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NFATc4: New hub in NASH development

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Non-Alcoholic Fatty Liver Disease, NAFLD, has emerged as one of the most frequent causes of chronic liver disease in our society and has became a major health problem in the world [1]. The problem is particularly present in Western countries, with an incidence of 20 and 30% among the population. NAFLD is a clinical syndrome that includes a wide spectrum of hepatic disorders from simple lipid accumulation in hepatocytes (steatosis or non-alcoholic fatty liver, NAFL) to non-alcoholic steatohepatitis (NASH). If NASH is not resolved, fibrosis develops and the disease can progress to severe stages such as cirrhosis, a high-risk condition for hepatocellular carcinoma (HCC). NAFLD is often associated to other pathologies such as obesity, the metabolic syndrome, insulin resistance, dyslipidemia, dysbiosis of the gut microbiome, type 2 diabetes, hypertension and cardiovascular disease [2, 3]. Excessive fat- and carbohydrates-enriched diets, lack of physical activity and genetic predispositions are key factors promoting NAFLD and associated pathologies.

From a mechanistic point of view, steatosis, the first stage of NAFLD, results from an imbalance between lipid anabolism (fatty acids uptake and *de novo* lipogenesis) and catabolism (export of VLDL and β-oxidation). With time, a persistent steatosis favors the intracellular accumulation of toxic lipid derivatives, ROS production, ER stress, mitochondrial dysfunction and hepatocytes death, all these pathological mechanisms contributing to NASH and fibrosis development [4]. A major energy source in hepatocytes is provided by the lipid catabolism through mitochondrial fatty acid oxidation (FAO). In FAO, fatty acids are mostly catabolized by mitochondrial β-oxidation in a series of steps tightly regulated. PPARα is a key regulator of mitochondrial β-oxidation [5], which positively regulates CPT1, an enzyme priming lipids for their translocation into mitochondria. PPARα also controls the transcription and activity of most of FAO-associated enzymes [5]. PPARα expression and activity are regulated by numerous factors including fatty acids and other lipid species, insulin, peroxisomes proliferators, microRNAs, protein kinase A/C, mTORC1 and AMPK signaling [5]. Recently, Meng and colleagues uncovered a new mechanism in NAFLD mediated by an increased expression in hepatocytes of the transcription factor NFATc4 (Nuclear Factor Of Activated T Cells 4), which negatively

regulates PPARα and osteopontin expression/activity, thereby importantly fostering steatosis development, as well as inflammatory responses and fibrosis in the liver.

The nuclear factor of activated T cells (NFAT) proteins are a family of transcription factors under the control of calcineurin, a Ca^{2+}-dependent phosphatase [6]. Various members of the NFAT family were shown to have fundamental roles in T/B lymphocytes during adaptive immunity (humoral immune responses, immunological tolerance, immune metabolism,…) [7] as well as in innate inflammatory responses of myeloid cells [8]. However, numerous reports now point to a key role of NFAT transcription factors in a wide panel of non-immune processes occurring in various cell type and organs. Related to NFATc4 in particular, this isoform was reported to play a pivotal role in cardiac hypertrophy [9] and in atrial natriuretic peptide production during transverse aortic constriction [10]. In the adipose tissue, NFATc4 was shown to be required for adipocyte differentiation [11], to promote secretion of inflammatory effectors [12] and to refrain adiponectin expression with obesity [13]. These deleterious effects of NFATc4 in obesity-associated metabolic disorders were further supported by the beneficial effect of its pharmacological inhibition, which prevented insulin resistance development and nephropathy induced by a high-fructose containing diet in rats [14]. Meng and colleagues in their study now expand the pathological role of NFATc4 activity in deregulated hepatic metabolism, inflammation and fibrosis by showing that NFATc4 expression in hepatocytes specifically promotes lipid accumulation, liver damage, macrophage recruitment and fibrosis through a paracrine activation of stellate cells. Authors could further show that nuclear translocation of NAFTc4 in hepatocytes occurs with NASH and fibrosis in humans through the analyses of patient biopsies. By feeding genetic mouse models of obesity/NAFLD with a fibrogenic diet, they could also demonstrate that NFATc4 is a key driver of the progression of obesity-associated steatosis toward inflammation and fibrosis.

As mentioned, NAFLD is associated with a plethora of molecular alterations in hepatocytes and non-parenchymal hepatic cells that all contribute to the development and progression of this pathology to severe stages. Transcription

factors such as NFATc4 are important signaling hubs controlling multiple cellular processes involved in NAFDL/NASH development and thus represent key molecular drivers of these diseases. With regard to NFATc4, Meng et al demonstrated that in mice at least two important factors, PPARα and osteopontin, are repressed by the increased activity of NFATc4 thereby promoting steatosis, inflammation and fibrosis in the liver. PPARα has a well-established role as a major regulator of fatty acid β-oxidation and immunity in the liver of mice [5]. However, human and mouse PPARα display structural and functional differences [15] and classical selective PPARα agonists (e.g. fenofibrates) were shown to be not sufficiently effective at safety doses for therapeutic purposes in NASH/fibrosis [16]. New research and clinical trials are now focusing on the development and testing of new PPARα agonists lacking adverse effects or dual inhibitors of PPARα/δ (e.g. Elafibranor), which are holding new promises in NASH/fibrosis treatment [16, 17]. Interestingly, authors also showed that PPARα inhibition by NFATc4 was not sufficient to promote inflammation and fibrosis. They thus search for other NFATc4 target and identify osteopontin as a pertinent factor induced by NFATc4 and capable, at least *in vitro*, to activate macrophages and stellate cells. Osteopontin was previously identified as a relevant signaling molecule activating hepatic stellate cells and as a promising biomarker for fibrosis and hepatocellular cancer [18]. However, in this study Meng et al could not evaluate the relative importance of NFATc4-induced osteopontin expression, as compared to other potential targets of NFATc4, in the progression of steatosis towards inflammation and fibrosis. Assessing quantitatively the relative role of PPARα and osteopontin inhibition, as well as those of other NFATc4 hepatic target, should provide important insights not only to fully understand the role of NFATc4 as a key driver of NASH, but also to identify new and relevant downstream NFATc4 effectors as potential new therapeutic targets.

The identification of NFATc4 as an important factor promoting NASH and its previously reported functions in immune cells raises also the question of its role in the progression of NASH toward HCC development with or without cirrhosis. Indeed, other members of the NFAT family were previously shown to display either tumor suppressive or oncogenic function through multiple mechanisms in various non-hepatic cancers [19]. Based on the study by Meng et al, we could

expect that inhibition of NFATc4 should likely refrain progression of hepatic metabolic disorders towards cancer development, or progression of established tumors towards malignancy. Future studies are now required to investigate HCC-related pathological mechanisms potentially under the control of NFATc4 such as for example deregulation of the lipid metabolism and inflammation, or other carcinogenic processes affected by NFAT members in other cancers such as cell proliferation, metastasis, drug resistance and modifications of the tumor microenvironment.

Of interest, several NFATc4 inhibitors have been developed and tested in specific cancers, but not in HCC [20], including immunosuppressive drugs specifically inhibiting calcineurin (cyclosporin A, FK506, Tacrolimus,..), a major regulator of NFAT members. These latter have clear anti-cancer activities but their clinical use needs caution. Indeed, when administered on a long term, they may increase tumour incidence or recurrence, including for HCC, likely due to their immunosuppressive activities, which affect the local immunosurveillance of tumours [21]. Beside a direct targeting of NFATc4, or of its upstream regulator calcineurin, other potential therapeutic options need also to be considered for liver diseases and cancer. These include for example targeting of other specific regulators of NFATc4 expression and activity. In this regard, Meng et al have identified in hepatocytes the LRRK2 protein and the non-coding RNA Nron as co-factors required to sequester NFATc4 in the cytoplasm and to prevent its translocation into the nucleus. Other mechanisms potentially druggable and regulating NFATc4 expression/activity such as post-transcriptional modifications (ubiquitination [22], sumoylation [23] or dimerization [24]), alternative splicing [25] or regulation by non-coding RNAs were not investigated in the study by Meng et al. but likely need further consideration to deepen our knowledge of the role of NFATc4 in NASH and cancer and to uncover new potential therapeutic tracks.

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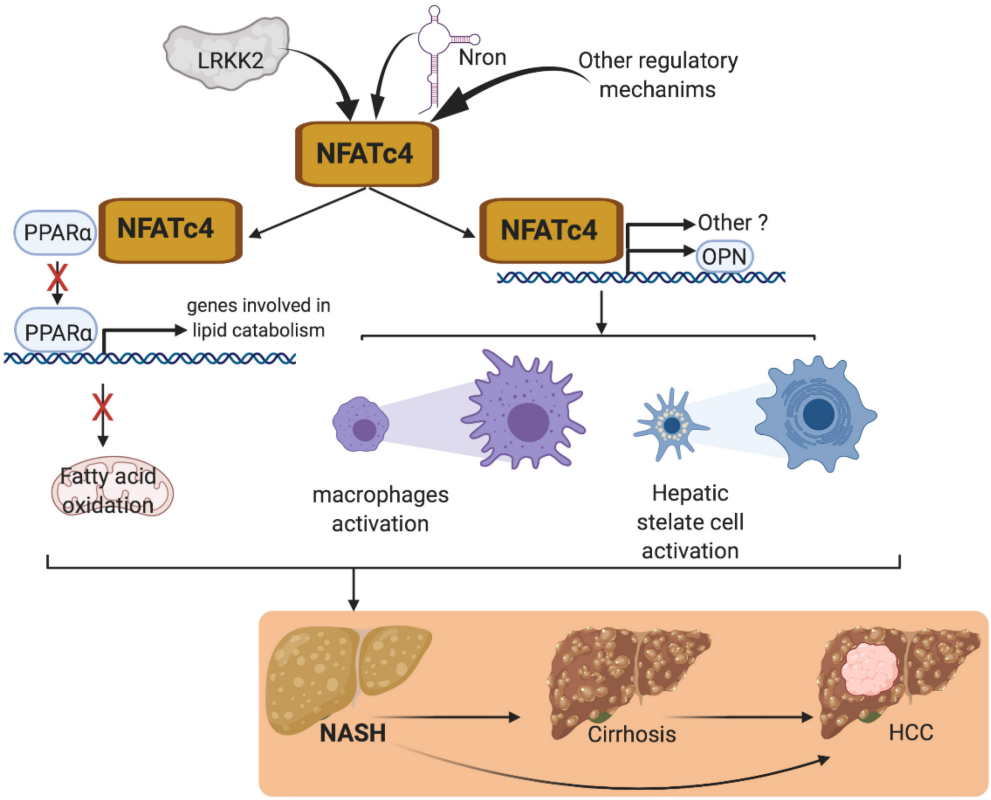
Figure legend

NFATc4 plays an essential role in NASH in one hand by sequestering PPARα and inhibiting its transcriptional activity thereby restraining fatty acid β-oxidation in hepatocytes - and in the other hand by triggering the expression of osteopontin, a hepatocyte-secreted factor promoting the activation of macrophages and stellate cells. Although calcineurin is the principal regulator of this transcriptional factor, LRRK2 and the Long non-coding RNA Nron have been implicated in NFATc4 activation with NASH.

Besides the previous role described for NFATc4 in immune processes, adipogenesis and cardiac hypertrophy, this transcription factor appears therefore also to represent a new signaling hub in NASH and a potential driver of carcinogenesis.


LRKK2
Nron
Other regulatory mechanims
NFATc4
PPARα
NFATc4
NFATc4
Other ?
OPN
PPARα
genes involved in lipid catabolism
Fatty acid oxidation
macrophages activation
Hepatic stelate cell activation
NASH
Cirrhosis
HCC