Glycoconj J (2015) 32:39–47
DOI 10.1007/s10719-014-9569-z

ORIGINAL ARTICLE

Expression of ST3Gal, ST6Gal, ST6GalNAc and ST8Sia in human hepatic carcinoma cell lines, HepG-2 and SMMC-7721 and normal hepatic cell line, L-02

Yan Zhang · Wenjin Zhao · Yujie Zhao · Qun He

Received: 22 September 2014 / Revised: 25 November 2014 / Accepted: 26 November 2014 / Published online: 9 January 2015

Abstract We measured ST3Gal, ST6Gal, ST6GalNAc and ST8Sia expression in human hepatic carcinoma cell lines, HepG-2 and SMMC-7721 and normal hepatic cell line, L-02 to reveal the relationship between hepatic carcinoma cell lines sialyltransferases expression and cell membrane sialic acid sugar chains. Membrane sialic acid sugar chains in L-02, HepG-2 and SMMC-7721 cell lines were measured with lectin microarrays to find expression profiles. Expression of 20 sialyltransferases was measured with DNA microarray. qRT-PCR and Western blot were used to verify DNA microarrays data. Siaα 2-3Galβ1-3[Siaα2-6GalNAc]α-R and Siaα 2-6Gal/GalNAc sugar chains in hepatic carcinoma cell lines, HepG-2 and SMMC-7721 were upregulated, and 7differentially expressed sialyltransferases were captured. ST3Gal-IV and ST6Gal I were overexpressed and ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAcII and ST6GalNAcVI were downregulated in HepG-2 and SMMC-7721 cell Lines, compared with control cell line. ST6GalNAc-IV and ST8sia expressions were not detected. Other sialyltransferases were not different among cell lines. Results from qRT-PCR and Western blot were consistent with DNA microarray. Overexpression of ST3Gal-IV and ST6Gal I in HepG-2 and SMMC-7721 cell lines may correlate with upregulation of Siaα 2-3Galβ1-3[Siaα2-6GalNAc]α-R and Siaα 2-6Gal/GalNAc sugar chains on cell membranes.

Key words Hepatic carcinoma · Sialic acid sugar chain · Sialyltransferase · Lectin microarray · DNA microarray

This work was supported by a grant from the National Natural Science Foundation of China (awarded to Qun He, Grant No. 20672144).

Y. Zhang
Department of Biochemistry, College of Basic Medicine, Shenyang Medical College, Shenyang 110034, Liaoning, China

W. Zhao
Center of Laboratory Technology, China Medical University, Shenyang 110001, Liaoning, China

Y. Zhang · Y. Zhao (✉)
Biochip Center, College of Basic Medicine, China Medical University, No.92, Beier Road, Shenyang, Liaoning Province 110001, China
e-mail: yjzhao@mail.cmu.edu.cn

Q. He (✉)
Key Laboratory of Cell Biology,Ministry of Education, Basic Medical School, China Medical University, No.92, Beier Road, Shenyang, Liaoning Province 110001, China
e-mail: hequn@mail.cmu.edu.cn

Introduction

Sugar chains on membrane surface not only participate in basic vital movements, but also contribute to cancer development [1]. Glycosyltransferase expression differences may give rise to changes in membrane sugar chains. Therefore, investigating glycosyltransferases may help to clarify how these contribute to tumorigenesis and progression. Sialic acids on tumor cell membrane are involved in tumorigenesis, progression, invasion and metastasis [2, 3]. At present, sialyltransferases can be further classified into four families according to their substrates and tissue distribution specificity: ST3Gal (α2, 3-ST), ST6Gal (α2, 6-ST), ST6GalNAc and ST8Sia (α2, 8-ST) families. All enzymes in the ST3Gal family that transfer a Neu5Ac residue inform the α2-3-linkage to terminal Gal residues found in glycoproteins or glycolipids. Enzymes in the ST6Gal family are only comprised of ST6Gal I and II, which both use Galß1→4GlcNAc-R as their acceptor substrate. Enzymes in the ST6GalNAc family catalyze the transfer of Neu5Ac residues in α2-6 linkages to the GalNAc residues found in O-glycosylproteins or in glycolipids. Enzymes in the ST8Sia family mediate the transfer of Neu5Ac residues in α2-8-linkage to other Neu5Ac residues found in glycoproteins and glycolipids [4].

Hepatic carcinoma is one of the most common and malignant tumors worldwide, but even after much investigation, its pathogenesis remains unclear. Researches showed that the expression and activity of cellular glycosyltransferases change during hepatoma formation and development, but the degree to which this contributes to tumorigenesis is still unknown. So far, in our previous studies, we've found that sugar chain number and category changes in hepatic carcinoma cell line membranes in mice and that sialic acid increase. These findings are related to cellular sialyltransferase expression [5]. In this study, we frist measured cell membrane sialic acid sugar chain expression in HepG-2 and SMMC-7721 cell lines with lectin microarrays, compared with control cell line. Next, we measured expression of 20 sialyltransferases in HepG-2 and SMMC-7721 cell lines using DNA microarray. These data suggest a relationship between sialyltransferase expression and sialic acid sugar chains in human hepatic carcinoma cell lines, HepG-2 and SMMC-7721. These findings may help to reveal how sialyltransferases participate in hepatic carcinoma tumorigenesis and development.

Materials and methods

Cell culture

Human L-02, HepG-2 and SMMC-7721 cells were cultured in RPMI 1640 medium with 10 % fetal bovine serum (FBS), at 37 °C in 5 % CO2. Medium was changed every 2 days and cultures were transferred every 3 days with 0.125 % trypsin.

Cell harvesting and fluorescent staining

Culture medium in the cell culture bottle was removed, and cells were washed twice with 2 ml of PBS. The cells were digested with 1 ml of 0.25 % collagenase (D-Hank's) for 3 min at 37 °C, and the mixture was removed by blowing into the microtube and centrifuging at 1,000 rpm for 5 min. The supernatant was removed, and the cells were resuspended in 2 ml PBS. Cells were rinsed two times. Next, cells were incubated with 10 μl acridine orange for 5 min and then centrifuged at 1,000 rpm for 5 min. The supernatant was removed and cells were rinsed with 2 ml PBS, then the cell concentration was adjusted to 105/ml.

Sugar chain meausrements

Lectin microarrays were produced by our laboratory as previously described [6]. Briefly, Putting the Lectin microarrays into the box containing PB (0.01 mol/L, pH 7.2) at 37 °C for 30 min(This process is called hydration), blocking with 0.5 % of casein solution for 5 min. Then rinses were done twice with PB for 5 min. The lectin microarray slide was overlaid with a PBS suspension of acridine orange-labeled cells. Slides were incubated 37 °C for 40 min and then washed twice with PBS (pH 7.2) for 5 min. Slides were then fixed with PBS containing 3 % of glutaraldehyde (PBS, pH7.2) for 10 min. Fluorescent intensity was measured by GeneTACTMLS IV (Gain 55, Black 0) to obtain expression profiles.

Sialyltransferase expression: total RNA isolation and fluorescent labeling

Total RNA was isolated separately from human L-02, HepG-2 and SMMC-7721 cell lines using TRIzol reagent (Invitrogen, Los Angeles, CA) according to the manufacturer's protocol and RNA concentrations were quantified via spectrometry. cDNA was reverse-transcribed using a Takara PrimeScript RT reagent kit (Takara, Japan) and fluorescently labeled by Cy5-dUTP (GE, Pittsburgh, PA). cDNA was cut into 100 bp long segments using restriction endonuclease CviKI-1 (Biolabs, N.Y. New Jersey).

Sialyltransferase expression

DNA microarrays were produced by our laboratory as previously described [6]. Sialyltransferases oligonucleotide probes were designed and synthesized by GenScript (Nanjing) Co., Ltd. (Table 1). Amino-modified oligonucleotide P442 (ttttttttttgctatgcctcatcttcttgttgggtaccaaa) was as the hybridization positive control and this could specifically bind to (NH2)P442(CY5) (Fig. 2d). DNA microarrays were washed with 1×prehybridization buffer solution (5×SSC, 5×Denhart, 1 % SDS, 100 μg/ml denatured salmon sperm DNA) for 15 min, blocked with 0.5 % of casein solution (PB, 0.01 mol/L, pH 7.2) for 10 min and then rinsed twice with 1× prehybridization buffer solution for 5 min. Each L-02, HepG-2 and SMMC-7721 cell line hybridization samples contained 7 μl cDNA (100 μg/μL), 1 μl (NH2) P442 (CY5) (100 μg/μL) and 2 μl1× hybridization buffer solution (0.15 mol/L KCl; 0.01 mol/L Tris Cl; and1mmol/L EDTA). The microarrays were incubated at 37 °C for 18 h and then washed twice with 1× cleaning solution (0.1×SSC, 0.1 % SDS) for 10 min. Fluorescent signal intensity was measured with GeneTACTMLS IV (Gain 60, Black 0) to quantify sialyltransferase expression.

Sialyltransferases mRNA expression measurement with qRT-PCR

cDNA was reverse-transcribed separately from human L-02, HepG-2 and SMMC-7721 cells using Takara PrimeScript RT reagent kit (Takara, Japan) according to the manufacturer's protocol as previously described. Sialyltransferases mRNA was measured with qRT-PCR using an SYBR Premix Ex Taq™ II kit (Takara, Japan). Amplification conditions were

Table 1 Sialyltransferases oligonucleotide probes

Code	Gene	Sequences (5′–3′)	Site(5′)	Tm(°C)
A	ST3GalI	CTTACCAAAAGAGCACTGAATTTCCTA	6411	64
B	ST3GalII	TTCCAATTCCGAAAGTAGAAAGAGTAAA	3680	64.8
C	ST3GalIII	CCACATGTTCTAGGTTTCAGCAACA	2075	65.2
D	ST3GalIV	CTACAACAAGAAGCAGACCATTCACTAC	995	64.2
E	ST3GalV	CAGTGGAGGCATTGATCGTGAA	1329	65.3
F	ST3GalVI	AAATCAGAAACCTAAACACCCAACAA	1179	64.5
G	ST6GalNAcI	TTCCAAGGGAACACTTGAACCAT	2083	64.4
H	ST6GalNAcII	CCTACCCCAGATGCTAAAGTGATTC	1721	64.5
I	ST6GalNAcIII	CACCAAAGGTTATGAAGAAGATGTCG	435	65.3
J	ST6GalNAcIV	CACCAGCATCATGACCTTGTGC	1487	65.6
K	ST6GalNAcV	ATCCTGAATGATGGTTGGAAATGG	1739	65.9
L	ST6GalNAcVI	TCTAGCAGGGAGGTTTTCCAACTG	2006	65.8
M	ST6GalI	GCCTTTAGTAGGGACCTGCTCTGT	3407	64.4
N	ST6GalII	AGGCGATGAAGGATTACCTGACC	737	65.4
O	ST8SiaI	GCCTTGGAGAAGTCTGATAATAGTATGTAAA	8719	64.9
P	ST8SiaII	GCACCATCAACTCAACAAGTCAGAA	4768	64.2
Q	ST8SiaIII	TGAACCCATACTGTGCAGAAAAGC	8993	65.3
R	ST8SiaIV	GCGACTATCTCCCCAAAACGG	215	65.4
S	ST8SiaV	AGTGAAGAAAGTGATCTCAAGAGGTCC	2227	64.8
T	ST8SiaVI	ACGCTCGAAGAGTCTAAAGCAAGAC	823	64.8
U	β-actin(positive control)	TCAGCAAGCAGGAGTATGACGAG	1123	64.2
V	NEU3(negative control)	CTAGAGGAATTGAGCAAAACAGAAGAA	2103	64.1
W	(NH2)P442	TTTTTGCTATGCCTCATCTTCTTGTTGGGTACCAAA	375	64.7

30 s at 95 °C followed by 40 cycles of 5 s at 95 °C, and then 20 s at 60 °C in a Light Cycler (Roche Diagnostics). Primer pairs for PCR are listed in Table 2 and these were designed and synthesized with GenScript (Nanjing) Co., Ltd. Each sample was analyzed for three times and expression of target genes was measured relative to GAPDH and calculated as $2^{-(Ct_{\text{Target gene}} - Ct_{\text{GAPDH}})}$.

Sialyltransferase protein expression

Cells were grown to 70 % confluence, washed twice with cold PBS and incubated in lysis buffer (20 mM Tris–HCl, pH 7.5, 1 % of Triton, 1 mM Na2EDTA, 1 mM EGTA, 150 mM NaCl, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na3VO4, 1 μg/mL leupeptin and 1 mM phenylmethylsulfonyl fluoride) for 15 min. Protein lysates were measured with a Pierce BCA Protein Assay Kit (Beyotime Institute of Biotechnology) and 40 μg of protein was separated by 12 % SDS–PAGE (Bio-Rad, Hercules, CA). Separated proteins were transferred to a PVDF membrane (Millipore Corporation, Billerica, MA) over 2 h by means of a wet electroblotting system in ice water. The membrane was blocked in 5 % of BSA and incubated overnight with the primary antibodies (rabbit anti-ST3Gal-I, rabbit anti-ST6GalNAc-II, rabbit anti-ST6Gal-III, rabbit anti-ST3Gal-IV, rabbit anti-ST3Gal-V and rabbit anti-ST3Gal-VI (1:500; Abcam, Boston, MA); mouse anti-β –actin (1:1,500; Santa Cruz, Texas). After several rinses in TBST, PVDF membranes were incubated with secondary antibodies (goat anti-rabbit and goat anti-mouse linked to horseradish peroxidase, 1:5,000) for 1 h at 37 °C. β-actin was used as an internal reference for relative quantification. Immunoreactive bands were visualized with ECL (Thermo,Boston, MA). Immunoblots were scanned with a densitometer and were quantified with Quantity One software (BioRad Bio-Rad, Hercules, CA).

Statistical analysis

All data are presented as means ± SD. The student's *t*-test was used to evaluate differences between various groups and statistical significance was set at $P<0.05$. All statistical analyses were conducted using SPSS for Windows version 16.0.

Results

Lectin microarrays for measuring cell surface sialic sugar chains

AAL (Aleuria aurantia), SNA (Sambucus nigra), MAH-I (Maackia amurensis-I) and WGA (Triticum unlgaris) were

Table 2 QRT-PCR primer sequences for analysis of sialyltransferases

Gene	Primer	Sequences
ST3GalI	Sense	5′- ACCACCGACCTGCAGTGGGT - 3′
	Antisense	5′- GGGGGCACGGGGACATAGGT -3′
ST3GalII	Sense	5′- TCCGATTCACCTATGCCCCAGT -3′
	Antisense	5′- TGCTCGGTCCACCTGTCGTGA -3′
ST3GalIII	Sense	5′- CCGCTGGACAAACCCTAGGCAC - 3′
	Antisense	5′- GGCTAGCTCGGCAGGCAGTTT -3′
ST3GalIV	Sense	5′- ACCTATGAGCTGCCCTTTGGGACT -3′
	Antisense	5′- GACACTCGAGGCTCTTTATGCTCTC -3′
ST3GalV	Sense	5′- TCAGTCAAGGTTCTGGGGCCGA -3′
	Antisense	5′- CCCGCCAAACTGACTTCATCGCA -3′
ST3GalVI	Sense	5′- GACCTTCGAGACATATTTCAGCTCG -3′
	Antisense	5′- TCCATTACCAACCACCACACACCT -3′
ST6GalI	Sense	5′- AGCGCTTCCTCAAAGACAGT -3′
	Antisense	5′- TCCGGATTCTGGTACCACTTTG-3′
ST6GalII	Sense	5′- GGGAAGAAGGCTGGTTCATTCT-3′
	Antisense	5′- GTGGTTTCATGGCAGGTCTCT-3′
ST6GalNAcI	Sense	5′- GTCCGCTACTTGCACTTCCT-3′
	Antisense	5′- GGTCTGTGCCTGAACCAGAA-3′
ST6GalNAcII	Sense	5′- GGGCTCTCTCACCAAGTCAT-3′
	Antisense	5′- CGGATACACTTTGGAGGGGT-3′
ST6GalNAcIII	Sense	5′- TATACGTGACCACAGAGAAGCG-3′
	Antisense	5′- TCACTCTGTACTGTCCTTCCCA-3′
ST6GalNAcIV	Sense	5′- CAAGAACCGGAGGCAGTCG-3′
	Antisense	5′- CACGAGCCGACCCTCTCTA-3′
ST6GalNAcV	Sense	5′- GGACCACAAGCCCCTGAAAAT-3′
	Antisense	5′- CTGGTCAATCTGGGAGCCTT-3′
ST6GalNAcVI	Sense	5′- GTGTTCCCCAACATGGAAGC-3′
	Antisense	5′- CGAATGAGACTTCTCCCTGTCC-3′
ST8SiaI	Sense	5′- GGTGGGAAATGGTGGGATTCT-3′
	Antisense	5′- AGGGAGATTGCATCGCATGAC-3′
ST8SiaII	Sense	5′- AAATCGGGAATTCGGGAGGC-3′
	Antisense	5′- CTGGTGATGAGGAGCCGTTT-3′
ST8SiaIII	Sense	5′- GCTCATCAGCTACGTGTCCC-3′
	Antisense	5′- GCGCAAATTGTGACCGGA-3′
ST8SiaIV	Sense	5′- GCGCAAATTGTGACCGGA-3′
	Antisense	5′- GTGCTGGAAGATTGAAGAGCC-3′
ST8SiaV	Sense	5′- GACTTCGTCTTCCGGTGCAA-3′
	Antisense	5′- TCACAGTGACCACATCCGTC-3′
ST8SiaVI	Sense	5′- TCCAGTGTCCCAGCCTTTTG-3′
	Antisense	5′- TGGTTGGGGGTAGGTTACAC-3′
GAPDH	Sense	5′- TTCTTTTGCGTCGCCAGCCGAG -3′
	Antisense	5′- CCAGGCGCCCAATACGACCAAA -3′

the lectin types fixed on lectin microarrays that specifically bound to sialic acids. BSA was used as a negative control. Figure 1a shows that MAH-I and SNA fluorescent intensities increased in hepatic carcinoma cell lines, hepG-2 and SMMC-7721 compared with control cell line. AAL and WGA were not seen different. According to lectin affinities (Table 3), Siaα2-3Galβ1-3[Siaα2-6GalNAc]α-R and Siaα 2-6Gal/GalNAc sialic acids were overexpressed on cell membranes in hepG-2 and SMMC-7721 cell lines compared with L02 cell line.

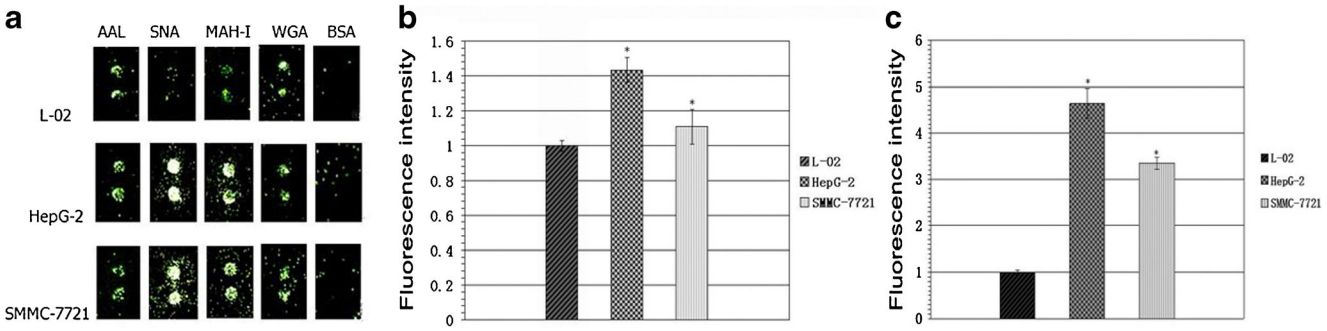

Fig. 1 Lectin microarrays to detect cell surface sialic sugar chains. **a** After fluorescent scanning by lectin microarrays (Gain 55, Black 0), 4 lectins have different fluorescent intensities. MAH-I and SNA fluorescent intensities are increased in hepG-2 and SMMC-7721 cell lines, compared with L02 cell line; while AAL and WGA showed no obvious difference. **b** Relative fluorescent intensities of MAH-I in L-02, HepG-2 and SMMC-7721 (*P<0.05, n=6). **c** Relative fluorescent intensities of SNA in L-02, HepG-2 and SMMC-7721 (*P<0.05, n=6)

Sialyltransferase expression in L-02, HepG-2 and SMMC-7721 cells

Figure 2 and Table 4 show that ST3Gal-IV and ST6Gal-I were upregulated and ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI were downregulated in hepG-2 and SMMC-7721 cell lines compared with L02 cell line and ST6GalNAc-IV and ST8sia were not detected. Other genes studied were not seen different.

Sialyltransferase mRNA expression

To verify DNA microarray studies, qRT-PCR was used to detect sialyltransferase mRNA expression in L-02, HepG-2 and SMMC-7721 cell lines. qRT-PCR data agreed with DNA microarray results. ST3GalI-V and ST6GalI were upregulated (Fig. 3a), while ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI were downregulated (Fig. 3b) in hepG-2 and SMMC-7721 cell lines, compared with L02 cell line. Other genes studied were not different (Table 4).

Sialyltransferase protein expression

To measure sialyltransferase protein expression, total protein extracted from L-02, HepG-2 and SMMC-7721 cell lines were western blotted. Figure 4 depicts upregulated ST3Gal-IV, ST6Gal-I and there was a reverse trend for ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI (Fig. 4). Other genes were not seen different and Western blot data agreed with qRT-PCR results.

ST3Gal-IV and ST6Gal-I protein expression is upregulated in hepG-2 and SMMC-7721 cell lines, compared with L02 cell line. ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI protein expression is opposite of that trend.

Discussion

Cell membrane carbohydrate expression is associated with malignant transformations. A family of important molecules related to aberrant glycosylation is sialic acids (SAs) and their derivatives. Sialylation is key to various biologic processes, such as cell-cell communication, cell-matrix interaction, adhesion and protein targeting. Cell surface SA are correlated with STgene mRNA. Previous evidence indicates that altered ST expression is significantly correlated with oncogenesis, tumor progression and lymph node metastases. Therefore, research into sialylation with respect to cancers will offer insight into modifying cancer behavior and treatment [4].

Here, we measured sialic acid expression on the cell surface of human hepatic carcinoma cell lines,HepG-2 and SMMC-7721 and a normal hepatic cell line, L-02 as control with lectin microarrays. Then, DNA microarrays were carried out to measure expression differences in sialyltransferases. There were 7 sialyltransferases expressed differently,

Table 3 Lectin affinity specificity and fluorescence intensity comparison

Lectin	Affinity specificity	L-02	HepG-2	SMMC-7721
AAL	Terminal αFuc and±Sia-Lex	++	++	++
MAH-I	Siaα 2-3Galβ1-3[Siaα2-6GalNAc]α-R	+	++	++
SNA	Siaα 2-6Gal/GalNAc	+/–	++	++
WGA	(GlcNAc)n and multivalent Sia	++	++	++

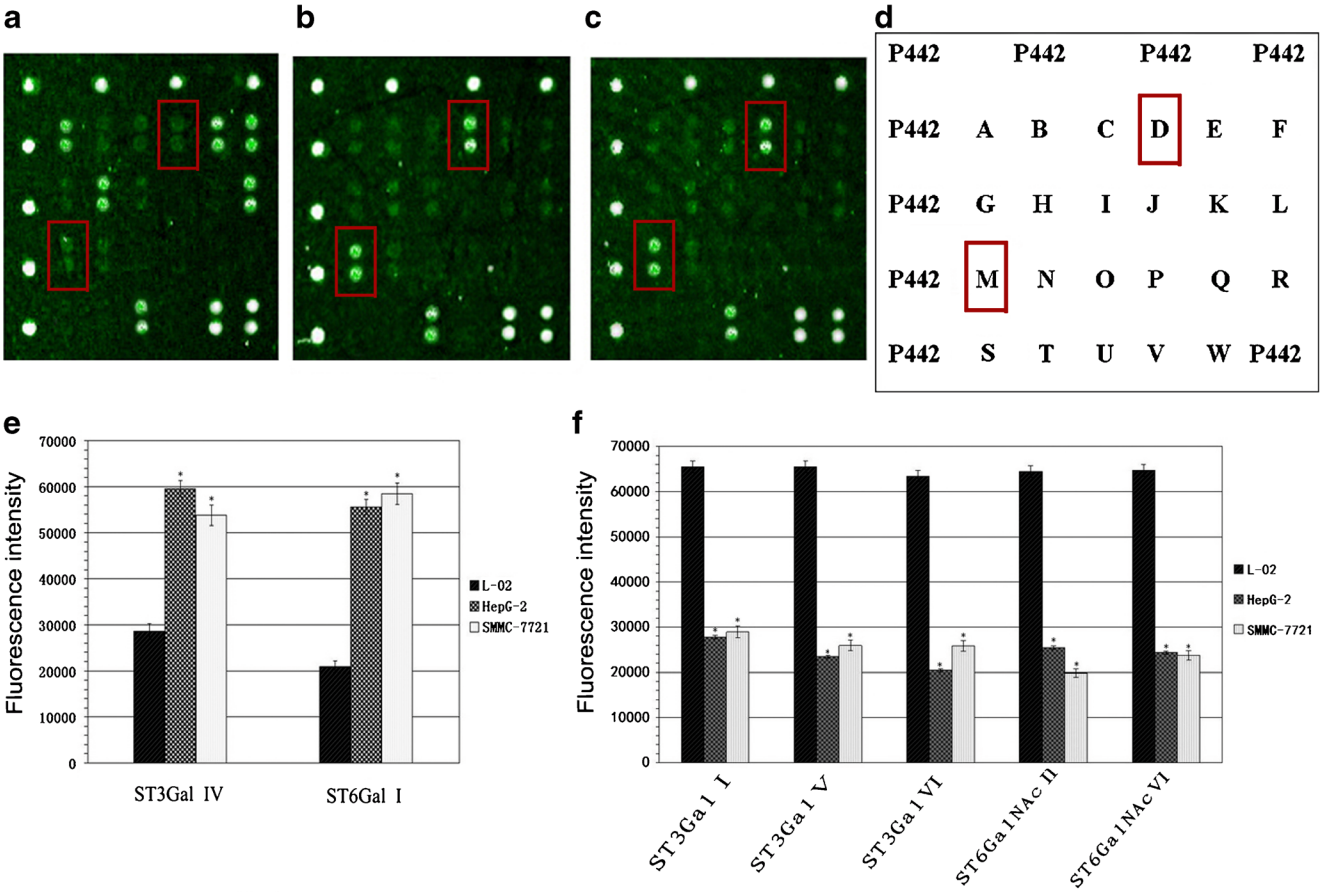

Fig. 2 Hybridization signal analysis of DNA microarrays. Fluorescent scanning of DNA microarrays by Gene TAC™ LS IV (Gain 60, Black 0). **a** L-02 ST3Gal-IV and ST6Gal-I points on DNA microarrays are circled in red. **b** HepG-2 ST3Gal-IV and ST6Gal-I points on DNA microarrays are circled in red. **c** SMMC-7721 ST3Gal-IV and ST6Gal-I points on DNA microarrays are circled in red. **d** DNA microarrays bitmap (capital letters represent 20 sialyltransferases in Table 1). In panel D, D is ST3Gal-IV, M is ST6Gal-I, corresponding points on the DNA microarrays are circled in red. ST3Gal-IV and ST6Gal-I are upregulated in hepG-2 and SMMC-7721 cell lines. However, ST3Gal-I (A in panel D), ST3Gal-V (E in panel D), ST3Gal-VI (F in panel D), ST6GalNAc-II (capital H in panel D) and ST6GalNAc-VI (L in panel D) contrast to this. **e** Relative fluorescent intensities of ST3Gal-IV and ST6Gal-Iin L-02, HepG-2 and SMMC-7721 cell lines (*$P<0.05$, $n=6$). **f** Relative fluorescent intensities of ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI in L-02, HepG-2 and SMMC-7721 cell lines (*$P<0.05$, $n=6$)

comprising 35 % of all sialyltransferases. Overexpression was increased by 10 % and downregulation was decreased by 25 %. Finally, qRT-PCR and Western blot confirmed these data were consistent with that from DNA microarrays.

All ST3Gal family enzymes transfer Neu5Ac residues in α2-3-linkage to terminal Gal residues found in glycoproteins or glycolipids [4]. ST3Gal-I is reported to first use Gal β1-3GalNAc as a substrate to synthesize O-glycans [7, 8]. T lymphocyte O-glycan sialylation is associated with ST3Gal-I knockout in the mouse [9]. In addition, ST3Gal-I promotes formation of breast carcinomas [10] and bladder carcinomas [11]. Here, we observed that ST3Gal-I expression was less in HepG-2 and SMMC-7721 cells than control cell, which may be due to tissue specificity of ST3Gal-I and carcinoma formation differences in tissues. ST3Gal-II is reported to be a synthetase of stage specificity embryonal antigen-4 which appears to be upregulated in colon carcinoma. ST3Gal-III can regulate cell movements and adhesion of pancreatic cancer and increase its metastasis potential *in vivo* [10]. Our results didn't show obvious difference in ST3Gal-II and ST3Gal-III expression in the hepatic cell lines. ST3Gal-IV uses Galβ1-4GlcNAc as a substrate in glycoproteins. N-glycan sialylation is associated with ST3Gal-IV knockout in the mouse as well [12, 13]. ST3Gal-IV was reported to be important in adhesion and migration of tumor cells. For instance, Soyasaponin-1 can inhibit ST3Gal-IV activity and decreases α2-3-sialic acid in MCF-7 breast cancer cells [14]. In our research, ST3Gal-IV was overexpressed in hepG-2 and SMMC-7721 cells, which was consistent with data reported in previous studies [14]. Thus, In view of data that Siaα2-3Galβ1-3 [Siaα2-6GalNAc]α-R sugar chains were upregulated in HepG-2 and SMMC-7721 cells, overexpression of ST3Gal-IV may be related to highly expressed Siaα 2-3Galβ1-3[Siaα2-6GalNAc]α-R cell membrane sugar chains. ST3Gal V is a synthetase of GM3 and is a glycosphingolipid, as well as participates in inducing cell

Table 4 Comparison between sialyltransferases and the DNA microarrays fluorescent scanning*

Official symbol	Official full name	L02	HepG	7721
ST3GalI	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	+++	+	+
ST3GalII	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	+	+	+
ST3GalIII	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	++	++	++
ST3GalIV	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	+	+++	+++
ST3GalV	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	+++	+	+
ST3GalVI	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	+++	+	+
ST6GalI	ST6Gal beta-galactosamide alpha-2,6-sialyltranferase 1	+	+++	+++
ST6GalII	ST6Gal beta-galactosamide alpha-2,6-sialyltranferase 2	+	+	+
ST6GalNAcI	ST6Gal(alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-*N*-acetylgalactosaminide alpha-2,6-sialyltransferase 1	+	+	+
ST6GalNAcII	ST6Gal(alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-*N*-acetylgalactosaminide alpha-2,6-sialyltransferase 2	+++	+	+
ST6GalNAcIII	ST6Gal(alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-*N*-acetylgalactosaminide alpha-2,6-sialyltransferase 3	+	+	+
ST6GalNAcV	ST6Gal(alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-*N*-acetylgalactosaminide alpha-2,6-sialyltransferase 5	+	+	+
ST6GalNAcVI	ST6Gal(alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-*N*-acetylgalactosaminide alpha-2,6-sialyltransferase 6	+++	+	+

*The undetected sialyltransferases are not listed in the table (+: Fluorescence Intensity≤31140; ++: Fluorescence Intensity is from 31140 to 58650; +++: Fluorescence Intensity≥58650)

differentiation, regulating proliferation, maintaining mortality; and plays roles in signal transduction and cell adhesion [15]. The relationship between ST3Gal V and hepatoma has not been reported to date. Decreased ST3Gal-V expression suggests that it may not be involved in cell membrane sugar chain increases, but participate in tumorigenesis by regulating GM3 concentration [16]. Thus, we will study ST3Gal-V function in hepatic tumorigenesis in the future. At present, few reports about ST3Gal-VI exist and the few that are available indicate that ST3Gal-VI expresses well in hepatoma [17]. Moreover, evidence suggests that knocking out ST3Gal-VI may induce abnormal glycosylation of cancer-related carbohydrate antigens and malignant phenotypes of gastric carcinoma [18].

The ST6Gal family is expressed in malignant tissues, especially in metastatic tissue. α2-6 sialyltransferase expression is regulated by the ras gene which is abnormal in many carcinomas and therefore, α2-6 sialyltransferase is regarded as a tumor marker [19]. Upregulation of ST6Gal-I occurs in many cancers and ST6Gal-I uses Galβ1→4GlcNAc as its substrate [20]. In addition, it controls sialylation of glycoproteins via α-2, 6 [18]. ST6Gal-I is reported to be upregulated in cervical cancer [21], gastric carcinoma [22] and transgenic hepatoma mouse models [23]. ST6Gal-I can inhibit apoptosis mediated by the death-receptor and promote proliferation via the sialylation of Fas (CD95). ST6Gal-I may also play a role in metastasis and prognosis of colon carcinoma [23]. We

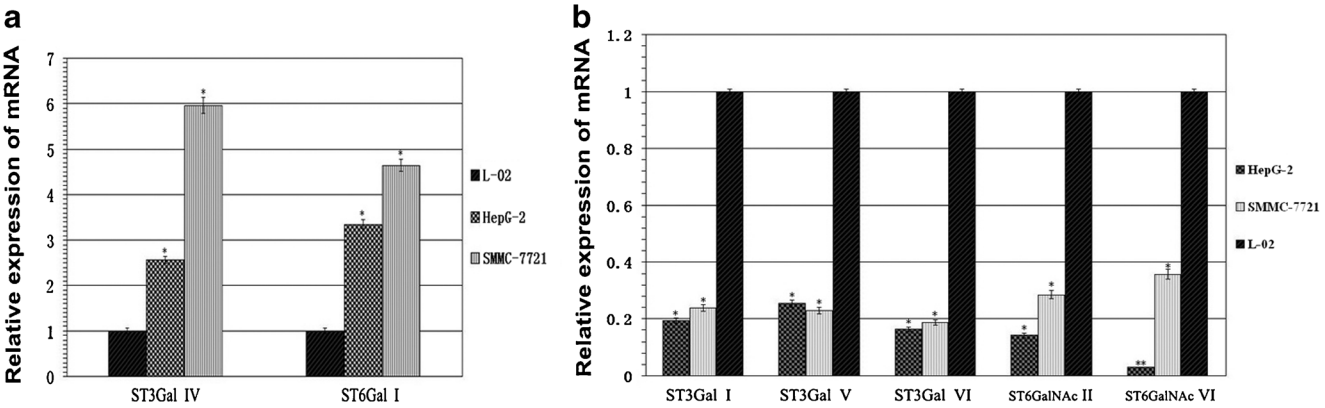

Fig. 3 mRNA expression of sialyltransferase. **a** ST3Gal-IV and ST6Gal-I mRNA expressions is upregulated in hepG-2 and SMMC-7721 cell lines, compared with L02 cells (*P<0.05, n=6). **b** ST3Gal-I, ST3Gal-V, ST3Gal-VI, ST6GalNAc-II and ST6GalNA-VI mRNA expression is downregulated in hepG-2 and SMMC-7721 compared with L02 (*P<0.05, **P<0.01, n=6)

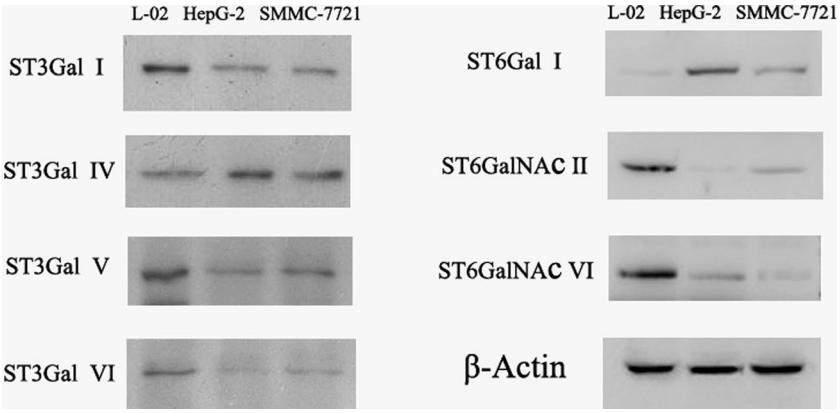

Fig. 4 Protein expression of ST3Gal-I, ST3Gal-IV, ST3Ga-V, ST3Gal-VI, ST6Gal-I, ST6GalNAc-II and ST6GalNA-VI in L-02, HepG-2 and SMMC-7721 cell lines

found that ST6Gal-I was overexpressed in hepG-2 and SMMC-7721 cell lines and these data combined with the fact that Siaα2-6Gal/GalNAc sugar chains in HepG-2 and SMMC-7721 cell lines were upregulated suggests that ST6Gal-I overexpression may be related to highly expressed Siaα2-6Gal/GalNAc sugar chains. They may be involved in hepatoma metastasis [19]. ST6Gal-III uses Galβ1→4GlcNAc as its substrate [23] and controls sialylation of glycoprotein via α2-6 connection [23]. So far, a few reports to describe ST6Gal-II suggest that it is related to expression of ST3Gal-III and ST3Gal-IV in ovarian carcinoma [24].

Enzymes of the ST6GalNAc family catalyze the transfer of Neu5Ac residues in α2-6 linkage to GalNAc residues found in O-glycosylproteins (ST6GalNAc-I, -II and -IV) or found in glycolipids (ST6GalNAc-III, -V and -VI) [23]. At present, no reports describe the relationship between the ST6GalNAc family and hepatocarcinoma. ST6GalNAc-I is reported to be upregulated in gastroenteric tumors [25], breast carcinoma [26]and colon cancer [27] but we found no difference in hepG-2 and SMMC-7721 cell lines, compared with control cell L02 cell lines. Over expression of ST6GalNAc-II results in poor prognosis in colon cancer [28] and we found that ST6GalNAc-II is dowregulated in hepG-2 and SMMC-7721 cell lines suggesting that ST6GalNAc-II is not involved in cell membrane sugar chain increases. ST6GalNAc-III has been reported to be found in normal renal cells [28], but this has not been confirmed. ST6GalNAc-III was not different among the three cell lines we detected and no reports about ST6GalNAc-IV exist in this regard. ST6GalNAc-V is a specific sialyltransferase of gangliosides, utilizing sialic glycolipids as its substrates [28]. Evidence suggests that it can inhibit growth of gliomas [26]. We observed that ST6GalNAc-V was not different in any cell lines. ST6GalNA-VI is expressed in normal renal cell lines, but was downregulated in renal cancer cell lines and cancer tissues and this may be related to inhibition of DSGG (disialylgalactosylgloboside) synthesis [28]. We found that ST6GalNA-VI is downregulated in hepG-2 and SMMC-7721 cell lines suggesting that ST6GalNA-VI is not involved in cell membrane sugar chain increases.

Enzymes of the ST8Sia family mediate the transfer of Neu5Ac residues in α2-8-linkage to other Neu5Ac residues found in glycoproteins and glycolipids. So far, there are few reports describe the role of ST8Sia in cancer [4]. ST8Sia I are overexpressed in neuroectoderm-derived malignant tumors, such as melanoma, glioblastoma, neuroblastoma and in estrogen receptor (ER) negative breast cancer, where they play a role in cell proliferation, migration, adhesion and angiogenesis [29]. ST8Sia-I was also found to be downregulated in lymphoblasts [30]. In our work, we didn't see the expression of 6 members in ST8Sia family.

In conclusion, ST3Gal-IV and ST6Gal-I were upregulated in hepG-2 and SMMC-7721 cell lines and these data coupled with the fact that Siaα2-3Galβ1-3 [Siaα2-6GalNAc]α-R and Siaα 2-6Gal/GalNAc sugar chains are upregulated in HepG-2 and SMMC-7721 cell lines suggest that overexpression of ST3Gal-IV and ST6Gal-I may be related to upregulation of Siaα2-3Galβ1-3[Siaα2-6GalNAc]α-R and Siaα2-6Gal/GalNAc sugar chains on cell membranes. These data may offer important clues for future studies into the mechanism of hepatic carcinoma formation [31].

Acknowledgments We are grateful to Professor Qun He for her assistance in this work. This work was supported by a grant from the National Natural Science Foundation of China (awarded to Qun He, Grant No. 20672144). We thank LetPub (www.letpub.com) for their linguistic assistance during the preparation of this manuscript.

Conflict of interest statement None declared

Funding This work was supported by a grant from the National Natural Science Foundation of China (awarded to Qun He, Grant No. 20672144).

References

1. Deng, W., Li, R., Ladisch, S.: Influence of cellular ganglioside depletion on tumor formation. J Natl Cancer Inst **92**(11), 912–917 (2000)
2. Schattenberg, J.M., Schuchmann, M., Galle, P.R.: Cell death and hepatocarcinogenesis: dysregulation of apoptosis signaling pathways. J Gastroenterol Hepatol **26**(1), 213–219 (2011)
3. Miyagi, T., Wada, T., Yamaguchi, K., Hata, K.: Sialidase and malignancy: a minireview. Glycoconj J **20**(3), 189–98 (2004)
4. Dall'Olio, F., Malagolini, N., Trinchera, M., et al.: Sialosignaling: sialyltransferases as engines of self-fueling loops in cancer progression. Biochim Biophys Acta **9**, 2752–64(2014) (1840)
5. Ma, R.H., Wang, D.Q., Pan, Z.C., et al.: Different expression of ST3Gal and ST6Gal mRNA between mouse hepatocellular carcinoma cell lines. Life Sci Res **15**(4), 323–327 (2011)
6. He, Q., Li, C.H., Pan, Z.C., et al.: Glycoprofiling investigation of hepatocellular carcinoma cell surface with lectin microarray. Prog Biochem Biophys **37**(3), 269–277 (2010)
7. Guo, C., Liu, Q.G., Zhang, L., et al.: Expression and clinical significance of p53, JunB and KAI1/CD82 in human hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int **8**(4), 389–96 (2009)
8. Lee, Y.C., Kurosawa, N., Hamamoto, T., et al.: Molecular cloning and expression of Galβ1, 3GalNAc α2, 3-sialyltransferase from mouse brain. Eur J Biochem **216**(2), 377–385 (1993)
9. Kono, M., Ohyama, Y., Lee, Y.C., et al.: Mouse βgalactoside α2,3-sialyltransferases: comparison of *in vitro* substrate specificities and tissue specific expression. Glycobiology **7**(4), 469–479 (1997)
10. Kornfeld, S.: Diseases of abnormal protein glycosylation: an emerging area. J Clin Invest **101**(7), 1293–1295 (1998)
11. Storey, E.L., Anderson, G.J., Mack, U., et al.: Desialylated transferrin as a serological marker of chronic excessive alcohol ingestion. Lancet **1**(8545), 1292–4 (1987)
12. Ellies, L.G., Ditto, D., Levy, G.G., et al.: Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands. Proc Natl Acad Sci U S A **99**(15), 10042–10047 (2002)
13. Hebert, D.N., Garman, S.C., Molinari, M.: The glycan code of the endoplasmic reticulum: asparagine-linked carbohydrate as protein maturation and quality-control tags. Trends Cell Biol **15**(14), 364–370 (2005)
14. Higai, K., Miyazaki, N., Azuma, Y., Matsumoto, K.: Interleukin-1beta induces sialyl Lewis X on hepatocellular carcinoma HuH-7 cells via enhanced expression of ST3Gal IV and FUT VI gene. FEBS Lett **580**(26), 6069–6075 (2006)
15. Chung, T.W., Kim, S.J., Choi, H.J., et al.: Ganglioside GM 3 inhibits VEGF/VEGFR-2-mediated angiogenesisDirect interaction of GM3 with VEGFR-2. Glycobiology **19**(3), 229–239 (2009)
16. Adriane Regina, T., Jose Nilson Dos, S., Kazuko, H., et al.: Ganglioside GM2-Tetraspanin CD82 complex inhibits met and its cross-talk with integrins, control of cell motility through Glycosynapse. J Biol Chem **282**(11), 8123–33 (2007)
17. Paulson, J.C., Colley, K.J.: Glycosyltransferases structure localization and control of cell type-specific glycosylation. J Biol Chem **264**(30), 17615–8 (1989)
18. Souady, J., Hülsewig, M., Distler, U., et al.: Differences in CD75s- and iso-CD75s-ganglioside content and altered mRNA expression of sialyltransferases ST6GalIand ST3GalVI in human hepatocellular carcinomas and nontumoral liver tissues. Glycobiology **21**(5), 584–94 (2011)
19. Korekane, H., Matsumoto, A., Ota, F., et al.: Involvement of ST6Gal I in the biosynthesis of a unique human colon cancer biomarker candidate alpha2,6-sialylated blood group type 2H (ST2H)antigen. J Biochem **148**(3), 359–370 (2010)
20. Paulson, J.C., Colley, K.J.: Glycosyltransferases structure localization and control of cell type-specific glycosylation. J Biol Chem **264**(30), 17615–17618 (1989)
21. Swindall, A.F., Bellis, S.L.: Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. J Biol Chem **286**(26), 22982–22990 (2011)
22. Milflores-Flores, L., Millán-Pérez, L., Santos-López, G., et al.: Characterization of P1 promoter activity of the beta-galactoside alpha 2,6-sialyltransferase I gene (siat 1) in cervical and hepatic cancer cell lines. J Biosci **37**(2), 259–267 (2012)
23. Dall'Olio, F., Chiricolo, M., D'Errico, A., et al.: Expression of beta-galactoside alpha2,6 sialyltransferase and of alpha2,6-sialylated glycoconjugates in normal human liver, hepatocarcinoma, and cirrhosis. Glycobiology **14**(1), 39–49 (2004)
24. Bouanene, H., Sahrawi, W., Mokni, M., et al.: Correlation between heterogeneous expression of Sialyltransferases and MUC16 in ovarian tumor tissues. Onkologie **34**(4), 165–169 (2011)
25. Marcos, N.T., Pinho, S., Grandela, C., et al.: Role of the human ST6GalNAc-I and ST6GalNAc-II in the synthesis of the cancer-associated sialyl-Tn antigen. Cancer Res **64**(19), 7050–7057 (2004)
26. Senda, M., Ito, A., Tsuchida, A., et al.: Identification and expression of a sialyltransferase responsible for the synthesis of disialylgalactosylgloboside in normal and malignant kidney cells: downregulation of ST6GalNAcVI in renal cancers. Biochem J **402**(3), 459–470 (2007)
27. Sewell, R., Bäckström, M., Dalziel, M., et al.: The ST6GalNAc-I sialyltransferase localizes throughout the Golgi and is responsible for the synthesis of the tumor-associated sialyl-Tn O-glycan in human breast cancer. J Biol Chem **281**(6), 3586–94 (2006)
28. Schneider, F., Kemmner, W., Haensch, W., et al.: Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. Cancer Res **61**(11), 4605–4611 (2001)
29. Kroes, R.A., He, H., Emmett, M.R., et al.: Overexpression of ST6GalNAcV, a ganglioside-specific alpha2,6-sialyltransferase, inhibits glioma growth *in vivo*. Proc Natl Acad Sci U S A **107**(28), 12646–12651 (2010)
30. Steenackers, A., Vanbeselaere, J., Cazet, A., et al.: Accumulation of unusual gangliosides G(Q3) and G(P3) in breast cancer cells expressing the G(D3) synthase. Molecules **17**(8), 9559–9572 (2012)
31. Dall'Olio, F., Malagolini, N., Trinchera, M., Chiricolo, M.: Sialosignaling:sialyltransferases as engines of self-fueling loops in cancer progression. Biochim Biophys Acta **9**, 2752–2764(2014) (1840)