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REVIEW

THE ROLE OF CONNEXIN43–SRC INTERACTION IN ASTROCYTOMAS: A MOLECULAR PUZZLE

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Abstract—Connexin43 (Cx43) as a building block of gap junction channels and hemichannels exerts important functions in astrocytes. When these cells acquire a malignant phenotype Cx43 protein but not mRNA levels are downregulated, being negligible in high-grade astrocytoma or glioblastoma multiforme, the *most common* and deadliest of *malignant* primary *brain tumors in adults*. Some microRNAs associated to glioma target Cx43 and could explain the lack of correlation between mRNA and protein levels of Cx43 found in some high-grade astrocytomas. More importantly, these microRNAs could be a promising therapeutic target. A great number of studies have confirmed the relationship between cancer and connexins that was proposed by Loewenstein more than 40 years ago, but these studies have also revealed that this is a very complex relationship. Indeed, restoring Cx43 to glioma cells reduces their rate of proliferation and their tumorigenicity but this tumor suppressor effect could be counterbalanced by its effects on invasiveness, adhesion and migration. The mechanisms underlying these effects suggest the participation of a great variety of proteins that bind to different regions of Cx43. The present review focuses on an intrinsically disordered region of the C-terminal domain of Cx43 in which converges the interaction of several proteins, including the proto-oncogene Src. We summarize data that indicate that Cx43–Src interaction inhibits the oncogenic activity of Src and promotes a conformational change in the structure of Cx43 that allosterically modifies the binding to other important signaling proteins. As a consequence, crucial cell functions, such as proliferation or migration, could be strongly affected. We propose that the knowledge of the structural basis of the antitumorigenic effect of Cx43 on astrocytomas could help to design new therapies against this incurable disease.

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Key words: connexin, Src, glioma, proliferation, glucose uptake, migration.

Contents

INTRODUCTION

The term 'glioma' comprises the majority of malignancies of the central nervous system and encompasses all tumors that are thought to be of glial cell origin. These include astrocytomas, oligodendrogliomas, ependymomas and mixed gliomas. Among them astrocytomas are the most frequent in adults and have been traditionally classified by the World Health Organization (WHO) into four histological grades (Louis et al., 2007). Grade I (pilocytic astrocytoma) and grade II (diffuse astrocytoma) are low-grade gliomas that usually grow slowly. Grade III (anaplastic astrocytoma) is a highly malignant glioma with increased cellularity, pleomorphism and atypical nuclei. Grade IV

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Abbreviations: Cx43, connexin43; Cx43CT, C-terminal domain of connexin43; GLUT-3, glucose transporter-3; GSC, glioma stem cells; HIF, hypoxia-inducible factor; HK2, type II hexokinase; IDP, intrinsically disordered protein; IDR, intrinsically disordered region; MAPK, mitogen-activated protein kinases; miRNA, microRNA; MTOC, microtubule organizing center; PDZ, postsynaptic density 95/disk-large/zona occludens; pRb, retinoblastoma protein; PTEN, phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase; PTPμ, protein tyrosine phosphatase μ; SH3, Src homology 3; TC-PTP, T-cell protein tyrosine phosphatase; WHO, World Health Organization; ZO-1, Zonula occludens-1.

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(glioblastoma multiforme) consists of poorly differentiated cells with microvascular proliferation and pseudopalisading necrosis. More recently, a gene expression-based molecular classification of glioblastoma into Proneural, Neural, Classical, and Mesenchymal subtypes has been proposed (Verhaak et al., 2010). Unfortunately, high-grade gliomas (WHO grade III and IV tumors) are the most common type of glioma in adults (Ostrom et al., 2014). Glioblastomas are rapidly progressive, very aggressive, diffusely infiltrate the adjacent brain tissue and are one of the most incurable forms of cancer in humans. Despite significant advances in diagnostics and therapeutics over the past decades, prognosis for patients with glioblastoma remains dismal, with a median survival of 16–19 months (Stupp et al., 2009), which indicates that a great research effort is required to propose new therapeutic strategies against this devastating disease.

A wide range of molecular alterations has been described in astrocytomas and includes genetic, epigenetic, transcriptomic, and microRNA (miRNA) changes (Purow and Schiff, 2009; Riemenschneider et al., 2010). In this review, we will focus on the alterations found in connexin43 (Cx43), an integral membrane protein encoded by GJA1 gene. Cx43 is the most abundant connexin in mammals, widely expressed in different tissues, including the central nervous system, where Cx43 is strongly expressed in astrocytes (Giaume et al., 1991). Cx43 assembles to form gap junction channels and hemichannels (Fig. 1A) that facilitate the behavior of astrocytes as cellular networks (Giaume et al., 2010) and the interchange of molecules between the cells and their extracellular medium (Bennett et al., 2003). Thus, Cx43 is highly expressed in astrocytes but this expression is downregulated when these cells acquire a malignant phenotype. It is well described that the levels of Cx43 protein inversely correlate with the degree of malignancy in astrocytomas. In fact, the levels of Cx43 protein in the vast majority of glioblastomas are negligible (Shinoura et al., 1996; Huang et al., 1999; Soroceanu et al., 2001; Pu et al., 2004; Caltabiano et al., 2010; Sin et al., 2012; Gielen et al., 2013).

Interestingly, restoring Cx43 to glioma cells reduces their rate of proliferation (Zhu et al., 1991; Huang et al., 1998) and their tumorigenicity (Yu et al., 2012). Although there are still many open questions to understand the mechanism by which Cx43 controls proliferation, most of the studies pinpoint the C-terminal domain of Cx43 (Cx43CT) responsible for the antiproliferative effect (Moorby and Patel, 2001; Zhang et al., 2003b). As recently reviewed (Naus and Laird, 2010; Sin et al., 2012), this tumor suppressor effect could be counterbalanced by its effects on invasiveness (Zhang et al., 2003a), adhesion (Elias et al., 2007) and migration (Matsuuchi and Naus, 2013). Several interesting reviews about the link of connexins with cancer, including astrocytomas have appeared in recent years (Mesnil et al., 2005; Vinken et al., 2006; Naus and Laird, 2010; Sin et al., 2012). Collectively, these studies have confirmed the relationship between cancer and connexins that was proposed by Loewenstein more than 40 years ago (Loewenstein and Kanno, 1966), but they have also revealed that this is a very complex relationship in which a great variety of molecular partners are participating. Therefore, the knowledge of the structural basis of the antitumorigenic effect of Cx43 on astrocytomas is necessary to design new therapies against this devastating disease. The present review focuses on a specific region of the Cx43CT in which converges the interaction of several partners, including the proto-oncogene Src. We summarize data that indicate that Cx43–Src interaction inhibits the oncogenic activity of Src and promotes a conformational change in the structure of Cx43 that allosterically modifies the binding to other important signaling proteins. As a consequence, crucial cell functions, such as proliferation or migration, could be strongly affected.

CX43 PROTEIN BUT NOT MRNA IS DOWNREGULATED IN HIGH-GRADE ASTROCYTOMAS

Importantly, in some of high-grade astrocytomas the levels of mRNA do not mirror with those of the protein (Caltabiano et al., 2010; Sin et al., 2012; Gielen et al., 2013). Thus, Caltabiano et al. analyzed 32 astrocyte tumors, and they found that 90% of the high-grade astrocytomas (7/7 grade III, 10/13 grade IV) demonstrated an intracytoplasmic positivity for Cx43 mRNA, despite the fact that 80% of the high-grade astrocyte tumors (5/7 grade III and 11/13 grade IV) demonstrated a marked reduction or negativity for Cx43 immunostaining labeling. Gielen et al. analyzed the glioma repository of The Cancer Genome Atlas (TCGA) to examine the DNA copy number and mRNA expression profile of Cx43/GJA1 in 372 grade III and IV astrocytomas. They found that Cx43/GJA1 has a tendency to be deleted in glioblastoma (11.3%) and that while 27.3% of glioblastoma exhibits a twofold downregulation of Cx43 mRNA, 20.3% of GBM show a twofold upregulation of Cx43 mRNA, compared to normal tissues. These authors performed a screen of a brain tumor tissue array and confirmed minimal Cx43 protein levels in glioblastomas.

Together, these data suggest that Cx43 mRNA levels in gliomas do not predict the levels of the protein. Consequently, the reduced levels of Cx43 protein found in high-grade glioma are not mainly due to a reduced genetic transcription, but to alterations in post-transcriptional mechanisms (Klotz, 2012). The inhibition of translation and/or the enhanced degradation of the protein could be the primary mechanism for Cx43 protein downregulation, as described for other key regulators of proliferation such as p27 (kip1), which is frequently decreased maintaining mRNA levels unchanged in many human cancers, including glioblastomas (Blain et al., 2003; Gillies and Lorimer, 2007).

Some miRNAs involved in gliomagenesis target Cx43

One of the cellular mechanisms responsible for post-transcriptional regulation is the synthesis of miRNAs, which are endogenous small noncoding transcripts of 21–23 nucleotides that downregulate gene expression by conducting mRNA degradation or by inhibiting protein

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