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BASIC AND TRANSLATIONAL—LIVER

Liver-Specific Deletion of Augmenter of Liver Regeneration Accelerates Development of Steatohepatitis and Hepatocellular Carcinoma in Mice

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See editorial on page 285.

BACKGROUND & AIMS: Augmenter of liver regeneration (ALR, encoded by *GFER*) is a widely distributed pleiotropic protein originally identified as a hepatic growth factor. However, little is known about its roles in hepatic physiology and pathology. We created mice with liver-specific deletion of ALR to study its function. **METHODS:** We developed mice with liver-specific deletion of ALR (ALR-L-KO) using the albumin-Cre/LoxP system. Liver tissues were collected from ALR-L-KO mice and $ALR^{floxed/floxed}$ mice (controls) and analyzed by histology, reverse-transcription polymerase chain reaction, immunohistochemistry, electron microscopy, and techniques to measure fibrosis and lipids. Liver tissues from patients with and without advanced liver disease were determined by immunoblot analysis. **RESULTS:** Two weeks after birth, livers of ALR-L-KO mice contained low levels of ALR and adenosine triphosphate (ATP); they had reduced mitochondrial respiratory function and increased oxidative stress, compared with livers from control mice, and had excessive steatosis, and hepatocyte apoptosis. Levels of carbamyl-palmitoyl transferase 1a and ATP synthase subunit ATP5G1 were reduced in livers of ALR-L-KO mice, indicating defects in mitochondrial fatty acid transport and ATP synthesis. Electron microscopy showed mitochondrial swelling with abnormalities in shapes and numbers of cristae. From weeks 2–4 after birth, levels of steatosis and apoptosis decreased in ALR-L-KO mice, and numbers of ALR-expressing cells increased, along with ATP levels. However, at weeks 4–8 after birth, livers became inflamed, with hepatocellular necrosis, ductular proliferation, and fibrosis; hepatocellular carcinoma developed by 1 year after birth in nearly 60% of the mice. Hepatic levels of ALR were also low in *ob/ob* mice and alcohol-fed mice with liver steatosis, compared with controls. Levels of ALR were lower in liver tissues from patients with advanced alcoholic liver disease and nonalcoholic steatohepatitis than in control liver tissues. **CONCLUSIONS:** We developed mice with liver-specific deletion of ALR, and showed that it is required for mitochondrial function and lipid homeostasis in the liver. ALR-L-KO mice provide a useful model for investigating the pathogenesis of steatohepatitis and its complications.

Keywords: Augmenter of Liver Regeneration; Mouse Model; NASH; ALD.

Augmenter of liver regeneration (ALR) (encoded by Gfer [growth factor ERV1 homolog of *Saccharomyces cerevisiae*]) protein was originally identified and purified from weanling and regenerating rat livers, and its gene cloned.[1–4] Expression of ALR in unmodified adult rat liver[5] suggested that it might also be functionally significant in this setting. ALR is known to function as a sulfhydryl oxidase,[6] cytochrome c reductase,[7,8] and inducer of cytosolic protein Fe/S maturation[9] and has additional effects such as suppression of hepatic natural killer cell (NK) cytotoxicity[10] and Kupffer cell activation.[11] Inhibition of ALR synthesis in cultured hepatocytes leads to mitochondrial dysfunction/damage and cell death.[12]

In order to dissect the roles that ALR plays in normal hepatocyte physiology, we generated liver-specific conditional ALR knockout (ALR-L-KO) mice. The data indicate that ALR is critical for mitochondrial function, lipid homeostasis, and cell survival, and abnormality in ALR gene function may be an important determinant in the development of steatohepatitis and its complications.

Abbreviations used in this paper: **ACACA, acetyl-CoA carboxylase; Adeno-Cre, adenovirus containing Cre recombinase; ALD, alcoholic liver disease; ALR, augmenter of liver regeneration; ASH, alcoholic steatohepatitis; ATP, adenosine triphosphate; CPT, carbamoyl palmitoyl transferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HPC, hepatic progenitor cell; KO, knockout; mRNA, messenger RNA; NASH, nonalcoholic steatohepatitis; NK, natural killer cell; ROS, reactive oxygen species; WT, wild type.**

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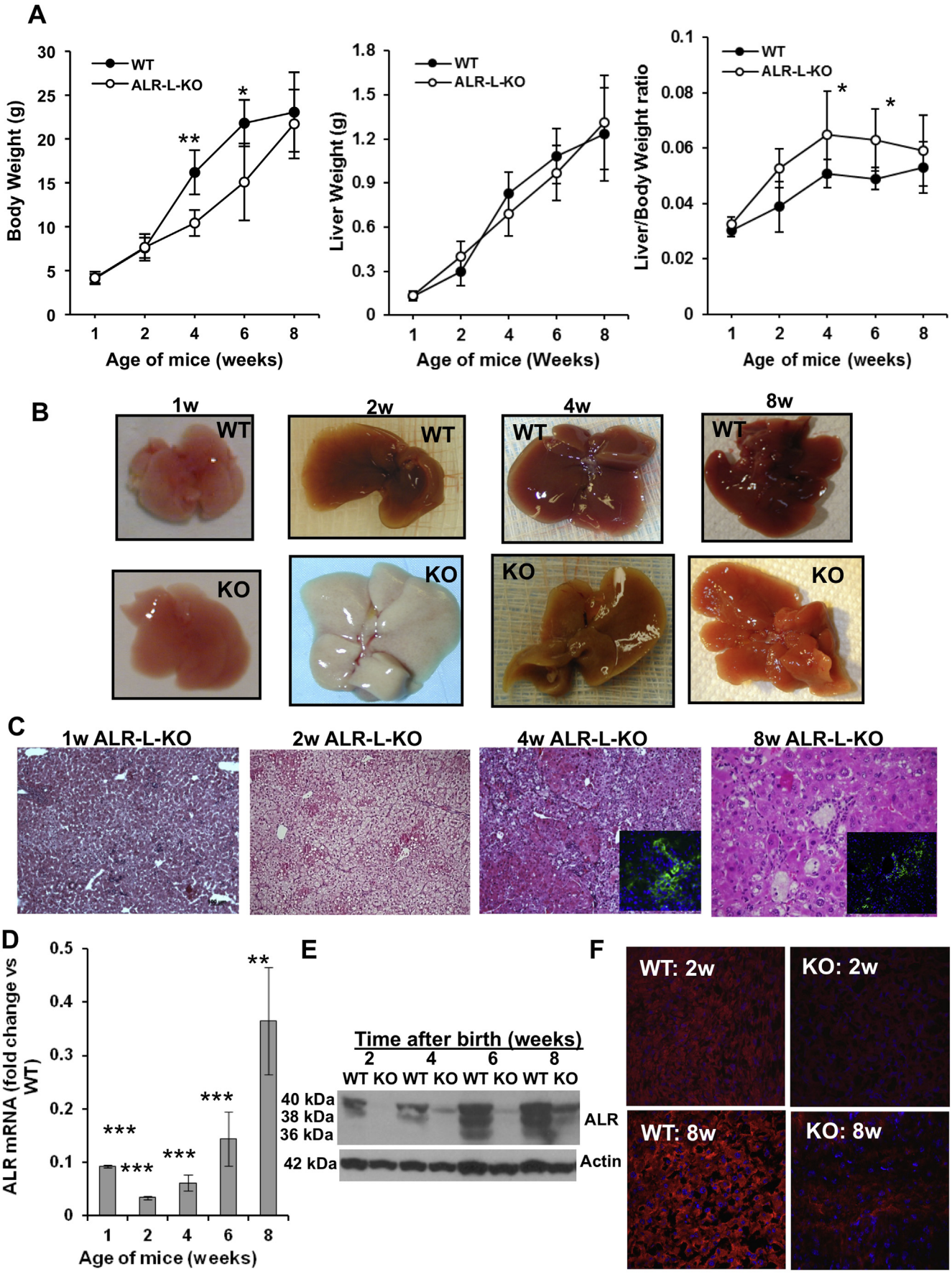
A
Body Weight (g)
WT
ALR-L-KO
Age of mice (weeks)
Liver Weight (g)
Age of mice (Weeks)
Liver/Body Weight ratio
Age of mice (weeks)
B
1w
2w
4w
8w
WT
KO
C
1w ALR-L-KO
2w ALR-L-KO
4w ALR-L-KO
8w ALR-L-KO
D
ALR mRNA (fold change vs WT)
Age of mice (weeks)
E
Time after birth (weeks)
2 4 6 8
WT KO WT KO WT KO WT KO
40 kDa
38 kDa
36 kDa
ALR
42 kDa
Actin
F
WT: 2w
KO: 2w
WT: 8w
KO: 8w