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Oncolytic vaccine virus harbouring the *IL-24* gene suppresses the growth of lung cancer by inducing apoptosis

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ABSTRACT

Lung cancer has an especially high incidence rate worldwide, and its resistance to cell death and chemotherapeutic drugs increases its intractability. The vaccinia virus has been shown to destroy neoplasm within a short time and disseminate rapidly and extensively as an enveloped virion throughout the circulatory system, and this virus has also demonstrated a strong ability to overexpress exogenous genes. Interleukin-24 (IL-24/mda-7) is an important cytokine that belongs to the activating caspase family and facilitates the inhibition of STAT3 when a cell enters the apoptosis pathway. In this study, we constructed a cancer-targeted vaccinia virus carrying the IL-24 gene knocked in the region of the viral thymidine kinase (TK) gene (VV-IL-24). Our results showed that VV-IL-24 efficiently infected and destroyed lung cancer cells via caspase-dependent apoptosis and decreased the expression of STAT3. *In vivo*, VV-IL-24 expressed IL-24 at a high level in the transplanted tumour, reduced STAT3 activity, and eventually led to apoptosis. In conclusion, we demonstrated that vv-IL-24 has the potential for use as a new human lung cancer treatment.

1. Introduction

Lung cancer has gradually become a major threat to human health because of the increases in its associated morbidity and mortality rates [1]. In China, the rate of lung cancer is significantly higher than that throughout the rest of the world. According to a research study conducted by the Harvard School of Public Health, more than 18 million people will die of lung cancer over the next 30 years. Therapeutic options for the treatment of locally advanced non-small cell lung cancer (NSCLC) have expanded over the past decade to include a range of targeted interventions, such as high-dose targeted thermal ablations, radiotherapy, and a growing platform of antibody and small molecule therapies and immunotherapies [2]. Although conventional chemotherapy drugs have played a major role in the treatment of lung cancer, they are ineffective against advanced cancers and drug-resistant tumours [3,4]. Thus, an increasing number of doctors and researchers have acknowledged that biological treatments represent a revolution in cancer research.

In 1987, Barker and Berk created the oncolytic virus ONXY-015 by knocking out 55 kd of genes in the E1B adenovirus [5]. In 1999, the *TK* and *B18R* genes were removed from JX594 [6], an oncolytic poxvirus, prior to its use in patients. In 2001, Xin-yuan Liu proposed the concept of a targeted cancer gene-virus therapy (CTGVT). On October 27, 2015, the United States Food and Drug Administration (FDA) approved a soluble tumour virus therapy developed by Amgen that uses VV-GM-CSF to treat melanoma lesions of the skin and lymph nodes that could not be removed completely by surgery [7–9]. A recombinant vaccinia virus that exhibits a perfect immune escape mechanism and satisfactory efficacy has demonstrated potential advantages in the biological treatment of cancer [10,11].

Over the past two decades, gene therapy has proven immensely popular for the treatment of various inherited and acquired disorders [12]. Gene therapy has been widely received as a potential oncological treatment strategy. Several genes have been used as effective anticancer genes in experimental contexts. Interleukin-24

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(IL-24/mda-7), a member of the Interleukin-10 family, has been identified in terminally differentiated melanoma cells [13]. IL-24 inhibits the growth of a variety of tumour cells but has no effect on normal cells, and it is closely linked to cell apoptosis and differentiation [14,15].

The signal transducer and activator of transcription (STAT) family of transcription factors plays crucial roles in regulating a number of diverse biological functions, including cell proliferation, differentiation, apoptosis, immunity, inflammatory response, and angiogenesis [16], and signal transducer and activator of transcription 3 (STAT3) is an important member of the STAT family. Recently, researchers have investigated the relationship between STAT3 activity and the malignancy of cancer.

In this study, we constructed a vaccinia virus carrying the *IL-24* gene inserted into the viral *thymidine kinase* gene (TK) locus by homologous recombination. VV-IL-24 exhibited strong carcinoma-specific cytotoxicity and significantly decreased the viability and malignancy of human lung cancer cells. VV-IL-24 induced the apoptosis of lung cancer cells by producing endoplasmic reticulum stress (ERS). *In vivo*, VV-IL-24 inhibited the growth of subcutaneous solid lung cancer tumours compared with the control group. Our findings suggest that VV-IL-24 is a potential anticancer agent.

2. Materials and methods

2.1. Cell culture

The human lung cancer cell lines A549 and H1299, the human normal lung cell line 16HBE, and the human embryonic kidney cell line HEK293 were purchased from the Shanghai Cell Collection (Shanghai, PRC). The mouse lung cancer cell line Lewis lung cell (LLC) was provided by Professor Ji Hongbin, a principle investigator of the Chinese Academy of Science (CAS, Shanghai, PRC). A549, H1299, 16HBE, HEK293 and LLC cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Shanghai, PRC) supplemented with 10% foetal bovine serum (FBS; GIBCO).

2.2. Construction, identification, and titration of VV-IL-24 and VV-Empty

The construction of the vaccinia virus carrying the *IL-24* gene and empty vector containing the *gpt* marker gene was conducted via homologous recombination between plasmids and the vaccinia WR stain. Recombination resulted in the insertion of the human IL-24 gene into the viral TK locus. Viral genomes were extracted from infected 293A cells and verified by PCR using IL-24 primers (Sense: 5′-TGTGGACTTTAGCCAGACCCTT-3′ and Antisense: 5′-GCTTTGGTCAGAGCTGCATCTA-3′), wild-type primers (Sense: 5′-AGACGATAAATTAATGATC-3′ and Antisense: 5′-GTTTGCCA-TACGCTCACAG-3′) and vector primers (Sense: 5′-GAAGGCAT-CAGTCGGCTTGCG-3′ and Antisense: 5′-CGCGCGTAATACGACTCACT-3′). The results were visualized by ethidium bromide agarose gel electrophoresis. After three freeze–thaw cycles, the viral titers were determined via the TCID50 method.

2.3. Western blot analysis

A western blot analysis was performed according to a previously described method [17,18]. The samples were subjected to electrophoresis on 12% SDS-polyacrylamide gels (Generay Biotech, Shanghai, PRC) and were transferred to PVDF membranes (Millipore, USA). The membranes were probed with the appropriate antibodies, including the primary antibody against IL-24 (GeneHunter, USA). Other primary antibodies used in this study were purchased from Cell Signaling Technology (CST). The blots were washed 3 times with TBST and labelled with peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (MultiSciences Biotech, PRC). All bands were visualized using a Superfine quantitative luminescence detector (Tanon, PRC) in conjunction with an ECL kit according to the manufacturer's instructions.

2.4. Cell viability assay

MTT (Amresco, USA) was used to determine the relative cell viability after treatment. The experiment was performed according to a previously described method [19]. The cells were cultured in 96-well plates and treated after adherence. After 4 h of incubation with MTT, the medium was removed and the cells were stained with 0.15 mL DMSO. The optical absorbance was determined at 490 nm using a microplate reader.

2.5. Flow cytometry

An apoptosis detection kit (BD Bioscience, San Diego, USA) was used to quantify cell apoptosis. The A549 cells were infected with 1 MOI VV-IL-24 and a vehicle as a control. The infected cells were harvested 24 h post infection and treated according to the manufacturer's instructions. The cells were then analysed using a FACS-Calibur platform (BD Bioscience, San Diego, USA). The experiment was repeated 4 times.

2.6. Animal experiments

All the animals used in this study were raised in the animal centre at Zhejiang Chinese Medical University. All animal pathological exams, including the immunohistochemical experiments, were performed in this centre. The results were quantified using Image-Pro Plus 6.0 software. All experiments conformed to the National Institutes of Health guidelines for the care and use of laboratory animals.

In the experiments, 5×10^6 A549 cells and Lewis cells were subcutaneously inoculated into nude mice and C57BL/6 mice, and 1×10^7 PFU VV-IL-24 was administered by intratumoural injection when the transplanted tumour size reached 60–80 mm^3. The vehicle and VV-Empty were also injected. The tumour volume was measured every three days until the volume approached the maximum permitted by the animal ethics guidelines.

2.7. Statistical analysis

All data are shown as the mean and standard deviation (SD). The data were analysed by Student's t-test using Graph Pad Prism 5 software. The results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Construction and characterization of VV-IL-24

Virotherapy is a novel strategy that avoids the problem of chemotherapy resistance, which often reduces the effectiveness of traditional therapies and leads to the incomplete eradication of tumours [19].

We constructed an expression cassette consisting of the *IL-24* gene and the *gpt* selection gene, whose expression was driven by the viral promoters p-se/l and p-7.5 k, respectively. These genes were inserted into the viral *TK* gene region (Fig. 1A). VV-Empty, a control virus, was constructed by replacing IL-24 with the empty vector pCB013. To estimate the correct formation and purity of our virus, specific primers were designed for a polymerase chain