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## Chronic inflammation as a molecular basis of nonalcoholic steatohepatitis: role of macrophages and fibroblasts in the liver

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### ABSTRACT

The pathological spectrum of nonalcoholic fatty liver disease includes simple steatosis and nonalcoholic steatohepatitis (NASH), the latter of which is the leading cause of cirrhosis and hepatocellular carcinoma. The available evidence shows that parenchymal cell injury and death trigger inflammation and tissue fibrosis. During the development of liver fibrosis, stromal cells dramatically changes in their cellular component and activation status responding to hepatocyte injury due to various etiologies. It is important to understand how cell death induces chronic inflammation and fibrosis, and the disease-specific macrophages and fibroblasts responsible for NASH development under metabolic stress. This review discusses recent progress in the understanding the pathogenesis of NASH, focusing on disease-specific macrophages and fibroblasts.

Keywords: chronic inflammation, macrophages, fibroblasts

#### Abbreviations:

FGF9: fibroblast growth factor 9

HCC: hepatocellular carcinoma

hCLS: hepatic crown-like structure

HFD: high-fat diet

MC4R: melanocortin 4 receptor

MCD: methionine and choline-deficient

NAFLD: nonalcoholic-alcoholic fatty liver disease

NASH: nonalcoholic-alcoholic steatohepatitis

WD: Western diet

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## INTRODUCTION

Chronic inflammation is now recognized as a molecular basis underlying metabolic syndrome, which is a constellation of visceral fat obesity, impaired glucose metabolism, dyslipidemia, and elevated blood pressure.<sup>1</sup> Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic phenotype of metabolic syndrome.<sup>2</sup> The pathological spectrum of nonalcoholic fatty liver disease includes simple steatosis and nonalcoholic steatohepatitis (NASH), the latter of which is characterized by chronic inflammation and fibrosis. NASH is a frequently diagnosed chronic liver disease, and is seen as the leading cause of cirrhosis and hepatocellular carcinoma (HCC).<sup>3-5</sup> During the course of NASH development, stromal cells dramatically changes in their cellular component and activation status responding to parenchymal cell (hepatocyte) injury or death.<sup>6</sup> This review discusses recent progress in the understanding NASH pathogenesis focusing on the disease-specific macrophages and fibroblasts.

## CLINICAL CHARACTERISTICS OF NAFLD/NASH

The prevalence of NAFLD has increased worldwide along with sedentary lifestyles and overnutrition. Although the prevalence varies with ethnicity, sex, age, comorbidities, and the diagnostic method, it is estimated that about 20%–30% of world population has NAFLD.<sup>7</sup> The natural history of NAFLD is poorly understood because invasive liver biopsy remains the gold-standard for the diagnosis of NASH. About 10–20% of NAFLD patients are supposed to have NASH and approximately 20% of those with NASH will develop advanced liver fibrosis, which increases the risk of extrahepatic complications, cirrhosis, and HCC.<sup>8,9</sup> The prevalence and severity of NAFLD is substantially increased in patients with type 2 diabetes, suggesting that insulin resistance is a shared pathophysiological variable and that type 2 diabetes promotes the progression of NAFLD.<sup>6,10</sup> Current evidence supports the importance of fibrosis as the strongest determinant of the increased risk of HCC and liver-related mortality.<sup>11-13</sup> A better understanding of how hepatic steatosis progresses to NASH and HCC, which will lead to improvement of overall outcomes.

Various serum biomarkers and combined indexes have been validated for the diagnosis of NASH and grading of disease severity, and imaging can be used to estimate liver stiffness. However, the available modalities do not have the sensitivity and specificity to distinguish NASH from hepatic steatosis.<sup>12</sup> The first-line clinical recommendation to manage NASH is lifestyle modification for weight loss, but achieving and maintaining weight loss is difficult and has shown limited effects in those with advanced liver fibrosis.<sup>8</sup> There are no approved medications for NASH, but clinical trials evaluating the benefits of interventions that target cell death, metabolic pathways, inflammatory mechanisms, the gut-liver axis, and fibrogenic pathways are ongoing.<sup>14</sup>

## ANIMAL MODELS OF NAFLD AND NASH

To understand the molecular mechanisms underlying the development of NASH, many attempts have been made to establish animal models of NASH/HCC.<sup>15,16</sup> Steatosis is readily induced by overnutrition with high-fat diet (HFD) feeding, or animal strains that have genetic defects in the regulation of appetite and energy expenditure such as *ob/ob* or *db/db* mice.<sup>16</sup> However, HFD-induced obesity leads to mild liver fibrosis only after long-term feeding, usually for more than 1 year, and leptin signal-deficient mice are resistant to liver fibrosis.<sup>17-19</sup> In contrast, chemically-induced liver fibrosis is not accompanied by obesity, insulin resistance, and hepatic

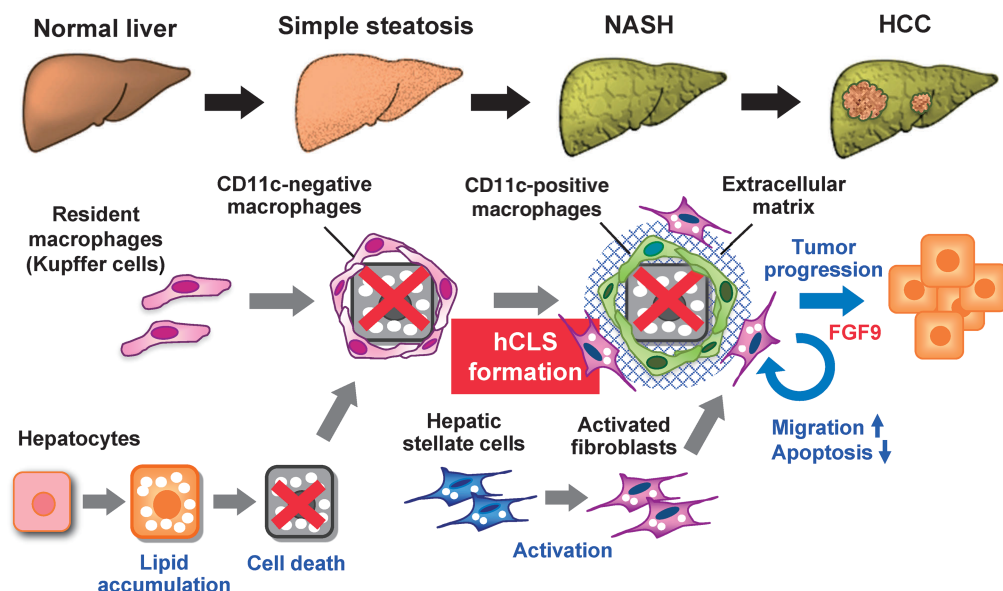
steatosis. Methionine and choline-deficient (MCD) diets can induce hepatic lipid accumulation and fibrosis, but the mice become cachexic and insulin sensitive.<sup>16</sup> In this regard, we have reported melanocortin 4 receptor-deficient (MC4R-KO) mice fed an HFD or Western diet (WD) as a novel rodent NASH model.<sup>20,21</sup> MC4R is a seven-transmembrane G protein-coupled receptor that is expressed in the hypothalamus and regulates food intake and body weight downstream of the leptin signaling.<sup>22</sup> MC4R-KO mice develop hyperphagic obesity and insulin resistance, and sequentially exhibit NASH-like liver phenotypes after 20 weeks of HFD or WD feeding and HCC after 1 year.<sup>20</sup> Because MC4R expression is primarily limited to the central nervous system and is not detected in the liver, it is possible that the hepatic phenotype in MC4R-KO mice results from metabolic derangements induced by hyperphagic obesity. A study by Nogueiras et al reported that MC4R signaling in the brain directly controlled lipid metabolism in the liver, and we previously reported that the central melanocortin system promoted peripheral monocyte chemotaxis.<sup>23,24</sup> Further investigation of the molecular mechanisms of the induction of NASH phenotypes by an HFD or WD in MC4R-KO mice is needed, but it appears to be a useful model of diet-induced steatosis, liver fibrosis, and HCC.

## HEPATOCTE DEATH-INDUCED INFLAMMATION AND FIBROSIS

The proposed molecular mechanisms of NASH include the two-hit hypothesis and the more recent multiple parallel-hit hypothesis that include excess lipid accumulation in the liver and inflammation caused by cytokines, oxidative stress, and endotoxin.<sup>25,26</sup> In contrast to simple benign hepatic steatosis, metabolic stress (lipotoxicity) that results in hepatocyte injury or death triggers the development of inflammation and fibrosis, and promotes disease progression.<sup>27</sup> Inflammation is necessary to clear cell debris and activate tissue repair programs. On the other hand, when cellular stress and injury persist chronically and repair programs are dysregulated, stromal cells including immune cells, fibroblasts, and hepatic sinusoidal cells, dramatically change in their cellular component and activation status, leading to tissue fibrosis and organ dysfunction. Several lines of evidence indicate that inhibition of hepatocyte death results in improvement of liver inflammation and fibrosis.<sup>28-30</sup> Using our NASH model, we found hepatic crown-like structures (hCLS) where CD11c-positive macrophages surround dead or dying hepatocytes that contain large lipid-droplets (Fig. 1).<sup>31,32</sup> Activated fibroblasts and increased collagen deposition are seen near hCLS, and the number of hCLS is positively correlated with the extent of liver fibrosis.<sup>31</sup> hCLS was observed in liver biopsy specimens from patients with NAFLD/NASH, but are rarely observed in patients with chronic viral hepatitis.<sup>31</sup> In comparison with NAFLD scoring system, the number of hCLS was positively associated the ballooning degeneration score, which reflects hepatocyte injury.<sup>31,32</sup> The clinical findings are consistent with experimental data showing that hepatocyte death triggers hCLS formation, which promotes inflammation and fibrosis.

## hCLS-MEDIATED LIVER FIBROSIS IN NASH

Macrophages are the major phagocytic cells in the liver, with proinflammatory and profibrotic activity, and they contribute to the pathogenesis of liver fibrosis in various etiologies. Recently, evidence has accumulated suggesting that macrophages are highly heterogenous in their origin and polarization, and activation status based on each etiology. For example Satoh et al reported a novel macrophage subset that was responsible for lung fibrosis.<sup>33</sup> In experimental models of liver fibrosis, large numbers of bone marrow-derived macrophages are recruited during the develop-



**Fig. 1** Potential role of CD11c-positive macrophages and activated fibroblasts in the progression from hepatic steatosis to NASH and HCC

During the development of NASH, resident macrophages (Kupffer cells) aggregate around dying or dead hepatocytes to constitute hCLS, and these macrophages become CD11c-positive in response to hepatocyte death, with unique gene expression profiles. Metabolic stress such as saturated fatty acids induces FGF9 expression in activated fibroblasts, thereby increasing cell migration and viability of fibroblasts and hepatoma cells, and thus promoting tumor growth.

ment of NASH and infiltrate the liver.<sup>34,35</sup> In addition, Kupffer cells, which are tissue-resident macrophages form hCLS, and become CD11c-positive in response to hepatocyte death (Fig. 1).<sup>32</sup> CD11c-positive macrophages have gene expression profiles distinct from the CD11c-negative macrophages that remain scattered in the liver. Depletion of CD11c-positive macrophages was found to abolish hCLS formation and fibrogenesis in MC4R-KO mice.<sup>32</sup> These findings indicate that CD11c-positive macrophages may be a NASH-specific macrophage subset that drives metabolic stress-induced liver fibrosis.

## CHARACTERIZATION OF ACTIVATED FIBROBLASTS IN NASH

During liver tissue remodeling caused by chronic inflammation, hepatic stellate cells (HSCs) transdifferentiate into activated fibroblasts or myofibroblasts with proliferative, migratory, and inflammatory properties. HSCs promote the formation of the extracellular matrix following activation by hepatocytes and other stromal cells.<sup>36,37</sup> The fibroblasts may have activation triggers unique to each etiology much like macrophages. The pathogenic role of profibrotic factors has been investigated mainly in liver fibrosis models without obesity and/or HCC development. In the MC4R-KO model, activated fibroblasts from mice fed a WD for 20 weeks had gene expression patterns that differed from those of fibroblasts from mice with carbon tetrachloride-induced fibrosis as a model without obesity or HCC development. Cancer-associated pathways were upregulated in activated fibroblasts even in MC4R-KO mice that had developed NASH.<sup>38</sup> Transcription of the

fibroblast growth factor 9 (FGF9) gene, the most upregulated gene in cancer-associated pathways, was induced by metabolic stress such as saturated fatty acids.<sup>38</sup> FGF9 expression was found to increase fibroblast and hepatoma cell migration and viability *in vitro* and promote tumor growth in an *in vivo* xenograft model (Fig. 1).<sup>38</sup> It is well known that cancer-associated fibroblasts affect the tumor microenvironment that promote tumor progression and metastasis.<sup>39</sup> The evidence indicates that activation of fibroblasts in NASH promotes proinflammatory and profibrogenic activity and creates a microenvironment promoting tumor progression.

## CONCLUSION

Considerable evidence shows that parenchymal cell injury or death trigger subsequent inflammation and tissue fibrosis. Review of recent evidence helps to understand how cell death induces chronic inflammation and fibrosis in NASH. The presence of disease-specific macrophages and fibroblasts is responsible for NASH development in response to metabolic stress. hCLS are sites of interaction between dead hepatocytes, macrophages, and fibroblasts that induce chronic inflammation and fibrogenesis. hCLS formation precedes the development of NASH and might be a histological marker of the progression from hepatic steatosis to NASH. hCLS formation may be novel target for preemptive medicine therapy.

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