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Suppression of human colon cancer tumors in nude mice by siRNA CD44 gene therapy

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Abstract

The expression of CD44, an adhesion protein associated with the tumor stem cell phenotype, is increased in most human malignant neoplasms. To further delineate the role of CD44 in colon cancer, we inhibited its expression using the siRNA method. HT-29, a human colon cancer cell line producing a large amount of CD44, was transfected with a construct producing a siRNA targeting a 19 mer sequence of the transmembrane domain of CD44 spanning between exon 16 and 17. Following stable transfection, siRNA CD44 resulted in over 75% inhibition of CD44 expression. The stable lines were less adhesive to hyaluronan and more susceptible to apoptosis induced by etoposide. siRNA CD44 clones formed a lower number and size of colonies in soft agar assays. A siRNA CD44 cell clone xenografted in nude mice generated tumors with a reduced tumor volume and wet weight, as compared to control vector clone. Intratumoral gene therapy with a polyethylenimine/siRNA CD44 plasmid DNA complex resulted in tumor growth suppression in nude mice. After siRNA CD44 intratumoral gene therapy, apoptosis was increased in the tumors when compared to the control vector group. In conclusion, based on this mouse xenograft model, siRNA targeting a discrete sequence of human CD44 may provide a potential therapeutic option for colon cancer.

Keywords: siRNA; CD44; Colon cancer; Gene therapy; Xenograft model; HT-29 cells

Introduction

The term RNAi was initially coined by Fire et al. (1998). RNAi process uses long double stranded (ds) RNA for gene silencing. To achieve gene silencing, the dsRNA is processed to 21–23 nucleotides small interfering RNAs (siRNAs) by ribonuclease III cleavage and these siRNAs serve as mediators of sequence specific mRNA degradation (Zamore et al., 2000). This phenomenon has been used to silence endogenous genes in a growing range of eukaryotes including plants, fungi and invertebrate and vertebrate animals (Hammond et al., 2001). siRNA is also used to silence human genes in cultured somatic cells (Elbashir et al., 2001). The discovery of the siRNA technology has thus greatly widened its scope to be considered in therapeutic approaches, including for cancer (reviewed in (Devi, 2006; Izquierdo, 2005; Karagiannis and El Osta, 2005) and recently in gene therapy by using nanotechnology (Heidel et al., 2007a,b; Hu-Lieskovan et al., 2005; Kommareddy and Amiji, 2007; Medarova et al., 2007; Song et al., 2005).

Cell–cell and cell–extracellular matrix adhesions are important cellular features involved in the integrity of epithelial structures, including the colon. Adhesion to the extracellular matrix has important effects on phenotypic features of epithelial cells such as gene regulation, cytoskeletal function, differentiation, cell migration, growth and apoptosis (Frisch and Francis, 1994). CD44, a ubiquitously expressed cell adhesion molecule, is unique in that it regulates both cell–cell and cell–matrix interactions in normal and transformed epithelial cells (reviewed in (Ponta et al., 2003; Rudzki et al., 1997). CD44 also functions as an anti-apoptotic protein (Lakshman et al., 2004a,b, 2005; Mielgo et al., 2006) and recent studies have shown that it is a characteristic phenotype of tumor stem cells (Jin et al., 2006; Krause et al., 2006; Shipitsin et al., 2007). A

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large number of CD44 isoforms generated by alternative splicing have been implicated in tumor growth and metastasis (Gunthert et al., 1991; Hofmann et al., 1991). Based on this observation, a number of *in vitro* studies have employed siRNA CD44 generated against sequences contained in exon 2 of CD44 to inhibit breast cancer cell invasion (Bourguignon et al., 2004), exon 14 to alter prostate cancer cell invasion (Omara-Opyene et al., 2004), exon 3 to block prostate and breast cancer cells adhesion to bone marrow endothelial cells (Draffin et al., 2004), exon 3 in RANTES activation and HIV-1 enhancement (Roscic-Mrkic et al., 2003) and exon 5 in hyaluronan regulation of ErB2 phosphorylation and signaling in carcinoma cells (Ghatak et al., 2005). Previously, it has been shown that using an antisense CD44s cDNA in human colon carcinoma cell line can lead to down regulation of tumor growth and metastasis in mice (Harada et al., 2001). In this study, we designed a siRNA CD44 targeting a sequence of human CD44 located close to the transmembrane region. The aim was to maximally inhibit both standard and variant CD44 isoform expression levels and to analyze the functional consequences of this inhibition. Using the above approach, we demonstrate that CD44 protein expression can be markedly inhibited by this siRNA construct. Furthermore, *in vivo* experiments showed that CD44 inhibition results in lower tumor volume in human colon cancer xenografts in nude mice. Finally, intratumoral gene therapy using siRNA CD44 plasmid DNA resulted in tumor growth suppression in a nude mouse model, with a concomitant increase in apoptosis.

Materials and methods

siRNA

siRNAs were synthesized by Dharmacon Inc. (Lafayette, CO). siRNA sequences targeting human CD44 corresponding to genomic sequence TTCCAGAATGGCTGATCAT were used in this study. This target sequence spans over exons 16 and 17 of human CD44 (Screaton et al., 1992).

Antibodies

Mouse anti-human CD44 (Monosan, Sanbio, Uden, The Netherlands), rabbit anti-cleaved caspase 3 and mouse anti-cleaved poly (ADP-ribose) polymerase (PARP) (Cell Signaling, Danvers, MA), mouse anti-human β-actin (Sigma-Aldrich, St. Louis, MO) and mouse anti-PCNA (Santa Cruz Biotechnology, Santa Cruz, CA) were used for the Western blot and immunohistochemistry experiments. Secondary antibodies consisted of: goat anti-mouse IgG, FITC-labeled anti-rabbit IgG (Jackson Immunological Research, West Grove, PA), HRP-labeled goat anti-rabbit IgG and HRP-labeled goat anti-mouse IgG (Promega, Madison, WI) and anti-rabbit-labeled polymer HRP (Dako, Carpinteria, CA).

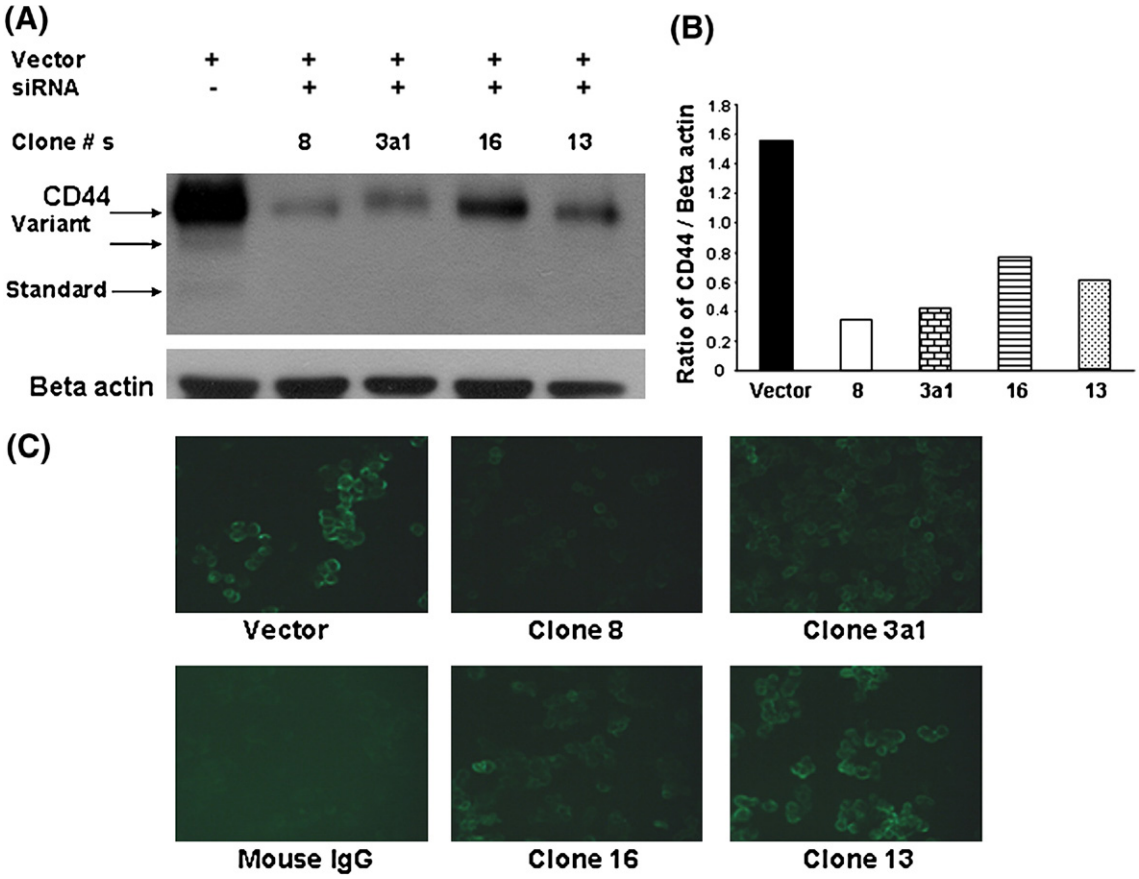

Fig. 1. Inhibition of CD44 expression following siRNA transfection. (A) Western blot analysis of CD44 in stably transfected CD44 siRNA clones shows marked decrease of variant isoform of CD44 with total loss of the standard CD44. (B) Densitometric analysis shows maximum loss of CD44 expression in clone 8 (78%) compared to clones 3a1 (72%), 16 (50%) and 13 (61%). (C) After siRNA transfection, loss of CD44 expression is shown by immunofluorescence. As compared to the vector control cells that display strong membrane expression of CD44, most siRNA transfected cells show decreased expression of CD44, with clone 8 exhibiting minimum expression. Mouse IgG was used as negative immunofluorescence control of empty vector cell (CD44 antibody, FITC fluorescence, 400×).