ABSTRACT

Glioblastoma multiforme (GBM) is the most common and lethal brain tumor. Its prognosis remains very poor, despite the use of combined treatments such as surgical resection, radiation and chemotherapy. The major limitations for the treatment of GBM are its high invasiveness, tumor recurrence and resistance to treatments. Therefore, gene therapy appears as a relevant strategy for its treatment. Thus, we have investigated the use of growth-arrest-specific 1 (Gas1) for the treatment of GBM. Gas1 is a tumor suppressor protein that inhibits glioma growth by inducing arrest and apoptosis of tumor cells. Moreover, we have shown that a soluble form of Gas1 acting in both autocrine and paracrine manners is also effective inhibiting tumor growth in animal models, indicating its potential as an adjuvant for the treatment of GBM.

Key words: Growth arrest specific 1; glioma; serine-threonine protein kinase; glial cell-derived neurotrophic factor; extracellular signal-regulated kinases and tumor

INTRODUCTION

Gliomas are the most frequent and aggressive tumors of the central nervous system (CNS) and current treatments have not improved their prognosis. In children and adolescents, tumors of the CNS are the most common and lethal,[1] and glioblastoma multiforme (GBM) is the most frequent malignant primary brain tumor.[2-5] Gliomas are the main neuroepithelial tumors of the CNS that originate from mature or precursor ectodermal-derived glial cells. The World Health Organization (WHO) has classified gliomas on 4 grades from I to IV (GI-GIV) according to the histological dedifferentiation and the expression of the KI-67 protein, which indicates the rate of proliferation. WHO-GI gliomas are considered as benign tumors, since the malignant features are only present on low-grade (WHO-GII) and high-grade (WHO-GIII and -GIV) gliomas; in this respect, WHO classification correlates with the prognosis of the patient, regardless of multimodal therapeutic treatments;[6,7] the 5-year life span rates after diagnosis are 50%, 30% and 5% for WHO-GII, -GIII and -GIV glioma patients, respectively.[8]

Tumors of glial origin are considered gliomas and are divided into: astrocytoma, oligodendroglioma, ependymoma, mixed gliomas and not otherwise specified.[2,3] On the other hand, The Cancer Genome Atlas (TCGA) designed a sub-classification of GBM based on their molecular signature [Table 1], which comprises: classical, mesenchymal, proneural and neural tumors.[8,9] Classical GBM are tumors that present high expression of the epidermal growth factor receptor (EGFR) and absence of tumor suppressor proteins such as p16 and p14. In mesenchymal GBM, the phosphatase and tensin homolog (PTEN) gene is mutated and loss of its activity leads to activation of the serine-threonine protein kinase (AKT) survival pathway. Additionally, this subtype expresses chitinase 3-like-1 and MET transcripts characteristics of mesenchymal cells and shows low expression of the transcript for the tumor suppressor protein neurofibromin 1. On the other hand, proneural GBM expresses oligodendrocyte transcripts NK2 homeobox 2 and oligodendrocyte transcription factor (OLIG2), as well as high levels of platelet-derived growth factor (PDGF) receptor, alpha polypeptide, and mutations of the dehydrogenase 1 and/or P53 genes. The neural GBM is the most dedifferentiated subtype because it expresses...
neuronal markers such as: negative regulatory factor (NEFK), γ-aminobutyric acid A receptor A, synaptotagmin 1 and the symporter K⁺: Cl⁻ (SLC12A5). The variability of the molecular signature of GBM suggests that the characterization of gliomas must be analyzed to devise specific treatments.

**CLINICAL MANAGEMENT OF GLIOMAS**

Advanced anaplasia in high-grade gliomas difficults the complete surgical resection of the tumor; as a consequence, tumor recurrences are unavoidable. On the other hand, postoperative radiotherapy has been the standard treatment for GBM. However, the survival after radiation is low and overall survival remains poor.[10-12]

Concomitant and adjuvant chemotherapy for high grade gliomas include alkylating agents that damage DNA, such as: carmustine, procarbazine, lomustine, vincristine and temozolomide (TMZ). Recently, it has been reported that both bevacinumab and cediranib prevent angiogenesis by inhibiting the vascular endothelial growth factor (VEGF) signaling pathway.[11-13] Moreover, TMZ is the drug of choice for the treatment of high-grade glioma. TMZ alkylates guanine, inducing the methylation of gene promoters and leading to apoptosis, when the mismatch repair system is intact.[14,15] This drug is well tolerated by most patients, furthermore it has a favorable safety profile that is associated with only mild side-effects compared with nitrosoureas.[10,16] The addition of chemotherapy to standard postoperative radiotherapy improves in 2.5 months the median survival relative to postoperative radiotherapy alone.[10-12] To enhance the effect of TMZ it has been proposed the use of lipid-based nanoparticles, which cross the blood brain barrier more efficiently causing an increment of brain levels of TMZ and reducing the adverse effects in other organs such as the heart and kidneys.[16-18] Despite the above GBMs that express high levels of O 6- methylguanine DNA methyltransferase (MGMT) protein are resistant to TMZ chemotherapy.[19-22] Small molecule inhibitors of MGMT exist, but their use in combination with TMZ is limited due to toxicity to peripheral organs.[23] Furthermore, the mutation in the mutS homolog (MSH) 6 mismatch repair gene facilitates resistance to TMZ and recurrence of GBM.[14] Until now, the surgical approach is still the most effective measure to treat gliomas, followed by radiotherapy and chemotherapy; however the clinical prognosis of the patients remains very poor. Therefore, new strategies and therapeutic agents should be investigated, based on the molecular characteristics of gliomas.

**MOLECULAR APPROACH AGAINST MULTI-RESISTANT GLIOMAS**

Resistance to various treatments and the recurrence of tumors has been attributed to the presence of a subpopulation

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**Table 1: Subclassification of GBM based on their molecular signature**

<table>
<thead>
<tr>
<th>GBM subclassification</th>
<th>Genes altered</th>
<th>Signal pathway</th>
<th>Status</th>
<th>Physiological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classical</strong></td>
<td>CDKN2A (p14/p16)</td>
<td>RB</td>
<td>Homozygote deletion</td>
<td>Cell cycle: G1/S transition</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>EGF/TNF-α</td>
<td>Overexpression and mutations</td>
<td>Cell Cycle</td>
</tr>
<tr>
<td></td>
<td>NESTIN</td>
<td></td>
<td>Overexpression</td>
<td>Neural stemness</td>
</tr>
<tr>
<td></td>
<td>NOTCH3, JAG1, LENG</td>
<td></td>
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<tr>
<td></td>
<td>SMO, GASI, GLI2</td>
<td>SHH</td>
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<tr>
<td><strong>Mesenchymal</strong></td>
<td>NF1</td>
<td>AKT</td>
<td>Deletion and/or mutation</td>
<td>Survival and proliferation pathways</td>
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<tr>
<td></td>
<td>PTEN</td>
<td></td>
<td>Mutation</td>
<td>Cell stress response</td>
</tr>
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<td></td>
<td>TRADD, RELB, NFRSF1A</td>
<td>NF-β/TNF superfamily</td>
<td>Overexpression</td>
<td>Mesenchymal transtion</td>
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<td></td>
<td>CH3IL1, MET</td>
<td>PDGF</td>
<td>Overexpression and mutation</td>
<td>Cell cycle and angiogenesis,</td>
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<td>Mutation</td>
<td>cytoplasmic NADPH production</td>
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<td></td>
<td>PDGFRA</td>
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<td></td>
<td>Survival and proliferation pathways</td>
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<tr>
<td></td>
<td>P53</td>
<td>P53</td>
<td>Mutation</td>
<td>Cell cycle: G1/S transition</td>
</tr>
<tr>
<td><strong>Proneural</strong></td>
<td>CDKN1A (p21)</td>
<td>SOX</td>
<td>Low expression</td>
<td>CNS cell fate determination</td>
</tr>
<tr>
<td></td>
<td>DCX, DLL3, ASCL1, TCF4</td>
<td>NXX2-2</td>
<td>Overexpression</td>
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<td></td>
<td>OLG2</td>
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<tr>
<td><strong>Neural</strong></td>
<td>NEFL</td>
<td>GABA</td>
<td>Expression</td>
<td>Neuronal markers</td>
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<td>GABRA1</td>
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<td>SYT1</td>
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of cells in gliomas with properties of stem cells, known as glioma-initiating cells (GICs). These cells express neural stem cell markers such as NESTIN, OLIG2, sex determining region Y-box 2 (SOX2) and fucosyltransferase 4. The classification of GICs is based on the expression of prominin-1 (CD133); the CD133+ GICs are more invasive than those that do not express the antigen, and constitute 3-29% of the glioma mass. Also, GICs from secondary than those that do not express the antigen, and constitute 3-29% of the glioma mass. Additionally, miR-181b-145 and -132 is related with TMZ resistance coupled with chemo- and radiotherapy. Moreover, to the formation of more aggressive tumors, resistant to facilitate the selection of multi-resistant GICs, leading to non-GICs cells, implicating the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). In this context, the use of classical therapies against gliomas, facilitate the selection of multi-resistant GICs, leading to the formation of more aggressive tumors, resistant to chemo- and radiotherapy. Moreover, in vitro and in vivo studies have shown that with the adequate stimulus, GICs differentiate to either neuronal or astrocytic cells, however many of these responses are deregulated in gliomas.

It is noteworthy that CD133+ GICs are predisposed to become resistant to chemo- and radiotherapy with respect to non-GICs cells, implicating the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). In this context, the use of classical therapies against gliomas, facilitate the selection of multi-resistant GICs, leading to non-GICs cells, implicating the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). In this context, the use of classical therapies against gliomas, facilitate the selection of multi-resistant GICs, leading to the formation of more aggressive tumors, resistant to chemo- and radiotherapy. Moreover, in vitro and in vivo studies have shown that with the adequate stimulus, GICs differentiate to either neuronal or astrocytic cells, however many of these responses are deregulated in gliomas.

Currently, the analysis of protein expression and of the transcriptome, including micro-RNAs (miRNAs), has helped to uncover molecular markers involved in the susceptibility or resistance to treatments. Indeed, differential expression patterns of miRNAs have been reported in high-grade gliomas. Even now, the association of miRNAs with the methylation of the MGMT promoter is still controversial since miRNAs are not considered as direct epigenetic regulators. However, overexpression of miR-222, -145 and -132 is related with TMZ resistance coupled with MGMT promoter methylation. Additionally, miR-181b and -181c are downregulated in patients that responded to radiotherapy and concomitant TMZ. However, sensitivity to chemotherapy is not only dependent of the presence of MGMT.

Screening of molecular changes after radiotherapy showed overexpression of miR-1, -125a, -144, -150, -151-5p, -221/22, -425 and -1285. The ectopic expression of miR-1, -125a, -150 and -425 increases cell survival and confers radiosensitivity through the induction of the cell cycle. On the other hand, TGF-α and -β, and the EGFR also contribute to radioresistance in classical-GBM. Thus, inhibiting TGF-β has been proposed as a treatment since it induces radiosensitivity in gliomas through decreasing the expression of miR-1 and -125a. Additionally, miR-221/222 downregulate PTEN, leading to activation of proteins that promote cell proliferation or prevent cell death, such as: AKT, B-cell lymphoma 2 (Bcl-2), Cyclin-D, matrix metallopeptidase 2 and 9. Interestingly, the phosphorylation of AKT is the main mechanism for developing radioresistance, regardless of the activity of its negative regulator PTEN. In summary, molecular analysis could help to reconsider whether conventional treatments are suitable, or therapeutic modifications should be adapted to the requirements of the patient.

**GROWTH-ARREST-SPECIFIC 1**

The balance between proliferation or growth arrest is regulated by several extrinsic and intrinsic factors. Cells can exit the cell cycle and enter in the non-proliferative phase, known as the G0 phase. Particularly, in the this phase six genes named growth-arrest-specific (Gas) genes are expressed, from 1 to 6. The gas1 transcript is the most abundant in NIH3T3 cells arrested in the G0 phase by deprivation of serum or high cell density. Gas1 induces growth arrest by inhibiting DNA synthesis in NIH3T3 cells when it is ectopically expressed.

**TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION**

Human and mouse gas1 genes are located in the long arms of chromosome 9 (9q21.3-q22) and chromosome 13 respectively, with 77.04% of homology between them. Gas1 is an intronless gene, suggesting that it probably originated from a retrotransposon.

There are few studies about the regulation of the gas1 gene, however it has been reported that Menin and Myb-like (coded by the men1 and dmp1 genes respectively), induce the transcriptional repression of gas1. Also, c-Myc and Src repress gas1 transcription, since they facilitate re-entering to the cell cycle. c-Myc protein requires the Myc-Box2 domain to be present on the N-terminus to repress the transcription of gas1. Furthermore, the basic Helix-loop-Helix leucine zipper domain located at the C-terminus of c-Myc is also necessary to induce the transcriptional repression of gas1, perhaps together with an accessory protein not yet identified.

Both c-Myc and Src are key components for the proliferation, growth, and survival of glioma cells. The expression of c-Myc closely correlates both with cellular dedifferentiation and the grade of malignancy since its activity induces the transcription of cyclin D1 and repression of the p21WAF1/CIP1 cyclin-dependent kinase inhibitor. Interestingly, the histone chaperone, Facilitate Chromatin Transcription protein complex (FACT) increases the transcription of Myc, a recent report showed that the downregulation or inhibition...
of FACT decreased the formation of metastasis and delayed tumor growth, it also proved to be an excellent cytotoxic adjuvant.[89] Thus, these data suggest that the inhibition both of FACT and Myc, could increase the anti-tumoral effect of Gas1. Moreover, transgenic mice that express Src under the transcriptional control of the glial fibrillary acidic protein develop hypervascularized glioblastomas with morphological and molecular characteristics of human GBM.[84,85]

On the other hand, estrogens like estradiol, induce their biological effects through binding to intra-cellular hormone-specific estrogen receptors (ERα and ERβ), and this binding produces a conformational change in the receptors, causing the activation of their transcriptional domains. Specifically, estradiol reduced the levels of gas1 mRNA, however it is not yet known whether the gas1 promoter has an estrogen response element.[86]

Little is known about the transcription factors that up-regulate gas1. For example the transcription factor Tbox5 increases the activity of the mouse gas1 promoter.[87] Moreover, microarray experiments indicate that gas1 could be a retinoic acid responsive gene.[88,89] Since both retinoic receptors and Gas1 are expressed during embryonic development,[76-74] we insinuate that retinoic acid may induce the expression of Gas1 to promote exit from the cell cycle and initiate the differentiation process.

Four miRNAs have been reported to interact with the human gas1 transcript: miR-34a-5p, -148a-3p, -130b-5p and -183-5p.[75,76] Only miR-34a, derived from the 5’ arm of the pre-miRNA sequence (miR-34a-5p), has been shown to downregulate the translation of Gas1 when the miRNA interacts with nucleotides located at position 812-832 from the 3’ untranslated region of gas1, preventing the activity of Gas1 on the phosphatidylinositol 3-kinase (PI3K)-AKT dependent cell survival pathway.[77,78] In fact, the repression of gas1 by miR-34a-5p promotes cell survival and proliferation, preventing apoptosis by reducing the cleavage of Caspase-3 on papillary thyroid carcinoma cell cultures.[77]

**GASI PROTEIN STRUCTURE AND EXPRESSION**

The nucleotide sequence of the gas1 of both the human and mouse genes reveals an open reading frame of 345 and 384 amino acids, respectively.[46] The proteins encoded by these genes undergo post-translational modifications in the endoplasmic reticulum consisting of an N-linked glycosylation, signal peptide cleavage and addition of a glycosylphosphatidylinositol (GPI) group at the C-terminal. The mature form of the Gas1 protein has a molecular mass of about 37 kDa and is anchored to the outer cell membrane by a GPI molecule.[49,51,79] The region of Gas1 from amino acid 182 to amino acid 234 is essential to induce growth arrest whereas neither the GPI nor the C-terminal domain are necessary for this function.[80]

We previously showed that Gas1 possesses significant structural homology with the glial cell-derived neurotrophic factor (GDNF) family of receptors (GFRAs). Gas1 has two domains, called D-N and D-C, which have high similarity to the D2 and D3 domains of the GFRAs. These domains have cysteines that participate in the formation of five disulfide bridges.[81,82] It is noteworthy to mention that Gas1 binds to RET in either the presence or the absence of GDNF.[83] Based on the above information we and other research groups showed that Gas1 inhibits the signaling pathway induced by GDNF, an aspect that we will discuss later.

Interestingly, it has been reported that a soluble form of Gas1 inhibits the proliferation of mesangial cells. Disintegrin and metalloproteinase (ADAM) 10 and 17 are responsible of cleaving the Gas1 GPI anchor.[84,85] In glioma cells, ADAM17 increases the shedding of soluble VEGF and activates the EGF-R-PI3K-AKT pathway, contributing to invasiveness, and angiogenesis.[86] For its part, ADAM10 promotes glioma cell migration by cleaving the adhesion molecule N-cadherin from the cell surface.[86] On the other hand, we constructed a lentiviral vector that produce a soluble and secretable form of Gas1 (tGas1), lacking the GPI consensus sequence. tGas1 induces cell arrest and apoptosis of GBM cells and inhibit glioma tumor growth in vivo.[87,88] This soluble form of Gas1 acts in both autocrine and paracrine manners. However, previous data suggests that the full form of Gas1 (with GPI) can have paracrine effects, since the GPI anchor of Gas1 could be cleaved by ADAM 10 and 17 in gliomas.

Gas1 is expressed during the early stages of development in the primitive streak, somites, heart, limb, otic vesicle, kidney, lung, muscle, gonads, brain and placenta.[51,70,72,73] Its expression is fundamental during embryonic development since Gas1 knockout (K.O.) mice die immediately after birth.[89-91] The K.O. mice develop several defects including decreased cell proliferation in cerebellum, morphological alterations in the gastrointestinal tract and microform holoprosencephaly associated with multiple craniofacial defects.[72,89-91] The defects in Gas1-/− mice, are associated with the loss of the signaling induced by Sonic hedgehog (Shh). Interestingly, some patients with holoprosencephaly present mutations in the Gas1 gene with or without additional mutations on the Shh gene.[89,92] During development, Gas1 has both negative and positive effects on cell proliferation, for example: in the limbs, Gas1 promotes the death of the interdigital tissue;[93] whereas it promotes proliferation of granular cell progenitors in the cerebellum.[94]
mainly found in neurons and in a more restricted manner in glial cells in different regions of the CNS of adult mice. Furthermore, the expression of Gas1 decreases when neural stem cells are differentiated to a glial phenotype. However, the role of Gas1 in glial cells is unknown in the adult CNS. In hippocampal neurons, Gas1 induces cell death after excitotoxic insults, inhibiting the signaling induced by GDNF. Nevertheless, during cerebellar development Gas1 induces the proliferation of cerebellar granule neuron progenitors in a Shh-dependent manner.

Gas1 PROMOTES Shh SIGNALING

Shh is a secreted and diffusible morphogen implicated in the development of tissues and organs, including the CNS. The receptor for Shh is Patched (Ptc) which constitutively inhibits Smoothened [Smo; Figure 1]. The binding of Shh-Ptc produces the disinhibition of Smo and allows its signaling. Downstream, Gli1, 2 and 3 proteins activate the transcription of genes such as N-myc, cyclin D and bcl-2 which promote cell proliferation. On the other hand, there are evidences of the interaction between Gas1 and Shh, and Indian hedgehog. This interaction was originally interpreted as antagonistic, however recently it has been shown that Gas1 promotes Shh signaling during the development of the neural tube and cerebellum.[89,96,102-104] Ptc and Gli are highly expressed in gliomas and are considered oncogenes. Therefore this would suggest that Gas1 could enhance the effect of Shh, inducing the proliferation of glioma and neuroblastoma cells, however we showed that Gas1 inhibits cell proliferation of glioma cells even in the presence of the Shh molecular machinery, which suggest that in tumors Gas1 inhibits the GDNF signaling pathway.

GAS1 INHIBITS THE SIGNALING INDUCED BY GDNF AND ARTEMIN

The GDNF family of ligands (GFLs), GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN), belong to a distant branch of the TGF-β superfamily. GFLs play a pivotal role in the differentiation and maintenance of both the central and the peripheral nervous system. The cellular responses to GFLs are mediated by a multicomponent receptor complex composed by GPI anchored co-receptors (GFRα1-4) and as ligand binding component the Ret receptor which is a tyrosine kinase. The co-receptors provide specificity for the binding of the ligand to the receptor complex; GDNF preferentially binds to GFRα1, NRTN to GFRα2, ARTN to GFRα3 and PSPN to GFRα4. Although there are promiscuity of the ligand-receptor interactions.

The binding of GDNF to GFRα-1 induces the recruitment of GDNF receptor α1 (GFRα1) and the subsequent activation of the tyrosine kinase Ret, which then recruits the adapter protein Shc, leading to the activation of downstream signaling pathways such as the Ras-Raf-MAPK and the PI3K-Akt pathways. The activation of these pathways promotes cell survival, proliferation and metastasis. Gas1, on the other hand, inhibits the signaling induced by GDNF and artemin.
of two RET proteins on lipid rafts.[109] Like other receptor tyrosine kinases, RET can activate various signaling pathways including ERK, PI3K/AKT, the p38 mitogen activated protein kinase and the c-Jun N-terminal kinase (JNK) pathways.[109,110] AKT is constitutively expressed in GBM cells and its activation induces uncontrolled growth, resistance to apoptosis, and enhanced tumor invasiveness.[111] By inactivating pro-apoptotic proteins as BAD and procaspase-9 [Figure 1], as well as the transcription factor forkhead box O (FOXO).[111] Thus, the inhibition of AKT is an important therapeutic target for the treatment of gliomas. The activation of AKT is regulated by PI3K, a member of the intracellular lipid kinase family, which catalyzes the generation of phosphatidylinositol-3,4,5-triphosphate (PIP3) from phosphatidylinositol-4,5-triphosphate (PIP2).[112] PIP3 recruits AKT to the plasma membrane where it is phosphorylated in Thr308 by phosphoinositide dependent kinase 1 and in Ser473 by PDK2, which results in the full activation of AKT.[113] On the other hand, the activation of the PI3K/AKT signaling pathway is reduced when PIP3 is dephosphorylated and converted to PIP2 by the activity of PTEN.[114] In neuroblastoma and glioma cells, Gas1 blocks cell cycle progression, inhibits proliferation and induces cell death by inhibiting the GDNF/AKT pathway.[107,108,115,116] We showed that Gas1 prevents the phosphorylation of Ret Tyr1062 and reduces the activation of AKT [Figure 1]. This leads to the translocation of BAD to the mitochondria and the release of cytochrome-C to the cytosol which in turn induces the activation of Caspases 9 and 3.[107,108,115-118] Recently Wang and et al.[106] demonstrated that Gas1 promotes excitotoxicity in dopaminergic neurons by inhibiting the GDNF signaling pathway.

AKT phosphorylates, activates, or inhibits a number of proteins that regulate several processes related with cell survival.[114,119] First, AKT has anti-apoptotic effects through the phosphorylation and inhibition of pro-apoptotic proteins, such as BAD, MDM2 and members of the FOX family. Second, AKT promotes the progression of the cell cycle by blocking the degradation of cyclin D and inactivating the inhibitors of the cell cycle p21 and p27. Finally, AKT activates the mammalian target of rapamycin (mTOR) kinase by inhibiting a complex formed by the tumor suppressor proteins tuberous sclerosis 1 and 2. In turn mTOR increases protein synthesis and cell proliferation.[114,119]

Additionality, we found that Gas1 inhibits cell growth through a RET-independent mechanism. Gas1 decreases the viability of MDA-MB-231 human breast cancer cells, interfering with the interaction between ARTN and GFRα3, leading to a decrement of the activation of ERK1/2.[120] In turn, the activation of the ERK pathway is triggered by a wide variety of receptor tyrosine kinases activated by growth factors and cytokines. ERK1/2 is activated by the small G protein Ras-Raf family members (Raf-1, A-Raf, B-Raf) followed by MEK1/2. ERK1/2 controls either cell survival or apoptosis by regulating the activity of anti- and pro-apoptotic transcription factors.[121] The phosphorylations of ERK 1/2 promote cell survival by enhancing the transcription and activity of the anti-apoptotic molecules Bcl-2, myeloid cell leukemia 1 and B-cell lymphoma-extra large.[122] Alternatively, ERK1/2 downregulate the expression and inhibit the activity of the pro-apoptotic protein Bcl2-interacting mediator. Moreover, under conditions of oxidative stress, ERK has pro-apoptotic effects;[123] however this process it is not well understood yet.
POTENTIAL THERAPEUTIC EFFECT OF GAS1 FOR THE TREATMENT OF GLIOMAS

Several studies suggest that GAS1 is a tumor suppressor and that its downregulation facilitates the uncontrolled growth of several types of cancer cells.[107,108,115-118,124,125] It is worth noting that the downregulation of GAS1 is associated with the progression of thyroid and prostate cancer and with a poor prognosis of survival.[126,127] Additionally, the loss of GAS1 increases the metastasis of breast, prostate and gastric cancers.[124,126] On the other hand, GAS1 has been proposed as a molecular marker for prostate cancer.[129] The mechanisms that regulate the expression of GAS1 in gliomas have not been identified yet; however these tumors express several transcription factors that negatively regulate GAS1 such as c-Myc and v-Src.[56,57]

We previously showed that GAS1 induces apoptosis and inhibits cell growth in glioma cell lines and human glioma primary cultures of low and high grade.[87,88,107,116-118] Furthermore, we demonstrated that GAS1 decreased the proliferation and induced apoptosis through the inhibition of AKT as well as the induction of apoptosis mediated by caspase 3, independently of the activity of p53 in C6 glioma cells and U373 human astrocytoma cells.[87,88,116-118] Interestingly, GAS1 produces death of glioma cells even in the presence of the molecular machinery of Shh,[92] suggesting that it acts through the GDNF pathway [Figure 2].

Gas1 binds to Ret in a manner independent of the presence of GDNF.[82] On the other hand, the expression and activity of GDNF and its receptor GFRα1 are increased by their soluble forms in gliomas.[130-133] Based on the above, we developed a lentiviral vector in which the expression of tGAS1 is inducible.[87,88] This soluble form of GAS1 acts both in autocrine and paracrine manners in GBM cells and inhibits glioma tumor growth in vivo. Subsequent to this study, we used neural stem cells as a vehicle to deliver tGAS1 into intracranial gliomas, since they have innate tropism towards tumors. We found that tGAS1 decreased tumor growth and increased the overall health and survival of nude mice implanted with GBM.[88]

There is evidence indicating that GAS1 is a metastasis suppressor in mouse 67NR breast cancer cells and B16-F0 melanoma cells.[128] Extracranial metastasis is a rare manifestation of GBM, this is probably due to the shortened survival of patients, that will not allow glioblastoma cells generate metastasis.[134] On the other hand, gliomas overexpress ERK1/2 and GDNF, molecules that promote migration and invasiveness of gliomas into the brain parenchyma.[108] There is evidence that GAS1 inhibits the migration of breast cancer cells by blocking ERK in a RET-independent manner.[120] Moreover, GAS1 decreased tumor vascularization in a breast cancer model.[120] All these suggest that GAS1 can be an important molecule to counter the migration of glioma cells. Additionally, GAS1 has a RGD domain which is essential for the binding and blockade of some integrins that promote the migration and invasiveness of gliomas.[49,135] It has been found that RGD-integrin antagonists can inhibit cell adhesion and angiogenesis.[115] On the other hand, it has been reported that integrin α5β1 (in the absence of attachment to fibronectin) decreased the proliferation of HT29 colon carcinoma cells by inducing the transcription of GAS1.[136] Until now, however, there is no evidence of a relationship between the RGD domain of GAS1 and cell migration [Figure 2].

The recurrence of gliomas that occurs after surgical resection, is attributed to the presence of GIC’s. Alternatively the activation of ERK is involved with the maintenance of the expression of MGMT and resistance to TMZ of GBM-GICs.[132] As previously mentioned GAS1 inhibits the activation of ERK1/2, thus it may promote the elimination of the GIC’s population [Figure 2]. It is relevant that the overexpression of GAS1 in human adenocarcinoma cells (A549) increases their sensibility to cisplatin, which inhibits proliferation and induces cell cycle arrest and apoptosis.[137,138] Also, the downregulation of GAS1 promotes resistance to epirubicin in human gastric cancer by regulating drug efflux and apoptosis.[139] On the other hand, it was reported that GAS1 could be an important biomarker for the prognosis of gastric cancer patients, since it was found that reduced or negative GAS1 expression is associated with shorter survival time and worse patient prognosis.[124] In conclusion, current data suggest that GAS1 is a potential adjuvant for the treatment of gliomas and other tumors. The use of GAS1 with current treatments may improve their efficacy.

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Conflicts of interest
There are no conflicts of interest.

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