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Research paper

Modified siRNA effectively silence inducible immunoproteasome subunits in NSO cells

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ABSTRACT

The pathogenesis of autoimmune and neurodegenerative diseases involves overexpression of inducible subunits of the immunoproteasome. However, the clinical application of inhibitors to inducible subunits of the immunoproteasome has been limited due to systemic toxicity. Here, we designed siRNAs that efficiently silence *LMP2*, *LMP7* and *MECL-1* gene expression. Inducible subunits of the immunoproteasome are complex siRNA targets because they have a long half-life; therefore, we introduced 2′-O-methyl modifications into nuclease-sensitive sites. This led to 90–95% silencing efficiency and prolonged silencing, eliminating the need for multiple transfections. Furthermore, we showed that in the absence of transfection reagent, siRNAs with lipophilic residues were able to penetrate cells more effectively and decrease the expression of inducible immunoproteasome subunits by 35% after 5 days. These results show that siRNA targeted to inducible immunoproteasome subunits have great potential for the development of novel therapeutics for autoimmune and neurodegenerative diseases.

1. Introduction

Proteasomes are large protein complexes that degrade misfolded proteins. This function is essential for many cellular processes such as transcription, cell cycle progression, differentiation, apoptosis, and the control of stress and immune responses [1,2]. The eukaryotic 26S proteasome consists of the multicatalytic 20S core proteasome associated with regulatory complexes such as PA700/19S or PA28/11S [3]. The 20S core proteasome is formed by α and β subunits. Seven different, but related α subunits form two outer rings, whereas seven different β subunits form two inner rings. β1, β2 and β5 subunits exhibit proteolytic activity. The constitutively expressed catalytic β subunits can be replaced by inducible β immunosubunits and multicatalytic endopeptidase complex-like 1 MECL-1 (β2i). Two inducible β immunosubunits are low molecular weight protein LMP2 (β1i) and LMP7 (β5i). Together, LMP2, LMP7 and MECL-1 form the immunoproteasome. β immunosubunits are constitutively expressed in immune cells and in other cells their expression is induced by inflammatory cytokines such as interferon γ (IFNγ) [4–6].

The cleavage specificity of the immunoproteasome differs from that of the constitutive proteasome. Compared with peptides produced by the constitutive proteasome, peptides produced by the immunoproteasome can bind to MHC class I molecules more efficiently and facilitate a stronger adaptive immune response [7,8]. Recent studies revealed a connection between immunoproteasome deregulation and the progression of neurodegenerative and autoimmune diseases [9–12]. Increased expression of LMP2 and LMP7 immunosubunints was observed in all brain compartments of mice with experimental autoimmune encephalomyelitis (EAE) [9]. Furthermore, immunosubunits LMP2 and MECl-1 were shown to be markedly upregulated in Sjögren's syndrome [10]. Increased expression of immunoproteasome subunits was also found in cases of neurodegenerative diseases including Huntington's disease [11] and Alzheimer's disease [12]. These observations support the role

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