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ORP150, a Major Target of Galectin-1 Pro-Angiogenic Effects in Human Hs683 Glioblastoma Xenografts

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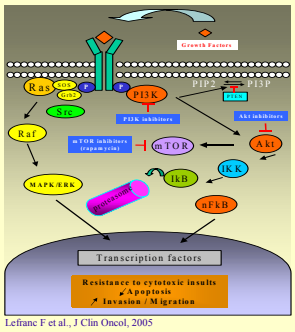
➔ **Glioblastomas (GBM) are the most common type of primary malignant brain tumors.**

➔ **Patients have an average life expectancy of one year based on the standard treatment of surgical resection followed by radiotherapy.**

➔ **GBM are associated with dismal prognoses because**

- **they diffusely infiltrate the brain parenchyma**
- **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…



Lefranc F et al., J Clin Oncol, 2005

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)

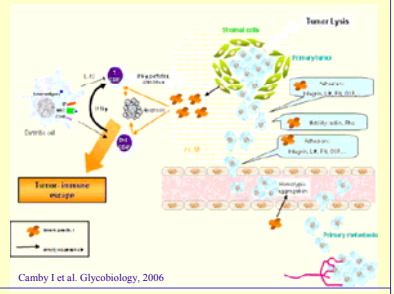
Galectin-1 is a potent modulator of GBM cell migration whose importance as a signaling molecule has already been highlighted

➔ **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.**

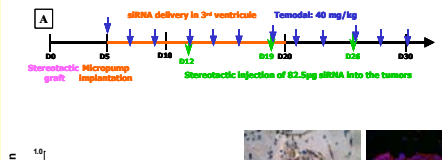
Camby et al., J Neuropathol Exp Neurol, 2002

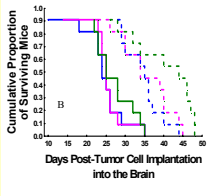


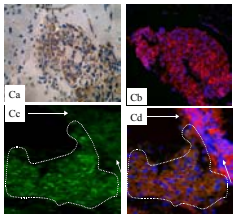
Camby I et al. Glycobiology, 2006

We investigated whether decreasing the expression levels of galectin-1 in human Hs683 GBM cells could increase their sensitivity to temozolomide.

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: Experimental protocol.

B: Results obtained in immuno-compromized mice bearing orthotopic human Hs683 GBM xenografts. Osmotic mini-pumps delivered vehicle (full blue line), scrambled siRNA (full pink line) or anti-galectin-1 siRNA (0.3 mg; full green line) for 2 weeks into the third ventricule. siRNA were also directly injected into the Hs683 orthotopic xenografts (**A**) on 3 occasions. Half of the mice received Temozolomide (hatched lines).

Ca and **Cb**: Examples of the galectin-1 expression pattern in Hs683 orthotopic xenograft by immunohistochemistry conventional and immunofluorescence.

Cc: Illustration of the penetration of the si-1 anti-galectin-1 siRNA-FITC into an Hs683 tumor.

Cd: Illustration of the corresponding galectin-1 expression in the same histological slide as used in **Cc**.

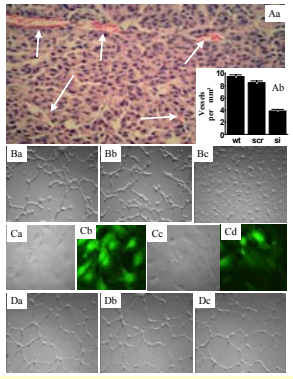
Decreasing Galectin-1 Expression in Hs683 GBM Cells Impairs In Vivo Angiogenesis an In Vitro Vasculogenic Mimicry

A: Typical morphological example (HE staining; G x 200) of the blood vessels (see the white arrows; **Aa**) that have been counted to determine the influence of decreasing Gal1 expression on Hs683 xenograft neoangiogenesis (**Ab**) in anti-Gal1 siRNA- (si) as compared to scrambled siRNA- (scr) transfected and wild-type (wt) Hs683 orthotopic xenografts (see Fig. 1B).

B: Wild-type (**Ba**) and scrambled siRNA- (**Bb**) transfected Hs683 cells develop vasculogenic mimicry processes *in vitro* when cultured on Matrigel®, while anti-Gal1 siRNA-transfected cells did not (**Bc**).

C: The anti-Gal1 siRNA used to decrease Gal1 expression in Hs683 GBM cells also decreased Gal1 expression in HUVEC cells (**Cd**, with **Cc** as bright field control) when compared to scrambled siRNA-transfected HUVEC cells (**Cb**, with **Ca** as bright field control).

D: While the anti-Gal1 siRNA significantly decreased Gal1 expression in HUVEC cells (**Cd**) as compared to wild-type (data not shown) and scrambled siRNA-transfected Hs683 cells (**Cb**), it did not prevent HUVEC capillary networking (**Dc**) when compared to wild-type (**Da**) and scrambled siRNA-transfected Hs683 cells (**Db**).



Decreasing Galectin-1 Expression in Hs683 GBM Cells Impairs Endoplasmic Reticulum Stress (ERS) Response and Reduces MDG1 and ORP150 Expression

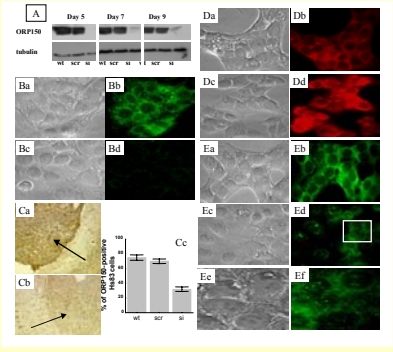
A: Western blotting analyses of ORP150 expression in wild type (wt), scrambled siRNA (scr) or anti-Gal1 siRNA (si) transfected (after 5, 7 or 9 days) Hs683 cells.

B: Immunofluorescence analyses (with bright field controls) of ORP150 in scrambled siRNA (**Ba**, **Bb**) or anti-Gal1 siRNA (**Bc**, **Bd**) transfected (after 5 days) Hs683 cells.

C: *In vivo* illustration of the immunohistochemical expression of ORP150 in a scrambled siRNA (**Ca**) or anti-Gal1 siRNA (**Cb**) transfected Hs683 GBM (black arrows). **Cc:** The ORP150 immunohistochemical expression has been quantitatively determined by means of computer-assisted microscopy.

D: Immunofluorescence analyses (with bright field controls) of VEGF in scrambled siRNA (**Da**, **Db**) or anti-Gal1 siRNA (**Dc**, **Dd**) transfected (after 5 days) Hs683 cells.

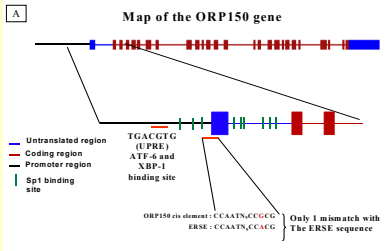
E: Immunofluorescence analyses (with bright field controls) of MDG1 expression in scrambled siRNA (**Ea, Eb**) or anti-Gal1 siRNA (**Ec-Ef**) transfected (after 5 days) Hs683 cells. **Ef** (and its control Ee) represents a magnification of the area delineated by the white square in **Ed**.

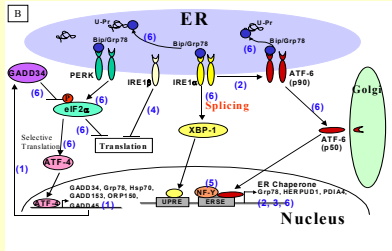


The endoplasmic reticulum stress and the unfolded proteins response Pathways

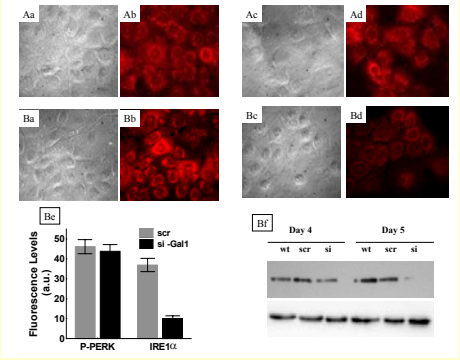
A: Map of the ORP150 gene depicted on the basis of data published by Wang Y et al., J Biol Chem 275, 2000; Kaneda S et al., J Biochem (Tokyo) 128, 2000; Yamamoto K et al., J Biochem (Tokyo) 136, 2004; and Zhang K and Kaufman RJ, Neurology 66, 2006.

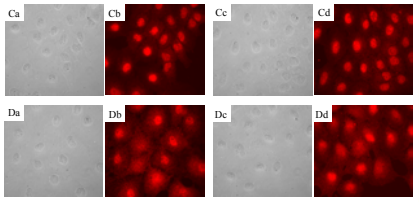
B: ER stress-related control of UPRE- and/or ERSE sequence-dependent (see Table 2) gene expression, depicted on the basis of data published by (1) Kokame K et al., J Biol Chem 276, 2001; (2) Wang Y et al., J Biol Chem 275, 2000; (3) Yamamoto K et al., J Biochem (Tokyo) 136, 2004; (4) Yoshida H et al., J Biol Chem 273, 1998, (5) Yoshida H et al., Mol Cell Biol 20, 2000; and (6) Zhang K and Kaufman RJ, Neurology 66, 2006.





Galectin-1 Could Modulate ORP150 Expression Through IRE1α





A: Immunofluorescence analyses (with bright field controls) of P-PERK in scrambled siRNA (**Aa**, **Ab**) or anti-Gal1 siRNA (**Ac**, **Ad**) transfected (after 5 days) Hs683 cells.

B: Immunofluorescence analyses (with bright field controls) of IRE1α in scrambled siRNA (**Ba**, **Bb**) or anti-Gal1 siRNA (**Bc**, **Bd**) transfected (after 5 days) Hs683 cells.

Bc: The P-PERK (Aa-Ad) and IRE1α (Ba-Bd) immunohistochemical expression has been quantitatively determined by means of computer-assisted microscopy. **Bf**: Western blotting analyses of IRE1α expression in wild type (wt), scrambled siRNA (scr) or anti-Gal1 siRNA (si) transfected after 4 or 5 days in Hs683 cells (loading control: β-actin).

C: Immunofluorescence analyses (with bright field controls) of XBP-1 in scrambled siRNA (**Ca**, **Cb**) or anti-Gal1 siRNA (**Cc**, **Cd**) transfected (after 5 days) Hs683 cells.

D: Immunofluorescence analyses (with bright field controls) of ATF6 in scrambled siRNA (**Da**, **Db**) or anti-Gal1 siRNA (**Dc**, **Dd**) transfected (after 5 days) Hs683 cells.

CONCLUSION

➔ **Decreasing galectin-1 expression in glioma cells impair angiogenesis through the regulation of ORP150 expression, which in turn control VEGF maturation**

➔ **Decreasing galectin-1 expression in glioma cells impairs endoplasmic reticulum stress (ERS) response**

➔ **Galectin-1 could modulate ORP150 expression through IRE1α**

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 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.