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Tbx3 is a downstream target of the Wnt/ß-catenin pathway and a critical mediator of ß-catenin survival functions in liver cancer

Legends to Supplementary Figures

Supplementary Figure 1

Distribution of GS expression levels in 55 human HCCs (left panel) and in 24 hepatoblastomas (right panel) according to ß-catenin status. Unlog values derived from Affymetrix microarray data are expressed in arbitrary units. In HCCs, mean expression values were significantly higher in tumors expressing mutant ß-catenin (MUT) and in a group of 6 cases with wt ß-catenin (WT, Wnt on) than in other tumors carrying wt ß-catenin (WT, Wnt off) and normal liver (NL) ($P < 0.0001$). Increase in GS expression in hepatoblastomas carrying mutant ß-catenin compared to other tumors and normal liver levels is also significant ($P < 0.011$). These data were validated by real time PCR.

Supplementary Figure 2

HuH7 hepatoma cells were infected with the lentiviral vector pTRIP-ß-catT41A or pTRIP-GFP as described previously (1). Cellular RNA was extracted at 48 h post-infection and analyzed by semi-quantitative RT-PCR, showing a marked increase of Tbx3 expression in cells expressing mutant ß-catenin. We verified that the myc-tagged ß-cateninT41A mutant was strongly expressed, and that other Wnt target genes such as MMP7 and IL-8 were upregulated together with Tbx3.

REFERENCE:

1. Levy L, Neuveut C, Renard CA, et al. Transcriptional Activation of Interleukin-8 by beta -Catenin-Tcf4. J Biol Chem 2002;277:42386-93

Supplementary Figure 3

The Tbx3 promoter is responsive to ß-catenin in 293 cells.

The ability of mutated, constitutively stabilized ß-catenin to activate the Tbx3 promoter was investigated in 293 cells, in which no alteration of the Wnt pathway has been evidenced. Cells were transfected with 0.5 µg of L-Tbx3-LUC and 1µg of T41A ß-catenin, along with 1 µg of Tcf4 or 2 µg of ΔNTcf4 vector. Luciferase activity was measured 48 h post transfection.

Supplementary Figure 4

Apoptotic effect of siRNA-mediated depletion of ß-catenin or Tbx3 in U2OS cells.

Cells were co-transfected with 2 µg of ß-cateninT41A vector or empty vector and 100 nM of siRNA for ß-catenin, Tbx3 or luciferase as indicated. After 24 h, cells were treated or not with 500 ng/ml doxorubicin and harvested 24 h later. Total protein extracts were analyzed by Western blot with anti-PARP antibody, and apoptosis was measured by quantification of cleaved/uncleaved (C/NC) PARP ratios using Syngene Photo Image System.

Note that Tbx3 siRNA, as well as ß-catenin siRNA, have apoptotic properties both in the presence and in the absence of doxorubicin treatment.