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# Augmenter of Liver Regeneration Links Mitochondrial Function to Steatohepatitis and Hepatocellular Carcinoma

**See "Liver-specific deletion of augmenter of liver regeneration accelerates development of steatohepatitis and hepatocellular carcinoma in mice," by Gandhi CR, Cahillet JR, Nalesnick MA, et al, on page 379.**

Steatohepatitis, an advanced stage of fatty liver disease that encompasses alcoholic and nonalcoholic steatohepatitis (NASH), can progress to liver cirrhosis and hepatocellular carcinoma (HCC). NASH is associated with obesity and type II diabetes, and the prevalence of NASH-induced HCC is a health concern worldwide. Therefore, a better understanding of the molecular players involved in NASH-HCC is of clinical relevance. Augmenter of liver regeneration (ALR encoded by *GFER*) is a hepatocyte growth factor originally identified in the regenerating liver of rats and dogs after partial hepatectomy.[1] ALR is mainly produced and secreted by hepatocytes and is the mammalian homolog of Erv, a sufhydryl oxidase of the mitochondrial intermembrane space, first described in yeasts. Along with Mia40, ALR functions in the import of nuclei-encoded Fe/S cluster proteins to the mitochondrial intermembrane space by an oxidation-dependent mechanism; consequently, ALR enhances oxidative phosphorylation capacity of liver mitochondria (Figure 1).[2–5] In this issue of *Gastroenterology*, Gandhi et al[6] provide a novel scenario in which ALR links mitochondrial function to steatohepatitis and HCC development, which illuminates our understanding for the role of ALR in liver pathophysiology.

To unravel the hepatic physiological function of ALR, Gandhi et al generated hepatocyte-specific, ALR-deleted mice (ALR-L-KO) by the albumin-Cre/LoxP system. Two-week-old ALR-L-KO mice exhibited low levels of adenosine triphosphate (ATP), reduced mitochondrial respiratory function, increased oxidative stress, reduced glutathione (GSH) levels, and increased Bax expression and caspase 3 activation, contributing to extensive hepatocyte apoptosis. Hepatic levels of carbamyl-palmitoyl-transferase 1a (CTP1a), ATP synthase subunit ATP5G1 and TFAM, a key activator of mitochondrial transcription, were reduced in ALR-L-KO mice, whereas electron microscopy showed mitochondrial swelling and abnormalities in the number and shape of cristae. Moreover, 2-week-old ALR-L-KO mice exhibited steatosis, increased liver triglycerides and cholesterol content and decreased expression of acetyl-coenzyme A carboxylase, sterol regulatory element-binding protein 1c, and peroxisome proliferator-activated receptor-$\alpha$, a regulator of mitochondrial fatty acid $\beta$-oxidation. These findings indicate that increased lipid accumulation was not owing to increased lipogenesis, but rather a consequence of impaired mitochondrial fatty acid $\beta$-oxidation, consistent with the reduced levels of CTP1a in ALR-L-KO mice. Thus, these data indicate a critical role for ALR in mitochondrial function, ATP synthesis, and fatty acid transport, which impacts on hepatocellular apoptosis and steatosis. The onset of liver injury and steatosis was not accompanied by inflammation at this early age as demonstrated by unchanged levels of inflammatory cytokines and minimal CD45 staining, although there was mild pericellular fibrosis and hepatic stellate cell activation ($\alpha$-smooth muscle actin [SMA] staining). These data indicate that, at 2 weeks after birth, the lack of ALR induces early stage steatohepatitis.

In general, deletion of target gene expression by the albumin–Cre-LoxP technology occurs progressively from 1 week after birth and takes several months to complete. The expression of ALR in the ALR-L-KO mice is minimal at 2 weeks of age. Quite intriguingly, liver ALR expression gradually increases in 4- and 8-week-old ALR-L-KO mice, although levels did not reach those found in wild-type mice, reflecting the re-expression of ALR in hepatocytes, the major cell source of ALR production and secretion. Native ALR is modified posttranscriptionally from a 22-kDa protein to 3 forms of 36-, 38-, and 40-kDa molecular weight.[7] The predominant form re-expressed in the ALR-L-KO mice was the 40-kDa isoform. The rebound in the 40-kDa form was sufficient to rescue mitochondrial impairments, and to normalize mitochondrial respiration, ATP levels, expression of TFAM and ATP5G1, and mitochondrial GSH depletion, which controls hepatocellular survival and sensitization to oxidative stress.[8,9] The improvement in mitochondrial function translated in reduced hepatic steatosis and normalization of triglycerides and cholesterol levels. Interestingly, TUNEL staining, caspase-3 activation, and hepatocyte apoptosis decreased again in 4- and 8-week-old ALR-L-KO mice, an

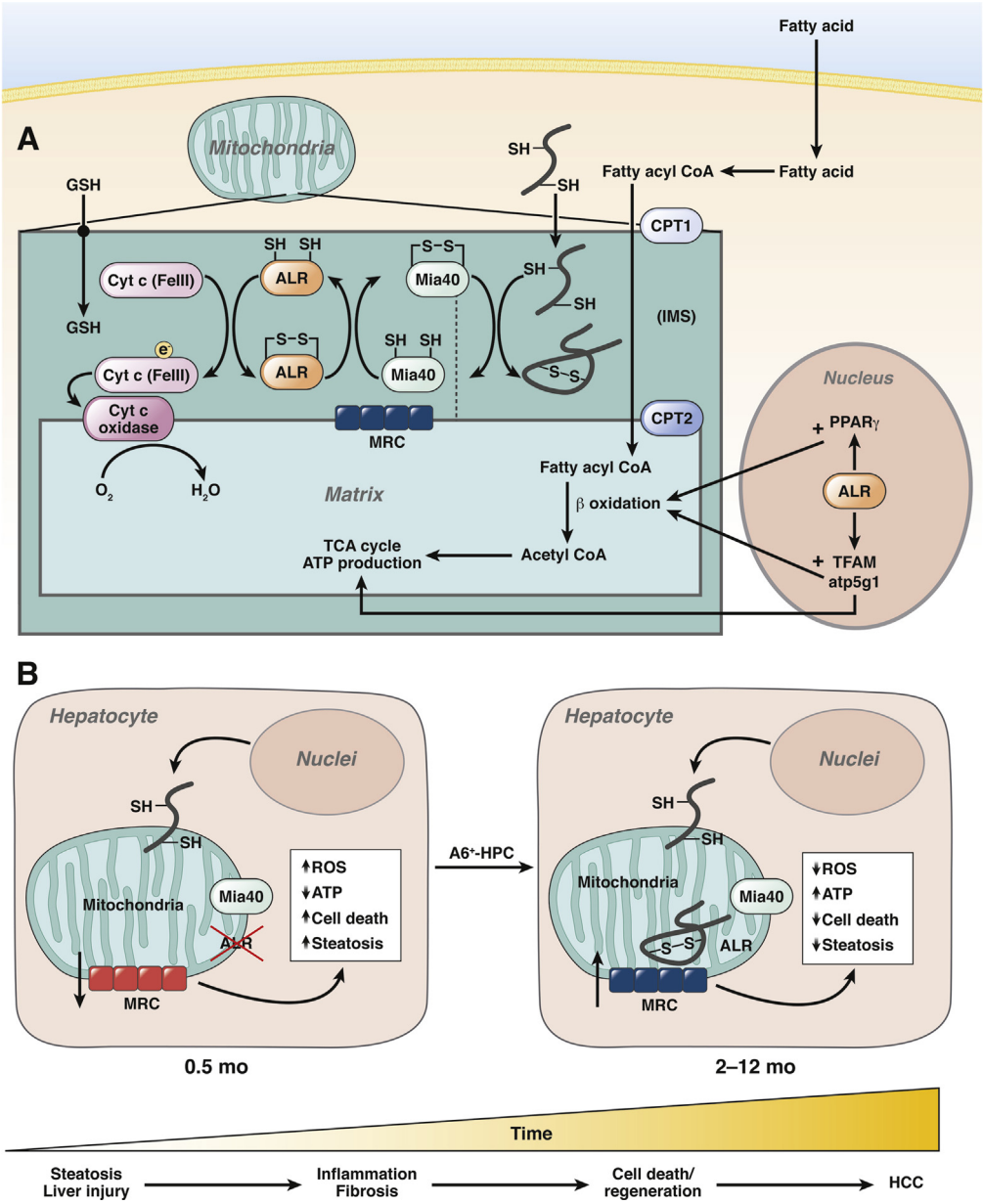


**Figure 1.** Role of augmenter of liver regeneration (ALR) in mitochondrial physiology and in the progression from steatohepatitis to hepatocellular carcinoma (HCC). (*A*) ALR plays a critical role in the transport of newly synthesized proteins across the mitochondrial outer membrane. In this function, Mia40 oxidizes incoming proteins and is reoxidized by ALR, which in turn transfers electrons to cytochrome c (Cyt c), which stimulates the conversion of oxygen to water by cytochrome c oxidase. Mia-40–mediated formation of disulfide bonds is accompanied by a proofreading step that involves glutathione (GSH). Mitochondrial GSH depletion, hence, reduces the oxidation rate of imported proteins, indicating that GSH improves oxidation efficiency. Moreover, ALR regulates fatty acid oxidation by regulating PPAR-$\gamma$ and expression of the fatty acid carrier CPT1a. (*B*) ALR-deleted mice (ALR-L-KO) as a model of nonalcoholic steatohepatitis (NASH)-HCC. Two weeks after birth, ALR-L-KO mice exhibit mitochondrial dysfunction, characterized by a defective mitochondrial respiration and oxygen consumption rate, particularly in state III, and presumably by impairing the mitochondrial respiratory chain assembly, which leads to excess reactive oxygen species (ROS), reduced adenosine triphosphate (ATP) levels, and hepatocellular apoptosis. Owing to the role of ALR in fatty acid $\beta$-oxidation, 2-week-old ALR-L-KO mice exhibit lipid accumulation and steatosis. During cycles of death and regeneration driven by $A6^{+}$-positive HPC dying hepatocytes are replaced by HPC-derived hepatocytes re-expressing ALR as detected in 8-week-old ALR-L-KO mice. This re-expression reduces ROS and cell death, increases ATP levels, and prevents steatosis. The reappearance of ALR in the context of inflammation at 12 months of age contributes to the protection of neoplastic hepatocytes fostering the development of HCC. CoA, coenzyme A.

outcome that was accompanied by increased Ki67 staining, indicative of proliferation, observed both in the parenchyma and in oval/biliary cells. These events paralleled an increase in portal and parenchymal inflammation reflected by enhanced CD45 staining, increased expression of proinflammatory cytokines, and expression of NKT cells (NKG2D)