Complex role of connexin 43 in astrocytic tumors and possible promotion of glioma-associated epileptic discharge (Review)

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Received October 20, 2016; Accepted June 19, 2017

DOI: 10.3892/mmr.2017.7618

Abstract. Connexin (Cx)43 is a multifunction protein which forms gap junction channels and hemi-channels. It also contains abundant binding domains which possess the ability to interact with certain Cx43-associated proteins and therefore serve a fundamental role in various physiological and pathological functions. However, the understanding of the association between cancer and Cx43 along with Cx43-gap junctions (GJ) remains unclear. All available data illustrate that Cx43 and its associated GJ serve important functions in cancers. The expression levels of Cx43 demonstrate a downward trend and an increase in the levels of malignancy, particularly in astrocytomas. The GJ intercellular communication activity in glioma cells can be adjusted via Cx43 phosphorylation and through the combination of Cx43 and its associated protein. Available evidence reveals Cx43 as a tumor-inhibiting factor that suppresses glioma growth and proliferation. However, its mechanism is also regarded as complicated and ambiguous. Furthermore, it is apparent that Cx43-GJ and the carboxyl tail may contribute to glioma growth and proliferation too. However, this valuable role could be weakened by its effects on migration and invasiveness. The detailed mechanism remains unclear and full of controversies. Cx43 can enhance the motor ability and invasiveness of astrocytic glioma cells. It is also able to influence glioma cells to detach from the tumor core to the peritumoral neocortex. This peritumoral region has recently been regarded as the basic focus of glioma-associated seizure. Thus, Cx43 may take part in the onset and development of glioma-associated epileptic discharge. In addition, change and increase of Cx43 expression in GJs has been observed in seizure perilesional tissue, which is associated with brain tumors. Cx43 or GJ/hemi-channels exert enduring effects in the promotion of glioma-associated epileptic release through direct mass effects and change of the tumor microenvironment. However, there are still a number of issues concerning this aspect that require further exploration. Cx43, as a potential treatment target against this incurable disease and its common symptom of epilepsy, requires further investigation.

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Key words: connexin 43, glioma, proliferation, motility, invasiveness, glioma-associated epilepsy

Contents

1. Introduction

Glioma accounts for the majority of central nervous system (CNS) malignancies. They are difficult to cure and always present a poor prognosis. Histologically, glioma can be divided into four classes: Astrocytomas, oligodendrogliomas, ependymomas and mixed gliomas; diffuse gliomas are the most common. According to the 2007 World Health Organisation CNS tumor classification (1), CNS gliomas are diagnosed as grade II (diffuse astrocytoma, oligodendroglioma and oligoastrocytic tumors), grade III (anaplastic astrocytoma, oligodendroglioma and oligoastrocytic tumors) and grade IV (glioblastoma multiforme; GBM). The evidence is that high grade gliomas (III and IV) are the most common, and GBM occupies ~30% of CNS gliomas (2). Although the therapeutic strategies of glioma involve continuous improvement and adjustment, the prognosis remains unsatisfactory. The Chinese Glioma Cooperative Group statistics (3) give a median general survival time (OS) of GBM at only 14.4 months, along with 5-year OS rates at 9%. Therefore, identification of the pathogenic mechanism of glioma is important, and novel therapeutic strategies to reduce the high mortality rates of CNS malignancies are required.

In the past, researchers have concentrated on exploring the molecular mechanism of glioma, which led to the discovery of isocitrate dehydrogenase 1, telomerase reverse transcriptase and various other molecules. A CNS glioma molecular classification has been suggested (3-5). The present review aimed

to focus on a widely-studied molecule, connexin (Cx)43, which is largely expressed in astrocytes and which participates in the construction of gap junctions (GJs) of astrocytes or astrocytes and neurons (6). Cx43 is a multifunctional protein which not only constructs gap junction channels and hemi-channels (7), but also contains numerous binding domains which can interrelate with various Cx43 linked proteins, thus serving an elemental role in several physiological and pathological functions (8). Cx43 has been reported to be involved in the inception of certain neurodegenerative diseases, including Alzheimer's and Parkinson's disease (9), epilepsy secondary to focal cortical dysplasia (FCD) (10) and amyotrophic lateral sclerosis (11), among others. The role of Cx43 in glioma has also been widely and consistently explored.

The present review aimed at introducing the role of Cx43 in glioma from the following aspects: i) Expression of Cx43 and glioma grade; ii) inhibition of glioma proliferation, but improvement in invasion and migration; iii) consideration of Cx43 and the possibility of its promoting glioma-associated epileptic discharge; and iv) disease diagnosis and therapy.

2. Structure and function

Cx43 is encoded by the GJA1 gene and is strongly expressed in astrocytes. Cx43 is an elemental membrane protein, which contains three intracellular regions, two extracellular loops together with multiple trans-membrane domains. The intracellular region is composed of N- and C-terminal (CT) domains along with a loop that links the trans-membrane domains. Cx43CT comprises of amino acids 232-381, a plurality of binding-domains and phosphorylation sites (12). The present aimed to review the functions of Cx43 and specifically to its functions in constructing GJs. Astrocyte Cx43 gathers adjacent to the central pore and forms connexons. Subsequently, it is coupled with neighbouring astrocytes or neurons through apposing connexons to form GJ channels, which may directly exchange the cytoplasm between coupled astrocytes and permit swapping of ions together with certain small molecules. Astrocyte Cx43 may also form membrane hemi-channels, which are directly involved in material exchange between the extracellular milieu and astrocytes or neurons (13-15).

From its special structure and character (forming GJ channels and hemi-channels), Cx43 can therefore serve important physiological and pathological functions in CNS through these two routes.

GJ channels and hemi-channels. Cx43 is highly expressed in astrocytes, lasting until adulthood. In relation to neurons, the typical feature of astrocyte GJs is to support the astrocytes in couple formation. This involves ions, amino acids, metabolites and certain small molecules to exchange through the cytomembrane between astrocytes in addition to extracellular milieu (16). The principal roles of astrocyte GJs are described below.

Potassium spatial buffering. When neurons are in an energized state, a large number of K^+ ions efflux into the intercellular space. Aggregation of extracellular milieu K^+ activates the inwardly rectifying K^+ channel, and there is an excessive intake of K^+, rapidly dispersed to adjacent astrocytes or neurons through GJs. Ultimately, the K^+ homeostasis of this coupling is maintained and is considered to be beneficial in maintaining the normal microenvironment, in addition to the electrical activity of neurons (17).

Signal transduction. Mediated by Cx43, astrocytes form functional group coupling astrocytes networks which may contribute to long-range signal transduction. External stimulation can spread through astrocytes via calcium waves to participate in neuromodulation (18,19). Astrocytes also contain an adenylate cycle and phosphoinositide courier delivery system, which can transmit signals through second messengers, including cyclic adenosine monophosphate (20).

Nutritional support. Astrocytes take in glucose which can be delivered to neurons through the GJ to contribute to metabolic regulation of neurons (21). Additionally, through the GJs formed by Cx43 between astrocytes and neurons, these two cell types may directly achieve material exchange along with signal transduction (22).

Specific binding domains and phosphorylation sites. Cx43 is a structurally complex protein in the C-terminal domains. There are certain binding domains which can interrelate with paired molecules to contribute to the building and regulation of cell architecture, polarity, mobility, invasion and growth (8,12,23,24). According to the reviews put forward by Giepmans (8) and Tabernero *et al* (12), the interactions of Cx43 when closely associated with proteins are summarized in Table I.

3. Expression of Cx43 and glioma grade

In standard physiological states, Cx43 is prominently expressed in astrocytes. However, when the cell becomes malignant, the expression of Cx43 is downregulated. Thus, Sin *et al* (25) suggested that decreased Cx43 expression is accompanied by greater proliferation and malignancy of tumors. By studying the expression of Cx43 in human glioma and normal tissue microarray slides, mainly by western blot analysis and immunohistochemical staining, Sin *et al* (26) and Ye *et al* (27) noted a reduced expression of Cx43 in the tumor center as the glioma malignancy increased. Grade I and II primary astrocyte gliomas may express an enhanced immunoreactivity compared with normal brain tissue. However, it lacks the distinct disrupting staining of normal astrocytes. In high grade glioma, the expression of Cx43 is commonly reduced compared with normal tissues. It is also decreased in the majority of GBM, where the expression of Cx43 protein is insignificant (12,26,27). However, Crespin *et al* (28) did not share this point of view. First, in spite of the modest inverse association between tumor grade and Cx43 expression, over half of glioblastomas still express Cx43. Secondly, the expression of Cx43 between grade II and III astrocytes gliomas is not significantly different. Additionally, the various expression levels of Cx43 between grade III astrocytoma and oligodendroglioma suggest that Cx43 can act as a marker in discriminating against grade III oligodendroglioma in addition to astrocytoma. In reality, expression of Cx43 differs within the same tumor. For instance, Cx43 is rarely labelled at the membranes and in the cytoplasm of GBM cells. Nevertheless, it is abundant at the plasma membrane of reactive astrocytes

Table I. Cx43-interacting proteins.

Protein and phosphorylation sites	Amino acids	Function	Cx43 interaction
ZO-1	379-382	Tight junctions, adherens junctions, cytoskeleton build, signal transduction	Inversely regulates gap Junctional communication and Hemichannel activity, prevents cytoplasmic localization and malignization
Src	247,265, 274-283	Phosphorylates Cx43 oncogenic activity	Inhibits Cx43-based GJC, Tumor suppression, but excludes the C-terminal tail for ZO-1 binding
Tubulin	234-262	Combines into dimers, assembles microtubules	Modulates cell polarity, Motility and directional cell migration
Cadherins, catenin and actin		Adherens junctions, β-catenin modulates Wnt-mediated gene transcription	Modulate cell motility
CK1, PKA MAPK, PKG, PKC		Phosphorylates Cx43	Upregulate Cx43 assembly Inhibit Cx43-based GJC

Cx, connexin; ZO, zonula occludens; CK, creatine kinase; PK, protein kinase; MAPK, mitogen-activated protein kinase; GJC, gap junction communication; Src, proto-oncogene tyrosine-protein kinase.

in the surrounding tumor mass (26,28). The notable features of these areas are tumor cell infiltration and reactive astrocytes (26,29). The peritumor cortex not infiltrated by glioma cells may increase Cx43 immunoreactivity and reactive astrocytes. However, this appearance is perhaps associated with the existence of epileptic seizures (30). Besides, this conclusion may not be valid; the origin of glioma associated seizure stemming from the peritumor area and infiltration by glioma cells has been widely accepted (31). Therefore, the above conclusion may require further elucidation. In addition, due to the driving factor of glioma pathogenesis partly being ascribed to cancer stem cells (CSCs), Hitomi *et al* (32) explored the expression levels of Cx43 in GBM glioma stem cells (GSCs). The results indicated that Cx43 is predominantly expressed in non-GSCs while Cx46 is expressed in CSCs. Yu *et al* (33) further identified lower expression of Cxs and the loss of GJ-like structures together with dysfunction of GJ intracellular function in GSCs.

4. Cx43 and glioma proliferation, invasion and migration

Glial tumors, as the most common supratentorial neoplasms, are particularly difficult to cure. This is made more difficult with a poor prognosis, largely due to tumor cell migration, invasion and proliferation. This section briefly introduces the role of Cx43 in glioma migration, invasion and proliferation in addition to its possible mechanism. Previous studies (12,25,34,35) focused more on the association between Cx43 and cancer, including astrocytic glioma. However, more recent studies have proposed novel insights. The present review aimed to examine the association of Cx43 and astrocytic glioma in light of previous reviews and new findings.

Inhibition of glioma growth and proliferation. Thus far, the majority of studies have indicated that Cx43, as a tumor suppressor factor, inhibits astrocytoma growth and proliferation in a variety of ways. Treatments that regulate Cx43 expression, including tolbutamide (36,37), selective β2-AR agonist (38), 17-β estradiol (E2) (39), ciliary neurotrophic factor (40) and low doses of γ-radiation (41) have been verified. Cx43 may therefore inhibit glioma growth and proliferation (Table II).

The specific mechanism of how Cx43 influences glioma proliferation remains to be elucidated. However, the following mechanisms may contribute to the regulation of Cx43 in glioma proliferation (Table II).

Affecting the GSC phenotype. GSCs are cells which possess the capability for self-renewal and are considered among the driving factors in glioma pathogenesis. Notably, Cx43 is mostly expressed in non-GSCs, and the expression of Cx43 in GSCs is low (32,33). When reconstituting Cx43 in GSCs, the tumorigenicity of GSCs is inhibited, while self-renewal and proliferation are delayed. Yu *et al* (33) described the interaction of Cx43 with epithelial cadherin as having an influence on CSC phenotype through the Wnt/β-Catenin signaling pathway; this may be a potential mechanism. Additionally, as a proto-oncogene, Scr and its interaction with Cx43 are also considered to be involved in glioma proliferation regulation. Tabernero *et al* (12) hypothesized c- proto-oncogene tyrosine-protein kinase (Src) inhibiting Src activity as the main initiator of Cx43. It has an effect on GSCs: Gangoso *et al* (42) transfected Cx43 to GSCs and identified that Ki-67-positive glioma cells decreased and expressed Cx43, while downregulating DNA-binding protein inhibitor (a transcriptional regulator) expression via inhibition of Src activity. Consequently, there was reduced (sex determining region Y)-box (Sox2) expression, downregulation of Sox2 and a reduction in GSC self-renewal (Fig. 1).

Intervention in cell metabolism. Cancer cells detect rapid proliferation by adapting to metabolic environmental changes. For glioblastoma, uptake of enough glucose or the

Table II. Cx43 and the regulation of glioma proliferation and growth.

Author (date)	Model	Regulated proteins	Regulatory mechanism Regulation of Cx43	Effect on cell growth and proliferation	(Refs.)
Moinfar *et al* (2016)	17-β estradiol (E2) treated-C6 cell	Estradiol Receptors	Cx43↓	Proliferation↑	(39)
Ye *et al* (2015)	PKC, MAPK, and PTK inhibitors treated-U251 cell	PKC, MAPK, and PTK	Cx43↓ and p-Cx43↓	Proliferation↓	(27)
Mostafavi *et al* (2014)	Selective β2-AR agonist treated-1321N1 astrocytoma cells	β2-AR, cAMP-Epac	Cx43↑	Proliferation↓	(38)
Jin *et al* (2013)	miR-125b-transfected U87 and U251 glioma cells		Cx43↓	Proliferation↑	(64)
Ghosh *et al* (2014)	Low doses of γ-radiation treated-U87 cells	ERK-1/2, p38-MAPK activation	Cx43↑and GJ↑	Proliferation↓	(41)
Hao *et al* (2012)	AS-miR-221/222 transfect U251 cells		Cx43↑	Proliferation↓	(65)
Zhang *et al* (2010)	Ad-bFGF-siRNA transfect U251 cells		Cx43↑	Proliferation↓	(67)
Ozog *et al* (2002)	CNTFR-α treated C6 glioma cells	CNTFRα	Cx43↑and GJ↑	Proliferation and growth↓	(40)
Sanchez-Alvarez *et al* (2001)	Tolbutamide-treated C6	p21, p27, Rbp,	Cx43↑and GJ↑	Proliferation and growth↓	(36)
Sanchez-Alvarez (2006)	glioma cells				(37)
P.A. Robe *et al* (2000)	TGF-β1-treated C6 glioma cells		p-Cx43↓and GJ↓	Proliferation↑	(66)
Effect on the GSCs phenotype					
Shi-Cang Yu *et al* (2012)	Reconstitution of Cx43 GSCs	E-Cadherin	Wnt/b-Catenin Pathway	Proliferation↓	(33)
Gangoso *et al* (2014)	Reconstitution of Cx43 GSCs	c-Src, Sox2, Id1, cadherin	c-Src activity↓→Id1↓→ Sox2↑→ GSC self-renewal↓	Proliferation↓	(42)
Intervention in cell metabolism					
Herrero-Gonzalez *et al* (2009)	Transfection of Cx43-siRNA astrocytes	Endothelin-1	glucose uptake ↑	Proliferation↑	(43)
Gang Li *et al* (2015)	Sprague-Dawley rats	Cx43 and AQP4	Cx43↑→edema		(44)
Kolar *et al* (2015)	GL261 glioma cells and mouse	Cx43 and Podoplanin	Podoplanin→ischemia		(45)
Wei Zhang *et al* (2003)	T98G-Cx43 cells	Cx43, GJ, VEGF	VEGF high expression in T98G-Cx43 cells		(46)
Ruochun Huang (2002)	Cx43-transfected cells U251	Cx43, MCP-1	Cx43 downregulates MCP-1, then inhibits angiogenesis	Proliferation↓	(47)

Table II. Continued.

Author (date)	Model	Regulated proteins	Regulatory mechanism Regulation of Cx43	Effect on cell growth and proliferation	(Refs.)
Participation in cell cycle regulation					
Sanchez-Alvarez *et al* (2006)	Tolbutamide treated C6	p21, p27, Rbp,	p21, p27↑→Rbp↓→	Proliferation and growth ↓	(37)
Tabernero *et al* (2006)	glioma cells	E2F, cyclin E	E2F↓→cyclin E↓		(51)
Geng Y *et al* (1996)					(52)
Regulated growth factor and proliferation related protein					
Sin *et al* (2008)	Cx43 expression C6 cell	CCN1↑		Proliferation ↑	(53)
Sin *et al* (2008)		CCN3↑		Proliferation ↓	(53)
Fu *et al* (2004)					(54)
Bradshaw *et al* (1993)		Osteopontin↑		Proliferation ↓	(55)
Bradshaw *et al* (1993)		IGFBP4↑, IGF-1↓,			(56)
Goldberg *et al* (2000)		IGFP↓, bFGFK, PDGF↓		Proliferation ↓	(57)
Xia *et al* (2003)					(58)
González-Sánchez *et al* (2016)	Cx43 expression C6 cell	PTEN, Csk	c-Scr activity↓	Proliferation ↓	(59)
Ghosh *et al* (2014)	Knock-down of Cx43 expression U87 cell	p38, ERK-1/2	p38, ERK-1/2 activity↓	Proliferation ↑	(41)

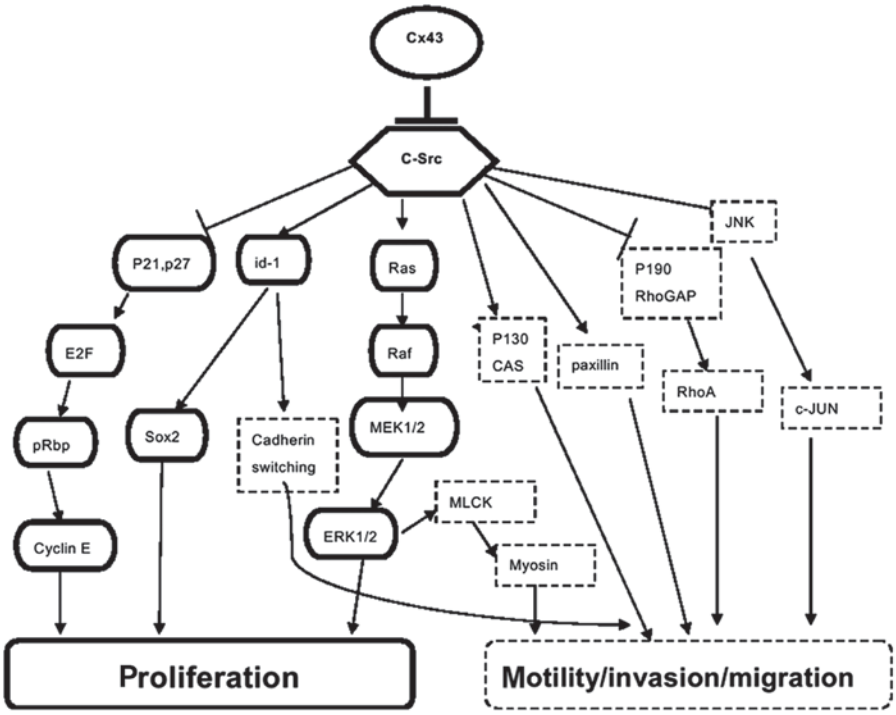

Figure 1. Cx43 interacts with c-Src and inhibits Src activity, sequentially modulating cell polarity, motility and invasion through several signaling pathways. Cx, connexin; c-Src, proto-oncogene tyrosine-protein kinase Src.

transformation of metabolism strategies to enable the cells to survive in a hypoxic tumor microenvironment is a key precondition for the growth and proliferation of GBM cells. Cx43 increases GJ channel and hemi-channel coupling, enabling the exchange of ions, amino acids, metabolites and certain small molecules through the cytomembrane and between astrocytes, together with the extracellular milieu. Notably, that inhibition of GJs or downregulation of Cx43 expression leads to an increase in glucose uptake (43). Accordingly, this is the main energy substance for glioma cells. Cx43 and its associated GJs are capable of influencing the peritumoral microenvironment of edema (44), ischemia (45) and angiogenesis (46,47), and of interfering with glioma cell metabolism. This may further influence glioma growth and proliferation. Equally, deletion of Cx43 in astrocytes has been observed to inhibit oligodendrocyte precursor cell proliferation by reducing matrix glucose levels (48).

Participation in cell cycle regulation. Cx43 may deter the cell cycle from G1 to S-phase or M-phase (49,50). It is also capable of rebuilding Cx43 in glioma cells which could delay the progression of cells from G0/G1 to S-phase (37). Cx43 has been observed to increase the expression of p21 and p27, and then weaken retinoblastoma phosphorylation (pRb) (37,51). pRb phosphorylation promotes the release of E2 transcription factor, which is associated with the expression of cyclin E (52). Cx43 possibly regulates the glioma cell cycle by decreasing pRb phosphorylation, subsequently inhibiting cyclin E expression.

Regulation of growth factor and proliferation-associated proteins. Several growth factors (GFs) may also affect the growth and proliferation of cells. Previous research indicates that Cx43 can regulate certain GF expression levels. For instance, Cx43-transfected glioblastoma cells (U251) downregulate the expression of MCP-1, a factor that can further promote angiogenesis, and then suppress glioma cell proliferation (47). Restored Cx43 expression in C6 glioma cells was also determined as being able to upregulate secretory proteins cysteine-rich angiogenic inducer (CCN)1 together with CCN3 expression (53). Notably, over-expression of CCN3 and its interaction with Cx43 are conducive to a decrease in glioma growth rate. Overexpression of CCN1 exhibits an opposite function (53,54). Similarly, Cx43 transfected to C6 rat glioma cells also regulates the expression of secreted proteins. For instance, it decreases insulin-like growth factor protein, basic fibroblast growth factor, platelet-derived growth factor, insulin-like growth factor 1 and N-methylpurine DNA glycosylase-E8 protein expression levels while increasing CCN3, insulin-like growth factor-binding protein 4 and osteopontin levels (55-58); this is the common outcome of suppressed glioma cell proliferation. Additionally, Cx43 adjusts certain kinase activities to affect the growth and proliferation of cells. For instance, Cx43 recruits phosphatase and tensin homolog and C-terminal Src kinase to inhibit c-Src activity (59). Additionally, c-Src equally combines with the C-terminal of Cx43 to reduce the oncogenic activity of c-Src (12,42,60). Cx43 can also modify the activity of other proliferation-associated proteins, including p38, extracellular signal-regulated kinases-1/2 (44) and zonula occludens (ZO)-1 (12), to affect the proliferation of glioma cells. Suzhi *et al* (61) noted that Cx43 could transfer microRNA (miR)-124-3p between coupling cells and improve the antiproliferative ability of miR-124-3p.

Regulation of gene expression. Cx43 regulates gene expression, perhaps as a potential mechanism that influences glioma cell proliferation. However, there are not enough relevant studies

to support this hypothesis. Dang *et al* (62) identified that the carboxyl-tail of Cx43 localizes to the nucleus and inhibits cell growth. Mennecier *et al* (63) also noted that Cx43 may enter into the nucleus of glioma cell lines. Thus, it may be hypothesized that Cx43 regulates gene expression directly or indirectly to affect the proliferation and growth of glioma cells.

Controversy of the effect of Cx43 on glioma cell invasion and migration. From the above discussion, it can be deduced that Cx43 is a tumor-suppression factor. However, this valuable role can be weakened by its effects on migration and invasiveness. The majority of the literature reports that Cx43 enhances glioma invasion (24,26,68,69), while certain studies report the inhibitory action of Cx43 in glioma invasion and migration (65,70,71). Although Cx43 is present in a lower expression state in a malignant glioma mass, a high expression of Cx43 is detected at the plasma membrane of the reactive astrocytes around the peritumor area (26,28), and in tumor cell infiltration and reactive astrocytes. In this area, malignant glioma cells form functional GJ communication between themselves and astrocytes (28,72), establishing a tight cell network (71). This may be the structural basis of the effect of Cx43 effect the invasion and migration of malignant glioma cells by GJ-dependent and independent mechanisms.

GJ-dependent mechanisms. Reduction of GJ activity has been reported to improve cell migration (71,73). However, more studies report that the overexpression of Cx43 encourages glioma cell migration and invasion in a GJ channel-dependent manner (69,72,74). Aftab *et al* (71) demonstrated that down-regulation of Cx43 expression in the U118 human glioma cell line is a way to increase migration by reducing cell-extracellular matrix adhesion, and change the migration pattern from collective to single cell. It was also demonstrated that Cx43-GJ serves more prominent roles in mediating migration and invasion behaviors compared with the C-terminal tail interaction. Functional GJ coupling also contributes to long-range signal transduction, and adjusts the formation of calcium waves (18,19,41,75). Additionally, it transmits signals through second messengers (20). Through these ways, Cx43-GJ may promote the transformation of malignant astrocytes by regulating a glioma-associated signaling pathway. Furthermore, Cx43 located in lipid raft microdomains can also regulate homocellular and heterocellular GJ communications between cancer and stroma cells, and can control the tumor phenotype (68,69). Consequently, such actions may influence glioma invasion behaviors. Additionally, a Cx43-constructed glioma-astrocyte GJ can modulate glioma invasive behavior by direct transfer of miRs (72). Cx43-GJ is involved in tumor microtube-mediated cell-to-cell communication and influences the motility of glioma cells (75).

GJ-independent mechanisms. Cx43 promotes glioma cell invasion through GJ-reliant mechanisms, which are not always recognized. Sin *et al* (26) suggested that astrocytic Cx43 may aid glioma cells to detach from the glioma core. However, Cx43 may mediate glioma invasion solely in a GJ-independent manner since the expression of Cx43-T154A has demonstrated no effect on glioma invasion (26). This conclusion contradicts the previously discussed findings in the present review. Certain Cx43-associated proteins merge with Cx43 extracellular loops or C-terminal regions to improve adhesive connections or to regulate cytoskeletal dynamics, which alter the structure of Cx43 to facilitate malignant glioma cell invasion and migration by independent mechanisms. A wound healing motility assay indicated that the C-terminal of Cx43 is required for Cx43-mediated C6 glioma cell motility (24). For instance, Cx43 interacts with ZO-1 protein, which could prevent the cytoplasmic localization and lead to glioma cell invasion (12,76). Cx43 interacts with other cytoskeleton proteins and tight junctions or adherens junctions associated with proteins, including tubulin, cadherins, catenin and actin, to modulate polarity, motility and directional migration of cells (12,24,77,78). In addition, Cx43 interacts with c-Src and inhibits Src activity, sequentially modulating cell polarity, motility and invasion through several signaling pathways (Fig. 1) (12,79,80). In brief, there is no consensus on the effect of Cx43 on glioma invasion and migration, and the detailed mechanism remains unclear.

5. Cx43 may promote glioma-associated epileptic discharge

The association between Cx43/-GJ and epilepsy has widely been studied in the last 20 years. Earlier studies failed to consider that Cx43 is associated with epilepsy, since expression of Cx43 had not identified significant differences between epileptogenic and nonepileptogenic tissues, in living tissue assays (81) and animal models (82). Nevertheless, the majority of studies have identified Cx43 as capable of participating in the genesis and development of certain types of epilepsy. For instance, Cx43 is increasingly expressed in the hippocampus tissue of patients with refractory temporal lobe epilepsy (83,84) and in FCD type IIB (10). Cx43/-GJ were also altered in either lithium pilocarpine-induced epilepsy (84) or in kainic-acid-induced status epilepticus models (85). Notably, the inhibition of the Cx43 GJ with carbenoxolone can shorten the duration of seizures and reduce the amplitude of the seizure discharges (86). In view of the above, Cx43 may be to be associated with the genesis and development of certain types of epilepsy, in addition to glioma-associated epilepsy.

Glioma-associated epilepsy may be defined as seizure which directly arises from the existence of supratentorial glioma. It is the presenting feature in ≤87% of low-grade gliomas and ≤50% of gliomas overall (87). Epilepsy is usually the initial symptom of glioma patients and a significant factor affecting their post-operative quality of life (88). However, the detailed mechanism of glioma-associated epilepsy remains to be elucidated. It may be a combination of direct mass effects and the change of the tumor microenvironment.

Overall, Cx43 is highly expressed in peritumoral astrocytes (29) which facilitate glioma cells detachment from the tumor core (26). Glioma cells invade the neocortex structure, a special peritumoral region where single neurons are bounded by very few or a single tumor cell (89). This peritumoral region has recently been considered as the basic focus of glioma-associated seizure (31,90,91). GJ changes (89) and the increase of Cx43 expression (30) have been identified in the perilesional tissue of seizures associated with brain tumors. GJ or Cx43-glial coupling may explain glioma-induced epileptogenesis (92). Cx43 and its associated GJ are capable of influencing the peritumoral microenvironment, including edema (44),

ischemia (45) and angiogenesis (46,47) which may induce epileptic discharge through direct effects of mass. Cx43-GJ is involved in the generation of sharp wave-ripple (34). It propagates neuronal activity through long-range signal transduction and Ca^{+} waves, then promotes a synchronized discharge of neurons (93). Cx43-GJ can also influence seizure discharge by regulating K^{+} redistribution and neuronal energy supply (94). Peritumoral reactive astrocytes can highly express Cx43. Cx43, in this context, may serve a predominant role in the regulation of neurotransmitters, including glutamate. First, Cx43-hem channels/GJ in astrocytes can control glutamate (95,96), release ATP (96) and sustain glutamatergic synaptic efficacy (97). Second, Cx43 knockdown may raise cortical glutamate transporter (GLT)-1 in addition to glutamate aspartate transporter (GLAST) protein expression levels, and control transcription and translation of glial glutamate transporters excitatory amino acid transporter (EAAT)-1 and EAAT-2 (98). Similarly, blocking the gap junction has been reported to suppress transcriptional activity of GLT-1 promoter, but increase GLAST gene transcription (99). The spinal astrocytic Cx43 has also been reported to be capable of activating N-methyl D-aspartate receptors (100) and elemental ionotropic glutamate receptors in the postsynaptic membrane (101). In essence, peritumoral Cx43 high immunoreactivity is mainly on the reactive astrocytes (29), and demonstrates Cx43 to be potentially associated with astrocyte reactivity. A recent study observed that reactive astrocytes not only limit glutamate uptake, but also inhibit the production of gamma-aminobutyric acid. Furthermore, this leads to a loss of inhibition and an increase in neuronal excitability (102). Even so, it can be hypothesized that Cx43 can possibly promote glioma-associated epileptic discharge through these aforementioned ways. The relevant studies remain scarce, and further studies are required to identify the exact mechanism of Cx43 in glioma-associated epilepsy.

In summary, the special microenvironment of glioma (tumor cell infiltration and high expression of Cx43 in reactive astrocytes) may identify why glioma patients present with epilepsy and why they possess a favorable prognosis but are prone to relapses (101). Cx43 also presents in temozolomide resistance and resistance to radiotherapy in glioblastoma cells. The peritumoral region has been considered the basic focus of glioma-associated seizure (31). It is hypothesized that early stage glioma cells highly express Cx43, and migrate and invade the host parenchyma with a low proliferation index (26).

6. Facilitating disease diagnosis and therapy

Investigating biomolecules is useful facilitate disease diagnosis and therapy. The value of Cx43 in glioma diagnosis and therapy is beginning to be recognized. Abakumova (103) demonstrated that the Cx43-targeted T1 contrast agent may efficiently visualize glioma C6 and its borders *in vitro* and *in vivo*. MAbE2Cx43 s was covalently associated with the Phthalosens derivative photosensitizer delivery of fluorescent agents to the glioma tissue. This may be valuable in demonstrating the optimal border and increase the extent of resection due to improved visualization of the glioma (104). Similarly, Cx43/-GJ may help brain tumor cells to interconnect a functional and resistant network (75), which confer temozolomide resistance (105-108) and radiotherapy resistance (75) in glioblastoma cells. The Cx43-antibody MAbE2Cx43 has been demonstrated to be potentially part of a combined therapy for poorly differentiated gliomas (108).

7. Conclusion

In regular physiological conditions, Cx43 is highly expressed in astrocytes. However, this expression is restrained when the malignant transformation of astrocytes and the levels of Cx43 are reduced, along with an increase in the degrees of malignancy in astrocytomas. The association between the expression of Cx43 and degrees of glioma implicates Cx43 as a tumor suppressor, inhibiting glioma cell proliferation. However, the majority of data have indicated that Cx43 may enhance the motor ability and invasiveness of astrocytic glioma cells, and to facilitate glioma cell separation from the tumor core to the surroundings. This can be interpreted as Cx43 in the early stages of glioma progression, with a relatively low proliferative index of glioma cells, predisposing glioma cells to migrate and integrate with the host parenchyma (26). Simultaneously, reactive astrocytes and the tumor cell invade into peritumoral tissue comprising the significant surrounding microenvironment, making an ideal environment for epileptic discharge. It is undoubtable that Cx43 or GJ /hemi-channels have a contribution in promoting glioma-associated epileptic discharge through direct mass effects and the change of tumor microenvironment, particularly the effect in excitatory neurotransmitter-glutamate regulation. Notably, Cx43 expression is considerably upregulated in astrocytes reactive due to tissue damage during surgery. This could promote tumor proliferation, in addition to migration (109), and then facilitate glioma recurrence following resection (110). This can partially explain post-operative epileptic seizures in glioma patients and those with no epilepsy prior to surgery.

Previously published reviews (12,25) have presented the roles of Cx43 in glioma proliferation in two mechanisms: GJ-dependent and GJ-independent. To the best of our knowledge, the present review was the first to introduce the exact mechanism of these functions and the roles of Cx43 in glioma-associated epilepsy. Certainly, there are still a number of challenges that require further exploration. If Cx43 is associated with the prognosis of glioma patients, then its potential as a treatment target requires further study. Peritoneal tissue which highly express Cx43 is involved in the incidence of glioma and is associated with epilepsy; thus, should be explored further.

In conclusion, the roles of Cx43 in glioma proliferation, in the present review, can be directed to the association between glioma and epilepsy. A number of identifiable challenges in this current review can be the subject of further studies. More importantly, future studies could also aid understanding of any other ways through which Cx43 and other expressions are associated with incidences of glioma and with epilepsy.

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