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Enhanced growth suppression of TERT-positive tumor cells by oncolytic adenovirus armed with CCL20 and CD40L

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ABSTRACT

Conditionally replicating adenoviruses (CRAds) selectively replicate in cancer cells and induce cell lysis, which represents a potential platform for cancer immunotherapy. The chemokine CCL20 exerts antitumor activity via chemoattraction of immature dendritic cells (DCs) and lymphocytes. However, the activation and maturation status of DCs is a limiting factor in the DCs -based immunity response. CD40L induces the phenotypic maturation of DCs, mediates DCs cytokine secretion, and increases the expression of FasL, which mediates apoptosis. We constructed a CCL20/CD40L co-expression CRAds (Ad-CCL20-CD40L) based on the AdEasy system. Ad-CCL20-CD40L was constructed from three plasmids, pGTE-CD40L, pShuttle-CMV-CCL20 and AdEasy-1, and was homologously recombined and propagated in the Escherichia coli strain BJ5183 and the packaging cell line HEK-293, respectively. Ad-CCL20-CD40L selectively replicates in TERT-positive tumor cells because the pGTE-CD40L plasmid contains the telomerase reverse transcriptase promoter (TERTp). Our results showed that Ad-CCL20-CD40L induced oncolytic effects and tumor-specific cytotoxicity of cytotoxic T lymphocytes (CTLs) in vitro. This study suggests that Ad-CCL20-CD40L can induce the antitumor immune response and that this platform can be modified to generate novel CRAds with other transgenes.

1. Introduction

Gene therapy has recently become an important approach for the treatment of cancers. The main challenge in cancer gene therapy is the control of vector security. Adenoviral vectors are commonly used for transgene expression in cancer gene therapy. These vectors offer several advantages over other vector systems, such as effective gene delivery and the ability to infect a variety of dividing and non-dividing cells [1–3]. Replication-deficient adenovirus vectors are usually based on adenovirus E1-E3 deletion mutants. Deletion of the E1 gene region permits the adenovirus vectors to accommodate larger inserts. These vectors are often propagated in E1-transformed 293 cells and cannot produce progeny viruses or offspring in other cells following infection. Thus, the traditional adenovirus (Ad) vectors (E1 deleted) have a relatively large cloning capacity and are safe. However, they have trouble penetrating whole tumor cells and tissues when equipped with cargo [4]. To overcome these challenges, optimal conditions for the proliferation of viral vectors have been developed and the concept of an oncolytic adenovirus has been investigated in recent years [5–7].

Oncolytic therapy is based on the use of conditionally replicating viruses that are selective for tumor cells. Oncolytic adenovirus vectors, also called conditionally replicating Ads (CRAds), can specifically replicate in cancer cells and lyse them [8–10]. The progeny viruses are released to the neighboring cancer cells, eventually lysing the infected cancer cells. CRAds do little harm to the normal cells as the viral replication process stops when they infect healthy cells. When CRAds are armed with therapeutic transgenes, transgene expression is increased along with the preferable replication of the CRAds in the tumor cells. Then, the therapeutic transgenes can spread along with the dissemination and replication of the adenovirus from the CRAd-infected tumor cells. Wang et al. [11] inserted the B7-1 (CD80) and GM-CSF genes into AdEasy-1. They found that treatment with oncolytic adenovirus combined with therapeutic transgenes enhanced the survival of mice bearing tumors. Similarly, Qin et al. [12] found an enhanced antitumor effect in breast cancer cells by the insertion of IL-24 into an oncolytic adenovirus. All of these results suggested that CRAds armed with

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therapeutic genes could be a feasible approach for enhancing the efficacy of oncolytic adenoviruses as tumor therapeutics. One approach, the construction of transcriptionally regulated oncolytic adenoviruses, has been used to successfully construct oncolytic adenoviruses. This approach uses tumor-specific promoters to control the expression of viral genes essential for transcription. Therefore, these viruses replicate in and kill tumor cells but not normal cells [13–15].

CRAd vectors are created and isolated from packaging cells by recombination between shuttle and backbone plasmids. However, purification of the CRAd vectors is indispensable, making the method inefficient and time consuming. The method of homologous recombination in bacteria improved the increasing the recombination efficiency. In this method, the homologous recombination of the shuttle and backbone plasmids occurs in Escherichia coli instead of in 293 cells. The recombinant plasmid is homogeneous as it originates from a single cluster of bacteria. Thus, the adenovirus propagated in the packaging cells with the recombinant plasmid is homogeneous and the purification of the plaques is dispensable. The AdEasy system exploits the high efficiency of recombination in a specific bacterial strain coupled with selectable markers to simplify recombinant vector production [16,17]. It has proven to be an efficient technology for generating recombinant adenoviruses in many laboratories [6,7,18,19]. In this study, we inserted the CCL20 and CD40L genes into an adenovirus vector and constructed a novel oncolytic adenovirus, Ad-CCL20-CD40L, using the AdEasy system. The novel oncolytic virus inhibits the growth of TERT-positive tumor cells and induces apoptosis. Combining the oncolytic effect with antitumor immune effects, Ad-CCL20-CD40L results in a remarkably improved efficacy against tumor cells. We believe that the antitumor effects of Ad-CCL20-CD40L could be further developed into a potential combination gene therapy strategy for cancer.

2. Materials and methods

2.1. Cell culture

The human prostate cancer cell line PC-3 M was obtained from the School of Basic Medicine, Jilin University, Changchun, China. The other cell lines were all purchased from the American Type Culture Collection (ATCC). The packaging cell line 293, fibroblast cell line BJ, prostate carcinoma cell lines PC3M, colon carcinoma cell line Caco-2, and osteosarcoma cell line U2OS were cultured in DMEM supplemented with 10% FBS. The colorectal adenocarcinoma cell line HT-29 was cultured in RPMI 1640 supplemented with 20% FBS. All of these culture media were supplemented with penicillin and streptomycin. All of the cells were maintained at 37 °C with 5% CO_2. The U2OS and BJ cells are known to be telomerase-negative, while the other cells possess telomerase activity.

2.2. Construction of recombinant adenovirus with transgenes CCL20 and CD40L

The plasmid JM17 was received from Microbix Biosystems, Inc., Ontario, Canada. The AdEasy-1 and pShuttle-CMV plasmids were purchased from Stratagene (La Jolla, CA). The pIRES Vector was purchased from BD Biosciences Clontech. The pGEM-T Easy vector was obtained from the Promega Corporation, Madison, WI. Ad-CD40L plasmid was constructed previously in our laboratory (extracellular domain of human CD40L). The coding sequence of E1B55K, internal ribosome entry site (IRES), Homo sapiens CD40 ligand (CD40L, extracellular domain of human CD40L), E1A gene coding region and the promoter of E1B, telomerase reverse transcriptase promoter (TERTp) and SV40pA fragments were amplified through PCR. After the PCR products were sequenced, they were sequentially inserted into the pGEM-T Easy plasmid. The generated plasmid was named pGTE-CD40L, and the primers, templates and restriction sites used to generate this plasmid are shown in Table 1. The Homo sapiens chemokine ligand 20 (CCL20) gene was cloned by PCR from human genomic DNA isolated from human peripheral blood leukocytes with the following primers: 5'-CCCAAGCTTATGTGCTGTACCAAGAGTTTG-3' and 5'-GCGGATATCTTACA TGTTCT TGACTTT-3'. Then, the CCL20 cDNA was inserted into the pShuttle-CMV plasmid at the HindIII and EcoRV restriction sites to generate pShuttle-CMV-CCL20. The fragment between the pShuttle-CMV-CCL20 MfeI sites was replaced with the fragment from pGTE-CD40L. We named this constructed plasmid pSC-CCL20-CD40L. The shuttle vector pSC-CCL20-CD40L and the adenovirus backbone plasmid pAdEasy-1 underwent homologous recombination in E. coli BJ 5183 following electroporation according to the AdEasy system manual. The identity of the recombinants was confirmed by kanamycin selection and restriction endonuclease digestion. The amplified fragments were verified by sequencing. The recombinant adenovirus plasmid was packaged in HEK-293 cells. The infective adenovirus virions were amplified in cells on plates. Recombinant adenovirus was harvested after 7 days and purified using a double CsCl density gradient ultracentrifugation. The transduction efficiency was confirmed by fluorescence microscopy and the multiplicity of infection was calculated from the infectious titers.

2.3. Determine the expression of CCL20 and CD40L

To detect the CCL20 and CD40L mRNAs in PC-3 M cells, the total cellular RNA was isolated from cells 24 h after Ad-CCL20-CD40L infection and reverse transcribed using the SuperScript™ III first-strand synthesis system for RT-PCR kit (Invitrogen) [20,21]. The cDNA was amplified using the following primers: for CCL20, 5'-GTCAGTGCTGCTACTCCACC TCT-3' and 5'-GTGTGAAAGATGATAGCATTGAT-3; for CD40L, 5'-CATA GAAGGTTGGACAAGATAG -3' and 5'-TCAGAGTTTGAGTAAGCCAAAG-3';

Table 1
The parameters of PCR for construction of pGTE-CD40L.

Fragment	Primers	Template	GenBank accession no.	Length of PCR product (bp)	Restriction enzyme
CCL20	F:CCCAAGCTTATGTGCTGTACCAAGAGTTTG R:GCGGATATCTTACATGTTCTTGACTTT	human genome	BC020698.1	306	HindIII EcoRV
SV40pA	F: CGCGGGCCCCAATTGTTGTTGTTAACTTGT R: CATGCCATGGGCGTTAAGATACATTGATGAG	pShuttle-CMV	---	159	ApaI NcoI
TERTp	F:CATGCCATGGTGGCCCCTCCCTCGGGTTAC R:CGGACTAGTCGCGGGGGTGGCCGGGGCCAGG	K562 genome	AF098956.1	474	NcoI SpeI
E1A-E1Bp	F:CGGACTAGTATGAGACATATTATCTGCCACGG R:TGCACTGCAGGAGGTCAGATGTAACCAAGATT	JM17	AY339865.1	1173	SpeI PstI
CD40L	F:AACTGCAGCATAGAAGGTTGGACAAGATAG R:CGCGTCGACTCAGAGTTTGAGTAAGCCAAAG	human genome	NM_000074.2	665	PstI SalI
IRES	F:ACCGTCGACAATTCCGCCCCTCTCCCTC R:GACCATATGCGGGTTGTGGCAAGCTTAT	pIRES Vector	---	614	SalI NdeI
E1B55K	F:CGGCATATGATGGAGCGAAGAAACCCATCTG R:GCCGAGCTCTCAATCTGTATCTTCATCGCT	JM17	AY339865.1	1509	NdeI SacI