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Effect of small interfering RNAs on matrix metalloproteinase 1 expression

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ABSTRACT

Three small double strand siRNAs (506-MMP1, 859-MMP1 and 891-MMP1), each contains 25–26 nucleotides, with high specific to human MMP1 were designed according to mRNA sequence of human MMP1 (NCBI, NM_002421). To monitor the MMP1 gene expression, the total RNAs of human skin fibroblast (Detroit 551, BCRC 60118) were extracted. One human matrix metalloproteinase 1 (MMP1) partial sequence cDNA, included all the three siRNA target sequences, amplified specifically via RT-PCR and PCR reactions, and three synthesized siRNA target DNAs were cloned individually into pAcGFP1-N3 with green fluorescent protein (GFP). These reporter plasmids were then transfected individually into malignant melanoma (MeWo, BCRC 60540) and the GFP was detected after 48 h. Fluorescence results indicated that the 859 siRNA revealed highest inhibitory ability (almost 90%), and was, accordingly, transfected into MeWo cells. According to the real-time quantitative PCR and western blot, the exhibition ability to silence MMP1 gene expression was 85–89%.

1. Introduction

Matrix metalloproteinase 1 (MMP1), the member of MMP family, is a kind of zinc and calcium-dependent endopeptidase and collagenase that are able to degrade essentially all extracelluar matrix (ECM) components, such as basement membranes, collagen, and fibronectin [23,16,24]. The human MMPs family, which consists of at least 26 proteases, can be divided into several subgroups according to their structure and substrate specificity [22,28]. These subfamilies include collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs (MT-MMPs), among others. MMPs play an important role in both physiological and pathological conditions, including tissue regeneration, wound repair, reproduction, arthritis, atherosclerosis, and autoimmune blistering disorders of the skin [3]. MMPs have also been implicated in carcinogenesis because of their ability to degrade ECM, which is a key event in cancer progression [7]. Growing evidence has shown that MMPs can facilitate tumor growth, invasion, and metastasis in various cancers [7]. The ECM is composed of collagen and elastin, and is very important for creating the cellular environments during morphogenesis, tissue repair and remodeling [28,16]. Degradation of ECM in skin tissue would cause skin wrinkle [8].

The human MMPs family, which consists of at least 26 proteases, can be divided into several subgroups according to their structure and substrate specificity [22,28]. These subfamilies include collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs (MT-MMPs). MMPs play an important role in both physiological and pathological conditions, including tissue regeneration, wound repair, reproduction, arthritis, atherosclerosis, and autoimmune blistering disorders of the skin [3]. It also plays an important role on the malignant tumor metastasis. The proliferation of host tumor cell is usually accompanied by simultaneous cancer cells migration that enable them to reach the target tissue [18]. During malignant tumor proliferation, the neoplastic cells firstly attach to the underlying basement membrane. After being degraded by proteases produced by malignant cells, tumor cells pass through the basement membrane, spread into adjacent connective tissue. They proliferate to form a metastasis in target tissue and induce angiogenesis [2].

MMP1 (collagenase-1), located on chromosome 11q22, is an important member of the MMP family that specifically degrades a

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major component of the ECM, type I collagen, as well as other fibrillar collagens of types II, III, V, and IX [13,32]. The MMP1 gene is expressed in various kinds of normal cells, often at low levels under physiological conditions. However, MMP1 gene expression increases dramatically in a large number of malignancies, including head and neck cancer [25]. It is worth to mention that MMP1 is very important to metastasis and invasion of tumor cells because of its capability of degrading fibrillar collagen type I and III [26], which consequently breaks the ECM molecules.

RNA interference (RNAi) is a phenomenon of sequence-specific post-transcriptional gene silencing (PTGS) that is a conserved biological response. It was first discovered by plant biologists in 1980, but its molecular mechanism kept unknown until the late 1990. Andrew Fire and Ceaig Mell studied the nematode, *Caenorhabditis elegans*, and found that it is a specific silencing of genes, highly homologous in sequence to the delivered double-stranded RNA (dsRNA) [6]. This kind of process was considered to be a defense mechanism against viral pathogens or uncontrolled transposon mobilization [19,31]. The mechanism of RNAi essentially involves the effectors, short (21–28 nucleotides) dsRNA, and then degrades the target mRNA. RNAi is mediated by siRNAs that are processed from long dsRNAs of exogenous or endogenous origin by a cytoplasmic ribonuclease-III type called dicer [21]. The resulting siRNAs are about 21–23 nucleotides (nt) long and then incorporated into nuclease complex, a RNA-inducing silencing complex (RISC). The antisense strand of siRNA serves as a template for RISC to recognize, then targets and cleaves the mRNA containing a sequence identical to that of the siRNA, which can consequently then rapidly degrade mRNA [29]. In this study, 3 small double strand siRNAs (506-MMP1, 859-MMP1 and 891-MMP1), each contains 25–26 nucleotides, with 30–50% of GC content and high specific to human MMP1 were designed and synthesized according to mRNA sequence of human MMP1 (NCBI, NM_002421) and factors affecting RNA interfering efficiency from previous studies [1,27,12,14,20]. To directly evaluate the silencing efficiency of three siRNAs in living cells, four target genes (a long one contained all three siRNA target DNAs and three individual target DNAs) – green fluorescent fusion protein (GFP) report plasmids were created and examined (Fig. 1). Finally, the quantitative PCR and western blot analyses were also carried out to evaluate the mRNA and protein expression quantity, respectively, of endogenous MMP1 in MeWo fibroblast cells.

2. Materials and methods

2.1. Cell line and cell culture

Human embryonic skin, Detroit 551 (BCRC 60118) and malignant melanoma of human skin, MeWo (BCRC 60540) were purchased from Bioresource Collection and Research Center (BCRC). The Detroit 551 cells were grown in a culture medium A [Minimum Essential Medium Alpha Medium powder (α-MEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS), 1.5 g/L sodium bicarbonate (Sigma)], while the MeWo cells were grown in the same culture medium A with 1.0 mM sodium pyruvate. All cells were maintained in a humidified 37 °C incubator with 5% CO_2.

2.2. RNA isolation and cDNA synthesis

Detroit 551 cells were incubated in 75 mL flask in cultured medium A for 3–4 days. Total RNA were isolated from Detroit 551 cells by using UltraspecII RNA™ isolation kit (Biotecx, Houston, TX) according to the manufacturer's instructions. The first strand

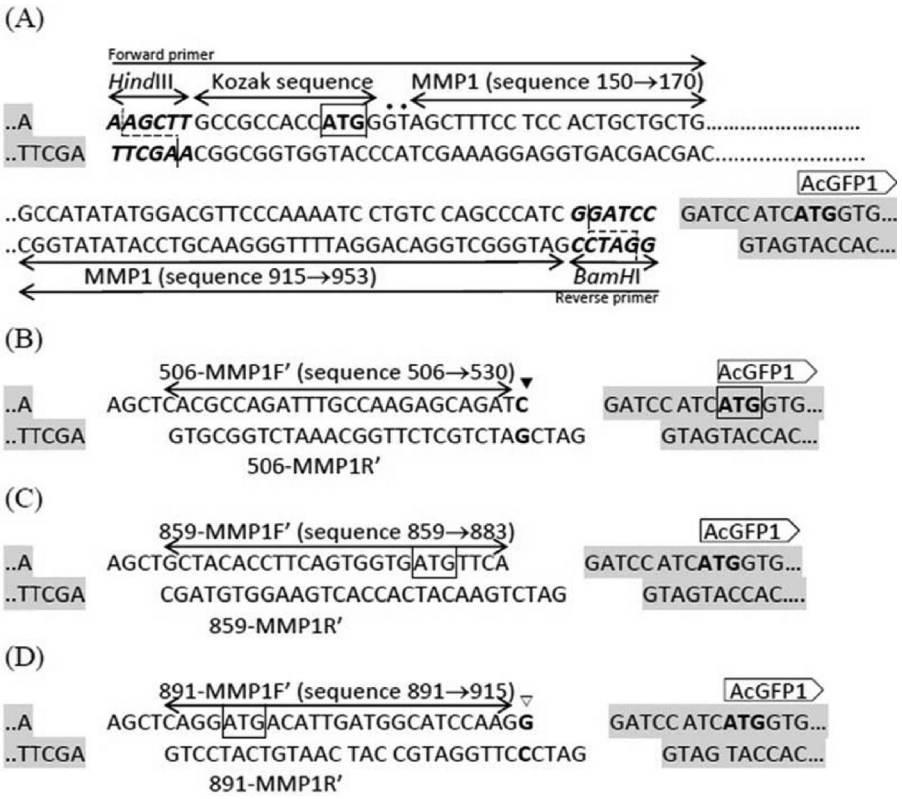

Fig. 1. Construction of MMP1-AcGFP1-N3 reporter plasmids. The sequence number was according to mRNA sequence of human MMP1 (NCBI, NM_002421). Bordered ATP indicated the translation initiation codons. Shading indicated the sticky ends of multiple cloning sites of pAcGFP1-N3 vector, which was cut by *Hind*III and *BamH*I restriction enzymes.