Research Article

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A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer

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Background & Aims: The lack of a preclinical model of progressive non-alcoholic steatohepatitis (NASH) that recapitulates human disease is a barrier to therapeutic development.

Methods: A stable isogenic cross between C57BL/6J (B6) and 129S1/SvImJ (S129) mice were fed a high fat diet with *ad libitum* consumption of glucose and fructose in physiologically relevant concentrations and compared to mice fed a chow diet and also to both parent strains.

Results: Following initiation of the obesogenic diet, B6/129 mice developed obesity, insulin resistance, hypertriglyceridemia and increased LDL-cholesterol. They sequentially also developed steatosis (4–8 weeks), steatohepatitis (16–24 weeks), progressive fibrosis (16 weeks onwards) and spontaneous hepatocellular cancer (HCC). There was a strong concordance between the pattern of pathway activation at a transcriptomic level between humans and mice with similar histological phenotypes (FDR 0.02 for early and 0.08 for late time points). Lipogenic, inflammatory and apoptotic signaling pathways activated in human NASH were also activated in these mice. The HCC gene signature resembled the S1 and S2 human subclasses of HCC (FDR 0.01 for both). Only the B6/129 mouse but not the parent strains recapitulated all of these aspects of human NAFLD.

Conclusions: We here describe a diet-induced animal model of non-alcoholic fatty liver disease (DIAMOND) that recapitulates the key physiological, metabolic, histologic, transcriptomic and cell-signaling changes seen in humans with progressive NASH.

Lay summary: We have developed a diet-induced mouse model of non-alcoholic steatohepatitis (NASH) and hepatic cancers in a cross between two mouse strains (129S1/SvImJ and C57Bl/6J). This model mimics all the physiological, metabolic, histological, transcriptomic gene signature and clinical endpoints of human NASH and can facilitate preclinical development of therapeutic targets for NASH.

Keywords: Steatosis; Fibrosis; Hepatocyte ballooning; Hepatocellular carcinoma; Drug therapy.

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; B6, C57BL/6J; CD, chow diet; CK-18, cytokeratin-18; DIAMOND, diet-induced animal model of non-alcoholic fatty liver disease; ER, endoplasmic reticulum; FDR, false discovery rate; GTT, glucose tolerance test; IRE-1, inositol-requiring enzyme-1; ITT, insulin tolerance test; JNK, c-Jun NH2-terminal kinase; LDL-c, low-density lipoprotein-cholesterol; MCD, methionine choline deficient; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NW, normal water; PERK, protein kinase R-like endoplasmic reticulum kinase; PUFA, polyunsaturated fatty acid; T2DM, type 2 diabetes mellitus; S129, 129S1/SvImJ; HCC, hepatocellular carcinoma, WD, Western diet; SW, sugar water.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as the leading cause of chronic liver disease in most parts of the Western world. NAFLD includes both a fatty liver and steatohepatitis which can progress through stages of fibrosis to cirrhosis and can be complicated by hepatocellular cancer [1]. There is currently no approved therapy for NASH.

There is a need to create animal models that recapitulate the physiology, histology, outcomes and transcriptomic changes seen in humans with NASH. While a large number of models have been described, they have several limitations [2] (Supplementary Table 1). These models are dissimilar to human NASH by either requiring specific gene knockout, non-physiological dietary manipulations or their lack of insulin resistance or liver histology

typical of human NASH [3]. Importantly, most models do not develop progressive fibrosis and do not lead to hepatocellular cancer [2,4]. While the Ossabaw pig develops lesions very consistent with human NASH [5], the cost and resources needed to use this model renders it challenging for widespread use. The C57BL/6J mice fed a high fat, high sugar diet develop steatohepatitis with fibrosis [6]. However, the severity of fibrosis is mild and the disease is not complicated by hepatocellular cancer, an increasingly recognized and clinically important end-point of the progression of NASH.

An ideal preclinical model for NASH should be relatively simple, triggered by the same causes as human disease (caloric excess), associated with the same risk factors as in humans (obesity, insulin resistance and dyslipidemia), and it should match human disease with respect to metabolic features, histology, outcomes, gene expression signature, lipid accumulation and activation of pathways relevant in humans. Importantly, it should also recapitulate the various histological stages of human disease. The development of hepatocellular carcinoma (HCC) in this setting should also be triggered by the disease state and not by administration of a chemical carcinogen. We here report a diet-induced animal model of non-alcoholic fatty liver disease (DIAMOND) using an isogenic strain derived from a cross of two common mouse strains, 129S1/SvImJ and C57BL/6J where a simple high fat diet accompanied by *ad libitum* consumption of water with a high fructose and glucose content (Western diet sugar water (WD SW)) sequentially induces steatosis, steatohepatitis, progressive fibrosis and HCC. The phenotype was noted serendipitously and refined to develop the isogenic strain and the dietary manipulations to reproducibly produce the disease phenotype.

Materials and methods

Animals

A unique, isogenic mouse strain derived from a C57BL/6J and 129S1/SvImJ background (B6/129) was created and maintained with inbreeding. Additional information on B6/129 origin are described in the Supplementary materials and methods section. Pure C57BL/6J (B6) or 129S1/SvImJ (S129) were purchased from Jackson Laboratory (Bar Harbor) and used as controls. All mice were housed in a 12 h light–12 h dark cycle in a 21–23 °C facility and were euthanized at varying time points following initiation of dietary intervention. All procedures were performed according to protocols approved by the Animal Care and Use Committee of Virginia Commonwealth University (IACUC AM 10154).

Dietary interventions

Male mice (8–12 weeks of age) were fed *ad libitum* a high fat diet, high carbohydrate diet (Western diet, WD) with 42% kcal from fat and containing 0.1% cholesterol (Harlan TD.88137) with a high fructose-glucose solution (SW, 23.1 g/L d-fructose + 18.9 g/L d-glucose), as previously described [6]. Control mice were fed a standard chow diet (CD, Harlan TD.7012) with normal water (NW).

Histological analysis

Liver histology was assessed using hematoxylin and eosin (H&E) stains in paraffin-embedded sections using standard commercially used methods. Fibrosis was assessed using both Masson's trichrome and Sirius Red stains in paraffin-embedded sections using established methodology [7]. The presence of steatosis was further confirmed used Oil-Red-O stains in frozen sections using standard methods [8]. The liver histology was evaluated by an expert liver pathologist (PB) who was blinded to the dietary condition. Histology was assessed using the NASH CRN and fatty liver inhibition of progression (FLIP) consortia criteria (see the Supplementary materials and methods section for details) [3].

A detailed description of biochemical, molecular, transcriptomic, bioinformatics and statistical analysis is also provided in the Supplementary materials and methods section.

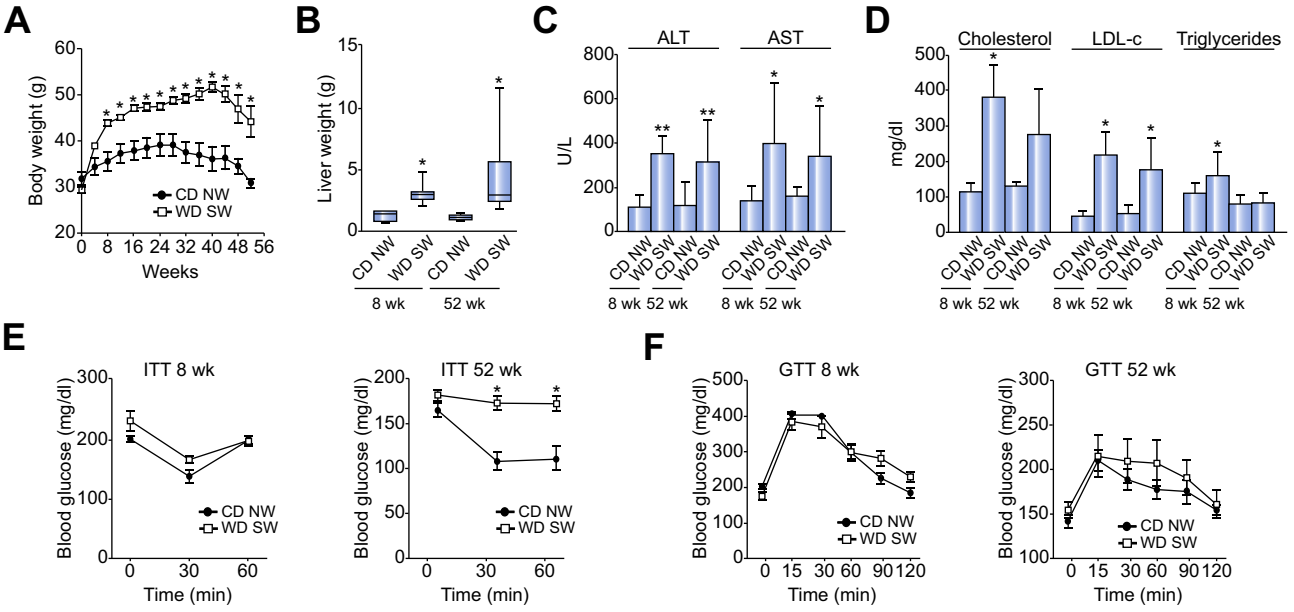

Fig. 1. DIAMOND mice develop obesity, liver injury, dyslipidemia and insulin resistance. B6/129 mice were fed a chow diet (CD NW) or high fructose/glucose, high fat Western Diet (WD SW) for up to 52 weeks. (A) Body weight change over time, (B) Liver weight, (C) serum ALT and AST levels, (D) serum cholesterol, LDL-c and triglycerides levels, (E) Insulin tolerance test (ITT) and (F) glucose tolerance test (GTT). Data are expressed as the mean ± SEM for 6–10 mice per group; $^{*}p$ <0.05 and $^{**}p$ <0.001, WD SW compared to CD NW.