REVIEW ARTICLE

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Translating scientific discovery: the need for preclinical models of nonalcoholic steatohepatitis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the Western world, affecting about 1/3 of the US general population and remaining as a significant cause of morbidity and mortality. The hallmark of the disease is the excessive accumulation of fat within the liver cells (hepatocytes), which eventually paves the way to cellular stress, injury and apoptosis. NAFLD is strongly associated with components of the metabolic syndrome and is fast emerging as a leading cause of liver transplant in the USA. Based on clinico-pathologic classification, NAFLD may present as isolated lipid collection (steatosis) within the hepatocytes (referred to as non-alcoholic fatty liver; NAFL); or as the more aggressive phenotype (known as non-alcoholic steatohepatitis; NASH). There are currently no regulatory agency-approved medication for NAFLD, despite the enormous work and resources that have gone into the study of this condition. Therefore, there remains a huge unmet need in developing and utilizing pre-clinical models that will recapitulate the disease condition in humans. In line with progress being made in developing appropriate disease models, this review highlights the cutting-edge preclinical in vitro and animal models that try to recapitulate the human disease pathophysiology and/or clinical manifestations.

Keywords Non-alcoholic fatty liver disease (NAFLD) · Non-alcoholic steatohepatitis (NASH) · Organotypic liver system · Lipitoxic system · Diet induced animal model of non-alcoholic fatty liver disease (DIAMOND) · Farnesoid X receptor (FXR)

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver-related morbidity and mortality worldwide and affects almost a third of all American adults [1]. It is strongly related to insulin resistance and its associated clinical conditions such as excess body weight, type 2 diabetes, hypertension, hypertriglyceridemia and low HDL-cholesterol (all of which constitute essential elements in the spectrum of the metabolic syndrome). The NAFLD disease spectrum has two phenotypes, namely, nonalcoholic fatty liver (NAFL) which is characterized by simple steatosis without obvious cellular injury; and nonalcoholic steatohepatitis (NASH), a more aggressive disease marked by cellular injury, inflammatory infiltrates and possible progression to fibrosis or cirrhosis [2]. NASH is increasing as an etiology for end-stage liver disease as well as for hepatocellular cancer-related liver transplantation; and is expected to surpass hepatitis C for this indication in the foreseeable future [3]. The hallmark lesion of NASH is hepatocellular ballooning, which is a marker of hepatocyte injury and constitutes one of the drivers of disease progression into fibrogenic remodeling [4, 5]. Liver biopsy remains the gold standard for the definitive diagnosis of NASH, even though there is significant on-going work at using non-invasive modalities for diagnosis [6].

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While data on incident rates are deficient, there are several reports that shed light on the prevalence of NAFLD. A fairly recent meta-analysis estimates the overall global prevalence of NAFLD (as diagnosed via imaging; but not biopsy) at about 25%, with the Middle East and South America (at approximately 32 and 30%, respectively) sharing the highest prevalence rates, while Africa (with about 13%) having the lowest [7]. However, in the specific case of NASH, given that liver biopsies cannot be routinely obtained at the population level, there is an inadequate direct assessment for true incidence or prevalence rates. Therefore, given the limitation in obtaining direct data on NASH, a rough estimate of prevalence in the general population, is thought to be in the range of 1.5–6.5% [7, 8].

The high prevalence of NAFLD in patients with the metabolic syndrome is likewise reciprocated by an increased risk of developing NAFLD in subjects bearing elements of the metabolic syndrome, giving credence to the bidirectional association that bind these two disease entities together [7, 9–11]. Patients with NAFLD have been shown to have higher overall mortality when compared with matched controls from the general population. However, the most common cause of death in NAFLD has been cardiovascular disease, irrespective of any metabolic comorbid conditions. Hence, the joint European Association for the Study of the Liver, European Association for the Study of Diabetes and the European Association for the Study of Obesity (EASL-EASD-EASO) guidelines recommend screening for NAFLD in all patients with persistent abnormal liver enzymes, as well as screening for metabolic syndrome in persons with steatosis, independent of liver enzymes. Likewise, they also recommend routine screening with liver enzymes and/or ultrasound for NAFLD in subjects with obesity or the metabolic syndrome [12]. In contrast, the American Association for the Study of the Liver Practice Guidance in 2017 does not recommend routine screening for NAFLD in high-risk individuals at this time. However, it calls for a high index of suspicion for NAFLD/NASH in subjects with type 2 diabetes and the use of non-invasive means to identify those at risk for advanced fibrosis (these practice guidance statements from the AASLD may be rendered obsolete by the association′s announced 2018 guidelines, which were close to publication at the time of original submission of this manuscript) [6].

Despite the significant toll that NAFLD has on health systems worldwide, the disease still remains without any approved therapies [13]. Therefore, there is an immediate unmet need to better understand the molecular drivers of the disease and its progression, and to develop methods for the best targeting and therapeutic strategies for individual patients. These require developing preclinical models that recapitulate as many aspects of human disease as possible,

and allow for translation of findings from simple models to human therapeutics [13, 14]. Here, we will review some recent advances that provide hope for future precision-medicine based approaches for the treatment of NASH.

Human in vitro organotypic models of NAFLD

While numerous animal models have been developed to mimic various aspects of this complex disease spectrum (reviewed below), the use of human primary liver cells to study NAFLD in a meaningful context continues to mature. To address this need, organotypic liver systems aim to incorporate the complexities of the native human liver in vitro, including three-dimensional (3D) architecture, multiple cell types, and the dynamic microenvironment stemming from blood flow through the sinusoids. The development of complex organotypic liver systems has been reviewed elsewhere, and this section of the review will focus on how these organotypic systems have been adapted for studying NAFL and NASH [15–19].

Though immortalized human cell lines, such as HUH7 and HepG2, as well as iPSC-derived hepatocytes, can be induced towards a steatotic phenotype in culture, multiple studies have shown that these cells poorly represent the native liver metabolic function, thus keeping primary human hepatocytes as the gold standard for in vitro studies [20–22]. In addition to hepatocytes, the addition of non-parenchymal cells (NPC), including human hepatic stellate cells (HSCs), resident inflammatory cells (e.g., Kupffer cells, macrophages), and/or sinusoidal endothelial cells (sECs), affect liver biology and are known to contribute to the progression of NAFLD [23–25]. Thus, these additional cell types enable the evaluation of NPC-driven disease mechanisms, such as inflammatory cytokine expression or stellate cell activation, which are hallmark features of NASH and fibrosis.

Diverse approaches have been utilized to combine these different cell types into 3D tissues in order to mimic the architecture found in the intact liver, including layered co-cultures, spheroids, micro-patterned surfaces, human precision cut liver slices (hPCLS), and bioprinting [15–19, 26]. Further, many of these tissues can be cultured within specialized devices (e.g. microfluidic chambers) to include aspects of flow and transport. Table 1 provides a summary of companies currently developing organotypic liver models who have developed perfused multicellular liver organotypic models.

Currently, most organotypic models have focused on drug safety and toxicity with few adaptations to mimic other aspects of NAFLD or NASH. In this context, the liver microenvironment includes a number of circulating risk

Table 1 Overview of liver organotypic systems

System:	HμRELflow	Regenemed	Emulate	Nortis SQL-SAL	Hepregen
System attributes					
Base technology	Microfluidic chip	Transwell cultures	Microfluidic chip	Microfluidic chip	Micropatterned cultures
Primary application(s)	Clearance, toxicity screen	Toxicity screen	Toxicity screen	Toxicity screen	Clearance, toxicity screen, disease modeling
Disease models	No	No	No	No	*Hepatitis B/C, malaria*
Separation of cell signals	No	No	No	No	No
Species	*Human, rat, dog, monkey*	*Human, rat*	*Human*	*Human*	*Human, rat, dog, monkey*
Liver tissue relevance					
Cell types	*Primary: hepatocytes + NPCs*	*Primary: hepatocytes + NPCs*	*Primary: hepatocytes + NPCs*	*Primary hepatocytes + NPC cell lines*	*Primary hepatocytes + 3T3 mouse fibroblasts*
Hemodynamic flow and transport	In development	No	*Yes*	*Yes*	No
Physiological milieu relevance					
Hormone/growth factors/fatty acids	No public data	No public data	No public data	No public data	Supraphysiological levels of insulin/glucose
Validation					
Morphology/liver function	*Yes*	*Yes*	No public data	*Yes*	*Yes*
CYP & transporter activity	*Yes*	*Yes*	No public data	*Yes*	*Yes*
Drug concentration responsiveness	10–50× C_{max}	10× C_{max}	No public data	10×–100× C_{max}	10×–100× C_{max}
Disease phenotype benchmark	No public data	No public data	No public data	No public data	No public data
NAFLD drug/target evaluation	No public data	No public data	No public data	No public data	No public data
References	hurelcorp.com [41]	regenemed.com [42]	emulatebio.com [43]	www.nortisbio.com [44]	hepregen.com [45–47]

System:	Insphero	Organovo ExVive	Human precision-cut liver slices	CN BIO LiverChip	HemoShear
System attributes					
Base technology	Hanging drop spheroids	3D bioprinting	Human tissue	Microfluidic chip	Cone-and-plate perfused cocultures
Primary application(s)	Toxicity screen, disease modeling	Toxicity screen, disease modeling	Disease, drug, toxicity mechanisms	Clearance, toxicity screen, disease modeling	Disease, drug, toxicity mechanisms
Disease models	*Drug-induced steatosis, viral hepatitis, cholestasis*	*Drug-induced steatosis, NAFLD, fibrosis*	*Based on tissue source (e.g., obese patients)*	*Hepatitis B/C, malaria, NAFLD*	*NAFLD, rare diseases*
Separation of cell signals	No	No	No	No	*Yes*
Species	*Human, rat, dog, monkey*	*Human*	*Human*	*Human*	*Human, rat*

Table 1 (continued)

System:	Insphero	Organovo ExVive	Human precision-cut liver slices	CN BIO LiverChip	HemoShear
Liver tissue relevance					
Cell types	*Primary: hepatocytes + NPCs*	*Primary: hepatocytes + NPCs*	*Primary: hepatocytes + NPCs*	*Primary: hepatocytes ± NPCs*	*Primary: hepatocytes + NPCs*
Hemodynamic flow and transport	No	No	No	*Yes*	*Yes*
Physiological milieu relevance					
Hormone/growth factors/fatty acids	No public data	No public data	Supraphysiological levels of glucose	*Physiological levels of insulin/glucose; supraphysiological levels of FFAs*	*Physiological levels of insulin/glucose/ FFAs*
Validation					
Morphology/liver function	*Yes*	*Yes*	No public data	*Yes*	*Yes*
CYP & transporter activity	*Yes*	*Yes*	No public data	*Yes*	*Yes*
Drug concentration responsiveness	25×–50× C_{max}	25× C_{max}	No public data	10×–100× C_{max}	1×–10× C_{max}
Disease phenotype benchmark	No public data	No public data	*Obese patient tissue source*	No public data	*Clinical NASH patient transcriptomics and lipidomics*
NAFLD drug/target evaluation	No public data	No public data	*Obeticholic acid, PPARα agonist*	*Pioglitazone, metformin*	*Obeticholic acid, elafibranor, ACC inhibitor, 15+ additional targets*
References	insphero.com [48, 49]	organovo.com [50]	[26, 51]	cn-bio.com [28, 52, 53]	hemoshear.com [31, 35, 54]

Attributes that provide a competitive advantage are italicized

factors that can be incorporated into the media formulation in vitro to promote a NASH-relevant phenotype. These risk factors include endotoxin, inflammatory cytokines, growth factors, glucose, insulin, and free fatty acid (FFA) exposure. In particular, FFAs are known to induce a lipotoxic phenotype and are the most commonly used stimuli in NAFLD in vitro models, as they may also lead to increased levels of additional factors, such as inflammatory cytokines [27].

Several groups are actively developing these models. For example, Organovo's bioprinting platform showed evidence of steatosis (drug-induced) and fibrosis, and is being developed as a NASH model. Kostrzewski et al. improved upon a previous microfluidics-based (designed by CellASIC) NAFLD model seeded with HepG2 cells, by incorporating primary human hepatocytes (no NPCs) into CN Bio's microfluidic liver platform [28, 29]. To generate a NAFLD-relevant phenotype, both studies exposed the hepatocytes to high concentrations of oleic acid (400–660 μM) and palmitic acid (200–330 μM)

combinations to create a "fat media". In keeping with histological observations in NAFLD patients, Kostrzewski et al. reported that the fat media increased lipid accumulation in the cells and altered gene and activity levels of many CYP P450 enzymes. Cytokine levels, such as IL8, were increased in fat media conditions, but other cytokines, such as IL6 were not detected, likely due to the absence of additional cell types, such as Kupffer cells or HSCs [28]. Pioglitazone, which was found in the PIVENS trial to ameliorate fat accumulation and other histological features of NASH in the clinical setting, reduced lipid accumulation in the lipid-loaded hepatocytes, but no additional effects were reported [30].

While the previous model incorporates NASH-associated risk factors in a flow-based system, the lack of additional cell types, functional endpoints and validation confine the model's potential use for target discovery. Feaver et al. recently developed a lipotoxic organotypic system for studying NAFL and NASH using the

HemoShear liver platform [31]. This lipotoxic system incorporates hemodynamic and transport conditions derived from the liver sinusoid in a 3D culture system that contains primary human hepatocytes, macrophages, and HSCs (Fig. 1). The components of the milieu were similar to those of Kostrzewski et al., using physiologically relevant levels of glucose, insulin, oleic acid (65 μM) and palmitic acid (45 μM) derived from circulating levels in NASH patients [32]. Under these lipotoxic conditions, increased intracellular lipid accumulation was observed in agreement with Kostrzewski et al.; and additional functional endpoints were evaluated, including evidence of insulin resistance, increased inflammatory and fibrosis biomarkers, and HSC activation (Fig. 2).

A key differentiator was the use of two-omic approaches to more deeply understand the lipotoxic phenotype and directly validate the response to human biopsies [31]. RNA sequencing and lipidomic metabolite signatures from the lipotoxic system and patient biopsies from control and histologically confirmed NASH patients were compared. There was a high degree of concordance between the gene pathways and lipidomic signature induced in the in vitro system and those from patient biopsies [33]. This is the only in vitro model to benchmark -omic responses to those observed in human biopsies at this time.

Lastly, an additional and vital validation step required for in vitro systems to be utilized in drug discovery is to provide evidence of clinical translation. As previously mentioned, Kostrzewski et al. evaluated the impact of pioglitazone and showed anti-steatotic effects. While hPCLS fail to incorporate flow aspects, disease-associated risk factors, and have short time windows before the tissue begins to decline in function, they excel at incorporating the full complement of liver cell types and are freshly isolated from the human liver.

Ljssennagger et al. used hPCLS to evaluate the farnesoid X receptor (FXR)-agonist, obeticholic acid (OCA, 1 μM for 24 h), which is currently in Phase 3 clinical trials for the treatment of NASH, and reported that gene expression profiling revealed on-target engagement of FXR [26]. Similarly, the lipotoxic system developed by Feaver et al. observed several clinically-relevant effects with 0.5-μM OCA treatment, including on-target effects and reductions in steatosis, inflammatory cytokines, fibrotic biomarkers, and triglycerides—all of which were observed in the Phase 2b clinical trial [31, 34, 35]. OCA was found in the clinic to raise LDL, a risk not observed in pre-clinical animal studies. Both hPCLS and the lipotoxic system reported transcriptional signatures that may explain the cholesterol endpoints observed in the clinical trials for OCA [26, 35]. Additionally, in the lipotoxic system, OCA treatment induced high secretion of ApoB (the primary apoprotein associated with LDL) and increased intracellular levels of cholesterol, suggesting dysregulation of this pathway, in agreement with what was observed in the clinic [31, 34]. Thus, while not perfect, substantial progress has been made in development of in vitro models that capture many aspects of NASH at a functional, molecular, transcriptomic and metabolomic levels that provide opportunities to test specific compounds, or combinations of compounds, for their anti-NASH efficacy.

Animal models of NAFLD/NASH

The liver has a substantial capacity to synthesize as well as store fat. It is therefore not surprising that a variety of manipulations targeting the metabolic machinery of the liver alters lipid flux and leads to accumulation of fat in the liver. This induces cell stress and may also lead to modest
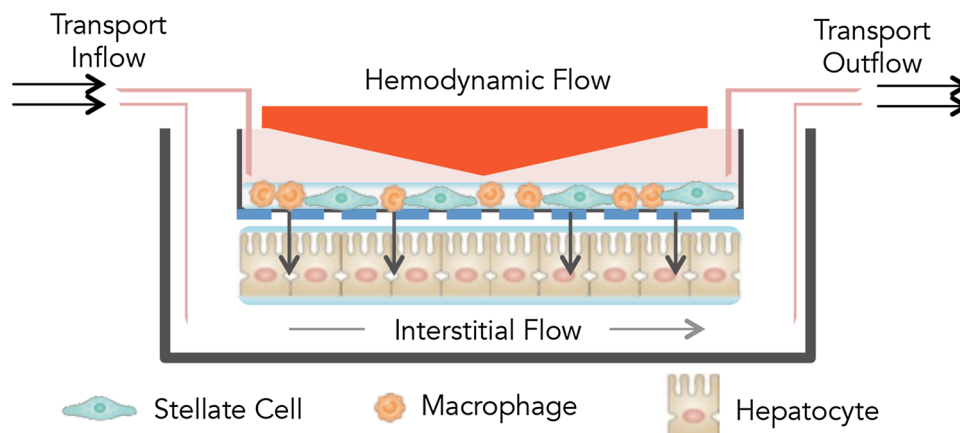

Fig. 1 Lipotoxic in vitro system utilizing liver sinusoidal hemodynamics in a 3D culture model. Liver sinusoidal hemodynamics are applied to the HemoShear human liver system described by Feaver et al. [23], which uses a cone-and-plate viscometer incorporated into a transwell multi-culture model. Non-parenchymal cells (NPCs, composed of human hepatic stellate cells and macrophages) are plated on the top of transwell while hepatocytes are plated on the bottom of the transwell. Rotation of the cone (orange triangle) imparts shear stress onto the transwell. Media is continually perfused to recapitulate interstitial flow, as indicated by the inflow and outflow ports

Fig. 2 Hepatocytes and non-parenchymal cells (NPCs) in the 3D lipotoxic in vitro system demonstrate increased intracellular lipid accumulation as well as inflammatory and fibrotic markers. Representative ×20 photomicrographs of hepatocytes (*HEPS*) and non-parenchymal cells (*NPCs*) cultured in the lipotoxic conditions described by Feaver et al. are shown [23]. Hepatocytes were marked by the polarization marker, E-cadherin, and intracellular lipid accumulation was stained by Nile Red. NPCs include hepatic stellate cells (reelin, *green*) and macrophages (CD68, *blue*). Activated stellate cells are indicated by smooth muscle actin-α (SMAA, *red*) staining
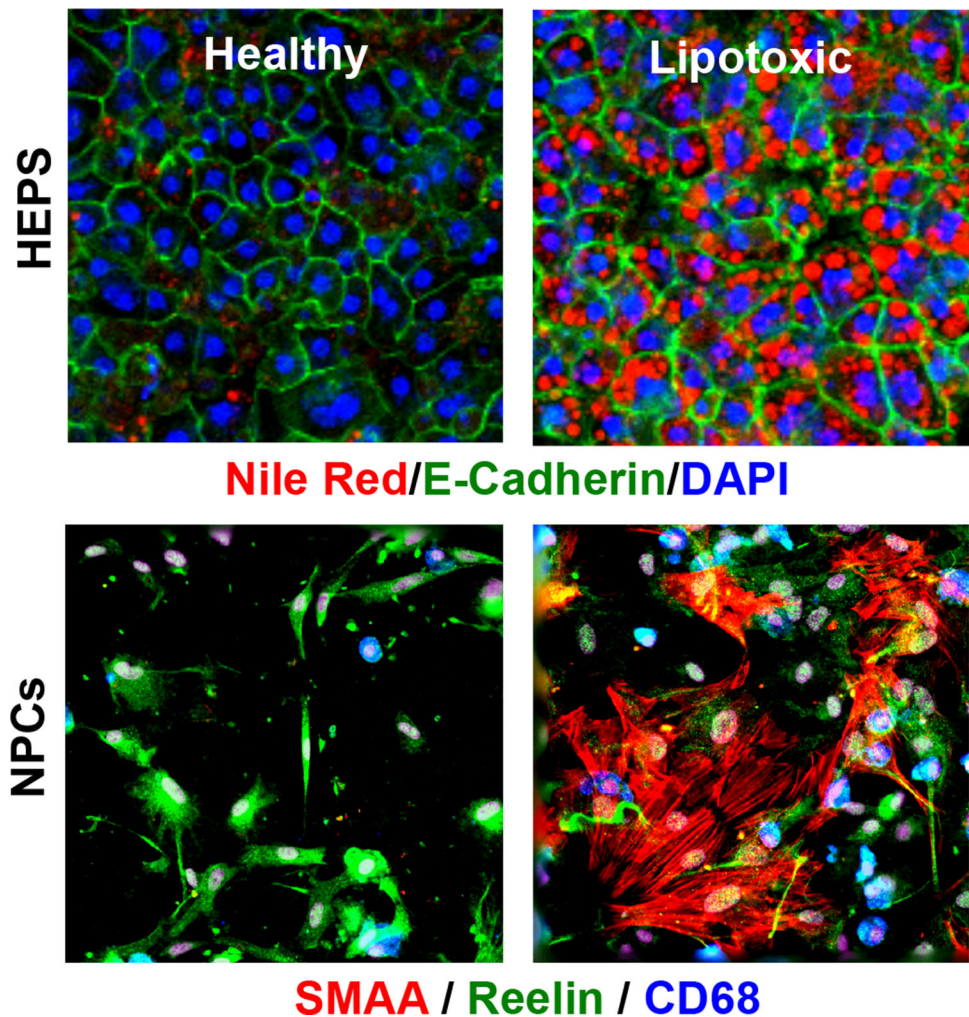
inflammation [36, 37]. These findings have fueled intense interest in studying pre-clinical models of human NAFLD. However, the great majority of animal models, especially those with specific gene knockouts or unusual diets do not come close to recapitulating human disease [38].

An ideal animal model must meet the following criteria in order to be considered representative of human disease. It must recapitulate human NAFLD by: (1) not requiring a specific gene knockout since human NAFLD is not marked by these gene-specific deficiencies, (2) induction by diet-induced obesity, (3) use of diets with at least a macronutrient composition similar to those consumed by most humans, (4) development of adiposity, insulin resistance and dyslipidemia similar to that in humans, (5) demonstrating a systemic inflammatory state with visceral adipose tissue inflammation and increased systemic inflammatory cytokines, (6) activation of cell signaling pathways that are relevant to human disease and disease progression, (7) transcriptomic concordance with human disease, (8) lipidomic concordance with human disease, and (9)

Fig. 3 Main histological features of DIAMOND mice are comparable to human NASH. Representative images of liver histology from DIAMOND mice (**a**, **c**, **e**, **g**, **i**, **k**) or human NASH (**b**, **d**, **f**, **h**, **j**, **l**) depicting steatosis (**a**, **b**; H&E; original magnification, ×5), Hepatocyte ballooning (**c**, **d**; H&E; original magnification, ×40), Mallory-Denk bodies (as indicated by *dark arrows*) (**e**, **f**; H&E; original magnification, ×40), Stage 1 fibrosis (**g**, **h**; Sirius Red; original magnification, ×20), Stage 2 fibrosis (**i**, **j**; Sirius Red; original magnification, ×20), Stage 3 fibrosis (**k**, **l**; Sirius Red; original magnification, ×10). *CV* central vein; *PT* portal tract. (Adapted from [14] with permission)

histological concordance with human disease and progression to advanced fibrosis and hepatocellular cancer.

A variety of animal models have been described using many species from zebrafish and sandflies to rodents and even large mammals. They generally involve either dietary manipulation or genetic manipulation. Diets have, however, been largely inconsistent with what is known in humans. For example, the majority of human diets are not choline-deficient [38], and while choline deficient diets can induce steatohepatitis, its translatability to the human state

| DIAMOND Mice | Human NASH |
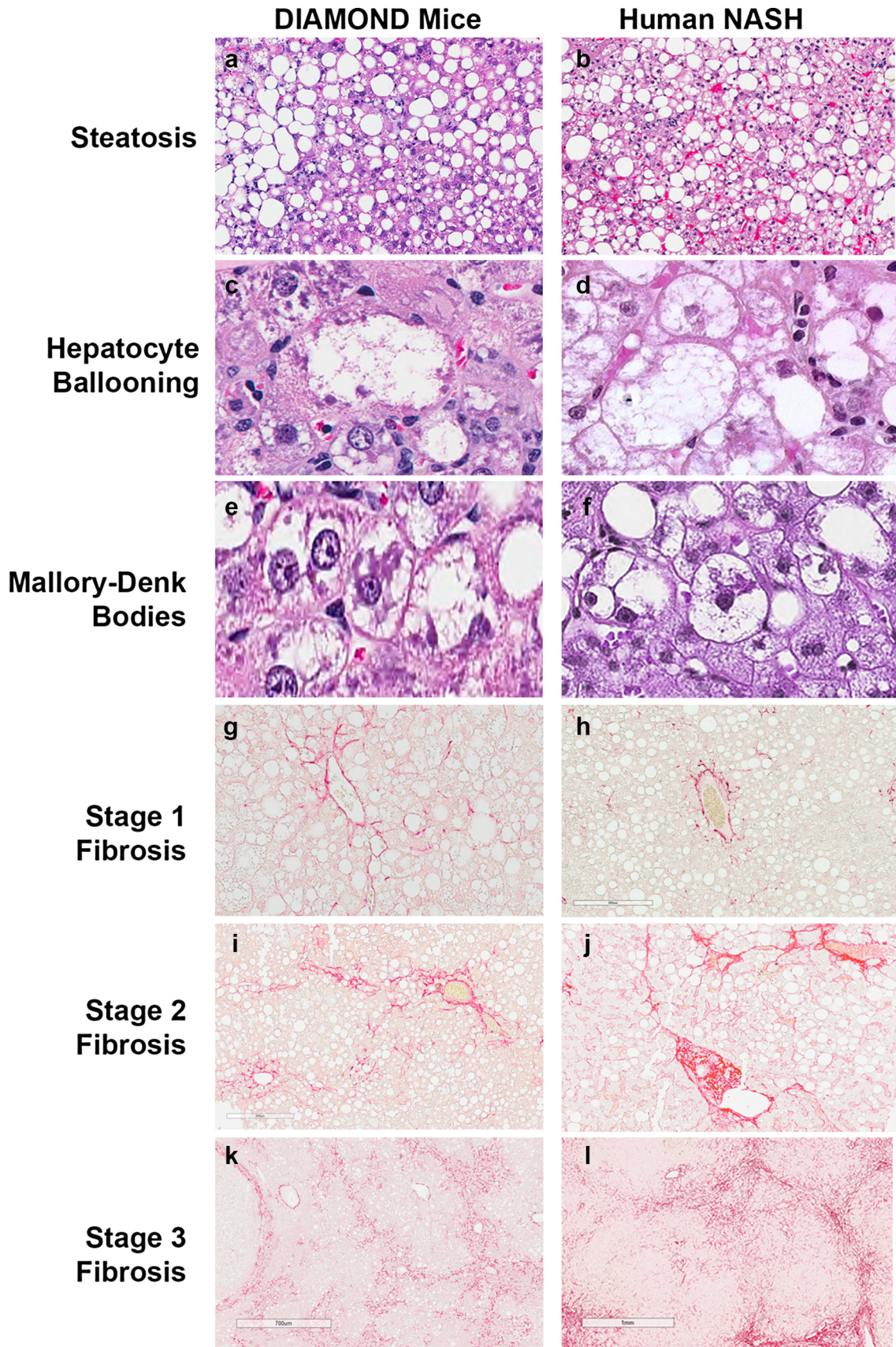
is low, especially since the animals do not develop insulin resistance and can actually lose weight. Other diets that include 2% cholesterol are simply unphysiologic. The ALIOS model and the high-fat diet with ad lib use of glucose/fructose described by Tetri et al. and others come closest to mimicking the average macronutrient composition of humans with NAFLD in the West [39, 40].

These limitations of existing models led us to work on a mouse model of NAFLD that, to a reasonable extent, answers to important elements of the criteria outlined above [14]. The discovery of this model was made by serendipity, but over the course of 10 years and extensive validation, we believe this model is perhaps the most rigorously evaluated and meets many of the quality metrics for an optimal representation of NAFLD. This Diet-Induced Animal Model Of NAFLD (DIAMOND) is an isogenic strain of a C57Bl/J and a S129/V mouse with 60% of genetic materials from the C57Bl/J. A key feature is that, while neither parent strain develops a phenotype, this mouse develops a consistent phenotype even after re-derivation in three different environments. Upon initiation of a high fat (40% calories from fat and 0.1% cholesterol) diet with ad lib glucose–fructose in drinking water, the mice sequentially develop fatty liver and then steatohepatitis with classical hepatocellular ballooning (Mallory–Denk bodies); and then progressive fibrosis resulting in bridging fibrosis and early nodule formation (Fig. 3). In some mice, this disease will progress to hepatocellular cancer, as in humans. Disease progression is generally accompanied by obesity, adipose tissue inflammation, elevated liver enzymes and activation of lipogenic,

oxidative stress, unfolded protein response, inflammatory and apoptotic pathways. At a transcriptomic and lipidomic level, it has a strong concordance with human NASH [14].

This model, which has close resemblance to human NAFLD with respect to the features noted above, provides an opportunity to evaluate the evolution of the disease and its progression to cirrhosis at a molecular level while longitudinal human transcriptomic data are awaited. Interrogation of the transcriptome identified early activation of metabolic pathways including PPAR-α and FXR, both known to be relevant in human disease. Interestingly, despite continuing on the same diet, with disease progression, there was a decrease in many metabolic pathways with the exception of FXR [14]. There was, however, progressive activation of inflammatory pathways. Furthermore, at a transcriptomic level, fibrogenic signaling peaked when the disease activity and inflammatory activation was the greatest. Together, these provide a molecular signature of disease evolution. Also, it demonstrated that, even though the histology showed steatohepatitis with some fibrosis, at different points in time, there was differential activation levels of metabolic, cell stress, apoptosis, inflammatory and fibrosis related pathways. If also true for the human state, this may provide insights into why only some patients respond to a given therapy.

As may be expected from any animal model, the DIAMOND also has its limitations, such as the prolonged period of time to develop steatosis (16 weeks) and bridging fibrosis (36 weeks) and the relatively high HCC frequency. Furthermore, and as a departure from the human disease state, there appears to be suppression of cholesterol

Fig. 4 Lipotoxic in vitro model comparing obeticholic acid and a TGFβR inhibitor (LY2109761). The effect of obeticholic acid (OCA, *dotted line*) alone, a TGFβR inhibitor (LY2109761, *dashed line*) alone, or a combination of both treatments (*solid line*) were evaluated on key signaling pathways associated with NASH in the Feaver et al. lipotoxic in vitro model [23]. The *axes* represent the average gene pathway fold change response relative to the lipotoxic vehicle (VEH) response (*gray*, set to equal 1) and a healthy control response (*light gray*, set to equal 0)
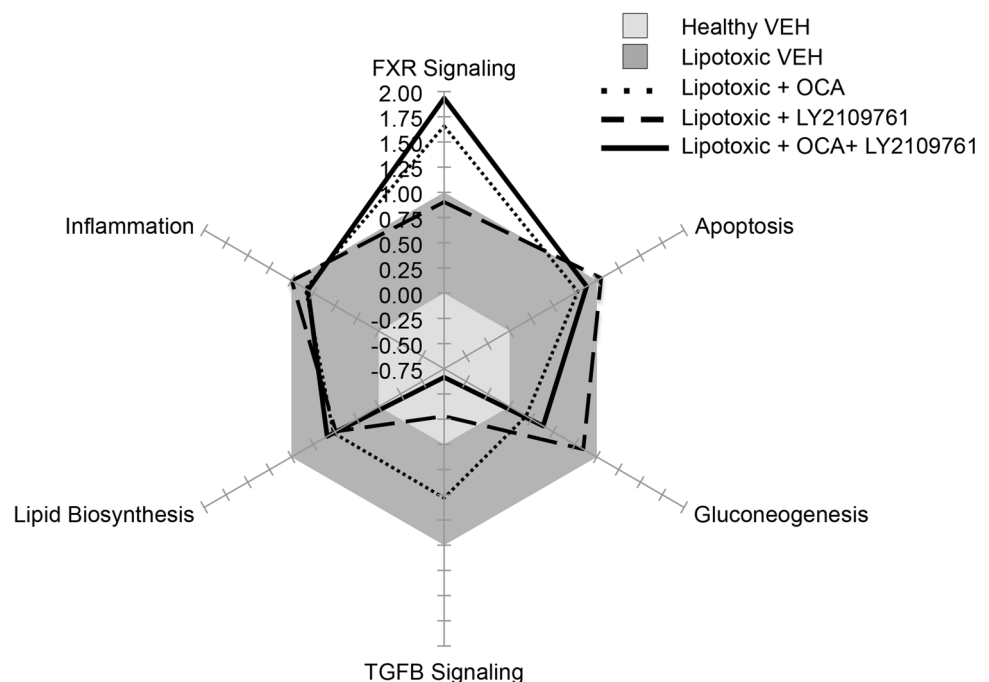
synthesis in the DIAMOND [14]. The true implication of this difference is yet unclear.

Translating from preclinical models to human therapeutics

Despite the high interest in NASH, there continues to be a lack of common therapeutic strategy, as evidenced by the over 30 different targets currently in development. This result likely stems from a lack of disease understanding and experimental models for identifying and validating targets. Preclinical models that show human translation can provide powerful platforms for gaining deeper mechanistic understanding of not only disease progression, but also therapeutic response mechanisms. The ability to develop in vitro and mouse models of NAFLD that recapitulate many features of human disease allow conceptualization of seamless drug development where the effects of one or more drugs on the key pathways relevant for disease progression in humans can be assessed. For example, recently a detailed description of FXR pharmacology has been described using such an approach in vitro [23, 27]. As a proof-of-concept, Fig. 4 illustrates the utility of in vitro systems for evaluating the impact of individual drugs or drug combinations (Feaver et al., unpublished data). The radar plot provides a visualization of how OCA, a TGFβR inhibitor (LY2109761), or the combination of both impact gene expression of specific pathways relative to vehicle controls in the lipotoxic system described by Feaver et al. above [31]. The impact of on-target signaling for each compound can be observed where OCA potently activated FXR signaling (Fig. 4, dotted line), while the TGFβR strongly inhibited TGFβ signaling (Fig. 4, dashed line). Used in combination (Fig. 4, solid line), an additive effect was observed yielding a therapeutic treatment that could simultaneously activate FXR and inhibit TGFβ. Thus, in vitro systems could aid in the discovery of drug/target combinations that may synergistically ameliorate key aspects of the NASH disease phenotype for future development.

There is currently an unmet need to assess the status of the hepatic transcriptome in ways that are easy to implement in clinical practice. There are ongoing efforts to reverse-engineer the transcriptomic profile to predict its impact on the metabolome and capture that signature in circulation. However, much more work needs to be done before these approaches become reality. It is hoped that, with continuing innovation and translation of scientific discovery in preclinical stages, as well as with the growing number of relevant animal models, the specific drivers of disease in a given patient will be readily identifiable and specific therapies can be brought to bear on these targets.

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