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厚生労働科学研究費補助金

創薬基盤推進研究事業

肝硬変・肝がん治療への応用を目的とした β-catenin依存性シグナルによる肝代謝機能制御機構の基礎的研究

（H22-政策創薬-一般-011）

平成23年度　総括研究報告書

研究代表者　関根　茂樹

平成 24（2012）年　5 月

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別添3

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肝硬変・肝がん治療への応用を目的とした β-catenin依存性シグナルによる肝代謝機能制御機構の基礎的研究

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研究代表者　関根　茂樹　　国立がん研究センター研究所

研究要旨

肝臓は多彩な代謝機能を担う臓器であり、これらの機能は多くのシグナル経路によって複雑に制御されている。申請者はこれまで主に肝細胞特異的β-cateninノックアウトマウスを用いてβ-cateninシグナルによる肝代謝機能制御を解析し、この経路が肝予備能に影響を与えている事、そして外来異物を含む種々の物質の代謝を制御している事が明らかにしつつある。さらに、β-cateninは肝小葉内で領域特異的な遺伝子発現制御に重要な働きを果たしている事から、肝小葉構造の破壊を伴う肝硬変組織においては、このシグナル経路の異常の存在が予想される。これまで肝硬変は主に肝実質細胞の減少により機能不全を来す疾患と考えられてきたが、肝小葉構築の破壊に伴うβ-cateninシグナル異常が相乗的に関わっているとすれば、このシグナルの制御による肝機能改善の可能性が期待される。一方、このシグナル経路は肝発がんにおいても重要な役割を果たしている事が報告されている。約30%の肝細胞がんにおいてβ-cateninをコードする*CTNNB1*遺伝子変異が見られ、その頻度は特にC型肝炎患者に発生する腫瘍で高率である事が知られている。このシグナル経路が代謝機能に与える役割を考慮すると、これらの腫瘍発生の背景となっているC型肝炎および、その発がん過程においてβ-cateninシグナルの異常が関わっている事、さらにβ-catenin遺伝子変異陽性の腫瘍は特徴的な代謝特性を有している事が予想される。本研究では、肝臓におけるβ-cateninシグナルの生理的機能と肝硬変、肝細胞がんにおける異常を総合的に明らかにし、このシグナル経路の制御を通じた肝機能の改善の可能性を探る。また、肝細胞がんにおけるβ-catenin遺伝子異常に伴う腫瘍特性を明らかにし、これを診断および個別改良への応用の可能性を検討する。

研究分担者

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研究所分子病理分野
ユニット長

A．研究目的

ウイルス性肝炎の終末像である肝硬変や肝細胞がんの治療については未だ対症療法や外科切除等が治療の重要な部分を占めており、新たな薬物治療の開発が望まれる。β-cateninは肝小葉内で領域特異的な遺伝子発現制御に重要な働きを果たしており、この制御を通じて種々の代謝機能の制御に関わっている事が明らかにされつつある。さらに、

30-40%の肝細胞がんではβ-cateninの変異の存在が知られている。本研究では肝細胞の代謝機能調節に関わるβ-cateninの役割に注目し、その肝硬変、肝細胞がんにおけるβ-cateninシグナル異常の病態への関わりを明らかにし、このシグナル制御を通じた、肝硬変における肝代謝能の改善や肝細胞がんの診断・個別化治療への応用のための基礎を築く事を目標とする。

B．研究方法

本研究では、マウスモデルの解析によりβ-catenin の生体肝における生理的機能を明らかにし、これを基盤として、肝硬変および肝細胞がん臨床検体の解析によるヒト疾患でのβ-catenin シグナル異常の病態への関わりを検索する。

遺伝子改変マウスとしては、肝細胞特異的β-catenin ノックアウトマウスおよびβ-catenin 依存性転写活性レポーターマウスを用いる。ノックアウトマウスは主に生体肝におけるβ-catenin による遺伝子発現制御と機能の解析に用いる。レポーターマウスは種々の病態における肝組織中のβ-catenin シグナルの変化の解析に用いる。

ヒト肝硬変および肝がん臨床検体におけるβ-catenin 下流遺伝子の発現を定量PCR および免疫組織化学染色で検索する。これに用いる肝硬変および肝がん臨床検体は既にcDNA 合成済みのものが約50症例、組織化学染色用の検体が約70症例確保されており、肝細胞がん検体についてはβ-cateninの遺伝子変異解析を終えたところである。これらの蒐集された検体のうち、約80%はウイルス性肝炎患者から得られたものである。

マウスモデルや臨床検体で明らかになったβ-catenin による下流遺伝子発現制御機構を明らかにする目的で、必要に応じて培養細胞を用いてin vitro の実験を行う。

（倫理面への配慮）

肝がん臨床検体の解析に関しては既に国立がんセンター倫理審査委員会の審査と承認を受けている。実施に当たっては「疫学研究に関する倫理指針」に基づいて行う。研究対象となる臨床検体については国立がんセンター中央病院での肝切除材料のうち、摘出標本のがん研究への利用に関して文書による患者の同意が得られている検体のみを用いる。解析にあたっては適切に匿名化を行い、患者情報の取り扱いに留意する。

動物実験に関しては既に国立がんセンター動物実験倫理委員会の審査を受け、認可を受けている。実施に当たっては「国立がんセンターにおける動物実験に関する指針」ならびに関連規定を遵守する。

C．研究結果

平成22年度までの研究において、主に肝細胞特異的β-cateninノックアウトマウスの解析からβ-catenin依存性シグナルが肝臓における胆汁酸代謝制御に重要な役割を果たしている事が明らかになった（文献1)。特にこのノックアウトマウスにおいてはSlco1b2等の血中から肝細胞への胆汁酸取り込みに関わるトランスポーターの著明な発現低下が認められた。この所見から、ヒトにおいてSlco1b2に最も相同性が高いとかんがえられている２つのトランスポーター、SLCO1B1, SLCO1B3の発現および*CTNNB1*遺伝子変異との相関をヒト肝細胞がんにおいて検索したところ、*CTNNB1*遺伝子変異を有する肝細胞がんにおいてSLCO1B3の高い発現を認めた。一方、*CTNNB1*遺伝子変異が陰性の肝細胞がんにおいては、その発現がほとんど消失していた（文献2)。

SLCO1B3は近年、画像診断において広く用いられるようになっている肝細胞特異的MRI造影剤であるGd-EOB-DTPAの主要なトランスポーターである事が報告されている。このため、*CTNNB1*遺伝子の変異とこの造影剤によるMRI信号増強効果の相関を調べた。この結果、*CTNNB1*遺伝子変異を有する肝細胞がんは、Gd-EOB-DTPAにより、有意に造影効果が認められることが明らかになった。

さらにこの所見は*CTNNB1*変異とMRI画像所見の相関を示すのみならず、β-catenin依存性シグナルと、この造影効果の相関を示唆するものでもあると考え、β-catenin依存性シグナルの異常が報告されており、画像診断上、肝細胞がんとの鑑別が困難である肝限局性結節性過形成の画像所見を検討したところ、β-catenin依存性シグナルの異常にともなって、造影剤の特徴的な取り込み像を示し、この所見が肝臓MRI診断において広く応用可能な知見である可能性が示唆された（文献3)。

これらSLCO1B3を含めたβ-catenin依存性シグナルの制御による下流遺伝子の調節に関わる機能を明らかにするため、β-cateninのプロモーター結合の網羅的解析を開始した。

D．考察

β-catenin依存性シグナルによる胆汁酸代謝制御の調節機能のマウスモデルにおける検索に基づいて、胆汁酸取り込みに関わるトランスポーターを同定し、そのヒト肝細胞がんにおける肝細胞特異的MRI造影剤の取り込みとの関連を明らかにした。

この所見は*CTNNB1*変異とMRI画像所見の相関を示すのみならず、β-catenin依存性シグナルと、この造影効果の相関を示すものとかんがえられ、β-catenin依存性シグナルの異常に関する知見が肝空間占拠性病変の画像診断に応用可能であることを示唆するものと考えられる。

一方、他の研究グループから、β-catenin依存性シグナルが肝臓における胆汁酸代謝制御はβ-cateninによる胆汁酸排泄能の低下による二次的なものであるとの報告がなされている。これは我々の得ている結果と矛盾するものであるが、その正否を含め、β-catenin依存性シグナルの肝臓における総体を明らかにするため、より網羅的な解析を行う必要があると考えられる。

E．結論

臨床例の解析から、ヒト肝細胞がんにおいてSLCO1B3は*CTNNB1*変異に相関しており、β-catenin依存性シグナルはヒト肝臓において、胆汁酸トランスポーターSLCO1B3の発現を誘導していると考えられる。この知見から、これまでに蓄積された種々の肝疾患におけるWnt/β-cateninシグナルの異常に関連する知見をGd-EOB-DTPAによる造影効果の理解へ応用が可能と考えられる。

今後、β-catenin依存性シグナルによる生理機能の制御をより総体的に理解するため、その下流遺伝子調節機構の網羅的な解析を進める。

F．健康危険情報

該当なし

G．研究発表

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2. 学会発表

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H．知的財産権の出願・登録状況

（予定を含む）

1. 特許取得　該当なし
2. 実用新案登録　該当なし
3. その他　該当なし

別添4

厚生労働科学研究費補助金（創薬基盤推進研究事業）

分担研究報告書

肝硬変・肝がん治療への応用を目的とした β-catenin依存性シグナルによる肝代謝機能制御機構の基礎的研究

（H22-政策創薬--一般-011）

研究分担者　関根　茂樹　　国立がん研究センター研究所

研究要旨

肝臓は多彩な代謝機能を担う臓器であり、これらの機能は多くのシグナル経路によって複雑に制御されている。申請者はこれまで主に肝細胞特異的β-cateninノックアウトマウスを用いてβ-cateninシグナルによる肝代謝機能制御を解析し、この経路が肝予備能に影響を与えている事、そして外来異物を含む種々の物質の代謝を制御している事が明らかにしつつある。さらに、β-cateninは肝小葉内で領域特異的な遺伝子発現制御に重要な働きを果たしている事から、肝小葉構造の破壊を伴う肝硬変組織においては、このシグナル経路の異常の存在が予想される。これまで肝硬変は主に肝実質細胞の減少により機能不全を来す疾患と考えられてきたが、肝小葉構築の破壊に伴うβ-cateninシグナル異常が相乗的に関わっているとすれば、このシグナルの制御による肝機能改善の可能性が期待される。一方、このシグナル経路は肝発がんにおいても重要な役割を果たしている事が報告されている。約30%の肝細胞がんにおいてβ-cateninをコードする*CTNNB1*遺伝子変異が見られ、その頻度は特にC型肝炎患者に発生する腫瘍で高率である事が知られている。このシグナル経路が代謝機能に与える役割を考慮すると、これらの腫瘍発生の背景となっているC型肝炎および、その発がん過程においてβ-cateninシグナルの異常が関わっている事、さらにβ-catenin遺伝子変異陽性の腫瘍は特徴的な代謝特性を有している事が予想される。本研究では、肝臓におけるβ-cateninシグナルの生理的機能と肝硬変、肝細胞がんにおける異常を総合的に明らかにし、このシグナル経路の制御を通じた肝機能の改善の可能性を探る。また、肝細胞がんにおけるβ-catenin遺伝子異常に伴う腫瘍特性を明らかにし、これを診断および個別改良への応用の可能性を検討する。

研究分担者

関根　茂樹　国立がん研究センター
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A．研究目的

ウイルス性肝炎の終末像である肝硬変や肝細胞がんの治療については未だ対症療法や外科切除等が治療の重要な部分を占めており、新たな薬物治療の開発が望まれる。β-cateninは肝小葉内で領域特異的な遺伝子発現制御に重要な働きを果たしており、この制御を通じて種々の代謝機能の制御に関わっている事が明らかにされつつある。さらに、30-40%の肝細胞がんではβ-cateninの変異の存在が知られている。本研究では肝細胞の代謝機能調節に関わるβ-cateninの役割に注目し、その肝硬変、

肝細胞がんにおけるβ-cateninシグナル異常の病態への関わりを明らかにし、このシグナル制御を通じた、肝硬変における肝代謝能の改善や肝細胞がんの診断・個別化治療への応用のための基礎を築く事を目標とする。

B．研究方法

本研究では、マウスモデルの解析によりβ-catenin の生体肝における生理的機能を明らかにし、これを基盤として、肝硬変および肝細胞がん臨床検体の解析によるヒト疾患でのβ-catenin シグナル異常の病態への関わりを検索する。

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C．研究結果

平成22年度までの研究において、主に肝細胞特異的β-cateninノックアウトマウスの解析からβ-catenin依存性シグナルが肝臓における胆汁酸代謝制御に重要な役割を果たしている事が明らかになった（文献1）。特にこのノックアウトマウスにおいてはSlco1b2等の血中から肝細胞への胆汁酸取り込みに関わるトランスポーターの著明な発現低下が認められた。この所見から、ヒトにおいてSlco1b2に最も相同性が高いとかんがえられている２つのトランスポーター、SLCO1B1, SLCO1B3の発現および*CTNNB1*遺伝子変異との相関をヒト肝細胞がんにおいて検索したところ、*CTNNB1*遺伝子変異を有する肝細胞がんにおいてSLCO1B3の高い発現を認めた。一方、*CTNNB1*遺伝子変異が陰性の肝細胞がんにおいては、その発現がほとんど消失していた（文献2）。

SLCO1B3は近年、画像診断において広く用いられるようになっている肝細胞特異的MRI造影剤であるGd-EOB-DTPAの主要なトランスポーターである事が報告されている。このため、*CTNNB1*遺伝子の変異とこの造影剤によるMRI信号増強効果の相関を調べた。この結果、*CTNNB1*遺伝子変異を有する肝細胞がんは、Gd-EOB-DTPAにより、有意に造影効果が認められることが明らかになった。

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これらSLCO1B3を含めたβ-catenin依存性シグナルの制御による下流遺伝子の調節に関わる機能を明らかにするため、β-cateninのプロモーター結合の網羅的解析を開始した。

D．考察

β-catenin依存性シグナルによる胆汁酸代謝制御の調節機能のマウスモデルにおける検索に基づいて、胆汁酸取り込みに関わるトランスポーターを同定し、そのヒト肝細胞がんにおける肝細胞特異的MRI造影剤の取り込みとの関連を明らかにした。

この所見は*CTNNB1*変異とMRI画像所見の相関を示すのみならず、β-catenin依存性シグナルと、この造影効果の相関を示すものとかんがえられ、β-catenin依存性シグナルの異常に関する知見が肝空間占拠性病変の画像診断に応用可能であることを示唆するものと考えられる。

一方、他の研究グループから、β-catenin依存性シグナルが肝臓における胆汁酸代謝制御はβ-cateninによる胆汁酸排泄能の低下による二次的なものであるとの報告がなされている。これは我々の得ている結果と矛盾するものであるが、その正否を含め、β-catenin依存性シグナルの肝臓における生理的機能の総体を明らかにするため、より網羅的な解析を行う必要があると考えられる。

E．結論

臨床例の解析から、ヒト肝細胞がんにおいてSLCO1B3は*CTNNB1*変異に相関しており、β-catenin依存性シグナルはヒト肝臓において、胆汁酸トランスポーターSLCO1B3の発現を誘導していると考えられる。この知見から、これまでに蓄積された種々の肝疾患におけるWnt/β-cateninシグナルの異常に関連する知見をGd-EOB-DTPAによる造影効果の理解へ応用が可能と考えられる。

今後、β-catenin依存性シグナルによる生理機能の制御をより総体的に理解するため、その下流遺伝子調節機構の網羅的な解析を進める。

F．健康危険情報

該当なし

G．研究発表

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2. 学会発表

関根茂樹．腫瘍発生に関わるβ-catenin 変異とその生理的機能の関連、第57回日本病理学会秋期特別総会、2011

H．知的財産権の出願・登録状況

（予定を含む）

4. 特許取得　該当なし

5. 実用新案登録　該当なし

6. その他　該当なし

別添５

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sekine S, Ogawa R, Ojima H, Kanai Y.	Overexpression of α-methylacyl-CoA racemase is associated with *CTNNB1* mutations in hepatocellular carcinomas	Histopathology	58	712-9	2011
Sekine S, Ogawa R, Ojima H, Kanai Y.	Expression of SLCO1B3 is associated with intratumoral cholestasis and *CTNNB1* mutations in hepatocellular carcinoma	Cancer Sci	102	1742-7	2011
Fujiwara H, Sekine S, Onaya H, Shimada K, Mikata R, Arai Y.	Ring-like enhancement of focal nodular hyperplasia with hepatobiliary-phase Gd-EOB-DTPA-enhanced magnetic resonance imaging: radiological-pathological correlation.	Jpn J Radiol	29	739-43	2011

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Overexpression of α-methylacyl-CoA racemase is associated with *CTNNB1* mutations in hepatocellular carcinomas

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Overexpression of α-methylacyl-CoA racemase is associated with *CTNNB1* mutations in hepatocellular carcinomas

Aims: α-Methylacyl-CoA racemase (AMACR) is expressed in the majority of hepatocellular carcinomas (HCCs) at variable levels, but the significance of AMACR overexpression remains elusive. The aim of this study was to investigate the relationship between AMACR expression and the presence of *CTNNB1* mutations in HCCs.
Methods and results: The expression of *AMACR* and *GLUL*, an established downstream target of β-catenin was examined in HCCs, by quantitative reverse transcription polymerase chain reaction (PCR), and the expression of their protein products by immunohistochemistry. The quantitative reverse transcription PCR analysis showed that the expression of *AMACR* was significantly higher in HCCs with *CTNNB1* mutations than in mutation-negative HCCs or normal livers, like the expression of *GLUL*. Immunohistochemistry also showed that strong AMACR protein expression was closely correlated with the presence of *CTNNB1* mutations. HCCs with *CTNNB1* mutations and those with AMACR overexpression frequently exhibited bile production.
Conclusions: The overexpression of AMACR was closely correlated with the presence of *CTNNB1* mutations in HCCs. AMACR is a putative target of β-catenin as well as an excellent immunohistochemically detectable marker of HCCs with *CTNNB1* mutations. As AMACR is physiologically involved in bile acid synthesis, the current observation implies a regulatory role of β-catenin in bile acid metabolism.

Keywords: AMACR, β-catenin, hepatocellular carcinoma

Abbreviations: AMACR, α-methylacyl-CoA racemase; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction

Introduction

α-Methylacyl-CoA racemase (AMACR) is an enzyme that is crucial for the β-oxidation of branched fatty acids and C27 bile acids.[1] Recent studies have shown the utility of AMACR expression in the histological diagnosis of prostatic cancer. AMACR was initially identified as a potential molecular marker of prostatic cancer in a microarray study.[2] As normal prostate expresses very low levels of AMACR, the immunohistochemical detection of AMACR is helpful in the diagnosis of prostatic cancers.[3,4] Furthermore, the inhibition of AMACR expression represses the growth of prostatic cancer cells *in vitro*,[5] suggesting that AMACR is not just a tumour marker, but is also directly involved in tumorigenesis.

On the other hand, the expression of AMACR is not limited to prostatic cancers. AMACR is also expressed in tumours of other organs, including liver, kidney and colorectal cancers.[6,7] With regard to liver cancer, AMACR expression has been reported in 77–100% of hepatocellular carcinomas (HCCs).[6–9] Even though HCCs express AMACR with a frequency comparable to that of prostatic cancers, AMACR expression cannot be directly used as a tumour marker in the liver, as it

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Table 1. Clinicopathological features of cases and results of mutational and immunohistochemical analysis

	Age (years)/ Gender	Histology	Viral infection	*CTNNB1* mutation		Immunohistochemistry			
				Nucleotide	Amino acid	AMACR	GS	β-Catenin	Bile
1	52/M	W/D	HCV	A95G	D32G	++	++	+	−
2	65/M	W/D	HCV	del94–141	del32–47	++	++	−	−
3	58/M	M/D	−	A121G	T41A	+	++	+	+
4	77/F	M/D	HCV	A95G	D32G	++	+	−	+
5	63/M	W/D	HCV	T104G	I35S	++	++	+	+
6	55/M	P/D	HCV	T109C	S37P	++	++	+	+
7	63/M	W/D	HCV	del114–125	del39–42	++	++	+	+
8	71/M	M/D	−	A107G/A126G	H36R/T42T	++	++	+	−
9	66/M	M/D	HCV	C134T	S45F	++	++	+	+
10	73/M	M/D	HCV	T104G	I35S	++	++	+	+
11	71/M	W/D	HCV	A107C	H36P	+	+	−	+
12	68/M	M/D	−	G101A	G34E	+	+	−	−
13	66/M	M/D	HBV, HCV	C110G	S37C	++	++	+	+
14	50/M	P/D	HCV	A95G	D32G	++	++	+	+
15	29/M	M/D	HBV	A107G	H36R	+	+	+	+
16	72/M	M/D	−	G101A	G34E	+	+	+	−
17	62/M	W/D	HBV	T133C	S45P	+	+	−	+
18	59/M	M/D	HBV	A121G	T41A	+	+	+	−
19	59/M	P/D	HBV	−	−	−	+	−	−
20	41/M	M/D	HBV	−	−	−	+	−	−
21	54/M	M/D	HBV	−	−	−	+	−	−
22	69/M	M/D	HCV	−	−	+	+	−	+
23	63/M	M/D	HBV, HCV	−	−	+	+	−	+
24	67/M	P/D	HCV	−	−	−	−	−	−
25	58/F	M/D	HBV	−	−	−	+	−	−
26	61/F	P/D	HCV	−	−	−	+	−	−
27	62/M	W/D	HCV	−	−	+	+	−	+
28	61/M	P/D	−	−	−	+	+	−	−
29	66/M	M/D	HBV	−	−	+	+	−	−
30	79/M	M/D	−	−	−	−	−	−	−
31	71/M	M/D	HCV	−	−	++	+	−	−

Table 1. (*Continued*)

	Age (years)/ Gender	Histology	Viral infection	*CTNNB1* mutation		Immunohistochemistry			Bile
				Nucleotide	Amino acid	AMACR	GS	β-Catenin	
32	70/M	M/D	HCV	–	–	+	+	–	–
33	76/M	P/D	–	–	–	+	–	–	–
34	58/M	M/D	HBV	–	–	+	+	–	–
35	56/M	P/D	HBV	–	–	+	+	–	–
36	70/M	P/D	–	–	–	+	+	–	–
37	36/M	P/D	HBV	–	–	–	+	–	–
38	50/M	P/D	HCV	–	–	–	–	+	–
39	59/M	M/D	HCV	–	–	+	+	–	–
40	60/M	P/D	HCV	–	–	–	–	–	–
41	72/F	P/D	HCV	–	–	+	+	–	–
42	68/M	P/D	HCV	–	–	ND	ND	ND	ND
43	68/M	W/D	HCV	–	–	ND	ND	ND	ND
44	75/M	W/D	HCV	–	–	ND	ND	ND	ND

AMACR, α-methylacyl-CoA racemase; F, female; GS, glutamine synthetase; HBV, hepatitis B virus; HCV, hepatitis C virus; M, male; M/D, moderately differentiated; ND, not done; P/D, poorly differentiated; W/D, well differentiated.
Samples 18 and 19 were derived from the same patient, as were samples 42 and 43.

is also expressed in non-neoplastic liver tissue.[6,8] Although some immunohistochemical studies have been performed, the clinicopathological significance of AMACR expression in HCCs remains controversial.[8,9]

CTNNB1 is a major oncogene in HCCs, and is mutated in approximately 30% of all cases.[10,11] β-Catenin, the protein product of *CTNNB1*, is involved in two distinct processes in cells: cell adhesion and the transduction of Wnt signalling. In the absence of active Wnt signalling, β-catenin is localized to the membrane in a complex with cadherins that mediates cell–cell adhesion. When the Wnt signalling pathway is activated, β-catenin is translocated to the nucleus, where it activates TCF-dependent transcription.[12] Oncogenic *CTNNB1* mutations lead to constitutively active T cell factor (TCF)-dependent transcription, and the dysregulated expression of β-catenin/TCF target genes is thought to induce cellular transformation.[13] A number of genes have been hitherto identified as targets of β-catenin-mediated signals in the liver, and are overexpressed in HCCs with *CTNNB1* mutations.[14–19]

Here, we examined the correlation between *CTNNB1* mutations and AMACR expression in a series of HCCs. We also examined the expression of a well-recognized target of β-catenin, glutamine synthetase, which is encoded by *GLUL*.[14,15,20]

Materials and methods

CASES

Forty-four surgically resected HCC samples obtained from 42 patients were analysed in this study. Eight non-neoplastic liver tissues obtained during resection of metastatic colorectal cancers were used as normal liver samples for comparison. All of the tissue samples were obtained from the National Cancer Centre Hospital, Tokyo, Japan. The clinicopathological features of the patients are listed in Table 1. This study was approved by the Ethics Committee of the National Cancer Centre, Tokyo, Japan.

QUANTITATIVE POLYMERASE CHAIN REACTION (PCR)

RNA extraction and the reverse transcription reaction were performed according to standard protocols. Quantitative PCR reactions were performed with SYBR Green PCR master mix (Applied Biosystems, Foster

City, CA, USA). The expression of *GUSB* was used for normalization, as previously described.[21] The primer sequences were as follows: *AMACR*, CGTCTGTGCAAGCGGTCGGA and TGGGCCCAGCTGGAGTTTCT; *GLUL*, GCCATGCGGGAGGAGAAT and ACTGGTGCCGCTTGCTTAGT; and *GUSB*, GGAATTTTGCCGATTTCATGA and CCGAGTGAAGATCCCCTTTTT. A *P*-value of <0.05 (Mann–Whitney *U*-test) was considered to be significant.

CTNNB1 MUTATIONAL ANALYSIS

PCR reactions were performed with the cDNA samples used for quantitative PCR as templates. A pair of primers encompassing the N-terminal region of *CTNNB1*, CCTGTTCCCCTGAGGGTATT and CAGGGAACATAGCAGCTCGT, was used. The PCR products were electrophoresed in an agarose gel and recovered with a QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). Isolated PCR products were sequenced bidirectionally with the same primers used for amplification.

IMMUNOHISTOCHEMISTRY

Liver tissue samples were fixed with 10% formalin, embedded in paraffin and cut into 4-μm-thick sections. Sections were subjected to haematoxylin and eosin and Hall's bile staining for evaluation of bile production in HCCs. Immunohistochemistry was performed using an indirect immunoperoxidase method. Antigen retrieval was performed by autoclaving in 10 mM citrate buffer (pH 6.0). The primary antibodies used were anti-AMACR (Clone 13H4; 1:400 dilution; Dako, Glostrup, Denmark), anti-β-catenin (Clone 14; 1:250 dilution; BD Biosciences, San Jose, CA, USA) and anti-glutamine synthetase (Clone 6; 1:1000 dilution; BD Biosciences). The signals were detected with peroxidase-labelled anti-mouse and anti-rabbit polymers (Histofine simple stain; Nichirei, Tokyo, Japan). 3,3′-Diaminobenzidine tetrahydrochloride was used as a chromogen.

The staining results for AMACR and glutamine synthetase were evaluated as diffuse strong expression (++), heterogeneous and/or weak expression (+), or no expression (−). Diffuse staining was defined as >80% of the cells showing homogeneous staining. Strong expression was defined as a staining intensity comparable to that of pericentral hepatocytes in normal liver. Staining for nuclear/cytoplasmic β-catenin was considered to be positive when more than 5% of the tumour cells exhibited evident nuclear and/or cytoplasmicimmunoreactivity.

Results

Sequencing analysis of *CTNNB1* identified mutations affecting the region encoding the casein kinase 1/glycogen synthase kinase-3β phosphorylation sites of β-catenin in 18 of 44 HCCs (41%; Table 1). These included 18 missense mutations and two in-frame deletions. One tumour with a missense mutation also had a silent mutation.

The quantitative reverse transcription PCR analysis clearly showed that tumours with *CTNNB1* mutations had elevated expression levels of *AMACR* as well as of *GLUL*, a known target of β-catenin,[14,15,20] as compared with normal liver tissues and HCCs without *CTNNB1* mutations (Figure 1). On the other hand, HCCs without *CTNNB1* mutations did not show significantly altered expression levels of *AMACR* or *GLUL*.

To test whether the elevated *AMACR* and *GLUL* expression levels resulted in the overexpression of their protein products, an immunohistochemical analysis of 41 corresponding tumours and seven normal liver tissue samples that were available for histological analysis was perfomed. Immunohistochemistry confirmed that AMACR overexpression was associated with the presence of *CTNNB1* mutations (Figure 2B,C,E,F; Table 2). Eleven of the 12 HCCs with diffuse and intense AMACR staining and seven of the 19 HCCs with heterogeneous and/or weak AMACR expression had *CTNNB1* mutations. In contrast, none

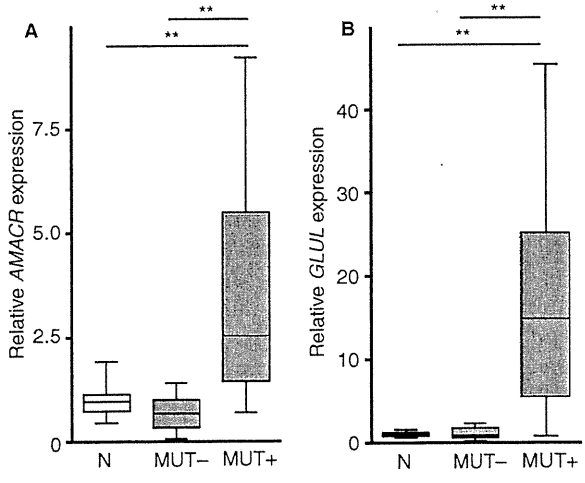

Figure 1. Expression of *AMACR* and *GLUL* in hepatocellular carcinomas. Box plot of *AMACR* and *GLUL* mRNA expression in normal liver (n = 8), hepatocellular carcinomas (HCCs) with wild-type *CTNNB1* (MUT−; n = 26) and HCCs with *CTNNB1* mutations (MUT+; n = 18), as determined using quantitative polymerase chain reaction. **P < 0.001 (Mann–Whitney U-test).

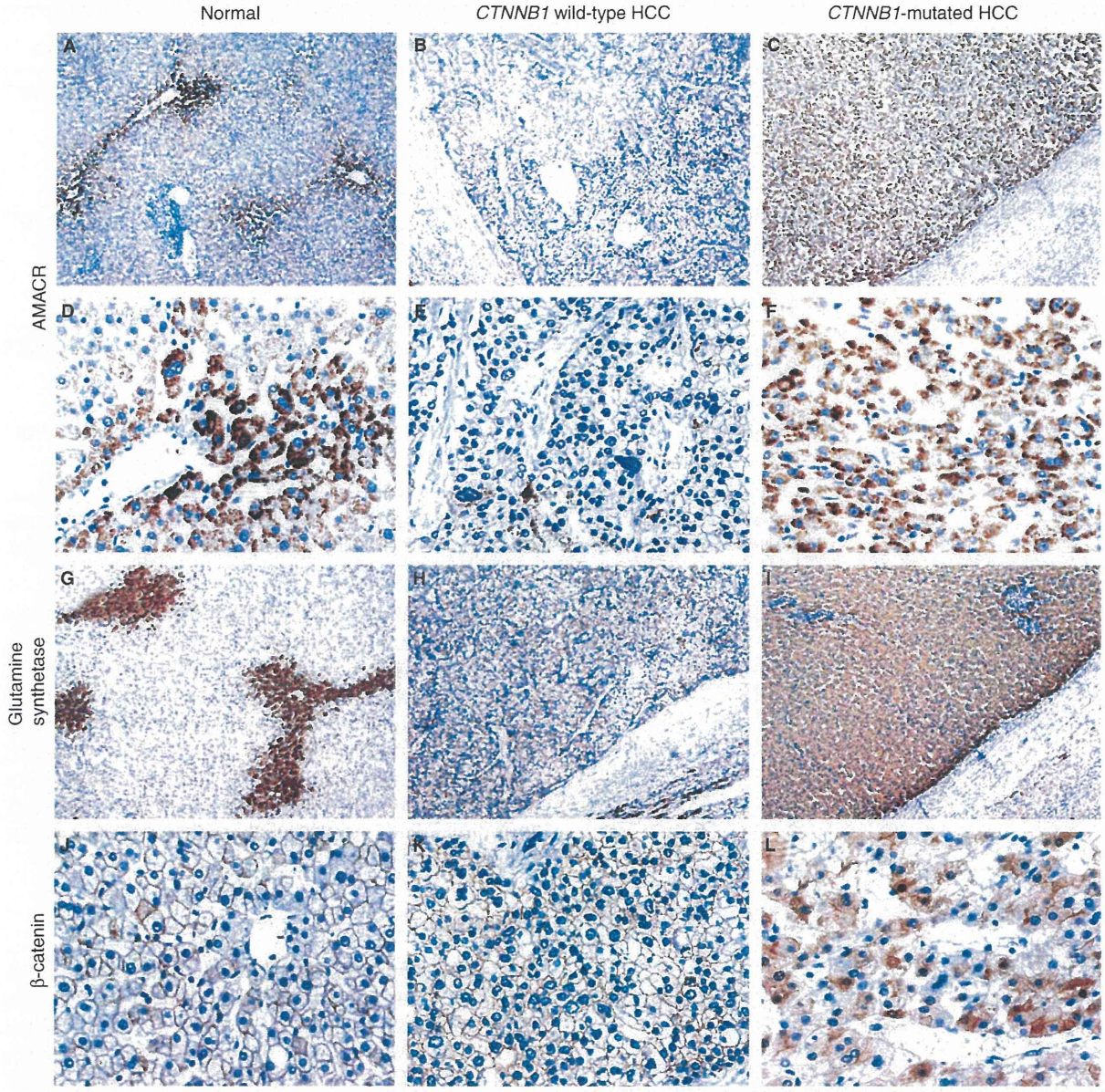

Figure 2. Expression of α-methylacyl-CoA racemase (AMACR), glutamine synthetase and β-catenin in hepatocellular carcinomas (HCCs) and normal liver. Immunohistochemistry for AMACR (**A–F**), glutamine synthetase (**G–I**) and β-catenin (**J–L**) in normal liver (**A,D,G,J**), HCCs without *CTNNB1* mutation (**B,E,H,K**) and HCCs with *CTNNB1* mutation (**C,F,I,L**).

of the AMACR-negative tumours had *CTNNB1* mutations. AMACR was consistently expressed in normal liver tissue: an expression gradient was observed, with the highest expression levels being seen in the proximal pericentral areas within the liver lobule (Figure 2A). The intracellular distributions of AMACR were cytoplasmic and granular, and a significant difference was not observed between the normal liver tissues and the HCCs (Figure 2D–F).

In agreement with previous studies, the diffuse and strong expression of glutamine synthetase, which is encoded by *GLUL*, was observed exclusively in tumours with *CTNNB1* mutations (Figure 2H,I). The staining results for glutamine synthetase were highly concordant with those for AMACR (Table 3). In the normal liver tissues, glutamine synthetase was exclusively expressed in a few layers of hepatocytes surrounding the central veins (Figure 2G). Nuclear/cytoplasmic

Table 2. Correlations among *CTNNB1* mutational status, immunohistochemistry for α-methylacyl-CoA racemase (AMACR), glutamine synthetase and β-catenin expression, and bile production in hepatocellular carcinomas

	Staining intensity	*CTNNB1* mutation (+)	*CTNNB1* mutation (−)	*P*-value
AMACR	++	11	1	6.4×10^{-5}
	+	7	12	
	−	0	10	
Glutamine synthetase	++	11	0	3.5×10^{-5}
	+	7	18	
	−	0	5	
Nuclear/cytoplasmic β-catenin	+	13	1	5.4×10^{-6}
	−	5	22	
Bile production	+	12	3	4.0×10^{-4}
	−	6	20	

The *P*-values indicate the correlation between the *CTNNB1* mutation status and the immunohistochemical expression of each of the proteins or bile production (chi-square test).

Table 3. Correlations among α-methylacyl-CoA racemase (AMACR), glutamine synthetase and β-catenin expression, and bile production in hepatocellular carcinomas

		AMACR			
	Staining intensity	++	+	−	*P*-value
Glutamine synthetase	++	10	1	0	4.2×10^{-7}
	+	2	17	6	
	−	0	1	4	
Nuclear/cytoplasmic β-catenin	+	9	4	1	1.5×10^{-3}
	−	3	15	9	
Bile production	+	8	7	0	5.3×10^{-3}
	−	4	12	10	

The *P*-values indicate the correlations among the immunohistochemical expression of AMACR and that of glutamine synthetase or β-catenin, or bile production (chi-square test).

β-catenin staining was observed in 12 of the 17 *CTNNB1* mutation-positive HCCs. In most cases, the nuclear staining was limited to focal areas within the tumours. In the normal liver tissues and HCCs without *CTNNB1* mutations, β-catenin staining was observed exclusively in the membranes (Figure 2J,K). Positive nuclear/cytoplasmic β-catenin staining was significantly correlated with AMACR expression (Table 3).

A recent study suggested that cholestasis might be useful as a marker for HCCs with *CTNNB1* mutations.[20] As AMACR is involved in bile acid metabolism in the liver, we histologically determined the presence of bile production in HCCs, and examined the correlations with *CTNNB1* mutations and AMACR expression (Figure 3). In agreement with the previous study, HCCs with bile production frequently harboured *CTNNB1* mutations and, as expected, overexpressed AMACR (Tables 2 and 3).

Discussion

Oncogenic mutations of *CTNNB1* result in the stabilization of β-catenin through the inhibition of proper proteosomal degradation. This leads to the abnormal accumulation and nuclear translocation of the protein and the constitutive activation of TCF-dependent transcription. Nuclear/cytoplasmic localization of β-catenin is therefore regarded as a hallmark of active β-catenin signalling.[12] As expected, nuclear and/or cytoplasmic β-catenin staining was closely correlated with the presence of *CTNNB1* mutations. However, in many instances, nuclear and/or cytoplasmic β-catenin was observed only in focal areas within the tumours with *CTNNB1* mutations. Therefore, it might be difficult to use immunohistochemistry for β-catenin to screen for *CTNNB1* mutation-positive HCCs when a limited amount of specimen is available.

So far, several β-catenin-regulated genes have been identified in the liver.[14–19] Among them, the

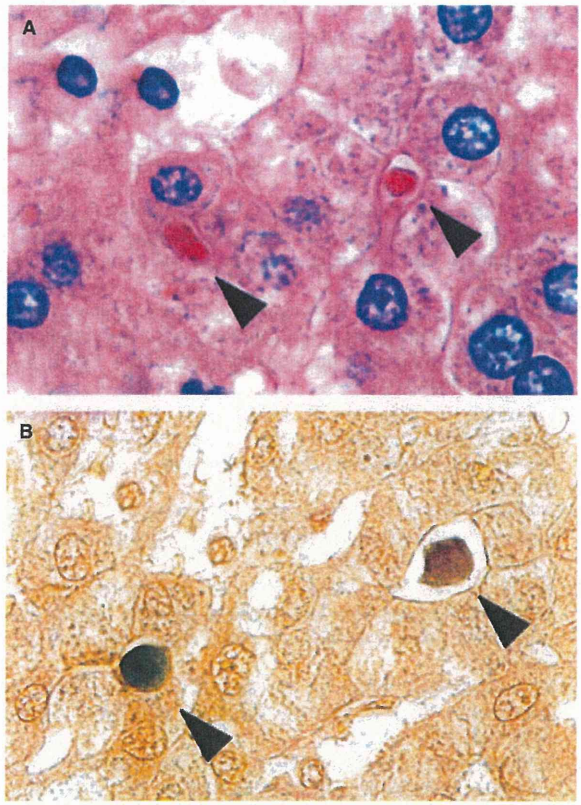

Figure 3. Bile production in hepatocellular carcinomas. Bile production (triangles) as determined using haematoxylin and eosin (**A**) and Hall's bile staining (**B**).

overexpression of glutamine synthetase has been reported to correlate well with the presence of *CTNNB1* mutations, and is regarded as an indicator of HCCs with *CTNNB1* mutations.[14,15,20] In agreement with these previous reports, *CTNNB1*-mutated HCCs showed significantly elevated levels of *GLUL* expression. At the same time, we found that *AMACR* overexpression is also highly correlated with the presence of *CTNNB1* mutations. The expression levels of *GLUL* and *AMACR* were also reflected by the expression of their protein products, which can be detected by immunohistochemistry. Diffuse and strong staining for AMACR and glutamine synthetase was observed almost exclusively in tumours with *CTNNB1* mutations. Conversely, none of the tumours negative for these proteins had *CTNNB1* mutations in the current series. We suggest that overexpression of AMACR might be a novel and excellent histological indicator of HCCs with *CTNNB1* mutations.

Bile production is occasionally observed in HCCs; notably, cholestasis has recently been suggested to be a feature of HCCs with *CTNNB1* mutations.[20] Audard *et al.*[20] noted a close correlation between strong glutamine synthetase expression and the presence of cholestasis, and suggested that cholestasis might be a marker of HCCs with *CTNNB1* mutations. In agreement with this hypothesis, tumour cholestasis was frequently associated with *CTNNB1* mutations and also with high AMACR expression in the present analysis. The biological significance of AMACR overexpression in cancers has been discussed in relation to its role in the β-oxidation of branched-chain fatty acids.[1] However, in addition to fatty acid metabolism, AMACR is also involved in bile acid synthesis in the liver, and is required for the conversion of C27 bile acids to C24 bile acids. C27 bile acids are more hydrophobic than their C24 products, and are more toxic to cells.[22–24] Considering the association between cholestasis and AMACR expression, AMACR might play a role in the processing of bile acid intermediates to avoid cell injury caused by the accumulation of C27 bile acids in *CTNNB1*-mutated HCCs.

In addition to its expression in HCCs, AMACR was also expressed in normal liver, predominantly in pericentral hepatocytes. Of note, recent studies have suggested that Wnt/β-catenin signalling plays a crucial role in the regulation of pericentral gene expression.[25] Wnt/β-catenin signalling is physiologically active in pericentral hepatocytes, and many of the previously identified β-catenin-regulated genes are localized to pericentral areas within the liver lobule.[25–28] Furthermore, Hailfinger *et al.*[26] demonstrated a similarity in the gene expression patterns of pericentral hepatocytes and *CTNNB1*-mutated HCCs, and suggested the common regulation of these genes by β-catenin-mediated signalling. Thus, the predominantly pericentral expression of AMACR implies that AMACR is also regulated by β-catenin-mediated signalling in normal liver. Overall, the present study suggests that AMACR is a target of β-catenin in the liver under both neoplastic and non-neoplastic conditions.

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Expression of SLCO1B3 is associated with intratumoral cholestasis and *CTNNB1* mutations in hepatocellular carcinoma

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Recent studies have shown that intratumoral cholestasis is a hallmark of *CTNNB1* mutations in hepatocellular carcinomas (HCC). Here, we analyzed the expressions of genes involved in bile acid and bilirubin metabolism and their correlation with the mutational status of *CTNNB1* in a series of HCC. The expressions of *CYP7A1* and *CYP27A1*, which encode rate-limiting enzymes in bile acid synthesis, were unaltered or only marginally increased in *CTNNB1*-mutated HCC compared with those in HCC with wild-type *CTNNB1*. Among the genes involved in bile acid and bilirubin transport, the expression of *SLCO1B3* was significantly elevated in HCC with *CTNNB1* mutations, whereas the expression of *ABCC4* was elevated in HCC with wild-type *CTNNB1*. Immunohistochemistry confirmed the frequent expression of SLCO1B3 in *CTNNB1*-mutated HCC at the protein level, but not in most HCC with wild-type *CTNNB1*. Immunohistochemistry for MRP4 (encoded by *ABCC4*) partly agreed with *ABCC4* expression, but most cases did not express detectable levels of MRP4. Notably, all HCC with bile accumulation, including those without *CTNNB1* mutations, expressed SLCO1B3, suggesting that SLCO1B3 expression, rather than *CTNNB1* mutation, is the critical determinant of intratumoral cholestasis. As SLCO1B3 is involved in the uptake of a number of chemotherapeutic and diagnostic agents, SLCO1B3 expression and the status of *CTNNB1* mutation might need to be considered in the drug delivery to HCC. (*Cancer Sci* 2011; 102: 1742–1747)

Bile acids are major components of bile and the liver plays a central role in their metabolism.[1–3] Bile acids are synthesized from cholesterol in the liver and secreted into bile. Bile plays essential roles in the absorption and excretion of lipid-soluble substances. In addition to widely recognized roles in lipid metabolism, bile acids act as a signaling molecule and modulate proliferation and energy metabolism in hepatocytes.[3,4] Mice deficient in the bile acid receptor FXR exhibit cholestasis and the spontaneous development of hepatocellular carcinomas (HCC), suggesting a potential linkage between bile acid signaling and hepatocarcinogenesis.[5,6]

Interestingly, recent reports have shown that HCC with activating *CTNNB1* mutations frequently exhibit cholestasis.[7,8] This observation implies that β-catenin regulates bile metabolism in HCC. Activating mutations of *CTNNB1*, encoding β-catenin, are present in 30–40% of HCC.[9,10] Physiologically, β-catenin acts as a transducer of the Wnt signaling pathway,[11] and mutation of *CTNNB1* leads to abnormal accumulation of β-catenin and constitutive activation of T-cell factor (TCF)-dependent transcription.[12] This results in the overexpression of β-catenin/TCF-regulated transcriptional targets in *CTNNB1*-mutated tumors and the promotion of tumorigenesis.

Based on these previous reports, we suspected that mutated β-catenin might coordinately induce genes critically involved in bile metabolism in HCC. To elucidate this issue, we examined the expressions of a list of genes that are involved in bile acid synthesis and transport in a series of HCC.

Materials and Methods

Cases. We examined 44 cases of HCC obtained from 42 patients; all of these tumors had been previously analyzed for *CTNNB1* mutations and the presence of intratumoral cholestasis.[8] Mutation analysis was done by direct sequencing of the N-terminal region of *CTNNB1* using cDNA samples. Intratumoral cholestasis was histologically determined by the presence of bile pigments on hematoxylin–eosin and Hall's bile acid staining. Eight non-tumoral liver tissues obtained during the resection of metastatic colorectal cancers were used as normal liver samples for comparison. All tissue samples were obtained at the National Cancer Center Hospital, Tokyo, Japan. The present study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan.

Quantitative RT-PCR. RNA extraction and reverse-transcription reactions were performed using standard protocols. Quantitative RT-PCR reactions were performed using SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA). The expression level of each gene was determined using *GUSB* as a standard, as previously described.[13] The primer sequences are shown in Table 1.

Immunohistochemistry. Among the tumors subjected to RT-PCR analysis, 41 lesions were available for histological analysis. Liver tissue samples were fixed in 10% buffered formalin, embedded in paraffin and cut into 4-μm-thick sections. Antigen retrieval was performed by autoclaving in 10 mmol/L of citrate buffer (pH 6.0) for 10 min. Anti-SLCO1B3 (1:250 dilution; Sigma, St Louis, MO, USA) and anti-ABCC4 antibodies (1:500 dilution; Abnova, Taipei, Taiwan) were used as the primary antibodies and the signals were detected using peroxidase-labeled anti-rabbit and anti-goat polymers (Histofine simple stain; Nichirei, Tokyo, Japan). 3-3′-Diaminobenzidine tetrahydrochloride was used as a chromogen. Normal liver tissue served as a positive control for SLCO1B3 and normal prostatic tissue was used as a positive control for ABCC4. The staining results were evaluated as follows: ++, diffuse (>50%) expression; +, focal (10–50%) expression; and −, no (<10%) expression.

Statistical analysis. For quantitative PCR analysis, statistical significance was confirmed using a two-tailed Mann–Whitney *U*-test. The Fisher–Freeman–Halton exact test was used to analyze each 2 × 3 table. $P < 0.05$ was considered statistically significant.

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Table 1. Primers used in the RT-PCR analysis

	Forward primer	Reverse primer
CYP7A1	GCTCTTTACCCACAGTTAATGC	TTGTCTTCCCGTTTTCATCA
CYP27A1	GATTGCAGAGCTGGAGATGC	CTCTTCAACTCCCCCGTCTC
HSD3B7	CTGGGCTGGTAGACGTGTTT	ACACAAGCCTCGATCACGTT
SLC10A1	AATGGACGGTGCAGACGCACT	AGGCCACATTGAGGATGGTGGAA
SLCO1A2	ACCAACGCAGGATCCATCAGAGTGT	ACCCAAAGGCAGGATGGGAGT
SLCO1B1	TGGAGGTGTTTTGACTGCTTTGCCA	ACAAGTGGATAAGGTCGATGTTGAATTTTCT
SLCO1B3	TGGTCCAGTCATTGGCTTTGCACT	AGCTCCAACCCAACGAGAGTCCT
ABCC2	CCAGGACCAAGAGATCCTCTACCAC	AAGGGCCAGCTCTATGGCTGCT
ABCC3	CTCTGGAGGCCTGTGCCTTGCTA	GCTGGCCCCCAGACAGGTTAATGC
ABCC4	GGTGGGCCTCTGGTACTGAAGC	TCCAGCTCCGGTTCTTCCCACAAT
ABCB11	AGATGACATGCTTGCGAGGACCT	AGCGTTGCCGGATGGAAGCC
NR0B2	CCTGAAAGGGACCATCCTCT	ACTTCACACAGCACCCAGTG
AXIN2	ACGCTGGCTCAGCTGGAGGA	ACAGCACCGCTGCTTTGGGG
GUS	GGAATTTTGCCGATTTCATGA	CCGAGTGAAGATCCCCTTTTT

Results

We first determined the expressions of genes encoding enzymes critical for bile acid synthesis, postulating that *CTNNB1*-mutated HCC show increased bile acid production. CYP7A1 and CYP27A1 are the rate-limiting enzymes of the classical and alternative pathways of bile acid synthesis, respectively.[3] HSD3B7 is another critical enzyme in bile acid synthesis and its mutation has been linked to a defect in bile acid synthesis.[14] The results showed that *CYP7A1* expression was increased in the HCC regardless of the *CTNNB1* mutation status (Fig. 1). *CYP27A1* expression was significantly but only marginally elevated in HCC with *CTNNB1* mutations compared with those without mutation. The *HSD3B7* expression level was unaltered between normal liver and the HCC. These results indicate that *CTNNB1* mutations do not induce genes for bile acid synthesis in HCC.

Next we examined four genes involved in bile acid and bilirubin uptake from portal blood. The expressions of three of the genes that were examined, *SLC10A1*, *SLCO1A2* and *SLCO1B1*, tended to be reduced in HCC, and no significant associations with the status of *CTNNB1* mutation were seen (Fig. 1). However, *SLCO1B3* expression was closely correlated with the presence of *CTNNB1* mutations. While HCC without *CTNNB1* mutations showed remarkably reduced *SLCO1B3* expression levels, *CTNNB1*-mutated HCC retained expression levels comparable with that observed in normal liver.

Two genes for canalicular transporters, *ABCC2* (encoding MRP2) and *ABCB11* (encoding BSEP), showed a modest increase in *CTNNB1*-mutated tumors. Between two basolateral efflux transporters, *ABCC3* (encoding MRP3) and *ABCC4* (encoding MRP4), *ABCC4* was significantly elevated in HCC with wild-type *CTNNB1*. Of note, expression of *NR0B2*, which mediates the feedback regulation of bile acid signaling,[15,16] did not differ between HCC with or without *CTNNB1* mutations.

The expressions of SLCO1B3 and MRP4 were further determined at the protein level using immunohistochemistry. SLCO1B3 expression in the HCC was membranous and consistent with the results of the RT-PCR analysis; the expression of SLCO1B3 was significantly correlated with the presence of *CTNNB1* mutations (Fig. 2, Table 2). SLCO1B3 was expressed in pericentral hepatocytes with a membranous pattern in normal liver (Fig. 3).

Immunohistochemistry for MRP4 showed diffuse expression in one case and focal staining in three cases (Figs 4,5). All four cases positive for MRP4 also showed high levels of *ABCC4* expression, indicating concordance between the mRNA and protein expression levels. However, negative or faint expression of MRP4 was observed in the other HCC. Non-neoplastic liver tissue did not express immunohistochemically detectable levels of

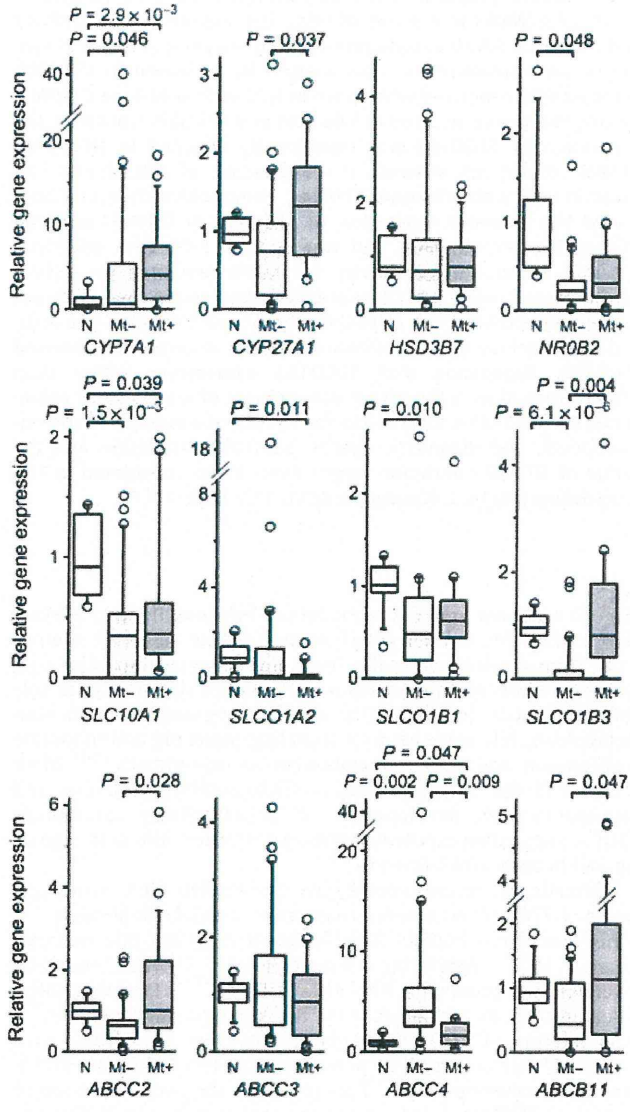

Fig. 1. Expression of genes related to bile acid metabolism. Box plots of the expression of genes related to bile acid metabolism in hepatocellular carcinomas (HCC). The expression of each gene was determined using quantitative RT-PCR with *GUSB* used as a reference. N, normal liver; Mt-, HCC with wild-type *CTNNB1*; Mt+, *CTNNB1*-mutated HCC.

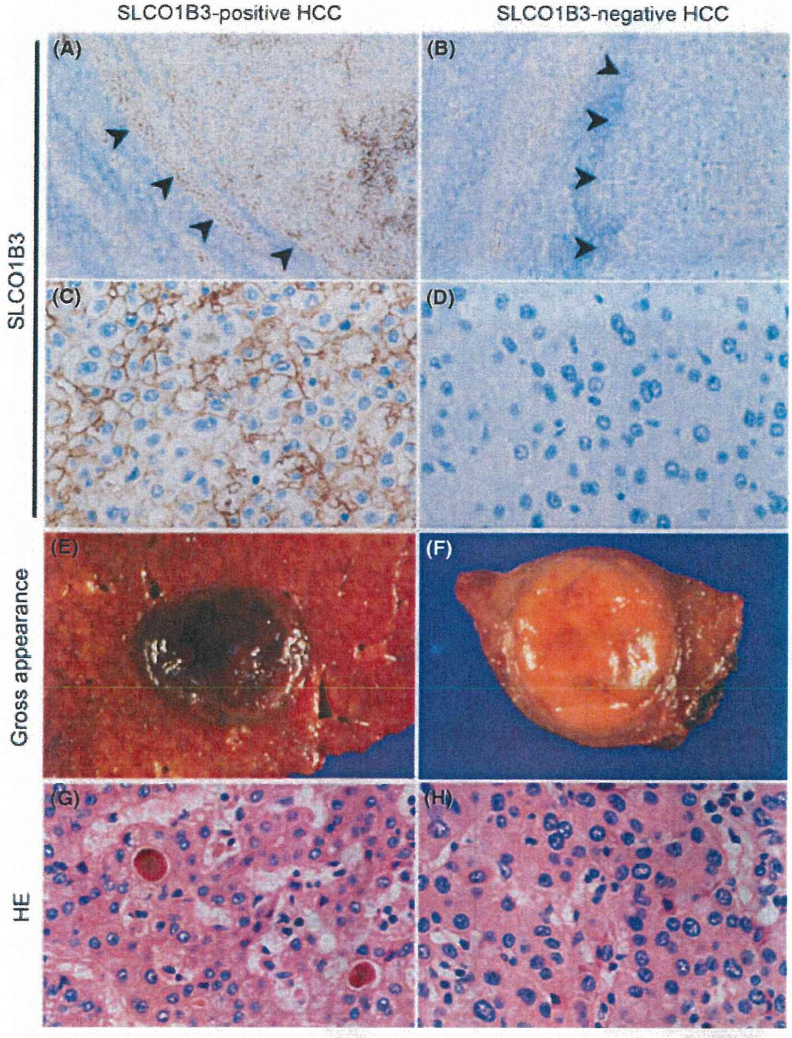

Fig. 2. SLCO1B3 expression, gross morphology and histology in hepatocellular carcinomas (HCC). Immunohistochemistry for SLCO1B3 (A–D), gross morphology (E,F) and histology (G,H) in a SLCO1B3-expressing HCC (A,C,E,G) and a SLCO1B3-negative HCC (B,D,F,H). Low-power views of SLCO1B3 staining (A,B). Areas of the tumor are indicated by arrowheads. Extensive SLCO1B3 expression in a case of cholestatic HCC (A) and almost completely negative staining in a case of non-cholestatic HCC (B). Focal SLCO1B3 expression is observed in non-neoplastic cirrhotic liver on the backgrounds of HCC (A,B). Magnified views showed membranous SLCO1B3 expression (C), and no SLCO1B3 staining (D). A SLCO1B3-expressing HCC has a greenish cholestatic appearance (E), whereas a SLCO1B3-negative HCC has a homogenous whitish appearance (F). A HE-stained section shows bile pigments in a SLCO1B3-expressing HCC (G) but not in a SLCO1B3-negative HCC (H).

MRP4. Prostatic tissues used for positive controls exhibited diffuse and strong membranous expression.

Next, we sought to determine the correlation between SLCO1B3 expression and intratumoral cholestasis. The results showed that HCC with SLCO1B3 expression frequently showed bile accumulation (Figs 2,5, Table 2). Remarkably, all three *CTNNB1* mutation-negative, cholestatic HCC expressed SLCO1B3, implying that the presence of bile accumulation is more closely correlated with SLCO1B3 expression than the mutational status of *CTNNB1*.

Table 2. Correlations among SLCO1B3 expression, *CTNNB1* mutation and cholestasis

	Total	SLCO1B3 immunohistochemistry			*P*-value
		++	+	–	
CTNNB1 mutation					
Present	18	11	4	3	6.8×10^{-4}
Absent	23	2	6	15	
Cholestasis					
Present	15	12	3	0	6.4×10^{-8}
Absent	26	1	7	18	

++, diffuse expression; +, focal expression; –, no expression.

While *CTNNB1*-mutations affecting N-terminal regions of β-catenin is the common cause of activation of β-catenin signaling in HCC, β-catenin signaling could potentially be activated by uncommon genetic alterations such as atypical *CTNNB1* mutations or *APC* mutations.[17,18] To exclude this possibility, we examined the expression of *AXIN2*, a ubiquitous target of β-catenin/TCF.[19,20] As expected, the expression of *AXIN2* was upregulated in *CTNNB1*-mutated HCC, but the levels of *AXIN2* expression were not significantly elevated in any of the *CTNNB1* mutation-negative HCC with SLCO1B3 expression (Fig. 5). This finding indicates that a minor subset of HCC express SLCO1B3 even in the absence of active β-catenin signaling.

Discussion

Based on the association between *CTNNB1* mutations and intratumoral cholestasis,[7,8] we postulated that active β-catenin signaling regulates bile acid metabolism in HCC. While previous analysis in a mouse model suggested that β-catenin induces bile acid synthesis genes under physiological conditions,[21] they were not upregulated in HCC with *CTNNB1* mutations. In contrast, the expression of SLCO1B3, a solute carrier organic anion transporter protein, was associated with the presence of

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