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Tumour Microtubes and Therapy Resistance in Gliomas Molecular Mechanisms and Therapeutic Opportunities

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Abstract: Gliomas are highly infiltrative primary brain tumours characterised by poor prognosis and frequent recurrence due to therapy resistance. Conventional treatments, including surgery, radiotherapy, and chemotherapy, are often ineffective in achieving long-term control, particularly in glioblastoma, the most aggressive subtype. Recent discoveries have revealed that glioma cells form extensive networks of actin- and myosin-rich membrane structures, termed tumour microtubes (TMs), which enable long-range intercellular communication, calcium wave propagation, and metabolic exchange via connexin 43 (Cx43) gap junctions. These networks facilitate tumour cell survival by supporting self-repair, maintaining calcium homeostasis, and conferring resistance to radiotherapy and temozolomide chemotherapy. GAP-43, a neuronal growth-associated protein, has been identified as a key driver of TM formation, linking glioma biology to neural developmental pathways. Targeting TM networks or Cx43-mediated signalling—through monoclonal antibodies, gap junction inhibitors, or peptide disruptors—has shown promise in preclinical models, particularly when combined with PI3K pathway inhibition to overcome temozolomide resistance. This review synthesises current molecular insights into TM biology, highlights their contribution to glioma therapy resistance, and discusses emerging strategies to translate these findings into effective, tumour-selective therapeutic approaches.

Keywords: glioma; astrocytoma; glioblastoma; tumour microtube; connexin 43; GAP-43; calcium wave; therapy resistance; radiotherapy; chemotherapy; intercellular communication; monoclonal antibody; PI3K pathway; temozolomide resistance

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1. Introduction

Gliomas, which include astrocytomas, oligodendrogliomas, and glioblastomas, represent a diverse group of malignant brain tumours arising from glial cells within the central nervous system. Their diffuse infiltration along blood vessels and axonal tracts renders complete surgical resection virtually impossible, necessitating reliance on radiotherapy and chemotherapy for disease control. Despite multimodal treatment, gliomas—especially glioblastomas—remain highly lethal, with median survival often measured in months. A major barrier to improved outcomes is the ability of glioma cells to resist therapeutic interventions and adapt to cellular stress, enabling recurrence even after initial tumour debulking and treatment.

The molecular and cellular mechanisms underlying this resistance have been a subject of intense investigation. While early research focused on genetic drivers, such as IDH1/2 mutations and 1p/19q chromosomal codeletion, more recent work has uncovered the critical role of intercellular communication in sustaining tumour survival. In 2015, Osswald et al. described a previously unrecognised form of tumour connectivity: long, stable, actin- and myosin-rich protrusions

termed tumour microtubes(TMs). These structures form extensive multicellular networks that allow glioma cells to exchange calcium signals, organelles, and metabolic resources, and to rapidly repair damage via nuclear transfer. TMs are particularly abundant in high-grade astrocytomas lacking 1p/19q codeletion, correlating with poor prognosis and pronounced therapy resistance.

This review examines the discovery, structure, and function of TMs in gliomas, with a focus on their molecular regulation by GAP-43 and functional integration via Cx43 gap junctions. It also explores how TMs contribute to radiotherapy and temozolomide resistance, and evaluates emerging therapeutic strategies aimed at disrupting TM networks or targeting Cx43-mediated signalling, with the goal of improving clinical outcomes for patients with these devastating tumours.

2. Therapy Resistance in Gliomas: Challenges and Molecular Insights

Gliomas, including astrocytoma and oligodendrogliomas, are malignant brain tumours that arise from glial cells in brain and spinal cord[1]. These tumours exhibit highly infiltrative growth patterns, spreading along blood vessels and nerve tracts, making complete surgical removal nearly impossible[2]. Consequently, glioma treatment relies on a combination of radiotherapy and chemotherapy. However, gliomas frequently develop therapy resistance, leading to recurrence and poor patient prognosis[3,4,5].

A major challenge in glioma treatment is understanding the molecular mechanisms underlying therapy resistance. Glioma cells use intercellular gap junction communication[6], but the precise mechanisms remained unclear. Osswald et al., (2015) aimed to investigate this process and its role in therapy resistance[7].

Gliomas are classified based on molecular markers, particularly IDH1/2 (Isocitrate Dehydrogenase) mutations and of 1p/19q chromosomal codeletion[8]. Oligodendrogliomas with 1p/19q codeletion respond better to treatment, whereas astrocytomas without this codeletion exhibit higher resistance[2]. Glioblastomas, the most aggressive gliomas, are IDH wildtype and highly treatment resistant[4,9]. The reasons behind this variation in therapy sensitivity remain a major research focus.

3. Membrane Extensions and Discovery of Tumour Microtubes

Membrane-bound intercellular connections have been identified in biological systems. In Drosophila, actin-rich protrusions called cytonemes facilitate long-range intercellular signalling[10]. In mammalian cells, tunnelling nanotubes (TNTs), actin-based projections enabling intercellular transfer of signals and organelles, were discovered by Rustom et al., (2004)[11]. This revealed a novel mode of cellular commination, expanding understanding beyond previously recognised mechanisms involving direct contact and signalling molecule. These raised the possibility that glioma cells might use similar structures for intercellular communication and therapy resistance.

Osswald et al., (2015) hypothesised that astrocytoma cells form long membrane extensions to establish intercellular networks[7]. They monitored tumour growth through a cranial window implanted in mice skulls and by using multiphoton laser-scanning microscopy (MPLSM), they observed that glioma cells extended long, thin protrusions containing actin and myosin into brain tissues. Further analysis using three-dimensional scanning electron microscopy (3D SEM) revealed that these extensions contained mitochondria, suggesting ATP production and vesicle trafficking. Unlike previously identified TNTs, these glioma-specific extensions were longer, thicker, and more stable, and followed axonal tracts for glioma cell invasion, leading to their designation as tumour microtubes (TMs)[7].

4. TMs in Human Astrocytomas

To determine if TMs exist in human gliomas, Osswald et al., (2015) analysed resected human glioma samples and

stained them with IDH1R132H mutation-specific antibodies to carry out immunohistochemistry analysis[7]. With precise identification of tumour-derived membrane extensions within the dense brain tissue, they found 63% of 1p/19q non-codeleted astrocytoma cells with intercellular TMs, whereas 0.7% codeleted oligodendroglioma cells exhibited similar structures (**Figure 1**). Further analysis revealed that TM formation varied by tumour type and grade, with longer TMs correlating with poorer prognosis[7]. These findings established TMs as defining features of gliomas.

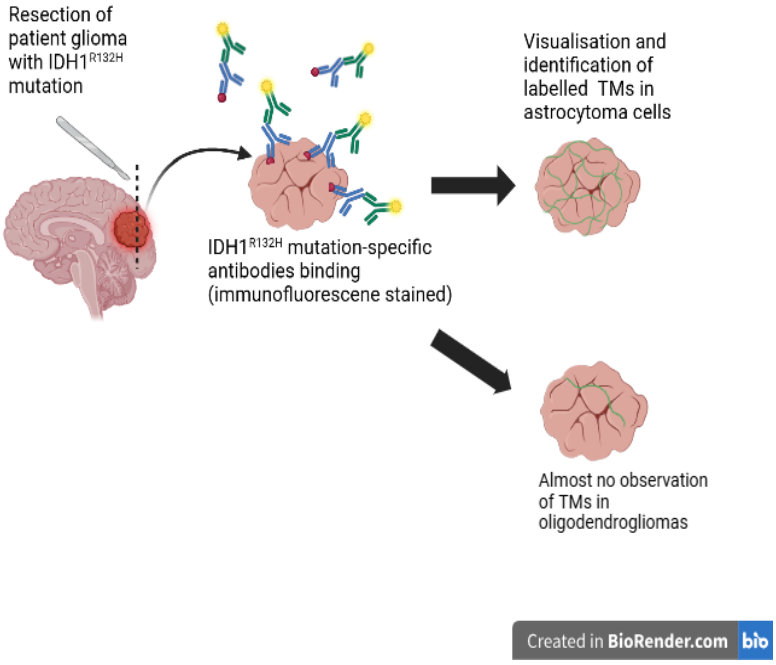

Figure 1. Identification of Tumour Microtubes (TMs) in Human Gliomas using IDH1 R132H Mutation-Specific Antibodies.

Patient glioma samples with IDH1 mutations were resected and analysed using immunohistochemistry (IHC) and immunofluorescence staining (green). IDH1 mutation specific antibodies were used to selectively bind glioma cells, enabling the visualization of TMs within brain tissue. TMs were identified in astrocytoma cells, but not in oligodendrogliomas. Labelled TMs were then analysed, providing evidence for their presence in human gliomas.

Own work, created in Biorender.com. Information from (Osswald et al., 2015)[7].

5. TM-mediated communication and the role of Connexin 43 (Cx43)

Given that intercellular calcium waves (ICWs) play a crucial role in astrocyte and neuronal communication[12], Osswald et al., (2015) hypothesised that TMs facilitate similar ICW propagation in gliomas[7]. Time-lapse imaging confirmed calcium waves travelled along TMs in glioblastoma stem-like cells (GBMSCs), indicating bidirectional communication along TMs. Heat maps showed TM-connected tumour cells exhibited synchronized calcium transients, but not in TM-unconnected cells. TM intersections with simultaneous calcium peaks act as relay sites, forming a tumour-wide communication network[7].

To investigate whether gap junctions mediate ICWs along TMs, Osswald et al., 2015 applied gap junction inhibitor carbenoxolone to GBMSCs and normal astrocytes, which significantly reduced ICW frequency in GBMSCs, but had minimal effect on normal astrocytes[7]. A dye transfer assay confirmed selective dye spread between TM-connected tumour cells, which was abolished upon gap junction blockage[7].

Analysis of glioma transcriptomic data from The Cancer Genome Atlas (TCFA) and confirmation by confocal microscopy revealed that connexin 43 (Cx43), a key protein forming gap junctions, was the only connexin highly

expressed at TM junctions in 1p/19q non-codeleted gliomas. 3D SEM imaging showed direct membrane contact at these junctions with ICW propagation. Knocking down Cx43 in GBMSCs using short hairpin RNA (shRNA) disrupted ICW propagation, reduced TM connectivity, and decreased tumour size in mouse models. Mice with Cx43-deficient tumours exhibited increased survival, suggesting that Cx43 is essential for TM network stability and glioma progression[7].

6. TM networks enable self-repair and radiation resistance

To test whether TM networks contribute to glioma survival, Osswald et al., (2015) selectively ablated single TM-connected GBMSCs using laser irradiation[7]. Following cell death, neighbouring tumour cells extended new TMs toward the damaged site, with a nucleus travelling through TMs to replace lost cells (**Figure 2**). Similarly, when multiple tumour cells were ablated, TM-connected cells rapidly repopulated the damaged area, whereas non-TM-connected cells exhibited minimal repair. In contrast, non-TM-connected cells exhibited minimal repair.

TMs also confer radiation resistance. Following radiotherapy, TM-connected cells were protected from apoptosis, whereas non-connected tumour cells died. Knockdown of Cx43 reduced this radioprotective effect, leading to non-TM-connected tumour regression. Researchers also found that basal intracellular calcium levels remained stable in TM-connected cells during radiation, whereas non-connected cells exhibited highly variable calcium levels, suggesting that TMs are involved in maintaining calcium homeostasis and redistributing calcium to mitigate cytotoxic effects of radiation[7]. These findings demonstrated that TMs are involved in tumour self-repair and resist radiotherapy. Disrupting TM formation or Cx43-mediated communication could be a therapeutic strategy.

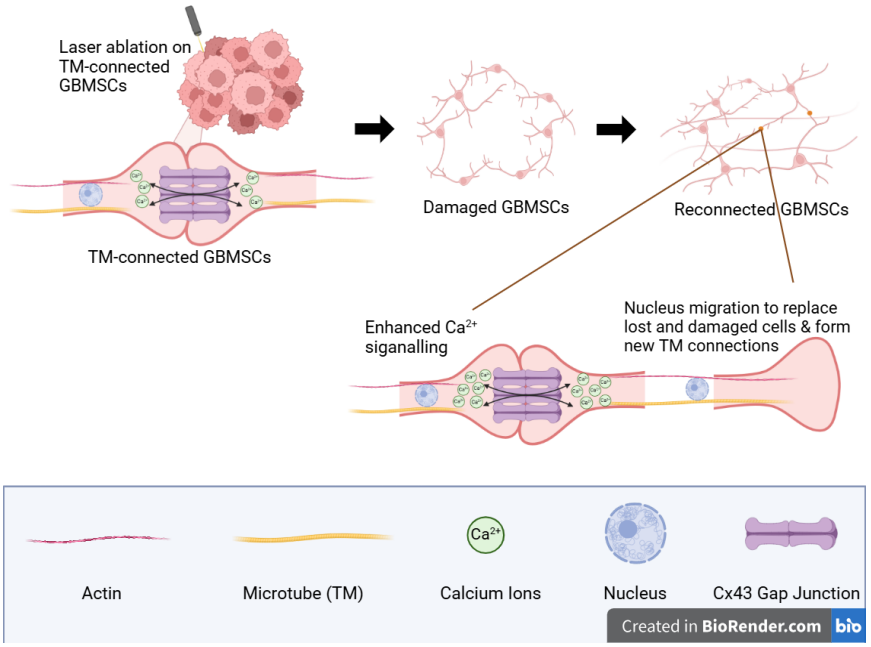

Figure 2. Tumour microtubes (TMs) facilitate glioblastoma repair and resistance to therapy. Laser ablation of TM-connected glioblastoma stem-like cells (GBMSCs) results in local cell loss. In response, surviving TM-connected cells enhance calcium signalling and initiate nuclear migration to replace lost cells, extending new TMs to re-establish network connectivity. This repair mechanism supports tumour survival following therapy and highlights TMs as a key driver of glioblastoma resistance.

Own work, created in Biorender.com. Information from (Osswald et al., 2015)[7].

7. Growth-associated protein 43 (GAP-43) as a key driver of TM formation

To identify the molecular drivers of TM formation to better understand their role in tumour progression and therapy

resistance, Osswald et al., (2015) compared transcriptomic profiles of 1p/19q non-codeleted astrocytoma and codeleted oligodendrogliomas. They found that GAP-43, a protein involved in neuronal axon growth, was overexpressed in astrocytomas, which is hypothesised as driver of TM formation[7]. To investigate its functional role, they engineered GBMSCs with GAP-43 knockdown. These GAP-43 deficient cells showed impaired TM formation, reduced Cx43 expression, and disrupted ICW propagation, leading to enhanced tumour regression after radiotherapy. Conversely, overexpressing GAP-43 in TM-lacking oligodendrogliomas induced a TM-rich phenotype with increased invasion and radiation resistance. These findings confirm GAP-43 as a key regulator of TM formation and therapy resistance[7].

8. Future Directions

8.1. Are TMs unique to glioblastomas?

TMs are well-characterised in gliomas, but evidence for their presence in other tumours is limited. However, TNT-like structures have been observed in various cancers cells, including bladder cancer[13], colon[14], ovarian[15], breast cancer[16], etc. Further studies should determine whether TMs contribute to therapy resistance in other malignancies and influence metastatic potential.

8.2. Targeting Cx43 in Glioblastoma

As discovered by Osswald et al., (2015), glioblastoma cells form extensive TM networks and gap junctions with astrocytes through Cx43, making them highly invasive[7]. Cx43-targeting therapies, such as the monoclonal antibody MAbE2Cx43, that targets the extracellular loop (E2) of Cx43, could disrupt TM networks and reduce glioma invasion[17]. By disrupting gap junction intercellular communication, MAbE2Cx43 inhibits the transfer of ICWs between glioma cells and astrocytes. In glioma-bearing rats, combing MAbE2Cx43 with radiotherapy significantly prolonged survival compared to standard radiotherapy or chemotherapy. However, delivering Cx43-targeting agents across the blood-brain barrier remains a challenge. Intraoperative or intrathecal administration may enhance drug delivery to brain lesions[17].

Cx43 inhibitors, including tonabersat[18] and meclofenamate[19], are under clinical trials for glioblastomas. These inhibitors enhance chemotherapy and radiotherapy effectiveness, but Cx43's widespread expression in the heart, brain, and skin raises concerns about off-target effects, including cardiac arrhythmias, neurological dysfunction, and impaired wound healing[20]. Future research should focus on tumour-specific Cx43-targeting strategies, such as phosphorylation site inhibitors or monoclonal antibodies, to maximise therapeutic efficacy while minimising toxicity.

8.3. Overcoming Temozolomide resistance

Temozolomide (TMZ) is the standard chemotherapy for glioblastoma, but at least 50% patients develop resistance driven by intercellular communication and DNA repair mechanisms[21,22,23].

In glioblastomas, after growth factors bind to their receptors, Cx43 selectively binds to the PI3K catalytic subunitβ (p110β/p85) to activate AKT kinase. Activated AKT phosphorylates and inhibits pro-apoptotic factors such as Bcl-2 (B-cell leukaemia/lymphoma 2 protein) from generating cell apoptosis[24,25], leading to TMZ resistance independent of methylguanine-DNA methyltransferase (MGMT), a well-known mediator of this resistance[23]. Targeting this Cx43-p110β/p85 interaction is a promising strategy (**Figure 3**). The Cx43 peptide inhibitor αCT1 disrupts this interaction, blocking PI3K/AKT activation and restoring glioblastoma sensitivity to TMZ. Preclinical studies have demonstrated that combining αCT1 with PI3K inhibitors (e.g. TGX-221 or GSK2636771) enhances tumour cell apoptosis, reduces tumour growth, leading to increased glioblastoma responsiveness to TMZ and prolongs survival in mouse models[23]. These findings support the clinical development of this combined therapy as a strategy to overcome TMZ resistance in glioblastoma.

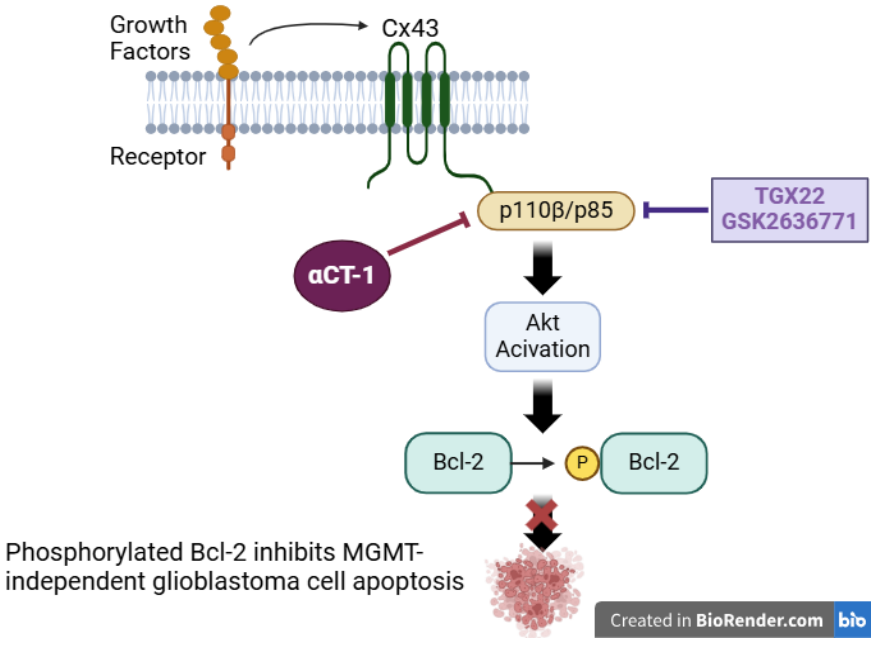

Figure 3. Cx43-mediated activation of PI3K/AKT pathway contributes to TMZ resistance in Glioblastoma.

Signals from growth factors activate Cx43, which binds to p110β/p85, leading to AKT activation. Activated AKT phosphorylates Bcl2, preventing apoptosis in MGMT-i ndependent glioblastomas. The Cx43-targeting peptide inhibitor αct1 disrupts this interaction, inhibiting PI3K/AKT signalling, leading to apoptosis. Additionally, PI3K inhibitors (TGX-221, GSK2636771) enhance apoptosis and sensitize glioblastoma cells to TMZ.

Own work, created in Biorender.com. Information from (Datta et al., 1997; Pridham et al., 2022)[23,25].

9. Conclusion

The discovery of TMs has advanced our understanding of glioma progression, invasiveness, and therapy resistance. These actin- and myosin-rich membrane extensions form stable, interconnected networks that facilitate long-range calcium signalling via Cx43 gap junctions. Through this structural and functional integration, glioma cells can maintain calcium homeostasis, coordinate stress responses, and rapidly repair damage through nuclear transfer and cell replacement.

The identification of GAP-43 as a critical regulator of TM formation links glioma biology to neuronal growth programs and offers a promising molecular target. Strategies to disrupt TMs or interfere with Cx43-mediated signalling—whether through monoclonal antibodies, small-molecule gap junction inhibitors, or peptide disruptors—show potential in preclinical models, especially when combined with PI3K pathway inhibition. Future research should determine whether TMs are unique to gliomas or a more general mechanism of therapy resistance across cancers. The development of tumour-selective Cx43 inhibitors will be key to translating these findings into safe and effective treatments with minimal off-target effects, potentially transforming the therapeutic landscape for this devastating disease.

Disclosure statement

The author declares no conflict of interest.

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