Expression of Fucosyltransferases Contributes to Melanoma Invasive Phenotype

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Abstract: During carcinogenesis aberrant N-glycosylation may lead to the development of subpopulations of tumor cells with altered adhesion properties and increased invasive potential. Biosynthesis of glycans and oligosaccharides is tissue–specific and developmentally regulated by number of glycosyltransferases of which fucosyl-, sialyl- and N-acetylglucosaminyltransferases often participate in synthesis of tumor type glycans.

We analyzed the expression of selected glycosyltransferases (real-time PCR): fucosyltransferases *FUT-1* and *FUT-4*, sialyltransferase *SIAT4C* and beta 1,6-N-acetylglucosaminyltransferase V (*MGAT-5*), in human melanoma cell lines: WM35 from primary tumor site and WM239, WM9, A375 from metastatic sites.

In parallel their proliferation (crystal violet test) and adhesion to fibronectin and collagen IV (BD Biocoat assay) was assessed.

Examined cell lines showed expression of all studied glycosyltransferases. The level of expression of fucosyltransferases was significantly higher in melanoma cell lines from metastatic site than from primary cell line: mRNA expression of *FUT-1* was 100 times higher in A375 melanoma cell line from metastatic site (A375, solid tumor) than in WM35 primary cell line. The expression of *FUT-4* in cell lines from metastatic sites: WM9 (lymph node) and WM239 (skin) was respectively 80 and 37 times higher than in WM 35 primary cell line. In all melanoma cell lines very low expression of *MGAT-5* and high expression of *SIAT4C* was observed. Melanoma cells bound both to fibronectin and to collagen IV. LTA (*Lotus tetragonolobus agglutinin*), the lectin that specifically recognizes fucose residue of glycans and 20mM L-fucose by itself significantly reduced adhesion of all studied cell lines, both primary and metastatic, to fibronectin (20-50 %) and to collagen IV (20-50 %). In addition LTA reduced the proliferation (20-30 %) of metastatic cell lines (A375, WM9, WM239) and did not affect the growth of primary cell line (WM35).

The results suggest that higher expression of fucosyltransferases (*FUT-1, FUT-4*) might be an important step in the formation of surface structures that facilitate metastasis of melanoma.

Key Words: Glycosyltransferases, real-time RT-PCR, melanoma, adhesion, proliferation.

INTRODUCTION

Malignant transformation is often associated with the alteration of glycoconjugates: glycolipids and glycans of glycoproteins expressed on plasma membrane [1, 2].

Biosynthesis of glycans is tissue specific and developmentally regulated by the activity of number of glycosyltransferases such as fucosyltransferases, sialyltransferases or galactosyltransferases. The most common alterations associated with cancer progression regarding protein glycosylation include the synthesis of β 1,6 GlcNAc branched and heavily sialylated as well as fucosylated complex glycans [2]. Formation of β 1, 6 branches on N-glycans is catalysed by β1, 6 Nacetylglucosaminyltransferase V (*MGAT-5*). Synthesis of glycans with sialic acid α2, 3 linked to to Galβ1,(3)4 GlcNAc structure on N-linked chains of glycoproteins is carried out by α2,3 sialyltransferases ST3 Gal-III (*SIAT3*) or ST3Gal-IV (*SIAT4C*).

Fucosyltransferases are classified into two main families based on acceptor specificity and their primary protein sequence: α1, 2- fucosyltransferases (*FUT-1* and *FUT-2*) and α1, 3- fucosyltransferases (*FU-T 3- FUT-7*) which are involved in the synthesis of H and Lewis related antigens, respectively. Fucosyltransferases play an important role in the biosynthesis of tumor associated antigens such as sialyl-Le^x, sialyl- Le^a, H antigenic determinant and Le^y [3, 4].

The expression of branched and sialylated or fucosylated complex type N-linked oligosaccharides in malignant tumor cells appears to be directly associated with their metastatic potential [1, 5]. Human cancer of breast, colon, bladder, and melanoma show increased levels of β1, 6GlcNAc branched N-glycans of tri- and tetra-antennary type, formed due to the increased activity of N-acetylglucosaminyltransferase V[2, 6]. The appearance of N-linked glycans with β1, 6GlcNAc branches correlates with metastasis and progression of tumor [6]. *MGAT-5* expression was found to be regulated by RAS - RAF - MAPK signaling pathway commonly activated in

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tumor cells. Malignant transformation of fibroblasts and epithelial cells was accompanied by increased β1,6 N-acetylglucosaminyl transferase V (*MGAT-5*) activity.

GlcNAc-TV expression promoted features of transformation including release from contact-inhibition of cell growth and reduced substratum adhesion and motility [5, 7, 8]. During the transition of cells from normal to malignant lesion, and subsequently to metastatic cancer, changes in intercellular communications provide tumor cells with ability to overcome cell-cell adhesion and microenviromental local control and to invade surrounding tissues and disperse to distant locations. The pattern of expression of cell adhesion molecules and their properties play a pivotal role in controlling the primary processes such as cell division, migration, proliferation, differentiation and death.

Sialoglycans on the surface of human colon cancer cell have been implicated in cellular adhesion and metastasis. The common structural motif among adhesion molecules in colon cells is the terminal NeuAcα2, 3Gal-R glycosidic epitope. β1 integrins with altered glycans are essential for cell adhesion to ECM proteins, β1, 6 GlcNAc branched structures of integrins, like α5β1 and α3β1 resulted in their reduced adhesion to fibronectin [9]. It has been recently shown in melanoma cell lines that adhesion molecules such as N-cadherin [10-11] and integrins [12,13] undergo altered glycosylation. Comparison of N-cadherin glycans from primary (WM35) and metastatic (WM9, WM239, A375) melanoma cell lines, based on MALDI MS studies, showed that N-cadherin from primary melanoma cells possesses high-mannose and biantennary complex type glycan, while N-cadherin from metastatic sites possessed mostly tri and tetra antennary, heavily fucosylated type glycans [11]. In this study we attempted to understand the relationship between the expression of selected glycosyltransferases and adhesive properties of investigated melanoma cells from primary and metastatic sites and possible role of fucosylation in tumor cell adhesion.

RESULTS

Quantitative mRNA Expression of Glycosyltransferases

The expression of glycosyltransferases was studied in human melanoma cell lines from primary tumor site (WM35) and from metastatic sites (WM239, WM9, and A375). Examined cell lines showed expression of fucosyltransferases I and IV, sialyltransferase and N-acetylglucosaminyltransferase V (Table **1**). In all melanoma cell lines very low expression of *MGAT-5* (C_T~30, Δc_T~17) and high expression of *SIAT4C* (C_T~20, Δc_T~5-6) was observed. An the contrary the expression of both fucosyltransferases varied between cell lines. The relative mRNA expression of fucosyltransferases was significantly higher in melanoma cell lines from metastatic sites than from primary site. The mRNA expression of *FUT-1* was about 100 times higher in A375 melanoma cell line than in WM35 Fig. (**1**).

The expression of *FUT-4* in cells from metastatic sites: WM9 (lymph node) and WM239 (skin) was respectively 80 and 37 times higher than in cells from primary tumor site Fig. (**1**).

Cell Adhesion to Extracellular Matrix Proteins

Cells from the all studied melanoma cell lines adhered to both fibronectin and collagen IV. However the efficiency of adhesion varied significantly between lines Fig. (**2**).

LTA, the lectin that specifically recognizes fucose residue on glycans and L-fucose significantly inhibited the adhesion of melanoma cells from all lines to both ECM proteins Fig. (**2**).

LTA lectin and 20mM L-fucose inhibited adhesion of melanoma cells to collagen IV respectively: 36% ($p<0.0001$) and 52 % ($p<0.0001$) in WM9, 33% ($p<0.0001$), 50% ($p<0.0001$) in WM 35. LTA lectin and 20mM L-fucose inhibited adhesion of melanoma cells to fibronectin respectively: 37% ($p<0.001$) and 22% ($p<0.001$) in WM9, 31%

Table 1. Expression of Glycosyltransferases in Melanoma Cell Lines

Gene	Relative cycle number Δc_T (comparison to GAPDH)							
	A375 n=6		WM9 n=6		WM239 n=6		WM35 n=6	
	c_T	Δc_T	c_T	Δc_T	c_T	Δc_T	c_T	Δc_T
FUT-1	28.283 ± 0.109	14.055**	35.052 ± 0.510	20.877***	34.820 ± 0.046	21.050***	36.020 ± 0.533	20.625***
FUT-4	30.754 ± 0.125	16.526**	25.279 ± 0.137	11.104	25.980 ± 0.146	12.210	32.834 ± 0.307	17.439**
SIAT4C	19.862 ± 0.121	5.634*	20.152 ± 0.124	5.977*	20.157 ± 0.123	6.387*	20.281 ± 0.076	4.886*
MGAT – 5	31.437 ± 0.156	17.209**	29.716 ± 0.393	15.541**	28.023 ± 0.270	14.253**	29.777 ± 0.073	14.382**
GAPDH	14.228 ± 0.193		14.175 ± 0.337		13.770 ± 0.105		15.395 ± 0.190	

* - high expression, ** - low expression, *** - very low expression, c_T-cycle number of crossing point, Δc_T -relative cycle number comparison to GAPDH.

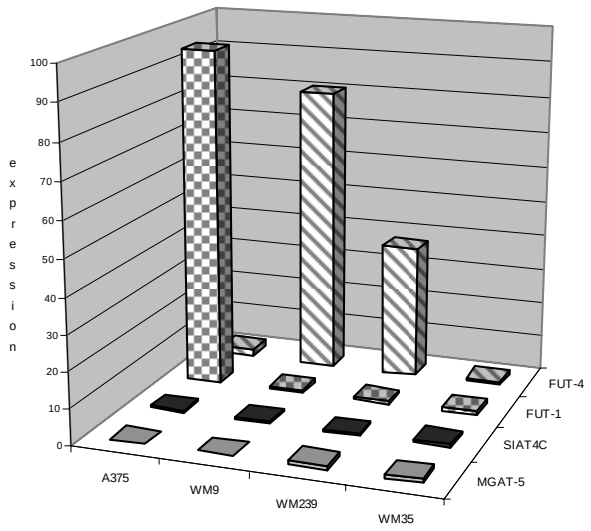

Fig. (1) Relative expression of glycosyltransferases (*MGAT-5*, *SIAT4C*, *FUT-1*, *FUT-4*) in metastatic melanoma lines (A375, WM9, WM239) in comparison to primary melanoma cell line (WM35).

($p<0.0001$), 31% ($p<0.0001$) in WM35. The inhibition of adhesion to both fibronectin and collagen IV was not so meaningful in case of highly metastatic A375 and skin metastatic WM239 melanoma cells.

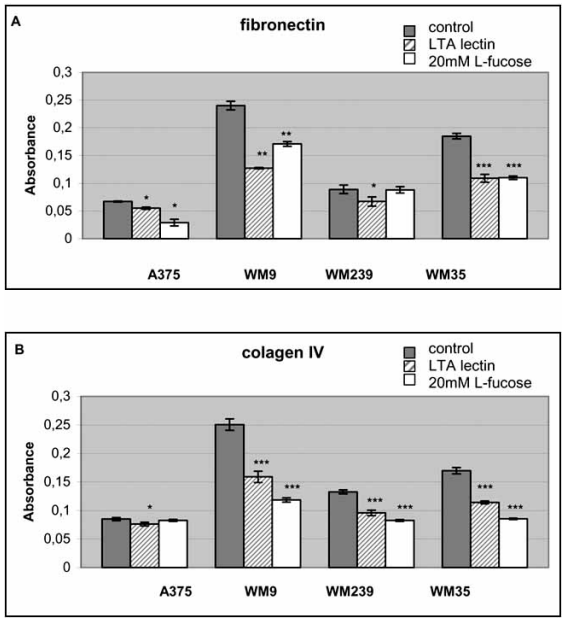

Fig. (2). Adhesion of human melanoma cell line to: fibronectin (A) collagen IV (B).

Values are expressed as mean ±standard deviation (6 wells in two independent experiments) *P<0.05 , **P<0.001, ***P<0.00001.

Cell Proliferation

LTA lectin inhibited proliferation of melanoma cell lines. The effect was observed for all metastatic cell lines. However the most profound inhibition was noticed in case of WM9 and WM239, their proliferation was inhibited after 48h respectively: 31% ($p<0.0001$) and 30% ($p<0.0001$) Fig. (**3**).

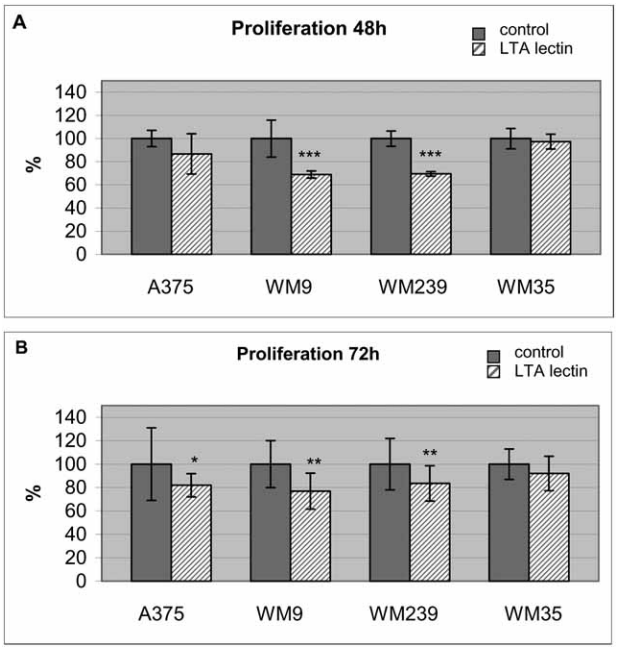

Fig. (3). Proliferation of human melanoma cell lines: 48h (A) 72h (B).

The measure of cell proliferation is absorbance of crystal violet at 600nm that was uptaken by proliferating cells.

Cell proliferation is presented as absorbance units (A) at 540nm. Values are expressed as mean ±standard deviation in 8 wells in two independent experiments *P<0.05, **P<0.001, ***P<0.00001.

DISCUSSION

Aberrant glycosylation is a hallmark of the malignant phenotype [1-3, 14]. Altered expression of glycosyltransferases in carcinoma tissue are prognostic factor and potential target for therapeutic approaches. The structural characterization of these altered glycoforms has identified carbohydrate motifs associated with tumor tissue. Generally, the most frequently observed cancer related changes in the pattern of glycosylation include the synthesis of highly branched, N-acetyllactosaminylated and heavily sialylated N-linked glycans [4, 8, 15, 16] as well as their altered fucosylation [3, 17]. Perhaps one of the critical steps in formation of invasive phenotype is the increased expression of respective glycosyltransferases: α2, 3- and α2, 6-sialyltransferases, α1, 2- and α1, 3-fucosyltransferase and N-acetylglucosaminyltransferase V.

The correlation of fucosylation patterns with adhesion events and various diseases led to intensive investigations on human fucosyltransferases and their regulation during developmental or pathological processes [18-20]. Fucosylated oligosaccharides occur throughout nature and many of them play a variety roles especially in a number of recognition processes [21]. The presence of terminal fucosylated struc-

tures was demonstrated by immunological methods in many glycans of animal and human tumors [3, 17]. Higher activity of α1,3- fucosyltransferase was indicated in tumors of colon, stomach, lung [4, 20, 22, 23]. In this study, for the first time to the best of our knowledge quantitative expression of some cancer related glycosyltransferases in human melanoma cell line was determined. We found very high expression of fucosyltransferases (*FUT-1, FUT-4*) in metastatic melanoma cell lines compared to their expression in primary melanoma cell line. The expression of α1, 3 – fucosyltransferase (*FUT-4)* from metastatic site was: in WM9 (lymph node) 80 times higher and, in WM239 (skin) 37 times higher than in primary cell line. In lung carcinoma the enhanced expression of FucT-IV and VII is related to a high metastatic potential and poor prognosis [24, 25]. Increased synthesis of sialyl Le^x structures by FucT-III is involved in colon cancer metastasis. Patients who express these structures have a significantly poorer disease free survival rate [26]. Different reports suggest increased FucT-III or FucT-IV activities in human intestinal cancers. In hepatocellular carcinoma the increased level of α1,6-fucosyltransferase and its products expression significantly correlate in low survival rate and bad clinical treatment prognosis [27]. α1, 2-fucosyltransferase (FucT-I) synthesize the H-antigenic determinant precursors for the A, B, Le^b and Le^y blood group determinants. Changes in these antigens have also been associated with cancer progression based on observation that high expression of H and Le^y antigens correlates with poor survival for some cancers [28]. Increased expression of α1, 2 fucosyltransferase correlated well with colon, gastric [4, 22] and prostate cancers progresion [29]. Also an increase in tumorigenicity of rat colon cells transfected with *FUT-1* has been reported [18]. The expression level of *FUT-1* mRNA in metastatic melanoma cell line A375 was 100 fold of its expression level in primary melanoma cells. This result remains in agreement with the overall view on the role of fucosylation in cancer progression.

Increased tumorigenity mediated by α1, 2-fucosylation is associated with increased resistance to apoptosis and escape from immune control as shown in case of rat colon carcinoma cells [19, 30]. Marker *et al.* (2001) [31] suggested specific role for *FUT-1* and H-type carbohydrate epitopes in regulating epithelial proliferation during branching morphogenesis in prostatic rat carcinoma. *FUT-1* was indicated as an important mediator of prostatic pathological growth and morphogenesis. *FUT-1* can modulate epithelial cell proliferation during prostatic branching morphogenesis [31]. According to Stelck *et al.* 1999 [32] fucose in α1,6-linkage regulates cell proliferation. Regarding reported here much higher expression of *FUT-1*, *FUT-4* and significant inhibition of proliferation and adhesion of melanoma cells to fibronectin and collagen IV, higher mRNA expression of fucosyltransferases (*FUT-1*) may be an important step in the formation of surface structures that facilitate metastasis of melanoma cells.

Addition of an exogenous sugar acceptor for α1, 2 fucosyltransferase to the cell medium resulted in suppression of α1, 2 fucosylated antigen expression on the tumor cells and increased susceptibility to anticancer treatment [33]. Our studies have also revealed that proliferation of melanoma cells was strongly dependent on a presence of fucosylated oligosaccharides structures since LTA – lectin specific for fucose residue inhibited significantly proliferation of metastatic cell lines but did not affect the growth of primary cell line (WM35). Similar trends were seen in previous studies [34, 11] carried out on WM 35 cell line.

It appears that α2,3- sialylation is important event in choise of a site of metastasis. According to Dimitroff *et al.* (1999) [15] in colon cancer cells the α2, 3-linked sialic acid bearing proteins are essential in mediating intercellular adhesion. Formation of glycans with α2, 3 linked sialic acid to Gal1, (3)4GlcNAc on N-linked chains of glycoproteins is carried out by α2, 3sialyltransferase ST3Gal III (*SIAT3*) or ST3Gal IV (*SIAT4C*). Expression of ST3Gal IV (*SIAT4C*) was significantly enhanced in gastric carcinoma tissue compared to normal tissue [22]. Increased expression of ST3 Gal III or IV may play a role in glial tumorigenesis [35]. Both ST6Gal I mRNA and ST3Gal III mRNA expression were significantly increased in patients with lymph node metastasis compared to cervix squamous cell carcinoma patients without lymph node metastasis [36]. We found very high level of ST3 Gal IV expression but no significant differences between various melanoma cell lines which may mean that extensive sialylation is a common feature of melanoma cells regardless on the stage of progresion.

Increased expression of GnT-V is observed during the oncogenesis of many cell types as a results of the stimulation of its transcription thought the ras signalling pathway [37]. Similar to sialylation we did not detect significant correlations between GnT-V expression and melanoma progression.

In summary the results of our study indicate that oligosaccharides with fucose may play an essential role in cellular adhesion and proliferation, and increased fucosylation catalyzed by some fucosyltransferase (*FUT-1, FUT-4*) could contribute to melanoma invasive phenotype.

MATERIALS AND METHODS

Materials

All standard chemicals of analytical grade were purchased from Sigma.

Cell Lines

Melanoma cell lines were obtained from the Department of Cancer Immunology, University School of Medical Sciences at Great-Poland Cancer Centre (Poznań, Poland). WM35 line was from the primary tumor site (radial growth phase) while WM9 was the lymph node metastatic line, WM239, the skin metastatic line (all established by Meenhard Herlyn, The Wistar Institute, Philadelphia, USA), and A375 (ATCC-CRL-1619, [38] the solid tumor metastatic line. Cells were cultured in the RPMI–1640 medium (Sigma) containing 10% foetal bovine serum (GibcoBRL^{TM}) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml; Polfa, Poland) at 37°C in a humidified atmosphere of 5% CO_2 in air.

RNA Isolation and cDNA Synthesis

Total RNA was extracted using RNeasy Mini Kit RNA Isolation System (QIAGEN). Its concentration was deter-

mined spectrophotometrically UV/VIS (Beckman). The sample (5μg) of total cellular RNA was reverse transcribed by reverse transcriptase Omniscript^{TM} (QIAGEN) with oligo dT_{23}. The reaction mixture contained 2.5μl 10 x RT-PCR buffer, 5mM dNTPs, 1 μl reverse transcriptase Omniscript, 2 μl oligo dT_{23} primer (10μM) and water in final volume of 22.5μl. RNA was incubated at 65°C for 5 minutes and with reaction mixture was incubated at 37°C for 1h, followed by inactivation of the enzyme at 95°C for 5 minutes.

Real-Time Analysis

Polymerase chain reaction (real-time PCR) on the basis of SYBR Green was performed to quantity *FUT-1, FUT-4, SIAT4C* and *MGAT-5* mRNA expressions (ABI PRISM 7700 PCR DNA Engine Opticon). Glycosyltransferase mRNA expression of each sample was determined in at least two independent experiments (independent RNA isolation).

Each PCR reaction mixture contained 2x QuantiTect SYBR Green PCR Master Mix 12.5 μl, 1.25 μl (10mM) of forward and reverse primer (Table **2**), 2μl cDNA sample and 8μ RNase-free water. PCR amplification of the identical sample was performed with both specific primers pairs for *FUT-1, FUT-4, SIAT4C* and *MGAT-5* gene and human glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) gene on the same reaction plate.

The PCR reaction was compromised at 40 cycles, consisting of cDNA denaturiation at 95 ^{0}C (15 sec) then annealing at 59 ^{0}C (30 sec) and extension at 72 ^{0}C (30 sec). The control reaction was performed simultaneously using the identical reaction mixture but without cDNA (negative control to test for contaminating DNA). The negative control contained all components including template RNA, except for the reverse transcriptase enzyme. Finally all samples were subjected to melting curve analysis in order to confirm specificity of the PCR products. Data analysis consisted of calculating the ratios of the crossing point (cycle number corresponding to the log linear phase of product amplifications) for glycosyltransferases amplifications in each sample to the *GAPDH* crossing point in the same sample. The row data were converted to relative expression levels of glycosyltransferase standarized by comparison with the levels of *GAPDH* mRNA determined under identical conditions. The differences between the mean C_T values of the gene of interest and housekeeping gene were denoted ΔC_T. Control wells containing SYBER Green PCR master mix and primers without sample cDNA emitted no fluorescence after 40 cycles.

Standard Curve. Real-Time PCR Amplification Efficiencies and Linearity

Real-time PCR efficiencies were calculated based on the slopes included in Opticon software [39]. The corresponding real time PCR efficiency (E) of one cycle in the expotential phase was calculated according to the equation $E= 10^{[-1/slope]}$ [40]. Investigated transcripts showed high real-time PCR efficiency rates: *FUT-1* 1.83; *FUT-4* 1.85; *SIAT4C* 1.85; *MGAT-5* 1.85; *GAPDH* 1.84, in the investigated range from 6.25 to 100 ng cDNA input (n=3) with high linearity R> 0.99 correlation.

Cells Adhesion Assay

Cells adhesion assays were performed using 96-well plate coated with extracellular matrix proteins: human fibronectin and mouse collagen IV (BD Biosciences). The plates were washed with the PBS buffer and non–specific binding site were blocked with 1% BSA for 1h at 37° C. Afterwards wells were washed again two times with the PBS buffer and once with serum-free media. Prior to adhesion assays cells were incubated in serum-free media for 30 min at 37° C in a humidified atmosphere containing 5% CO_2. Subsequently cells were trypsynized and finally washed with serum–free media.

The adhesion of melanoma (A375, WM9, WM239 and WM35) to ECM proteins (fibronectin and colagen IV) were performed for cells treated either with (i) LTA, the lectin specific to α-fucose of N-glycans (Sigma, 5μg/ml RPMI 1640) or (ii) L-fucose (Fluka, 20mM in RPMI 1640) incubated with inhibitor prior to the assay at 37°C for 1 hour. Afterwards cells were seeded (5x 10^4 cells in 100 μl volume) into the wells covered with fibronectin or collagen IV and left for 1h in 37°C. Finally cells were washed three times

Table 2. Glycosyltransferases and Primers Used for Their Amplification

Lp	GENE	PRIMERS (F – forward, R- reverse)	Temp [°C]
1	Fucosyltransferase I *FUT-1*	F: 5'-CACGAAGCCCCCGTTTT-3' R: 5'-TGGGAGGTGTCGATGTTTTCTT-3'	59.0
2	Fucosyltransferase IV *FUT-4*	F: 5'-CAGTGGCCCGCTACAAGTTC-3' R: 5'-GCCAGAGCTTCTCGGTGATATAA-3'	59.0
3	α2,3-Sialyltransferase III Gal –IV *SIAT4C*	F: 5'-GATAAGAAGCGGGTGCGAAA-3' R: 5'-TCCGAATCTGTTTAGGATTGACATC-3'	59.0
4	β1,6N-acetylglucosaminyltransferase V *MGAT-5*	F: 5'-TCAACAATCAGGAGGAAGTAGAGGAT-3' R: 5'-TTCATATGGCATGTATGGCTCAAT-3'	59.0
5	Glyceraldehyde-3-phosphate-dehydrogenase *GAPDH*	F: 5'-GCCAGCCGAGCCACATC -3' R: 5'-CCAGGCGCCCAATACGA -3'	59.0

with the PBS and the adherent cells were fixed with 200 µl 96% ethanol for 10 min. at room temperature, followed by washing with PBS (3 times) and adherent cells were stained with crystal violet (100 µl , 0,1%) for 15 min.

After staining cells were washed with water and treated with 50 µl 0,5 % Triton X-100 for overnight. Absorbance of crystal violet proportional to number of adherent cells was measured at 600 nm after 18h lasting gentle wash-out procedure. The adhesion was compared with that of untreated control melanoma cells.

Cell Proliferation Assay

The proliferation of cells was assessed with the crystal violet test.

For LTA blocking experiments cells were incubated with the lectin (Sigma, 5µg/ml RPMI 1640 medium) for 1h at 37° C. The cell were plated at dencity $1x10^4$ cell/well for A375 or WM9 and $2x10^4$ for WM239 or WM35 in 96 well plates. After 48h or 72h incubation at 37°C the medium was replaced and cell were washed with PBS. Afterwards cells were fixed with 200µl 96% ethanol for 15 min and stained with 0.5% crystal violet, washed with water and air dried. After subsequent 30 min incubation of cells with elution solution (1:1 0.1 M Na^+ citrate, pH 4.2 to 100% ethanol 200 µl per well) sample were measured at 540 nm on a plate reader (BIO-TEK).

Cytotoxicity Assay

The cytotoxicity of LTA lectin and 20mM L-fucose was assayed by the determination of activity of lactate dehydrogenase (LDH) released by treated cells to medium (Cytotoxicity Detection Kit LDH, Roche, Germany). LTA lectin and L-fucose (20mM) showed no cytotoxic effect during 72h treatment of all melanoma cells (data not shown).

Statistics

The significance of the differences between mean values were calculated using t-students test (*$p<0.05$, **$p<0.001$, ***$p<0.00001$).

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ABBEREVIATIONS

LTA = Lotus tetragonolobus agglutinin

ECM = Extracellular matrix

BSA = Bovine serum albumin

GAPDH = Glyceraldehydes-3 phosphate dehydrogenase

GlcNAc = N-acetylglucosamine

GnT-V = N-acetylglucosaminyltransferase V

FucT-I = Fucosyltransferase I

FucT-IV = Fucosyltransferase IV

LDH = Lactate dehydrogenase

PBS = Phosphate saline buffer

TBS = Tris-HCl buffer

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