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研究課題名（和文）Development of combined cellular and molecular target-directed therapies for glioblastoma

研究課題名（英文）Development of combined cellular and molecular target-directed therapies for glioblastoma

研究代表者

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研究成果の概要（和文）：膠芽腫は最悪性の脳原発希少がんであり、新しい治療法の開発が望まれている。最近、間葉系幹細胞が膠芽腫局所で抗腫瘍効果を示すことが報告された。我々はGSK3βのがん促進作用を発見し、その阻害による膠芽腫治療効果を実証してきた。近年、 GSK3β は間葉系幹細胞に抑制的に作用することが報告されている。そこで、膠芽腫細胞と動物モデルを対象に、 GSK3β阻害剤と間葉系幹細胞移植の治療効果を調べた。その結果、膠芽腫の幹細胞性腫瘍細胞に対する間葉系幹細胞の治療的作用はGSK3β阻害剤の併用により増強された。本研究の結果は、間葉系幹細胞移植とGSK3β阻害剤の併用による新たな膠芽腫治療法の開発に有用である。

研究成果の概要（英文）：We investigated whether mesenchymal stem cells (MSCs) transplantation benefits to treatment of glioblastoma (GBM) by enhancing anti-tumor effect in combination with GSK3β inhibitors and temozolomide (TMZ). Our experiments showed that transplantation of MSCs and GSK3β inhibition synergizes for treatment of experimental GBM and that MSCs co-cultured with GBM cells participate in regulation of GBM stemness similar to that observed under GSK3β inhibition. We also examined the interaction between patient-derived GBM stem-like cells and adipose tissue-derived MSCs in the presence and absence of GSK3β inhibition and TMZ and its influence on GBM stemness. We found that the interaction of MSCs with GBM stem-like cells is enhanced by a combined treatment by GSK3β inhibitor. These observations encouraged us to continue investigation of the biological mechanisms by which MSCs regulate GBM stemness under the control by GSK3β addressing the role of cell-to-cell interactions and local signals.

研究分野：　Neuro-oncology

キーワード：　glioblastoma　temozolomide　mesenchymal stem cells　GSK 3β

１．研究開始当初の背景

Glioblastoma (GBM) is the most frequent malignant tumor of the brain and is highly unresponsive to the currently available anticancer treatments. The proliferative and invasive activity of GBM (N Engl J Med 2008;359:942) hinders curable surgical intervention, and makes GBM highly resistant to radiation and chemotherapy (J Clin Oncol 2005;23:2411) with median patient survival showing little improvement over the past 30 years (Lancet 2002;359:1011). In human GBM acquisition of a pro-inflammatory phenotype is relevant for malignant progression due to coordinated overexpression of a panel of pro-inflammatory genes (N Engl J Med 2001; 344:114, J Neuroinflamm 2011;8:32). Consequently, there is an urgent need to develop new treatment modalities represented by molecular target-directed therapies.

Glycogen synthase kinase 3β (GSK3β) is a multi-functional protein kinase that regulates various cellular pathways depending on its substrates for phosphorylation. GSK3β regulates a range of cellular processes including energy metabolism, transcription control and cell fate determination. It has been implicated in chronic diseases including cancer (Neurochem Res 2007; 32:577). We have recently demonstrated that GSK3β promotes GBM cell survival and proliferation (Clin Cancer Res 2009;15;887, Carcinogenesis 2013;34:2206). Previously it was reported that GSK3β attenuates pluripotency in normal stem cells (Nat Med 2004;10:55). Therefore, investigating mechanisms underlying potential oncogenic role of GSK3β provides new insights into molecular pathways leading to carcinogenesis as well as development of novel strategies for GBM treatment with little adverse effects.

O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation is an independent favorable prognostic factor in patients with GBM and it is implicated in chemosensitivity to an alkylating agent temozolomide (TMZ). Patients with a methylated MGMT promoter benefit from TMZ therapy (N Engl J Med 2005;352:997). Recently I have clarified the molecular mechanisms underlying combined effect of GSK3β inhibition and TMZ against human GBM cells, demonstrating that GSK3β inhibition enhances the effect of TMZ by decreasing MGMT expression via c-Myc-mediated MGMT promoter methylation (Carcinogenesis 2013;34:2206). Based on our laboratory studies, the clinical trial of GSK3β inhibitors for GBM treatment (UMIN000005111) is currently undertaken in our University Hospital.

Mesenchymal stem cells (MSCs) are a mixed population of adult stem cells that can renew itself and differentiate to generate bone, cartilage, adipose and fibrous connective tissue. MSCs are capable of neurogenic trans-differentiation, giving rise to neurons, astrocytes, or oligodendrocytes (Science 2000;290:1179). They are easily cultured and available for autologous transplantation. Thus, in the view of MSC plasticity, their application for cell-based therapies in neurosurgery seems to be associated with less ethical problems and comparative studies with neural stem cell transplantation are of high scientific interest. The applicant has previously developed the patent-pending approach for culture conditions for bone marrow mesenchymal stem and progenitor cells. This supports efficient cell proliferation and confirms the possibility for neural trans-differentiation of MSCs (Cell Cultures News-bulletin 2009;24:52). It has been shown that MSCs migrate toward GBM cells *in vitro* and suppress their growth, inhibit angiogenesis and decrease migration of GBM cells (Cancer Res 2005;65:3307, PLoS One 2010;5:e1035, Oncol Rep 2010;23:1561, Stem Cells 2013;31:146).

GSK3β inhibition stimulates proliferation and prevents differentiation of MSCs; which is opposite to the effects observed in GBM cells (Carcinogenesis 2013;34:2206, PLoS One 2012; 7:e31502, BMC Cell Biol 2008;9:11). GSK3β promotes inflammation by stimulation of the production of a number of pro-inflammatory cytokines, such as interleukin-6, IL-1β and tumor necrosis factor, following stimulation of several types of Toll-like receptors. Moreover, GSK3β reduces the production of the anti-inflammatory cytokine IL-10 and promote angiogenesis in GBM inducing migration of endothelial cells (Nat Immunol 2005:6:777, Cancer Res 2011;71: 5374). From other side, MSCs reduce inflammation decreasing IL-6 and IL-1β expression (Am J Respir Crit Care Med 2010; 182:1047).

２．研究の目的

Our research group has demonstrated GSK3β as a promising therapeutic target in human GBM, the most refractory cancer. It has been shown that MSCs exert therapeutic effect against GBM and that GSK3β negatively regulates stemness of MSCs. Based on these knowledge, this study attempted to explore whether transplantation of MSCs in combination with GSK3β inhibition and TMZ benefits to treatment of GBM and to investigate underlying biological mechanism. This approach provides scientific basis for development of a novel, combined cell-based and molecular target-directed therapy for GBM.

３．研究の方法

For in vitro study, we isolated GBM stem-like cell culture from patients' GBM tumors, MSCs from human adipose tissues and C57BL mice bone marrow, and primary neural stem cell cultures from C57BL mice. Molecular characteristics of these cells were examined by flow cytometry to confirm the phenotypes of the respective cells (CD14, CD29, CD31, CD34, CD44, CD45, CD90, CD105).

GBM stem-like cells and MSCs were labeled with a fluorescent linker to provide fate mapping of the cells in consequent co-culture experiments and after transplantation to *in vivo* GBM model. We then examined effects of MSCs, GSK3β inhibition, TMZ and various combination of them against GBM stem-like cells by cell proliferation assay (isobologram method, median dose-effect analysis), time-lapse microscopy and by evaluation of MGMT promoter methylation status and gene expression. To address our working hypothesis of interaction between GBM stem-like cells and MSCs via GSK3β-mediated signaling, we investigate (a) c-Myc and O6-methylguanine DNA methyltransferase (MGMT) expression and MGMT promoter methylation; and (b) c-Myc and DNA (cytosine-5)-methyltransferase (DNMT) 3A binding to MGMT promoter according to our previous study (Carcinoenesis 2013;34:2206).

For animal model study, we examined effects and underlying mechanism of intracranial trans-plantation of fluorescence-labeled MSCs against GBM in C57BL mice bearing mouse GBM cells generated by using GBM line EPNT-5 (Institute for Cytology, Saint-Petersburg). This cell line was induced in C57BL mouse by 7,12-dimethyl-benzanthracene and adequately represents human GBM. We observed survival as well as consequence of neurological state and cognitive functions by open field analysis in mice following transplantation of the respective cells. We evaluated morphology and cellular interactions phenomena in GBM locus in this GBM model by histological and luminescent microscopy of brain cryosections, respectively. I have investigated the effects of co-culture with mice MSCs on mice EPNT-5 GBM cells by fluorescent microscopy.

４．研究成果

Studies in vitro and in animal model were carried out complementary to explore whether MSCs transplantation benefits to treatment of GBM by enhancing anti-tumor effect in combination with GSK3β inhibitors and TMZ. Our experiments showed that transplantation of MSCs and GSK3β inhibition synergizes for treatment of experimental GBM and that MSCs co-cultured with GBM cells participated in regulation of GBM stemness phenotype similar to that observed under GSK3β inhibition.

We examined the interaction between patient-derived GBM stem-like cells and adipose tissue-derived MSCs in the presence and absence of GSK3β inhibition and TMZ and its influence on GBM stemness phenotype. I found that the interaction of MSCs with GBM stem-like cells is enhanced by a combined treatment by GSK3β inhibitor. I have also observed by confocal time-lapse microscopy frequent phagocytosis-like cellular reactions enhanced by GSK3β inhibition of the patient-derived MSCs when they were interacting with primary GBM-SCs. These observations encouraged us to continue investigation of the biological mechanisms by which MSCs regulate GBM stemness phenotype under the control by GSK3β, addressing the role of cell-to-cell interactions including phagocytosis and local signals.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計１　件）

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〔図書〕（計　0 件）

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○出願状況（計　0 件）

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〔その他〕
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