Experimental study on the treatment of intracerebral glioma xenograft with human cytokine-induced killer cells

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1. Introduction

Malignant glioma is the most common primary brain tumor in adults. Despite aggressive treatments including surgical resection, radiotherapy and cytotoxic chemotherapy, 50% of the patients die within a year, 90% within 2 years and only very few survive for 5 years [1]. Therefore, novel therapeutic approaches to prolong survival are needed. Immunotherapy is a novel treatment for tumors that has been investigated in different types of tumors including brain tumors. In recent years, the application of cell-mediated immunity in the treatment of malignant tumor has achieved encouraging results and immune effector cell plays an important role in immune therapy against tumor. Numerous studies have demonstrated that compared with traditional lymphokine-activated killer cells (LAKs), tumor infiltrating lymphocyte (TIL) and cytotoxic T lymphocyte (CTL), cytokine-induced killer cells (CIKs) have many advantages such as enhanced cell proliferation and cytotoxic activity, an enlarged anti-tumor spectrum, more strong cytotoxicity toward drug-resistant tumor cell lines and resistance to Fas-mediated apoptosis, lower hemopoietic toxicity [2–6]. In recent years, the better curative effects of CIKs on many solid tumors have been observed in many studies [7–9].

In this paper, we studied the biological characteristics of CIKs and cytotoxic activities of CIKs and LAKs against human glioma cell lines U251 and U87 in vitro. By transplanting U251 tumor tissue into mouse brain, a rapid and stable intracranial glioma model was established in our study. Intracranial tumors of 1–2 mm in diameter could be detected by a 1.5T clinical MRI system with good image resolution. Using this experimental model, the therapeutic effect of CIKs in vivo was tested. CIKs are a kind of highly effective immune cells which have a strong suppressive effect on growth of in vitro and in vivo glioma. CIKs are likely to be used in clinical adoptive immunotherapy of intracerebral glioma.

Abstract

Objective. To investigate the phenotype changes and proliferation activities of cytokine-induced killer cells (CIKs) and lymphokine-activated killer cells (LAKs) from healthy donor, and the cytotoxicities of CIKs and LAKs to human in vitro glioma cell lines U251 and U87. Therapy of CIK intratumoral injection was evaluated in nude mouse models. Methods. CIK cells were induced from peripheral blood mononuclear cells (PBMC) of healthy donors with multiple cytokines. Phenotype analysis of CIKs and LAKs was performed with flow cytometer (FCM). The specific cytotoxicities of CIKs and LAKs against cell line U251 and U87 were determined by LDH method. After intracerebral injection of CIKs, the distribution of CIKs and the inflammatory reaction of their surrounding brain tissue were observed through continuous pathological sections. In vivo anti-tumor activity of CIKs was evaluated in athymic nude mice with intracerebral xenotransplanted U251 glioma by MRI. Results. Amount of CIKs was increased (49.83 ± 2.04) times and double positive cells, CD3+CD56+ cells, were increased from (3.36 ± 1.85%) to (44.07 ± 14.14%) with elevated absolute amount over 1000 times after 2 week culture. In vitro experiments demonstrated that compared with LAK, CIKs possessed more obvious cytotoxic activity to U251 and U87. In vivo experiments showed that there was no severe inflammatory reaction in brain tissue. CIKs can markedly inhibit intracranial xenotransplanted glioma growth by intracranial injection (P<0.01). Conclusion. CIKs are a kind of highly effective immune cells which have a strong suppressive effect on growth for in vitro and in vivo glioma. Local injection of CIKs does not produce severe damage to normal brain tissue and is likely to be used in clinical adoptive immunotherapy of intracerebral glioma.
2. Materials and methods

2.1. Cells and nude mice

Human glioma cell lines U251 and U87 were purchased from Cell Culture Center, Institute of Basic Medical Sciences. Forty-five six-week-old Balb/c nude mice of either sex weighing between 18 and 22 g were purchased from Animal Center, Chinese Academy of Medical Sciences and maintained under specific pathogen free (SPF) conditions.

2.2. Main reagents

Recombinant human IL-2 was from Peking Shuangyih Pharmaceutical Company Limited. Mouse anti-human CD3Ab and recombinant human IL-1α were purchased from Institute of Biotechnology, Academy of Military Medical Sciences. Recombinant human INF-γ and RPMI-1640, as well as fetal calf serum, were obtained from Gibco BRL (USA) and MTI from Sigma Company (USA). CD3-FITC, CD8-FITC, CD45RO-FITC, CD3-RPE, CD8-FITC and CD45-RPE were purchased from BD Pharmingen Company.

2.3. Preparation of CIKs and LAKs

Peripheral blood mononuclear cells (PBMC) were collected from volunteers with Baxter CS-300 blood cell separator and adjusted to a final cell concentration of (1–3) × 10⁸/ml with RPMI-1640 culture fluid containing 10% fetal calf serum, then were placed in a 75 cm² culture flask and were finally cultured in a humidified 5% CO₂ incubator at 37 °C. On the first day of the culture, 100 ng/ml of anti-human CD3 monoclonal antibody, 100 U/ml of recombinant human IL-1α and 1000 U/ml of recombinant human INF-γ were added, and on the second day of the culture 300 U/ml of recombinant human IL-2 was added. Fresh culture fluid replaced the old one once every 3 days, and at the same time recombinant human IL-2 and INF-γ were added in proportion to maintain their concentration. CIK cells were harvested 12–14 days after culture. LAKs were cultured for 7 days in the same complete medium supplemented only with 1000 U/ml rHIL-2. Viabilities of CIKs and LAKs were determined by trypan blue staining.

2.4. Determination of phenotypes of CIKs and LAKs with flow cytometer (FCM)

CIKs cultured for 12–14 days and LAKs cultured for 7 days were harvested and washed with PBS once, then were placed in a U-shaped 96-well plate at more than 5 × 10⁵ cells per well. Twenty microliters of fluorescent antibodies to different phenotypes was, respectively, added and incubated in the dark at 4 °C for 30–60 min, then washed twice with PBS and 100 μl of 1% paraformaldehyde was added into every well to fix, finally phenotype was determined with Beckman–Coulter XL100 FCM. At the same time, phenotype of PBMC was also determined as control.

2.5. Assessment of CIK and LAK cytotoxic activity to in vitro human glioma cell lines U251 and U87

CIK and LAK cytotoxic activity to human in vitro glioma cell lines U251 and U87 was detected by the tests of release of lactate dehydrogenase (LDH). After general digestion and washing cells with PBS, target cells were adjusted at 1 × 10⁶/ml and effector cells at 4 × 10⁴/ml. According to different ratios of effector cells to tumor cells (40:1, 20:1, 10:1), 100 μl of different concentrations of effector cells and tumor cells was, respectively, dropped in 96 culture wells, simultaneously effector cell control, target cell maximal release, target cell spontaneous release, volume correct and base-line control wells were set. Ninety-six-well plate was placed in a humidified 5% CO₂ incubator for culture for 3 h at 37 °C, after that, was taken out, then 20 μl of lysate was, respectively, added into maximal release and volume correct wells and continued to incubate for 1 h. Ninety-six-well plate was centrifuged at the rate of 1000 r/min for 4 min, and then supernatant of each well was transferred into another 96-well plate and 50 μl of reaction substrate was added into each well for incubating at room temperature for 20–30 min. Fifty microliters of stopping solution was added into each well and pigment granules were broken up with shaker. Finally, the 96-well plate was placed on enzyme calibration system for determining absorbance at the wavelength of 492 nm. The specific cytotoxicity was calculated by the following formula: specific cytotoxicity = [(experimental counts – effector control counts – target spontaneous counts)/(target maximal counts – target spontaneous counts)] × 100%.

2.6. Influence of CIKs on the normal brain tissue

Supernatant was removed following centrifugation of CIKs. CIKs were first washed twice with serum-free medium by centrifugation, then stained with 0.4% of trypan blue to count living cell ratio of more than 95%, and finally prepared with normal saline into cell suspension and adjusted at a concentration of 1 × 10⁶/ml. Twenty nude mice were anesthetized by abdominal injection of 0.2% pentobarbital sodium in prone position. Head skin was sterilized and surgical field was fixed with sterile surgical membrane. Puncture needle can pierce skull because it is very thin. A microsyringe pierced into brain tissue through the point of right 2.5 mm from cranial midline and back 2 mm from arcuate suture on the frontal part at a depth of 4 mm, then returned back at a length of 1 mm and 5 μl of CIK suspension was injected within 20 min, finally the microsyringe stayed for 2 min and was drawn. Without the use of antibiotics and after anesthesia resuscitation, nude mice were placed in sterile box. Nude mice physiological behaviors including ingestion, walk and sleep were observed. All twenty nude mice did not die. On the 1st, 2nd, 3rd, 4th, 5th, 7th and 10th day after operation, two nude mice were, respectively, killed by cervical vertebrae dislocation and the whole brain tissue was removed. The infiltration depth of CIK cell and inflammatory reaction surrounding brain tissue were observed by continuous pathological sections. The other 6 mice continue to be fed and observed, and then 3 mice were, respectively, killed in a month and two after operation.

2.7. Establishment of nude mouse brain glioma model

Establishment of subcutaneous xenotransplanted tumor model by cell inoculation: 0.2 ml of U251 cell suspension containing 1 × 10⁷ cells of U251 cells was injected into groin of 5 nude mice. Subcutaneous xenotransplanted tumor was excised when its diameter was 1 cm. After connective and necrotic tissues were removed, tumor was cut into many pieces with a diameter of about 1.5 mm for further use.

Establishment of intracranial xenotransplanted tumor model by tissue inoculation: anesthesia and surgical preparation were as previously described. Head skin was incised for exposing skull. A hole with a diameter of 3 mm was made with micro-electrical drill in the place of right 2.5 mm from cranial midline and back 2 mm from arcuate suture on the frontal part. A modified sterile trocar pumped a small piece of tumor, pierced into brain tissue at a depth of 4 mm, then returned back at a length of 1 mm, finally was drawn after the small piece of tumor was injected. Head skin was sutured with 4–0atraumatic suture. After a week of tumor tissue inoculation, brain MRI showed that the diameter of intracranial planting tumor reached 2 mm in 18 nude mice which entered experimental group.
2.8. MRI observation of intracranial tumor

The air in MRI examination room was sterilized with ultraviolet radiation and a sterile towel was spread on the examination platform. MRI was performed on a 1.5T superconductive MRI system (Signa Excite II; General Electric Medical system, Wis) with a 3 in. coil. Axial, sagittal and coronal T2-weighted fast spin-echo images were acquired (TR/TE, 2200/97; slice thickness, 2 mm; gap, 0.2 mm; NEX, 6; FOV, 70 × 70 mm; matrix, 128 × 128).

2.9. Grouping and growth characteristics of xenotransplanted tumor

CIK cell suspension was prepared as previously described. Five microliters/time/mouse was injected into tumor with micro-syringe twice a week for 4 weeks. Eighteen nude mice with xenotransplanted tumor were divided into three groups (each group with 6 mice): CIK-1 group received 6 × 10^6 CIKs per mouse, CIK-2 group 3 × 10^6 CIKs and control group 5 μl of normal saline.

The size of the tumor was observed with MRI once a week and tumor volume was calculated according to V (tumor volume) = L × W^2/2 (L is the longest diameter of the tumor and W is the widest diameter of the tumor). Nude mice were killed by cervical vertebrae dislocation and brain was drawn 28 days after treatment. The tumor inhibition rate was calculated according to the formula: tumor inhibition rate = (1 – tumor volume in experimental group/ tumor volume in control group) × 100%.

2.10. Pathological examination of tumor specimen

Tumor specimen was fixed with 10% neutral formaldehyde solution for 24 h, dehydrated in gradient alcohol, underwent transparency management with dimethylbenzene, was embedded in paraffin, sectioned at a thickness of 3–4 μm and stained with HE.

2.11. Statistical analysis

Statistical analyses for all data were performed with the SPSS 10.0 statistical software. T test or χ² test were used. Statistical significance was established at P<0.05.

3. Results

3.1. Proliferation and phenotype analysis of CIKs and LAKs

When cultured for 4, 7 and 10 days, total amount of CIKs was increased by (2.04 ± 0.24), (4.98 ± 0.22) and (21.62 ± 2.03) times, respectively. Rapid growth period of CIKs is in 11–14 days after culture, during which period the total amount of CIKs reached (5.0–7.8) × 10^8 with a mean value of (6.7 ± 0.74) × 10^8 and absolute counting of CIK cell was increased by (44.8–53.4) times with a mean value of (49.83 ± 2.04) times. Living cell ratio was more than 95% by examination of trypan blue staining.

CIKs consist of a group of different kind cells and main effector cell is CD3^+CD56^+ cell which accounted for 3.36 ± 1.85% in PBMC. When cultured with multiple cytokines for 7 days, CD3^+CD56^+ cells accounted for 8.42 ± 1.82%, and when cultured for 14 days, CD3^+CD56^+ cells accounted for 44.07 ± 14.14% and according to the total number of CIK cell proliferation the absolute counting of CD3^+CD56^+ cell was increased by over 1000 times. The amounts of CD3^+CD8^+ and CD3^+CD4^+ of CIKs were, respectively, increased by over 100 times. FCM results showed that the expression of CD3^+, CD3^+CD4^+, CD3^+CD8^+ and CD3^+CD56^+ of CIKs was all obviously increased, but proportions of memory T cell (CD45RO^+/CD8^+ and NK cell (CD3^-CD16^+CD56^+) were not markedly changed (Fig. 1).

Phenotypic analysis of LAK 7 days after culture indicates that percentage of CD3^-CD16^+CD56^+ was 46.93 ± 14.71%. In brief, the most important subpopulation of CIK is CD3^+CD56^+, main part of LAK is NK cell.

3.2. Analysis for the cytotoxic activities of CIKs and LAKs to human in vitro glioma cell lines U251 and U87

At E/T ratio of 40:1, 20:1 and 10:1, mean values of cytotoxicity of CIK against U251 and U87 were 45.8 ± 9.6%, 29.7 ± 7.2% and 19.7 ± 4.3%, and 51.2 ± 8.2%, 26.5 ± 6.4% and 17.3 ± 3.8%, respectively, and those of LAK against U251 and U87 were 29.3 ± 4.9%, 21.6 ± 3.3% and 9.2 ± 1.7%, and 27.7 ± 7.2%, 16.3 ± 3.8% and 10.4 ± 2.1%, respectively. CIKs showed more effective role in glioma tumor cell line lysis than LAKs at different E/T ratios with P<0.05 (Fig. 2).

3.3. Influence of CIKs on brain tissue

Ingestion, walk and sleep of nude mice given CIK cells were normal. Pathological sections showed that most CIKs were confined within the area of 500 μm around puncture needle passage, brain cells around puncture needle passage mildly swelled, edema area was localized, there was no obvious necrocytosis, brain tissue outside edema area was normal. With the extension of time, brain tissue edema area gradually extended and reached peak until 7th day, but the edema on 10th day after injection of CIK returned to the level on the fourth day. Continuous pathological sections in 1 and 2 months after injection showed no needle passage and CIK cell infiltration (Fig. 3).

3.4. Xenotransplanted tumor growth curve and tumor inhibition rate in three groups

Alteration of tumor volume can be dynamically observed with brain MRI (Fig. 4). After the treatment of injecting CIKs, CIK cell groups showed obvious suppressive effect on tumor growth, and there were markedly statistical differences between two therapeutic groups and control group in tumor volume 2 weeks after treatment (P<0.01). Although the group with high dose of CIK cell showed stronger suppressive effect, there was no statistical difference between the two CIK groups (Fig. 5). On 7th, 14th, 21st, and 28th day after treatment, tumor inhibition rate in CIK-1 group was 27.8%, 45.00%, 40.40% and 44.20% and in CIK-2 group 24.1%, 39.0%, 29.6% and 32.3%, respectively.

3.5. Pathological examination

HE staining sections in control group showed tumor cells were of round shape and polygonal with uniform distribution and compact arrangement. Cell nucleus appeared rounded and oval with rich chromatin and deep dyeing. Mitosis was common. Less cytoplasm and intercellular substance, as well as slight eosinophilia, were seen. Invasive growth of tumor to the surrounding brain tissue was present. Compared with control group, tumor cells in CIK-1 and CIK-2 group were obviously decreased, and infiltration of many lymphocyte-like cells and necrocytosis were apparent (Fig. 6).

4. Discussion

CIKs consist of a group of different kind of cells obtained from PBMC by the stimulation of multiple cytokines. Double positive cell, CD3^+CD56^+ derived from T cells [10], is the most important effector cell in CIKs which simultaneously expresses surface marker CD56 antigen of T cell and NK cell, so that it possesses either the strong anti-tumor effects of T cell or the non-major histocompatibility complex (MHC)-restricted anti-tumor advantage of NK cell. CIK is a more powerful immunologic effector cell
than other immunocytes. Based on CIK advantages in cell number and cytotoxicity, the total lytic unit (TLU) of CIKs is 73 times the TLU of LAKs [10]. CIKs-mediated cytotoxicity was not restricted by MHC gene products, so it showed more effective cytotoxicity against NK sensitive and non-sensitive solid tumor cell lines [3,4,6,8,10–14]. CIKs possessed a higher level of cytotoxicity than LAKs toward drug-resistant tumor cell lines [3]. A variety of malignancies express FasL, which can induce apoptosis of effector lymphocytes and may inhibit cellular immunity. CIKs are suitable for immunotherapy against FasL-positive tumors [5]. The immunosuppressive drugs cyclosporine (CsA) and FK506 inhibited anti-C3D3-mediated degranulation, but did not affect cytotoxicity of CIKs against tumor target cells [12]. CIKs did not show obviously toxic effect on hematopoietic stem cells [6,11]. CIKs exhibited increased levels of cytoplasmic interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), γ-interferon (IFN-γ) and perforin [13,14].

Fig. 1. The phenotypic analysis of PBMC, CIK and LAK. The phenotypic characteristics of PBMCs, CIKs and LAKs are displayed. All cell samples were stained with FITC-labeled CD3, PE-labeled CD4, CD8 and CD56. (1) The typical flow cytometry graphs of PBMCs, CIKs and LAKs. (2) Phenotypic analyses of PBMC and CIKs 12–14 days after culture indicate that percentages of CD3+, CD3+/CD4+, CD3+/CD8+ and CD56+ cell subsets are increased from 50.39 ± 9.28%, 29.34 ± 4.62%, 20.07 ± 6.18%, 3.36 ± 1.85% and 14.43 ± 5.12% to 83.24 ± 10.69%, 46.39 ± 12.49%, 37.55 ± 15.59%, 44.07 ± 14.14% and 35.08 ± 15.23%, respectively, with P values < 0.01. However, percentages of CD3+/CD16+/CD56+, CD14+, CD20+ cell subsets are decreased from 13.52 ± 7.3%, 18.37 ± 11.68% and 13.76 ± 6.34% to 5.44 ± 3.83%, 4.69 ± 1.81% and 7.58 ± 2.7%, respectively, with P values < 0.05. Phenotypic analysis of LAK 7 days after culture indicates that percentages of CD3+, CD3+/CD4+, CD3+/CD8+, CD3+/CD56+, CD25+, CD20+, CD3+/CD16+/CD56+ and CD14+ cell are 62.75 ± 7.05%, 37.83 ± 9.46%, 10.26 ± 1.68%, 29.79 ± 11.09%, 31.78 ± 12.39%, 9.73 ± 2.28%, 46.93 ± 14.71% and 5.24 ± 1.32%, respectively.

Fig. 2. Cytotoxicity of CIKs and LAKs. Cytotoxicity of CIKs and LAKs against two different glioma cell lines (U251, U87) at different effector-to-target ratios in vitro is examined using a nonradioactive cytotoxic test. All experiments are performed in triplicate. CIKs show more effective lysis of glioma tumor cell lines than LAKs at each E/T ratio. At E/T ratio of 40:1, 20:1 and 10:1, mean values of cytotoxicity of CIKs and LAKs against U251 are 45.8 ± 9.6% vs 29.3 ± 4.9%, 29.7 ± 7.2% vs 21.6 ± 3.3% and 19.7 ± 4.3% vs 9.2 ± 1.7%, respectively, with P values < 0.05. Those of CIKs and LAKs against U87 are 51.2 ± 8.2% vs 27.7 ± 7.2%, 26.5 ± 6.4% vs 16.3 ± 3.8% and 17.3 ± 3.8% vs 10.4 ± 2.1%, respectively, with P values < 0.05.
Fig. 3. Pathological observation of CIKs distribution in brain tissue (HE staining, A × 200, B × 100, C × 100). Arrow indicates edema area of brain tissue around needle pathway. (3A) The figure shows that the reaction of surrounding brain tissue is mild and edema was not obvious on the 1st day after CIKs injection. There is not also obvious inflammation reaction of the brain tissue near the injection place. (3B) Results of the pathologic observation show that there is a little edema on the 4th day after CIKs injection. (3C) The pathologic results are similar to those of (B) and the brain tissue is mildly swelled up and slightly bleeding on the 7th day after CIKs injection. There is not serious necrosis after injection.

Some studies have indicated anti-tumor mechanisms of CIKs including direct cytotoxicity to tumor cells, tumor cell apoptosis induced by CIK cells through FasL expression, and the increase in secretion of multiple cytokines to produce direct suppressive effect and to indirectly kill tumor cells by regulating immune response system. Studies on clinical treatment of solid tumors have demonstrated that CIKs can not only directly kill tumor cells but also regulate and enhance immunologic function to inhibit tumor growth and recurrence [8,9].

Fig. 4. Intracranial glioma engrafted animal model and MRI scan. MRI was performed on a 1.5 T superconductive MRI system (Signa Excite II; General Electric Medical system, Wix) with a 3 in. coil. Axial, sagittal and coronal T2-weighted fast spin-echo images were acquired (TR/TE, 2200/97; slice thickness, 2 mm; gap, 0.2 mm; NEX, 6; FOV, 70 × 70 mm; matrix, 128 × 128). (1) One nude mouse in control group at the 4th week. (A) and (B) show that we have founded a successful intracranial glioma engrafted animal model. The arrows indicate the tumor tissue. (C) and (D) are the three-dimensional MRI scan of intracranial engrafted glioma. (2) A mouse in CIK-2 group at the 4th week.
The characteristics that glioma easily recurred in local area and distant metastasis is rare suggest that local treatment is very important. Direct cytoxicity of CIKs to tumor cells and the effects of CIKs on tumor cell apoptosis are greatly associated with the ratio of CIKs to tumor cells, so that the local presence of numerous immune cells is the basis of tumor local immunotherapy. A great deal of peripheral blood immune cells cannot concentrate around tumor to kill tumor cells because of blood–brain barrier, so that the clinical curative effect by giving a lot of intravenous immune cells for the treatment of glioma is not satisfactory. Since the first paper about phase I clinical trials of IL-2 and LAKs used in the treatment of recurrent glioma was reported by Jacobs et al. in 1986, although local application of LAKs for treating glioma has been used in some clinical practice for a long time and achieved some effects, total curative effect is not optimistic [15–17]. The main causes include insufficient LAKs, poor cytoxicity, short duration and bad specificity. Severe blood vessel transudation syndrome, neurotoxicity, cerebral edema and disorder of consciousness can occur in application of massive dose of IL-2, so that IL-2 has to be reduced even stopped. In the subsequent studies about the treatment of glioma with TIL cells [18,19], since separating TIL cells from tissue and obtaining enough TIL cells are difficult widespread use in clinical practice has been limited. Studies in other solid tumors demonstrated that CIK cell is superior to LAK and TIL cell in many aspects including in vitro proliferation, cytoxic activity, in vivo survival time and no need of numerous IL-2 [2–6]. The local treatment of glioma with CIK cells is a research direction which is going very well.

Infiltration of numerous lymphocytes in brain tissue is rare due to blood–brain barrier. Our experimental results showed that after CIK cells were injected into local brain tissue, the alteration of biological behaviors of experimental mice was not obvious, severe inflammation and necrosis of surrounding brain tissue were not observed, majority of CIKs were confined within the area of 500 μm around puncture needle passage, and with the extension of time, distant infiltration was not observed.

According to “soil-seed” theory, survival of glioma cell requires specific metabolic environment which can be provided only by central nervous system. Jaworski et al. inoculated the same glioma cell lines into brain tissue and abdominal cavity, respectively, and discovered that the expression of BEHAB appeared obviously different in xenotransplanted tumors which suggested that there are different biological behavior characteristics between orthotopic and heterotopic transplantation glioma [20]. Intracranial orthotopic
model can better exhibit glioma biological characteristics. Models established by stereotactically injecting cell suspension into the brain tissue of mice [20] were complex, time-consuming and unpractical because it was very difficult to deal with a large number of animals within a short period. By transplanting U251 tumor tissue into brains of mice, a quick and reliable intracranial glioma model was established in our experiment. The mice brain tumors can be measured and followed up sequentially by serial MR imaging, so that the therapeutic effect of CIK on glioma in vivo can be tested in living mice, avoiding killing mice. Since the skull of nude mice is very thin, with the increase of tumor size, the skull rose gradually and no mouse died of cerebral hernia.

Magnetic resonance imaging (MRI) is an emerging technique that provides a powerful, non-invasive tool for in vivo studies of cancer therapy in animal models, epically in intracranial model. Some studies in rodent used a high-field MRI method to increase the throughput of imaging [21,22]. Though high quality images would be got with 3–7 T MRI system and the therapeutic effect of CIK on glioma in vivo can be tested in living mice, avoiding killing mice.

Our experimental results indicated that compared with LAKs, CIK cells from healthy human possess more rapid proliferation, high anti-tumor activity and strong cytotoxicity against glioma cell lines U251 and U87 in vitro. CIKs can still maintain a certain anti-tumor activity at lower ratio of CIKs to tumor cells, and with the elevated ratio of CIK cells to tumor cells, anti-tumor activity is gradually increased. Using our easy and dependable orthotopic glioma model which was established through transplanting U251 tumor tissue and followed by MRI, we demonstrated that injection of CIKs into nude mouse glioma tumor could effectively inhibit tumor growth and tumor inhibition rate achieved 44.2%. Our results provide experimental basis for further study on the treatment of glioma with CIK cells. Our experimental results indicated that CIKs have stronger direct cytotoxic effects on glioma cells, suggesting that CIKs are likely to become new adoptive immune cells to exert strong effect on the treatment of brain glioma and become a novel accessory treatment following surgery, radiotherapy and chemotherapy.

References
