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·Minireview·

Dendritic cell-based immunotherapy for malignant glioma

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Abstract: The immunotherapy for malignant glioma faces unique difficult, due to some anatomical and immunological characteristics including the existence of blood brain barrier, the absence of lymphatic tissues and dendritic cells (DCs) in the central nervous system (CNS) parenchyma, and the presence of an immunosuppressive microenvironment. Therefore, immunotherapeutic approaches will not be beneficial unless the compromised immune status in malignant glioma patients is overcome. DC-based immunotherapy, vaccinating cancer patients with DCs pulsed with various tumor antigens, is one of the most promising immunotherapeutic approaches for treatment of malignant glioma because it seems able to overcome, at least partially, the immunosuppressive state associated with primary malignancies. The preparation of DCs, choice of antigen, and route and schedule of administration are improving and optimizing with rapid development of molecular biology and gene engineering technology. DC vaccination in humans, after a number of pre-clinical models and clinical trials, would increase the clinical benefits for malignant glioma immunotherapy.

Keywords: dendritic cell; immunotherapy; malignant glioma

Despite malignant gliomas are located in the central nervous system (CNS), a site which is described as "immune privileged", numerous animal model experiments and clinical trials have demonstrated that they can be effectively targeted by activated immune system following various immunotherapeutic strategies. Immunotherapy is designed to aid immune system to recognize and destroy tumor cells in order to eliminate the tumor burden. One of the most promising immunotherapeutic approaches for treatment of malignant glioma is vaccination with dendritic cells (DCs) pulsed with tumor antigens. This review will highlight recent advances of DC-based immunotherapy for malignant glioma.

1 Challenges in malignant glioma immunotherapy

Both the anatomical characteristics of CNS and the immune milieu of malignant glioma create a pessimistic scenario that effective immunotherapy faces many challenges. Firstly, the brain parenchyma is not only protected by the blood brain barrier (BBB), but also lack of lymphatic tissues and DCs. So the immunoreaction of brain parenchyma is different from that outside the central nervous system, in which various antigens can be phagocytosed and transported to peripheral lymphoid organs to elicit an adaptive immune response[1-5]. Furthermore, there is a complete absence of lymphatic structures, and noticeable absence of immune cells in the brain parenchyma of higher vertebrates. Neutrophils, the largest population of circulating leukocytes involved in innate immunity, are rarely detected in either cerebrospinal fluid (CSF) or brain parenchyma[6,7]. DCs have not been detected in the normal brain[6,8] although they are observed in the meninges and choroids plexi[2,3,9,10]. These suggest that the complete monitoring for foreign agents is deficient and which in turn limits the response of immune system to antigens in the CNS.

Secondly, the presence of malignant glioma adds another set of challenges by creating an immunosuppressive environment in which tumor can evade monitoring and is favorable to their continued growth. Within the glioma mass,

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through the secretion of interleukin-10 (IL-10), prostaglandin E2 (PGE2) and tumor growth factor α (TGF-α)[11], T-cell development, proliferation, activation, and cytotoxic T-cell are inhibited, major histocompatibility complex (MHC) expression is down regulated, and antigen presentation to $CD4^+$ T cells decreased[12]. These evidences indicate that the environment created by malignant glioma cells actively limits the ability of immune system to eliminate tumor, so immunotherapy for malignant glioma faces unique difficult.

2 A better understanding of DC biology for malignant glioma immunotherapy

As described above, immunotherapeutic approaches would not benefit unless the compromised immune status in malignant glioma patients is overcome. Cellular immunotherapies, such as adoptive transfer of lymphokine-activated killer cells (LAK), tumor-infiltrating lymphocytes (TIL), and tumor-sensitized cytotoxic T lymphocyte (CTL), as well as vaccination with irradiated tumor cells have been performed, but there were limited effects in glioma patients[13]. Tumor-antigen pulsed DCs, which are used as vaccines in vivo, offer the unique feature of presenting tumor specific peptides in the context of an efficient antigen processing and presenting machinery and a multitude of costimulatory molecules, thus stimulating immune effector cells in a very potent way.

There are usually two pathways to activate T cells. One is direct way, by which tumor antigen is directly presented to T cells and subsequently activates the antigen-specific T cell. Another is indirect pathway, utilizing specialized potent antigen presenting cells (APCs) to present antigen and activate T cells. DCs are the most potent APCs known to date. Upon the stimulation of inflammatory mediators and pathogens, immature DC resided in the peripheral tissues efficiently uptake and process antigens, migrate to the T cell areas of lymphoid organs and mature. Mature DCs lose their antigen-capturing capacity and present processed endogenous and exogenous antigens to naive T cells in a human leucocyte antigen (HLA)-restricted manner. CTL are thought to be the main effector cells in tumor rejection. DCs' infiltration into tumors has been associated with prolonged survival and reduced metastasizing capacity in cancer patients[14].

DC-based immunotherapy refers to vaccinating cancer patients with DCs pulsed with various tumor antigens. Theoretically, co-injection of DCs and co-stimulatory molecules or DC growth factors will not only stimulate various effector immune cells, but also improve antigen presentation to DCs. Accumulative evidences demonstrate that immunotherapy with DC seems able to overcome, at least partially, the immunosuppressive state associated with primary malignancies. DC therapy proved to be safe in both animal models and clinical trials. No serious side effects and no evidence of autoimmune toxicity were observed until now. So DC-based immunotherapy for various malignancies is now gaining increasing attentions.

3 Dendritic cell-based immunotherapy for malignant glioma

There are various successful reports describing the efficacy of DC-based vaccination for malignant glioma both in extracranial experimental neoplastic models and clincal trials. Liau and his colleagues[15] described the optimistic use of tumor-peptide pulsed DC vaccination for treatment of established intracranial gliomas in rats. Yu *et al*.[16] have successfully performed a DC vaccine treatment in a phase I clinical trial involving patients with malignant glioma. DCs from patients' peripheral blood mononuclear cells (PBMCs) were pulsed *ex vivo* with autologous tumor cell surface peptides which were isolated by acid-elution. Nine patients (two with grade III astrocytoma, seven with glioblastoma) received DC vaccine following surgical resection and external beam radiotherapy. Result showed that the patients in the study group had a longer survival time than the age and gender matched controls who had undergone the surgical resection and external beam radiotherapy but without DC immunization. This result indicated that DC-based immunotherapy for malignant gioma patients was safe and effective, thus encouraged the expansion of this study into phase II and III clinical trials. Now key problems in the study of DC vaccination for malignant gioma are as follows.

3.1 Source and maturation of DCs In general, DCs are large-scale isolated, expanded and induced maturation *in vivo*. Proliferating $CD34^+$ progenitor cells and non-proliferating $CD14^+$ monocytic precursors from peripheral blood (PBMC) are the main souse of immature DCs[17-21]. Furthermore, spleen-generated DCs are also included in DC-precursors preparation[22]. Differences in their T cell priming capacity seemed to be mainly due to varying culture conditions[23]. Despite DCs

from different origins can stimulate antitumor immune responses, the choice of the optimal precursor cells for generation of clinically administered DC vaccines does need to be further examined. Accumulative evidences demonstrate that cultured mature DCs are superior to immature DCs in eliciting both protective immunity and CTL responses against established tumors *in vivo*[24]. Thus some cytokines and chemokines such as GM-CSF, IL-4, IL-13, CD40L, TNF-α and IL-2 should be co-cultured to promote the differentiation and maturation of DCs[25,26]. Recently, it has been reported[27] that DCs were expanded 20-fold *in vivo* by using Fems-like tyrosine 3 ligand (Flt-3) for treatment of cancer patients.

3.2 Choice of Antigen The most important point in DC-based immunotherapy research is how to pulse DCs with appropriate peptide or antigen and then boost the tumor-specific CTL responses. A multitude of antigen-loading strategies have adopted effectively up to now, including cytokines, tumor-associated antigens, tumor cell lysate[17,20,28], tumor-derived mRNA[18], intact tumor cells[19,22], apoptotic body, and so on.

As critical participants in the processes such as proliferation, differentiation, and antigen presentation of DCs, some cytokines combined with DCs could improve the vaccinal efficacy in theory. Transgenes, such as immunostimulatory cytokines (e.g. IL-12, IL-15, IL-18, GM-CSF) and costimulatory molecules (e.g. B7-1, ICAM-1), have been delivered by viral and non-viral vectors into DCs, and substantially improve the priming of CTL and $CD4^+$ T helper cells[29,30].

Specific recognition of tumor cells by infiltrating CTL is mediated by MHC-restricted presentation of antigens by glioma cells. A patient who was immunized with DCs pulsed with allogeneic MHC class I-matched glioblastoma peptides had shown a limited immunological response[21]. In malignant gliomas, only few tumor associated antigens representing targets for CTL were described, such as MAGE-1, MAGE-3 and SART3[31,32]. After being delivered into DCs by viral and non-viral vectors, transgenes tumor-associated antigens (e.g. MART-1, MAGE-3) produced by genetic engineering means showed substantial enhancement in their immunostimulatory capacity[29,30].

In fact, use of DCs loaded with a panel of tumor-associated antigens (such as tumor cell lysate or a mixture of peptides eluted from tumor cell membranes) seems to be a more promising therapy than use of DCs pulsed with defined tumor peptides. Liu *et al*.[33] showed the efficiency of DCs in treating intracerebral glioma in their rat C6 glioma model. From the next day after intracranial implantation of C6 cells (1×10^5), SD rats received three-weekly intratumoral injections of bone-marrow generated DCs which were pulsed *ex vivo* with acid-eluted antigens from C6 glioma cells. Results indicated that tumor group had many more necrotic cells than control group ($P < 0.01$), and DCs pulsed with the acid-eluted piptides derived from autologous tumors could promote tumor necrosis. An *in vitro* research also showed that immunization with DCs pulsed with allogeneic glioma-association antigen by freeze-melt method could produce a significant immunological response[34].

Transfection of tumor-derived cDNA or mRNA into DC via gene engineering technology is also one of the most promising approaches to pulse DC vaccines. Yamanaka R and his colleagues[18] utilized genetically modified DCs which were pulsed with Semilili Forest Virus (SFV)-mediated complementary DNA to enhance the antitumor immune response. In their model, the glioma RNA and the glioma lysate induced equal CTL response in DC-vaccinated mice, but the SFV-mediated glioma cDNA had a stronger stimulatory capacity and resulted in cures of glioma-bearing animals. Moreover, cDNA can be easily obtained and amplified by RT-PCR, which make DC therapy overcome the dilemma that large amounts of tumor tissue are required for preparation of tumor lysate, tumor mRNA, and acid-eluted membrane peptides, while only small quantity of tumor tissue is available.

Recently, hybrid cells produced by fusions of tumor and autologous DCs have demonstrated remarkable efficacy in stimulating the antitumor immune response of extra-cranial neoplasms in both preclinical and clinical studies. Akasaki *et al*.[19] studied the antitumor immunity conferred by fusions of dendritic cells and glioma cells in a mouse brain tumor model. Administration of fusion cells (FCs) alone had limited effects on survival rate of the mice bearing brain tumors; which was remarkably prolonged, however, by combined administration of FCs and recombinant interleukin-12 (rIL-12). Coadministration of FCs and rIL-12 also improved the CTL activity against glioma cells compared with only administration of FCs or rIL-12. These data support the therapeutic effect of combining FC-based vaccine therapy and rIL-12 administration. Kikuchi T and his colleagues investigated the safety and clinical response to the immunotherapy using fusion of dendritic and glioma cells in a clinical trial involving

eight malignant glioma patients[35]. DCs were generated from peripheral blood and cultured autologous glioma cells were established from surgical specimens in each case. The fusion cells of dendritic and glioma cells were prepared with polyethylene glycol. The fusion efficiency ranged from 9.2% to 35.3% (mean, 21.9%). All patients received the immunization of FCs every three weeks, totally 3 to 7 times. FCs were injected intradermally close to a cervical lymph node. The percentage of CD16- and CD56-positive cells in peripheral blood lymphocytes slightly increased after immunization in 4 out of 5 cases investigated. Peripheral blood mononuclear cells were incubated with irradiated autologous glioma or U87MG cells and supernatants were harvested. In 6 cases analyzed, the concentration of interferon-gamma in the supernatant increased after immunization. There were two partial responses and no serious adverse effects. Although the results of the phase I clinical trial of fusion cells indicated that this treatment induced immune responses safely, a statistically significant treatment-associated response rate, due to limited sample population, still unable to be established. Thus further evaluation of the role of adjuvant cytokines is necessary.

3.3 Route and Schedule of Administration Efficient T cell priming *in vivo* requires migration of DCs to the T cell areas of lymphoid organs. So the route and schedule of vaccine administration seems to be of critical importance. Up to now, various possible administration routes including intravenous injection (i.v.), intradermal injection (i.d.), subcutaneous injection (s.c.), intraperitoneal injection (i.p.), and intranodal injection (i.n.) were used to augment the tumor-specific immunity of DC vaccinations. Increasing data suggests that tumor antigen-specific T cell responses could be elicited in all cancer patients who treated by DC vaccinations either via a single route or via a combination of i.v., s.c., i.d. and i.n. administration[36]. Ali S *et al.*[37] developed a novel approach, intracranial injection, attempting to increase DC's infiltration into glioma. Results demonstrated that the infiltration of macrophages and $CD8^+$ T-cells into tumor increased largely and the survival rate in rat model improved significantly. Although the optimal schedule for DC immunotherapy is still under investigation, a consensus that DC vaccines should be administered repeatedly has reached, as booster immunizations was shown to increase antigen-specific T cell responses strongly[38]. Akasaki *et al.*[19] showed that tumor-specific CTL-responses increased significantly if glioma-bearing animals received two times of DC injections instead of only one time ($P < 0.001$). Prolonged survival rates and even cure of tumor-bearing animals were achieved by either s.c., i.p. (animal studies) or i.d. (human trials) injection of DCs, implying that all the three routes enable DCs to get in close with effector T cells, e.g. migrating to draining lymph nodes[38].

4 Conclusion

As an adjuvant treatment, the use of dendritic-cell based immunotherapy now is still limited in the situations when open resection of tumor is inaccessible. With the rapid development of molecular biology and gene engineering technology, mechanisms of DCs in the setting of glioma-induced immunosuppression will be better understood. Furthermore, the preparation, immunotherapeutic efficiency, and constancy of DC vaccination will be optimized and improved, and elimination of major side-effects for safer clinical application can be expected.

References:

[1] Cartmell T, Southgate T, Rees GS, Castro MG, Lowenstein PR, Luheshi GN. Interleukin-1 mediates a rapid inflammatory response after injection of adenoviral vectors into the brain. J Neurosci 1999, 19: 1517-1523.

[2] Harling-Berg CJ, Park TJ, Knopf PM. Role of the cervical lymphatics in the Th2-type hierarchy of CNS immune regulation. J Neuroimmunol 1999, 101: 111-127.

[3] Perry VH. A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. J Neuroimmunol 1998, 90: 113-121.

[4] Stevenson PG, Freeman S, Bangham CR, Hawke S. Virus dissemination through the brain parenchyma without immunologic control. J Immunol 1997, 159: 1876-1884.

[5] Thomas CE, Schiedner G, Kochanek S, Castro MG, Lowenstein PR. Peripheral infection with adenovirus causes unexpected long-term brain inflammation in animals injected intracranially with first-generation, but not with high-capacity, adenovirus vectors: toward realistic long-term neurological gene therapy for chronic diseases. Proc Nat Acad Sci USA 2000, 97: 7482-7487.

[6] Ransohoff RM, Kivisakk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol 2003, 3: 569-581.

[7] Perry VH, Andersson PB. The inflammatory response in the CNS. Neuropathol Appl Neurobiol 1992, 18: 454-459.

[8] Lowenstein PR. Immunology of viral-vector-mediated gene trans-

fer into the brain: an evolutionary and developmental perspective. Trends Immunol 2002, 23: 23-30.

[9] Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by CNS antigen- presenting cells: different roles for microglia and astrocytes. Immunol Today 2000, 21: 141-147.

[10] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998, 392: 245-252.

[11] Dix AR, Brooks WH, Roszman TL, Morford LA. Immune defects observed in patients with primary malignant brain tumors. J Neuroimmunol 1999, 100: 216-232.

[12] Zuber P, Kuppner MC, De Tribolet N. Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. Eur J Immunol 1988, 18: 1623-1626.

[13] Pollack IF, Okada H, Chambers WH. Exploitation of immune mechanisms in the treatment of central nervous system cancer. Semin Pediatr Neurol 2000, 7: 131-143.

[14] Zeid NA, Muller HK. S100 positive dendritic cells in human lung tumors associated with cell differentiation and enhanced survival. Pathology 1993, 25: 338-343.

[15] Liau LM, Black KL, Prins RM, Sykes SN, DiPatre PL, Cloughesy TF, *et al.* Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. J Neurosurg 1999, 90: 1115-1124.

[16] Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, *et al*. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res 2001, 61: 842-847.

[17] Heimberger AB, Crotty LE, Archer GE, McLendon RE, Friedman A, Dranoff G, *et al*. Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. J Neuroimmunol 2000, 103: 16-25.

[18] Yamanaka R, Zullo SA, Tanaka R, Blaese M, Xanthopoulos K. Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semiliki Forest virus-mediated complementary DNA. J Neurosurg 2001, 94: 474-481.

[19] Akasaki Y, Kikuchi T, Homma S, Abe T, Kofe D, Ohno T. Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. J Immunother 2001, 24: 106-113.

[20] Ni H, Spellman SR, Jean WC, Hall WA, Low WC. Immunization with dendritic cells pulsed with tumor extract increases survival of mice bearing intracranial gliomas. J Neurooncol 2001, 51: 1-9.

[21] Liau LM, Black KL, Martin NA, Sykes SN, Bronstein JM, Jouben-Steele L, *et al*. Treatment of a glioblastoma patient by vaccination with autologous dendritic cells pulsed with allogeneic major histocompatibility complex class I-matched tumor peptides. Neurosurg Focus 2000, 9: e8.

[22] Siesjö P, Visse E, Sjogren HO. Cure of established, intracerebral rat gliomas induced by therapeutic immunizations with tumor cells and purified APC or adjuvant IFN-γ treatment. J Immunother Emphasis Tumor Immunol 1996, 19: 334-345.

[23] Ferlazzo G, Klein J, Paliard X, Wei WZ, Galy A. Dendriticcells generated from $CD34^+$ progenitor cells with flt3 ligand, c-kit ligand, GM-CSF, IL-4, and TNF-alpha are functional antigen-presenting cells resembling mature monocytederived dendritic cells. J Immunother 2000, 23: 48-58.

[24] Labeur MS, Roters B, Pers B, Mehling A, Luger TA, Schwarz T, *et al*. Generation of tumor immunity by bone marrow-derived dendritic cells correlates with dendritic cell maturation stage. J Immunol 1999, 162: 168-175.

[25] Schreurs MW, Eggert AA, Punt CJ, Figdor CG, Adema GJ. Dendritic cell-based vaccines: from mouse models to clinical cancer immunotherapy. Crit Rev Oncog 2000, 11: 1-17.

[26] Thurner B, Roder C, Dieckmann D, Heuer M, Kruse M, Glaser A, *et al*. Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. J Immunol Methods 1999, 223: 1-15.

[27] Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, *et al*. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci USA 2001, 98: 8809-8814.

[28] Yu JS, Wheeler CJ, Zeltzer PM. Dendritic cell immunotherapy for patients with glioblastoma multiforme and anaplastic astrocytoma. Proc Am Assoc Cancer Res 2001, 42: 1478. (Abstract)

[29] Kirk CJ, Mule JJ. Gene-modified dendritic cells for use in tumor vaccines. Hum Gene Ther 2000, 11: 797-806.

[30] Jenne L, Schuler G, Steinkasserer A. Viral vectors for dendritic cell-based immunotherapy. Trends Immunol 2001, 22: 102-107.

[31] Chi DD, Merchant RE, Rand R, Conrad AJ, Garrison D, Turner R, *et al*. Molecular detection of tumor-associated antigens shared by human cutaneous melanomas and gliomas. Am J Pathol 1997, 150: 2143-2152.

[32] Murayama K, Kobayashi T, Imaizumi T, Matsunaga K, Kuramoto T, Shigemori M, *et al*. Expression of the SART3 tumor-rejection antigen in brain tumors and induction of cytotoxic T lymphocytes by its peptides. J Immunother 2000, 23: 511-518.

[33] Liu FS, Wang ZC, Li JH, Lin S, Ren M, Xiao Q, *et al*. Experimental study of bone-marrow-derived dendritic cells pulsed with tumor antigens in the treatment for intracranial gliomas. Chin J Neurosurg 2002, 18: 91-95. (Chinese, English abstract)

[34] Jiao B, Li C, Zhang Q, Bai B, Geng S. *In vitro* experiment research of the antiglioma activity of the glioma vaccine made from dendritic cell. Chin J Neurosurg Dis Res 2005, 4: 486-491.

[35] Kikuchi T, Akasaki Y, Abe T, Fukuda T, Saotome H, Ryan JL, *et al*. Vaccination of gliom a patients with fusions of dendritic and glioma cells and recombinant human interleukin-12. J Immunother 2004, 27: 452-459.

[36] Eggert AA, Schreurs MW, Boerman OC, Oyen WJ, de Boer AJ,

Punt CJ, *et al*. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. Cancer Res 1999, 59: 3340-3345.

[37] Ali S, Curtin JF, Zirger JM, Xiong W, King GD, Barcia C, *et al*. Inflammatory and anti-glioma effects of an adenovirus expressing human soluble Fms-like tyrosine kinase 3 ligand (hsFlt3L): treatment with hsFlt3L inhibits intracranial glioma progression. Mol Ther 2004, 10: 1071-1084.

[38] Dhodapkar MV, Krasovsky J, Steinman RM, Bhardwaj N. Mature dendritic cells boost functionally superior $CD8^+$ T-cell in humans without foreign helper epitopes. J Clin Invest 2000, 105: R9-R14.

恶性神经胶质瘤的树突细胞免疫疗法

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摘要：中枢神经系统存在血脑屏障且缺乏淋巴组织和树突细胞，加之肿瘤周围建立的免疫抑制微环境，都使神经系统恶性胶质瘤的免疫治疗面临许多特殊的困难。以树突细胞为基础的免疫治疗是指树突细胞肿瘤疫苗的接种治疗，它可以部分或全部改善胶质瘤患者神经系统的免疫状况，因此可以成为有效的治疗方法。随着分子生物学和基因工程技术的发展，树突细胞肿瘤疫苗的制备、优化和应用技术得到了很大提高，为其未来临床应用奠定了良好的基础 。

关键词：树突细胞；免疫治疗；恶性胶质瘤