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Title	GREB1 induced by Wnt signaling promotes development of hepatoblastoma by suppressing TGFβ signaling
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Citation	大阪大学, 2020, 博士論文
Version Type	
URL	https://hdl.handle.net/11094/76248
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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

肝芽腫では約90％の高頻度でβ-カテニンに遺伝子変異が生じて、Wntシグナルが活性化される。肝芽腫において、Wntシグナルにより誘導される下流遺伝子を探索して、GREB1（Growth Regulation By Estrogen In Breast Cancer 1）を同定し、肝芽腫の約90％の患者においてGREB1が過剰に発現することを見出した。GREB1を発現する肝芽腫細胞でGREB1の発現を抑制すると、細胞の増殖が阻害され、細胞死が誘導された。また、GREB1は、核内でTGFβシグナルの構成転写因子であるSmad2/3と結合し、Smad2/3と転写共役因子p300の相互作用を阻害した。その結果、TGFβシグナルの細胞増殖抑制作用が阻害され、肝芽腫の増殖が促進するという分子メカニズムが解明された。さらに、本研究で開発したGREB1に対する修飾型アンチセンス核酸が肝芽腫の形成を阻害する効果を有したことから、今回の発見は肝芽腫の新たな分子標的治療薬の開発に貢献することが期待される。

以上により本論文は学位論文に値すると考えられる

論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	山道 拓
論文題名 Title	GREB1 induced by Wnt signaling promotes development of hepatoblastoma by suppressing TGFβ signaling (Wntシグナルの標的遺伝子であるGREB1はTGFβシグナルを抑制することで肝芽腫の腫瘍形成を促進する)

論文内容の要旨

〔目　的(Purpose)〕

Hepatoblastoma (HB) is predominant hepatic neoplasm in infants and young children, with an incidence of a few cases per 1 million children. HB has the highest association with mutational activation of β-catenin. However, the underlying mechanism by which Wnt/β-catenin signaling induces HB tumor formation is unknown. In this study, we identified growth regulation by estrogen in breast cancer 1 (GREB1) as a previously unknown target gene of Wnt/β-catenin signaling and analyzed its function and mechanism in HB development.

〔方法 (Methods)〕

RNA-sequencing analyses were performed in HepG2 cells with β-catenin siRNA to screen novel downstream target genes of Wnt/β-catenin signaling. Expression of GREB1 was examined immunohistochemically in HB tissues (n=11) and using a public dataset of patients with HB. We investigated the involvement of GREB1 in HB cell proliferation and survival by using siRNA or CRISPR-Cas9 system. Downstream mechanisms regulating cell proliferation by GREB1 were analyzed by molecular biological methods. Using our newly established model mice harboring HB-like tumor induced by forced expression of β-catenin, YAP, and c-Met (BYM), GREB1 expression and the effects of shRNA-mediated GREB1 knockdown on liver tumor formation were investigated. To examine the feasibility of GREB1 as a therapeutic target, we synthesized an amido-bridged nucleic acid (AmNA)-modified antisense oligonucleotides (ASOs) that target human GREB1 and injected ASOs into mice with HepG2 induced liver tumors.

〔成績(Results)〕

We identified GREB1 as a downstream target of Wnt/β-catenin signaling in human HB. GREB1 was overexpressed in the nucleus of tumor lesions of 10/11 HB tissues (90.9%). Analyses using a public dataset of HB patients showed that the region-specific expression pattern of GREB1 in HB tissue tended to be positively correlated with the accumulation of β-catenin in consecutive sections. In addition, there was a significant positive correlation between expression levels of GREB1 and those of target genes of Wnt/β-catenin signaling, such as Axin2, DKK1, NKD1, and glutamine synthetase (GS) in HB. GREB1 depletion decreased HepG2 cell proliferation and survival. GREB1 knockdown also decreased protein expression of cell cycle markers, including cyclinA, cyclinB, and phosphorylated histoneH3. The public dataset revealed a positive correlation between mRNA expression levels of GREB1 and MKI67, as well as GMNN and PCNA, which are involved in cell cycle progression. GREB1 interacted with Smad2/3 and competed with p300, resulting in the inhibition of TGFβ-dependent cytostasis. In our conditions of hydrodynamics transfection, the combination of ΔNβ-catenin, YAPS127A, and c-Met induced large and multiple mouse liver nodules throughout the liver surface. The nodules upregulated GREB1 expression along with HB specific markers and GREB1 knockdown by shRNA in BYM mice suppressed expression of HB marker genes and tumor formation. GREB1 depletion by ASOs suppressed HepG2-derived liver tumor formation, decreased the number of Ki-67-positive cells and also increased the number of apoptotic tumor cells. GREB1 expression was inhibited and PAI-1 gene expression tended to be increased by GREB1 ASOs in HB liver tumors.

〔総　括(Conclusion)〕

These findings uncover a previously unrecognized involvement of Wnt/β-catenin-GREB1-Smads axis in HB pathogenesis, suggesting that GREB1 represents a potential therapeutic target in β-catenin-driven HB. GREB1 ASOs may be a good candidate for molecularly targeted therapy for HB.