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ScienceDirect
Cancer Letters 271 (2008) 105–116
CANCER Letters
www.elsevier.com/locate/canlet

Adenovirus-mediated ING4 expression suppresses lung carcinoma cell growth via induction of cell cycle alteration and apoptosis and inhibition of tumor invasion and angiogenesis

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Received 5 January 2008; received in revised form 5 February 2008; accepted 27 May 2008

Abstract

Previous studies demonstrated that ING4 as a novel member of ING (inhibitor of growth) family has potential effect on tumor inhibition via multiple pathways. However, adenovirus-mediated ING4 expression in inhibition of human tumors has not been reported. To explore its therapeutic effect on human lung carcinoma, we constructed a recombinant adenoviral vector Ad-ING4 expressing the humanized ING4 gene derived from murine ING4 with two amino acid modifications at residue 66 (Arg to Lys) and 156 (Ala to Thr) by site-directed mutagenesis. We demonstrated that Ad-ING4-mediated transfection of A549 human lung carcinoma cells induced cell apoptosis, altered cell cycle with S phase reduction and G2/M phase arrest, suppressed cell invasiveness, and down-regulated IL-6, IL-8, MMP-2, and MMP-9 expression of transfected tumor cells. In athymic mice bearing A549 lung tumors, intratumoral injections of Ad-ING4 suppressed the tumor growth and reduced the tumor microvessel formation. Therefore, Ad-ING4 may be useful in gene therapy of human lung carcinoma.

Keywords: ING4; Adenoviral vector; Humanized; Vector modification; Lung carcinoma

1. Introduction

Members of ING (inhibitor of growth) gene family have been proven as candidate tumor suppressor genes involving in tumor cell apoptosis, cell cycle regulation, and DNA repair. For example, ING1 gene encodes three different isoforms, p47ING1, p33ING1, and p27ING1, by alternative splicing [1,2]. ING1 and ING2 can negatively regulate cell proliferation in a p53-dependent manner through induction of G1 phase arrest of cell cycle and apoptosis [3–5]. ING3 can activate p53-transactivated promoters and induces a decreased population of cells in S phase and apoptosis [6]. Allelic loss and

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doi:10.1016/j.canlet.2008.05.050

reduced expression of ING3 was also found in human head and neck cancers [7]. ING4 has a plant homeodomain (PHD)-finger motif [8] transcriptional regulator involving in chromatin remodeling [9] in its COOH-terminal region and a potential bipartite nuclear localization signal (NLS) [10] in its middle region. ING4 can reduce cell population in S phase of cell cycle and induce apoptosis in a p53-dependent manner, along with the increased p21 expression in RKO cells [11] and induce significant G2/M arrest of cell cycle and enhance chemosensitivity to doxorubicin and etoposide in HepG2 cells [12]. ING4 can also suppress the brain tumor growth and angiogenesis by associating with p65 (RelA) subunit of NF-κB [13], the loss of contact inhibition elicited by MYCN or MYC [14], the activation of hypoxia inducible factor (HIF) through a physical interaction with HIF prolyl hydroxylase (HPH)-2C in a chromatin-dependent manner [15], and regulate tumor cell growth and motility [16]. In addition, it has also been found that ING4 was down-regulated in human glioblastoma cells [13], deleted in human breast cancer cells [14] and head and neck squamous cell carcinomas [17]. Therefore, ING4 is a potent tumor-suppressing agent that exerts its tumor-suppressive effect via multiple pathways in different tumor types.

Lung cancer is the most common malignancy in the world and is the leading cause of cancer-related mortality [18]. Surgery is the most effective therapeutic modality for lung cancer patients, but the post-operative prognosis is not satisfactory [19]. Adenovirus is one of the most promising vectors for cancer gene therapy [20]. A great deal of data have been accumulated, with non-replicating adenoviral vectors suggesting a reasonable safety profile in humans. Recent studies have shown that ING4 could negatively regulate the tumor cell growth in different model systems [11–13]. However, adenovirus-mediated ING4 expression in application of lung cancer gene therapy has not been reported.

In this study, we obtained the humanized ING4 gene based upon our previously cloned murine ING4 gene by site-directed mutagenesis and constructed two kinds of humanized ING4 recombinant adenoviral vector, Ad-ING4-GFP and Ad-ING4-ΔGFP in which GFP marker gene had been removed; we assessed the potential therapeutic effect of Ad-ING4 against a lung carcinoma cell line A549 *in vitro* and *in vivo* in animal model and also elucidated its potential mechanism.

2. Materials and methods

2.1. Cell lines, reagents, and mice

The pAdTrack-CMV and pAdEasy-1 vectors and human embryonic kidney cell line QBI-293A were kindly provided by Prof. Jiang Zhong, Fudan University (Shanghai, China). The A549 human lung carcinoma cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD). QBI-293A and A549 cells were cultured in RPMI1640 (GIBCO, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT). Lipofectamine2000 was purchased from Invitrogen (Shanghai, China). The reverse transcriptase polymerase MuMLV was purchased from MBI (Shanghai, China). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit was purchased from Sigma (Shanghai, China). The Annexin-V–FITC/PI apoptosis detection kit was purchased from BD Biosciences (Shanghai, China). The polyclonal goat anti-ING4 and the monoclonal anti-CD34 antibodies were purchased from Abcam (Shanghai, China) and Santa Cruz (Shanghai, China), respectively. A Transwell system and Matrigel were obtained from Corning and BD Biosciences (Shanghai, China), respectively. The SuperEnhanced chemiluminescence detection and enzyme-linked immunosorbnent assay (ELISA) kits were purchased from Applygen Technologies Inc. (Beijing, China) and Jingmei (Shanghai, China), respectively. The male athymic nude mice were obtained from Shanghai Experimental Animal Center (Shanghai, China) and maintained in the animal facility at Soochow University according to the animal research committee's guidelines of Soochow University.

2.2. Humanization of murine ING4 gene by site-directed mutagenesis

By analyzing the reported protein sequences of murine (GenPept: NP-579923) and human (GenPept: NP-057246) ING4, we found that there were merely two codon differences at residue 66 (Arg in murine ING4 and Lys in human ING4) and residue 156 (Ala in murine ING4 and Thr in human ING4). Based upon our previously cloned murine ING4 gene, we constructed a novel ING4 gene coding for human ING4 protein through humanization of the murine ING4 gene by site-directed