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The effects of chitosan-based materials on glioma: Recent advances in its applications for diagnosis and treatment

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Abstract

Glioma is known as the most common primary brain tumor occurring in adolescents and is considered as a lethal disease worldwide. Despite the advancements in presently available therapeutic approaches (i.e. radiation therapy and chemotherapy), the rate of amelioration in glioma patients is still low. In this regard, it seems that there is a need for reconsidering and enhancing current therapies and/or discovering novel therapeutic platforms. Chitosan is a natural polysaccharide with several beneficial characteristics, including biocompatibility, biodegradability, and low toxicity. Without causing toxic effects on healthy cells, chitosan nanoparticles are attractive targets in cancer therapy which lead to the sustained release and enhanced internalization of chemotherapeutic drugs as well as higher cytotoxicity for cancer cells. Hence, these properties turn it into a suitable candidate for the treatment of various cancers, including glioma. In the viewpoint of glioma, cancer inhibition is possible through targeting glioma-associated signaling pathways and molecules such as MMP-9, VEGF, TRAIL and nuclear factor-κB by chitosan and its derivatives. Moreover, it has been acknowledged that chitosan and its derivatives can be applied as a delivery system for carrying a diverse range of therapeutic agents to the tumor site. Besides the anti-glioma effects of chitosan and its derivatives, these molecules can be utilized for culturing glioma cancer cells; providing a better understanding of glioma pathogenesis. Furthermore, it is documented that 3D chitosan scaffolds are potential targets that offer advantageous drug screening platforms. Herein, we summarized the anti-glioma effects of chitosan and also its utilization as drug delivery systems in the treatment of glioma.

Keywords: Chitosan, glioma, drug delivery, scaffold, cell culture

1. Glioma

Annually, 27,000 new cases of malignant glial tumors and 1000 new cases of malignant ependymal tumors are diagnosed as glioma, according to Europe guidelines which are provided for the diagnosis, prognosis, and treatment of the most common adult brain tumors [1]. Indeed, one of the most life-threatening types of adult brain tumor is glioblastoma multiforme (GBM) which is accounted for about 80% of all primary malignant central nervous system (CNS) tumors [2, 3]. GBM and anaplastic glioma are the most frequent glial tumors, comprising more than 50% and 10% of the total glioma, respectively [4]. Several risk factors are related to the incidence of GBM. Among them, age is associated with a higher incidence and mortality rate in GBM. The average age of diagnosed patients is 64 years old [5]. Moreover, the incidence of GBM is lower in women compared to men [6].

While glioma is mainly the most frequent intrinsic CNS tumor, it inclines to develop metastatic forms and represents poor responses to chemotherapy and radiotherapy with undesirable outcomes [7]. Gliomas encompass two principal subgroups: Diffuse glioma is one of the subgroups of glioma that is defined by wide-ranging infiltrative growth into the surrounding parenchyma of CNS. Non-diffuse glioma is another subtype that shows a more circumscribed growth pattern that is characterized by pilocytic astrocytoma and ependymomas, which are rather common signs of this subgroup [8]. Despite the extensive efforts that have been made internationally in clinical oncology, treatment of GBM is still challenging [9-11]. At present, the mainstay of treatment for glioma is surgical resection followed by concurrent chemotherapy and radiotherapy. The treatment of patients with gliomas, particularly GBM, has been improved greatly through the mentioned treatments. However, the results of these treatments are not

promising. Common anticancer therapies may lead to drug resistance and subsequent recurrence or metastasis of cancer. The median survival time of patients diagnosed with GBM ranges from 12 to 15 months; meanwhile, the 5-year survival rate has been less than 5% [12]. Therefore, new treating strategies are essential to combat glioma and improve the survival rates of patients who suffer from glioma.

2. Risk factors of glioma

Exposure to a high dose of ionizing radiation is the only confirmed risk factor of GBM up to now [13-15]. Radiation exposure accounted for more than 116 cases of GBM which have been reported since the 1960s. It has been estimated that the overall risk of developing GBM after radiation therapy is 2.5% [16]. Findings of experimental animal studies have shown that some pesticides and other agricultural chemicals, such as organochlorides and alkylureas combined with copper sulfates are capable of inducing cancer, as well. However, case-control and cohort studies of agricultural workers have reported equal negative or positive findings related to the risk for brain tumors [17]. A meta-analysis has shown that taking vitamin C may reduce the risk of glioma [18]. Few studies have shown that ovarian steroid hormones play a possible role in the development of GBM [19]. Also, it is reported that long-term consumption of hormonal contraceptives may lead to a higher risk of glioma [20]. Occupational risk factors, severe head injury, exposure to the pesticide, smoking, dietary risk factors, cell phones, and electromagnetic field are environmental factors that have no conclusive association with GBM [13, 15, 21-23]. However, studies indicated that average amounts of weekly exposure to carbon tetrachloride have been related to a higher risk of glioma in people who are occupationally exposed to it [24]. Nelson et al. also reported that occupational exposure to carbon tetrachloride and sugar intake

are two risk factors for the development of glioma [25]. Some investigations reported that diabetes mellitus is inversely associated with the risk of glioma [26-28]. Zhao et al. [29] also demonstrated that there is an association between diabetes mellitus and reduced risk of glioma. Besides, they reported that males and Caucasians with diabetes mellitus have a lower risk of glioma [29]. Based on some studies, the effect of infection and allergic diseases on GBM is possibly protective, which may due to the initiation of immune surveillance mechanism [14, 23]. A study has shown that the risk of developing glioma is reduced by 40% in people who suffer from allergies [30]. Also, gliomas are found to run in families but its susceptibility gene has not been identified yet [14]. Reports indicated that the incidence of some specific subtypes of glioma can be increased by inherited monogenic Mendelian syndromes such as Li–Fraumeni syndrome and Lynch syndrome, which are associated with GBM. Tuberous sclerosis, neurofibromatosis type 1 and type 2 are also related to multiple types of glioma including giant cell astrocytoma, astrocytoma, optic nerve glioma, and ependymoma. Furthermore, Ollier disease (Maffucci syndrome) and melanoma-neural system tumor syndrome have been reported to be involved in the incidence of all gliomas [31]. GBM shows a high degree of both genomic and spatial heterogeneity [32]. Changes in some genes are highly associated with GBM including RB1, TP53, NF1, PTEN, IDH1, EGFR, PIK3R1, and PIK3CA [33-35]. Additionally, patients with mutations in their L2HGDH gene, which leads to L-2-hydroxyglutaric aciduria, have been observed to have a higher incidence of brain tumors [32]. IDH1 mutation is observed to be present in several secondary GBM. Whereas, evidence expressed that it is not present in primary GBM. Neomorphic enzymatic activity of IDH1 results in the generation of 2-hydroxyglutarate that is an oncometabolite. 2-hydroxyglutarate production leads to some cell function alterations which relate it to epigenome and development of GBM [36]. Gene mutation is also able to alter

some signaling pathways such as dysregulation of growth factors, inactivation of Rb and p53, and activation of phosphoinositide 3-kinase; thereby, participate in GBM pathogenesis [32].

3. Chitin and its derivative

Chitin, poly (β-(1–4)-poly-N-acetyl-D-glucosamine), is a biopolymer that is synthesized by several organisms [37]. After cellulose, it is the second most abundant polysaccharide that is widely distributed in nature. Chitin is the main structural compound in the cell wall of fungi and exoskeleton of crustaceans, such as crabs and shrimps. Chitin is found in nature as ordered microfibrils. Chitin is only usable in the field of biomedicine after converting to its derivatives, especially chitosan. [38]. Both chitin and chitosan are biocompatible, biodegradable, and non-toxic biopolymers. Also, they have antimicrobial and hydrating effects. Furthermore, chitosan, along with other molecules, is used to culture cancer cells [39]. Because of these unique properties, chitosan has recently received considerable attention in the biomedical field [40].

4. Roles of chitosan in biological processes

Chitosan has various biological benefits, including antimicrobial [41], anti-tumor [42], and immune-promoting activities. Studies have shown that chitosan can increase IL-2-mediated expansion of leukocytes in tumors and tumor-draining lymph nodes by 40% and 100%, respectively. Immuno-phenotyping studies demonstrated that chitosan co-formulation causes an increase in the IL-12-induced populations of important effectors, such as CD8+IFN-γ+ and NKT cells, in tumors and dendritic cell populations of the tumor-draining lymph nodes [42]. Chitin and chitosan oligosaccharides can regulate the inflammatory activities in macrophages [43]. Moreover, chitosan promotes dendritic cell maturation by inducing type I interferons (IFNs) and

enhances antigen-specific T helper 1 (Th1) responses in a type I IFN receptor-dependent manner [44]. In gingival fibroblasts, chitosan has shown an anti-inflammatory activity *via* reducing the production of prostaglandin E-2 (PGE-2) though downregulating the c-Jun N-terminal kinase (JNK) signaling pathway. Also, chitosan suppresses the adipogenesis in 3T3-L1 adipocytes. However, the effect of chitosan molecular weight on inflammatory activity is still questionable for researchers and some reverse effects have been observed [45].

5. Anti-tumor activities of chitosan

Novel studies indicate that there are certain subpopulations of cancer cells in a tumor from which the tumor can originate. These cells, which are called cancer stem cells (CSCs), have multiple similar characteristics to stem cells [46, 47]. These cells have been shown some features, including a higher ability of migration that is associated with invasion and metastasis [48]. Besides, they remain at a slow-cycling/quiescent state, which leads to resistance against anti-proliferative drugs [49]. Some certain surface markers such as CD133, EpCAM, and CD44 can be used for CSC identification and isolation [50]. CSCs have the ability of self-renewal that provides maintenance of CSC pools as well as differentiation into heterogeneous progeny cancer cells [51]. Signaling cascades within CSCs, such as Notch, STAT3, and Wnt/β-catenin, to are not regulated to maintain their stem cell properties [52]. Hence, targeting CSCs and some specific signaling pathways which are essential for tumor cells can provide novel and promising therapeutic strategies [55]. Chitosan cross-linked with other molecules is being used for culturing cancer cells [39, 56]. For instance, the CD133+ GBM CSC population may be enriched by porous chitosan-alginate scaffolds [57]. Also, breast cancer stemness may be increased by electrospun polycaprolactone-chitosan scaffolds [58]. However, the mechanisms of interactions

between cells and biomaterials are not yet well-known. Some investigations indicated that chitosan membranes and hyaluronan (HA) grafted chitosan (CSHA) membranes could increase the stemness of mesenchymal stem cells (MSCs) [59]. The main ligand of CD44 receptor that is aberrantly expressed on the surface of CSCs is HA [60]. Likely through the interaction of HA and CD44 receptor, CSHA membranes promote the aggressiveness of lung cancer cells. However, the influence of chitosan itself on some cancer cells remains to be elucidated [61]. In a recent study by Rao et al. it is demonstrated that chitosan nanoparticles could bind to CSCs *via* CD44 receptor, a major target gene of Wnt signaling [62, 63]. This finding indicated that the chemical properties of chitosan are somehow similar to HA.

Chang et al. [64] recently demonstrated that chitosan itself enhances the CSC-related characteristics and tumor progression of not only $CD44^{positive}$ colon cancer cells but also $CD44^{negative}$ HCC cells. They observed that chitosan alone could increase cancer cell stemness properties and tumor progression. Meanwhile, they showed that chitosan and CSHA could induce diverse morphology in various cancer cells [64]. Several studies have been revealed that SW480 cells are not able to form spheroids and they fail to aggregate on chitosan and CSHA. HT29, DLD-1 and HCT116 are all CD44-positive cells which can aggregate on CSHA membranes [64-66]. Thus, chitosan as a suitable platform that has similar properties with ECM may be useful for studies concerning CSC biology as well as drug screening.

6. Application of chitosan as a drug delivery system for glioma

As we mentioned earlier, chitosan is a biodegradable and biocompatible agent which is used in the pharmaceutical industry. Within the past two decades, chitosan has been used for delivering

various therapeutic agents in nanoparticle forms [69]. There are several methods for preparing chitosan nanoparticles, such as emulsion solvent diffusion, nanoprecipitation, and emulsion cross-linking [69]. Utilization of nanomatrial-based drugs is associated with various advantages i.e., targeting drug to specific sites in the body, enhanced bioavailability by improving aqueous solubility, increasing resistance time in the body and passing the blood-brain barrier [70]. Recently, findings have shown that a combination of chitosan and nanotechnology may lead to overcoming the challenges we are currently facing in delivering drugs [70]. Herein, we take a look into recent studies concerning with chitosan-based drug delivery systems that are used for treating glioma.

Chitosan-coated poly(lactide-co-glycolic acid) nanoparticles that are modified with polyethylene glycol and loaded with paclitaxel and R-flurbiprofen have shown efficient delivery of drugs to the tumor site. Moreover, these nanoparticles have higher cytotoxic effects against glioma due to the combination of anti-inflammatory and antitumor agents [71]. In C6 glioma cells, silibinin-loaded chitosan nanoparticles provide sustained release of the drugs while increasing the expression levels of Bax and caspase3, two essential parts of apoptosis [72]. Turabee et al. [73] found that hydrogel of N, N, N trimethyl chitosan combined with pluronic F127 provides a sustained release of docetaxel. In GBM cells, this delivery method has a more effective killing ability than free docetaxel or docetaxel-loaded pluronic F127 [73]. Another study showed that chitosan coating changes the surface charge of core-shell polymeric nanoparticles to positive values, which enhances the nanoparticles internalization [74]. Besides, these nanoparticles that were loaded with docetaxel have shown higher cytotoxicity in comparison with docetaxel alone [74]. Poly-l-arginine-chitosan-triphosphate matrix nanoparticle loaded with doxorubicin and superparamagnetic iron oxide is a potential delivery system for diagnostic and therapeutic

purposes in GBM [75]. It is revealed that increasing the concentration of iron that is used in this method results in a decline in times of T_2 relaxation of MRI [75]. Chitosan-capped gold nanoparticles have been shown to cause selective cytotoxicity for GBM stem cells without affecting normal cells [76]. Unlike uncoated nanoparticles, nanoparticles composed of chitosan exhibit a high accumulation in cells within the lysosomes and cytosol as well as near the nucleus [76]. Sharma et al. [77] designed a nanoformulation consisted of polyamidoamine dendrimer and chitosan for delivering temozolomide to GBM. They reported that this delivery method is more efficient than temozolomide alone since the concentration of the drug was doubled in the brain with this method [77]. Chitosan nanoparticles with an outer shell of 1,3β-Glucan have been used for delivering paclitaxel to malignant GBM [78]. Findings showed that this platform provides multiple benefits including improved drug bioavailability, overcoming systemic toxicity, decreasing hemolytic properties, and more cytotoxicity against glioma cancer cells [78].

7. Application of chitosan in culturing glioma cells to study the biology of these cells and develop therapies

Despite the ability of patient-derived xenografts of GBM that provide a similar behavior as an *in vivo* tumor characteristic, these xenografts are reported to be costly and time-consuming [79]. In the other hand, testing potential anti-tumor agents require *in vitro* models which provide a suitable microenvironment for glioma [80]. Furthermore, pre-clinical studies of drug screening that use 2D culturing methods are not much effective in patients [81]. Chitosan is an excellent candidate biomaterial for designing scaffolds, which enhances tissue regeneration and tissue engineering [82].

It is reported that 3D chitosan-alginate scaffold can be used as a beneficial microenvironment for glioma since human glioma cells exhibit higher malignancy when they are cultured in chitosan-alginate scaffolds [83]. This report showed that chitosan scaffolds, which have properties similar to the extracellular matrix, provide an environment for glioma cells to show a phenotype more similar to *in vivo* condition [83]. A study showed that culturing GBM cells on chitosan-polycaprolactone polyblend nanofibers results in an upregulation of genes related to invasiveness, such as Twist, STAT3, Snail, β-catenin, and TGF-β [80]. Moreover, the cultured cells present the same migration profile as *in vivo* cells [80]. Chitosan and HA have been used for synthesizing a 3D scaffold [84]. While monolayer and flat epithelioid cells are grown in 2D adherent cultures, GBM cells have been observed to form ovoid cells clusters in the pores of chitosan-hyaluronic acid scaffolds [84]. Besides, cells that grow on these scaffolds indicated remarkable features, including higher expression levels of genes related to EMT, exhibiting an undifferentiated phenotype, and higher expression levels of genes related to hypoxia-induced oxidative stress [84]. It is found that chitosan and chitosan-hyaluronic acid scaffolds can form glioma cell spheroids [85]. Scaffold-grown cells present a higher expression of biomarkers associated with glioma stem cells in comparison with common 2D monolayers [85]. Noteworthy, cells that were cultured on chitosan-HA scaffold have a higher ability of tumorigenicity *in vivo* compared to 2D-cultured cells [85]. HIF-1α, Nestin, Musashi-1, GFAP, and CD44 which are stem-like characteristics of GBM cells have been observed to be upregulated in chitosan-HA scaffolds compared with 2D-cultured cells [81]. Besides, 3D-cultured cells showed higher resistance to chemotherapeutic drugs [81].

8. Possible effects of chitosan on signaling pathways involved in glioma

Studies reported that some signaling pathways are involved in the pathogenesis and development of glioma. Chitosan has been observed to have a beneficial effect in the treatment of cancer cells *via* involving different signaling pathways [86-88]. Herein, we investigate the possible effects of chitosan on some signaling pathways that are involved in glioma. However, the exact roles of chitosan in glioma signaling pathways are remained to be elucidated.

Various factors contribute to the regulation of signaling pathways and maintenance of GBM malignant cells such as increased metabolism rate and reactive oxygen species (ROS) [89]. ROS activates the transcription factor nuclear factor-κB (NF-κB). Subsequently, NF-κB activates the expression of genes involved in tumor growth and development [90]. Formation of amyloid β, which is induced by oxidative stress, and cytotoxicity are prevented by chitosan in NT2 neurons. This prevention occurs through two transcription factors: NF-κB and Nrf2. Hence, chitosan might be considered as an additional therapeutic strategy to combat neural demise in Alzheimer's disease as well as other diseases that are associated with oxidative stress. Therefore, chitosan has the potential to be used for both preventing and treating diseases of CNS [91]. Interestingly, inhibiting the NF-KB reduces matrix metalloproteinase-2 (MMP-2) and MMP-9 expression [90]. MMPs are enzymes that have various roles in the destruction of the extracellular matrix and serve as important factors in physiological and malignant processes [92]. Based on the evidence, the upregulation of MMP expression is a critical cause of tumor growth and inhibition of anti-tumor processes [93]. Current studies approved the nutraceutical value of two water-soluble derivatives of chitosan and chitin, carboxymethyl-chitosan and carboxymethyl-chitin. These derivatives serve as potent antioxidants and MMP inhibitor; leading to the alleviations of radical-induced oxidative damage [94]. Chitosan polymer has also been used for

delivering a bioactive compound with neuroprotective effects, eugenol, to glioma cells. Findings showed that eugenol-loaded chitosan is capable of the inhibiting protein expression of NF-κB as well as reducing MMP-9 and urokinase-type plasminogen activator. Moreover, this nanopolymer significantly decreased the expression of VEGF [95]. Human tumor necrosis factor α- related apoptosis- inducing ligand (TRAIL) is one of the TNF cytokine superfamily members. TRAIL forms a homotrimer that crosslinks death receptors on the cell surface; resulting in downstream signaling of apoptosis [96, 97]. While the majority of GBM express death receptors [98], studies have shown that TRAIL can be a potential target for glioma treatment [99]. Wang et al. [100] designed an iron oxide nanoparticle coated with chitosan–polyethylene glycol–polyethyleneimine copolymer and chlorotoxin to provide a delivery system of plasmid DNA encoding TRAIL into GBM. They observed that TRAIL was successfully delivered into human T98G GBM cells. The results suggested that this drug delivery system is a potential candidate to combat against GBM [100].

9. Conclusions

Considering statistics, the high rate of mortality, and the low life quality of glioma patients, it seems that common therapies are not satisfying enough. Besides, there are several challenges in the study of the glioma cells as well as developing new therapies for this cancer. Chitosan, which has been used against various cancers, has recently attracted the attention of glioma-related researches. Chitosan nanoparticles have been suggested to have anti-tumor characteristics against glioma cells as evidenced by their effects on several signaling pathways and molecules. A variety of glioma chemotherapeutic drugs has been loaded into chitosan nanoparticles. These nanoparticles are reported to be more effective than pure therapeutic drugs due to multiple

features, including the sustained release of the drug, enhanced internalization of the drug, and higher cytotoxicity on cancer cells. Since 2D-cultured cells have different characteristics from the cells grown *in vivo*, this culturing method is not much effective for screening drugs. Findings demonstrate that 3D chitosan scaffolds are potential targets for providing a drug screening platform **(Fig.1).** Moreover, culturing glioma cells on chitosan-based scaffolds leads to a better understanding of glioma stem cell biology. Altogether, we believe chitosan is a promising agent that can be used as a sufficient drug delivery system for treatment of glioma as well as circumventing obstacles existing in the studying of the glioma cells and drug screening.

Abbreviations

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Legend to Figure:

Figure 1. Schematic representation of chitosan applications for culturing glioma cells and drugs delivery. (A) Studies reported that 3D chitosan scaffolds are more effective for culturing glioma cells compared to 2D monolayer cultures. These scaffolds provide a platform for drug screening and studying the biology of glioma cells. (B) Findings suggest that chitosan nanoparticles are potential targets for delivering glioma therapeutic drugs.

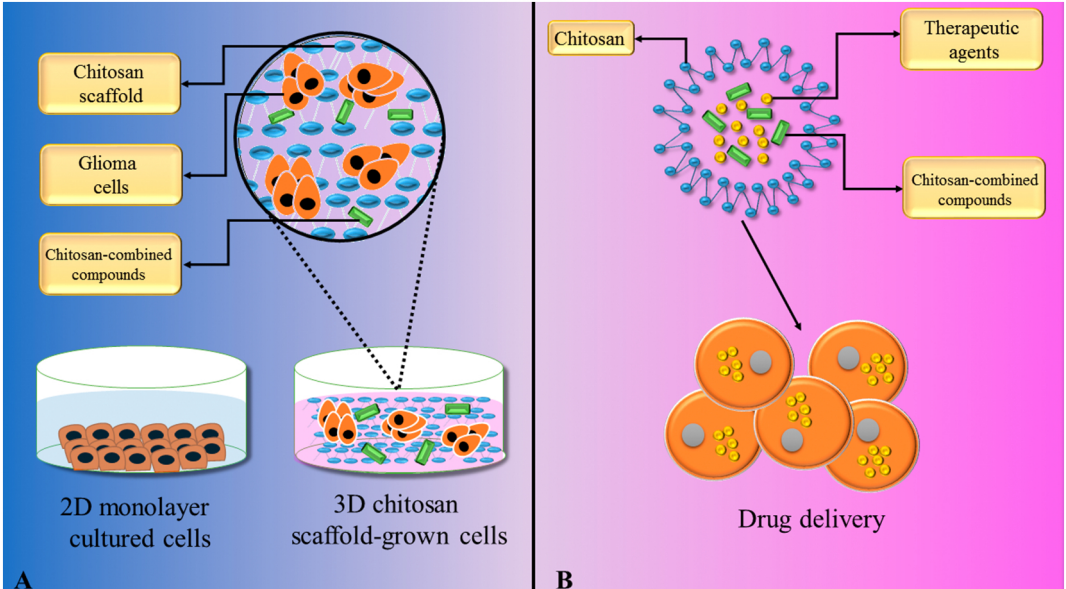
Chitosan scaffold
Glioma cells
Chitosan-combined compounds
2D monolayer cultured cells
3D chitosan scaffold-grown cells
A
Chitosan
Therapeutic agents
Chitosan-combined compounds
Drug delivery
B

Figure 1