A Novel Splice Variant of GLI1 That Promotes Glioblastoma Cell Migration and Invasion

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Abstract

The family of GLI zinc finger transcription factors regulates the expression of genes involved in many important cellular processes, notably embryonal development and cellular differentiation. The glioma-associated oncogene homologue 1 (GLI1) isoform, in particular, has attracted much attention because of its frequent activation in many human cancers and its interactions with other signaling pathways, such as those mediated by K-Ras, transforming growth factor-β, epidermal growth factor receptor, and protein kinase A. Here, we report the identification of a novel truncated GLI1 splice variant, tGLI1, with an in-frame deletion of 123 bases (41 codons) spanning the entire exon 3 and part of exon 4 of the GLI1 gene. Expression of tGLI1 is undetectable in normal cells but is high in glioblastoma multiforme (GBM) and other cancer cells. Although tGLI1 undergoes nuclear translocation and transactivates GLI1-binding sites similar to GLI1, unlike GLI1, it is associated with increased motility and invasiveness of GBM cells. Using microarray analysis, we showed >100 genes to be differentially expressed in tGLI1-expressing compared with GLI1-expressing GBM cells, although both cell types expressed equal levels of known GLI1-regulated genes, such as PTHC1. We further showed one of the tGLI1 up-regulated genes, CD24, an invasion-associated gene, to be required for the migratory and invasive phenotype of GBM cells. These data provide conclusive evidence for a novel gain-of-function GLI1 splice variant that promotes migration and invasiveness of GBM cells and open up a new research paradigm on the role of the GLI1 pathway in malignancy. [Cancer Res 2009; 69(17):6790–8]

Introduction

Glioma-associated oncogene homologue 1, GLI1, was first identified as an amplified gene in a human glioblastoma multiforme (GBM; ref. 1) and later shown to be a member of the Kruppel family of zinc finger transcription factors (2). GLI1 and two other members of the GLI family are nuclear mediators of the Hedgehog signaling pathway that regulates genes involved in early development of the central nervous system and in the malignant process in a number of tumor types (3, 4). Hedgehog signaling is activated following binding of the secreted Sonic Hedgehog (Shh) ligand to its receptor PTCH, an inhibitor of Smoothened. Shh binding to PTCH derepresses Smoothened, which, in turn, activates the release of GLI1 from cytoplasmic sequestration mediated by a protein complex that includes Sufu (4, 5). The released GLI1 translocates to the cell nucleus, where it binds to a consensus GLI1-binding element in target genes resulting in their activation (2).

Although the GLI1 gene was first isolated from a human GBM (1) and the Hedgehog–GLI1 pathway is frequently activated in malignant gliomas (3, 6), the role of GLI1 in the biology of GBM remains poorly understood. In the course of our studies to gain a better understanding of the biology of malignant gliomas, we undertook the functional and structural characterization of the GLI1 gene in GBM. The results led to the identification of a previously unknown truncated GLI1 splice variant, tGLI1, in which the entire exon 3 and part of exon 4 of the GLI1 gene, corresponding to 41 codons and representing amino acid residues 34 to 74 are deleted. We showed that this novel truncated GLI1 is expressed in most GBM cells, but not in normal brain and other normal cells, and that it is a gain-of-function variant of the GLI1 transcription factor that positively regulates the migratory and invasive phenotype of GBM cells and may thus be associated with the aggressiveness of these tumors.

Materials and Methods

Reagents, cell lines, xenografts, and primary tumor specimens. All chemicals were purchased from Sigma unless otherwise stated. cDNAs of normal tissues and genomic DNAs from peripheral leukocytes were from BioChain. Human GBM cell lines were established in our laboratory from primary specimens (7), with the exception of U87MG, T98G, U373MG, U138MG, and CRL1718, which were from the American Type Culture Collection. Primary GBM specimens were generous gifts from Dr. Balveen Kaur (Ohio State University). All small interfering RNAs (siRNA) were purchased from Dharmaco and the sequences are 5′-GAAACAACAACUGGAAACUU-3′ (human CD24 siRNA), 5′-GGUGUAUUGCCUUGAUULUC-3′ (human MEST siRNA), and 5′-UGGUUUAACUGCUGCAAU-3′ (nontargeting siRNA).

Plasmids. The GLI1-binding sites–driven luciferase construct, 8x3′Gli-BS Luc, was generously provided by Dr. Hiroshi Sasaki (Osaka University; ref. 8). Reporter constructs pCD24-1.2kb-Luc and pCD24-0.3kb-Luc were generous gifts from Genentech (9) and Dr. Tsuyoshi Fukushima (University of Miyazaki), respectively (10).

Immunoblotting. This was performed as described previously (11, 12). Antibodies used included mouse monoclonal antibodies against flag-tag (Sigma), β-actin (Sigma), α-tubulin (Sigma), and lamin B (EMD), rabbit polyclonal GLI1 (H300; Santa Cruz Biotechnology) and CD24 (FL-80; Santa Cruz Biotechnology) antibodies, and goat polyclonal GLI1 antibody (C-18; Santa Cruz Biotechnology).

Cell proliferation assay. This was conducted, as we described previously (11, 12), using the CellTiter Blue Cell Viability Assay Kit (Promega), a fluorescent method that is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Briefly, the cells were seeded on 96-well cell culture plates

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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(4 × 10^3 per well), and at 0, 24, 48, and 72 h, the number of viable cells was determined.  

**Assay of glioma cell migration.** The scratch wound assay (10) was used to determine glioma cell migratory activity. Briefly, tumor cells seeded in 6-well culture plates were carefully scratched with a fine pipette tip to create a gap. Images of the scratched cell monolayers were taken at ×10 magnification and used to compute gap width using the AXIO 4.0 software attached to the microscope. For each gap, the average width of three measurements (top, middle, and bottom) of the microscopic field was computed.

**Quantitation of glioma cell invasiveness.** For this, the InnoCyte Quantitative Cell Invasion Assay (EMD) was used, according to the manufacturer's instructions. Briefly, 1.75 × 10^5 cells were placed in the top chamber of rehydrated inserts with an 8 μm pore size polycarbonate membrane coated with a uniform layer of basement membrane matrix on the top surface. Following incubation for 24 h, the medium in the top chamber was discarded and the inserts were placed in fresh wells containing fluorescent calcine-AM cell staining/detachment buffer. Aliquots of the fluorescence-stained dislodged cells were transferred to duplicate wells of a 96-well cell culture plate and the fluorescence was measured. The invasive tumor cells on the inserts were stained in 0.5% crystal violet.

**GeneChip DNA microarray.** Total RNA extracted from the three U87MG stable transfectants were used to examine their gene expression profile. This was conducted in the DNA Microarray Core Facility at Duke Institute of Genome Science & Policy using the human Genome U133 Plus 2.0 Array GeneChips (Affymetrix) containing >47,000 gene transcripts.

**Chromatin immunoprecipitation assay.** This was conducted using a Chromatin Immunoprecipitation Assay Kit (Upstate) as we described previously (13). A rabbit polyclonal GLI1 antibody (H300; Santa Cruz Biotechnology) that recognizes the COOH-terminal domain (amino acids 781-1,080 present in both GLI1 and tGLI1 proteins) of the human GLI1 proteins was used in these experiments. Primer sequences for amplifying the CD24 promoter are 5’-GCTATTGTGGCTTTCCTGGT-3’ (forward) and 5’-GCTGGGTGCTTGGAGAAC-3’ (reverse).

**Xenografts, immunohistochemistry, and H&E staining.** GBM xenografts (three per group) were generated by s.c. implantation of U87MG-GLI1 and U87MG-tGLI1 cells into the right flanks of female nude mice (National Cancer Institute-Frederick). A total of 5 × 10^5 cells were used per inoculation. Tumors were excised, −30 days post-inoculation, embedded in paraffin, sectioned into 5 μm microsections, and subjected to immunohistochemistry and H&E staining. After deparaffinization, immunohistochemistry was conducted as described previously (11, 14) using a mouse anti-CD24 monoclonal antibody (Neomarkers; Ab-2; 1:10) and a rabbit anti-GLI1 polyclonal antibody (H300; Santa Cruz Biotechnology; 1:75). For H&E staining, deparaffinized tumor sections were stained with Mayer's hematoxylin solution (Sigma; 15 min), washed in water, stained with 0.5% eosin Y alcoholic solution (EMD; 2 min), dehydrated in alcohol, and mounted using xylene-based mounting medium (Vector Laboratories).

**Results**

**Identification of a novel GLI1 splicing variant.** Analysis of multiple GLI1 cDNA clones from cells of a GBM cell line showed consistently the presence of two GLI1 transcripts. Nucleotide sequencing showed the larger transcript to be wild-type GLI1, whereas the smaller transcript corresponded to a truncated GLI1, tGLI1 (Fig. 1A and B), which contains an in-frame deletion of 123 bases, spanning nucleotides 179 to 301, encompassing the entire exon 3 and part of exon 4 of the GLI1 gene. The deleted region encodes 41 codons corresponding to amino acid residues 34 to 74. tGLI1 retains the major GLI1 functional domains, including the degron degradation signals, the Sufu-binding domains, the nuclear localization signal, the zinc finger DNA-binding domains, and the transactivation domain. To further characterize the nature of this novel GLI1 variant, we PCR amplified exons 2 to 4 of the GLI1 gene from genomic DNA from 15 GBM cell lines, 2 normal human astrocyte cell lines, and peripheral blood leukocytes from 48 normal adults. The nucleotide sequences of the amplified genomic DNA showed no deletion in the GLI1 gene in any of the specimens, confirming that tGLI1 is a product of post-transcriptional alternative splicing of the GLI1 mRNA.

**tGLI1 is frequently expressed in GBM but not in normal tissues.** The expression of tGLI1 and GLI1 transcripts in human GBM was determined using cell lines, xenografts, and primary specimens. The results of reverse transcription-PCR (RT-PCR) of exons 1 to 4 of GLI1 and subsequent nucleotide sequencing of the cDNAs are summarized in Fig. 1C. The majority (67%) of GBM cell lines, xenografts, and primary specimens expressed comparable levels of tGLI1 and GLI1. In addition to the GBM, tGLI1 was also highly expressed in human breast cancer cells (Supplementary Fig. S1). In contrast, tGLI1 was undetectable in normal brain tissues (Fig. 1D, left) or other normal tissues (Fig. 1D, right). Interestingly, the GBM cells we analyzed did not express a recently reported GLI1ΔN variant that has been shown to contain a relatively large NH2-terminal deletion of amino acid residues 1 to 128 and to be expressed predominantly in normal tissues and to possess a weaker transcriptional activity compared with wild-type GLI1 (15). Together, these results provide strong evidence that tGLI1 expression is a predominant characteristic of human GBM.

**tGLI1 protein retains the ability to transactivate consensus GLI1-binding sites and undergo nuclear translocalization.** For these studies, we created three U87MG stable transfectant lines, U87MG-vector, U87MG-GLI1, and U87MG-tGLI1, which express the control vector, GLI1, and tGLI1, respectively. RT-PCR (Fig. 2A, left) confirmed expression of GLI1 in U87MG-GLI1 cells and tGLI1 in U87MG-tGLI1 cells, whereas immunoblotting showed that both cell lines expressed the full-length proteins (Fig. 2A, right). The levels of GLI1 and tGLI1 transcripts in GLI1/tGLI1-expressing cells were comparable with those observed in a primary GBM. Using these U87MG stable transfectants, we showed that GLI1 and tGLI1 similarly activated GLI1-binding sites cloned into a luciferase reporter, 8xGli-BS Luc (8), in both the absence and the presence of the Shh ligand (Fig. 2B). The observed modest transcriptional induction by Shh is consistent with a previous study (3) reporting that GLI1 is constitutively activated in GBM. However, another study (6) reported that the Shh pathway is activated in primary grade 2 and anaplastic astrocytomas but not in GBM. These observations suggest that the state of the Shh pathway in GBM remains unclear. We further showed that tGLI1 is localized in the nucleus similar to GLI1, as indicated by nuclear fractionation/immunoblotting and immunofluorescence staining/confocal microscopy (Fig. 2C). In the former analysis, fractionation efficiency was indicated by the absence of the nuclear protein, lamin B, in the nonnuclear fractions and the absence of the cytosolic protein, α-tubulin, in the nuclear fractions. In the immunofluorescence staining/confocal microscopy, a flag antibody was used to detect flag-tagged GLI1/tGLI1 proteins (green) and propidium iodide was used to stain nuclei (red). The yellow merged signals indicate nuclear tGLI1/GLI1. The nuclear presence of tGLI1 is consistent with the fact that it contains an intact nuclear localization signal (Fig. 1A). The three stable transfectant lines had similar growth rates (Fig. 2D). Together, these results show that tGLI1 retains the ability to transactivate consensus GLI1-binding sites and to undergo nuclear localization similar to wild-type GLI1.

**tGLI1 has a higher propensity to promote the migration and invasion of GBM cells than GLI1.** Figure 3A summarizes the results of the scratch wound migration assay and shows that
U87MG-tGLI1 cells migrated at a significantly higher rate than both control U87MG-vector and U87MG-GLI1 cells. The average gap width after time, t, relative to that at time zero (t₀) was used as a migratory index, Iₘ. Iₘ values at t₂₄ were 12.7%, 17.3%, and 100% for U87MG-vector, U87MG-GLI1, and U87MG-tGLI1 cells, respectively (Fig. 3A).

The differential effects of GLI1 and tGLI1 on invasiveness of GBM cells was examined using a quantitative fluorescence invasion assay (Fig. 3B, top) and crystal violet staining of cells in the Transwell assay (bottom). In both assays, we showed that U87MG-tGLI1 cells were significantly more invasive than U87MG-GLI1 and U87MG-vector cells. Both proliferation and invasiveness of the cells were determined and used to compute an invasion/proliferation ratio as a quantitative measure of net invasiveness. Similar observations were found in the T98G GBM cells transiently transfected with the control, GLI1, and tGLI1 expression vectors (Fig. 3C and D). The growth curve for T98G cells over 72 h also showed no significant difference in proliferation under these conditions, indicating that the observed increase in migration and invasiveness was not due to increased proliferation. Together, these results show a higher propensity of tGLI1 relative to GLI1 to promote migration and invasiveness in GBM cells.

**tGLI1 protein binds to the CD24 promoter and activates gene expression.** To gain further insight into the molecular mechanisms underlying tGLI1-mediated GBM cell migration and invasion, we examined the gene expression profiles of U87MG-tGLI1 cells and compared them with those of the U87MG-vector and U87MG-GLI1 cells. The results showed 75 genes to be expressed at a significantly higher level and 26 genes to be more suppressed in U87MG-tGLI1 cells compared with...
U87MG-vector and U87MG-GLI1 cells (Fig. 4A; Supplementary Tables; Supplementary Fig. S2). Interestingly, the levels of well-known GLI1 target genes, such as 

**PTCH1**, were higher in both U87MG-GLI1 and U87MG-tGLI1 cells compared with U87MG-vector cells. Also, U87MG-tGLI1 cells, but not U87MG-GLI1 or U87MG-vector cells, showed significantly higher levels of expression of the migration-associated gene, 

**CD24**. Because CD24 has been shown to recruit adhesion molecules to lipid rafts, thereby contributing to tumor cell migration, dissemination, and metastasis (16, 17), we focused on it to gain insight into the role of tGLI1 in the observed migratory and invasive phenotype of GBM cells. RT-PCR (Fig. 4B), quantitative RT-PCR (Fig. 4C), and immunoblotting (Fig. 4D) showed CD24 to be expressed at a significantly higher level in U87MG-tGLI1 cells than in U87MG-vector and U87MG-GLI1 cells. Subsequent Web-based motif searches, including TFSearch and TESS, showed no putative GLI1-binding sites in the human 

**CD24** promoter. Both U87MG-GLI1 and U87MG-tGLI1 cell lines expressed the 

**PTCH1** gene at equivalent levels, in contrast with U87MG-vector cells (Fig. 4B). This is consistent with the results of microarray and our findings (Figs. 1A and 2B) showing that tGLI1 and GLI1 exhibit a similar ability to activate GLI1 target genes. In addition to 

**CD24**, we examined levels of 

**MEST** gene expression because DNA microarray identified 

**MEST** to be a potential tGLI1-regulated gene with the highest tGLI1/GLI1 ratio (260.2; Supplementary Table SI). As shown in Supplementary Fig. S3A and B, MEST gene transcripts and promoter activity were significantly higher in U87MG-tGLI1 cells compared with U87MG-vector and U87MG-GLI1 cells.

Analysis of protein-DNA binding using the chromatin immunoprecipitation assay showed that tGLI1, but not GLI1, binds to the 

**CD24** promoter (Fig. 5A). Binding specificity was shown by the absence of any signals in the negative immunoprecipitation controls using rabbit IgG. The results showed a significantly higher binding affinity of tGLI1 to the 

**CD24** promoter compared with...
GLI1 ($P = 0.012$; Fig. 5B). To further characterize the region within the CD24 promoter required for tGLI1-mediated transcriptional activation, five reporter constructs carrying successively truncated (1.2, 0.3, 0.25, 0.2, and 0.14 kb) CD24 promoter were used. As shown in Fig. 5C, deletion of the 0.91 kb region (nucleotides -1,167 to -253) did not substantially alter activity of the CD24 promoter and did not abolish tGLI1-mediated transcriptional activation, indicating that this region is not targeted by tGLI1. In contrast, CD24 promoter activity was decreased to basal level with deletion of the 0.06 kb region (nucleotides -207 to -141), suggesting that this region is required for tGLI1-mediated induction of CD24 gene expression.

Using U87MG-GLI1 and U87MG-tGLI1 xenografts established in the flanks of nude mice, we further found CD24 expression to be significantly higher in U87MG-tGLI1 tumors than U87MG-GLI1 tumors to be more invasive than U87MG-GLI1 counterparts, as shown by their increased infiltration into the smooth muscles. RT-PCR (Fig. 5D, bottom) confirms expression of GLI1 and tGLI1 transcripts in U87MG-GLI1 and U87MG-tGLI1 xenografts, respectively. Collectively, these results show that tGLI1 leads to a unique gene expression profile and that it transcriptionally activates the promigratory CD24 gene in GBM cells.

CD24 expression is required for tGLI1-mediated GBM cell migration and invasiveness. We found that CD24-specific siRNA significantly down-regulated CD24 expression, but not MEST (Fig. 6A, top), in U87MG-tGLI1 cells and reduced their migration to the level observed in U87MG-GLI1 cells (Fig. 6A, bottom). Similarly, the results of the invasion assay indicate that CD24 siRNA significantly decreased the net invasiveness of U87MG-tGLI1 cells by 3.15-fold (Fig. 6B). In contrast, MEST siRNA did not affect the invasiveness of
U87MG-tGLI1 cells (Supplementary Fig. S3C and D). To complement the siRNA experiments, U87MG cells were transiently transfected with the CD24-expressing vector; in addition to the expected increased CD24 expression (Fig. 6C, left), there was a significant increase in the migration and net invasiveness of the cells, as shown by the scratch wound (Fig. 6C, right) and cell invasion (Fig. 6D) assays, respectively. Modulations of CD24 expression did not affect the proliferation of these GBM cells (Supplementary Fig. S4A and B).

Figure 4. tGLI1 leads to a unique expression profile and activates CD24 expression. A, differential gene expression patterns of U87MG-tGLI1 cells compared with U87MG-vector and U87MG-GLI1 cells. GeneChip DNA microarray and subsequent data analyses were conducted to compare gene expression profiles of the U87MG transfectant lines. Cluster analysis included 75 genes that were expressed at significantly higher levels (>2-fold; \( P < 0.05 \)) in U87MG-tGLI1 cells than U87MG-vector and U87MG-GLI1 cells. A color scale (bottom) shows the range of gene expression. Arrows, CD24 and MEST genes that were validated in subsequent studies. B to D, CD24 expression is up-regulated in tGLI1-expressing GBM cells. RT-PCR (B), quantitative RT-PCR (C), and immunoblotting (D) were conducted to determine CD24 expression level in U87MG stable transfectants. Compared with U87MG-vector cells, U87MG-tGLI1 and U87MG-GLI1 cells expressed higher levels of PTCH1, a well-known GLI1 target gene. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Together, the results of these transcriptional knockdown and overexpression studies indicate that CD24 is required for tGLI1-mediated increase in GBM cell migration and invasiveness.

**Discussion**

We report the discovery of tGLI1, a novel truncated splice variant of the transcription factor GLI1, resulting from deletion of 41 codons of the GLI1 gene. tGLI1 retains all of the known functional domains of wild-type GLI1. Although not expressed in normal cells, tGLI1 is highly expressed in cell lines, xenografts, and primary specimens of GBM. GBM cells engineered to express tGLI1 were significantly more migratory and invasive than their isogenic wild-type GLI1 containing counterparts. We showed that CD24, an invasion-associated gene, is a specific transcriptional target of tGLI1 and that CD24 expression is required for tGLI1-mediated GBM cell migration and invasion.

The tGLI1 splice variant identified in this study differs significantly in structure, expression patterns, and physiological function from another recently described GLI1 splice variant, GLI1\(\Delta N\) (15). First, tGLI1 not only contains a comparatively small deletion (41 codons encoding amino acid residues 34-74) but also retains all the known regulatory and functional domains of GLI1. In contrast, GLI1\(\Delta N\) contains a large NH\(_2\)-terminal truncation of 128 amino acid residues resulting in a loss of the degron degradation signals and the NH\(_2\)-terminal Sufu-binding domain of GLI1. In addition to the structural differences, the expression patterns of tGLI1 differ from that of GLI1\(\Delta N\), with the former being highly expressed in malignant gliomas but not in normal cells, whereas the latter is expressed predominantly in normal cells but not in GBM.
With regard to their physiologic function, tGLI1 behaves as a gain-of-function gene; in contrast, GLI1ΔN is a significantly weaker transcriptional regulator compared with GLI1.

Our findings showing that the migration/metastasis-associated CD24 gene is a direct transcriptional target of tGLI1 is the first evidence linking the Hedgehog signaling to the CD24 pathway. This finding is significant given that CD24 is overexpressed in various tumor types and has been shown to be involved in tumor cell migration, invasion, and metastasis (16, 18). The fact that GBMs highly express CD24 is consistent with previous reports that suppression of CD24 expression reduced migration and invasiveness of C6 rat glioma and human GBM cells (10, 19). Despite previous reports implicating CD24 in the proliferation of breast, colon, and cervical cancer cells (16, 20, 21), our results indicate that CD24 may not be a significant regulator of in vitro growth of GBM cells. The effects of CD24 on tumor growth may be tumor type dependent.

GLI1 plays a central integrative role in various cell signaling pathways, such as those of Hedgehog, transforming growth factor-β, epidermal growth factor receptor, protein kinase A, and K-RAS, and thereby mediates several important cellular processes involved in normal development, oncogenesis, tumor proliferation, and progression (22–24). The gain-of-function of tGLI1 in regulating CD24 transcriptional activity and promoting GBM cell migration and invasion, its GBM-specific expression pattern, and the fact that it retains all the functional domains of GLI1,

Figure 6. CD24 expression is required for tGLI1-mediated GBM cell migration and invasiveness. A and B, suppression of CD24 expression reduces migratory ability and invasiveness of U87MG-tGLI1 cells. U87MG-tGLI1 cells were transfected with CD24-specific and control nonspecific siRNAs, whereas U87MG-GLI1 cells were only exposed to control siRNA. RT-PCR and immunoblotting (A, top) showed a significant down-regulation of CD24 expression in the CD24 siRNA-treated cells. Forty-eight hours following transfection, cells were examined for proliferation and migration using the scratch wound assay (A, bottom) and for invasiveness (B), using invasion assay. Net invasiveness was determined by computing the ratio of invasiveness over proliferation. To note, the exposure to the transfection reagent and siRNA may have caused tumor cells to appear less confluent than the untransfected counterparts in Fig. 3A and B. C and D, increased CD24 expression promotes GBM cell migration and invasiveness. U87MG cells with an undetectable level of CD24 were transfected with control or CD24 vectors. RT-PCR for CD24 transcripts showed increased CD24 expression in CD24 vector-transfected cells but not in those with control vector, as shown by RT-PCR and immunoblotting (C, left). Forty-eight hours following transfection, the cells were examined for proliferation. There was no effect of CD24 overexpression on proliferation of U87MG cells. In contrast, there was a significant increase in the migratory ability (C, right; scratch wound assay) and invasiveness (D; invasion assay) of the CD24-overexpressing U87MG cells compared with control cells. Net invasiveness was determined by computing the ratio of invasiveness over proliferation.
collectively, suggest that tGLI1 may be a more important mediator of GBM cellular physiology and behavior than GLI1. These results are significant given that GBM is the most frequent and deadliest brain cancer in adults and is highly infiltrative and resistant to therapy (25–27). Also, our findings provide a rationale for further investigations of tGLI1 in other tumors known to have active Hedgehog signaling and to be highly metastatic. The discovery of tGLI1 is, thus, highly significant and is likely to open up novel concepts of the role of GLI1 in tumor biology and may provide the basis for novel treatment strategies.

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Disclosure of Potential Conflicts of Interest

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