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ORIGINAL RESEARCH

Hepatocyte-Specific Deletion of Mouse Lamin A/C Leads to Male-Selective Steatohepatitis

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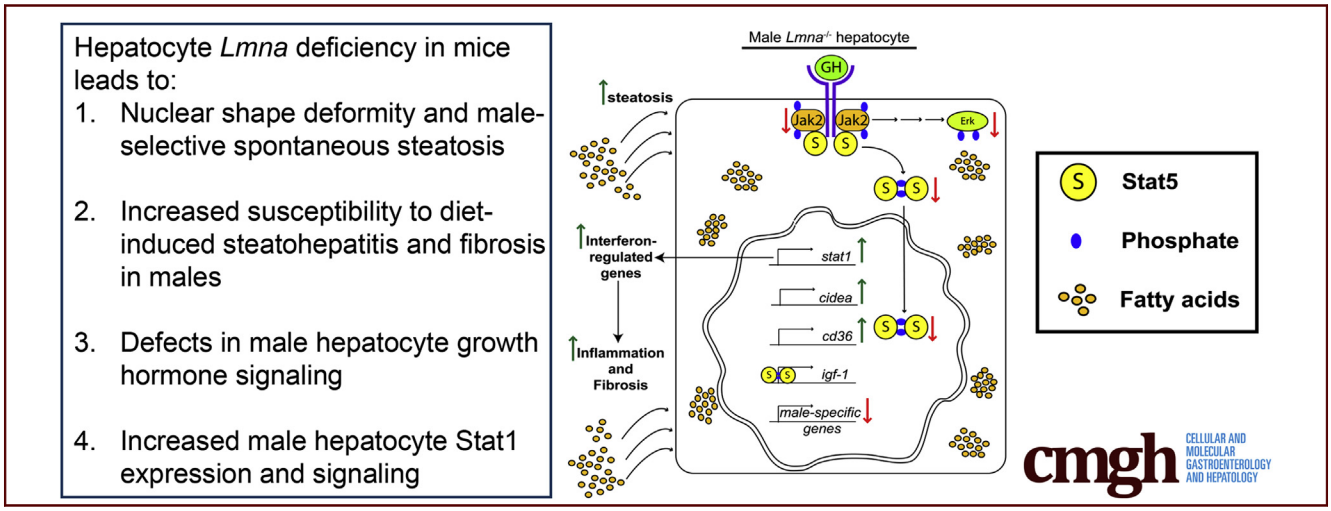

SUMMARY

The nuclear intermediate filament protein lamin A/C acts cell-autonomously in hepatocytes as a positive regulator of growth hormone signaling and maintains hepatocyte homeostasis and nuclear shape. Lamin A/C absence leads to male-selective fatty liver disease and predisposes to nonalcoholic steatohepatitis and fibrosis.

BACKGROUND & AIMS: Lamins are nuclear intermediate filament proteins that comprise the major components of the nuclear lamina. Mutations in *LMNA*, which encodes lamins A/C, cause laminopathies, including lipodystrophy, cardiomyopathy, and premature aging syndromes. However, the role of lamins in the liver is unknown, and it is unclear whether laminopathy-associated liver disease is caused by primary hepatocyte defects or systemic alterations.

METHODS: To address these questions, we generated mice carrying a hepatocyte-specific deletion of *Lmna* (knockout [KO] mice) and characterized the KO liver and primary hepatocyte phenotypes by immunoblotting, immunohistochemistry, microarray analysis, quantitative real-time polymerase chain reaction, and Oil Red O and Picrosirius red staining.

RESULTS: KO hepatocytes manifested abnormal nuclear morphology, and KO mice showed reduced body mass. KO mice developed spontaneous male-selective hepatosteatosis with increased susceptibility to high-fat diet–induced steatohepatitis and fibrosis. The hepatosteatosis was associated with up-regulated transcription of genes encoding lipid transporters, lipid biosynthetic enzymes, lipid droplet-associated proteins, and interferon-regulated genes. Hepatic *Lmna* deficiency led to enhanced signal transducer and activator of transcription 1 (Stat1) expression and blocked growth hormone–mediated Janus kinase 2 (Jak2), signal transducer and activator of transcription 5 (Stat5), and extracellular signal–regulated kinase (Erk) signaling.

CONCLUSIONS: Lamin A/C acts cell-autonomously to maintain hepatocyte homeostasis and nuclear shape and buffers against male-selective steatohepatitis by positively regulating growth hormone signaling and negatively regulating Stat1 expression. Lamins are potential genetic modifiers for predisposition to steatohepatitis and liver fibrosis. The microarray data can be found in the Gene Expression Omnibus repository (accession number: GSE93643). *(Cell Mol Gastroenterol Hepatol 2017;4:365–383; http://dx.doi.org/10.1016/j.jcmgh.2017.06.005)*

Keywords: Nonalcoholic Fatty Liver Disease; Laminopathy; Growth Hormone Signaling; Lipodystrophy; Fibrosis.

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Nuclear lamins are type V intermediate filament proteins that play important roles in maintaining nuclear stability and regulating gene expression, and they also serve as signaling scaffolds at the inner nuclear membrane.[1,2] Lamins are classified based on their isoelectric points and sequence homology as A- or B-type lamins. *LMNA* (in human beings; *Lmna* in mice) encodes the alternatively spliced lamins A and C, and lamins B1 and B2 are encoded by *LMNB1* and *LMNB2*, respectively.[3–5]

Lamin B1 is expressed ubiquitously, whereas lamin A/C is expressed postnatally in differentiated cells, including cardiac and skeletal muscle, adipocytes, and hepatocytes.[6–8] Lamin A/C binds to chromatin, lamina-associated proteins, and signaling mediators, such as extracellular signal–regulated kinase (Erk), c-Fos, retinoblastoma protein, and sterol regulatory element-binding protein-1c, to regulate gene expression and cell signaling.[9–11] Mutations in lamin A/C cause several human diseases, termed *laminopathies*, that can affect muscle (eg, Emery–Dreifuss muscular dystrophy), adipose tissue, liver (eg, Dunnigan familial partial lipodystrophy [FPLD2]), bone (eg, mandibuloacral dysplasia), or multiple tissues (eg, Hutchinson–Gilford progeria syndrome), depending on the site of the mutation.[12,13]

FPLD2 (OMIM 151660) is characterized by partial lipodystrophy and metabolic syndrome, including insulin resistance, glucose intolerance, and hypertriglyceridemia.[14–17] Hepatosteatosis occurs in many patients with FPLD2 and other lipodystrophies and may progress to steatohepatitis.[18,19]

The mechanism by which lipodystrophy-associated lamin mutations promote hepatic steatosis and metabolic syndrome is unclear. One possibility is that lamin A/C is dispensable in hepatocytes and, if so, the liver simply serves as a storage depot for excess fatty acids shunted into the circulation as a result of adipose tissue loss. Alternatively, FPLD2-associated lamin A/C variants in hepatocytes might directly promote fatty acid uptake and/or lipogenesis in the liver.

Previous characterization of laminopathy mouse models focused on adipose and muscle tissues. For example, characterization of mice transgenic for a *LMNA* mutation that causes FPLD2 focused primarily on its effect on adipose tissue, using the adipocyte protein 2 enhancer, which directs overexpression to adipose tissues, and showed a defect in adipose tissue renewal.[20] In addition, assessment of total-body *Lmna*-deficient mice showed normal fat distribution and metabolism in heterozygous mice. However, complete characterization of a potential FPLD2 phenotype in homozygous mice was prevented by severe muscular dystrophy that became lethal by 8 weeks of age.[21,22]

To characterize the role of lamin A/C specifically in the liver, we generated mice carrying hepatocyte-specific deletion of *Lmna*, using mice carrying a *Lmna* allele with loxP sites.[23] We found that hepatocyte lamin A/C deficiency induced spontaneous liver injury and steatosis and led to markedly increased susceptibility to steatohepatitis upon feeding a high-fat diet (HFD) in a male-specific manner that correlated with the up-regulation of genes encoding fatty acid binding proteins, lipogenic enzymes, and lipid transporters. Notably, lamin A/C deficiency disrupted hepatic growth hormone (GH)-receptor signaling through the Janus kinase 2 (Jak2), Erk, and signal transducer and activator of transcription (Stat) 5 axes, leading to dysregulation of downstream Stat5-dependent gene transcription, with up-regulated expression of Stat1 and downstream interferon-regulated genes. Hepatic GH signaling regulates liver metabolism and gender-specific hepatic gene expression in rodents and human beings.[24–30] Our findings show that lamins help maintain hepatocyte homeostasis cell-autonomously and regulate hepatic growth hormone signaling. Lamin A/C genetic variants potentially may contribute to the development and/or progression of nonalcoholic fatty liver disease (NAFLD), which is becoming a major global liver disease.[31]

Materials and Methods

Antibodies

The antibodies used were as follows: lamin A/C (H-110; Santa Cruz Biotechnology, Santa Cruz, CA); lamin B1 (ab16048; Abcam, Cambridge, UK); phosphorylated Stat5 (9314S), total Stat5 (9363S), phosphorylated Jak2 (3771S), total Erk (clone L34F12), phosphorylated Erk (4370P), total Akt (clone 40D4), total Jak2 (clone D2E12), phosphorylated Akt (4058S), total Stat1 (clone D1K9Y), and phosphorylated Stat1 (clones D4A7 and 58D6) (Cell Signaling Technology, Danvers, MA); pan-actin (Ab-5, 1:2500 dilution; ThermoFisher Scientific, Wayne, MI); and CD45 (clone 30-F11, 1:200 dilution; BD Biosciences, San Jose, CA). All antibodies were used at a 1:1000 dilution unless specified otherwise.

Mouse Experiments

Mouse experiments were performed in accordance with guidelines outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health, and with approval from the University of Michigan Institutional Animal Care and Use Committee. C57BL/6 mice with a floxed allele of *Lmna*[23] were crossed to C57BL/6 albumin-Cre mice[32] to generate C57BL/6 offspring with hepatocyte-specific deletion of exons 10 and 11 of *Lmna* and littermate control mice that either lacked the floxed *Lmna* allele or the albumin-Cre transgene. Both male and female mice were

Abbreviations used in this paper: **Erk, extracellular signal–regulated kinase; FPLD2, Dunnigan familial partial lipodystrophy; GH, growth hormone; Het, heterozygous; HFD, high-fat diet; Igf1, insulin-like growth factor 1; Jak2, Janus kinase 2; KO, knockout; % liver weight, liver percentage of body mass; NAFLD, nonalcoholic fatty liver disease; ND, normal diet; PBS, phosphate-buffered saline; qPCR, quantitative polymerase chain reaction; Stat, signal transducer and activator of transcription; WT, wild type.**

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