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Galectin-1 Regulates p53 and Is Implicated in Glioma Cell Resistance to Cytotoxic Drugs

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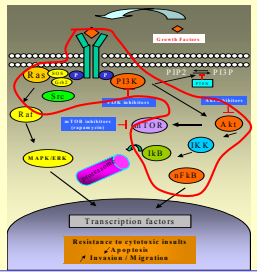
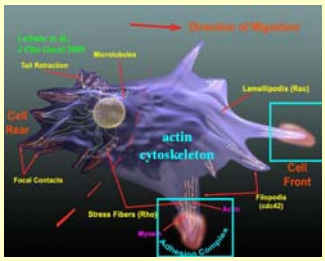
➔ Glioblastomas (GBM) are the most common type of primary malignant brain tumor.

➔ Patients have an average life expectancy of one year on the basis of the standard treatment of surgical section followed by radiotherapy.

➔ GBM are associated with dismal prognoses because they diffusely infiltrate the brain parenchyma, and because migrating malignant glioma cells are resistant to apoptosis, and thus to pro-apoptotic cytotoxic drugs due to constitutive activation of distinct anti-apoptotic signaling pathways including: PI3-K, Akt/PKB (PTEN), mTOR, NF-kappaB, etc…





Temozolomide displays actual efficacy against malignant gliomas (Stupp et al., N Engl J Med 2005) because it is a pro-autophagic drug, not a pro-apoptotic one (Kanzawa et al., Cell Death Differ 2004)

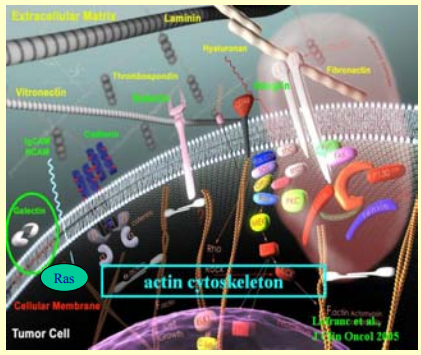
Galectin-1 is a potent modulator of GBM cell migration and a close partner of Ras whose importance as a signaling molecule in the case of GBMs has already been highlighted

➔ Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration.
Camby et al., Brain Pathol, 2001

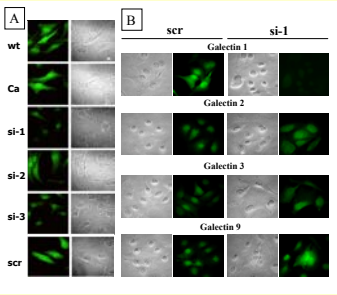
➔ Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases.
Camby et al., J Neuropathol Exp Neurol, 2002

➔ Galectin-1: A small protein with major functions.
Camby et al., Glycobiology 2006, in press.

We investigated whether decreasing the levels of expression of galectin-1 in human Hs683 GBM cells could increase their sensitivity to the pro-autophagic effects of temozolomide.
An anti-galectin-1 siRNA approach was employed to decrease the expression levels of galectin-1 in human Hs683 GBM cells.



Designing Anti-Galectin-1 siRNAs



Human galectin-1 (accession number NM_002305, 526 bp) with indication of the location of three putative siRNA sequences: si-1 (first underlined bold sequence below), si-2 (second underlined sequence below) and si-3 (third underlined bold sequence below).

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1 atctctctcg ggtggagtcc ttctgacagc tggtgcgcct gcccgggaac atcctcctgg
61 actcaatcat ggcttgtggt ctggtcgcca gcaacctgaa tctcaaacct ggaggagtgc
121 ttcgagtgcg aggcgaggtg gctcctgacg ctaagagctt cgtgctgaac ctgggcaaag
181 acagcaacaa cctgtgcctg cacttcaacc ctcgcttcaa cgcccacggc gacgccaaca
241 ccatcgtgtg caacagcaag gacggcggtg cctgggggac cgagcagcgg gaggctgtct
301 ttcccttcca gcctggaagt gttgcagagg tgtgcattac cactgaccag gccaacctga
361 ccgtcaagct gccagatgga tacgaattca agttcccaaa ccgcctcaac ctggaggcca
481 agccagccca tggccccaaa taaaggcagc tgcctctgct cccctg (SEQ ID No:69)
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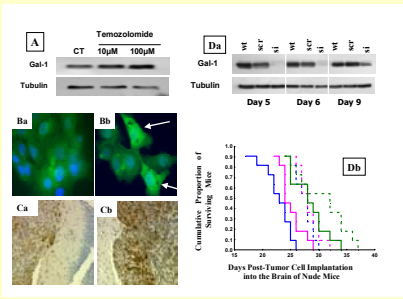
A: The expression of Gal1 (green fluorescence) detected by immunofluorescence in Hs683 GBM cells transfected for 5 days with si-1, si-2, si-3, scrambled siRNA (scr) or with calcium phosphate transfection buffer (Ca) compared to that of wild-type (wt) Hs683 cells.

B: Expression of Gal1, Gal2, Gal3 and Gal9 detected by immunofluorescence in Hs683 GBM cells transfected for 5 days with si-1 anti-Gal1 siRNA or with the scr siRNA.

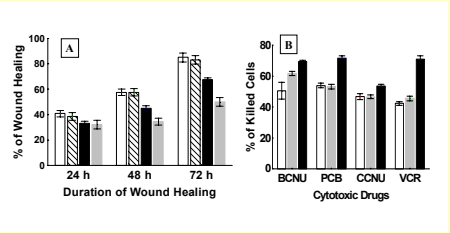
Temozolomide Increases Galectin-1 Expression in Human Hs683 GBM Cells *In Vitro* and *In Vivo*



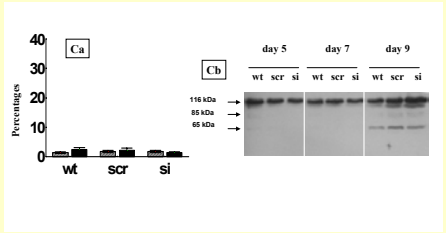
A: Western blot analyses of Gal1 expression in Hs683 GBM cells left untreated (CT) or treated with 10µM and 100µM temozolomide for 72h.
B: Immunofluorescence staining to reveal galectin-1 expression (green fluorescence) in wild type Hs683 cells left untreated (**Ba**) or treated with 10µM temozolomide for 72h (**Bb**).
C: *In vivo* expression of Gal1 detected by immunohistochemistry in the GBM of Hs683 cell-bearing immuno-compromized mice left untreated (**Ca**) or treated with temozolomide (40mg/kg i.v. three times a week for three weeks; **Cb**).
Da: Western blotting process controls confirming *in vitro* reduced Gal1 expression 5 to 9 days post transfection with anti-Gal1 siRNA (si: see Fig. 1) of Hs683. **Db:** Survival of immuno-compromized mice after brain grafts of i) wild type Hs683 cells (blue line), ii) Hs683 cells transfected *in vitro* with scrambled siRNA (pink line) or iii) anti-Gal1 siRNA (green line). The mice bearing the orthotopic Hs683 xenografts (11 mice per experimental group) were left untreated (full lines) or were treated with temozolomide (Temodal; hatched lines) at 40 mg/kg i.v. (tail vein) three times a week (each Monday, Wednesday and Friday) for three consecutive weeks, with treatment starting on the 5th day post-tumor graft.

Decreasing Galectin-1 Expression in Hs683 GBM cells Increases the Survival of Hs683 GBM Orthotopic Xenograft-Bearing Immuno-Compromized Mice and Improves the Therapeutic Benefits of Temozolomide

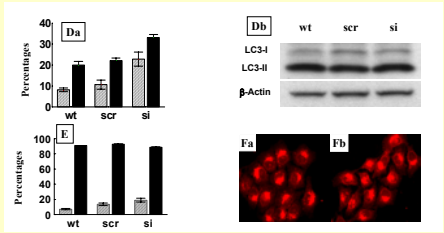
Decreasing Galectin-1 Expression in Hs683 GBM cells Increases the *In Vitro* Anti-Tumor Effects of cytotoxic Drugs



A: Quantitative determination (computer-assisted videomicroscopy) of the ability of Hs683 GBM cells to colonize the "mechanical" wound made at 0 hour on a cell population grown to confluence (Scratch wound assay). Open bars: cells were transfected with scrambled siRNA and left untreated. Blue bars: cells were transfected with scrambled siRNA and treated with 10µM temozolomide. Black bars: cells were transfected with anti-Gal1 siRNA and left untreated. Grey bars: cells were transfected with anti-Gal1 siRNA and treated with 10µM temozolomide.
B: Percentages of killed cells assessed by the colorimetric MTT assay in Hs683 GBM cells: left untreated (open bars), transfected with scrambled siRNA (grey bars) or transfected with the anti-Gal1 siRNA (black bars) and treated with BCNU, PCB, CCNU (10µM) or VCR (10nM) for 72h.



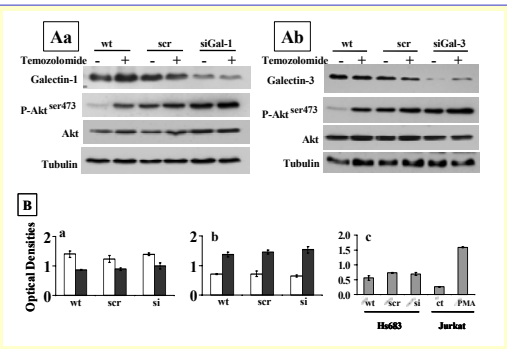
C: Apoptosis measurements by means of flow cytometry analyses by TUNEL (**Ca**) or PARP cleavage analyses (**Cb**) in wt, scr and anti-Gal1 transfected Hs683 cells (si). The open bars in **Ca** represent Hs683 cells left untreated, while black bars represent cells treated for 72h with 10µM temozolomide.



Da: Flow cytometry analyses of acridine orange staining measuring the red fluorescence in wild type (wt), scrambled siRNA- (scr) and anti-Ga1 siRNA (si) transfected Hs683 GBM cells treated for 72h with 10µM temozolomide (black bars) or left untreated (open bars). **Db:** LC3 western blotting analyses of i) wt, scr or anti-Gal1 (si) transfected Hs683 cells.
E: Flow cytometry analyses of acridine orange staining measuring the green fluorescence in wild type (wt), scrambled siRNA- (scr) and anti-Ga1 siRNA (si) transfected Hs683 GBM cells treated for 72h with 10µM temozolomide (black bars) or left untreated (open bars).
F: Immunofluorescence analyses of cathepsin B expression and localization in scr (**Fa**) and anti-Gal1 siRNA (si: **Fb**) transfected Hs683 cells.

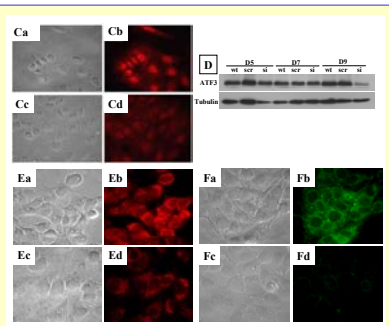
Decreasing Galectin-1 Expression in Hs683 GBM cells Does Not Induce Apoptosis, Autophagy or LMP

Decreasing Galectin-1 Expression in Hs683 GBM cells Does Not Impair Classical Pathways Involved in resistance to Chemotherapy but Impairs the Endoplasmic Reticulum Stress (ERS) Response



A: Western blots illustrating the expression and phosphorylation levels of Akt in Hs683 cells that have been i) left untreated (wt) or ii) transfected with scrambled siRNA (scr) or iii) transfected with anti-Gal1 siRNA (si) or anti-galectin-3 siRNA.

B: Illustration of the effects of Gal1 depletion (si; see Fig. 1) as compared to controls (wt and scr Hs683 cells) on the expression level (open bars in **Ba** and **Bb**) or the level of phosphorylation (black bars in **Ba** and **Bb**) of Src (**Ba**), PI3K-p85 (**Bb**) and NF-κB activation (p65/RelA DNA binding activity) (**Bc**).



C: Immunofluorescence analyses (with bright field controls) of DUSP5 expression in scr (**Ca-Cb**) and anti-Gal1 (**Cc-Cd**) siRNA transfected Hs683 cells.
D: Western blots illustrating the expression levels of ATF3 in wt, scr and anti-Gal1 siRNA (si) transfected Hs683 cells.
E: Immunofluorescence analyses (with bright field controls) of HERP expression in scr (**Ea-Eb**) and anti-Gal1 (**Ec-Ed**) siRNA transfected Hs683 cells.
F: Immunofluorescence analyses (with bright field controls) of ORP150 expression in scr (**Fa-Fb**) and anti-Gal1 (**Fc-Fd**) siRNA transfected Hs683 cells.

CONCLUSION

➔ **Temozolomide treatment increases galectin-1 expression**
➔ **Decreasing galectin-1 expression in glioma cells increases the anti-tumor effects of chemotherapeutical agents**
➔ **Decreasing galectin-1 expression does not induce apoptotic or autophagic features**
➔ **Decreasing galectin-1 expression weaken glioma cell defenses by impairing their ERS response**

Taken together, these effects seem to reinforce the therapeutic benefit of temozolomide in *in vivo* glioblastoma models.

The novel aspects of galectin-1-related function in the ERS response may be amenable to therapeutic manipulation either by the *in vivo* delivery of anti-galectin-1 siRNA as demonstrated here, or through compounds suppressing galectin-1.