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Galectin-1 knocking down in human U87 glioblastoma cells alters their gene expression pattern ☆

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Abstract

We have previously reported that (i) progression of malignancy in patients bearing astrocytic tumors correlates with increased tumor levels of galectin-1; (ii) in vitro addition of purified galectin-1 to U87 human glioblastoma cells enhances tumor cell motility; and (iii) conversely, knocking down galectin-1 expression in this cell line by stable transfection with antisense galectin-1 mRNA impairs motility and delays mortality after their intracranial grafting to nude mice. We here used cDNA microarray analysis to compare the effect on gene expression of stable transfection with antisense galectin-1 vector to mock-transfected and wild-type cells. Among the 631 spots probing genes potentially involved in cancer that were valid for analysis on all the arrays the expression of 86 genes was increased at least 2-fold. Confirmation of increased protein levels was provided by immunocytochemistry for $p21^{waf/cip1}$, cullin-2, p53, ADAM-15, and MAP-2. Major differences in the expression patterns of ADAM-15 and the actin stress fiber organization were also observed. U87 cells stably deficient for galectin-1 expression were significantly less motile than control. We conclude that the stable inhibition of galectin-1 expression alters the expression of a number of genes that either directly or indirectly influence adhesion, motility and invasion of human glioblastoma cells.

Keywords: Galectin-1; Glioblastoma; Microarray; Migration; ADAM-15; Actin cytoskeleton; Cullin-2; p53; $p21^{waf}$; MAP-2

Most of the cell-adhesion molecules and ECM proteins that favor the migration of tumor astrocytes are glycosylated and, in particular, bear considerable amounts of β-galactosyl residues and poly-*N*-acetyllactosamine chains, which are ligands for galectins [1,2]. Galectins, originally named galactose-specific lectins, are synthesized in the cytosol without a signal peptide and may be released from cells by non-conventional secretory mechanism(s) that bypass the endoplasmic reticulum and the Golgi complex (see [1], for review). Galectins form homodimers or oligomers that can readily bridge *N*- and *O*-glycans as well as glycolipids present on cell surfaces with similar glycans in the ECM [1]. Although most galectins have been described as extracellular actors, intracellular functions have also been reported [1].

Galectin-1 appears to be the most important member of this family in physiological brain processes [3]. Indeed, in patients with human glial tumors, the expression patterns of galectin-1 and -3, but not of -8, correlate with the grade of malignancy [4–7]. In

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addition, a low level of galectin-1 expression in tumors is associated with patients' unusually long survival periods [4]. Conversely, high levels of galectin-1 expression have been observed for highly invasive tumoral astrocyte, both in the case of human surgical samples and of animal models [4,7]. In vitro, the addition of galectin-1 to culture medium of U87 cells increased cell motility [6,7]. These effects were associated with the reorganization of the actin cytoskeleton and the increased expression of the small GTPase, RhoA [7]. We engineered human U87 glioblastoma cells constitutively expressing reduced levels of galectin-1 (U87/G1$^-$) by means of stable transfection of an expression vector for the antisense mRNA of galectin-1 [7]. In vivo, intracranial grafting of galectin-1 deficient U87 cells into nude mice led to much longer survival in comparison with mice grafted with control cells [7].

In the present study, we used a comparative cDNA microarray to screen which set of genes could explain, at least partly, why galectin-1 deficient U87 cells are less aggressive than their wild-type and mock-transfected counterpart. For selected gene products, quantitative immunocytochemical analysis ascertained that up-regulated gene expression translated into enhanced protein levels. Taken together, our results reveal that galectin-1 is a pleiotropic regulator of the malignant phenotype in the human U87 glioblastoma cell model.

Materials and methods

Cell lines and reagents

The U87 wild-type (U87/wt) glioblastoma cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and propagated as monolayers [4]. The U87 cells knockdown for galectin-1 expression (U87/G1$^-$) and the corresponding mock (U87/mock) cell lines were obtained by stable transfection with antisense galectin-1 or mock vectors. As previously reported, U87/G1$^-$ cells expressed about five times less galectin-1 than their wt or mock-transfected counterparts [7]. To ensure specificities in view of the high level of similarity between galectins, we checked by an in vitro dimerization assay that the antisense galectin-1 mRNA, produced by its expression vector, did not hybridize to other galectin mRNA (data not shown). To exclude any contamination problems between the cell lines, DNA fingerprinting was carried out to verify that both U87-transfected cell lines originated from the parental U87/wt cell line. The nine microsatellites tested showed identical profiles in the three cell lines, so confirming that the transfectants in fact originated from the parental U87wt cell line (data not shown).

Goat polyclonal anti-ADAM-15 (C-20) was from Santa Cruz Biotechnology (SanverTECH, Boechout, Belgium) was used at 2 μg/ml and the mouse monoclonal anti-p21$^{waf/cip1}$ (Ab-5; HZ52; 0.2 μg/ml), anti-MAP-2a, b antibodies (AB-2, clone AP20; 0.2 μg/ml) and rabbit polyclonal anti-cullin-2 antibody (Ab-1; 10 μg/ml) originated from NeoMarkers (Fremont, CA, USA).

Gene expression analysis by cDNA microarrays

Total RNA extraction. Total RNA was extracted from non-confluent cells with TriPure (Roche, Mannheim, Germany) according to the manufacturer's instructions and was treated with DNase-I (Invitrogen, Life Technologies). The quantity of the extracted RNA was measured by spectrophotometry at 260 nm (Beckman–Coulter DU640, Analis, Ghent, Belgium); its integrity was assessed by the RNA 6000 LabChip kit (Agilent, Technologies, Massy, France) and analyzed with the Agilent 2100 bioanalyzer as well as by gel electrophoresis [8]. These checks were completed by the analysis of the β-actin gene expression by means of a standard 25 cycles RT-PCR method with 200 ng of loaded cDNA [8].

cDNA arrays hybridization. The arrays used in this study were from Ipsogen (Marseilles, France). Each V1.0 cDNA nylon cancer Chip membrane contained 1771 different clones of genes known to be involved in cancer and 201 clones with unknown functions; some clones were double spotted, giving a total of 2305 spots. Re-sequenced clones from the IMAGE consortium database (http://image.llnl.gov) were represented on the chips in the form of cDNA PCR fragments. Background measurement was carried out on the basis of spotted poly(A) sequences, PCR negative controls, non-relevant cDNAs and empty spots (a total of 350 additional spots per membrane). After membrane hybridization (with vector probe hybridization and ^{33}P-labeled cDNAs), imaging and quantification were carried with a Fuji-BAS5000 scanner and AIDA image analyzer software (Raytest Benelux, Tilburg, NL). Two different samples of RNA were processed independently for the three cell lines under study.

cDNA microarray data analysis. As the first step, the values obtained were preprocessed to compare the multiple arrays. Only the 631 spots which exhibited intensity values above the background level on each array were considered further, and the values from a given array were normalized to the median intensity corresponding to this set of valid spots (i.e., the median set to 1). As the second step, the levels of significance to reliably detect up- or down-regulation were established as described [9]. This approach enabled the significant detection up- (>2.33) or down- (<0.4) regulated genes.

The analysis showed that whereas that 91 spots were significantly overexpressed, none of the 631 genes valid for analysis in the U87/G1$^-$ cell line underwent any significant decrease in their level of expression as compared to control (wt and mock). Some of the genes in the microarray were probed by two different spots corresponding to two different IMAGE clones. As illustrated in Tables 1 and 3, the reproducibility obtained for the duplicate measurements was adequate. Because of these duplicates, the 91 overexpressed spots related

Table 1
cDNA microarray analysis

Gene name	Code	Image ID	Ratio
cyclin d3	ccnd3	327182	2.78]
cyclin d3	ccnd3	358749	2.36]
cullin-2	cul2	286287	2.60
nedd8 ubiquitin-like protein	nedd8	1086357	2.41
proliferating cell nuclear antigen	pcna	232941	2.53]
proliferating cell nuclear antigen	pcna	789182	2.64]
cdk inhibitor p21$^{waf/cip1}$	cdkn1a	152524	3.56]
cdk inhibitor p21$^{waf/cip1}$	cdkn1a	291705	3.04]
tp53	Tp53	236338	2.34
cyclin g2	ccng2	21505	3.14
bag1	bag1	469256	2.78
caspase 1	casp1	120106	2.56

Overexpression of genes involved in the regulation of cell cycle and apoptosis.
Image ID: clone identification number in the I.M.A.G.E. consortium database. Ratio: ratio between the expression level of U87/G1$^-$ and CT (U87/wt and U87/mock). A significant gene overexpression is reached when ratio >2.33. For details see Materials and methods.] indicate reproducibility between duplicate spots.

Table 2
cDNA microarray analysis

Gene name (synonyms)	Code	Image ID	Ratio
ECM			
thrombospondin-1	thbs1	160963	2.64
extracellular matrix-1	ecm1	301122	2.64
Adhesion			
ADAM-15	ADAM15	713782	3.30
CD147 (EMMPRIN, basigin)	Bsg	756533	2.57
integrin α7	itga7	628743	3.57
integrin α9	itga9	1086306	2.41
cadherin-6 (k-cadherin)	cdh6	739155	2.98
tetraspanin-3 (TM4SF3, CO-029)	Tm4sf3	509731	2.93
catenin β1[a]	ctnnb1	774754	2.86
transmembrane glycoprotein	Gpnmb	773330	6.06
Cytoskeleton			
actin-capping protein	Capg	1012666	2.36
microtubule-associated protein-2	map2	347503	2.77
tubulin β5	tubb5	171684	3.15

Overexpression of genes involved in the control of cell migration. Same presentation as in Table 1.

[a] Catenin β1 acts also as a pivot between cell adhesion and Wnt signaling, as a transcription factor and as a ligand for galectin-3 [31].

to 86 different genes. These are listed in Tables 1–3. As detailed in the Results and discussion, 22 of them attracted our attention because of their potential involvement in the control of cell growth and migration.

Fluorescent detection of protein expression

Protein expression in the cells cultured on glass coverslips was revealed by immunofluorescence as described elsewhere [8]. The binding of the primary antibodies was detected by Alexa Fluor 488- or 594-conjugated secondary antibodies (10 μg/ml, Molecular Probes, Eugene, OR, USA). For controls, primary antibodies were replaced by nonspecific serum or slides were incubated with secondary antibodies alone. The organization of the actin cytoskeleton was demonstrated by fluorescent phallacidin-conjugated with Alexa Fluor488; G-actin was simultaneously stained with Alexa Fluor 594-conjugated DNAse-I (Molecular Probes) as described elsewhere [7,8]. Fluorescent images acquisition was carried out with a computer-assisted fluorescent microscope (Olympus AX70, Omnilabo, Antwerp, Belgium) equipped with a Megaview2 digital camera and the analySIS software (Soft Imaging System, Munster, Germany).

Motility assay

Cell motility was quantified by a videomicroscopy cell tracking device detailed elsewhere [7,10]. The greatest linear distance covered by each cell was semi-automatically calculated from their recorded trajectories, so as to yield the maximum relative distance from the point of origin (MRDO variable). All experiments were performed over 24 h. A minimum of 1000 cells were analyzed for each experimental condition.

Statistical analysis

All the values shown are means ± standard error of the mean (SEM). The statistical significance of the comparisons between the different experimental groups was tested by the non-parametric Mann–Whitney U test and is indicated by $^{***}p < 0.001$.

Results

Altered gene and protein expression in cells expressing a reduced level of galectin-1

Among the 86 genes over expressed in the U87 cells knock-down for galectin-1 expression, some gene products attracted our attention because of their potential involvement in the complex processes of tumor aggressiveness, i.e., cell growth and cell migration. Nine of these gene products known to be involved in the control of the cell cycle and apoptosis were overexpressed in the U87/G1$^-$ as compared to the wt and mock-transfected cells (Table 1). Overexpression was confirmed by fluorescence microscopy for p21$^{waf/cip1}$ (Figs. 1A and B), p53 (Figs. 1C and D), and cullin-2 (Figs. 1E and F). Ten gene products involved in cell adhesion were also overexpressed, namely, extracellular matrix interaction (thrombospondin-1 and extracellular matrix protein-1), matrix digestion (ADAM-15), and its control (EMMPRIN); cell surface adhesion (integrin α7 and α9, cadherin-6) or interaction with the latter (tetraspanin-3 and catenin β1). The increased abundance of ADAM-15 in U87/G1$^-$ was confirmed by computer-assisted quantitative immunofluorescence microscopy (data not shown). Interesting pattern differences were noticed (Figs. 1G and H). For example, the ADAM-15 protein had no preferred site in the U87/wt cells (Fig. 1G) but showed clear peripheral labeling and accumulated at cell–cell contact sites in the U87/G1$^-$ cells, where it was more abundant (Fig. 1H).

Another set of three gene products are involved in the cytoskeleton. They include the actin-capping-protein, capG, as well as the β5 tubulin isoform and the microtubule-associated protein MAP-2. CapG overexpression predicts modifications to the actin cytoskeleton dynamics. The level of polymerized (fibrillar) actin in relation to monomeric (globular) actin was thus further characterized by fluorescence microscopy. As shown in Figs. 2A and B, whereas the pool of fibrillar actin labeled with phallacidin decreased markedly in U87/G1$^-$ as compared to the wt cells, there was no detectable change in the pool of actin monomer labeled with DNAse-I. This result was confirmed by the quantification of the red and green fluorescent intensities of 100 cells in each experimental condition: a ~70% decreased in the intensity of the green fluorescence was observed in the U87/G1$^-$ cells when compared to the parental U87/wt (data not shown). The increased level of MAP-2 in the U87/G1$^-$ cells was also confirmed directly at protein level by immunofluorescence analysis (Figs. 2C and D). These modifications to the levels and patterns of expression of proteins known to be involved in the control of cell migration predicted altered U87/G1$^-$ motility as compared to control. A significant decrease in the level of

Table 3
cDNA microarray analysis

Gene name	Code	Image ID	Ratio
aldehyde deshydrogenase 3	aldh3a1	525221	2.47
atpase 6 d	atp6d	320610	2.37
b-cell translocation gene 1	btg1	382760	2.56
cd 63	cd63	125552	2.78
cytochrome *c* assembly protein 11	cox11	23707	2.65
cytochrome *c* assembly protein 5b	cox5b	549361	2.51
cytochrome *c* assembly protein 6a1	cox6a1	512910	2.68
cytochrome *c* assembly protein 7b	cox7b	566862	2.55
collapsin response mediator 1	crmp1	878280	2.38
distal less homeobox 2	dlx2	1486752	2.52
dual specificity phosphatase 6	dusp6	40851	2.89
dishevelled 3	dvl3	1932820	2.74
platelet derived-endothelial cell growth factor 1	ecgf1	1944256	3.85
translation elongation factor 1alpha1	eef1a1	1683100	2.91
translation elongation factor 4a1	eif4a1	46171	2.35
ephrin receptor x1	ephx1	49995	2.34
erbb2ip	erbb2ip	595089	2.42
frizzled 5	fzd5	2254555	2.89
galactose 1 phosphate uridyllyltransferase	galt	68972	2.34
GAPDH	gapd	153607	2.52
GAPDH	gapd	50117	2.38
glutathione s tranferase theta 1	gstt1	1173851	2.34
gw128	gw128	262546	2.50
protein kinase h11	h11	359191	2.78
hypoxia inducible factor 1a	hif1a	142934	2.96
3-hydroxy-3-methylglutaryl-coenzyme a reductase	hmgcr	125682	3.47
hepatocyte nuclear factor 3a	hnf3a	84786	2.42
interferon inducible protein	ifi30	856447	2.39
interferon related regulator 1	ifrd1	882483	5.77
inhibin beta b	inhbb	730012	2.80
jun d	jund	175421	2.68
kiss-1 metastasis suppressor	kiss1	812955	2.57
kynureinase	kynu	252515	2.60
lim and sh3 protein 1	lasp1	592598	2.71
leukotriene a4 hydrolase	lta4h	814095	2.34
malate dehydrogenase1, nad	mdh1	53316	2.49
midkine	mdk	309009	2.94
mucine 1	muc1	840687	2.48
nuclear receptor coactivator 4	ncoa4	25858	2.81
nuclease sensitive element binding protein	nsep1	211623	2.82
nuclease sensitive element binding protein	nsep1	324561	2.88
plexin b1	plxnb1	289743	2.61
protein phosphatase 1a (catalytic subunit)	ppp1ca	1422791	2.42
tyrosine phosphatase 1a	ptpra	21477	2.48
retinoblastoma binding protein2	rbbp2	509544	2.48
S100A6	s100a6	512420	2.85
scaffold attachment factor b	safb	42280	2.76
carrier protein	slc2a1	207358	2.80
swi/snf related, actin dependent regulator of chromatin	smarce1	338519	2.50
nuclear antigen sp100	sp100	429357	2.38
stac	stac	470379	3.11
stromal interaction molecule 1	stim1	41993	2.90
tbx3- isoprotein	tbx3	131589	2.76
homeobox 11-like 2	tlx3	1860115	2.64
u5 snmp specific protein—116 kDa	u5–116 kDa	1013232	2.36
unknown	unknown	1691084	2.82
unknown	unknown	33133	2.82
unknown	unknown	44478	3.60
unknown	unknown	545138	4.03
unknown	unknown	599882	3.53
unknown	unknown	655774	2.87
unknown	unknown	785765	3.22
ubiquitin specific protease 4	usp4	544818	3.59

Table 3 (*continued*)

Gene name	Code	Image ID	Ratio
ubiquitin specific protease 8	usp8	1091323	2.41
tyr/trp monooxygenase	ywhaq	487913	2.39
tyr/trp monooxygenase	ywhaz	229706	2.41

Overexpression of genes involved in other biological functions. Same presentation as in Table 1.

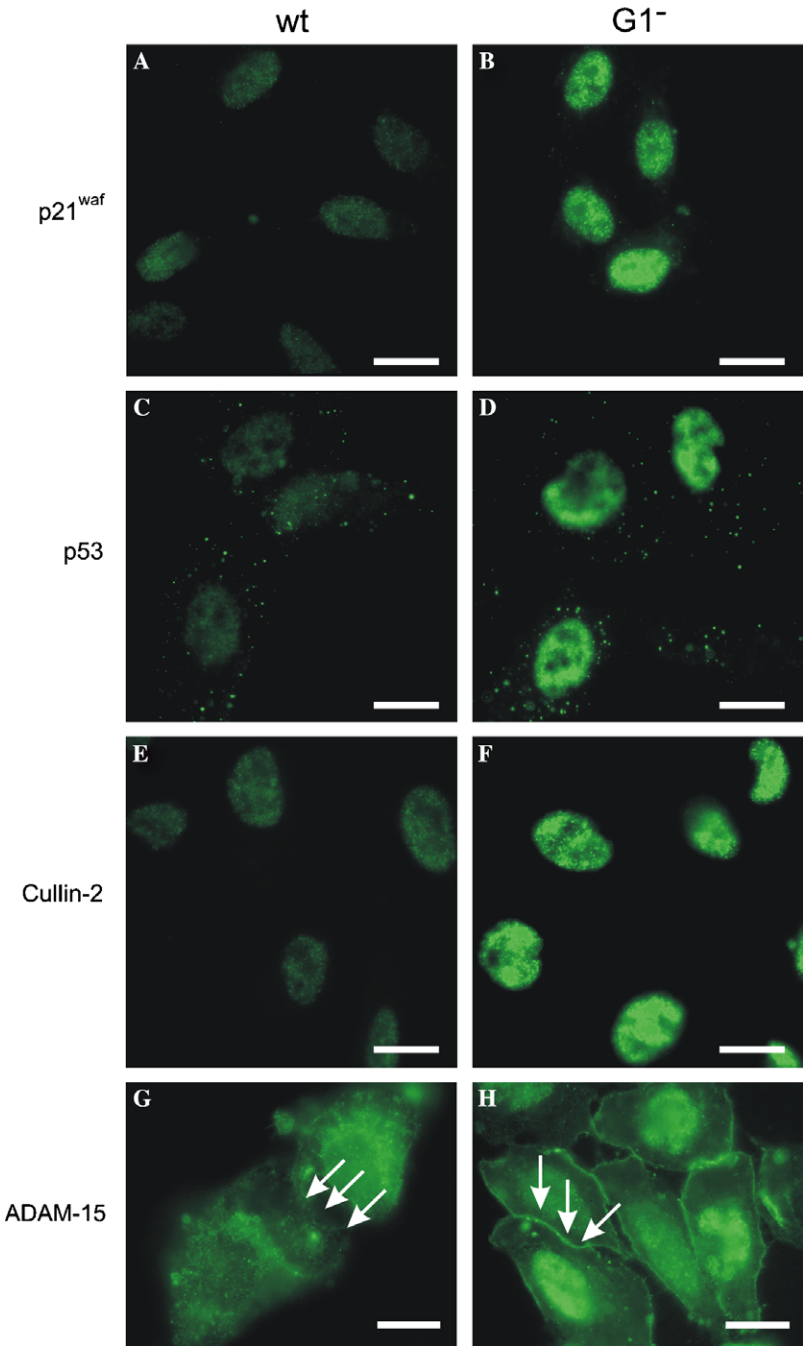

Fig. 1. Expression level of gene products: immunofluorescence evidence. Magnification bars: 25 μm.

U87/G1$^-$ cell motility was in fact evidenced in the case of cells cultured on Matrigel- or fibronectin-coated substrates, but not in the case of laminin- or vitronectin-coated ones (Fig. 2E).

Discussion

Tumor aggressiveness in general, and that of high-grade astrocytic tumors in particular, depend not only

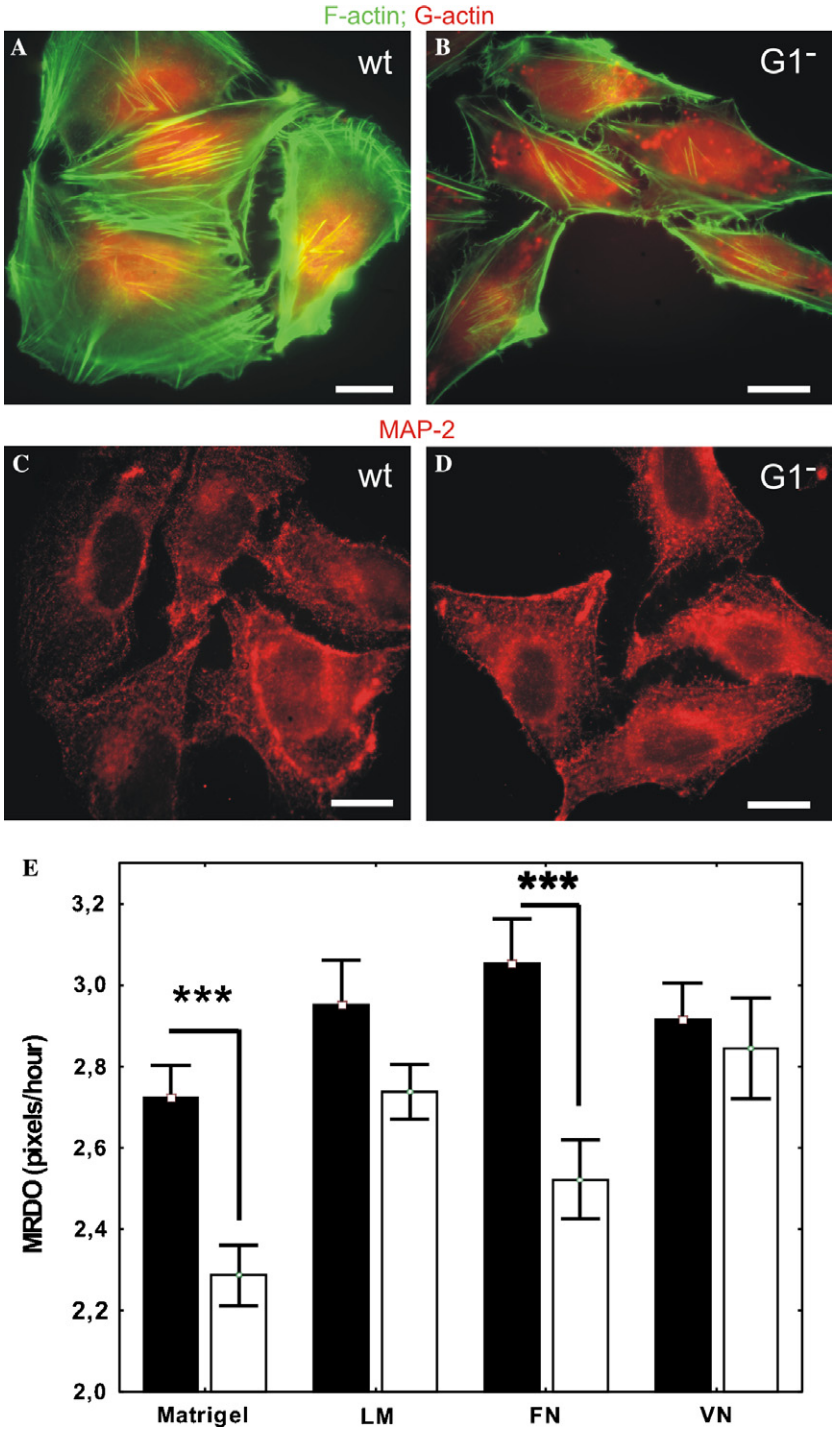

Fig. 2. Expression and function of gene products related to cell motility: evidence from immunofluorescence. (A,B) F-actin (polymeric, green) and G-actin (monomeric, red) were evidenced in U87/wt (A) and U87/G1$^-$ cell lines (B) by fluorescent probes. Magnification bars: 25 μm. (C,D) Immunofluorescence pattern of MAP-2. Magnification bars: 25 μm. (E) Motility of the U87wt (filled bars) and the U87/G1$^-$ cells (open bars) as evaluated by quantitative videomicroscopy. The maximum relative distance from the point of origin (MRDO) traveled by living cells growing in vitro on a substrate coated with Matrigel, laminin (LM), fibronectin (FN), and vitronectin (VN) was recorded for at least 1000 cells per condition. $^{***}p < 0.001$.

on an imbalance between cell proliferation and cell death favoring growth, but also on a higher propensity of tumor cells to detach from the primary tumor site to migrate, and to invade the surrounding parenchyma [2]. Several of these steps involve extracellular proteolysis. By immunohistochemical analysis of specimens from patient, from animal studies and from in vitro studies of glioblastoma cell lines as well as by SAGE screening of invasive cells, the picture has emerged that galectin-1 is of critical importance in astrocytic tumor cell growth

and invasion [4,6,7,11,12]. We have therefore analyzed stable galectin-1 antisense transfectants of human U87 glioblastoma cells for gene expression in detail; cDNA microarray analysis revealed that a set of genes generally regarded as involved in the regulation of cell cycle and apoptosis is enhanced in U87 cells deficient in galectin-1. Among these, the overexpression of p53, $p21^{waf/cip1}$ and cullin-2 was confirmed at protein level.

Ras (which has importance for p21) is associated with crucial roles within the progression of malignancy of astrocytic tumors [13] and its biological activity necessitates membrane anchorage that depends on the Ras farnesyl moiety and is strengthened by Ras/galectin-1 interactions [14]. Galectin-1 also acts as a negative growth regulator in neuroblastoma cells [15], while it does not display such a role in tumor astrocytes (data not shown). In fact, the cell density-dependent growth inhibition of human SK-N-MC neuroblastoma cells is initiated by increased ganglioside sialidase activity leading to elevated cell surface presentation of ganglioside GM1, a ligand of galectin-1 [15]. Galectin-1 is thus a probable effector in the sialidase-dependent growth control of SK-N-MC neuroblastoma cells [15]. Astrocytic tumor cells seem to display weak, if any, amounts of ganglioside GM1, while oligodendroglial tumor cells do it [16].

Integration of our present study with our previous one [7] provides further evidence that U87 tumor astrocytes with galectin-1 knocking down are characterized by modified adhesive and motile properties as compared to parental or mock-transfected cells. Binding to a permissive ECM and integrin-dependent focal adhesion dynamics is critical for cell migration. To migrate, cells must not only repeatedly adhere to a substrate and detach themselves from it in a coordinated manner; they also need to generate a tensile force in the direction of movement. The actin cytoskeleton and integrin-dependent protein complexes that anchor the actin to specific sites on the cell membrane generate this force. Hikita et al. [17] have demonstrated for example that secretion of galectin-3 by epithelial cells is required for the correct polymerization of ECM components such as hensin (a glycoprotein responsible for the polarization of intercalated columnar epithelial cells). The present study shows that the mRNA expression of α9 integrin was increased in U87 cells expressing a reduced amount of galectin-1. We are now investigating in detail how α9 integrin is involved in galectin-1-related glioblastoma cell adhesiveness. It has recently been reported that galectin-3 knocking down expression induces an overexpression of α6 integrin with increased cell motility in glioma cells migrating on laminin [18].

Dynamic actin remodelling is also essential for invasion. An association between a decrease in the pool of fibrillar actin and a decrease in cell motility is not uncommon and has been reported in tumor astrocytes [19]. In the present study, the decreased cell motility of U87 cells expressing a reduced amount of galectin-1 was paralleled by obvious disturbances in actin polymerization without any detectable changes in the pool of globular actin. The galectin-1-related re-organization of the actin cytoskeleton has also been described for T-cells [20] and tumor astrocytes [7]. The highly dynamic remodelling of the actin cytoskeleton required for cell motility is under the control of numerous actin-binding proteins [21]. One of these is CapG, a member of the gelsolin family that exclusively caps the barbed ends of actin filaments without the severing activity of gelsolin [21]. CapG is a key controller in the regulation of actin polymerization and cell motility [21]. The significant increase in the expression of *CapG* in U87 cells deficient in galectin-1 provides additional support for the view that defects in the dynamics of actin polymerization/depolymerization have profound effects on cell motility and adhesion. Indeed, increased levels of activated CapG are expected to induce the increased capping of the barbed ends of actin filaments, thereby causing net filament depolymerization and impairing actin-based motility [22].

Although microtubules do not directly contribute to the generation of the forces that drive the cell movements of most cell types, they are key partners in the polarization of actin-based motility [23]. In U87 cells deficient in galectin-1 we found that the mRNA of the tubulin β5 isoform and the microtubule-associated protein-2 (MAP-2) are both overexpressed as compared to U87/wt cells. Particularly interesting is the increased expression of microtubule-associated protein-2 (MAP-2), a member of the family of structural MAPs that bind microtubules and is believed to play an important part in the regulation of microtubule polymerization and stabilization [24]. By interacting with the carboxy-terminus of tubulin, these MAPs stabilize microtubules and cross-link them to actin [24]. It is interesting to note that some tubulin isoforms are ligands for galectin-2 [25].

The present report also shows that the reduced expression of galectin-1 in U87 glioblastoma cells brings about an increased expression of *cadherin-6*, *tetraspanin-3*, *ADAM-15*, and *CD147* mRNAs. Interestingly, alterations to E-cadherin glycosylation with subsequent modifications to its interaction with lectins suppress lung metastasis formation and are associated with a reduced level of tyrosine phosphorylation of β-catenin (also listed in Table 2) that may play a central role in cell motility [26,27]. Numerous reports have described the role of tetraspanins in both cell–cell and cell–ECM adhesion. Although tetraspanins may have pro-migratory activities, most studies of this family of proteins have emphasized their negative effect on tumor cell motility and invasiveness (for a review see [28]). The ADAM family of membrane-anchorage glycoproteins encompasses a

catalytically active metalloproteinase domain and a disintegrin domain (for a review see [29]) and may thus be involved both in the proteolytic cleavage of cell-surface proteins and in integrin-mediated cell adhesion (including α9β1/ADAM-15 interactions) via RGD-dependent and -independent binding [30].

In conclusion, this report points to several potential mechanisms whereby the level of galectin-1 expression affects glioblastoma malignancy either directly or indirectly. The cDNA microarray unravelled a complex pattern of related genes whose expression is enhanced in galectin-1 deficient cells. These properties may result from the multifunctionality of galectin-1 and are likely to have a profound influence on the infiltration patterns of malignant astrocytes.

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References

[1] H.-J. Gabius, H.-C. Siebert, S. André, J. Jiménez-Barbero, H. Rüdiger, Chemical biology of the sugar code, Chembiochem 5 (2004) 740–764.
[2] F. Lefranc, J. Brotchi, R. Kiss, Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis, J. Clin. Oncol. 23 (2005) 2411–2422.
[3] B.S. Stillman, P.S. Mischel, L.G. Baum, New roles for galectins in brain tumors—from prognostic markers to therapeutic targets, Brain Pathol. 15 (2005) 124–132.
[4] I. Camby, N. Belot, S. Rorive, F. Lefranc, C.A. Maurage, A. Lahm, H. Kaltner, Y. Hadari, M.-M. Ruchoux, J. Brotchi, Y. Zick, I. Salmon, H.-J. Gabius, R. Kiss, Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration, Brain Pathol. 11 (2001) 12–26.
[5] L. Gordower, C. Decaestecker, Y. Kacem, A. Lemmers, J. Gusman, M. Burchert, A. Danguy, H.-J. Gabius, I. Salmon, R. Kiss, I. Camby, Galectin-3 and galectin-3-binding site expression in human adult astrocytic tumours and related angiogenesis, Neuropathol. Appl. Neurobiol. 25 (1999) 319–330.
[6] S. Rorive, N. Belot, C. Decaestecker, F. Lefranc, L. Gordower, S. Micik, C.A. Maurage, H. Kaltner, M.-M. Ruchoux, A. Danguy, H.-J. Gabius, I. Salmon, R. Kiss, I. Camby, Galectin-1 is highly expressed in human gliomas with relevance for modulation of invasion of tumor astrocytes into the brain parenchyma, Glia 33 (2001) 241–255, Erratum Glia 33 (2001) 166.
[7] I. Camby, N. Belot, F. Lefranc, N. Sadeghi, Y. de Launoit, H. Kaltner, S. Musette, F. Darro, A. Danguy, I. Salmon, H.-J. Gabius, R. Kiss, Galectin-1 modulates human glioblastoma cell migration into the brain though modifications to the actin cytoskeleton and the levels of expression of small GTPases, J. Neuropathol. Exp. Neurol. 61 (2002) 585–596.
[8] F. Lefranc, T. Mijatovic, V. Mathieu, S. Rorive, C. Decaestecker, O. Debeir, J. Brotchi, P. Van Ham, I. Salmon, R. Kiss, Characterization of gastrin-induced proangiogenic effects in vivo in orthotopic U373 experimental human glioblastomas and in vitro in human umbilical vein endothelial cells, Clin. Cancer Res. 10 (2004) 8250–8265.
[9] M. Remmelink, T. Mijatovic, A. Gustin, A. Mathieu, K. Rombaut, R. Kiss, I. Salmon, C. Decaestecker, Identification by means of cDNA microarray analyses of gene expression modifications in squamous non-small cell lung cancers as compared to normal bronchial epithelial tissue, Int. J. Oncol. 26 (2005) 247–258.
[10] O. Debeir, P. Van Ham, R. Kiss, C. Decaestecker, Tracking of migrating cells under phase-contrast video microscopy with combined mean-shift processes, IEEE Trans. Med. Imaging 24 (2005) 697–711.
[11] J.M. Gunnersen, V. Spirkoska, P.E. Smith, R.A. Danks, S.S. Tan, Growth and migration markers of rat C6 glioma cells identified by serial analysis of gene expression, Glia 32 (2000) 146–154.
[12] K. Yamaoka, K. Mishima, Y. Nagashima, A. Asai, Y. Sanai, T. Kirino, Expression of galectin-1 mRNA correlates with the malignant potential of human gliomas and expression of antisense galectin-1 inhibits the growth of 9 glioma cells, J. Neurosci. Res. 59 (1997) 722–730.
[13] P.S. Mischel, T.F. Cloughesy, Targeted molecular therapy of GBM, Brain Pathol. 13 (2003) 52–61.
[14] B. Rotblat, H. Niv, H. Kaltner, H.J. Gabius, Y. Kloog, Galectin-1 (L11A) predicted from a computed galectin-1 farnesyl-binding pocket selectively inhibits Ras-GTP, Cancer Res. 64 (2004) 3112–3118.
[15] J. Kopitz, C. von Reitzenstein, S. Andre, H. Kaltner, J. Uhl, V. Eheman, M. Cantz, H.J. Gabius, Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3, J. Biol. Chem. 276 (2001) 35917–35923.
[16] B. Popko, D.K. Pearl, D.M. Walker, T.C. Comas, K.D. Baerwald, P.C. Burger, B.W. Scheithauer, A.J. Yates, Molecular markers that identify human astrocytomas and oligodendrogliomas, J. Neuropathol. Exp. Neurol. 61 (2002) 329–338.
[17] C. Hikita, S. Vijayakumar, J. Takito, H. Erdjument-Bromage, P. Tempst, Q. Al-Awqati, Induction of terminal differentiation in epithelial cells requires polymerization of hensin by galectin-3, J. Cell Biol. 151 (2000) 1235–1246.
[18] C. Debray, P. Vereecken, N. Belot, P. Teillard, J.-P. Brion, M. Pandolfo, R. Pochet, Multifaceted role of galectin-3 on human glioblastoma cell motility, Biochem. Biophys. Res. Commun. 325 (2005) 1393–1398.
[19] F. Lefranc, I. Camby, N. Belot, E. Bruyneel, C. Chaboteaux, J. Brotchi, M. Mareel, I. Salmon, R. Kiss, Gastrin significantly modifies the migratory abilities of experimental glioma cells, Lab. Invest. 82 (2002) 1241–1252.
[20] G.A. Rabinovich, A. Ariel, R. Hershkoviz, J. Hirabayashi, K.I. Kasai, O. Lider, Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1, Immunology 97 (1999) 100–106.
[21] T.D. Pollard, L. Blanchoin, R.D. Mullins, Molecular mechanisms controlling actin filament dynamics in nonmuscle cells, Annu. Rev. Biophys. Biomol. Struct. 29 (2000) 545–576.
[22] W. Witke, W. Li, D.J. Kwiatkowski, F.S. Southwick, Comparison of CapG and gelsolin-null macrophages, demonstration of a unique role for capG in receptor-mediated ruffling, phagocytosis, and vesicle rocketing, J. Cell Biol. 154 (2001) 775–784.
[23] T. Wittmann, C.M. Waterman-Storer, Cell motility:can Rho GTPases and microtubules point the way? J. Cell Sci. 114 (2001) 3795–3803.

[24] R.B. Maccioni, V. Cambiazo, Role of microtubule-associated proteins in the control of microtubule assembly, Physiol. Rev. 75 (1995) 835–864.
[25] K. Ozaki, K. Inoue, H. Sato, A. Iida, Y. Ohnishi, A. Sekine, H. Sato, K. Odashiro, M. Nobuyoshi, M. Hori, Y. Nakamura, T. Tanaka, Functional variation in LGALS2 confers risk of myocardial infarction and regulates lymphotoxin-alpha secretion in vitro, Nature 429 (2004) 72–75.
[26] M. Yoshimura, A. Nishikawa, Y. Ihara, S.I. Taniguchi, N. Taniguchi, Suppression of lung metastasis of B16 mouse melanoma by *N*-acetyl-glucosaminyltransferase III gene transfection, Proc. Natl. Acad. Sci. USA 92 (1995) 8754–8758.
[27] T. Kitada, E. Miyoshi, K. Noda, S. Higashiyama, H. Ihara, N. Matsuura, N. Hayashi, S. Kawata, Y. Matsuzawa, N. Taniguchi, The addition of bisecting *N*-acetylglucosamine residues to E-cadherin down-regulates the tyrosine phosphorylation of beta-catenin, J. Biol. Chem. 276 (2001) 475–480.
[28] F. Berditchevski, Complexes of tetraspanins with integrins, more than meets the eye, J. Cell Sci. 114 (2001) 4143–4151.
[29] P. Primakoff, D.G. Myles, The ADAM gene family. Surface proteins with adhesion and protease activity, Trends Genet. 16 (2000) 83–87.
[30] K. Eto, W. Puzon-McLaughlin, D. Sheppard, A. Sehara-Fujisawa, X.P. Zhang, Y. Takada, RGD-independent binding of integrin $\alpha 9\beta 1$ to the ADAM-12 and -15 disintegrin domains mediates cell–cell interaction, J. Biol. Chem. 275 (2000) 34922–34930.
[31] T. Shimura, Y. Takenaka, S. Tsutsumi, V. Hogan, A. Kikuchi, A. Raz, Galectin-3, a novel binding partner of beta-catenin, Cancer Res. 64 (2004) 6363–6367.