Decreasing Galectin-1 Expression in Human Hs683 Glioblastoma Cells Impairs their Response to Endoplasmic Reticulum Stress

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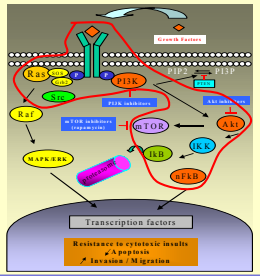
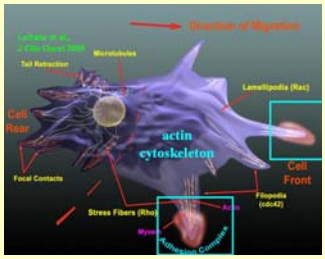
- Glioblastomas (GBM) are the most common type of primary malignant brain tumor.
- Patients have an average life expectancy of one year on the basis of the standard treatment of surgical resection followed by radiotherapy.
- GBM are associated with dismal prognoses both because they diffusely infiltrate the brain parenchyma, and,

Migrating glioma cells are protected against apoptosis because the constitutive activation of



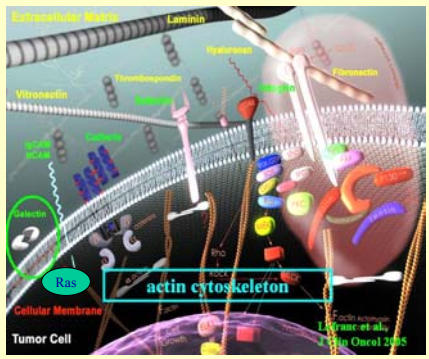


Temozolomide displays actual efficacy against malignant gliomas (Stupp et al., N Engl J Med 2005) because it is a pro-autophagic drug, not a pro-apoptotic one (Kanzawa et al., Cell Death Differ 2004)

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

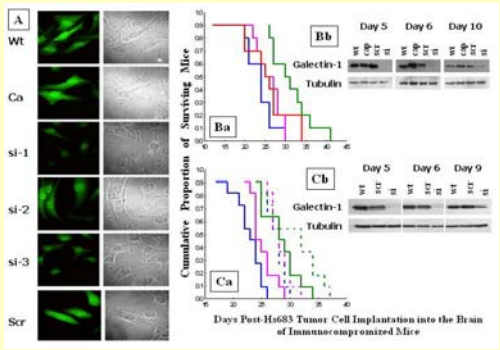
- Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration.
  Camby et al., Brain Pathol, 2001
- Galectin-1 modulates human glioblastoma cell migration into the brain 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 *in vitro* and *in vivo*.



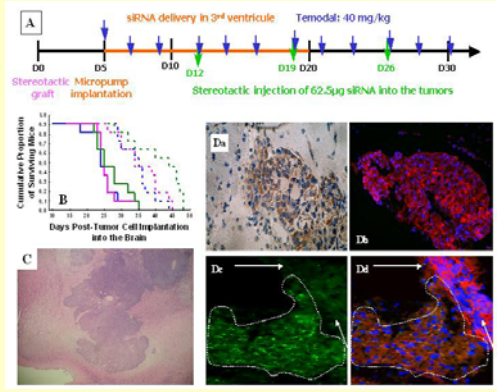
Decreasing the Levels of Expression of Galectin-1 in Human Hs683 GBM Cells Increases the Survival of Hs683 GBM Orthotopic Xenograft-Bearing Immunocompromised Mice

A: The expression of galectin-1 detected by immunofluorescence in Hs683 GBM cells transfected for 5 days with si-1, si-2, si-3 or with a scrambled siRNA (Scr) or with calcium phosphate (transfection agent: Ca) compared to that of wild-type (wt Hs683) cells.
Ba: Survival of immunocompromized mice after brain grafts of i) Wt Hs683 cells (blue line), ii) Hs683 cells cultured with the transfection agent only (red line), iii) Hs683 cells first transfected *in vitro* with scrambled siRNA (pink line) or iv) anti-galectin-1 (si-1) siRNA (green line).
Bb and Cb: Western blotting process controls confirming *in vitro* reduced galectin-1 expression after 5 to 9/10 days transfection with anti-galectin-1 si-1 siRNA of Hs683 cells. The anti-galectin-1 siRNA si-1 was subsequently used in all investigations.
Ca: Experiment similar to Ba above except half of each group of mice (i) wild-type: blue line, ii) scrambled siRNA: pink line and iii) anti-galectin-1 siRNA: green line) were left untreated (full lines) and the other half treated with temozolomide (Temodal; hatched lines) as detailed in Figure 2A.

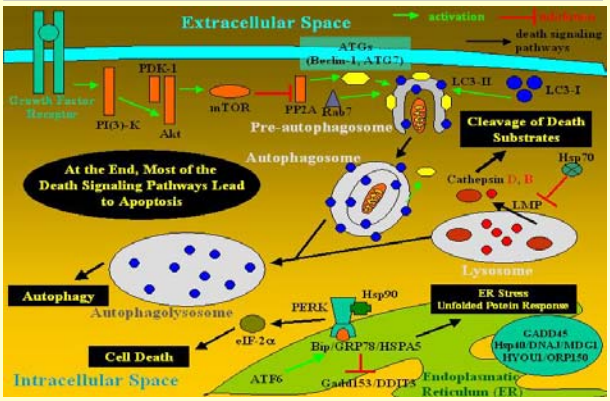


The *In Vivo* Delivery of an Anti-Galectin-1 siRNA to Hs683 GBM Orthotopic Xenograft-Bearing Immunocompromized Mice Increases the Therapeutic Benefit of Temozolomide

A-B: Description of the experimental protocols used and results obtained in immuno-compromized mice bearing orthotopic human Hs683 GBM xenografts in their brains. Wild type Hs683 cells were grafted orthotopically on day 0 (**A**). Osmotic mini-pumps delivered vehicle (full blue line in **B**), scrambled siRNA (0.3 mg; full pink line in **B**) or anti-galectin-1 siRNA (0.3 mg; full green line in **B**) for 2 weeks into the third ventricule. Vehicle, scrambled siRNA and si-1 siRNA were also directly injected into the Hs683 orthotopic xenografts (**A**) on 3 occasions. Half of the mice each received 12 i.v. (tail vein) injections of either 50µl saline (control) or 50µl of a solution containing Temozolomide to permit dosing at 40 mg/kg (hatched lines in **B**).
C: Typical example of the development of an Hs683 orthotopic xenograft in the brain of a nude mouse.
Da: Conventional immunohistochemistry and **Db:** immunofluorescence immunohistochemistry: examples of the pattern of galectin-1 expression in the same Hs683 orthotopic xenograft. **Dc:** Illustration of the penetration of the si-1 anti-galectin-1 siRNA-FITC into an Hs683 tumor (green fluorescence).
Dd: Illustration of the corresponding galectin-1 expression (red fluorescence) in the same histological slide as used in Dc.



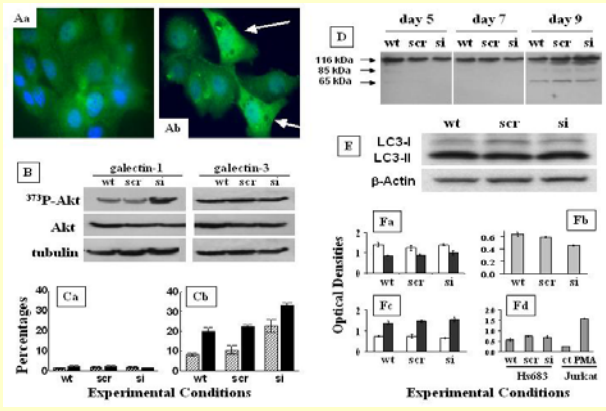
Interlinks Between Apoptosis (Type I PCD) and Autophagic Cell Death (type II PCD)



Conditions of starvation (low-oxygen and low-nutrient conditions in the central area of a tumor mass) and subsequent ATP depletion or sub-lethal damage (after cancer therapies) induce autophagy in the tumor cells to survive. The process ultimately results in autophagic cell death (type II PCD) if the stress imposed on the cells is sustained (point of no return). Endoplasmic reticulum (ER) stress (ERS) and unfolded protein response (UPR) processes participates in cancer cell defenses against various adverses biological effects induced by hypoxia or cytotoxic insults for example.

Temozolomide Increases Galectin-1 Expression whose the Decrease Induces Akt-Associated Kinase Activity in Hs683 GBM Cells

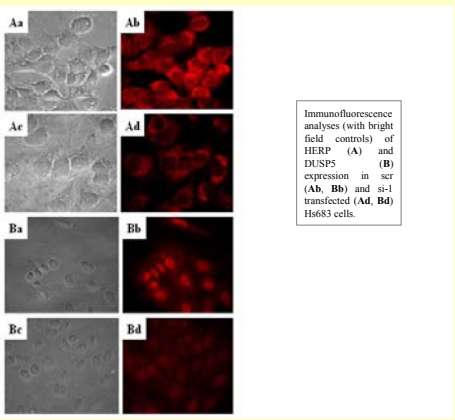
Decreasing Galectin-1 Expression in Hs683 GBM Cells Does not Induce Apoptosis or Autophagy



A: Wild type Hs683 cells left untreated (**Aa**) or treated with 10 µM temozolomide for 72h (**Ab**) with immunostaining to reveal galectin-1 expression (green fluorescence).
B: Western blots illustrating the levels of activity and the levels of expression of Akt in Hs683 cells that have been i) left untreated (wt) or ii) transfected with scrambled siRNA (scr) or iii) transfected with anti-galectin-1 (si-1) or anti-galectin-3 siRNA.
C: Flow cytometry analyses for apoptosis (TUNEL; **Ca**) and acridine orange (**Cb**) measurements in wt, scr or si-1 transfected Hs683 cells left untreated (hatched bars) or treated for 72h with 10 µM Temozolomide (black bars).
D: PARP cleavage analyses in wt, scr and si-1 transfected Hs683 cells.
E: LC3 western blotting analyses of i) wt, scr or si-1 transfected Hs683 cells.
F: Illustration of the effects of galectin-1 depletion (si-1) as compared to controls (wt and scr Hs683 cells) on the expression level of (the open bars in **Fa** and **Fc**) or the level of phosphorylation (the black bars in **Fa** and **Fc**) of Src (**Fa**), EGFR (**Fb**), PI3K-p85 (**Fc**) and NF-κB activation (p65/Rel1 DNA binding activity) (**Fd**).

Decreasing the Level of Galectin-1 Expression in Hs683 GBM Cells Impairs Responses to Endoplasmic Reticulum Stress (ERS)

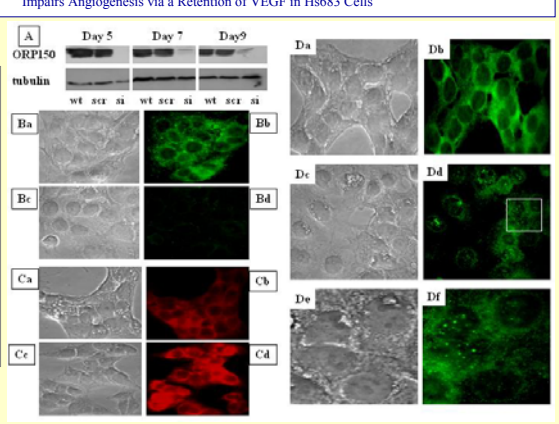
Galectin-1 Reduction in Hs683 GBM Cells Reduces HERP and DUSP5 Expression



Immunofluorescence analyses (with bright field controls) of HERP (**A**) and DUSP5 (**B**) expression in scr (**Ab**, **Bb**) and si-1 transfected (**Ad**, **Bd**) Hs683 cells.

Transient Galectin-1 Reduction in Hs683 GBM Cells Seriously Reduces MDG1 and ORP150 Expression, a Feature Which Impairs Angiogenesis via a Retention of VEGF in Hs683 Cells

A: Western blotting analyses of ORP150 expression in wild type (wt), scrambled siRNA- (scr) or anti-galectin-1 siRNA (si-1) transfected (for 5 days) Hs683 cells.
B-C: Immunofluorescence analyses (with bright field controls) of ORP150 (B) and VEGF (C) in scrambled siRNA- (**Bb**, **Cb**) or anti-galectin-1 siRNA (**Bd**, **Cd**) transfected (for 5 days) Hs683 cells.
D: Immunofluorescence analyses (with bright field controls) of MDG1 expression in scrambled siRNA- (scr) (**Db**) or anti-galectin-1 siRNA (si-1) (**Dd**) transfected (for 5 days) Hs683 cells. **Df** (and its control De) represents a magnification of the area delineated by the white square in **Dd**.



Decreasing the levels of galectin-1 expression in gliomas can thus:

i) **contribute to decreasing the levels of migration of individual GBM cells diffusely invading the brain parenchyma,**
ii) **weaken glioma cell defenses by weakening their ERS pattern and**
iii) **impair angiogenesis. Taken together these effects were seen to reinforce the therapeutic benefit in glioblastoma models *in vivo* of the pro-autophagic drug temozolomide, which is the standard chemotherapeutic treatment for GBM patients.**

The novel aspects of galectin-1-related function in the ER stress response that are highlighted in the present study may be amenable to therapeutic manipulation either by the *in vivo* delivery of anti-galectin-1 siRNA as demonstrated here or through compounds which suppress galectin-1.

The *in vivo* delivery of anti-galectin-1 siRNA could be performed directly in the case of GBMs by means of Ommaya reservoirs, thus minimizing/avoiding systemic release/exposure and potential hepatotoxicity.