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Modulation of endogenous VEGF-A expression by artificial transcription factors

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ABSTRACT

The vascular endothelial growth factor A (VEGF-A) gene is an attractive therapeutic target because both activation and repression of the gene are useful for treatment or cure of many diseases related to abnormal angiogenesis. To modulate the endogenous gene expression artificially, we previously designed a six-finger AZP to recognize a 19-bp DNA in the VEGF-A gene, and fused the AZP with a nuclear localization signal and a repressor domain to generate an artificial transcription factor (ATF). Using the ATF, we demonstrated efficient modulation of the VEGF-A expression. In the present study, we evaluate the ability of the ATF to modulate the gene expression in more detail. First we examined the ability of the ATF under hypoxia condition. ELISA of the VEGF-A protein in the culture medium revealed that the gene-delivered ATF repressed the expression rate of the VEGF-A gene under hypoxia condition.

INTRODUCTION

The vascular endothelial growth factor A (VEGF-A) is a pivotal stimulator of angiogenesis because its binding to VEGF receptors has been shown to promote endothelial cell migration and proliferation, two key features required for the developement of new blood vessels.[1] In addition, VEGF-A is known as vascular permeability factor, based on its ability to induce vascular leakage when injected in the guinea-pig skin.[2] An increase in vascular permeability is a crucial step in angiogenesis associated with tumours and wounds. [3] VEGF-A levels are dramatically increased by hypoxia,[4,5] triggering angiogenesis and microvascular permeability.[6,7] Treatments that reduce VEGF-A levels may prevent angiogenesis associated with tumour growth, rheumatoid, or diabetic retinopathy.[8] In contrast, treatments that increase VEGF-A levels may stimulate neovascularization to treat ischemia or wound healing.

Previously, we designed a six-finger AZP to recognize a 19-bp DNA in the VEGF-A gene by using our nondegenerated recognition code[9] and demonstrated that artificial transcription factors containing the AZP modulated the endogenous VEGF-A gene successfully.[10] In the present study, we evaluate the ability of the ATFs to modulate the gene expression in more detail. We first examine whether or not an ATF generated for repression represses the expression rate of the VEGF-A gene under hypoxia condition, such as in a 1% O_2 atmosphere.

RESULTS AND DISCUSSION

The six-finger was designed to bind to the 19-bp DNA, 5'-GGGGCTGGGGGCGGTGTCT-3', in the VEGF-A gene (Fig. 1) and fused to a nuclear localization signal from the simian virus 40 large T antigen and a Krüppel-assoiciated box (KRAB) domain of KOX1 (residues 1–75)[11] to generate the ATF for the repression of VEGF-A.[10] The corresponding mammalian expression plasmid was constructed by cloning the ATF-encoding DNA into a pcDNA3.1 (Invitrogen). The construct was verified by DNA sequencing.

Ability of the ATF to repress the VEGF-A expression was evaluated to measure amounts of VEGF-A protein in the culture medium by ELISA. A total of 2×10^4 HEK293 cells per well was plated onto a poly(D-lysine)-coated 96-

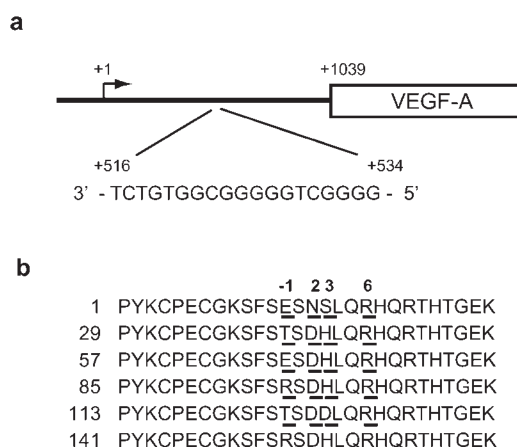

Fig.1 AZP designed for VEGF. (a) DNA sequence of the AZP target for modulation of VEGF-A expression. The 19-bp target is located from +516 to +534 (relative to the transcriptional start site, +1) in the 5' UTR. (b) Amino acid sequence of the six-finger AZP used for the 19-bp recognition. The underlined amino acids in each finger domain show recognition amino acids at positions at -1, 2, 3 and 6 in the α-helix of the finger domain. These amino acids were chosen from our recognition code table.[9]

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well plate (BD Biosciences) with 100 μl of DMEM supplemented with 0.1 mM nonessential amino acids and 10% FBS (Invitrogen) and incubated in a 5% CO_2–95% air incubator at 37°C. One day later, the ATF expression plasmid was cotranfected with a pCMV-β-galactosidase plasmid (Clontech) by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. After incubation for 12 h in a 5% CO_2–95% air incubator at 37°C, the culture medium was changed to 100 μl of fresh DMEM supplemented with 0.1 mM nonessential amino acids and 10% FBS, and the cells were incubated for 24 to 72 h in a 1% O_2–5% CO_2–94% N_2 incubator at 37°C. The culture medium was then collected, and the VEGF-A protein concentration was quantitated by using a human VEGF-A ELISA kit (R & D Systems) according to the manufacturer's instruction. Under all conditions examined, the ATF reduced the expression rate of the endogenous VEGF-A gene. Even after incubation for 72 h under hypoxia condition, the ATF reduced the expression rate to <20% of the control.

We also prepared cell-permeable ATFs [designated designed regulatory proteins (DRPs)], in which the ATF was fused to cell-penetrating peptides (CPPs), for modulation of VEGF-A expression. Evaluation of the DRPs under hypoxia condition is in progress.

CONCLUSION

By precisely regulating concentration of oxygen during incubation of mammalian cells, we demonstrated that the ATF repressed expression of the endogenous VEGF-A under hypoxia conditions. Thus we hope that the ATF will inhibit or reduce growth rate of tumours in vivo.

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