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Epigenetic inactivation of transforming growth factor-β1 target gene HEYL, a novel tumor suppressor, is involved in the P53-induced apoptotic pathway in hepatocellular carcinoma.

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Author information

Abstract

AIM: Hairy/enhancer-of-split related with YRPW motif-like (**HEYL**) protein was first identified as a transcriptional repressor. It is a downstream **gene** of the Notch and **transforming growth factor**-β pathways. Little is known about its role in the pathogenesis of **hepatocellular carcinoma** (HCC).

METHODS: Eighty surgically resected paired HCC and adjacent non-cancerous tissues were analyzed for **HEYL** expression by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC). HCC cells were transfected with pHEYL-EGFP vector to overexpress the **HEYL gene** or infected with specific shHEYL lentiviral vector to silence **HEYL gene** expression. **HEYL** expressional analysis and functional characterization were assessed by 3-(4 5-dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide assays, flow cytometry, RT-qPCR, western blotting and methylation-specific PCR.

RESULTS: We determined that **HEYL** expression was inactivated in more than 75% of HCC. In addition, overexpression of **HEYL** in SK-Hep 1 cells caused apoptosis by the cleavage of caspase 3 and poly (ADP-ribose) polymerase. We discovered that **HEYL** apoptosis was preceded by serine 15 phosphorylation and accumulation of P53. Molecular analysis revealed that **HEYL** overexpression led to increased p16, p19, p21, p27 and Bad protein expression, and reduced c-Myc, Bcl-2 and Cyclin B1 expression. **Epigenetic** silencing of **HEYL** expression by DNA hypermethylation in HCC directly correlated with loss of **HEYL** expression in HCC.

CONCLUSION: HEYL is frequently downregulated by promoter methylation in HCC. **HEYL** may be a **tumor suppressor** of liver carcinogenesis through upregulation of P53 **gene** expression and activation of P53-mediated apoptosis.

KEYWORDS: HEYL; P53; apoptosis; **hepatocellular carcinoma**; promoter hypermethylation

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