Involvement of Intracellular Cholesterol in Temozolomide-Induced Glioblastoma Cell Death

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Abstract

Glioblastoma (GBM) still carries a poor prognosis due to the refractoriness against antitumor drugs. Temozolomide (TMZ), one of the few standard therapy drugs against GBM worldwide, has only limited effect due to acquired TMZ resistance of GBM. Therefore, development of novel therapeutic methods to overcome the TMZ resistance of GBM is urgent. The brain is the most cholesterol-rich organ in the human body, so modulation of cholesterol in tumor cells originating from the brain including GBM may be a tumor-specific therapeutic strategy including enhancement of TMZ effects. The unique lipid metabolism of glioma has recently been reported, but the involvement of intracellular cholesterol in TMZ therapy is yet to be fully elucidated. This review summarizes the effect of modulation of intracellular cholesterol level on cancer therapy including GBM treatment and the implications for TMZ therapy. Our recent findings about the involvement of intracellular cholesterol in TMZ-induced GBM cell death are described.

Key words: glioblastoma, temozolomide, intracellular cholesterol level

Introduction

Glioblastoma (GBM) is a highly aggressive and refractory tumor of the central nervous system. Median overall survival is still only around 15 months despite improvements in surgical technique, development of various chemotherapeutic regimens, and advances in the development of irradiation devices in recent years.1

Temozolomide (TMZ) is an alkylating agent currently used for the standard therapy against GBM and has demonstrated better therapeutic outcome compared with previous anticancer drugs.2–5 However, prolonged treatment with TMZ often results in the development of TMZ resistance and acute relapse with subsequent poor clinical outcome.6,7 Therefore, novel therapeutic methods to treat TMZ-resistant GBM are urgently required.

Tumor-specific intracellular metabolites such as amino acids, glucose, lipids, or oxygen carriers have recently become novel therapeutic targets.8 Cellular metabolisms within the central nervous system are known to involve higher lipid contents compared with other systemic organs.9 Therefore, intracellular cholesterol metabolism has become a novel therapeutic target of GBM.10,11 However, little is known about the relationship between GBM biology and cholesterol metabolism.

The present review focuses on the involvement of intracellular cholesterol metabolism in GBM cell death, especially the relationship between intracellular cholesterol and TMZ-induced GBM cell death.

Differences in Lipid Metabolism Characteristics between Normal Brain Tissue and GBM

The brain is the most cholesterol-rich organ of the body, containing 20% of total body cholesterol.9
However, the brain cholesterol pool is independent of the peripheral cholesterol metabolism. Cholesterol cannot pass through the blood brain barrier from blood vessels, so almost all brain cholesterol is synthesized de novo. Moreover, the cholesterol metabolism in GBM is different from that in normal brain tissue. Cholesterol is mainly synthesized in astrocytes from glucose, glutamine, or acetate-derived acetyl-coenzyme A, and supplied to the surrounding cells in normal brain tissue. Excess intracellular cholesterol is eliminated by promoting cholesterol efflux transporters such as ABCA1 and uptake is suppressed through degeneration of low density protein receptor (LDLR). These negative feedback mechanisms, which complement suppression of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, maintain cholesterol homeostasis in normal brain tissue. In contrast, de novo synthesis of cholesterol is suppressed in GBM, so the cells depend on the supply of exogenous cholesterol by upregulating LDLR expression. Therefore, the administration of statins, a type of HMG-CoA reductase inhibitor used for the treatment of hypercholesterolemia, may not cause clinical improvement in patients with GBM.

Regulation of Tumor Cell Biology by Intracellular Cholesterol

Intracellular cholesterol has been known to regulate the biology of tumor cells including GBM and also drawn attention as the therapeutic target against these tumors. For example, increased cholesterol consumption is involved in the positive regulation of GBM cell biology. On the other hand, intracellular free cholesterol overload has been reported to induce apoptotic and/or autophagic cell death in cancer cells by induction of endoplasmic reticulum (ER) stress, inactivation of extracellular signal-regulated kinases, or induction of death receptor 5 (DR5), a death receptor localized in the plasma membrane.

Effects of Simultaneous Treatment with Cholesterol Depleting Agents and Anti-cancer Drugs Active against GBM Cells

Excess cholesterol is converted to oxysterol in normal astrocytes or neurons, and oxysterol acts as an endogenous ligand for liver X receptor to trigger cholesterol efflux through ABCA1 and suppression of LDLR. In GBM cells, liver X receptor agonists induce cell death by decreasing intracellular cholesterol through ABCA1-dependent cholesterol efflux and LDLR degeneration. Methyl-beta-cyclodextrin (MβCD), a highly water soluble cyclic heptasaccharide consisting of α- and β-glucopyranose units, has been reported as the most effective agent for the depletion of cholesterol from cells among the various cholesterol-depleting agents. Recently, MβCD was shown to have antitumor effects both in vivo and in vitro by depleting membrane cholesterol. Moreover, combination treatment of MβCD and conventional chemotherapeutic drugs is more effective than either monotherapy in several cancer cells. Statins, the HMG-CoA reductase inhibitors clinically used as anti-hypercholesterolemia drugs, also show antitumor activities. For example, statins can trigger G1 arrest in some transformed cells in vitro, and the arrest response is mediated by modulation of the activity, expression and/or distribution of cyclin-dependent kinase 2, p21, and p27. However, these findings applied on very high concentrations of statin compared with clinical plasma concentration used as the anti-hypercholesterolemia drug. In the case of GBM, one past report demonstrated statin treatment enhances anti-GBM effect of TMZ in vitro, but a large dose of statins was also used on this study compared with clinical concentration. In addition, our recent report demonstrated statins with clinical concentration rather suppress TMZ-induced GBM cell death. Therefore, it is suggested the antitumor effect of statins against GBM is various and might depend on its concentration of use.

Upregulation of Intracellular Cholesterol Level in GBM Cells Enhances TMZ-Induced GBM Cell Death through DR5-Mediated Extrinsic Apoptotic Pathway Activation

As mentioned above, cholesterol is clearly important in the regulation of tumor biology. We have recently focused on molecular mechanism which regulates acquired TMZ resistance of GBM that is one of the critical problem in clinical GBM therapy and anticipated about involvement of intracellular cholesterol modulation. To investigate this idea, we have established a clone of the human GBM cell line with acquired TMZ resistance. Intracellular cholesterol level of TMZ-resistant clones (U251-R cell) is significantly lower than that of TMZ-sensitive clones (U251-Con cell) in the U251 MG human GBM cell line (Fig. 1A). Therefore, we hypothesized that TMZ resistance of GBM cells might be regulated by the intracellular cholesterol level. Our investigation found that the intracellular cholesterol level of U251-Con cells was increased by single TMZ treatment and fluctuated after addition of...
Fig. 1 Intracellular cholesterol level regulates the sensitivity of glioblastoma (GBM) cells against temozolomide (TMZ; 400 μM)-induced cell death. (A) Intracellular cholesterol levels of TMZ-sensitive clone of U251 MG human GBM cell line (U251-Con cells) continuously treated by vehicle (dimethyl sulfoxide) and clone of U251-Con cells with acquired TMZ resistance after continuous TMZ treatment (U251-R cells) showing that total intracellular cholesterol was significantly lower in U251-R cells compared with U251-Con cells. (B) In vitro intracellular cholesterol measurement after TMZ, methyl-beta-cyclodextrin (MβCD; 0.5 mM) or water-soluble cholesterol (Chol; 20 μg/ml) treatment showing intracellular cholesterol level was upregulated by single TMZ treatment and fluctuated according to addition of MβCD and Chol. (C) Under the same conditions, cell death rate and immunoblotting of cleavage of lamin A/C showed almost the same trends as intracellular cholesterol level of U251-Con cells. (D) Combination of TMZ and Chol dramatically enhanced cell death induction in U251-R cells compared with single TMZ treatment. lamin A/C: apoptosis marker cleaved by active effector caspases. *P < 0.01. Reproduced with permission from the *Biochem Biophys Res Commun* (495: 1292–1299, 2018) © 2018, Elsevier B.V.
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cholesterol scavenger/inducer (Fig. 1B). Cell death rate and immunoblotting of cleavage of lamin A/C, an apoptosis marker, showed the same trends as the intracellular cholesterol level of the cells (Fig. 1C). More importantly, intracellular cholesterol loading also reversed resistance against TMZ-induced cell death of U251-R cells (Fig. 1D).

The key to the molecular mechanism is DR5, which is one of the plasma membrane-localized death receptor family members highly expressed in GBM. DR5 regulates cell death signaling and the cellular biology of tumor cells including GBM. Generally, stimulation of DR5 by its ligand, tumor necrosis factor-related apoptosis-inducing ligand or the Apo-2 ligand, induces clustering of DR5 and recruitment of adaptor molecule Fas-associated death domain (FADD) to the cytoplasmic death domain of DR5. In turn, caspase-8 associates with FADD and forms the death-inducing signaling complex which results in the auto-catalytic activation of procaspase-8. Caspase-8 then activates downstream effector caspases such as caspase-3. These processes are called the extrinsic apoptotic pathway (summarized in Fig. 2). In addition, the plasma membrane lipid raft, the cholesterol-rich microdomain which regulates activation of various signaling molecules localized in the plasma membrane, also has crucial roles in these DR5-mediated molecular processes in several cancer systems. In our reported system, TMZ exposure of GBM cells induced accumulation and activation of DR5 in the plasma membrane lipid raft, and intracellular loading of cholesterol, the main component of the lipid raft, enhanced these processes which resulted in increased TMZ-induced GBM cell death. One of the suggested mechanism which induces augmented expression of DR5 and ligand-independent DR5-mediated cell death upon treatment with TMZ or intracellular cholesterol loading in GBM cells is ER stress. Not only intracellular cholesterol loading but also high dose TMZ is reported to induce ER stress to GBM cells, and ER stress is known to upregulate DR5 expression and induce ligand-independent DR5 activation in GBM cells (Fig. 3).

Very importantly, this DR5-mediated extrinsic apoptosis cascade-activation induced by TMZ and cholesterol loading was also critical for regulation of acquired TMZ resistance of U251-R cells in our study. When considering about molecular mechanism which induce acquired TMZ resistance of GBM cells, gain of accelerated DNA repair is already well-known. Hence, we have previously checked about expression of DNA break maker gamma-H2AX in U251-Con cells and U251-R cells and found kinetics of gamma-H2AX expression after TMZ treatment in U251-R cells did not altered compared with that of U251-Con cell (data not shown). Therefore, it is suggested another mechanism which might involve regulation of intracellular cholesterol metabolism would be also important for gain of TMZ resistance in GBM cells.

Conclusion

The involvement of cholesterol in tumor cell biology has recently been recognized, but the underlying molecular mechanism is complex and not yet fully explored. Our review suggests that control of intracellular cholesterol has potential as a novel therapeutic target for GBM therapy even the case of GBM with acquired TMZ resistance. In addition, it is also suggested there is possibility that patients with GBM taking anti-hypercholesterolemia drugs such as statins may have poor outcome during TMZ treatment (summarized in Fig. 3).
Conflicts of Interest Disclosure

We have no potential conflicts of interest. All authors have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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Author Contributions

Conceived and designed the experiments: YY, AT. Performed the experiments: YY. Contributed reagents/materials/analysis tools: NS, KI, HN, KM. Wrote the paper: YY, AT.

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