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Antitumor effect and underlying mechanism of RGD-modified adenovirus mediated IL-24 expression on myeloid leukemia cells

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ABSTRACT

Interleukin-24 (IL-24), a member of the IL-10 cytokine gene family, causes growth suppression and apoptosis in various solid tumor cells. However, the effects of IL-24 on hematopoietic malignant cells have not been extensively explored. In this report, we constructed an RGD-engineered recombinant adenoviral vector, Ad.RGD-IL-24, and assessed its effects on human myeloid leukemia cells. Ad vector mediates gene transfer into leukemia cells with high efficiency. Ectopic over-expression of IL-24 has significant growth inhibition and differentiation inducement effects on these cells. Treatment with Ad.RGD-IL-24 could potentially induce leukemia cells' cell-cycle arrest. In addition, IL-24 expression could significantly induce apoptosis of the THP-1 cells. Ad.RGD-IL-24 had a potent effect on the up-regulation of the expression of GRP78/Bip, GADD34 and Bax, down-regulation of the expression of Bcl-2 and Mcl-1, and induced the activation of Caspase-3, which may be responsible for its apoptosis-inducing effect on THP-1 cells. Furthermore, IL-24 expression could retard transplanted leukemia cell tumor growth in vivo in athymic nude mice. These findings showed the marked antitumor activity of IL-24 and provided potential perspectives in designing therapeutic or vaccine strategies in immuno-gene therapy of myeloid leukemia.

1. Introduction

IL-24 was identified and cloned using the differentiation induction subtraction hybridization approach after treating HO-1 human melanoma cells with IFN-beta and mezerein. Reports of the loss of IL-24 expression during the pathologic progression of melanomas, and of the significant correlation between this loss and tumor invasion, suggest that IL-24 may function as a tumor suppressor in melanoma. Expression of IL-24 is mainly restricted to the spleen, thymus and immune cells, including Th2 cells, B cells, natural killer (NK) cells, dendritic cells, monocytes and melanocytes. The protein product of IL-24 can exert its antitumor effect by binding to its two receptor complexes: IL-20R1/IL-20R2 and IL-22R1/IL-20R2. In addition, IL-24 has at least two separate functions. At low concentration, it mostly acts as a cytokine. However, over-expression of IL-24 at the supra-physiological level shows an irreversible cancer cell growth inhibitory function and cell-cycle arrest, reversal of the malignant phenotype, and terminal differentiation. IL-24 has a broad range of antitumor properties, including an ability to selectively induce apoptosis in cancer cells, stimulate antitumor immune response, inhibit angiogenesis, and induce multipronged 'bystander' activity eliminating both primary and distant tumors [1–4]. In addition to cancer-specific cytotoxicity, IL-24 also synergizes with radiation and chemotherapeutic agents, further amplifying the potential therapeutic applications of this novel cytokine. When introduced into tumor cells by gene transduction, IL-24 has a potent intracellular mode of action and is active through a receptor-independent mechanism, making it an ideal candidate for anticancer gene therapy [5–7]. Recently, our group has successfully cloned the IL-24 gene and demonstrated that IL-24 exhibits potent tumor suppressive activity against a wide variety of human cancers, including those derived from the pancreas, prostate, breast, gastrointestinal tract, and lung, with no significant toxicity to normal cells [8–11]. IL-24 is currently being studied in clinical trials for its potential therapeutic usefulness. Studies document that IL-24 is well tolerated and demonstrate the evidence of significant clinical activity.

Myeloid leukemia is a heterogeneous group of blood cancers characterized by increased, uncontrolled proliferation of hematopoietic progenitors. Although IL-24 has been extensively studied in epithelial tumors, the therapeutic potential of this cytokine has been rarely explored in hematologic malignancies. Therefore, it is worthwhile to investigate the effects of over-expression of IL-24 on leukemic cells. Many hematopoietic malignant cells lack IL-20R1/IL-20R2 and IL-22R1/IL-20R2 receptor complexes. To produce an optimum therapeutic response using IL-24, defining ways of ensuring efficient delivery of this

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molecule to the leukemic cells are of critical importance. Recombinant adenovirus (Ad) vectors are attractive vehicles for gene transfer to a wide variety of cell types. Adenoviral vectors harboring therapeutic genes have been used successfully for gene transfer in vitro and in vivo. However, one of the limitations in the use of Ad vectors for gene transfer is the inefficient gene transfer into cells lacking in coxsackie-adenovirus receptors (CARs), such as some types of leukemia cells [12]. Adenovirus infection requires two sequential steps. The first involves the attachment of the C-terminal knob domain of the fiber protein to the CAR on the cell surface. After binding to CARs, virus internalization via receptor-mediated endocytosis takes place through the interaction of the RGD motif of the penton bases with the host cell receptors, avb3 and avb5 integrin.

Arginine–glycine–aspartic acid (RGD) sequence is a conserved recognition motif between integrins and their ligands. RGD can bind selectively to avb3 integrin [13,14]. The expression of avb3 integrin is significantly up-regulated in some leukemia cells, but is minimally expressed on quiescent endothelial cells. Because avb3 integrin is a specific marker for neovasculature and angiogenic cells, it has emerged as a promising target for cancer therapy [15]. To improve the efficiency of transduction, we constructed an RGD-engineered recombinant adenoviral vector, Ad.RGD-IL-24, expressing the tumor suppressor IL-24. We investigated the therapeutic effect of Ad.RGD-IL-24 against human myeloid leukemia cells. Our study revealed novel functions of this antitumor gene and characterized IL-24 as a promising agent for further clinical development for hematologic malignancy therapy.

2. Materials and methods

2.1. Cell lines, reagents, and mice

Human myeloid leukemia cell lines, K562, THP-1 and MEG-01, purchased from the American Type Culture Collection (ATCC, Rockville, MD), were cultured in RP1640 with 10% FCS. The adenoviral transfer plasmid (pAdTrack-CMV) expressing green fluorescent protein, BJ5183 bacteria, and QBI-293A human embryonic kidney cell line were kindly provided by Dr. Jiang Zhong (Department of Microbiology, College of Life Science, Fudan University, Shanghai, China). The recombinant backbone vector, pAd-RGD, was provided by Prof. Albert Deisseroth (Sidney Kimmel Cancer Center, Canada). MuMLV reverse transcriptase polymerase, GoTaq® qPCR Master Mix, was purchased from Promega. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit was purchased from Sigma. Caspase-3 colorimetric assay kit and the Bradford protein assay kit were purchased from Nanjing KeyGen Biotech. Co., Ltd. China. The polyclonal rabbit anti-IL-24 antibody was purchased from Santa Cruz. Polyvinylidene difluoride membrane (PVDF) was purchased from Millipore. PE-conjugated antibodies specific for CD41, CD14, and CD11b were obtained from eBioscience. CD36-PE antibody was purchased from Miltenyi Biotec. The Annexin V-APC/7-AAD apoptosis detection kit was purchased from BD Biosciences. The athymic nude mice were purchased from Shanghai Experimental Animal Center (Shanghai, China) and maintained in the animal facility at Medical Biotechnology Institute according to the animal research committee's guidelines of Soochow University.

2.2. Construction of recombinant adenoviral vectors

The sense primer (5′-ATGGA TATCA TGCAG GGCCA AGAAT TCCAC TT-3′) and the antisense primer (5′-GCACT CGAGT CAGAG CTTGT AGAAT TTC-3′) were used for cloning the human IL-24 gene directly from the RNA of IFN-γ-treated human peripheral blood mononuclear cells (PBMC) by using reverse transcription-polymerase chain reaction (RT-PCR) as previously described [16]. The cloned IL-24 cDNA fragment was ligated into the pAdTrack-GFP vector expressing green fluorescent protein (GFP) to form pAdTrack-IL-24 expressing both GFP and IL-24. The recombinant backbone vector pAd-RGD, modified by RGD-4C and pAdTrack-IL-24 or pAdTrack-GFP vector DNA, linealized with PmeI digestion and was cotransfected into the bacteria BJ5183 cells. Resultant pAd.RGD-IL-24 and pAd.RGD-GFP plasmid vectors were purified from the transfected BJ5183 cells, linealized by PacI digestion, and transfected into QBI-293A cells by lipofectamine (Sigma, CA, USA), leading to the formation of the recombinant adenoviruses Ad.RGD-GFP (expressing GFP) and Ad.RGD-IL-24 (expressing both GFP and IL-24). The AdVs were amplified in QBI-293A cells, purified by cesium chloride ultracentrifugation, and stored at −80 °C.

2.3. Infection of leukemia cells

The K562, THP-1 and MEG-01 cells were cultured in 10% FCS RPMI 1640 medium with 100 U/ml penicillin and 100 μg/ml streptomycin. The medium was changed every 3 days. Before infection, the cells were extensively washed and cultured in RPMI 1640 medium without FCS. To assess the optimal multiplicity of infection (MOI) for maximal infection and transgene expression, the K562, THP-1, and MEG-01 leukemia cells were infected with Ad.RGD-GFP and Ad.RGD-IL-24 at various MOIs (25, 50, 100 and 200, respectively). Five hours later, 10% FCS was added into the medium and infected leukemia cells were then incubated at 37 °C in a humidified atmosphere of 5% CO_2 at a density of 3×10^6 cells/ml. The adenoviral infection efficiency was examined according to GFP expression by fluorescence microscopy.

2.4. RT-PCR

The IL-24 mRNA expression in the K562, THP-1, and MEG-01 cells after Ad.RGD-IL-24 infection was determined by RT-PCR analysis. In brief, the K562, THP-1, and MEG-01 cells were infected with recombinant adenoviruses for 48 h and then collected to extract the total RNA with Trizol Reagent (Invitrogen, Carlsbad, CA, USA). The first cDNA strand was synthesized using Oligo $d(T)_{18}$. The PCR primers specific for IL-24 were described above. PCR primers specific for β-actin were: the sense primer, 5′-TTCTTTGCAGCTCCTTCGTTGCCG-3′, and the antisense primer, 5′-TGGATGGCTACGTACATGGCTGGG-3′. The PCR conditions comprised 1 cycle at 94 °C (3 min), followed by 35 cycles at 94 °C (50 s), 58 °C (50 s), and 72 °C (55 s) in a PTC-100 system (MJ Research). PCR products were analyzed in 1% agarose gel electrophoresis with ethidium bromide staining.

2.5. Western blotting analysis

As described above, the leukemia cells were infected, harvested, and washed with cold phosphate-buffered saline (PBS) and lysed in lysis buffer for preparation of total cellular lysates. The protein concentration was determined by the Bradford method using spectrophotometer. Total cellular lysates (100 μg per lane) were loaded on 12% acrylamide gels, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subsequently transferred onto PVDF. Blocked with 5% (w/v) non-fat dry milk in Tris-buffered saline containing 0.05% Tween-20, the membrane was incubated with primary antibodies specific for IL-24 (1:200) and β-actin (1:1000) as an internal control for 12 h at 4 °C, followed by incubation with horseradish peroxidase-conjugated secondary antibody for another 1 h at 20 °C. The membrane was then washed and developed using Supersignal West Pico chemiluminescence detection kit (Thermo Pierce) according to the manufacturer's instructions. The protein bands were visualized after exposure of the membranes to Kodak X-ray film.

2.6. Wright–Giemsa staining

To examine the functional relevance of IL-24 with myeloid differentiation, cellular morphologic changes associated with differentiation were detected with Wright–Giemsa staining. Cells infected with Ad.RGD-IL-24