Online Supplement:

Methods

Cell culture and transfection assays

Human HCC cell lines HepG2 and Hep3B were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% vol/vol fetal bovine serum (FBS). As indicated, NF-κB inhibitor (sc-3080, Santa Cruz) was added to the medium 6 hours before harvesting cells at a concentration of 1 or 10 μg/ml. Small interfering RNA (siRNA) duplex oligo targeting *CTNNB1* mRNA or a nonspecific duplex oligo (Ambion, Inc., Austin, TX) at final concentration of 50 nM, or *CTNNB1* (ISIS102708) or control antisense (ISIS Pharmaceuticals Inc., California) at a concentration of 50 nM, were transfected using Lipofectamine (Invitrogen, Carlsbad, CA). Cells were harvested at 24, 48 or 72 hours for protein extraction and additional analysis. All experiments were done in triplicates and representative results are reported.

For the thymidine incorporation assay, thymidine [^{3}H] (2.5 μCi/ml) was added to medium, 6 hrs after the transfection with control or β-catenin siRNA (50 nM) and β-catenin or control antisense (50 nM). Cells were next treated with Gleevec (10 μM) 24 hrs after the transfection. Cells were harvested after 24 hrs and assayed for thymidine incorporation as described [1].

Histology, IHC, IP and WB

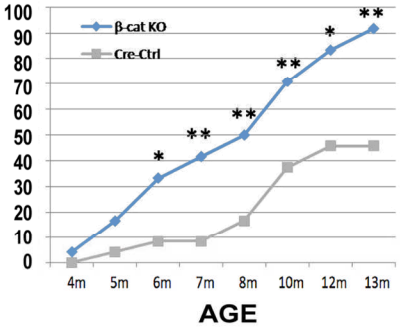
Livers were fixed in 10% neutral formalin for 48 hours. 4 μm thick paraffin sections were stained with H&E for histology and quantification of hepatic lesions including foci, adenoma, and carcinomas. The tumor numbers were assessed and area occupied by tumors was measured using ImageJ software (NIH). IHC was performed as described previously [2]. Primary antibodies were against β-catenin (Santa Cruz, 1:50 dilution), PCNA (Santa Cruz, 1:200 dilution), phospho-Akt (Thr308) (Santa Cruz, 1:400 dilution), PDGFRα (Santa Cruz, 1:200 dilution) and AFP (Santa Cruz, 1:200 dilution).

Apoptotic nuclei were detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining using ApopTag Peroxidase kit (Intergen Company, Purchase, NY). The positive cells were counted in 10 low-power fields (50X) in at least 3 independent knockout and control livers.

IP and WB were performed as described previously [3, 4]. All western blots were done using three different samples for same timepoint and representative data is included in the results. To be able to directly compare and contrast various KO and WT liver samples between various gels probed for the same antibody, two common samples were included in each gel, which assisted in determining equivalent exposure times of the autoradiographs. Antibodies were against CD14, CD45, PDGFRα, PDGFRβ, Akt, p-Akt (Thr 308), p-Akt (Ser 473), C-Myc, Cyclin-D1, GAPDH, Actin, GS, GSK3β, p-GSK3β (Ser9), Met, HGF, PCNA, p-Tyr (PY20), PIK3CA, NF-κB p65 and p50, Fas and TRAF1 (Santa Cruz); β-catenin (BD biosciences); EGFR, PDK1, p-PDK1 (Ser 241), p-PIK3CA (Tyr458/Tyr199) (Cell Signaling); and Cox-2 (Cayman chemicals).

REFERENCES:

1. Zeng G, Apte U, Cieply B, Singh S, Monga SP. siRNA-mediated beta-catenin knockdown in human hepatoma cells results in decreased growth and survival. Neoplasia 2007;9:951-9.
2. Tan X, Yuan Y, Zeng G, Apte U, Thompson MD, Cieply B, Stolz DB, Michalopoulos GK, Kaestner KH, Monga SP. Beta-catenin deletion in hepatoblasts disrupts hepatic morphogenesis and survival during mouse development. Hepatology 2008;47:1667-79.
3. Micsenyi A, Tan X, Sneddon T, Luo JH, Michalopoulos GK, Monga SP. Beta-catenin is temporally regulated during normal liver development. Gastroenterology 2004;126:1134-46.
4. Tan X, Behari J, Cieply B, Michalopoulos GK, Monga SP. Conditional deletion of beta-catenin reveals its role in liver growth and regeneration. Gastroenterology 2006;131:1561-72.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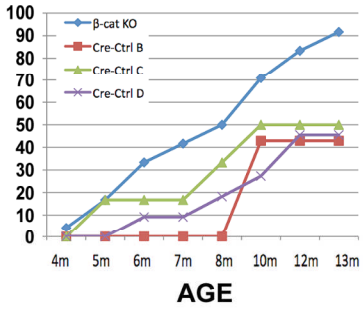
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