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SHORT COMMUNICATION

Pannexin2 as a novel growth regulator in C6 glioma cells

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Connexins (Cxs), the gap junction proteins, have been found to be downregulated in many types of cancers including gliomas. By restoring gap junctional communication in cancer cell models, the neoplastic phenotype can be reversed, suggesting Cxs are tumor suppressors. Pannexin2 (Panx2) is a member of the novel gap junction protein family, Panxs, and it has been proposed as a brain-specific protein. Recently, gene array analysis showed an overall reduction of Panx2 in gliomas, and a direct correlation was observed between Panx2 expression and post-diagnosis survival in patients. In this study, we explored the potential inverse correlation between Panx2 and glioma oncogenicity. A decrease or absence of Panx2 expression in a panel of human glioma cell lines was found, whereas an appreciable amount of Panx2 was detected in both human brain and astrocytes. Stable Panx2 expression revealed a flattened morphology and increased cell–cell contacts in rat C6 glioma cells similar to Panx1. However, in contrast to Panx1 and Panx3, Panx2 was predominately detected in the cytoplasm in vesicle-like patterns but not at the plasma membrane. Coexpression of Panx2 and Panx1 did not show colocalization of both Panxs. Strikingly, restoration of Panx2 expression significantly reduced *in vitro* oncogenicity parameters, including monolayer saturation density and anchorage-independent growth, as well as *in vivo* tumor growth. This study suggests a role of aberrant Panx2 expression during gliomagenesis, and that Panx2 independently functions as a negative growth regulator without Panx1.
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Keywords: Panx2; pannexins; gap junction; intercellular communication; C6 glioma

Introduction

Gap junctions are membrane channels that connect the cytosols of adjacent cells, allowing the direct passage of small molecules <1200 Da (Loewenstein, 1987). A lack of cell–cell communication has been shown in neoplasms including gliomas (Mesnil, 2002; Naus, 2002), and restoration of gap junctional intercellular communication in cancer cell models has been found to reverse the transformed phenotypes and to reduce proliferation (Naus *et al*., 2005). Later studies have also shown a tumor-suppressive role of connexins (Cxs), the canonical gap junction proteins, through protein–protein interactions, further supporting its function as a tumor suppressor (Trosko and Ruch, 2002; Naus *et al*., 2005).

Pannexins (Panxs) are a novel family of gap junction proteins recently identified as the mammalian homologs of the invertebrate gap junction proteins, innexins. To date, three Panx members have been identified in vertebrates: Panx1, Panx2 and Panx3. A functional role of Panx1 has been shown in intracellular Ca^{2+} release, propagation of Ca^{2+} waves in blood vessel endothelial cells, inflammatory response, ischemic cell death and taste cell signaling, advocating the importance of Panx1 under both normal and pathological conditions (Locovei *et al*., 2006; Pelegrin and Surprenant, 2006, 2007; Thompson *et al*., 2006; Huang *et al*., 2007; Romanov *et al*., 2007). In addition, we showed a reversion of transformed phenotypes of C6 gliomas when Panx1 was overexpressed (Lai *et al*., 2007), suggesting a tumor-suppressive role of Panxs in neoplasms. However, Panx2 function remains largely undiscovered, and its role in neoplasm has not been examined.

Panx2 transcripts, unlike Panx1, are particularly abundant in the brain in rodents and presumably brain specific in humans (Bruzzone *et al*., 2003; Baranova *et al*., 2004; Vogt *et al*., 2005; Ray *et al*., 2006). In parallel to the loss of Panx2 transcript in C6 gliomas when compared with its normal counterpart, rat primary astrocytes (Lai *et al*., 2007), high-throughput cDNA microarray analysis of human brain tumor samples has shown an overall reduction of Panx2 gene expression in gliomas (Litvin *et al*., 2006). A direct correlation was also found between Panx2 upregulation and post-diagnosis survival in patients with glial tumors using the brain cancer gene expression database REMBRANDT (Repository of Molecular Brain Neoplasia Data, http://rembrandt.nci.nih.gov/rembrandt) (Litvin *et al*., 2006). Moreover, human *Panx2* is located within chromosomal region 22q13.3, where deletions are often found in human astrocytomas and ependymomas (Ino *et al*., 1999; Oskam *et al*., 2000; Hu *et al*., 2004; Ray *et al*., 2006). Thus, *Panx2* expression may be commonly altered during glioma development. Altogether, the findings suggest a role of aberrant Panx2 in gliomagenesis and oncogenicity.

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Results and discussion

Reduced Panx2 expression in human glioma cells

To investigate whether aberrant Panx2 expression occurs in gliomas, a panel of human glioma cell lines was examined using western blot analysis and showed a reduction or absence of Panx2 protein when compared with human astrocytes (Figure 1a). The observation is in agreement with the result from gene array analysis of human brain tumor samples in which gliomas have an overall decrease of Panx2 expression (Litvin *et al*., 2006). A notable level of Panx2 expression was detected in the human brain, advocating a role of Panx2 in the brain (Vogt *et al*., 2005; Ray *et al*., 2006; Zappala *et al*., 2007). An appreciable amount of Panx2 protein was also found in human astrocytes. This is in contrast to the observation made in rats in which Panx2 was only detected in the neurons and reactive astrocytes, but not in normal astrocytes (Vogt *et al*., 2005; Zappala *et al*., 2007). The disparity could be a result of species differences and warrants further investigation to determine the type(s) of astrocytes expressing Panx2 in humans. Overall, this finding suggests an inverse correlation between Panx2 expression and glioma oncogenicity.

Panx2 expression alters cellular morphology

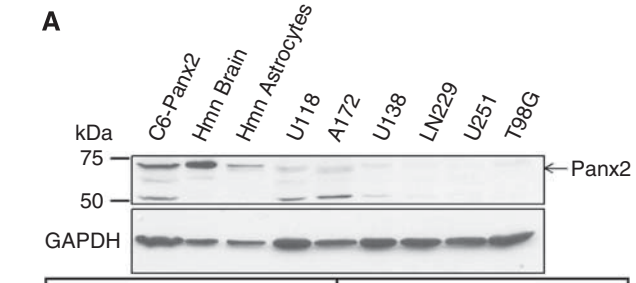
Conversely, we investigated whether restoring Panx2 expression could suppress glioma growth. Using a well-established glioma cell line, rat C6 cells, we investigated the effect of Panx2 expression on the transformed phenotype. C6 cells are considered to be of astrocytic origin and poorly coupled by gap junctions because of their limited Cx43 expression (Naus *et al*., 1991; Barth 1998). In addition, a recent study showed most of C6 cells as cancer stem cells with clonogenic, self-renewal and tumorigenic capacities (Zheng *et al*., 2007). Furthermore, our recent report determined C6 gliomas to be devoid of Panx transcripts (Lai *et al*., 2007), which together makes it an ideal model system to study the effect of Panx2 expression on glioma oncogenicity. Several clones with varying expression levels were successfully generated for Panx2-HA and Panx2-EGFP stable transfectants to study the potential correlation between Panx2 and oncogenicity (Figure 1b and c). Clones for the control transfectants, EGFP, were previously created at the same time with tagged Panx2 transfectants (Lai *et al*., 2007). Endoplasmic reticulum stress is an artifact commonly observed in the over-expression system, perturbing cell growth and survival (Lee, 2005). To address this possibility, stable control- and Panx transfectants were examined for glucose-regulated protein (GRP78/BiP), an endoplasmic reticulum stress marker, and showed no evident increase in expression (Lee, 2005) (Supplementary Figure 1a). On stable transfection, a noteworthy flattened morphology was observed in Panx2-expressing cells (Figure 1d). Interestingly, the flattened morphology was moderate when compared with that of Panx1-expressing cells (Figure 1d). Moreover, Panx2-EGFP signal was observed in the cytoplasm, which is distinctly different from predominately plasma membrane-located Panx1 (Lai *et al*., 2007).

Panx2 is not associated with the plasma membrane

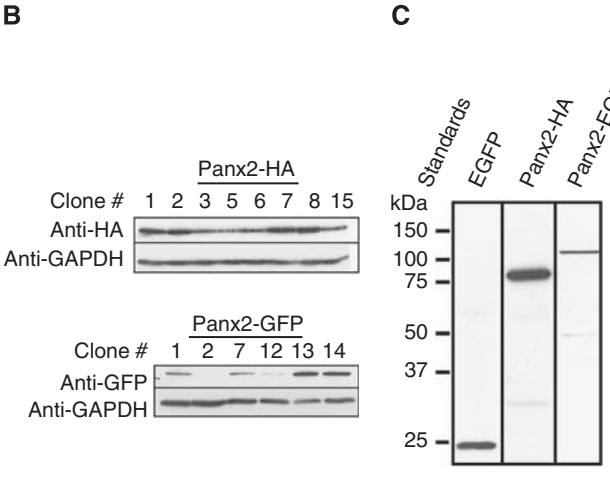
To further examine the intracellular location of Panx2, a crude membrane fractionation assay identified Panx2-HA and Panx2-EGFP only in the membrane-enriched fraction, suggesting that Panx2 is in membrane-bound vesicles and/or organelles (Figure 1e). We then stained the cell membranes with wheat germ agglutinin, and detected Panx2-EGFP predominately around the perinuclear region in a vesicle-like pattern, but not at the plasma membrane nor at areas of cell–cell contact (Figure 2a). Using specific markers, Panx2 showed a prominent signal overlap with the endoplasmic reticulum but only limited with the Golgi apparatus (Supplementary Figure 1b). Importantly, Panx2 did not appear to aggregate in either organelles, indicating that its trafficking was not encumbered as a result of over-expression; previous studies have shown an aberration in Cx trafficking because of its overexpression (Skerrett *et al*., 2004; Das *et al*., 2005; Thomas *et al*., 2005). One possible explanation for Panx2 distinctive intracellular localization is that it was actively trafficked to the plasma membrane but promptly internalized for degradation, possibly through the endolysosomal pathway previously shown for Cx43 (Laing *et al*., 1997; Qin *et al*., 2003; Berthoud *et al*., 2004). However, Panx2 did not appear colocalized to early endosomes and lysosomes, showing that Panx2 was not being actively degraded in the cytoplasm (Supplementary Figure 1c). It was also unlikely that Panx2 was being actively degraded by the proteasomal degradation pathway, as it was not readily detected in the cytosol-enriched fraction (Figure 1e). Several recent studies have reported Cx40 and Cx43 located in the mitochondria of cardiomyocytes, resembling Panx2 subcellular pattern (Li *et al*., 2002; Boengler *et al*., 2005; Schulz *et al*., 2007). However, co-labeling of mitochondria and Panx2 showed no apparent colocalization (Supplementary Figure 2). Importantly, recent studies have shown a similar expression pattern of endogenous Panx2 in neuronal cells, reactive astrocytes after hypoxia, as well as in spiral ganglion neurons of the cochlea, where Panx2 was detected in the cytoplasm and especially prominent at the perinuclear region (Zappala *et al*., 2007; Wang *et al*., 2008). Together, the current finding indicates Panx2 is primarily located within the cytosol and not at the plasma membrane in C6 cells.

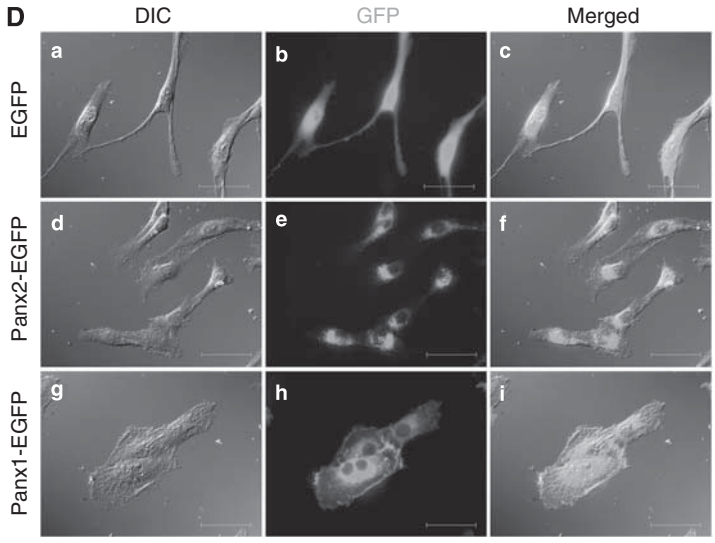
Panx2 does not colocalize with Panx1 at the plasma membrane

The subcellular localization pattern of Panx2 is consistent with the finding that Panx2 alone was not able to form either hemichannels or intercellular channels in the *Xenopus* oocyte system on the cell surface (Bruzzone *et al*., 2003). Thus, we speculated that Panx2 can be recruited to the plasma membrane by Panx1 as previously suggested to elicit its function (Bruzzone *et al*., 2003, 2005). Yet, when the two Panxs (tagged or



Name of Cell Line	Tumor Type
U118	Glioblastoma
A172	Glioblastoma
U138	Glioblastoma
LN-229	Glioblastoma
U251	Glioma
T98G	Glioblastoma multiforme





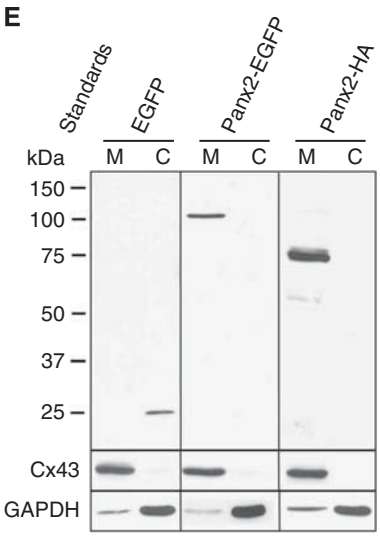
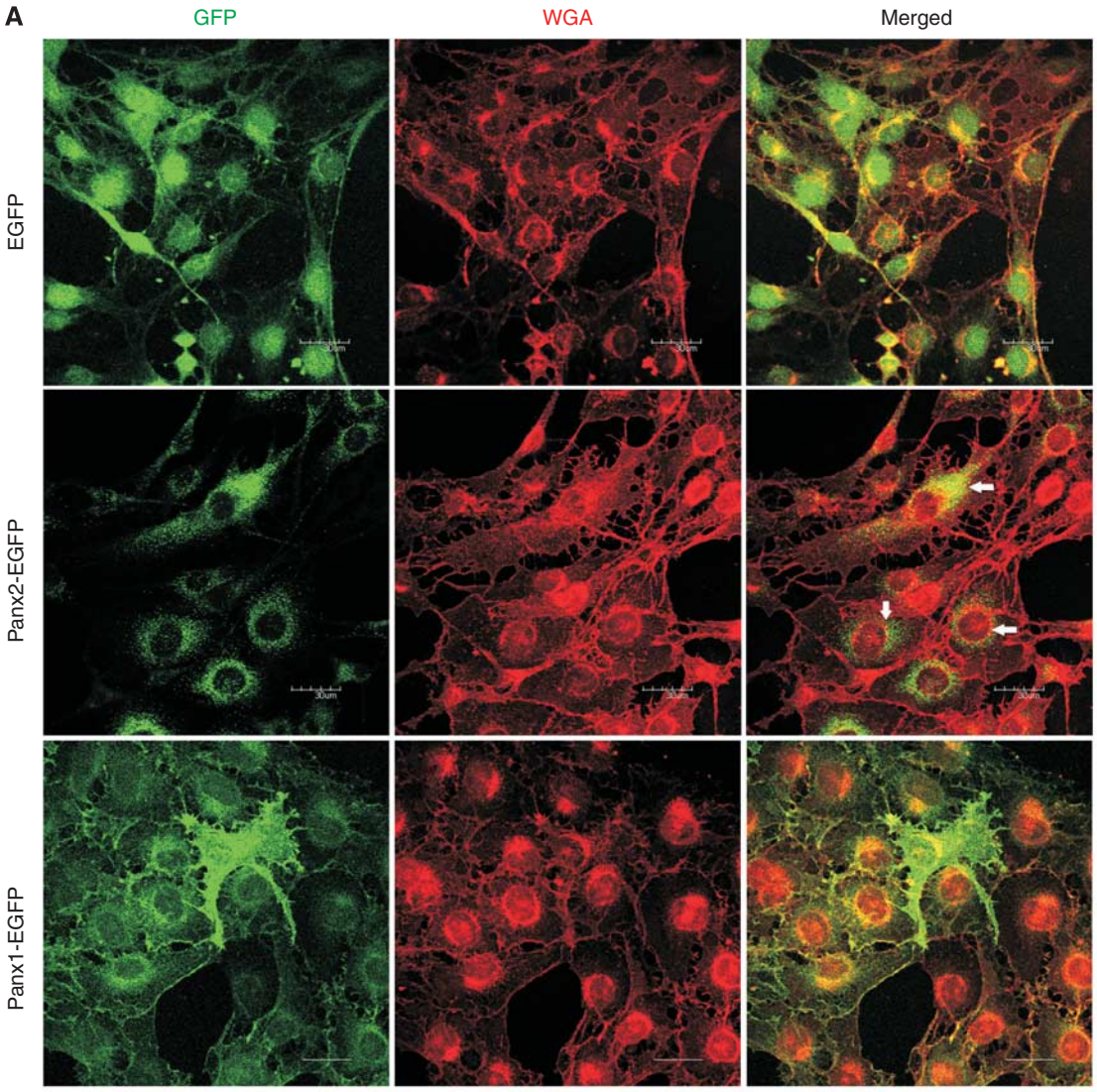
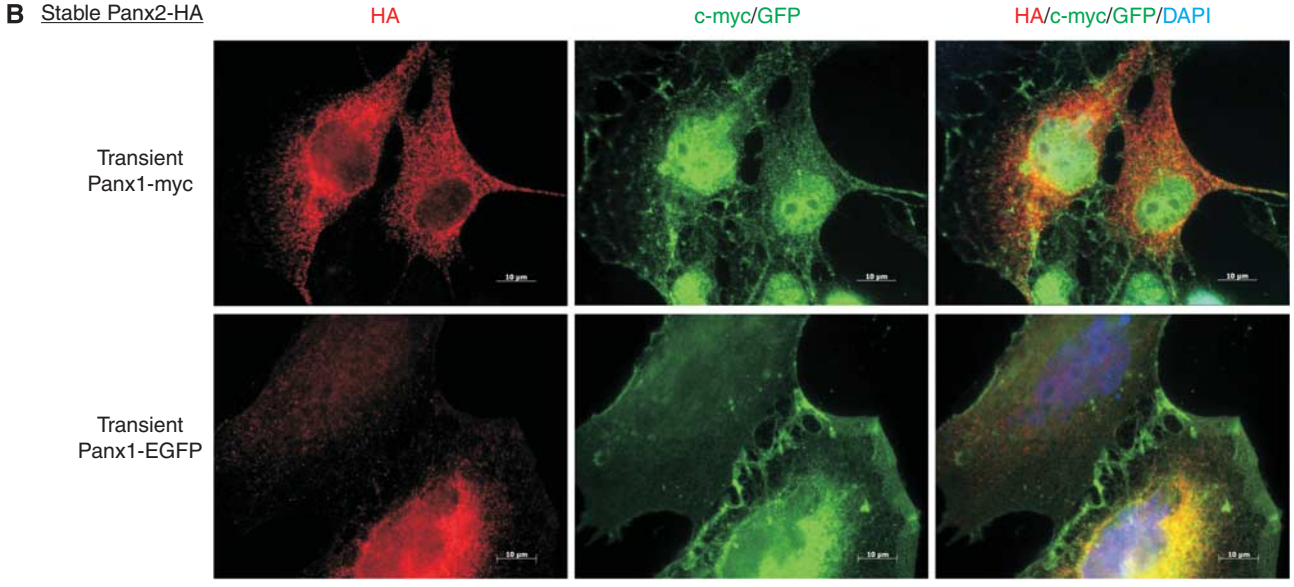


Figure 1 Pannexin2 (Panx2) expression is reduced in human glioma cell lines, and alters cell morphology in stable Panx2-transfected C6 cells. (**A**) Using anti-Panx2 antibody (Aviva Systems Biology, San Diego, CA, USA), western blotting of total protein extracted from a panel of human gliomas (American Type Culture Collection, Manassas, VA, USA), human brain (Zyagen, San Diego, CA, USA), astrocytes (ScienCell Research Lab, Carlsbad, CA, USA) and stable Panx2-transfected C6 cells showed very limited or negligible Panx2 signal in the human glioma cells. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as a loading control (Cedarlane Lab, Hornby, ON, Canada). The 50-kDa band observed in Panx2-expressing C6 cells and some other glioma lines is nonspecific (see Supplementary Figure 5). (**B**) C6 cells were co-transfected with Murine Stem Cell Virus vectors encoding a puromycin resistance gene (pMSCVpuro) (Clontech Laboratories, Mountain View, CA, USA) and the plasmid of interest (*EGFP*, *Rattus norvegicus Panx2* tagged with human influenza hemagglutinin tag (Panx2-HA) or *enhanced green fluorescence protein* reporter gene (Panx2-EGFP) at carboxy terminus) using Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) as previously described (Bruzzone *et al*., 2005; Lai *et al*., 2007). Co-transfectants were selected with 3 μg/ml puromycin, and clones were subsequently isolated. Cell lysates from selected clones were probed with anti-HA (Cedarlane Lab, Hornby, ON, Canada) and anti-green fluorescent protein (GFP) antibodies (Stressgen, Ann Arbor, MI, USA). The following clones were selected for further experimentation: Panx2-HA clone 3 and 8; Panx2-EGFP clone 1 and 13. (**C**) A representative immunoblot showing specificity of exogenous protein expression using EGFP clone 5, Panx2-HA clone 8 and Panx2-EGFP clone1 (EGFP 25 kDa, Panx2-HA 73 kDa, Panx2-EGFP 98 kDa). (**D**) Live-cell imaging with differential interference contrast (DIC) (a, d, g) shows Panx2-EGFP cells (b, e, h) exhibiting a moderate flattened morphology and greater cell–cell contacts. (c, f, i: merged images) By contrast, Panx1-EGFP displayed the most flattened morphology between the samples. Bar, 50 μm. (**E**) Crude membrane fractionation assay (Lai *et al*., 2007) shows EGFP in the cytosol fraction (C), whereas Panx2-HA and Panx2-EGFP were only found in the membrane-enriched fraction (M). To verify fraction specificity, immunoblots were probed for Cx43 (Sigma-Aldrich, St Louis, MO, USA) and GAPDH (CedarLane Lab); Cx43 were only detected in membrane-enriched fraction, whereas GAPDH were predominately found in the cytosol-enriched fractions.

untagged) were coexpressed, while Panx1 was found at the plasma membrane as previously reported (Lai *et al*., 2007), Panx2 localization remained unchanged (Figure 2b). The difference in findings suggests that Panx1/2 channel formation is not present in C6 gliomas. Collectively, our findings suggest that Panx2 exists in an individual entity, likely in the form of membrane-bound vesicles.


A
GFP
WGA
Merged
EGFP
Panx2-EGFP
Panx1-EGFP


B
Stable Panx2-HA
HA
c-myc/GFP
HA/c-myc/GFP/DAPI
Transient Panx1-myc
Transient Panx1-EGFP

Panx2 reduces oncogenicity of C6 gliomas

Next, we set to determine the effect of restoring Panx2 expression on the oncogenicity parameters in C6 gliomas. Using a growth curve assay, a significant retardation in proliferative rate was discovered in the stable Panx2 transfectants (Figure 3a). As no significant increase in apoptotic nuclei was observed in cells seeded on coverslips in parallel to the growth curve assay (data not shown), the growth reduction by Panx2 is proposed to occur through suppression of the cell cycle, but not cell death. In addition, Panx2 elicited a consistent reduction in saturation density (Figure 3a), which coincides with the flattened morphology of Panx2 transfectants; fewer cells of increased size are required to grow to confluence. A similar growth suppression with human glioma cells was also observed (Supplementary Figure 3). As Panx2 was only found within the cells but not at the plasma membrane like Panx1, and given that further examination also showed that Panx2 does not affect expression of Panx1, Cx43, as well as gap junctional intercellular communication (Supplementary Figure 4), Panx2 may induce its phenotype through a pathway distinct from Panx1. The notion is supported by the transwell assay in which Panx2, in contrast to Panx1, showed no clear effect on cell motility (Figure 3b). Anchorage-independent growth is another hallmark of cancer and can be assessed by the formation of colonies in soft agar. It is noted that both Panx2-HA and Panx2-EGFP transfectants drastically suppressed colony formation efficiency and anchorage-independent growth (Figure 4a and b). This suggests that Panx2 functions in a channel-independent approach. The proposition is strengthened by the soft agar assay as no cell–cell communication was present in the initial phase of the experiment when single cells were seeded in the soft agar. To further examine whether Panx2 elicits tumor-suppressive effects *in vivo*, tumorigenicity assay was conducted and both Panx2-expressing cells exhibited a strikingly reduced average tumor size when compared with the control. Findings reported here suggest Panx2 expression restores anchorage dependence of C6 gliomas on tethering to the extracellular matrix (ECM). Whether this dependence is through an alteration of the expression of the cell surface receptors, integrins, glioma-derived ECM (that is, vitronectin, tenascin-C), and/or the constitutive activity of survival pathways (that is, PI3K/Akt, MEK/ERK, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)), remains to be elucidated (D'Abaco and Kaye, 2007; Chiarugi and Giannoni, 2008).

In summary, the unique subcellular location of Panx2 suggests its functions through pathway(s) independent from gap junction channels. As Panx2 has a 358 amino acid C-terminal tail, which is more than twice the size of that of Cx43 (151 amino acid),

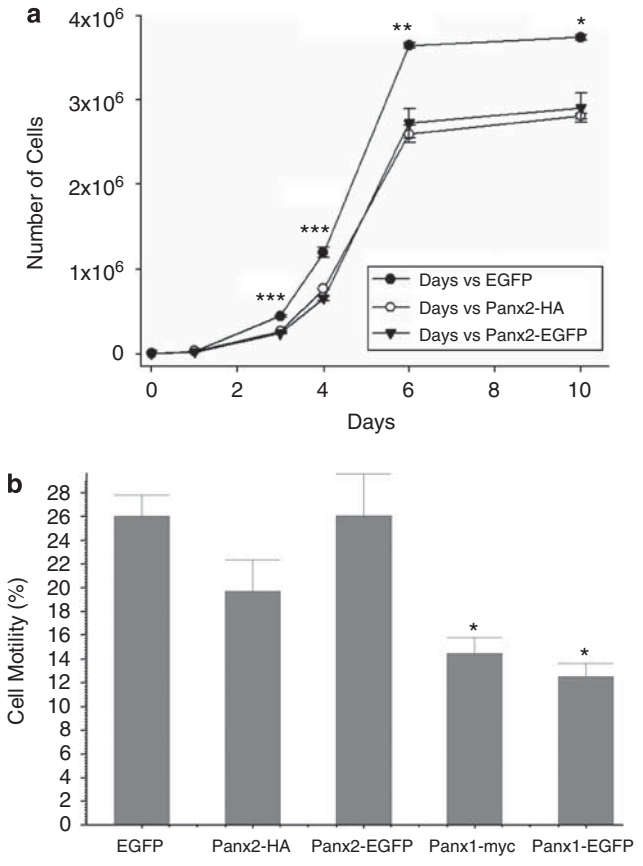


Figure 3 Pannexin2 (Panx2) reduces cell proliferation and saturation density, but not motility. (**a**) Growth curve assay shows a dramatic reduction in saturation density of Panx2-HA and Panx2-EGFP from day 6 to 10. The Panx2 transfectants also exhibited a reduced proliferative rate compared to the control from day 3 to 4. In all, 10 000 cells per well were seeded in 12-well plates, and the number of cells per well were counted on days 1, 3–4, 6 and 10 using a Z1 Coulter Particle Counter (Beckman Coulter, Mississauga, ON, Canada). Day 0 denotes the day of seeding (*$P<0.01$, **$P<0.01$, ***$P<0.001$ compared with control). Cells seeded on coverslips in parallel to the growth curve assay were stained with 4,6-diamidino-2-phenylindole (DAPI), and no significant increase in apoptotic nuclei was observed between the samples (data not shown). (**b**) Transwell assay reveals no decrease in cell motility of the Panx2 transfectants, whereas the Panx1 transfectants exhibited a significant retardation. A total of 75 000 cells were seeded and incubated for 14 h in duplicates in both the top of a transwell insert (BD Biocoat 8.0 μm inserts in 24-well plates; BD Biosciences, Mississauga, ON, Canada) and a separate well without the transwell insert. Cell motility was determined as number of traversed cells (bottom of the insert)/number of total cells (separately seeded well) (*$P<0.05$, compared with control).

Figure 2 Pannexin2 (Panx2) is not associated with the plasma membrane. (**A**) Confocal images showing Panx2-EGFP signal in the cytoplasm and especially at the perinuclear region (arrows). Cell membranes were permeabilized and stained with Alexa 594-conjugated wheat germ agglutinin (Invitrogen). a–c: EGFP control; d–f: Panx2-EGFP; g–i: Panx1-EGFP. Bar, 30 μm. (**B**) Coexpression of Panx2 and Panx1 by transiently transfecting stable Panx2-HA transfectants with Panx1-myc (top) or Panx1-EGFP (bottom) using Lipofectamine 2000 showed no apparent colocalization between the two proteins at the plasma membrane or in the cytoplasm. Exogenous expression was immunolabeled with mouse anti-HA (Cedarland Lab) or rabbit anti-myc (Abcam, Cambridge, MA, USA) antibodies followed by Alexa 568- or 488-conjugated anti-mouse or anti-rabbit IgG (Invitrogen), respectively. Nuclei were visualized by 4,6-diamidino-2-phenylindole (DAPI). Bar, 10 μm.

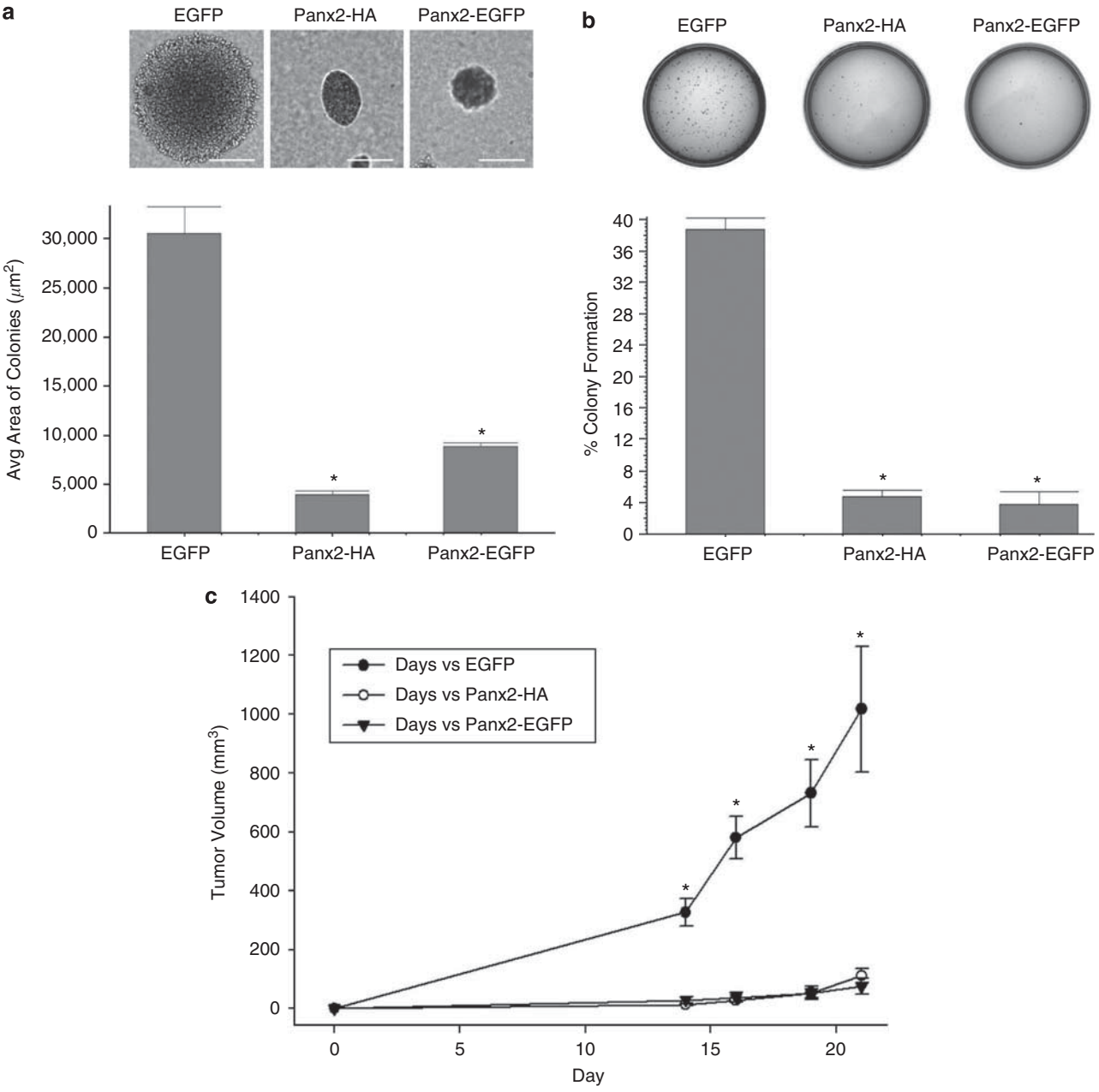


Figure 4 Pannexin2 (Panx2) suppresses anchorage-independent growth and *in vivo* tumor formation. (**a**, **b**) Panx2 dramatically retards anchorage-independent growth. (**a**) Top: sample images of individual colonies in the soft agar. Bottom: large colonies were consistently observed in the control (EGFP, 30 576.78 ± 2 671.3 μm^2), whereas only small colonies were found in the Panx2 transfectants (Panx2-HA, 3 895.91 ± 385.51 μm^2; Panx2-EGFP, 8 812.03 ± 400.61 μm^2). Bar, 200 μm. (**b**) Top: sample plates from the soft agar assay. Bottom: a significant reduction in the efficiency of colony formation of the stable Panx2 transfectants (Panx2-HA, 4.72 ± 0.81%; Panx2-EGFP, 3.69 ± 1.68%) when compared with the control (EGFP, 38.67 ± 1.50%) was observed. Soft agar assay was carried out as previously described (Lai *et al*., 2007) (*$P<0.001$ compared with control). (**c**) Tumorigenicity assay shows a significant reduction in the tumor size of the stable Panx2-transfected C6 cells beginning on day 14 and consistently observed until day 21 (*$P<0.001$). Nude mice injected with the control cells (EGFP) were killed on day 21 as the tumor size had reached 1000 mm^3. In contrast, mice injected with Panx2-expressing cells did not reach similar tumor size until an average of 12 days later (Panx2-HA: one of five mice on day 28, two of five mice on day 30, one of five mice on day 32 and 35; Panx2-EGFP: one of five mice on days 30, 32 and 35, two of five on day 37). Day 0 denotes the day of injection. Five 6 to 8-week-old female immunodeficient CrTac: NCr-Foxn1^{nu} mice (Taconic, Hudson, NY, USA) were injected at two sites on the flank/upper hips (500 000 cells per site) for each sample, and monitored for tumor growth as previously described (Lai *et al*., 2007).

we envision that the long Panx2 C-terminal tail may be advantageous in providing potential sites for intracellular protein–protein interaction to mediate Panx2 functions. This study provides the first report on the tumor-suppressive effects of Panx2 and implies Panx2 also functions in glioma stem cells, as a recent study showed that C6 cell line was mainly composed of cancer stem cells with perpetual self-renewal ability (Zheng

et al., 2007). In conclusion, Panx2 may serve as a prospective therapeutic target against glial tumors and warrant further investigation.

Conflict of Interest

The authors declare no conflict of interest.

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References

Baranova A, Ivanov D, Petrash N, Pestova A, Skoblov M, Kelmanson I *et al*. (2004). The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. *Genomics* **83**: 706–716.

Barth RF. (1998). Rat brain tumor models in experimental neuro-oncology: the 9L, C6, T9, F98, RG2 (D74), RT-2 and CNS-1 gliomas. *J Neurooncol* **36**: 91–102.

Berthoud VM, Minogue PJ, Laing JG, Beyer EC. (2004). Pathways for degradation of connexins and gap junctions. *Cardiovasc Res* **62**: 256–267.

Boengler K, Dodoni G, Rodriguez-Sinovas A, Cabestrero A, Ruiz-Meana M, Gres P *et al*. (2005). Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. *Cardiovasc Res* **67**: 234–244.

Bruzzone R, Barbe MT, Jakob NJ, Monyer H. (2005). Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in Xenopus oocytes. *J Neurochem* **92**: 1033–1043.

Bruzzone R, Hormuzdi SG, Barbe MT, Herb A, Monyer H. (2003). Pannexins, a family of gap junction proteins expressed in brain. *Proc Natl Acad Sci USA* **100**: 13644–13649.

Chiarugi P, Giannoni E. (2008). Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* **76**: 1352–1364.

D'Abaco GM, Kaye AH. (2007). Integrin-linked kinase: a potential therapeutic target for the treatment of glioma. *J Clin Neurosci* **14**: 1041–1048.

Das SJ, Das S, Koval M. (2005). Regulation of connexin43 oligomerization is saturable. *Cell Commun Adhes* **12**: 237–247.

Hu J, Jiang CC, Ng HK, Pang JC, Tong CY, Chen SQ. (2004). A genome-wide allelotype study of primary and corresponding recurrent glioblastoma multiforme in one patient. *Chin Med J (Engl)* **117**: 456–458.

Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD. (2007). The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. *Proc Natl Acad Sci USA* **104**: 6436–6441.

Ino Y, Silver JS, Blazejewski L, Nishikawa R, Matsutani M, von Deimling A *et al*. (1999). Common regions of deletion on chromosome 22q12.3-q13.1 and 22q13.2 in human astrocytomas appear related to malignancy grade. *J Neuropathol Exp Neurol* **58**: 881–885.

Lai CP, Bechberger JF, Thompson RJ, MacVicar BA, Bruzzone R, Naus CC. (2007). Tumor-suppressive effects of pannexin 1 in C6 glioma cells. *Cancer Res* **67**: 1545–1554.

Laing JG, Tadros PN, Westphale EM, Beyer EC. (1997). Degradation of connexin43 gap junctions involves both the proteasome and the lysosome. *Exp Cell Res* **236**: 482–492.

Lee AS. (2005). The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. *Methods* **35**: 373–381.

Li H, Brodsky S, Kumari S, Valiunas V, Brink P, Kaide J *et al*. (2002). Paradoxical overexpression and translocation of connexin43 in homocysteine-treated endothelial cells. *Am J Physiol Heart Circ Physiol* **282**: H2124–H2133.

Litvin O, Tiunova A, Connell-Alberts Y, Panchin Y, Baranova A. (2006). What is hidden in the pannexin treasure trove: the sneak peek and the guesswork. *J Cell Mol Med* **10**: 613–634.

Locovei S, Bao L, Dahl G. (2006). Pannexin 1 in erythrocytes: function without a gap. *Proc Natl Acad Sci USA* **103**: 7655–7659.

Loewenstein WR. (1987). The cell-to-cell channel of gap junctions. *Cell* **48**: 725–726.

Mesnil M. (2002). Connexins and cancer. *Biol Cell* **94**: 493–500.

Naus CC. (2002). Gap junctions and tumour progression. *Can J Physiol Pharmacol* **80**: 136–141.

Naus CC, Bechberger JF, Caveney S, Wilson JX. (1991). Expression of gap junction genes in astrocytes and C6 glioma cells. *Neurosci Lett* **126**: 33–36.

Naus CC, Goldberg GS, Sin WC. (2005). Connexins in growth control and cancer. In: Winterhager E (ed). *Gap Junctions in Development and Disease*. Springer-Verlag: Heidelberg, pp. 253–273.

Oskam NT, Bijleveld EH, Hulsebos TJ. (2000). A region of common deletion in 22q13.3 in human glioma associated with astrocytoma progression. *Int J Cancer* **85**: 336–339.

Pelegrin P, Surprenant A. (2006). Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J* **25**: 5071–5082.

Pelegrin P, Surprenant A. (2007). Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. *J Biol Chem* **282**: 2386–2394.

Qin H, Shao Q, Igdoura SA, Alaoui-Jamali MA, Laird DW. (2003). Lysosomal and proteasomal degradation play distinct roles in the life cycle of Cx43 in gap junctional intercellular communication-deficient and -competent breast tumor cells. *J Biol Chem* **278**: 30005–30014.

Ray A, Zoidl G, Wahle P, Dermietzel R. (2006). Pannexin expression in the cerebellum. *Cerebellum* **5**: 189–192.

Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS. (2007). Afferent neurotransmission mediated by hemichannels in mammalian taste cells. *EMBO J* **26**: 657–667.

Schulz R, Boengler K, Totzeck A, Luo Y, Garcia-Dorado D, Heusch G. (2007). Connexin 43 in ischemic pre- and postconditioning. *Heart Fail Rev* **12**: 261–266.

Skerrett IM, Di WL, Kasperek EM, Kelsell DP, Nicholson BJ. (2004). Aberrant gating, but a normal expression pattern, underlies the recessive phenotype of the deafness mutant Connexin26M34T. *FASEB J* **18**: 860–862.

Thomas T, Jordan K, Simek J, Shao Q, Jedeszko C, Walton P *et al*. (2005). Mechanisms of Cx43 and Cx26 transport to the plasma membrane and gap junction regeneration. *J Cell Sci* **118**: 4451–4462.

Thompson RJ, Zhou N, MacVicar BA. (2006). Ischemia opens neuronal gap junction hemichannels. *Science* **312**: 924–927.

Trosko JE, Ruch RJ. (2002). Gap junctions as targets for cancer chemoprevention and chemotherapy. *Curr Drug Targets* **3**: 465–482.

Vogt A, Hormuzdi SG, Monyer H. (2005). Pannexin1 and Pannexin2 expression in the developing and mature rat brain. *Brain Res Mol Brain Res* **141**: 113–120.

Wang XH, Streeter M, Liu YP, Zhao HB. (2008). Identification and characterization of pannexin expression in the mammalian cochlea. *J Comp Neurol* **512**: 336–346.

Zappala A, Li VG, Serapide MF, Pellitteri R, Falchi M, La Delia F *et al*. (2007). Expression of pannexin2 protein in healthy and ischemized brain of adult rats. *Neuroscience* **148**: 653–667.

Zheng X, Shen G, Yang X, Liu W. (2007). Most C6 cells are cancer stem cells: evidence from clonal and population analyses. *Cancer Res* **67**: 3691–3697.

Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)