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GM-CSF Promotes DC Proliferation by Increasing pAkt to Treat Glioblastoma

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Abstract. Glioblastoma (GBM) is a kind of highly invasive, rapidly progressing and low survival primary brain tumor, and there are almost no particularly effective treatment methods for glioblastoma so far. This article aims to test the therapeutic effect of granulocyte-macrophage colony stimulating factor (GM-CSF) on GBM. These experiments focus on the effect of GM-CSF on pAkt, DC proliferation and tumor size under different concentrations and treatment durations. The proposal highlights the feasibility of treating GBM with GM-CSF, providing a new treatment approach and support human health.

Keywords: Glioblastoma, treat, GM-CSF, pAkt, DC

1. Introduction

Glioblastoma (GBM) is a highly invasive tumor that can affect the central nervous system in adults [1]. Although there has been progress in treatment methods, the median survival time of GBM patients is still very short, typically between 14.6 and 16.7 months [1]. Because of its invasive nature, it is easy for GBM to spread within brain tissue, causing the surgical impossibility of complete resection [2]. After the surgery, a combined treatment of radiotherapy and chemotherapy is usually performed [3]. Temozolomide (TMZ), as an oral alkylating prodrug which is involved in standard chemotherapy for GBM, can lead to DNA purine base methylation to induce cancer cell death [2]. However, compared to radiotherapy alone, the median survival time for patients with combination therapy can only increase by 2.5months [2]. At the same time, patients are prone to developing resistance to TMZ, which can diminish the effectiveness of the treatment [2]. Even worse, it is almost unavoidable for tumor to recur [3]. The existing treatment methods cannot meet the treatment needs of GBM. So, finding new ways to treat it is significant.

In recent years, immunotherapy has become a new way to treat cancers and a novel approach in the management of glioma [4]. As a kind of antigen-presenting cells (APCs) [5], DCs play an important role in treating cancer. The ability of conventional DCs to activate T-cells leads to the foundation for the 'cancer immunity cycle' [5]. Conventional DCs (cDCs) which are related to cancer can endocytose dead cancer cells or cellular debris and transport cancer-related antigens to draining lymph node to promote T-cells production and activation [5]. Furthermore, cancer cells can be killed through T-cell action [5]. However, tumors have the ability to suppress immunity by effecting DCs [5]. For example, the cross-talk between GBM cells and tumor-associated

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macrophages results in releasing a few kinds of interleukins (IL; e.g., IL-6) [6] that can suppress DC differentiation and activation [5]. As a result, it is necessary to promote DCs proliferation.

Granulocyte-macrophage colony stimulating factor (GM-CSF), a type of cytokine, can regulate the proliferation and/or activation of myeloid cells, including DCs, in vitro [7]. The article aims to investigate and test the effect of GM-CSF on promoting DCs proliferation and leading to the decrease of tumor size in vivo.

This article will test its effectiveness from the perspective of phospho-Akt. Akt is a kind of serine/threonine protein kinase [8]. It has been tested that GM-CSF can stimulate the phosphorylation of Akt [9]. Particularly, phosphorylated Akt (pAkt) can activate mechanisms such as the mammalian target of rapamycin (mTOR, a kinase regulated by dopamine and glutamate receptors [10]) to promote cell proliferation [8]. mTOR can form the catalytic subunits of two different protein complexes, namely mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) [11]. Among them, the activation of mTORC2 can be promoted by the partial activation of Akt, while mTORC2 can act on Akt in reverse, phosphorylating and fully activating it [11]. After that, Akt can promote cell proliferation through the phosphorylation and suppression of some critical substrates, such as the mTORC1 inhibitor TSC2, the metabolic regulator GSK3β and FoxO1/3a transcription factors [11]. So GM-CSF may promote DCs proliferation by increasing pAkt.

In this article, other related proteins are used to compare their killing effect of U251 cells with GM-CSF, for example, Flt3L. Known as human soluble FMS-like tyrosine kinase 3 ligand, Flt3L can stimulate DCs proliferation [12]. King GD, et al. have verified that after injecting adenoviral vectors which can express Flt3L into the brain of rats, the number of plasmacytoid dendritic cells (pDCs, a type of dendritic cell subsets [5]) that can secrete interferon alpha within the parenchyma has a specific increase, further contributing to the improvement of survival when treatment is received within three days after tumor implantation [12]. It can be inferred that Flt3L plays a significant role in DCs proliferation and cancer treatment. Therefore, we will compare its therapeutic effect on GBM with GM-CSF's. At the same time, PBS will be used in experiments as negative control. Phosphate-buffered saline (PBS) is a kind of buffer solution which is typically used in biological research, including a buffer system based on potassium dihydrogen phosphate and disodium phosphate [13]. It is useful to maintain a constant pH of the solution, generally, 7.4 [13].

2. Hypothesis

Predict that increasing concentrations and treatment durations with GM-CSF in U251 glioblastoma xenograft mice increases dendritic cell proliferation by increasing phospho-Akt and leading to decreased size of tumor.

3. Materials and methods

3.1. Adenoviral vectors

Cultivate the first-generation replication-defective recombinant adenovirus type 5 vectors (Ad) that can express GM-CSF (AdGM-CSF) or Flt3L (AdFlt3L) under the transcriptional control of the human cytomegalovirus intermediate early promoter embedded within the E1 region [12]. Expand the cultivation of adenoviral vectors.

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3.2. FACS

FACS (Fluorescence activated Cell Sorting) can be used for cell sorting. The specific content is to stain the cells with the specific fluorescent dyes and put them in sample tubes. Then cells will enter a flow chamber filled with sheath fluid. After that, they will be arranged in a row and sprayed out from the nozzle of the flow chamber, forming a column of cells perpendicular to the incident laser beam. Cells will be excited by the laser and produce fluorescence. Finally, the optical system will collect signals, and the computer system will collect and analyze various measured signals, making statistical analysis of various indicators.

3.3. Western Blot

Western Blot is a method based on the specific binding of antigens and antibodies to detect a certain protein in complex samples. Its basic principle is to separate the mixed antigen samples on the gel plate by electrophoresis, then take the fixed matrix membrane and stick it to gel. Then the single antigen component in the gel is transferred to the imprint paper and solidified under the natural adsorption force of the imprint paper, electric field force or other external force. Finally, immune coverage techniques are applied to detect and analyze antigen immobilized matrix membranes.

3.4. Experimental design

Set up control experiments. Set AdFlt3L group as positive control group and set PBS group as negative control group. Each group should include at least 36 model rats to ensure the normal conduct of parallel experiments.

Set a concentration gradient for both AdGM-CSF and AdFlt3L. The specific concentration gradient data is as follows: $1*10^6$ IU, $1*10^7$ IU, $1*10^8$ IU, $1*10^9$ IU and $1*10^{10}$ IU.

We will euthanize the corresponding rats at around 10 a.m. after a 2-hour fast just before the injection to determine the initial value. On the 5^{th} ,10^{th} ,15^{th} ,20^{th} and 25^{th} days after injection, we will euthanize the corresponding rats at around 10 a.m. after a 2-hour fast and do the further experiments.

3.5. Establish U251 glioblastoma xenograft mice model and set control groups

Under sterile conditions, male Lewis rats (230g-260g) will be injected into the right striatum (+1mm bregma, +3mm lateral, and -4mm from the dura) with 6000 luciferase-labeled U251 cells [12]. Feed rats normally for 10 days. The baseline tumor size will be measured at around 10 a.m. after a 2-hour fast. Also, in subsequent experiments, any the size of tumor measurement by weight will be conducted under these conditions. After 10 days, divide the model rats into three groups and use the same drill hole to deliver the same volume of PBS (negative control), AdFlt3L (positive control) and AdGM-CSF (set a concentration gradient for both AdGM-CSF and AdFlt3L at the same time, like $1*10^6$ IU, $1*10^7$ IU, $1*10^8$ IU, $1*10^9$ IU and $1*10^{10}$ IU) within the tumor mass using the same coordinates as those used for implantation of U251 cells.

3.6. pAkt measured by Western Blot

Randomly select half of the rats from each concentration ($1*10^6$ IU, $1*10^7$ IU, $1*10^8$ IU, $1*10^9$ IU and $1*10^{10}$ IU) of all three groups (AdGM-CSF group, AdFlt3L group and PBS group). On the 5^{th} ,10^{th} ,15^{th} ,20^{th} and 25^{th} days after injection, euthanize the rats. Quickly obtain spleen or tumor draining lymph nodes and place them in PBS that is pre cooled on ice and contains phosphatase

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inhibitors. Operate the entire process in a low-temperature environment. Then the tissue will be mechanically ground to prepare a single-cell suspension through a 70um sieve. Perform MACS positive sorting (>90% purity) using CD11c microspheres to achieve DC enrichment. After that, the sorted DCs are directly lysed in RIPA lysis buffer which contains 1*protease/phosphatase inhibitor. Subsequently, protein testing will be conducted. Quantify protein concentration using BCA method. Then take 30ug of protein for 10% SDS-PAGE electrophoresis and wet transfer to PVDF membrane. After blocking with 5% BSA for 1 hour, incubate the primary antibodies (anti pAkt (Ser473,1:1000) and anti-total Akt (1:2000)) overnight at 4 ° C and the secondary antibody at room temperature for 1 hour, which is labeled by HRP (1:5000). Finally, signal capture is performed and the activation degree of each group's pathway is analyzed by standardizing the pAkt/total Akt ratio.

3.7. DCs proliferation measured by CD11c FACS

Select the remaining half of the rats from each concentration ($1*10^6$ IU, $1*10^7$ IU, $1*10^8$ IU, $1*10^9$ IU and $1*10^{10}$ IU) of all three groups (AdGM-CSF group, AdFlt3L group and PBS group). On the 5^{th} ,10^{th} ,15^{th} ,20^{th} and 25^{th} days after injection, euthanize the rats. Quickly obtain spleen or tumor draining lymph nodes. Prepare single-cell suspension and remove red blood cells by using red blood cell lysis method in an ice bath. Stain cells with fluorescent antibody combination CD11c+MHC-II+Lin- in the dark on the ice for 20 minutes. Wash and remove unbound antibodies. Then perform CD11c FACS and calculate the proportion and absolute number of selected DCs.

3.8. The size of tumor measured by weight

After each euthanasia in all the experiments mentioned above, under the premise of low-temperature operation throughout the process, the rats will be subjected to craniotomy for brain extraction, and the tumor tissue will be quickly weighed after being separated using microanatomical dissection.

3.9. Statistical analysis

Every experiment will be repeated at least three times. The data will be reported as the form of mean ± standard. One-way ANOVA will be employed to process data obtained from Western Blot analysis of pAkt, FACS analysis of DC proliferation and weight analysis of tumor size. After that, Tukey's post-hoc test will be used to determine if there are significant differences between two or more groups. In the Western Blot experiment, after ECL chemiluminescence development, ImageJ will be used to quantify the grayscale values of stripes. When the significant level is $p<0.05$, the result has statistical significance.

4. Results

4.1. Combination of possible results

Table 1. The combination of possible results

Combination of possible results (CR)	GM-CSF increases pAkt increases by Western Blot?	GM-CSF increases DCs by CD11c FACS?	GM-CSF decreases tumor size by weight?	Support of hypothesis
CR1	+	+	+	Yes
CR2	+	+	-	Partial

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Table 1. (continued)

CR3	+	-	+	Partial
CR4	-	+	+	Partial
CR5	-	-	+	Partial
CR6	-	+	-	Partial
CR7	+	-	-	Partial
CR8	-	-	-	No

Table legend: "+" indicates that the phenomenon observed in the experiments of AdGM-CSF group is similar to the positive control (AdFlt3L) and opposite to the negative control (PBS) , and it has statistical significance; "-" indicates that the phenomenon observed in the experiments of AdGM-CSF group is not similar to the positive control (AdFlt3L) and not opposite to the negative control (PBS), or it even doesn't have statistical significance.

According to Table 1, after the injection of AdGM-CSF, the experimental group will produce different combinations of possible results compared to the corresponding control group. The specific description of the combinations of possible results is as follows:

CR1: After the injection of AdGM-CSF, pAkt increases, DCs proliferate and the size of tumor decreases.

CR2: After the injection of AdGM-CSF, pAkt increases, DCs proliferate, but the size of tumor does not decrease.

CR3: After the injection of AdGM-CSF, pAkt increases while DCs do not proliferate, and the size of tumor decreases.

CR4: After the injection of AdGM-CSF, pAkt does not increase, but DCs proliferate and the size of tumor decreases.

CR5: After the injection of AdGM-CSF, pAkt does not increase and DCs do not proliferate, but the size of tumor decreases.

CR6: After the injection of AdGM-CSF, pAkt does not increase but DCs proliferate. However, the size of tumor does not decrease.

CR7: After the injection of AdGM-CSF, pAkt increases but DCs do not proliferate. Also, the size of tumor does not decrease.

CR8: After the injection of AdGM-CSF, pAkt does not increases, DCs do not proliferate and the size of tumor does not decrease.

4.2. Possible results for the variables of concentration and treatment duration

4.2.1. Concentration

PR1: With the increasing concentration, pAkt increases compared with the corresponding negative control group.

PR2: With the increasing concentration, pAkt decreases compared with the corresponding negative control group.

PR3: With the increasing concentration, pAkt remains unchanged compared with the corresponding negative control group.

PR4: With the increasing concentration, DCs increase compared with the corresponding negative control group.

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PR5: With the increasing concentration, DCs decrease compared with the corresponding negative control group.

PR6: With the increasing concentration, DCs remain unchanged compared with the corresponding negative control group.

PR7: With the increasing concentration, tumor size decreases compared with the corresponding negative control group.

PR8: With the increasing concentration, tumor size increases compared with the corresponding negative control group.

PR9: With the increasing concentration, tumor size remains unchanged compared with the corresponding negative control group.

4.2.2. Treatment duration

PR10: With the increasing treatment duration, pAkt increases compared with the corresponding negative control group.

PR11: With the increasing treatment duration, pAkt decreases compared with the corresponding negative control group.

PR12: With the increasing treatment duration, pAkt remains unchanged compared with the corresponding negative control group.

PR13: With the increasing treatment duration, DCs increase compared with the corresponding negative control group.

PR14: With the increasing treatment duration, DCs decrease compared with the corresponding negative control group.

PR15: With the increasing treatment duration, DCs remain unchanged compared with the corresponding negative control group.

PR16: With the increasing treatment duration, tumor size decreases compared with the corresponding negative control group.

PR17: With the increasing treatment duration, tumor size increases compared with the corresponding negative control group.

PR18: With the increasing treatment duration, tumor size remains unchanged compared with the corresponding negative control group.

5. Discussion

The purpose of the experiments is to test the treatment effects of GM-CSF on GBM by detecting pAkt,DC proliferation and tumor size. Eight combinations of possible results listed in the table will be discussed.

The result from CR1 fully support the hypothesis that GM-CSF can increase pAkt, thereby promoting DC proliferation, and enhance immunity to reduce tumor size, achieving therapeutic effects on GBM. This is consistent with the content of the article by Yan WL et al. that GM-CSF can promote DC proliferation and recruitment, and has therapeutic effects on cancer [14]. The result may be explained that GM-CSF can stimulate pAkt in DCs, then pAkt activate the mechanisms like mTOR, and the mTOR signaling pathways control cell growth and mitotic divisions through metabolic energy generated from substances like glucose, lipid and protein [15] to promote DC proliferation. And DCs can present antigens to T-cells, prime immature T-cells, activate memory T-cells and B cells [5], which enhance immune effect and may lead to a reduction in tumor size. The result once again emphasizes that GM-CSF has an important role in cancer immunotherapy and has

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great research potential. Also, compare the results with the positive control. If the tumor size is smaller than the positive control, it shows that GM-CSF is more effective in treating GBM than Flt3L. On the contrary, it indicates that the treatment effect is worse than Flt3L, but there is still therapeutic effect. At the same time, pay attention to the difference between the results and the negative control. The tumor size is smaller than the negative control, and that shows GM-CSF have the ability to treat GBM and the operation is correct. The further experiments may focus on the specific pathway of GM-CSF inducing increase in pAkt, the possibility and side effects of long-term treatment with GM-CSF.

In CR2, although pAkt and DCs are increased, tumor size does not decrease. This result partially supports the hypothesis. The phenomenon can be explained that GM-CSF has the ability to increase pAkt to increase DCs. However, at the same time, it may increase pAkt to activate Akt in glioblastoma cells too. After that, Akt activation will promote mTOR activity, and PI3K/AKT/mTOR pathway activation can promote cell proliferation, playing a significant role in oncogenic transformation and apoptosis prevention [16]. So, tumor cells increase and the tumor size increase. Compared with the positive control, this result indicates that GM-CSF is less effective in treating GBM than Flt3L. Compared with the negative control, the tumor size remains larger than the negative control, and that indicates that GM-CSF can cause severe immune suppression and has the ability to promote tumor growth. In the future, it is possible and significant to do experiments on the combination therapy of GM-CSF and other drugs to inhibit tumor cell proliferation signaling pathway and enhance tumor immunity.

The result from CR3 partially supports the hypothesis. GM-CSF can increase pAkt in DCs, but that does not cause the proliferation of DC. Yang ZY et al. explained that pAkt can also promote the survival and motility of cells through mechanisms like activation of mammalian target of rapamycin [8]. So, the result may be explained that after GM-CSF stimulates pAkt, pAkt may influence the function of mTOR and then extend DC survival or influence DC recruitment to increase DCs in the tumor microenvironment to promote immune function. If the tumor size is smaller than the positive control, it shows that GM-CSF is more effective in treating GBM than Flt3L. On the contrary, it indicates that the treatment effect is worse than Flt3L, but there is still therapeutic effect. At the same time, pay attention to the difference between the results and the negative control. The tumor size is smaller than the negative control. This shows GM-CSF have the ability to treat GBM and the operation is correct. In the experiments in the future, it is helpful to test whether there is an increase in DC survival and changes in DC content in the tumor microenvironment after treating by GM-CSF.

In CR4, the result partially supports the hypothesis and provide a possibility that GM-CSF can increase DCs through other methods, maybe increasing STAT3. STAT3 can upregulate the expression of genes that can promote antiapoptosis, like Bcl-2 and Bcl-xL, and cell cycle progression, like cyclin D1 [17]. Then cell proliferation will be promoted by relevant proteins. The increase of DC promotes immunity and lead to the decrease of the tumor size. This may explain the result. Also, Tormo AJ et al. explained that signal transducer and activator of transcription 3 (STAT3) is significant for DC progenitor expansion and DC differentiation [18]. For control trials, if the tumor size is smaller than the positive control, it shows that GM-CSF is more effective in treating GBM than Flt3L. On the contrary, it indicates that the treatment effect is worse than Flt3L, but there is still therapeutic effect. At the same time, pay attention to the difference between the results and the negative control. The tumor size is smaller than the negative control, and it shows GM-CSF have the ability to treat GBM and the operation is correct. In the future, it is feasible to conduct experiments on the effect of GM-CSF on increasing STAT3 to promote DC proliferation.

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In CR5, the result partially supports the hypothesis. It shows that GM-CSF has the therapeutic effects on GBM, but the effects don't come from DC proliferation and GM-CSF cannot promote the increase of pAkt. So, GM-CSF has other pathways to kill tumor cells. Yan WL et al. explained that GM-CSF has the immune independent effect on antitumor growth, and this effect had been confirmed in colon cancer cell line overexpressing GM-CSF and its heterodimer receptors implanted subcutaneously into immunodeficient nude mice [14]. Also, STAT3 phosphorylation can promote ferroptosis and the release of NET, which in turn help DC antigen presentation [19], further activating T-cells and enhancing immunity to kill tumor cells. Both of the above explanations may be the reasons for the result. And for control experiments, if the tumor size is smaller than the positive control, it shows that GM-CSF is more effective in treating GBM than Flt3L. On the contrary, it indicates that the treatment effect is worse than Flt3L, but there is still therapeutic effect. At the same time, pay attention to the comparison between the results and the negative control. The tumor size is smaller than the negative control. That shows GM-CSF have the ability to treat GBM and the operation is correct. In the future, we may use immunodeficient mice for further experiments to test the ability and effectiveness of GM-CSF to directly kill tumor cells without relying on immunity.

For CR6, the result partially supports the hypothesis. GM-CSF is tested that it can increase DCs without increasing pAkt. However, at the same time, it has little effect on inhibiting tumor growth. As a result, on the one hand, it can promote DC proliferation to enhance immunity; on the other hand, it may also promote the effects of immunosuppressive factors. And the result shows that the promotion of immunosuppressive factors is greater than the promotion of immunity, leading to no reduction in tumor size. The result may be explained as follows: GM-CSF can promote the proliferation of DC through other ways, for example, STAT3. STAT3 can upregulate the expression of genes that can promote ant apoptosis, like Bcl-2 and Bcl-xL, and cell cycle progression like cyclin D1 [17], which will promote cell proliferation. So that can lead to the increase of DC. However, it may increase pAkt to activate Akt in glioblastoma cells too. After that, Akt activation will promote mTOR activity, and PI3K/AKT/mTOR pathway activation can promote cell proliferation, playing a significant role in oncogenic transformation and apoptosis prevention [16], causing tumor growth. If the promotion of immunosuppressive factors like those mentioned above are greater than the promotion of immunity, the result will be caused. Compared with the positive control, this result indicates that GM-CSF is less effective in treating GBM than Flt3L. Compared with the negative control, the tumor size remains larger than the negative control. It indicates that GM-CSF can cause severe immune suppression and have the ability to promote tumor growth. As a result, further experiments will focus on the other ways that GM-CSF promotes DC proliferation, like increasing STAT3. Also, it is necessary to do experiments to compare the impact of GM-CSF on immune suppression and tumor treatment, and determine whether GM-CSF is suitable as a drug for treatment.

The result of CR7 can only partially support the hypothesis. It shows that GM-CSF can only increase pAkt, and have no effect on DC proliferation and tumor size reduction. GM-CSF can increase pAkt, but pAkt may have other impacts and don't promote DC proliferation. The result may be due to GM-CSF inhibiting the production of downstream response factors of pAkt, such as mTOR, resulting in an inability to promote DC proliferation even with an increase in pAkt, thereby suppressing tumor immunity. GM-CSF may also directly suppress immunity, leading to a failure to reduce tumor size. Treatment with excess GM-CSF may lead to prevent the activation of DCs. GM-CSF can increase the production of Foxp3+ Tregs and Gr-1+CD11b+ myeloid derived suppressor cells, thereby inhibiting the function of antigen-specific T-cells [14] and further inhibit the

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immunity, causing the tumor growth. All of the above may be explanations for the result. Compared with the positive control, this result indicates that GM-CSF is less effective in treating GBM than Flt3L. Compared with the negative control, the tumor size remains larger than the negative control. It indicates that GM-CSF can cause severe immune suppression and has the ability to promote tumor growth. So future experiments will focus on testing whether GM-CSF can inhibit other related factors besides pAkt in the DC proliferation pathway, such as PI3K/AKT/mTOR pathway, and its immune suppression.

For CR8, all parameters cannot support the hypothesis. This phenomenon may be explained that GM-CSF will cause immunosuppression that will stimulate the proliferation and survival of tumor cells. It may be explained that excessive treatment with GM-CSF can prevent the activation of DCs. Also, GM-CSF can increase the production of Foxp3+ Tregs and Gr-1+CD11b+ myeloid derived suppressor cells, thereby inhibiting the function of antigen-specific T-cells [14] and further inhibit the immunity, causing the tumor growth. Also, GM-CSF may also directly suppress immunity, leading to a failure to reduce tumor size. Considering the negative control, it may be also explained that the concentration, treatment duration, operation or the experiments environment have some problems, leading to this result. The result indicates that in the future experiments, processing conditions may need to be reset and immunosuppression may need to be considered.

In addition, the concentration and treatment duration also play an important role.

For the concentration, for PR1, it indicates that the higher the concentration of GM-CSF, the more pAkt can be stimulated to be produced. For PR2, it indicates that high concentrations of GM-CSF inhibit the production of pAkt. Additionally, the result from PR3 shows that the concentration of GM-CSF has no impact on pAkt. For PR4, it indicates that the higher the concentration of GM-CSF, the more DCs can proliferate. The result from PR5 indicates that high concentration of GM-CSF inhibits DC proliferation. Also, for PR6, it shows that the concentration of GM-CSF has no influence on DC proliferation. The result from PR7 indicates that the higher the concentration of GM-CSF, the better the therapeutic effect on GBM. On the contrary, for PR8, it indicates that high concentration of GM-CSF has immunosuppressive effects and can cause tumor development. This result fundamentally overturns the hypothesis. Also, for PR9, it shows that GM-CSF has a certain therapeutic effect but can only inhibit tumor development, which means it cannot thoroughly treat or even cure GBM patients. At the same time, it shows that the concentration may have little impact on the therapeutic effect. Compared with the positive control, we can compare the therapeutic effects of GM-CSF and Flt3L on GBM at different concentrations based on tumor size and find drug that is more suitable for using at different concentrations: the smaller the tumor size is, the better the therapeutic effect is. At the same time, focus on the comparison between the results of AdGM-CSF group and negative control. If the tumor size of AdGM-CSF group is smaller than negative control, it demonstrates that at the corresponding concentration, the use of GM-CSF is beneficial to treating GBM. However, if the tumor size of AdGM-CSF group is larger than negative control, it shows that at this concentration, GM-CSF not only has no effect on treating GBM, but also promotes tumor growth. So, we must consider the immune suppression. In addition, in the future, the experiments will focus on narrowing concentration gradient and increasing the density of concentration gradient to find the optimal concentration for treatment.

For the treatment duration, in PR10, the result indicates that the longer the treatment duration of GM-CSF, the more pAkt can be stimulated to be produced. On the contrary, the result from PR11 indicates that long treatment duration of GM-CSF inhibits the production of pAkt. Additionally, for PR12, it shows that the treatment duration of GM-CSF has no impact on pAkt. For PR13, it indicates that the longer the treatment duration of GM-CSF, the more DCs can proliferate. On the

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contrary, in PR14, the result indicates that long treatment duration of GM-CSF inhibits DC proliferation. Also, the result from PR15 demonstrates that despite different treatment duration, GM-CSF has little effect on DC proliferation. For PR16, it indicates that the longer the treatment duration of GM-CSF, the better the therapeutic effect on GBM. In PR17, the result indicates that long treatment duration of GM-CSF enhances its immunosuppressive effects, thereby facilitating tumor development. Also, the result from PR18 shows that GM-CSF has a certain therapeutic effect but can only inhibit tumor development, which means it cannot cure GBM. At the same time, it demonstrates that the treatment duration has little impact on the therapeutic effect. Compared with the positive control, we can compare the therapeutic effects of GM-CSF and Flt3L on GBM at different treatment duration based on tumor size and find drug that is more suitable for using at different treatment duration: the smaller the tumor size is, the better the therapeutic effect is. At the same time, the comparison between the results of AdGM-CSF group and negative control is also important. If the tumor size of AdGM-CSF group is smaller than negative control, it demonstrates that at the corresponding treatment duration, GM-CSF has therapeutic impact on GBM. However, if the tumor size of AdGM-CSF group is larger than negative control, it shows that at this treatment duration, GM-CSF promotes tumor growth and may have immune suppression that is harmful to treatment. In addition, in the future, the experiments will focus on narrowing treatment duration gradient and increasing the density of treatment duration gradient to find the optimal treatment duration for treatment. At the same time, it is significant to consider drug combination therapy to inhibit the side effects like immune suppression phenomenon that may be caused by long-term treatment of GM-CSF.

Additionally, future research will focus on the therapeutic principle and effect of GM-CSF combined with other drugs in the treatment of GBM so as to discover new and efficient treatment methods to meet the need of patients.

6. Conclusion

The article tests the therapeutic effect of GM-CSF on GBM through three aspects: pAkt, DC proliferation and tumor size. Eight different combinations of possible results show the possibility of using GM-CSF for the treatment of GBM, and reveals the possibility and necessity of further studying its treatment mechanism, the consequences of long-term treatment with GM-CSF and the combination therapy with other drugs. With the effort of this article, new ways of treatment on GBM will be further researched, and GM-CSF's treatment status for cancer will shift from adjuvant to main therapeutic drug. Also, it is beneficial to the health of all humanity.

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