Mini Review

Potential biochemical therapy of glioma cancer

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Abstract

Glioma is a highly invasive, rapidly spreading form of brain cancer that is resistant to surgical and medical treatment. The recent progress made in intracellular and ion channels of glioma cells provide a potential new approach for biochemical therapy of brain tumor. In this paper, we reviewed clinical data on chemotherapy by temozolomide and results from new studies on voltage-gated potassium channels, large-conductance Ca2+-activated K+ channels, volume-activated chloride channels, glioma-specific chloride channel and their modulators. These new findings may represent future directions for brain tumor studies and treatment.

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The malignant transformation of astrocytes, oligodendrocytes or their progenitor cells gives rise to tumors that are collectively called gliomas [1]. Gliomas account for 40–60 percent of primary brain tumors including astrocytoma, glioblastomam, lymphoma, meningioma, schwannoma [2]. Malignant gliomas are notoriously resistant to currently available therapies because these cancer cells can’t be induced to undergo apoptosis upon anticancer treatment [3]. Invasion of tumor cells into normal tissue is thought to be a complicated process, consisting of cell interactions with extracellular matrix (ECM) and with adjacent cells [4]. Gliomas are a disease can occur in all age groups, especially for elderly population. While a small percentage of patients are genetically predisposed to develop glioblastomas, the disease occurs sporadically with no known underlying cause.

Traditional therapy of brain tumors includes surgery, radiation therapy, chemotherapy, and immunotherapy (Fig. 1). Despite these treatments, the median survival time is less than 15 months [4]. Recent studies on temozolomide [5–8], voltage-gated potassium channels (Kv1.3 and Kv1.5) [9], large-conductance Ca2+-activated K+ channels (BK channels) [10,11], volume-activated chloride channels [12], and glioma-specific chloride channel (GCC, a voltage-activated channel) [13–15] suggest some potential approaches to treatment of gliomas. In this paper, we reviewed clinical data on chemotherapy by temozolomide and results from new studies on voltage-gated potassium channels, large-conductance Ca2+-activated K+ channels, volume-activated chloride channels, glioma-specific chloride channel and their modulators. These new findings may represent future directions for brain tumor studies and treatment.

Temozolomide—a new beginning of chemotherapy for glioma

In the same issue of the New England Journal of Medicine, four reports [5–8] highlight the growing importance of chemotherapy in treating malignant brain tumors. Temozolomide (C6H6N6O2, TMZ, Fig. 2) marks the first advance in treating glioblastoma in three frustrating
decades of trying to control this disease. TMZ was synthesized from 5-amino-imidazole-4-carboxamide hydrochloride salt by diazotization in polar solvent cyclisation with methyl isocyanate in the presence of catalyst with reaction time 20 h [16]. TMZ is a small molecule prodrug with a molecular weight of 194 Da, readily absorbed in the digestive tract. It is also able to cross the blood–brain barrier because of its lipophilicity. TMZ is not active until it is converted to the active form, 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) exposure to physiologic pH. TMZ is extremely stable at the acidic pH of the stomach. Once in contact with the slightly basic pH of the blood and tissues, TMZ spontaneously undergoes hydrolysis to the active metabolite MTIC, which rapidly breaks down to form the reactive methyldiazonium ion. The cytotoxicity of MTIC is due to the alkylation of DNA at the O6 and N7 positions of guanine [17].

O6-Methylguanine-DNA methyltransferase (MGMT) is an enzyme that repairs the DNA damage caused by alkylating agents such as TMZ and its metabolite. The damage to DNA by MTIC results in apoptotic cell death. Methylation of the promoter of MGMT turns off transcription of the gene, reducing the intracellular level of MGMT and thereby inhibiting the DNA repair mechanism. In principle, therefore, interference with MGMT expression and activity is a key factor for the antitumor effect of the alkylating agent [5]. A recent clinical study on radiotherapy and temozolomide by the European Organization for research and treatment of Cancer and National Cancer Institute of Canada [6] involved 573 adults with glioblastoma across Canada and in 12 European countries. Eighty-four percent of patients had undergone debulking surgery. At a median follow-up of 28 months, the median survival time was 14.6 months for radiotherapy plus TMZ group and 12.1 months for radiotherapy alone group. The two-year survival rate was 26.5 percent with radiotherapy plus TMZ and 10.4 percent with radiotherapy alone. In a companion translational study [7], methylation of the MGMT promoter, which results in gene silencing, is associated with a striking survival benefit in patients treated with radiotherapy plus TMZ. The results showed that patients with glioblastoma containing a methylated MGMT promoter benefited from temozolomide treatment. Among patients whose tumor contained a methylated MGMT promoter, a survival benefit was observed in patients treated with temozolomide and radiotherapy. Their median survival time was 21.7 months compared with 15.3 months for those who were treated with radiotherapy [7]. Additional studies, including TMZ combined with radiotherapy [18] and TMZ com-
bined with lomustine and radiotherapy [19], showed that the combination therapy had acceptable toxicity and yielded promising survival data in patients with newly diagnosed GBM. MGMT gene-promoter methylation was a strong predictor of survival.

Recent work by Rutkowski et al. [8] addressed the treatment of medulloblastoma in very young children. The results of IQ tests (the Colored Progressive Matrices test) in children treated with chemotherapy alone were significantly higher than the results of the test who received radiotherapy but did not receive intraventricular methotrexate. So, postoperative chemotherapy alone is a promising treatment for medulloblastoma in young children without metastases.

$K^+$ and $Cl^-$ ion channel blockers—future directions of glioma therapy

Human glioma cells express a variety of ion channels, including of voltage-gated $K^+$ currents, voltage-gated Na$^+$ currents, Ca$^{2+}$-activated $K^+$ currents, voltage-gated Cl$^-$ currents and volume-regulated Cl$^-$ currents [10]. Glioma cells can adjust their cell shape and cell volume to facilitate invasion into narrow spaces. These changes require secretion of Cl$^-$ ions along with either $K^+$ or Na$^+$ to allow water loss and cell shrinkage. The expression of large-conductance, Ca$^{2+}$-activated K$^+$ channels (BK) and voltage-gated K$^+$ channel (Kv) by glioma cells is of particular interest because these channels are related to the degree of differentiation and proliferative state of retinal glial (Muller) cells [11,20]. At the same time, the up-regulation of voltage-gated Cl$^-$ channels represents an adaptive feature in human glioma cells [21]. Along this line, chlorotoxin, a voltage-gated Cl$^-$ channels blocker, was recently evaluated as a potential therapeutic agent in clinical treatment of brain tumor.

Modulation of $K^+$ channels in glioma cells’ migration

Large-conductance Ca$^{2+}$-activated K$^+$ channels (BK channels) are highly expressed in human glioma cells. They play an important role in glial cell proliferation [10]. BK channels is triggered by membrane depolarization and enhanced by an increase in [Ca$^{2+}$] [10,22]. Intracellular Ca$^{2+}$ fluctuations are known to modulate neuronal migration [23]. The BK Channels in glioma cells were active at typical resting potentials with [Ca$^{2+}$] near 1 $\mu$M [11]. Current study [10] has showed that phloretin and its related substance (NS1619) are potent activators of BK channels in 1321N1 human glioma cells and that these substances inhibit cell migration. Moreover, both the activation of ion channel currents and the inhibition of migration were abolished by the specific BK channel blockers iberiotoxin (a 37 amino acid polypeptide isolated from the venom of the Indian red scorpion Buthus tamulus, the sequence is QFTDVDCSVS KECSVCKDL FGVDRGKCMG KKCRCYQ, Accession No. P24663) [24] and paxilline (C$_{27}$H$_{33}$NO$_4$, Fig. 3) (www.fermentek.co.il/paxilline.htm). Hence, the BK channel activation slows glioma cell migration. In another report [9], several human glioma samples expressing different voltage-gated K$^+$ channel (Kv) subtypes (Kv1.3 and Kv1.5) were investigated using reverse transcriptase-PCR. Increase in cell cycle-related Kv1.3 and Kv1.5 expression has been observed in proliferating cells and blockage of these channels by the results in a decrease in proliferation rates of these cells in normal glia [23,26]. The results showed that Kv1.5 expression level correlated with glioma entities and malignancy grades, i.e. expression was high in astrocytomas, moderate in oligodendroglomas, and low in glioblastomas. However no such correlation was evident for Kv1.3 expression. Further studies on the K$^+$ channels in physiological processes, such as Ca$^{2+}$ entry, voltage and volume regulation, are required to further understand the mechanism by which K$^+$ channels modulate glioma cell migration [10].

Modulation of $Cl^-$ channels in glioma cells’ migration

Cl$^-$ channels are vitally important in regulating cell volume. In glioma cells, volume-activated and voltage-activated chloride channels and their blockers have been widely studied in recent years [12–15,21,27–30].

Ransom et al. [12] used in vitro model to study glioma cell invasion (transwell migration assay) and patch-clamp techniques to investigate the role of voltage-activated Cl$^-$ currents in glioma cell invasion. Results of patch-clamp recordings suggested that $I_{Cl,vol}$ was activated during cell movement. Inhibition of $I_{Cl,vol}$ with NPPB (5-nitro-2-(3-phenylpropylamino)-benzoate, an Cl$^-$ channel inhibitor) reduced chemotactic migration in a dose-dependent fashion with an IC$_{50}$ of 27 $\mu$M. It has been suggested that $I_{Cl,vol}$ modulate cell shape and volume required for glioma cell migration through brain tissue.

Using whole-cell patch-clamp recordings, Ullrich et al. [21,31] identified large voltage-activated chloride currents that were selectivity expressed in glioma cells from 23 patient biopsies and these currents were sensitive to several Cl$^-$ channel blockers including chlorotoxin (Cltx, a 36 amino acid peptide isolated from Leiturus quinquestriatus venom, the sequence is MCMPCFTTDH QMARK

![Chemical structure of paxilline](www.fermentek.co.il/paxilline.htm)
CDDCC GGKGRGKCYG PQCLCR, Accession No. P45639), tetraethyl ammonium chloride (TEA), and tamoxifen (a selective estrogen receptor modulator for treating breast cancer). Several studies were performed to investigate the molecular mechanisms of Cl− channels and their blockers (especially Cltx, an inhibitor of small-conductance Cl− channels) in preventing glioma cells’ invasion. Using native and recombinant 125I-labeled Cltx, Soroceanu et al. [13] demonstrated that binding of Cltx to glioma cells is specific and involves high affinity (Kd = 4.2 nM) and low affinity binding sites. These data suggest that Cltx and Cltx-conjugated molecules may serve as glioma-specific markers with diagnostic and therapeutic potentials. Vast majority (74 out of 79) of primary human brain tumors were found to bind Cltx, with greater than 90% Cltx-positive cells in each section. Moreover, high percentage of peripheral neuroectodermal tumors (31 out of 34) examined showed specific Cltx binding [27].

Interestingly, in addition to Cltx-sensitive chloride channel, matrix metalloproteinase-2 (MMP-2) was isolated and identified as a second Cltx receptor on the surface of glioma cells [28]. In this study, 6XHis tagged Cltx was expressed in and purified from *Escherichia coli*. Results from matrigel invasion assay demonstrated that Cltx inhibited the enzymatic activity of MMP-2. MMP-2 is involved in cell migration and specifically up-regulated in gliomas. However, there is no interaction was observed between Cltx and MMP-1, -3 and -9, although these proteinases are also expressed in malignant glioma cells.

Recently, a clinical study on safety, bio-distribution, and dosimetry of intracavitary-administered iodine-131-Cltx (also named as 131I-TM-601) in patients with recurrent glioma were performed [30]. The results showed that 131I-TM-601 bound to the tumor periphery and demonstrated long-term retention on the tumor with minimal uptake by any other organ system. A single dose of 10 mCi 131I-TM-601 was well tolerated for 0.25 to 1.0 mg TM-601 and may have an antitumoral effect.

Conclusions

For nearly 20 years, surgery combined with radiation therapy has been used as the standard treatment for malignant brain tumors [32,33]. With the exciting advances in neuroscience and molecular mechanism of invasive migration, temozolomide and K+,Cl− ion channel modulators were developed as potential biochemical therapeutics in treating glioma cancer. It’s clear that these adjuvant treatment will lead to newer and more effective methods and strategies for brain tumor control in the future.

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