Tunneling Nanotubes for Glioblastoma Treatment

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Communication between cells helps tumors acquire resistance to chemotherapy and makes the struggle against cancer more challenging. Tunneling nanotubes (TNTs) are long channels able to connect both nearby and distant cells, contributing to a more malignant phenotype. This finding might be useful in designing novel strategies of drug delivery exploiting these systems of connection. This would be particularly important to reach tumor niches, where glioblastoma stem cells proliferate and provoke immune escape, thereby increasing metastatic potential and tumor recurrence a few months after surgical resection of the primary mass. Along with the direct inhibition of TNT formation, TNT analysis, and targeting strategies might be useful in providing innovative tools for the treatment of this tumor.

Keywords: tunneling nanotubes ; glioblastoma ; stem cells ; tumor microenvironment ; drug delivery ; nanoparticles

1. Introduction

Glioma is the most common primary tumor of the central nervous system, with an annual incidence of approximately 6 cases per 100,000 individuals worldwide and with approximately 50% of them being classified as glioblastoma (GBM). GBM is the most aggressive form of glioma, with a median lifespan from time of diagnosis to death of approximately 15 months.

Based on its histological appearance, GBM has been traditionally classified as an astrocytoma, though the precise cell type from which the disease originates is still a controversial issue. Some experts argue that the GBM origin is a subpopulation of neural stem cells, while others claim that it derives from the transformation of differentiated astrocytes [1]. Regardless, little progress has been made in GBM therapy, with no change in the standard of care for almost 20 years [2]. The current therapeutic approach for newly diagnosed GBM patients is based on surgery, followed by temozolomide chemotherapy and radiotherapy in combination with corticosteroids. In addition, in 2015, a noninvasive technique based on the application of alternating electrical fields (tumor treating fields, TTF) was approved as an adjuvant therapy for newly diagnosed GBM [3]. GBM is assigned WHO grade IV [4], and recently, the classification has been refined, with diagnosis based not only on histology but also on several molecular markers such as isocitrate dehydrogenase (IDH) and epidermal growth factor receptor (EGFR) [5]. Unlike other cancers, GBM remains confined in the brain without any systemic spread [6]. However, almost every GBM recurs, and recurrent tumors are chemotherapy-resistant, with higher invasiveness and aggressiveness compared with the original tumor [7][8][9]. Consequently, there is no standard treatment for recurrent GBM, partially due to poor biological knowledge of the disease [10][11]. Recent studies on tumor heterogeneity suggest that residual tumor cells after whole total tumor resection share only 60–80% of their mutations with the primary tumor and differ significantly in terms of gene expression profile, microenvironment, and extent of immune cell infiltration [12][13]. Additionally, cancer stem cells play a pivotal role in GBM recurrence, though there is no generally accepted definition of them within GBM and how they specifically contribute to therapy resistance and tumor recurrence has not been clarified.

2. Main Cell Types Interacting in Glioblastoma Microenvironment

Along with TAMs, CD4 + T and CD8 + T cells are capable of influencing tumorigenesis by receiving inhibitory signals from other TME cells and cancer cells, which lead to immune exhaustion and tumor tolerance [14].

It has been also suggested that, in brain tumors, dendritic cells (DCs) recognize and present tumor-derived antigens inside the brain tissue or in the draining lymphoid stations in order to boost a T effector cell response against cancer cells [15][16]. These cells are normally not present in the healthy brain parenchyma. However, during tumorigenesis, they can reach the brain tissue via afferent lymphatic vessels and/or endothelial venules [17][18][19]. Drainage of tumor antigens into cervical lymph nodes has been observed in animal models via the glymphatic system [20][21]. The glymphatic system is a functional meningeal system located in the dura mater, which allows for the passage of molecules and immune cells into the deep cervical lymph nodes [22][23][24], where internal recirculation mechanisms involve the cerebrospinal fluid and

interstitial fluid [25]. Moreover, cervical lymph nodes may have the property to modulate the immune response to tumor antigens toward either tolerance or reactivity [26]. Even if the specific role of DCs in the GBM environment is not yet elucidated, it is accepted that they have a pivotal role in antitumor immunity [27][28][29].

In addition, GSCs transdifferentiate into pericytes and contribute to the vascular structure [30][31]. Another phenomenon, called “vasculogenic mimicry”, takes place in GBM, where GSCs differentiate into endothelial-like cells, forming vessel-like structures. These structures are able to supply the tumor cells with nutrients and oxygen [32][33]. GSCs can also communicate with immune cells, promoting the establishment of a suppressive TME and thus allowing for tumor immune escape and progression [30]. In return, GAMs promote GSCs metabolic pathways to gain energy [34]. Furthermore, GSCs directly regulate immune cells, leading to the activation of T regs , the inhibition of cytotoxic T cell proliferation, and the induction of cytotoxic T cell apoptosis [35][36]. In summary, GSCs present in these niches preserve their phenotypic plasticity, protect themselves from the immune system, facilitate GBM metastasis, and are resistant to commonly employed cancer therapies. This is one of the main reasons why targeted therapy has not demonstrated efficacy in phase 3 clinical trials against GBM so far.

Astrocytes, essential components in the structure and function of the blood–brain barrier (BBB), have been shown to support tumor angiogenesis via multiple mechanisms including secretion of angiogenic and growth factors, such as VEGF, and protein carriers, such as insulin and albumin. Astrocytes surrounding GBM commonly undergo functional and phenotypical changes through astrogliosis, a process in which reactive astrocytes secrete a large number of soluble factors that promote GBM invasiveness, proliferation, and migration [37]. In turn, tumor cells suppress p53 expression in astrocytes, thus promoting GBM cell survival through modulation of the extracellular matrix composition [38][39].

3. Tunneling Nanotubes in Glioblastoma

In terms of functional analysis, Civita et al. demonstrated the trafficking of mitochondria from astrocytes to GBM cells via F-actin TNT structures using both 2D and 3D in vitro GBM co-culture models, indicating that contact communication between non-neoplastic astrocytes and tumor cells may occur [40]. Interestingly, we recently showed that TNTs formed by healthy astrocytes or by GBM cells display structural differences in terms of length and thickness, which reflect different transport efficiencies [41]. A recent paper showed that irradiated U87 GBM cells quickly establish a network of cell-to-cell connections with high TNT content in comparison with non-irradiated cells, suggesting that the TNT formation may be also a consequence of treatment [42].

In vivo, in a syngeneic astrocytoma mouse model, it has been shown that many tumor cells extend ultra-long membrane protrusions and use TMs and TNTs as routes for brain invasion, proliferation, and interconnection over long distances [43].

TNT cell-to-cell communication allows tumor cells to acquire new abilities, such as enhanced plasticity, migratory phenotypes, angiogenic ability, and therapy resistance, which can contribute to cancer aggressiveness, invasiveness, and recurrence [44][45].

In co-culture experiments, astrocytes surrounding U87 GBM cells enhanced TNT formation toward tumor cells and exploited this physical connection to transfer undamaged mitochondria, useful molecules, or energy substrates to GBM cells in order to modulate cell behavior and response to cytotoxic agents [40].

4. TNTs as a Novel Strategy to Enhance Tumor Drug Delivery

A more creative alternative would be the exploitation of these membranous cellular structures as a novel Trojan horse strategy to strengthen tumor drug delivery. Consequently, the exchange of drug delivery systems between cells composing the TME via TNTs comes as an interesting and advantageous opportunity to reach tumor cells, which usually escape current therapies. Moreover, exploiting the capability of TNTs to make physical contact with even distant cells indicates that they could be used to reach metastatic cells located in different tumor niches.

Some studies related to the ability of different cells to interchange nanoparticles through TNTs are available [46]. It has been shown that fluorescently labelled silica nanoparticles can be transferred between tumor cells by TNTs [47]. Franco S et al. demonstrated the intercellular trafficking of mesoporus silica nanoparticles along TNTs and TMs between macrophages and cancer cells in vitro and in vivo [48]. A recent paper from our group reported the exchange of multifunctional liposomes between human GBM cells and healthy astrocytes in vitro. Interestingly, the TNT-mediated transport of liposomes was more efficient between tumor cells compared with healthy astrocytes [41].

This highlights the structural differences in TNTs formed between tumor and healthy cells, which reflect a different rate of material exchange, which can be used to improve the precision of treatments. These findings support the exploitation of TNTs for cell-to-cell transfer of drug delivery systems to maximize treatment efficacy and efficiency.

In the field of GBM treatment, GSCs represent a challenge due to their resistance to commonly employed anticancer drugs. Moreover, this issue is worsened by the localization of these cells in tumor niches, often far away from the tumor mass. Therefore, the combination of long TNTs formation from cancer cells that colonize normal tissue [49] and their intercellular transport ability makes their targeting a valuable approach for the prevention and treatment of GBM recurrence. In addition, the possibility of TNTs formation between different cell types, e.g., between tumor and immune cells, and the structural differences between TNTs formed by tumor vs. healthy cells could be exploited to boost the precision of nanocarrier delivery.

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