Modulation of P2X7R in the homing of EPCs to brain glioma

Poster No.: C-0638
Congress: ECR 2014
Type: Scientific Exhibit
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Keywords: CNS, Neuroradiology brain, Molecular imaging, MR, Laboratory tests, Cancer
DOI: 10.1594/ecr2014/C-0638

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Aims and objectives

Endothelial progenitor cells (EPCs) contributed to tumor-related angiogenesis in the manner of paracrine or postnatal neovasculature, which is related to vasculogenesis. Glioma is the most common primary tumor in central nervous system and one of the hypervascular tumors in human. During the progression of glioma, a large number of angiogenesis are formed. Our previous study found that most of the newborn microvessels are located in the peritumor area, where also is the prior location of the transplanted EPCs (1). Meanwhile, previous study also confirmed that MRI can reveal the distribution of the magnetically labeled EPCs in glioma tissue in a short period (1,2). However, with the extension of time, MRI could not detect the transplanted cells since the concentration of USPIO was lower in the glioma tissue which was resulted from the death of EPCs. Therefore, how to enhance the proliferation and targeting ability of transplanted EPCs is the most important problem to resolve for using EPCs as a vector.

There are high concentration ATP in the extracellular milieu resulted from ruined neuron or hyperplastic glial in the development of brain glioma. ATP not only act as an intracellular energy source, but also as the natural ligand of P2 receptors. The P2X<sup>7</sup> receptor (P2X<sub>7</sub>R) is widely distributed, as most bone marrow cells including mononuclear cells, are reported to express the P2X<sub>7</sub>R (3). The activation of P2X<sub>7</sub>R could modulate cell proliferation, differentiation, apoptosis and migration. So, it is possible that activation of P2X<sub>7</sub>R could promote the proliferation and targeting ability of EPCs. To test the hypothesis, the present study determined the effect of P2X<sub>7</sub>R activation on proliferation and targeting ability of EPCs.
Methods and materials

The whole research was composed of in vitro and in vivo study. The in vitro experiments were conducted on cultured rat spleen-derived EPCs. For in vivo experiments, rat brain glioma model was used.

Healthy SD rat spleen derived-mononuclear cells were isolated by density gradient centrifugation, after 3 days cultured, non-attached cells were washed away by DPBS and then attached cells were cultured for 7 days, the medium was changed every 3 days. EPCs were characterized as adherent cells were double positive for Dil-acLDL uptake and lectin binding by immunofluorescence staining. Meanwhile, EPCs were also identified by special morphological characteristics, such as colony, line-like or tube-like structure.

P2X$_7$R expression in EPCs was detected by western blot. Meanwhile, the distribution of P2X$_7$R in EPCs was evaluated by immunofluorescence staining. Calcium imaging in EPCs after BzATP stimulation with different concentration in the presence or absence of BBG were detected by fluorescence. The EPCs were divided into three groups including control group, BzATP intervention group and BzATP+BBG intervention group to reveal effect of P2X$_7$R on the proliferation of EPCs. In order to confirm the influence of P2X$_7$R on apoptosis of EPCs, the 7 days cultured EPCs were stimulated with BzATP in the presence or absence of BBG. Then, the apoptosis level of treated cells was measured by immunofluorescence staining for Annexin V or DAPI. The migration of EPCs with BzATP stimulation in the presence or absence of BBG was assessed by transwell migration assay. Brain glioma model was constructed using stereotaxic apparatus in healthy SD rat. The distribution of USPIO labeled-EPCs in the glioma tissue was evaluated by in vivo MR imaging and Perls staining.
Results

The lately-isolated MNCs were small and round, after 3 days cultured, attached cells become bigger and more lucency. With the extension of time, the attached cells increased, appeared more spindle cells and exhibited the typical cobblestone morphology. After 7 days cultured, fluorescence microscope confirmed that 90% of the adherent cells were double positive for Dil-acLDL uptake and lectin binding.

Both western blot and immunofluorescence confirmed the expression of P2X7R in rat spleen-derived endothelial progenitor cells. BzATP stimulation induced a significant increase of intracellular \([\text{Ca}^{2+}]_i\) in EPCs in the manner of dose-dependent. While, BBG co-culture inhibited BzATP-induced \([\text{Ca}^{2+}]_i\) increase. P2X7R activation induced by BzATP stimulation enhanced the proliferation of EPCs which was inhibited by BBG. Although Annexin and PI staining demonstrated that the BzATP could induced more cells showing apoptosis feature, the difference between the cells with or without treatment was not significant. The migration of EPCs was promoted by BzATP stimulation, which could be reversed by BBG intervention. The MRI detected glioma growth in the cerebral parenchyma, which showed high intensity in \(T_2\)-weighted imaging (\(T_2\)WI) as well as enhancement at 10 days post-injection of C6 glioma cells. \(T_2\)WI revealed the low signal intensity in the periphery of tumor at 1 days post-transplantation of USPIO labeled-EPCs. Perls staining showed that there were numerous blue-stained cells accumulating in the tumor neovasculature region. Compared with the glioma tissue from the control group, there were less blue-stained cells in glioma tissue with BBG treatment.
Fig. 1: Western blot and immunofluorescence staining verified the expression of P2X7 receptor in rat spleen-derived endothelial progenitor cells.

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Fig. 2: BzATP induced [Ca2+]i elevation in a dose-dependent manner. # P<0.05, ## P<0.01 vs. BzATP+BBG. ** P<0.01 vs. BzATP(10µM).

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**Fig. 3:** Susceptibility weighted imaging (B) and T2 * sequence (C) demonstrated the distribution of USPIO-labeled EPCs in glioma tissue. A, T2 weighted imaging; D, T2 map.

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Fig. 4: Perls blue staining of glioma tissue. Representative images revealed the accumulation of USPIO- labeled endothelial progenitor cells at the periphery of the glioma (black arrows; top). The blue-stained cells were quantified (bottom). ##P##0.01 vs. Control.

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Conclusion

P2X$_7$R express in rat spleen-derived EPCs, activation of P2X$_7$R in EPCs could promote the proliferation and targeting ability of the cells. P2X$_7$R suppression could inhibit the accumulation of exogenous EPCs in glioma tissue, which suggests that is possible to change the targeting ability of EPCs via modulating P2X$_7$R. According to these findings, activation of P2X$_7$R can make the EPCs do better as a targeting and tracing probe for MR imaging through enhancing cell proliferation and targeting ability of EPCs.
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