The Effects of Magnetically Labeled Rat Spleen-originated Endothelial Progenitor Cells on Growth of Glioma in Vivo:

An Experimental Study

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Rationale and Objectives: The aim of this study was to investigate the effects of exogenous endothelial progenitor cells (EPCs) on the growth and invasiveness of glioma in vivo to provide an experimental basis for the value and safety of using magnetically labeled EPCs as target vectors to detect early infiltration of glioma.

Materials and Methods: EPCs were collected from the spleens of healthy Sprague-Dawley rats, made EPCs conditioned medium after identification. Four models of Sprague-Dawley rat glioma (60 rats in total) were established as a control and three experimental groups (group A, B, and C). In the control group, orthotopic transplantation of C6 glioma cells was performed. Compared to the control group, EPCs conditioned medium was added in group A and P7228-labeled EPCs were added in group B. In group C, P7228-labeled EPCs were transplanted via the tail vein. Magnetic resonance imaging and perfusion-weighted imaging were performed on several days. Tumor microvascular density and vascular endothelial growth factor expression were determined through immunohistochemistry.

Results: In group C, hypointense areas were detected at the periphery of the tumor on the first day after transplantation of EPCs, and more hypointense areas were found inside the tumor over time. Tumor size in all four groups developed significantly with increasing time ($P < .01$), but there was no marked difference among these groups at the same time ($P > .05$). No remarkable differences in microvascular density and cells positive for vascular endothelial growth factor were found at the same time among the four groups ($P > .05$).

Conclusions: Both magnetic resonance imaging and immunohistochemical findings confirmed that exogenous EPCs could not affect the biologic behavior of C6 glioma cells in vivo through a paracrine effect or by direct cellular interaction. Therefore, exogenous EPCs could not exert significant promoting effects on glioma growth.

Key Words: Endothelial progenitor cells; glioma model; cell labeling; spleen; superparamagnetic iron oxide.

Endothelial progenitor cells (EPCs), one kind of precursor cells that can proliferate and differentiate into mature endothelial cells in vitro, have sound proliferating and differentiating capacity. They not only participate in vasculogenesis at the embryonic stage but also play an important role in postnatal neovasculogenesis (1–3). EPCs can home in to a tumor site and incorporate into the tumor's vascular endothelium. They can secrete angiogenic factors that promote tumor neovasculature. In light of the homing feature of EPCs and the biocompatibility of iron oxide–labeled nanomaterial molecular probes, the use of magnetically labeled EPCs as a magnetic resonance contrast agent to detect early brain glioma is promising. However, whether transplanted exogenous EPCs can promote the development of glioma has not been clearly clarified.

In this study, we performed orthotopic transplantation and transplantation via the tail vein of P7228-labeled EPCs and used magnetic resonance imaging (MRI) to determine the distribution of EPCs in glioma tissue and their effects on the biologic behavior of tumors, such as growth and invasion, to provide an experimental basis for evaluating the clinical safety of using magnetically labeled EPCs in diagnosing early brain glioma.

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MATERIALS AND METHODS

The use of laboratory animals was in compliance with the guideline of National Institute of Health. All animal experiments were approved by the Animal Use Subcommittee.

Isolation, Culture, and Identification of Rat Spleen-originated EPCs and Preparation of EPCs Conditioned Medium

According to the method introduced by Fang et al (4), spleen-derived mononuclear cells from healthy Sprague-Dawley rats (obtained from the Experimental Animal Center of Daping Hospital, Chongqing, China) were isolated by density gradient centrifugation and plated onto a culture flask (Corning, Inc, Corning, NY). After 3 days, the cells were washed three times with phosphate-buffered saline to remove nonattached cells, and the attached cells were maintained at 37°C and 5% carbon dioxide in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA), containing 20% fetal bovine serum (Hyclone, Logan, AR). The medium was changed every 3 days. When the cells were at the subconfluent level, the cells were identified via DiI-labeled acetylated low-density lipoprotein uptake and fluoroisothiocyanate labeled lectin–1 (Sigma, St Louis, MO) binding assay, visualized by laser scanning confocal microscopy. To determine cell surface markers, cells were fixed with 4% paraformaldehyde, incubated with antibodies CD31 and CD34, and determined by laser scanning confocal microscopy.

The cells were maintained in DMEM until they were 60% to 70% confluent. The medium in the flask was changed and centrifuged at 800*g* for 8 minutes to obtain the supernatant, the cells were maintained in new DMEM for 3 to 4 days, and the process was repeated and the supernatant collected. The supernatant collected from the cells cultured at the same time was mixed and filtered; the resulting supernatant was termed EPCs conditioned medium.

Labeling EPCs with P7228

P7228 129 μL (Guerbet Asia Pacific, Hong Kong, China) and poly-L-lysine (PLL) 2.25 μL (Sigma) working solution was extracted, and DMEM was added to a volume of 2 mL to produce the desired concentrations of P7228 and PLL (37.5 and 2.25 μg/mL, respectively). The mixture was then vibrated at room temperature for 60 minutes, and the culture medium in the culture flask was changed with the above P7228-PLL complex labeling solution. The EPCs were maintained in the labeling solution at 5% carbon dioxide and 37°C overnight. Prussian blue staining was performed every other day to determine the rate of cell labeling.

Establishment of Glioma Model

C6 glioma cells (Cell Bank, Chinese Academy of Sciences, Shanghai, China) were maintained in DMEM/F_{12} containing 10% fetal bovine serum until 80% confluent, and the cells were then harvested in 0.25% trypsin/ethylenediamine tetra-acetic acid (Hyclone). The P7228-labeled cells were harvested in 0.25% trypsin/ethylenediamine tetra-acetic acid after being maintained in labeling solution for 24 hours.

Sixty female Sprague-Dawley rats weighing 210 ± 10 g were randomized into the control group and three experimental groups (groups A, B, and C). The rats were anesthetized with 10% chloral hydrate and positioned on the stereotactic apparatus (Midwest Group, Palo Alto, CA). According to the Baker method, a microsyringe of cell suspension was inoculated in the right caudate nucleus through a 1-mm burr hole at 1 mm in front of the bregma and 3 mm right of the midline. For the control group, the cell suspension containing 10 μL C6 glioma cells, group A containing 10 μL glioma cells and EPCs conditioned medium of equal volume, group B containing 10 μL glioma cells and P7228-labeled EPCs, and group C inoculated 10 μL glioma cells in the right caudate; meanwhile, the labeled equivalent EPCs were transplanted via the tail vein. After injection, the burr hole was filled with bone wax (Ethicon, Gargrave, United Kingdom), the skin incision was sewn, and the rats were caged according to group membership.

MRI Scanning for Rats with Glioma

A 1.5-T dual-gradient superconductive MRI system (GE Medical Systems, Milwaukee, WI) with 3-inch encircling coils, with gadolinium diethylenetriamine penta-acetic acid (Beijing Beilu Medical & Chemical Corporation, Beijing, China) as a contrast agent, were used. In the four groups, the gliomas were scanned on days 3, 7, 10, 15, 20, and 25 days. Group C was scanned on days 1, 3, 5, 7, and 9 after the transplantation of P7228-labeled EPCs. The routine MRI protocol included a spin-echo T1-weighted sequence (repetition time, 240 ms; echo time, 20 ms; field of view, 8 × 8; slice thickness, 2.5 mm; slice gap, 0.2 mm; matrix size, 192 × 160; number of signals acquired, 6) and a fast relaxation fast spin T2-weighted sequence (repetition time, 2360 ms; echo time,: 86.7 ms; field of view, 6 × 6; slice thickness, 2.0 mm; slice gap, 0.2 mm; matrix size, 192 × 160; number of signals acquired, 12). For perfusion-weighted imaging (PWI), the gradient echo was used in combination with echo-planar imaging (repetition time, 1500 ms; echo time, 40 ms; field of view, 6 × 6; slice thickness, 2.0 mm; slice gap, 0.2 mm; matrix size, 64 × 64; number of signals acquired, 1; flip angle, 90°). Enhanced T1-weighted imaging was performed using the same parameters as for the spin-echo T1-weighted sequence to scan the gliomas with reference to the PWI localizing line. One rat with a glioma from each group was sacrificed at each time point after scanning, tissue was fixed, and immunohistochemistry was performed.

Image Analysis

Original images were processed using GE Advantage Workstation version 4.3 (GE Medical Systems). The dynamic susceptibility contrast (DSC) perfusion data were managed with Functool version 4.5.5 for calculation to formulate the