A single intratumoral injection of a fiber-mutant adenoviral vector encoding interleukin 12 induces remarkable anti-tumor and anti-metastatic activity in mice with Meth-A fibrosarcoma ☆

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Abstract

Cytokine-encoding viral vectors are considered to be promising in cancer gene immunotherapy. Interleukin 12 (IL-12) has been used widely for anti-tumor treatment, but the administration route and tumor characteristics strongly influence therapeutic efficiency. Meth-A fibrosarcoma has been demonstrated to be insensitive to IL-12 treatment via systemic administration. In the present study, we developed an IL-12-encoding fiber-mutant adenoviral vector (AdRGD-IL-12) that showed enhanced gene transfection efficiency in Meth-A tumor cells, and the production of IL-12 p70 in the culture supernatant from transfected cells was confirmed by ELISA. In therapeutic experiments, a single low-dose (2×10^7 plaque-forming units) intratumoral injection of AdRGD-IL-12 elicited pronounced anti-tumor activity and notably prolonged the survival of Meth-A fibrosarcoma-bearing mice. Immunohistochemical staining revealed that the IL-12 vector induced the accumulation of T cells in tumor tissue. Furthermore, intratumoral administration of the vector induced an anti-metastasis effect as well as long-term specific immunity against syngeneic tumor challenge.
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The immunostimulating cytokine interleukin 12 (IL-12), a heterodimeric protein composed of two disulfide-linked subunits, is secreted by dendritic cells as well as macrophages and is a key mediator of immunity [1,2]. A variety of studies have focused on the use of IL-12 in cancer therapy and, in these experiments, IL-12 has exhibited potent anti-tumor activity in a number of tumor models [3–5]. IL-12 acts on T and natural killer (NK) cells by enhancing the generation and activity of cytotoxic T lymphocytes and inducing the proliferation and production of cytokines, especially interferon-γ [6]. In addition, IL-12 inhibits

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tumor angiogenesis mainly through IFN-γ-dependent production of the chemokine interferon-inducible protein-10 (IP-10) [7].

Several mechanisms of the anti-tumor activity of IL-12 have been identified, and each contributes differently to the overall therapeutic outcome in a given tumor model [8–10]. Further, some tumor models, such as Meth-A and MCH-1A1 cells, are resistant to treatment with systemically administered IL-12 [11,12]. For example, intraperitoneal administration of murine recombinant IL-12 failed to inhibit the growth of Meth-A fibrosarcoma, even at a dosage of 500 ng daily for 3 days [11]. Compared with so-called IL-12-sensitive tumor cells such as OV-HM ovarian carcinoma and CSA1M fibrosarcoma, which both exhibited notable tumor regression after IL-12-stimulated T-cell infiltration into tumor tissues, Meth-A and MCH-1-A1 tumors lacked similar accumulation of immune cells [12]. Furthermore, otherwise exciting tumor regression results from preclinical studies were moderated by the severe adverse effects that occurred after systemic administration of IL-12 in murine models [13]. The clinical development of IL-12 as a single recombinant protein for systemic therapy has been tempered by pronounced toxicity and disappointing anti-tumor effects [14].

Intratumoral administration of IL-12 may offer several potential advantages over systemic dosing, such as delivery of the gene directly to the tissue of interest and avoidance of the drawbacks of systemic delivery, including the induction of toxicity, acute allergic reactions, and other adverse effects due to the encoded gene [15]. The results of one clinical trial suggest that intratumoral injection of $\leqslant 3 \times 10^{12}$ viral particles of an IL-12-encoding adenoviral vector in patients with advanced gastrointestinal malignancies is feasible and well tolerated [16].

In the present study, we constructed a recombinant adenovirus (Ad) vector that encoded IL-12 (AdRGD-IL-12); the gene transfection efficiency of AdRGD-IL-12 was higher than that of a conventional Ad vector. We also investigated the feasibility of using a single intratumoral injection of AdRGD-IL-12 to provide effective cancer treatment for primary and metastatic Meth-A fibrosarcoma. Furthermore, immunostaining was used to measure the postinjection infiltration of immune cells into tumor tissue.

Materials and methods

Cell lines and animals. Meth-A fibrosarcoma cells (BALB/c origin) were kindly provided by Dr. Hiromi Fujiwara (School of Medicine, Osaka University, Osaka, Japan) and were maintained by intraperitoneal passage in syngeneic BALB/c mice. Human embryonic kidney (HEK) 293 cells were cultured in DMEM supplemented with 10% FBS. BALB/c female mice were obtained from SLC (Hamamatsu, Japan) and used at 6–8 weeks of age. All of the experimental procedures were performed in accordance with the Osaka University guidelines for the welfare of animals in studies of experimental neoplasia.

Vector construction. The replication-deficient AdRGD vector was based on the adenovirus serotype 5 backbone with deletions of E1/E3 region. The RGD sequence for αv-integrin targeting was inserted into the HI loop of the fiber knob by using a two-step method, as previously described [17]. AdRGD-Luc, which is identical to the AdRGD-IL-12 vectors but with the substitution of the luciferase gene expression cassette for the cytokine, was used as negative control vector in the present study. The replication-deficient AdRGD-IL-12, which carries the murine IL-12 gene derived from mIL-12 BIA/pBluescript II KS(−) [18] (kindly provided by Prof. Hiroshi Yamamoto, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan), was constructed by an improved in vitro ligation method using pAdHM15-RGD [19,20]. The expression cassette, which was designed to be transcribed in order from the IL-12 p35 cDNA through the internal ribosome entry site sequence to the IL-12 p40 cDNA under the control of the cytomegalovirus promoter, was inserted into the E1-deletion region of the E1/E3-deleted Ad vector (Fig. 1). All vectors were propagated in HEK293 cells, purified by two rounds of CsCl gradient centrifugation, dialyzed with phosphate-buffered saline (PBS) containing 10% glycerol, and stored at −80 °C. The number of viral particles in vector stock was determined spectrophotometrically by the method of Maizel et al. [21]. Titers (tissue culture infectious dose$_{50}$; TCID$_{50}$) of infective AdRGD particles were evaluated by the endpoint dilution method using HEK293 cells and expressed as plaque-forming units (PFU).

Gene expression by AdRGD-Luc or conventional Ad-Luc in Meth-A cells. Meth-A cells were plated in 96-well plates at a density of 2×10^3 cells/well and incubated with Ad-Luc or AdRGD-Luc at concentrations of 1250, 2500, 5000, or 10,000 viral particles/cell for 1.5 h. Cells were then washed with PBS and cultured for an additional 48 h. Subsequently, the cells were washed, collected, and lysed with Luciferase Cell Culture Lysis buffer (Promega, USA), and their luciferase activity was measured by the Luciferase Assay System (Promega,
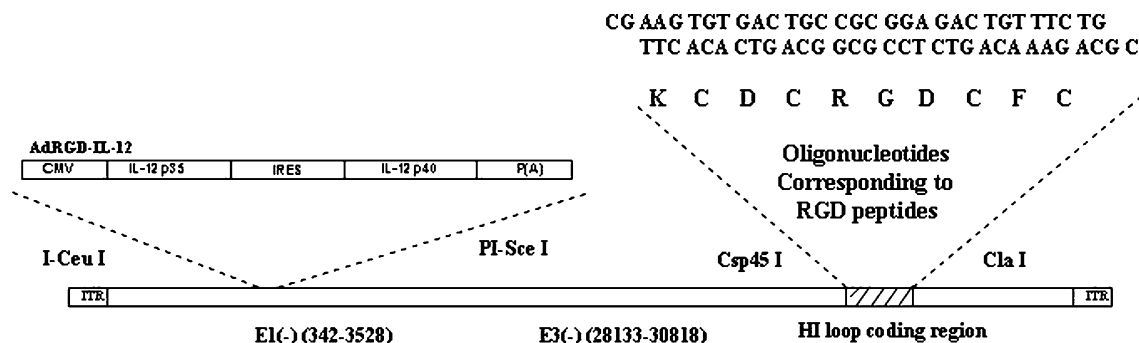

Fig. 1. Construction of IL-12 encoding fiber-mutant adenoviral vector.