Indian Hedgehog links obesity to development of hepatocellular carcinoma

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Abstract

Obesity increases the risk of hepatocellular carcinoma (HCC), but precise identification and characterization of druggable oncogenic pathways that contribute to the progression of NAFLD to HCC, and hence to the increased incidence and aggressiveness of HCC in obese individuals is lacking. In this regard, we demonstrate that the Indian Hedgehog (Ihh) signaling pathway is upregulated in the fatty livers of mice consuming a high fat diet, and furthermore sustained in HCC tumors specifically within the context of a NAFLD microenvironment. Using a diet-induced mouse model of HCC wherein only obese mice develop HCC, targeted ablation of hepatocyte-secreted Ihh results in a decreased tumor burden and lower grade tumors. Ihh activation regulates the transdifferentiation of ciliated stellate cells and proliferation of Epcam+ ductal cells to promote fibrosis. Mechanistically, increased expression of hitherto uncharacterized effectors of Hh pathway, namely Myc and Tgf-β2 is critical to the observed physiology. This pro-tumorigenic response is driven by increased expression of Wnt5a to effect a poorly-differentiated and invasive tumor phenotype. Wnt5a secreted from activated stellate cells act on Ror2-expressing hepatocytes. We further demonstrate that Wnt5a expression is also elevated in poorly-differentiated HCC cells, suggesting that these ligands are also able to function in an autocrine positive feedback manner to sustain poorlydifferentiated tumors. Taken together, our study provides a mechanistic understanding for how Ihh signaling promotes HCC tumorigenesis specifically in obese mice. We propose that therapeutic targeting of the Hh pathway offers benefit for patients with dietary / NAFLD-driven steatotic HCC.

Figure

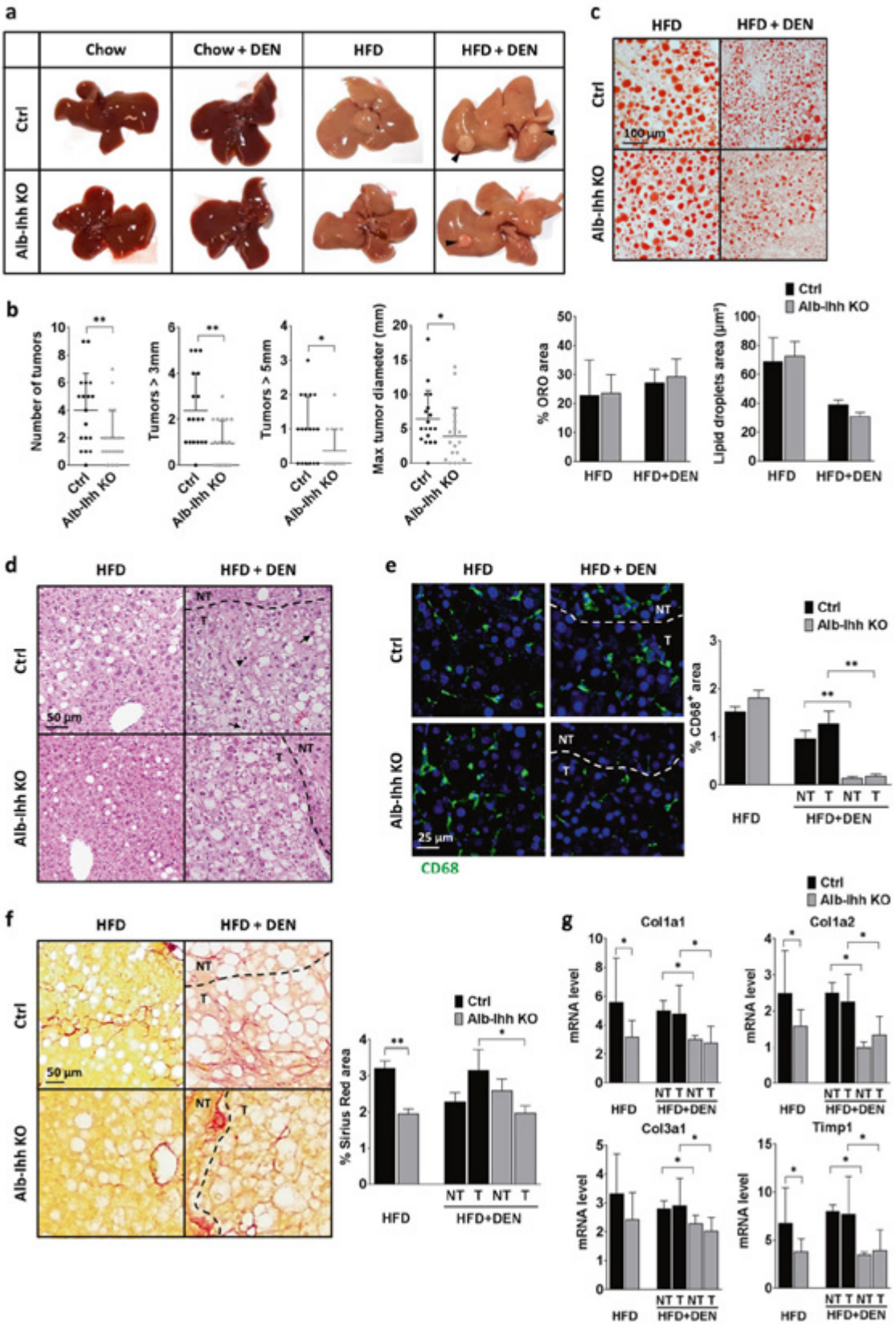
a
Chow
Chow + DEN
HFD
HFD + DEN
Ctrl
Alb-Ihh KO
c
HFD
HFD + DEN
Ctrl
Alb-Ihh KO
100 µm
b
Number of tumors
Tumors > 3mm
Tumors > 5mm
Max tumor diameter (mm)
Ctrl
Alb-Ihh KO
% ORO area
Lipid droplets area (µm²)
HFD
HFD+DEN
d
HFD
HFD + DEN
Ctrl
Alb-Ihh KO
NT
T
50 µm
e
HFD
HFD + DEN
Ctrl
Alb-Ihh KO
NT
T
25 µm
CD68
% CD68⁺ area
NT T NT T
HFD
HFD+DEN
f
HFD
HFD + DEN
Ctrl
Alb-Ihh KO
NT
T
50 µm
% Sirius Red area
g
Col1a1
Col1a2
Col3a1
Timp1
mRNA level
NT T NT T
HFD
HFD+DEN

Figure Legend: Hepatocyte-derived Ihh promotes obesity-driven HCC development. **a** Macroscopic images of livers of *Ihh*fl/+ Ctrl and *Alb-cre*; *Ihh*fl/fl KO mice fed chow or HFD with / without DEN administration (Ctrl – Chow, *n* = 2; Chow + DEN, *n* = 3; HFD, *n* = 11; HFD + DEN, *n* = 18. KO – Chow, *n* = 2; Chow + DEN, *n* = 7; HFD, *n* = 14; HFD + DEN, n = 18). Black arrowhead indicates visible tumor. **b** Tumor burden in livers of DEN-administered Ctrl and KO mice kept on HFD. **c** Oil Red O staining of livers of FD and HFD + DEN mice. The histograms indicate the mean area (%) that is positive for Oil Red O staining, and the mean size (μm2) of each lipid droplet. **d** H&E staining of livers of Ctrl and KO mice. Arrowhead indicates a Mallory body, arrows mark examples of cells different in size to non-tumor hepatocytes. **e** Immunostaining of steatotic livers (HFD) and HCCs (HFD + DEN) with CD68. The histogram indicates the mean area (%) that is positive for CD68 staining. A minimum of 4 random images each from at least 9 mice per treatment group was used for quantification. **f** Sirius Red staining of livers of HFD and HFD + DEN-treated mice. The histogram indicates the mean area (%) that is positive for Sirius Red staining. **g** Quantification of expression levels of fibrosis markers in livers of HFD and HFD + DEN-treated mice. NT, nontumor; T, tumor (HCC). Data, means ± SD. *$p < 0.05$; **$p < 0.01$