**, Cortez-Pinto H. Non-alcoholic fatty liver disease: what the clinician needs to know. *World J Gastroenterol* 2014; **20**: 12956-12980 [PMID: 25278691 DOI: 10.3748/wjg.v20.i36.12956]

26 **Lumeng CN**, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest* 2011; **121**: 2111-2117 [PMID: 21633179 DOI: 10.1172/jci57132]

27 **Dalmas E**, Clément K, Guerre-Millo M. Defining macrophage phenotype and function in adipose tissue. *Trends Immunol* 2011; **32**: 307-314 [PMID: 21616718 DOI: 10.1016/j.it.2011.04.008]

28 **Sears B**, Perry M. The role of fatty acids in insulin resistance. *Lipids in Health and Disease* 2015; **14**: 1-9 [PMID: 26415887 DOI: 10.1186/s12944-015-0123-1]

29 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]

30 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]

31 **Peverill W**, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci* 2014; **15**: 8591-8638 [PMID: 24830559 DOI: 10.3390/ijms15058591]

32 **Czaja AJ**. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. *World J Gastroenterol* 2014; **20**: 2515-2532 [PMID: 24627588 DOI: 10.3748/wjg.v20.i10.2515]

33 **Wallace K**, Burt AD, Wright MC. Liver fibrosis. *Biochem J* 2008; **411**: 1-18 [PMID: 18333835 DOI: 10.1042/bj20071570]

34 **Pinzani M**, Marra F. Cytokine receptors and signaling in hepatic stellate cells. *Semin Liver Dis* 2001; **21**: 397-416 [PMID: 11586468 DOI: 10.1055/s-2001-17554]

35 **Lee UE**, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 195-206 [PMID: 21497738 DOI: 10.1016/j.bpg.2011.02.005]

36 **Marvie P**, Lisbonne M, L'helgoualc'h A, Rauch M, Turlin B, Preisser L, Bourd-Boittin K, Théret N, Gascan H, Piquet-Pellorce C,

Samson M. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med* 2010; **14**: 1726-1739 [PMID: 19508382 DOI: 10.1111/j.1582-4934.2009.00801.x]

37 **Takahashi Y**, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2300-2308 [PMID: 22654421 DOI: 10.3748/wjg.v18.i19.2300]

38 **Nakamura A**, Terauchi Y. Lessons from mouse models of high-fat diet-induced NAFLD. *Int J Mol Sci* 2013; **14**: 21240-21257 [PMID: 24284392 DOI: 10.3390/ijms141121240]

39 **Jovicic N**, Jeftic I, Jovanovic I, Radosavljevic G, Arsenijevic N, Lukic ML, Pejnovic N. Differential Immunometabolic Phenotype in Th1 and Th2 Dominant Mouse Strains in Response to High-Fat Feeding. *PLoS One* 2015; **10**: e0134089 [PMID: 26218873 DOI: 10.1371/journal.pone.0134089]

40 **More SK**, Chiplunkar SV, Kalraiya RD. Galectin-3-induced cell spreading and motility relies on distinct signaling mechanisms compared to fibronectin. *Mol Cell Biochem* 2016; **416**: 179-191 [PMID: 27130204 DOI: 10.1007/s11010-016-2706-1]

41 **Inohara H**, Akahani S, Raz A. Galectin-3 stimulates cell proliferation. *Exp Cell Res* 1998; **245**: 294-302 [PMID: 9851870 DOI: 10.1006/excr.1998.4253]

42 **Moutsatsos IK**, Wade M, Schindler M, Wang JL. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc Natl Acad Sci USA* 1987; **84**: 6452-6456 [PMID: 3306680]

43 **Nakahara S**, Oka N, Raz A. On the role of galectin-3 in cancer apoptosis. *Apoptosis* 2005; **10**: 267-275 [PMID: 15843888 DOI: 10.1007/s10495-005-0801-y]

44 **Inohara H**, Raz A. Functional evidence that cell surface galectin-3 mediates homotypic cell adhesion. *Cancer Res* 1995; **55**: 3267-3271 [PMID: 7542167]

45 **Di Lella S**, Sundblad V, Cerliani JP, Guardia CM, Estrin DA, Vasta GR, Rabinovich GA. When galectins recognize glycans: from biochemistry to physiology and back again. *Biochemistry* 2011; **50**: 7842-7857 [PMID: 21848324 DOI: 10.1021/bi201121m]

46 **Zhu W**, Sano H, Nagai R, Fukuhara K, Miyazaki A, Horiuchi S. The role of galectin-3 in endocytosis of advanced glycation end products and modified low density lipoproteins. *Biochem Biophys Res Commun* 2001; **280**: 1183-1188 [PMID: 11162652 DOI: 10.1006/bbrc.2001.4256]

47 **Li Y**, Komai-Koma M, Gilchrist DS, Hsu DK, Liu FT, Springall T, Xu D. Galectin-3 is a negative regulator of lipopolysaccharide-mediated inflammation. *J Immunol* 2008; **181**: 2781-2789 [PMID: 18684969]

48 **Radosavljevic G**, Volarevic V, Jovanovic I, Milovanovic M, Pejnovic N, Arsenijevic N, Hsu DK, Lukic ML. The roles of Galectin-3 in autoimmunity and tumor progression. *Immunol Res* 2012; **52**: 100-110 [PMID: 22418727 DOI: 10.1007/s12026-012-8286-6]

49 **Volarevic V**, Milovanovic M, Ljujic B, Pejnovic N, Arsenijevic N, Nilsson U, Leffler H, Lukic ML. Galectin-3 deficiency prevents concanavalin A-induced hepatitis in mice. *Hepatology* 2012; **55**: 1954-1964 [PMID: 22213244 DOI: 10.1002/hep.25542]

50 **Dragomir AC**, Sun R, Choi H, Laskin JD, Laskin DL. Role of galectin-3 in classical and alternative macrophage activation in the liver following acetaminophen intoxication. *J Immunol* 2012; **189**: 5934-5941 [PMID: 23175698 DOI: 10.4049/jimmunol.1201851]

51 **Pang J**, Rhodes DH, Pini M, Akasheh RT, Castellanos KJ, Cabay RJ, Cooper D, Perretti M, Fantuzzi G. Increased adiposity, dysregulated glucose metabolism and systemic inflammation in Galectin-3 KO mice. *PLoS One* 2013; **8**: e57915 [PMID: 23451284 DOI: 10.1371/journal.pone.0057915]

52 **Menini S**, Iacobini C, Blasetti Fantauzzi C, Pesce CM, Pugliese G. Role of Galectin-3 in Obesity and Impaired Glucose Homeostasis. *Oxid Med Cell Longev* 2016; **2016**: 9618092 [PMID: 26770660 DOI: 10.1155/2016/9618092]

53 **Jeftic I**, Jovicic N, Pantic J, Arsenijevic N, Lukic ML, Pejnovic N. Galectin-3 Ablation Enhances Liver Steatosis, but Attenuates Inflammation and IL-33-Dependent Fibrosis in Obesogenic Mouse Model of Nonalcoholic Steatohepatitis. *Mol Med* 2015; **21**: 453-465 [PMID: 26018806 DOI: 10.2119/molmed.2014.00178]

54 **Nomoto K**, Tsuneyama K, Abdel Aziz HO, Takahashi H, Murai Y, Cui ZG, Fujimoto M, Kato I, Hiraga K, Hsu DK, Liu FT, Takano Y. Disrupted galectin-3 causes non-alcoholic fatty liver disease in male mice. *J Pathol* 2006; **210**: 469-477 [PMID: 17029217 DOI: 10.1002/path.2065]

55 **Nomoto K**, Nishida T, Nakanishi Y, Fujimoto M, Takasaki I, Tabuchi Y, Tsuneyama K. Deficiency in galectin-3 promotes hepatic injury in CDAA diet-induced nonalcoholic fatty liver disease. *ScientificWorldJournal* 2012; **2012**: 959824 [PMID: 22593713 DOI: 10.1100/2012/959824]

56 **Iacobini C**, Menini S, Ricci C, Blasetti Fantauzzi C, Scipioni A, Salvi L, Cordone S, Delucchi F, Serino M, Federici M, Pricci F, Pugliese G. Galectin-3 ablation protects mice from diet-induced NASH: a major scavenging role for galectin-3 in liver. *J Hepatol* 2011; **54**: 975-983 [PMID: 21145823 DOI: 10.1016/j.jhep.2010.09.020]

57 **Smedsrød B**, Melkko J, Araki N, Sano H, Horiuchi S. Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. *Biochem J* 1997; **322**: 567-573 [PMID: 9065778]

58 **Negre-Salvayre A**, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol* 2008; **153**: 6-20 [PMID: 17643134 DOI: 10.1038/sj.bjp.0707395]

59 **Henderson NC**, Mackinnon AC, Farnworth SL, Poirier F, Russo FP, Iredale JP, Haslett C, Simpson KJ, Sethi T. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. *Proc Natl Acad Sci USA* 2006; **103**: 5060-5065 [PMID: 16549783 DOI: 10.1073/pnas.0511167103]

60 **Kavanaugh D**, Kane M, Joshi L, Hickey RM. Detection of galectin-3 interaction with commensal bacteria. *Appl Environ Microbiol* 2013; **79**: 3507-3510 [PMID: 23524672 DOI: 10.1128/aem.03694-12]

61 **Negrin KA**, Roth Flach RJ, DiStefano MT, Matevossian A, Friedline RH, Jung D, Kim JK, Czech MP. IL-1 signaling in obesity-induced hepatic lipogenesis and steatosis. *PLoS One* 2014; **9**: e107265 [PMID: 25216251 DOI: 10.1371/journal.pone.0107265]

62 **Milovanovic M**, Volarevic V, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, Lukic ML. IL-33/ST2 axis in inflammation and immunopathology. *Immunol Res* 2012; **52**: 89-99 [PMID: 22392053 DOI: 10.1007/s12026-012-8283-9]

63 **Miller AM**, Xu D, Asquith DL, Denby L, Li Y, Sattar N, Baker AH, McInnes IB, Liew FY. IL-33 reduces the development of atherosclerosis. *J Exp Med* 2008; **205**: 339-346 [PMID: 18268038 DOI: 10.1084/jem.20071868]

64 **McHedlidze T**, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, Voehringer D, McKenzie AN, Neurath MF, Pflanz S, Wirtz S. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* 2013; **39**: 357-371 [PMID: 23954132 DOI: 10.1016/j.immuni.2013.07.018]

65 **Gao Y**, Liu Y, Yang M, Guo X, Zhang M, Li H, Li J, Zhao J. IL-33 treatment attenuated diet-induced hepatic steatosis but aggravated hepatic fibrosis. *Oncotarget* 2016; **7**: 33649-33661 [PMID: 27172901 DOI: 10.18632/oncotarget.9259]

66 **Bergis D**, Kassis V, Ranglack A, Koeberle V, Piiper A, Kronenberger B, Zeuzem S, Waidmann O, Radeke HH. High Serum Levels of the Interleukin-33 Receptor Soluble ST2 as a Negative Prognostic Factor in Hepatocellular Carcinoma. *Transl Oncol* 2013; **6**: 311-318 [PMID: 23730411]

67 **Oztas E**, Kuzu UB, Zengin NI, Kalkan IH, Onder FO, Yildiz H, Celik HT, Akdogan M, Kilic MY, Koksal AS, Odemis B, Suna N, Kayacetin E. Can Serum ST2 Levels Be Used as a Marker of Fibrosis in Chronic Hepatitis B Infection? *Medicine* (Baltimore) 2015; **94**: e1889 [PMID: 26632683 DOI: 10.1097/md.0000000000001889]

68 **Lok DJ**, Lok SI, Bruggink-André de la Porte PW, Badings E,

Lipsic E, van Wijngaarden J, de Boer RA, van Veldhuisen DJ, van der Meer P. Galectin-3 is an independent marker for ventricular remodeling and mortality in patients with chronic heart failure. *Clin Res Cardiol* 2013; **102**: 103-110 [PMID: 22886030 DOI: 10.1007/s00392-012-0500-y]

69 **Bayes-Genis A**, de Antonio M, Vila J, Peñafiel J, Galán A, Barallat J, Zamora E, Urrutia A, Lupón J. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol* 2014; **63**: 158-166 [PMID: 24076531 DOI: 10.1016/j.jacc.2013.07.087]

70 **Januzzi JL**, Pascual-Figal D, Daniels LB. ST2 testing for chronic heart failure therapy monitoring: the International ST2 Consensus Panel. *Am J Cardiol* 2015; **115**: 70B-75B [PMID: 25670638 DOI: 10.1016/j.amjcard.2015.01.044]

71 **de Oliveira SA**, de Freitas Souza BS, Sá Barreto EP, Kaneto CM, Neto HA, Azevedo CM, Guimarães ET, de Freitas LA, Ribeiro-Dos-Santos R, Soares MB. Reduction of galectin-3 expression and liver fibrosis after cell therapy in a mouse model of cirrhosis. *Cytotherapy* 2012; **14**: 339-349 [PMID: 22149185 DOI: 10.3109/14653249.2011.637668]

72 **Traber PG**, Chou H, Zomer E, Hong F, Klyosov A, Fiel MI, Friedman SL. Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioacetamide-induced liver disease. *PLoS One* 2013; **8**: e75361 [PMID: 24130706 DOI: 10.1371/journal.pone.0075361]

73 **Li LC**, Li J, Gao J. Functions of galectin-3 and its role in fibrotic diseases. *J Pharmacol Exp Ther* 2014; **351**: 336-343 [PMID: 25194021 DOI: 10.1124/jpet.114.218370]

74 **MacKinnon AC**, Farnworth SL, Hodkinson PS, Henderson NC, Atkinson KM, Leffler H, Nilsson UJ, Haslett C, Forbes SJ, Sethi T. Regulation of alternative macrophage activation by galectin-3. *J Immunol* 2008; **180**: 2650-2658 [PMID: 18250477]

75 **Burguillos MA**, Svensson M, Schulte T, Boza-Serrano A, Garcia-Quintanilla A, Kavanagh E, Santiago M, Viceconte N, Oliva-Martin MJ, Osman AM, Salomonsson E, Amar L, Persson A, Blomgren K, Achour A, Englund E, Leffler H, Venero JL, Joseph B, Deierborg T. Microglia-Secreted Galectin-3 Acts as a Toll-like Receptor 4 Ligand and Contributes to Microglial Activation. *Cell Rep* 2015; Epub ahead of print [PMID: 25753426 DOI: 10.1016/j.celrep.2015.02.012]

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