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Lecture

解　説

キメラ抗原レセプター(CAR)遺伝子導入T細胞によるがん治療*

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Key Words：chimeric antigen receptor(CAR), gene-modified T cells, adoptive immunotherapy

はじめに

がんワクチン療法や養子免疫療法，免疫チェックポイント阻害療法など，がんに対する免疫療法の開発において近年多くの進展がみられている．それらの中には有意な臨床効果を示す治療法もあり，抗cytotoxic T-lymphocyte antigen 4(CTLA-4)抗体のように薬剤として認可されたものも存在する．このように，現在研究や開発が進んでいるがん免疫療法の中で，最も期待されている手法の一つがキメラ抗原受容体(chimeric antigen receptor；CAR)を利用した遺伝子改変型T細胞療法である．CARとはがん細胞上の標的分子を認識する一本鎖抗体とT細胞の活性化に必要なシグナル配列を融合した分子であり，現在欧米で実施されているCAR発現T細胞を用いた臨床試験では，進行期血液造血器腫瘍に対してきわめて優れた治療効果が認められている．本総説では，CAR-T細胞療法の現状と課題について概説するとともに，われわれの技術改良により開発された，さらに有用性の高い新規CAR-T細胞療法についても紹介する．

CARの開発

CARの構造は，がん細胞の表面抗原を認識するモノクローナル抗体由来の軽鎖(V_L)と重鎖(V_H)の可変領域を結合させた一本鎖抗体(single chain variable fragment；scFv)と，膜貫通ドメインおよびT細胞を活性化するために必要な細胞内シグナル伝達ドメインを融合して作製される(図1)．CD3ζ鎖のみをシグナル伝達ドメインに用いたCARを第一世代CAR，CD3ζ鎖に加えてT細胞の共刺激分子であるCD28のシグナル伝達ドメインを組み込んだものを第二世代CAR，さらに4-1BBなどの別の共刺激分子のシグナル伝達ドメインを組み込んだCARを第三世代CARと呼ぶ．共刺激シグナルのない第一世代に比べ，第二世代，第三世代のCAR-T細胞は増殖能やサイトカイン産生能，細胞傷害活性が高く，生体内での長期間生存能力が示されている[1)~5)]．標的とするがん表面抗原の選択，scFvの親和性やヒト化，共シグナル分子の数や選択など，抗腫瘍効果をもたらすために最適なCARの構成デザインに関しては，現在世界中で積極的な検討が進んでいる．

T細胞にCARを発現させるための遺伝子導入技術としては，レンチウイルスあるいはレトロウイルスベクター，トランスポゾン，mRNAエレクトロポレーションなど，複数の手法が利用されている．レンチ・レトロウイルスベクターやトランスポゾンの使用では，導入遺伝子はホスト細胞のゲノムへ取り込まれるため，長期間かつ安定に発現することができる[6)~8)]．一方，mRNAエレクトロポレーション法ではCAR遺伝子発現は一過性であるが，ウイルスベクター導入に伴う潜在的なゲノム変異のリスクを回避できる利点がある[9)10)]．

* Chimeric antigen receptor(CAR)-expressing T cells for cancer immunotherapy.

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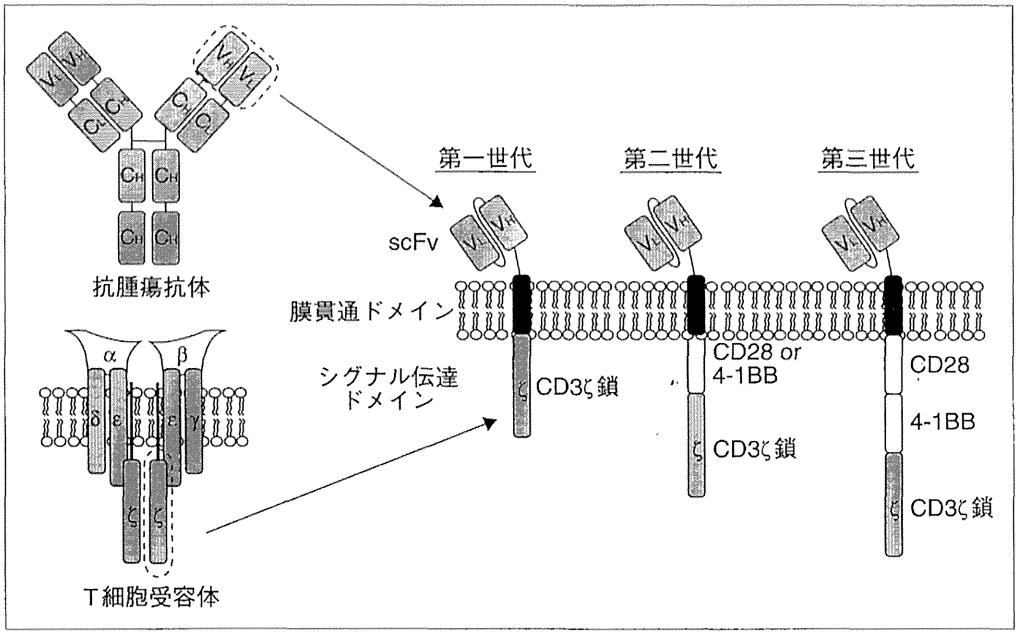

図1　キメラ抗原受容体(CAR)の構造
CARはがん細胞上の抗原に対する抗体(抗腫瘍抗体)の可変領域より作製された一本鎖抗体と細胞膜貫通ドメイン，および細胞内シグナル伝達ドメインの融合により形成された人工受容体である．細胞内シグナル伝達ドメインは，T細胞受容体に会合するCD3ζ鎖と刺激性共シグナル分子であるCD28や4-1BBの組み合わせにより形成される．

CAR-T細胞療法による臨床試験の現状

現在，CAR-T療法において最も臨床試験が進行しているのはCD19を発現するB細胞系造血器腫瘍を標的としたものである．CD19は非ホジキンリンパ腫や急性リンパ性白血病(acute lymphoid leukemia；ALL)，慢性リンパ性白血病(chronic lymphocytic leukemia；CLL)などで発現する一方，造血幹細胞や非B細胞系造血細胞，非リンパ組織には発現しない．CD19を標的としたCAR-T細胞を用いた臨床試験では，治療抵抗性の再発性リンパ腫や白血病に対する優れた臨床効果を複数のグループが報告している[3)11)~15)]．これらの報告では，scFvの配列，遺伝子導入の方法，輸注CAR-T細胞数，標的腫瘍の型などは必ずしも同一ではないが，第二世代CARが主に使用されていること，化学療法などによる前処置が行われていることは共通している．前処置による内在性免疫細胞の除去によりhomeostatic proliferationが誘導され，また免疫抑制環境が修正されることにより，移入したCAR-T細胞の増殖やエフェクター機能が増強し，優れた治療効果が誘導されたと考えられる[16)17)]．

CD19を標的としたCAR-T細胞による臨床試験を積極的に推進している米国ペンシルバニア大学の報告では，進行した成人CLL3例と再発化学療法抵抗性の小児ALL2例に対して，シクロホスファミドなどによる前処置の後にCAR-T細胞を投与することで優れた臨床効果が得られている[12)14)15)]．さらに最近では，より多くの症例数で検討が行われ，成人CLLでは32例中15人が治療反応性を示し，そのうち7例で最長3年間の完全寛解が認められている．また，小児ALLでは22例中19例，成人ALLでは5例全例で完全寛解が誘導されている．このように，CAR-T細胞療法は難治性の造血器腫瘍患者に対して高い奏効率を認めており，今後化学療法や造血幹細胞移植との組み合わせも含め，新たな治療選択肢の一つとして非常に期待されている．

現在のCAR-T細胞療法における課題

CAR-T細胞療法に伴う有害事象として，発熱，悪寒，倦怠感，筋肉痛，血圧低下などのサイトカイン放出症候群，腫瘍溶解症候群，CD19を標

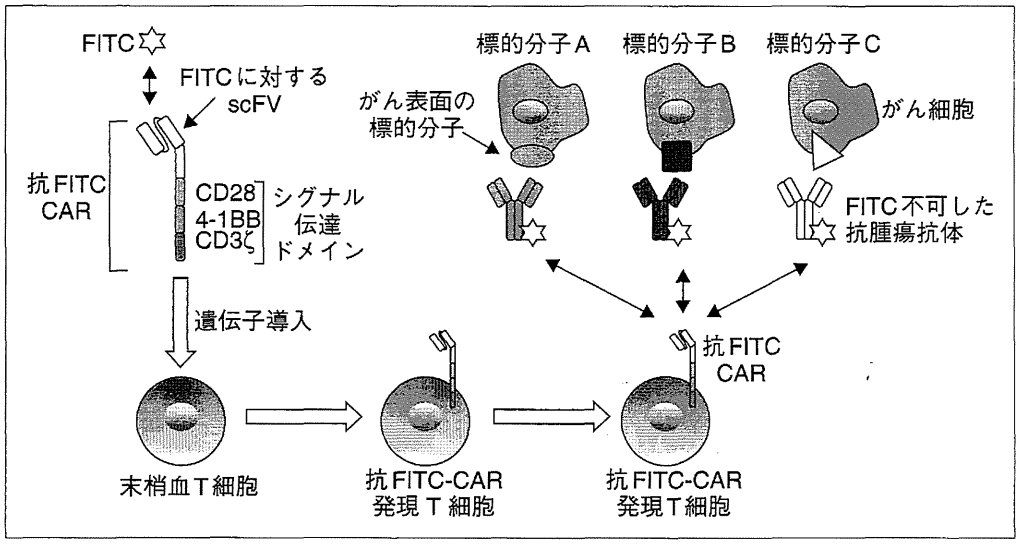

図2　複数の標的分子を認識できるCAR-T細胞技術
FITCを認識するCARの遺伝子導入によりFITC認識CAR-T細胞を作製すると同時に，がん細胞上の標的分子に対する抗体にFITC付加したものを作製する．これらを同時投与することにより，がんに対する傷害活性を誘導できる．このシステムでは，複数のFITC付加抗腫瘍抗体を使用することにより，がん細胞上の複数の標的分子を認識し，傷害することが可能である．

的とした場合におけるB細胞の長期欠損などが存在する．特に，B細胞系造血器腫瘍に対してCD19認識CD28/CD3ζ型CAR-T細胞を投与した臨床試験では，残存腫瘍細胞量に比例して重度なサイトカイン放出症候群が認められた[18)19)]．サイトカイン放出症候群については，ステロイドや抗interleukin(IL)-6受容体抗体，tumor necrosis factor(TNF)阻害剤の投与により制御可能な場合も多いが，ときに死亡に至るような重篤な場合もあるため，細心の注意が必要である．また，治療開始後，比較的遅い時期に腫瘍溶解症候群が認められる場合がある[14)]．長期のB細胞欠損に対しては，精製イムノグロブリンの投与による抗体補填が必要となる．

CAR-T細胞療法における重要な課題の一つはon-target off-tumor toxicityである．これは，たとえ微量であっても標的分子ががん以外の正常組織にも発現している場合，それに対してCAR-T細胞が傷害活性を示すことにより発生する有害事象である．たとえば，ErB2特異的なCAR-T細胞を投与後，呼吸障害や肺浸潤を認め，多臓器不全により死亡した例が報告されており，これは肺上皮細胞における微量のErB2発現が原因と考えられている[20)]．したがって，CAR-T細胞療法では，がん細胞にきわめて高い特異性で発現している分子を標的抗原とすることが重要である．

CAR-T細胞療法での有害事象に対する対応策として，CAR-T細胞を必要に応じて速やかに除去するための自殺遺伝子あるいは中和抗体の標的分子をあらかじめ遺伝子導入しておく，という方法が考案されている．たとえば，薬剤誘導性に発現するcaspase9遺伝子を導入したCAR-T細胞は，薬剤投与により速やかにアポトーシスにより除去されることが動物モデルで確認されている[21)22)]．また，CD20やEGFRを導入したCAR-T細胞は，これらの分子に対する抗体の投与により除去できることが報告されており，このような手法のヒトへの臨床応用が期待されている[23)~25)]．

CAR-T細胞療法の効果や有用性の向上を目指した技術改良

現在のCAR-T細胞療法は，がん種により異なるものの，単一のがん関連分子を標的としており，標的分子の発現がheterogeneousであるがん種では，その効力が不十分である可能性が高い．また，治療に応じて，標的分子を欠失，変異したがん細胞が選択的に増殖する危険性を含んでいる．そこで筆者らは，このような問題点を克服

するための技術改良に取り組み，複数の標的分子を同時に認識できる新規CAR-T細胞技術を開発した．この手法は，がん細胞上の標的分子を認識する抗体にFITC(fluorescein isothiocynate)を付加する一方で，FITCを認識するCAR-T細胞を作製し，これらを同時投与することで抗体を介したがん細胞傷害活性を誘導するものである(図2)[26]．このシステムでは，複数のFITC付加抗体と併用することで，単一のFITC認識CAR-T細胞が複数のがん標的分子を同時に攻撃することが可能である．また，個々の標的分子に応じてCARコンストラクトを構築する必要がなく，さまざまな抗体との組み合わせでユニバーサルに応用可能であり，その汎用性がきわめて高いことが利点である．

われわれの検討では，FITC認識CAR-T細胞はFITC付加した抗EGFR抗体存在下ではEGFR陽性膵がん細胞を傷害する一方，同一の細胞がFITC付加した抗Her2抗体存在下ではHer2陽性乳がん細胞を傷害することが示された[26]．さらに，マウスモデルを用いた実験では，FITC付加した抗腫瘍抗体とFITC認識CAR-T細胞の投与により，あらかじめ生着した固形腫瘍の完全拒絶およびマウスの長期生存が認められた．これらのデータはFITC認識CAR-T細胞療法の高い汎用性，有用性および優れたがん治療効果を示すものである．

おわりに

CAR-T細胞療法は，B細胞系の造血器悪性腫瘍をはじめとする進行がんに対して非常に高い奏効率を示しており，抗PD-1/PD-L1抗体などのチェックポイント阻害剤に加えて，今後のがん免疫療法を担う革新的な治療法である．一方，最適なCARコンストラクトの開発，がん特異的に発現する標的抗原の選択，サイトカイン放出症候群やon-target off-tumor toxicityのコントロールなどのさまざまな課題が存在する．さらに，固形がんに対してどの程度の臨床効果を示すのかはいまだ十分に検証されていない．今後さらなる研究成果の蓄積により，最少の副作用で最大限のがん治療効果をもたらすようなCAR-T細胞療法の確立が期待される．

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Glypican 3 Expression in Pediatric Malignant Solid Tumors

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Abstract

Purpose Glypican 3 (GPC3) is one of the cell surface heparan sulfate proteoglycans that binds to the cell membrane, and it is known as an oncofetal protein in adult malignant tumors. Clinical trials using a GPC3 peptide vaccine have already been started in Japan as a new immunotherapy for hepatocellular carcinoma in adult patients. To investigate the possibility of GPC3 immunotherapy for pediatric malignant tumors, we assessed the expression of GPC3 in pediatric malignant tumors.

Methods Immunohistochemically, the GPC3 expression was examined in 159 pediatric solid tumors, including 35 cases of neuroblastoma, 30 cases of Wilms tumor, 10 cases of hepatoblastoma, 25 cases of germ cell tumors, 56 cases of rhabdomyosarcoma, and 3 cases of other tumors. In addition, to clarify the physiological expression during the fetal to neoinfantile period, autopsy specimens of subjects without any neoplastic diseases were assessed in 9 fetal cases and 21 neoinfantile cases. The serum levels of GPC3 were also analyzed using specimens obtained from 53 subjects by the sandwich enzyme-linked immunosorbent assay method.

Results Histologically, a high rate of GPC3 expression was noted in 10 (90.9%) of the 11 subjects with yolk sac tumors and 6 (60.0%) of the 10 subjects with hepatoblastoma. In addition, 9 (30.0%) of the 30 subjects with Wilms tumors and 14 (25.0%) of the 56 subjects with rhabdomyosarcoma were positive for the expression of GPC3. Concerning autopsy specimens, most of the 23 subjects younger than 7 months showed positive findings in the liver (94.7%) and kidney (81.8%). Two subjects (100%) with yolk sac tumors and six (75.0%) of the eight subjects with hepatoblastoma serologically demonstrated a high rate of positive expression. Concerning the distribution of the serum GPC3 level according to age, 8 (80.0%) of the 10 subjects younger than 1 year showed a positive finding, while only 16 (37.3%) of the 43 subjects older than 1 year showed a positive finding.

Conclusion Most cases of hepatoblastoma and yolk sac tumor, and some cases of other tumors were found to express GPC3 either histologically or serologically. On the other hand, GPC3 was physiologically expressed during the fetal and neoinfantile period under 1 year of age. Although, more preliminary data and experience are required,

Keywords
- glypican 3
- tumor marker
- immunotherapy

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patients older than 1 year that show a positive finding for GPC3 are considered to be appropriate candidates to receive the new immunotherapy using GPC3 peptide vaccination.

Introduction

Glypican 3 (GPC3) is a cell surface heparan sulfate proteoglycan that is linked to the extracytoplasmic cell-surface membrane by a glycosylphosphatidylinositol anchor.[1] GPC3 is associated with cell growth, development, and the responses to various growth factors.[2] Gonzalez et al described its role as a negative regulator of inhibitory growth factors.[3] GPC3 inactivation has been found to be responsible for X-linked Simpson–Golabi–Behmel (SGB) overgrowth syndrome. In SGB syndrome, 10 to 20% of the patients described have an embryonal malignancy, including hepatoblastoma, neuroblastoma, gonadoblastoma, Wilms tumor, or hepatocellular carcinoma.[4]

Recent studies have shown that there is an overexpression of GPC3 in hepatocellular carcinoma, and has its usefulness as a novel diagnostic marker in many series.[5] Furthermore, the expression of GPC3 has also been reported in other malignant tumors, such as malignant melanoma,[8] clear cell adenocarcinoma of the ovary,[9] and malignant germ cell tumors in adult subjects.[10] Ota et al reported the immunoreactivity of adult testicular tumors, including a yolk sac tumor, teratoma, and choriocarcinoma, as well as a seminoma and embryonal carcinomas. The author demonstrated a high rate of immunoreactivity for the yolk sac tumor.[10]

GPC3 expression has not yet been widely analyzed in pediatric tumors and the roles of GPC3 expression are still unclear. The expression of GPC3 mRNA in several cell lines, including those derived from neuroblastomas, Wilms tumors, and hepatoblastomas, has been reported.[11,12] In addition, Zynger et al examined 65 cases of hepatoblastoma by immunohistochemistry and all subjects exhibited a positive reaction.[13] Zynger et al speculated that GPC3 has a role in the tumorigenesis of hepatoblastoma.

In this study, we analyzed the expression of GPC3 in pediatric malignant solid tumors and assessed the clinical implications of its expression.

Materials and Methods

The immunohistochemical studies examined 159 pediatric solid tumors, including 35 cases of neuroblastoma, 30 cases of Wilms tumor, 10 cases of hepatoblastoma, 25 cases of germ cell tumors (11 yolk sac tumors, 4 immature teratomas, and 10 mature teratomas), and 56 cases of rhabdomyosarcoma and 3 cases of other tumors (2 undifferentiated sarcomas and 1 case of Ewing sarcoma) treated at our institution. The serum levels of GCP3 were also analyzed in samples obtained from 53 subjects, including 13 cases with neuroblastoma, 10 cases of Wilms tumor, 8 cases of hepatoblastoma, 16 cases of germ cell tumors (2 cases with yolk sac tumors, 4 cases with immature teratomas, and 10 cases with mature teratomas), 3 cases of rhabdomyosarcoma, and 3 cases of other tumors by the sandwich enzyme-linked immunosorbent assay (ELISA) method using a GPC3 ELISA kit (Bio Mosaics, Burlington, Vermont, United States).

In addition, to clarify the physiological expression during the fetal to neoinfantile period, autopsy specimens from subjects without any neoplastic disease were assessed by immunohistochemistry. These included samples from 9 fetal cases (age, 19–41 weeks) and 21 neoinfantile cases.

For the immunohistochemical analysis the streptavidin-biotin-peroxidase method (Histofine SAB-PO Kit, Nichirei, Tokyo, Japan) was used. A GPC3 monoclonal antibody (Bio Mosaics) was used at 1:200 dilution.

The serum levels of GPC3 were analyzed by a sandwich ELISA method using an ELISA kit. The samples were diluted at 1:4 and 100 µL of samples or of GPC3 standards were pipetted into the appropriate wells. Covered wells were incubated overnight at 2 to 8°C. After washing the wells five times with wash buffer, 200 µL of a biotin-conjugated anti-GPC3 antibody was pipetted into each well. After overnight incubation, the wells were washed with buffer and 200 µL of streptavidin-horseradish peroxidase conjugated diluents were added to each well. After 30 minutes of incubation, 200 µL of tetramethylbenzidine substrate solution was added to each well for 30 minutes. After these procedures, the absorbance of each well was analyzed by a spectrophotometric plate reader. Based on the data of healthy adult subjects with the standard deviation, the cut-off level for GPC3 was defined as 178 ng/mL in this study.

The patient's parents provided consent for obtaining tumor and tissue preservation and for the subsequent biological analyses. This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan on July 30, 2003.

Results

Histologically, a high rate of GPC3 expression was noted in 10 (90.9%) of the 11 subjects with yolk sac tumors and in 6 (60.0%) of the 10 subjects with hepatoblastoma (►**Table 1** and ►**Figs. 1a, b**). In addition, 9 (30.0%) of the 30 subjects with Wilms tumor (►**Fig. 1c**), 14 (25.0%) of the 56 subjects with rhabdomyosarcoma, and 1 (2.9%) of the 35 subjects with neuroblastoma were positive for the expression of GPC3.

Similarly, 2 subjects (100%) with yolk sac tumors and 6 (75.0%) of the 8 subjects with hepatoblastoma serologically demonstrated a high rate of positive expression, while 1 (33.3%) of the 3 subjects with rhabdomyosarcoma, 4 (30.7%) of the 13 subjects with neuroblastoma, and 1

Table 1 The results of the immunohistochemical and serological analysis of glypican 3

Histology		GPC3	
		Tissue GPC3 (immunohistochemistry)	Serum GPC3 (ELISA)
HB	Hepatoblastoma	6/10 (60.0%)	6/8 (75.0%)
NBs	Neuroblastoma	1/35 (2.9%)	4/13 (27.3%)
WT	Wilms tumor	9/30 (30.0%)	1/10 (10.0%)
RMS	Rhabdomyosarcoma	14/56 (25.0%)	1/3 (33.3%)
GCT	Yolk sac tumor	10/11 (90.9%)	2/2 (100%)
	Immature teratoma	1/4 (25.0%)	3/4 (75.0%)
	Mature teratoma	0/10 (0.0%)	5/10 (50.0%)
Others	Undifferentiated sarcoma	1/2 (50%)	2/2 (100%)
	Ewing sarcoma	0/1 (0.0%)	0/1 (0.0%)
Total number		159	53

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GCT, germ cell tumor; GPC3, glypican 3; HB, hepatoblastoma; NBs, neuroblastoma and associated tumor; RMS, rhabdomyosarcoma; WT, Wilms tumor.

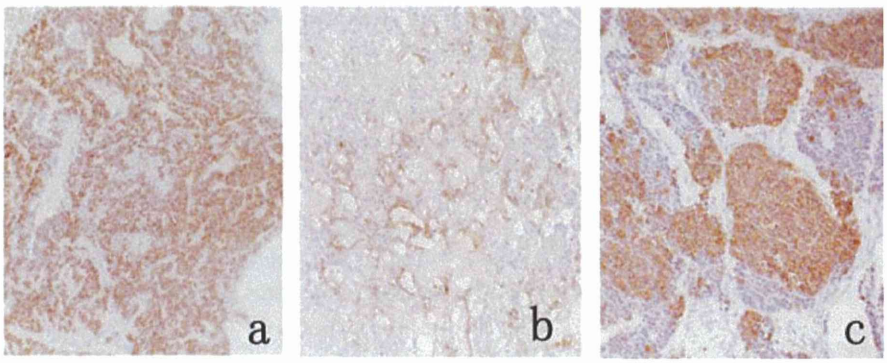

Fig. 1 Immunohistochemical findings: (a) hepatoblastoma, 2-year old; (b) yolk sac tumor, 12-year old; (c) Wilms tumor, 3-year old.

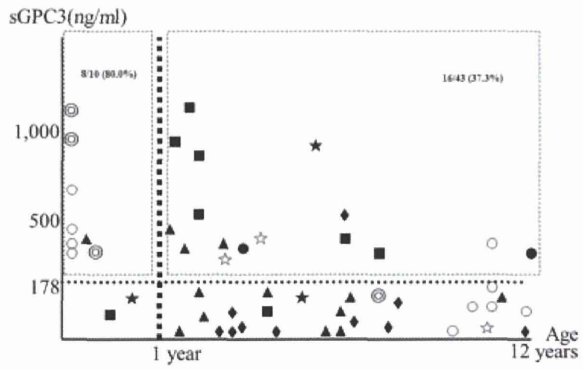

Fig. 2 The distribution of the serum glypican 3 (GPC3) levels according to age. Samples from 8/10 (80.0%) patients younger than 1 year were serologically GPC3-positive, while only samples from 16/43 (37.2%) patients older than 1 year were serologically GPC3-positive. ■, hepatoblastoma; ▲, neuroblastoma and associated tumors; ♦, Wilms tumor; germ cell tumor; ●, yolk sac tumor; ◎, immature teratoma; ○, mature teratoma; ★, rhabdomyosarcoma; ☆, others.

(10.0%) of the 10 subjects with Wilms tumor demonstrated a positive finding (►**Fig. 2**).

Concerning the distribution of the serum GPC3 level according to age, 8 (80.0%) of the 10 subjects younger than 1 year showed a positive finding. In contrast, only 16 (37.2%) of the 43 subjects older than 1 year showed a positive finding.

Concerning the autopsy specimens, most of the 23 subjects younger than 7 months (including 9 fetal and 14 neoinfantile subjects) showed positive findings in the liver (94.7%) and kidney (81.8%) (►**Table 2**) (►**Figs. 3a–d**). The other six subjects older than 1 year did not demonstrate a positive finding in any organ.

The clinical course of one representative case was as follows (►**Fig. 4**). The subject had an undifferentiated sarcoma that was diagnosed when the patient was 4 years and 6 months old. The serum α-fetoprotein (AFP) level was low, the serum GPC3 level was 334 ng/mL, and the biopsy specimen was immunohistochemically positive for GPC3. The level of serum GPC3 normalized following preoperative intensive

Table 2 The results of the immunohistochemical analysis for autopsy specimens

Age	CNS	Heart	Lung	Liver	Kidney	Pancreas	Spleen	Adrenal gland	Thymus	GI tract
19 wk	–	ND	–	+	+	+	–	ND	ND	–
19 wk	–	ND	–	ND	+	ND	–	ND	–	ND
21 wk	ND	–	–	+	+	–	–	–	ND	ND
21 wk	–	–	–	+	+	–	–	ND	–	–
24 wk	–	–	–	+	+	ND	–	ND	ND	–
24 wk	–	–	–	+	+	ND	–	ND	ND	–
32 wk	–	–	–	+	+	–	–	–	–	–
38 wk	–	–	–	+	+	ND	–	–	+	–
41 wk	–	–	–	+	ND	ND	–	–	–	ND
0 d	–	–	–	+	+	+	–	–	–	–
0 d	ND	–	–	+	+	–	–	–	–	–
0 d	–	–	–	ND	+	ND	–	ND	ND	–
0 d	–	–	–	ND	–	ND	–	ND	ND	–
0 d	ND	–	–	+	+	–	–	–	ND	–
1 d	ND	–	–	+	+	ND	–	–	–	ND
12 d	ND	–	ND	+	–	–	–	–	–	–
14 d	–	–	–	ND	+	+	–	–	–	–
1 mo	ND	–	–	+	–	–	–	–	–	–
3 mo	ND	–	–	+	–	–	–	ND	–	ND
4 mo	ND	–	–	+	+	–	–	ND	–	ND
6 mo	ND	–	–	+	+	–	–	–	ND	–
6 mo	ND	ND	–	–	+	–	–	–	ND	–
7 mo	ND	–	–	+	+	–	–	ND	–	ND
8 mo	ND	–	–	ND	ND	ND	ND	ND	ND	ND
1 y 0 mo	–	–	–	ND	–	–	ND	–	–	ND
1 y 0 mo	–	–	–	–	–	–	–	–	ND	–
1 y 4 mo	–	–	–	ND	–	–	–	–	ND	–
9 y	–	ND	ND	ND	ND	ND	ND	ND	ND	ND
9 y	ND	–	–	ND	ND	ND	ND	ND	–	ND
10 y	–	–	–	–	–	–	–	–	ND	–

Abbreviations: CNS, central nervous system; +, positive; –, negative; ND, not done.

chemotherapy and was maintained within a normal range thereafter. Furthermore, the specimen obtained by radical surgery showed no viable tumor cells and the tissue was immunohistochemically negative for GPC3. After the treatment, the patient has survived for 5 years without any events and the patient's GPC3 level is normal. In this case, the serum GPC3 level was useful as an independent tumor marker.

Discussion

In recent years, several authors have reported the diagnostic value of the serum GPC3 level in hepatocellular carcinomas and other kinds of malignant tumors in adults. In the field of GPC3 research, the expression levels in fetal tissue have been discussed by several authors. Immunohistochemically, three cases of fetal liver tissue were found to be positive, although the benign pediatric liver was negative.[13] In this way, GPC3 has been considered to be a kind of oncofetal protein and to be associated with tumorigenesis in pediatric malignant tumors. However, the clinical implications of the GPC3 expression levels as a diagnostic marker or for the monitoring of tumor progression or curability have not yet been sufficiently analyzed.

From the current data, most hepatoblastomas and yolk sac tumors showed positive findings for both serum and tissue GPC3. In most subjects younger than 1 year, there was a tendency toward a higher level of GPC3 expression compared with subjects older than 1 year. In particular, newborn patients with germ cell tumors, including mature and immature teratomas, exhibited a high level of serum GPC3. Based

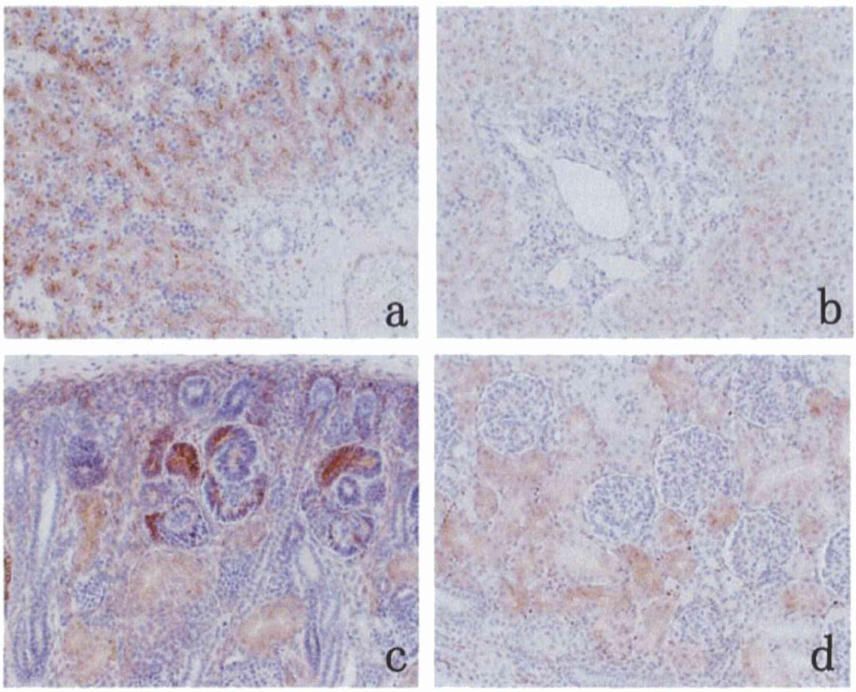

Fig. 3 Immunohistochemical findings for autopsy specimens: (a) fetal liver, 19 weeks, strongly positive; (b) infantile liver, 7 months, moderately positive; (c) fetal kidney, 19 weeks, strongly positive; (d) infantile kidney, 7 months, moderately positive.

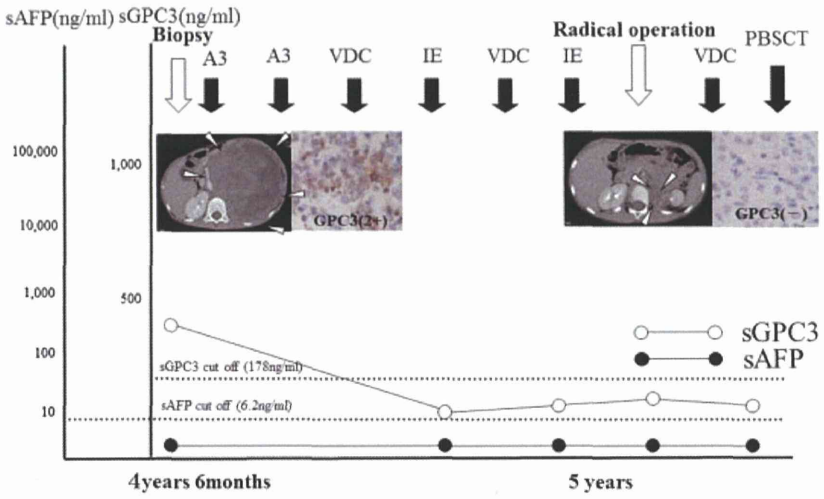

Fig. 4 Clinical course of one case with undifferentiated sarcoma. A3, vincristine + THP-adriamycin + cyclophosphamide + cisplatin; IE, ifosfamide + etoposide; sAFP, serum α-fetoprotein; sGPC3, serum glypican 3; VDC, vincristine + doxorubicin + cyclophosphamide.

on the results of autopsy specimens, we can speculate that GPC3 expression can be observed from the fetal to early neoinfantile period for younger than 1 year regardless of whether malignancy is present. For the subjects older than 1 year, the data from patients who were positive for serum GPC3 but negative for serum AFP imply that GPC3 may be an independent novel tumor marker.

The role of GPC3 as a novel tumor marker for hepatocellular carcinoma in adults has been widely debated in recent studies. A trial using GPC3-targeted immunotherapy for the prevention of cancer development and recurrence has already begun.[14] The same trial protocol would be acceptable to treat and prevent pediatric malignant tumors. However, the number of this series is small, more preliminary data and experience are required to conclude this suitability for immunotherapy.

Conclusion

Most cases of hepatoblastoma, yolk sac tumors and some cases of neuroblastoma, Wilms tumor, and rhabdomyosarcoma were found to express GPC3 either histologically or serologically. On the other hand, GPC3 was also physiologically expressed during the fetal and neoinfantile period in

subjects younger than 1 year. Because the patients older than 1 year who show a positive finding for GPC3 are considered to be appropriate candidates to receive the new immunotherapy using the GPC3 peptide vaccination.

Conflict of Interest

None.

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