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Prevention of chemotherapy-induced alopecia by the anti-death FNK protein

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Abstract

Many anticancer drugs attack rapidly dividing cells, including not only malignant cells but also hair follicle cells, and induce alopecia. Chemotherapy-induced alopecia (CIA) is an emotionally distressing side effect of cancer chemotherapy. There is currently no useful preventive therapy for CIA. We have previously constructed anti-death rFNK protein from rat Bcl-x_L by site-directed mutagenesis to strengthen cytoprotective activity. When fused to the protein transduction domain (PTD) of HIV/Tat, the fusion protein PTD (TAT)-rFNK successfully entered cells from the outside in vitro and in vivo to exhibit anti-death activity against apoptosis and necrosis. Here, we show that topical application of FNK protected against CIA in a newborn rat model. The protective activity against hair-loss was observed in 30–1000 nM TAT-rFNK administrative groups in a dose-dependent manner. Furthermore, a human version of FNK (hFNK) fused to other PTD peptides exhibited a protective ability. These results suggest that PTD-FNK possesses protective activity against CIA and is not restricted to a sequence of PTD peptides or species of FNK. Thus, PTD-FNK represents potential to develop a useful method for preventing CIA in cancer patients.

Keywords: Alopecia; Hair loss; Cell death; Bcl-x_L; Protein therapy; Protein transduction domain

Introduction

Chemotherapy-induced alopecia (CIA) is one of the most common and psychologically distressing side effects of cancer chemotherapy. Although many methods have been proposed for decades, progress has been insufficient for the prevention or treatment. Many anti-cancer drugs induce apoptosis in hair follicles and cause hair loss (Schilli et al., 1998; Botchkarev et al., 2000; Selleri et al., 2004; Hendrix et al., 2005; Kim et al., 2006). Although the underlying molecular mechanism(s) of the hair follicle apoptosis induced by chemotherapy is poorly understood, the p53, Fas and *c*-kit signaling pathways were recently shown to be involved in apoptosis (Botchkarev et al., 2000; Sharov et al., 2003, 2004). On the other hand, a wide variety of agents have been reported to exhibit a protective effect on CIA in various rodent models, including 1, 25-dihydroxyvitamin D_3 (Jimenez and Yunis, 1992b), a bacteria-derived biologic response modifier ImuVert (Hussein et al., 1990), an anti-oxidant *N*-acetylcysteine (D'Agostini et al., 1998), a combination of ImuVert and *N*-acetylcysteine (Jimenez et al., 1992a), a cytokine interleukin 1 (Hussein, 1991; Jimenez et al., 1992b), an immunosuppressant cyclosporine A (Paus et al., 1994; Hussein et al., 1995), a hypertrichotic agent minoxidil (Hussein, 1995), a monoclonal antibody against doxorubicin (Balsari et al., 1994), a soybean-derived immunostimulating peptide soymetide-4 (Tsuruki et al., 2005), an α-lactalbumin-derived immunostimulating peptide Gly-Leu-Phe (Tsuruki and Yoshikawa, 2005), an immunomodulator AS101 (Sredni et al., 1996), a FPRL1 receptor agonist peptide MMK-1 (Tsuruki and Yoshikawa, 2006), an apoptosis inhibitor M50054 (2,2'-methylenebis) (Tsuda et al., 2001), prostaglandins (Malkinson et al., 1993), epidermal, fibroblast and keratinocyte growth factors (Jimenez and Yunis, 1992a; Braun et al., 2006), and a segment polarity gene product sonic hedgehog (Sato et al.,

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2001), although not all are reported to have anti-apoptotic activity. It is likely that hair loss is caused by not only apoptosis but also other mechanism(s).

Green et al. and Frankel et al. found that the transcriptional activator of transcription (TAT) protein from human immunodeficiency virus-1 (HIV-1) possesses a unique ability to enter cells from the extracellular environment (Green and Loewenstein, 1988; Frankel and Pabo, 1988). The domain that mediates the translocation of the protein was identified 11 amino acid residues, and termed protein transduction domain (PTD) or cell-penetrating peptide (CPP). We have previously made anti-death rFNK protein, which was constructed from rat Bcl-x_L by site-directed mutagenesis (Y22*F*/Q26*N*/R165*K*) to strengthen cytoprotective activity (Asoh et al., 2000), and fused to the protein transduction domain (PTD) of HIV-1/Tat protein (Asoh et al., 2002; Snyder and Dowdy, 2005), and showed that PTD (TAT)-rFNK rapidly entered cultured cells or reached chondrocytes in slice cultures of cartilage when added into culture media (Asoh et al., 2000, 2002; Ozaki et al., 2004; Asoh et al., 2005) and was delivered into the liver and brain when injected i.p. into mice (Asoh et al., 2002, 2005). In our previous studies, TAT-rFNK successfully protected chondrocytes from death induced by NO and anti-Fas antibody (Ozaki et al., 2004), reduced ischemic injury (Asoh et al., 2002; Nagai et al., 2007; Arakawa et al., 2007), mitigated carbon tetrachloride-induced liver injury (Asoh et al., 2005), protected cells from death induced by freezing and thawing (Sudo et al., 2005) and aminoglycoside toxicity (Kashio et al., 2007), and improved the transplantation efficiency of bone marrow mononuclear cells (Tara et al., 2007). Lately we found that TAT-rFNK prevented necrosis and acute hepatic injury with zonal death induced by carbon tetrachloride. It suggests that TAT-rFNK protects against cell death from both apoptosis and necrosis, and has great potential for clinical applications to prevent cell death (Asoh et al., 2005).

Here, we show that the topical application of rFNK significantly protected hair follicle cells from CIA in a newborn rat model. The protective activity against hair loss was observed in TAT-rFNK administrative groups in a dose-dependent manner. Furthermore, a human version of FNK (hFNK) was also examined, where hFNK was fused with various peptides carrying protein-transduction activity (TAT, R9, K2R7, and R7G6). All these proteins exhibited protection activity against CIA.

Materials and methods

CIA model

Wistar rats (10 day-old) were purchased from Nippon SLC (Hamamatsu, Shizuoka, Japan). Rats were fed ad libitum and housed under a 12-hour light cycle. Alopecia was induced by intraperitoneal injection of etoposide (3 mg/kg, a single treatment) on rat pups (13 day-old). Animal protocols were approved by the Animal Care and Use Committee of Nippon Medical School.

Chemicals

Etoposide was purchased from Sigma (Sigma-Aldrich Japan, Tokyo, Japan) and dissolved in dimethyl sulfoxide (DMSO) at the concentration of 20 mg/ml and further diluted with saline (Otsuka Normal Saline, Otsuka Pharmaceutical Co.-Ltd., Tokyo, Japan) at use.

Construction of human FNK

Five amino acid residues are different between human and rat Bcl-x_L proteins, as follows: Gly (human) and Glu (rat) at residues 40, Ser (human) and Pro (rat) at residues 43, Met (human) and Arg (rat) at residues 45, Ala (human) and Ser (rat) at residues 168, and Glu (human) and Asp (rat) at residues 193 (GeneBank Accession No. L20121 for human Bcl-x_L and Accession No. U72350 for rat Bcl-x_L). To construct a human FNK version, pEF1BOS ratFNK (Asoh et al., 2000), in which ratFNK was inserted at the Xba I site of pEF1BOS, was used as a template for PCR-based site-directed mutagenesis. PCR was independently performed to obtain four PCR products using following four primer pairs: the first is a pair of 5'-primer EF1α-2 (5'-GGGGTTTTATGCGATGGAGT-3'; the nucleotide sequence of the vector upstream of the FNK coding region) and 3'-primer 3970 (5'-TTCcGaTTCAGTgcCTTCTGGGGCTTCAGTC-3'; the codons underlined produce P43S and E40G substitutions) to produce fragment 1, the second is a pair of 5'-primer 3971 (5'-GgcACTGAAtCgGAAAtGGAGACCCCCAGTGC: the codons underlined produce E40G, P43S, and R45M substitutions and the 5'-end half is complementary to the 5'-end half of the primer 3970) and 3'-primer 3974 (5'-GATCCAggcTGCAATCTTACTCACCAA-3'; the codon underlined produces S168A substitution) to produce fragment 2, the third is a pair of 5'-primer 3973 (5'-GATTGCAgccTGGATGGCCACCTACCTG-3'; the codon underlined produces S168A substitution, and the 5'-end half is complementary to the 5'-end half of primer 3974) and 3'-end primer 3975 (5'-CCCGTAGAGtTCCACAAAAGTGTCCCAG-3'; the codon underlined produces D193E substitution) to produce fragment 3, and the fourth is a pair of 5'-primer 3976 (5'-GTGGAaCTCTACGGGAACAATGCA-3'; the codon underlined produces D193E substitution, and the 5'-end half is complementary to the 5'-end half of primer 3975) and 3'-primer 3932 (5'-GATGGGGAACACTGCTGTTTA-3'; the nucleotide sequence of the vector downstream of the FNK coding region) to produce fragment 4. Fragments 1 and 2 were mixed and annealed to synthesize the complementary strand, followed by amplification using 5'-primer EF1α-2 and 3'-primer 3974 to produce fragment 1/2. Fragments 3 and 4 were also mixed and annealed to synthesize the complementary strand, followed by amplification using 5'-primer 3973 and 3'-primer 3032 to produce fragment 3/4. Fragments 1/2 and 3/4 were mixed and annealed to synthesize the complementary strand, followed by amplification using 5'-primer EF1α-2 and 3'-primer 3032 to produce a full length of the human FNK coding sequence. After the final PCR product was cloned into the Xba I site of