Construction, Identification and Anti-bladder Tumor Effect of High Targeting, Dual Regulated Oncolytic Adenovirus Vector Expressing the Staphylococcus Aureus Enterotoxin A Gene *

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ABSTRACT Objective: To construct the expression of superantigen staphylococcus enterotoxin A gene high targeting, dual regulated replication-selective oncolytic adenovirus SG502-SEA and contrasting group the non-proliferation oncolytic adenovirus DC318-SEA of gene, and investigate the potential anti-tumor effect on bladder. Methods: After double-digestion by SpeI and SalI, superantigen SEA gene section was cloned into the vector of non-proliferation oncolytic adenovirus vector pSG218, and named the accurately identified advenovirus vector as pDC318-SEA. By the same method, the superantigen SEA gene segment was cloned into the dual regulated specific proliferous oncolytic adenovirus vector pSG502, and named the accurately identified adenovirus vector as pSG502-SEA. The two virus vectors carrying SEA gene and the virus framework plasmid PPE3-ccdB were cotransfect the above into 293 cells. After cotransaction 9~14d appears virus plaque. After the virus plaque purification for three times, extract the adenovirus DNA and use PCR for identification. The adenovirus, identified to be correct, is named as DC318-SEA and SG502-SEA respectively. After large amplification, the adenovirus was purified through cesium chloride gradient centrifugation, and then the virus titer was measured. Results: Through PCR and enzyme cutting identification, the virus vectors can express the SEA gene, and the virus titer is 2.5×10^{10} pfu/mL. Conclusion: The dual regulated, high targeting replication-selective oncolytic adenovirus SG502-SEA and non-proliferous oncolytic adenovirus DC318-SEA of contrasting group carrying SEA were construct successfully.

Key words: Dual regulated; Superantigen SEA; Oncolytic adenovirus

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Introduction

As a new antitumor treatment mode, superantigen is researched and developed rapidly in recent years, and the research mainly focuses on the direct antitumor effect of superantigen, targeting antitumor effect of superantigen, and the gene therapy of superantigen and so on[1-3]. At present, the most effective one is the 5T4Fab-SEA targeting superantigen which has been subject to phase III clinic test for treatment of cancer [4]. The staphylococcus aureus intestinotoxin A (SEA) is the superantigen receiving most research at present[5]. The previous experiences have independently used the superantigen and the superantigen guided by the bladder cancer monoclonal antibody for the experiment and research of controlling tumor of bladder and also achieved a relatively sound effect. In order to improve the targeting property of the superantigen application and increase the cancer suppression effect, based on the previous clone of the superantigen SEA gene segment and verification on the consistency between the superantigen SEA gene segment and the gene order reported by GenBank [6], this study cloned the superantigen SEA gene into the SG502 special oncolytic adenovirus plasmid under the dual regulation of human telomerase reverse transcriptase gene (hTERT) promoter and the hypoxia-responsive element (HIF-1) and packed it to the complete virus.

1 Materials and methods

1.1 Materials

Virus vector pSG218, PENTR12-Linker, PPE3-ccdB, SG502, and HEK293 cells were all purchased from Virus and Gene Therapy Center of Shanghai Eastern Hepatobiliary Surgery Hospital. The products such as gel extraction kit, PCR product extraction kit, plasmid DNA preparation kit and the virus DNA extraction kit all belonged to Qiagen Company. LipofectAmine2000 kit was purchased from Gibco brl. DNA ligase SolutionI and Taq enzyme were purchased from TakaRa Company.

1.2 Construction of the non-proliferative oncolytic adenovirus vector pDC318-SEA carrying SEA gene

Connect the superantigen SEA gene segment introducing the SpeI and Sa1I restriction enzyme cutting sites to the non-proliferative oncolytic adenovirus shuttle plasmid pSG218 being subject to double enzyme cutting of SpeI and Sa1I. Name the non-proliferati-

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ve oncolytic adenovirus vector, identified to be correct, as PDC 318 -SEA.

1.3 Reconstruction, amplification and titration measurement of non-proliferative oncolytic adenovirus DC318-SEA carrying SEA gene

Conduct cotransfection for non-proliferative oncolytic adenovirus vector pDC318-SEA and virus framework plasmid pPE3-ccdB into 293 cells (It is the epithelial cell system of human kidney, therefore it is easier to be transfected and is the common cell strain for expressing and researching the foreign gene). After cotranfaction, 9~14d appears virus plaque. After the virus plaque purification for three times, the adenovirus DNA was extracted and PCR was used for identification. The adenovirus, identified to be correct, is named as DC318-SEA. It is required to increase up to the required virus quantity repeatedly, use 50% tissue culture infective dose method to measure the virus titer.

1.4 Reconstruction, amplification and titration measurement of hTERT/ HIF-1 dual regulated proliferative oncolytic adenovirus vector Psg502-SEA

The non-proliferative oncolytic adenovirus vector plasmid carrying SEA gene, which has received the dual enzyme cutting of SpeI and SalI, connects the superantigen SEA gene segment to the proliferative oncolytic adenovirus shuttle plasmid, which also has received the dual enzyme cutting of SpeI and SalI. The proliferative oncolytic adenovirus vector carrying SEA gene, which was identified to be correct, is named as PENTR12-SEA. The proliferative oncolytic adenovirus vector PENTR12-SEA carring SEA gene and the virus framework plasmid PPE 3 were reconstructed, and then the recombinant adenovirus-SEA and plasmid PPE3-SEA was obtain. Cotransfect for the dual regulated proliferative oncolytic adenovirus pSG502 of PPE3-SEA and hTERT/ HIF-1 to 293 cells. After cotransaction, 9~14d appears virus plaque. After the virus plaque purification for three times, extract the adenovirus DNA and use PCR for identification. The hTERT/ HIF-1 dual regulated oncolytic adenovirus, identified to be correct, is named as SG502-SEA, namely, the hTERT/ HIF-1 dual regulated special proliferative adenovirus carrying SEA gene.

1.5 Amplification and titration measurement of hTERT/ HIF-1 dual regulated proliferative oncolytic adenovirus SG502-SEA carrying the superantigen SEA gene

20mL 10%FBS/DMEM grows to 80%-90% 293 cells in the 75cm^2 culture bottle, and then use 15mL 2%FBS/DMEM instead. Take 0.5ul firstly amplified virus preservation solution (virus plaque infection to be obtained through 24 holes), carefully add the virus mixture, and shake it for three times slowly along the crossed direction. After cultivation for 48 hours in the 5% CO_2 couveuse under 37℃, then collect the virus supernatant or cell precipitation. Add AD buffer preservation solution to the precipitated cell, and freeze and melt it for three times (from -80℃ to 37℃). Then they were centrified at 600g for 20 minutes, later the supernatant was extract, and then freeze and melt them for three times (from -80℃ to 37℃). Then they were centrified at 600g for 20 minutes, later remove the cell debris, collect the supernatant. It is required to increase upto the required virus quantity repeatedly, use 50% tissue culture infective dose method to measure the virus titer.

2 Results

2.1 Identification of non-proliferative oncolytic adenovirus vector superantigen pDC318-SEA carrying the superantigen SEA gene

SEA was used as primer to amplify the pDC318-SEA vector, Fig.1 showed the SEA gene segment was 771 bp After SpeI+SalI conducts dual enzy3926bp pSG218 plasmid segment, which indicated that pDC318-SEA contains SEA gene segment, Fig.2 showed the 771bp gene segment and pSG218 gene segment. Fig.3 showed the constructed non-proliferative adenovirus DC318-SEA structure carrying the superantigen SEA gene.

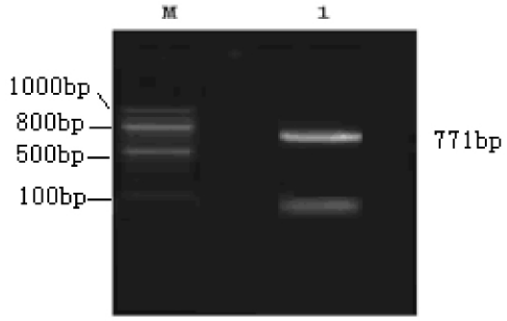

Fig.1 Electrophoresis results of pDC318-SEA after being amplified through SEA primer PCR
M :3000bp DNA markers ;1: amplified SEA gene segment

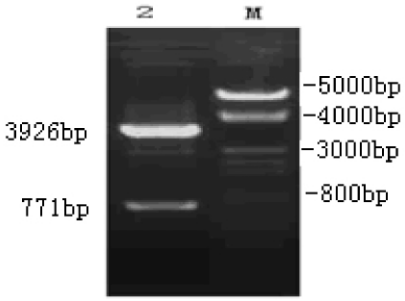

Fig.2 Electrophoresis results of pDC318-SEA after receiving the dual enzyme cutting of SpeI and SalI :M :3000bp DNA markers ;2 :SEA gene segment and pSG218 plasmid segment

Fig.3 Structure drawing of constructed non-proliferative adenovirus DC318-SEA carrying superantigen SEA gen

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2.2 Identification of the hTERT/ HIF-1 dual regulated proliferative oncolytic adenovirus vector pSG502-SEA carrying superantigen SEA gene

See structure drawing 4 for the dual regulated proliferative oncolytic adenovirus pSG502-SEA vector structure. Use SEA primer to amplify the reconstructed and packed dual regulated oncolytic adenovirus SG502-SEA, it is available to see the SEA gene segment with the size of 771bp, shown in Figure 5. Use the hTERT promoter primer to amplify the virus SG502-SEA and DC318-SEA synchronously, it is available to see the 261bp hTERT promoter gene in the SG502-SEA, while the DC318-SEA does not include the hTERT promoter gene, shown in Fig.6.

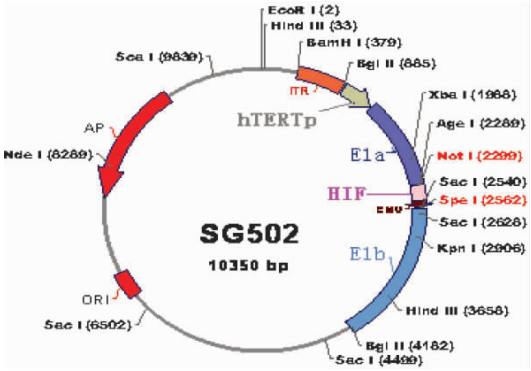

Fig.4 Structure drawing of constructed hTERT and HIF-1dual regulated proliferative adenovirus gene vector carrying superantigen SEA gene

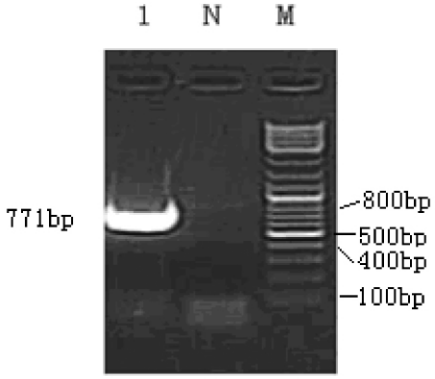

Fig.5 Electrophoresis results of SG502-SEA after being amplified through SEA primer PCR :M :3000bp DNA markers ;N :Negative control ;1 : Amplified SEA gene segment

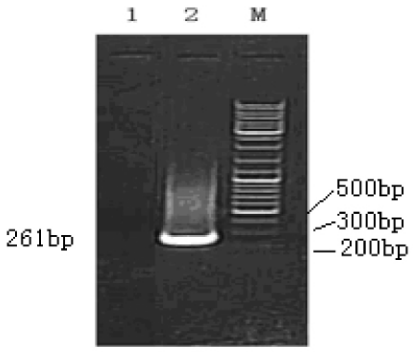

Fig.6 Electrophoresis results of SG502-SEA and DC318-SEA after being amplified by the hTERT promoter primer PCR :M: 3000bp DNA markers ; 1: There is no hTERT promoter gene in DC318-SEA; 2 :There is hTERT promoter gene with the size of 261 bp in SG502-SEA

2.3 Measurement of virus titre

DC318-SEA and SG502-SEA are in small amplification. After a certain quantity is amplified, conduct ultracentrifugal purification concentration. The virus titre reaches 2.5×10^{10} pfu/ml.

3 Discussion

We have systematically researched the proliferation condition and antitumor effect of superantigen SEA, anti-human bladder carcinoma monoclonal antibody BDI-1Fab section, and SEA uncoupling protein external stimulus T lymphocyte post CTL, the CTL cell activated by SEA has a strong killing effect out of the body of E-J and BIU-87 bladder tumor cell. The animal experiment also shows that SEA and the uncoupling protein of the monoclonal antibody have a good inhibition effect for the growth of living tumor, and the tumor inhibition rate is respectively 84.7% and 95.2%[7-9].

However, no matter independently use the superantigen for anti-tumor treatment or use the targeting superantigen anti-tumor treatment of the antibody at present; we still have many problems needed to be solved [10]. CTL with SAg mediate is mainly for killing the tumor cell with MHC-Ⅱ in positive property, while its killing effect for the MHC-Ⅱ negative tumor cell is relatively weaker [11]. But, the tumor cell MHC-Ⅱ's positive rate is low generally and has obvious heterogeneity. Therefore, the independent SAg anti-tumor effect is not ideal, and it is difficult to avoid the toxic and side effect on the MHC-II positive normal cell of the body [12, 13]. T cell activated by the combined protein monoclonal antibody and superantigen is SEA reactive, but not the special T cell of the tumor [14, 15]. In addition, not all the bladder cancers will express the related antigen of bladder tumor, so the fusion protein of such superantigenmonoclonal antibody is still in lack of the wide-adaptability and part icularity of anti-tumor. The in ductive targeting anti-tumor immune response is not so satisfactory.

As for the gene treatment, the key measure is to adopt one proper efficient vector and guide it to the targeting organ [16,17]. Currently, during the research of tumor gene treatment vector, the virus vector takes a leading position [18-20]. The tumor specialty proliferous oncolytic adenovirus (RSOAds) vector is highly concerned by people because of the following advantages: ①. RSOAds can infect the tumor cell and also can duplicate, proliferate, crack and kill the tumor cell and then release more viruses, thereby infecting other tumor cells nearby, then the tumor cells will be killed through dissolution in a large scale, so as to form chain reaction; ② The duplication and proliferation of. RSOAds is only restricted to tumor cells, in this way, it is available to reduce the damage on the normal host tissue, so as to improve the treatment effect [21,22].

RSOAds can be effectively constructed through the following two methods: (1) Duplicate the necessary virus in the normal cells, and eliminate the unnecessary virus gene in the tumor cells. (2) Control the gene necessary for the virus duplication by using the

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tumor specific promoter [23]. E1A and E1B genes are necessary when the viruses are duplicated in the normal cells, but unnecessary in the tumor cells, among which, E1A gene is the gene expressed earliest after the adenovirus infects the cells [24-26]. The method used in this subject is to use the tumor specific promoter (namely, the human telomerase reverse transcriptase (hTERT) promoter and hypoxia-responsive element (hypoxia-inducible factor-1，HIF-1) promoter) to respectively control the expression of virus E1A and E1B genes, so as to make them expressed in the tumor cells in a more specific manner, while not expressed in the normal cells, thereby making the virus can only be duplicated and proliferated in the tumor cells and achieving the purpose of killing the tumor cells in a targeting method.

The telomerase is under the activated condition in most malignant cells, but has no activity or very low activity in the normal cells, and it is the most broad-spectrum tumor biomolecule mark reported at present. Human telomerase reverse transcriptase is the catalytic subunit necessary for keeping the activity in the telomere, whose expression regulation is on the transcription level. Use the telomerase reverse transcriptase promoter to regulate the necessary gene for virus duplication and proliferation, according to the theory, it can make the virus proliferated in the tumor cells selectively with the telomerase in positive property. Many researches show that hTERT is in an efficient expression in 92% bladder cancer cells, but nearly has no expression in the normal cells [27]. If hTERT promoter is used for replacing the self promoter of the E1A gene, which can realize that the E1A gene expression is only restricted in the tumor cells, but not expressed in the normal cells. Hypoxia inducible factor-1 widely exists in many tumors and its expression is restricted by the regulation of anoxia, tumor gene and many factors, which can regulate the generation of related tumor vessels. HIF-1 can combine with the special positioning points of a series of hypoxia response elements, thereby starting the transcriptional expression of the targeting gene, which plays a very important role in the respects of tumor generation, development, apoptosis, infiltration and transfer [28]. Some researches proof that HIF-1 is in a high expression in a series of human solid tumors including bladder cancer, brain cancer, breast cancer, prostatic cancer and renal carcinoma, but has no expression in the adjacent normal tissues or stroma cells [29].

Therefore, the construction of oncolytic adenovirus under dual regulation of hTERT and HIF-1 double promoters can target the bladder tumor cells in a more specific manner, so as to protect the normal tissues greatly. Qi Zhang uses the human telomerase promoter and hypoxia-responsive element to regulate the EIA and EIB genes of the adenovirus respectively. The constructed virus vector has a stronger targeting property and can infect specially and kill the tumor cells, but has a very small influence on the normal cells. If two anti-cancer genes having supplementary effect or synergistic effect are inserted in the cloning sites at the same time, the targeting property of the virus can be kept on the original basis, but also the anti-tumor effect of the gene is increased [30].

References

[1] Katsuhiko O, Wataru N, Hidehito K, et al. High Affinity of Interaction between Superantigen and T Cell Receptor Vβ Molecules Induces a High Level and Prolonged Expansion of Superantigen-reactive CD4+ T Cells[J]. Biol. Chem, 2010, 285: 30427-30435

[2] Yakup T, Alfonso MF, Kulachelvy R, et al. Superantigen-Activated Regulatory T Cells Inhibit the Migration of Innate Immune Cells and the Differentiation of Naive T Cells[J]. Immunol, 2009, 183: 2946-2956

[3] Pawan KS, Ranu A, Dev VK, et al. Construction of a Single-Chain Variable-Fragment Antibody against the Superantigen Staphylococcal Enterotoxin B[J]. Appl. Envir. Microbiol, 2010, 76: 8184-8191

[4] Shaw DM, Connolly NB, Patel PM, et al. A phase II study of a 5T4 oncofoetal antigen tumour-targeted superantigen (ABR-214936) therapy in patients with advanced renal cell carcinoma [J]. Br J Cancer, 2007, 96:567-574

[5] Xiu F, Cai Z, Yang Y, et al. Surface anchorage of superantigen SEA promotes induction of specific antitumor immune response by tumor-derived exosomes [J]. J Mol Med, 2007, 85(5): 511-521

[6] Hao L, Han CH, Wang YM, et al. Clone and Identification of Superantigen Staphylococcus Aureus Enterotoxin A Gene [J]. Journal of Xuzhou Medical College, 2009, 29(2):74-76

[7] Han CH, Tian J, Zhen BZ. Research on Killing Effect of Superantigen Induced Destruction T Cell for Bladder Tumor inside and outside of the Body [J]. Chinese Journal of Oncology, 2000, 22: 392-392

[8] Hao L, Li HF, Han CH, et al. Research on Controlling Bladder Tumor of Superantigen SEA Combined Dendritic Cell Induction Specific Property [J]. Chinese Journal of Experimental Surgery, 2008, 25 (12) : 1586-1570

[9] Gong Z, Han CH, Hao L, et al. Preparation and in-vitro bioactivity of a novel superantigen conjugate targeting bladder carcinoma [J]. Journal of Pharmacy and Pharmacology, 2009, 61(3):869-875

[10] Wang S, Li Y, Xiong H,et al. A broad-spectrum inhibitory peptide against staphylococcal enterotoxin superantigen SEA, SEB and SEC [J]. Immunol Lett, 2008, 121: 167-172

[11] Xu M, Wang X, Cai Y, et al. An engineered superantigen SEC2 exhibits promising antitumor activity and low toxicity [J]. Cancer Immunol Immunother, 2011, 60(5): 705-713

[12] Zhou P, Liang P, Dong B, et al. Long-term results of a phase II clinical trial of superantigen therapy with staphylococcal enterotoxin C after microwave ablation in hepatocellular carcinoma [J]. Int J Hyperthermia, 2011, 27(2): 132-139

[13] Walenkamp AM, Boer IG, Bestebroer J, et al. Staphylococcal superantigen-like 10 inhibits CXCL12-induced human tumor cell migration [J]. Neoplasia, 2009, 11(4): 333-344

[14] Miyakawa H, Mason RP, Jiang J, et al. Lipid-derived free radical production in superantigen-induced interstitial pneumonia [J]. Free Radic Biol Med, 2009, 47(3): 241-249

[15] Han XJ, Dong BW, Liang P, et al. Local cellular immune response induced by ultrasound-guided tumor bed superantigen injection after percutaneous microwave coagulation therapy for liver cancer [J]. Chinese Journal of Qncology, 2009, 31(8): 602-606

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[16] Vincenzo C, Sari P, Iulia D, et al. Oncolytic Adenovirus Coding for Granulocyte Macrophage Colony-Stimulating Factor Induces Antitumoral Immunity in Cancer Patients [J]. Cancer Res, 2010, 70: 4297-4309

[17] Debanjan D, Jacqueline FS, Karoly T, et al. Effect of Preexisting Immunity on Oncolytic Adenovirus Vector INGN 007 Antitumor Efficacy in Immunocompetent and Immunosuppressed Syrian Hamsters [J]. J. Virol., Mar 2009, 83: 2130-2139

[18] Guo ZS, Thorne SH, Bartlett DL. Oncolytic virotherapy: molecular targets in tumor-selective replication and vector cell-mediated delivery of oncolytic viruses [J]. Biochim Biophys Acta, 2008, 1785(2): 217-231

[19] Hsieh JL, Wu CL, Lai MD, et al. Hepatitis B Virus X Protein Sensitizes Hepatocellular Carcinoma Cell to Cytolysis Induced by E1B-deleted Adenovirus through the Disruption of p53 Function [J]. Clin Cancer Res, 2003, 9 (l) :338-345

[20] Terao S, Shirakawa T, Kubo S, et al. Midkine promoter-based conditionally replicative adenovirus for targeting midkine-expressing human bladder cancer model[J]. Urology, 2007, 70(5):1009-1013

[21] Raki M, Sarkioja M, Desmond RA, et al. Oncolytic adenovirus Ad5/3-delta24 and chemotherapy for treatment of orthotopic ovarian cancer [J].Gynecologic Oncol, 2008,108(1):166-172

[22] Eduardo GC, Daniela RM, Maria VL, et al. A Novel A33 Promoter-Based Conditionally Replicative Adenovirus Suppresses Tumor Growth and Eradicates Hepatic Metastases in Human Colon Cancer Models [J]. Clin. Cancer Res, 2009, 15: 3037-3049

[23] Cheong SC, Wang Y, Meng JH, et al. E1A-expressing adenoviral E3B mutants act synergistically with chemotherapeutics in immunocompetent tumor models [J]. Cancer Gene Ther, 2008, 15(1): 40-50

[24] Fiona JM, Craig M, Julian B, et al. Use of Macrophages to Target Therapeutic Adenovirus to Human Prostate Tumors [J]. Cancer Res, 2011, 71: 1805-1815

[25] Petri N, Sari P, Sophie E, et al. Oncolytic Adenovirus ICOVIR-7 in Patients with Advanced and Refractory Solid Tumors [J]. Clin. Cancer Res, 2010, 16: 3035-3043

[26] Kristopher JK, Ronald AA, Gene PS, et al. Conditionally Replicating Adenovirus Expressing TIMP2 for Ovarian Cancer Therapy [J]. Clin. Cancer Res., 2011, 17: 538-549

[27] Zou L, Zhang P, Luo C, et al. ShRNA-targeted hTERT suppress cell proliferation of bladder cancer by inhibiting telomerase activity [J]. Cancer Chemother Pharmacol, 2006, 57:328-334

[28] KangAe L, David ZQ, Sergio, et al. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells [J]. PNAS, 2009, 106: 2353-2358

[29] Nadaoka J, Horikawa Y, Saito M, et a1. Prognostic significance of HIF-1 alpha polymorphisms in transitional cell carcinoma of the bladder[J]. Int J Cancer, 2008, 122: 1297-1302

[30] Zhang Q, Wu MC, Li YM, et al. A novel replication-competent adenovirus CNHK500 in the treatment of heptocellular carcinoma[J]. Chinese-German J Clin Oncol, 2004, 3:70-74

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荷载金黄色葡萄球菌肠毒素 A 基因的双调控溶瘤腺病毒载体的构建、鉴定及其抗膀胱肿瘤作用 *

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摘要 目的：构建表达超抗原金黄色葡萄球菌肠毒素 A 基因的双调控选择增殖型溶瘤腺病毒 SG502-SEA 及对照组携带超抗原 SEA 基因的非增殖溶瘤腺病毒 DC318-SEA，并探讨其潜在的抗膀胱肿瘤作用。方法：将超抗原 SEA 基因片段经 SpeI 和 SalI 双酶切后，克隆入非增殖溶瘤腺病毒载体 pSG218 中，将鉴定正确的腺病毒载体命名为 pDC318-SEA。同样方法将超抗原 SEA 基因片段克隆入双调控特异性增殖溶瘤腺病毒载体 pSG502 中，将鉴定正确的腺病毒载体命名为 pSG502-SEA。将以上两种携带 SEA 基因的病毒载体与病毒骨架质粒 PPE3-ccdB 共转染 293 细胞，9~14d 出现病毒空斑，经过 3 次病毒空斑纯化，提取腺病毒 DNA，应用 PCR 进行鉴定，经鉴定正确的腺病毒分别命名为 DC318 - SEA 和 SG502-SEA。大量扩增后，氯化铯梯度离心纯化腺病毒，测病毒滴度。结果：经 PCR 及酶切鉴定，SEA 基因成功克隆到两病毒载体中，可以表达 SEA 基因，且病毒滴度为 2.5×10^{10}pfu/ml。结论：成功构建表达超抗原 SEA 基因的双调控增殖型溶瘤腺病毒 SG502-SEA 及对照组携带超抗原 SEA 基因的非增殖溶瘤腺病毒 DC318-SEA，为下一步抗膀胱肿瘤体内外实验奠定基础。

关键词 超抗原 腺病毒 膀胱癌

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