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A Novel Regulation Method of mRNA by Recruiting Regnase-1 Using Binding Motif-Antisense Oligonucleotide Conjugates

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ABSTRACT

In the present study, a novel regulation method of mRNA using RNA stem-loop motif (SL) which is recognized by Regnase-1, an endoribonuclease responsible for suppression of inflammatory mRNAs, is described. Antisense oligonucleotides (ASO) linked to SL, ASO-SLs targeting *KRAS* mRNA codon 12 were prepared and suppression of *KRAS* mRNA by ASO-SLs was evaluated. ASO-3SL containing SL linked to 2'-OMeRNA ASO targeting *KRASwt* at its 3'-end suppressed $KRAS^{wt}$ mRNA 49.2% at 100nM and 66.8% at 1μM, respectively. On the other hand, ASO-5SL containing SL linked to 2'-OMeRNA ASO at its 5'-end did not affect $KRAS^{wt}$ expression at all at 1μM.

Introduction

Endoribonuclease involved in various biological functions such as cellular inflammatory response and immune homeostasis, glial differentiation of neuro-progenitor cells, cell death of cardiomyocytes, adipogenesis and angiogenesis. Functions as an endoribonuclease involved in mRNA decay (Interleukin-1-inducible MCPIP protein has structural and functional properties of RNase and participates in degradation of IL-1beta mRNA. (FEBS J. 2009, 276:7386-7399)

Regnase-1, MCPIP1 (Monocyte chemotactic protein-1-induced protein-1) ZC3H12A (AY920403)

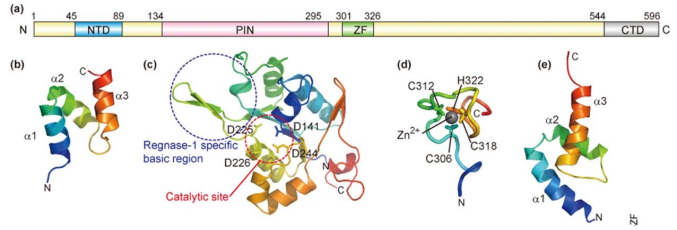

Figure 1: Structural and Function of Regnase-1.
From: Structural basis for the regulation of enzymatic activity of Regnase-1 by domain-domain interactions (F Inagaki et al, Scientific Reports 2016, 6: 22324)

Regnase-1 modulates the inflammatory response by promoting the degradation of a set of translationally active cytokine-induced inflammation-related mRNAs, such as IL6 and IL12B, during the early phase of inflammation (MCPIP1 endoribonuclease activity negatively regulates interleukin-17-mediated signaling and inflammation. Immunity 43:475-487 (2015). Regnase-1 prevents aberrant T-cell-mediated immune reaction by degradation of multiple mRNAs controlling T-cell activation, such as those encoding cytokines (IL6 and IL2), cell surface receptors (ICOS, TNFRSF4 and TNFR2) and transcription factor (REL) (Figure 2).

Figure 2. Degradation of Inflammatory mRNA by Regnase-1

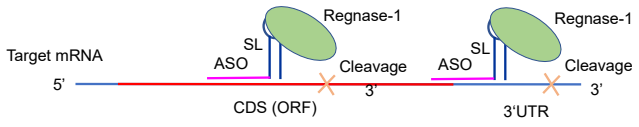

Figure 3. Recruitment of Regnase-1 and mRNA Cleavage by ASO-SL Conjugate

Results and Discussions

In the present study, we aimed to modulate Regnase-1 activity using Regnase-1 binding stem-loop motif (SL) linked to antisense oligonucleotide (ASO). We prepared two types of SL-ASO targeting *KRAS* codon 12 and 13, 2'-OMeRNA ASO linked to RNA SL at the 3'-end (ASO-3SL) and 2'-OMeRNA ASO linked to RNA SL at the 5'-end (ASO-5SL) as well as 2'-OMeRNA ASO and siRNA.

ASO: 5'-(CCUACGCCACCAGCUCCAACU)m-3'
siRNAwt: 3'-(AC)mCUCGACCACCGCAUCCGUU-5'
5'-GAGCUGGUGGCGUAGGCAA(GA)m-3'
siRNAG12D: 3'-(AC)mCUCGACUACCGCAUCCGUU-5'
5'-GAGCUGAUGGCGUAGGCAA(GA)m-3'
ASO-3SL: 5'-(CCUACGCCACCAGCUCCAACU)m-SL-3'
ASO-5SL: 5'-SL-(CCUACGCCACCAGCUCCAACU)m-3'

Suppression efficiencies of *KRASwt* mRNA in HeLa cells by ASO-3SL and ASO-5SL were evaluated by quantification of KRAS mRNA by RT-PCR and compared with those of ASO, siRNAwt and siRNAG12D (Figure 3).

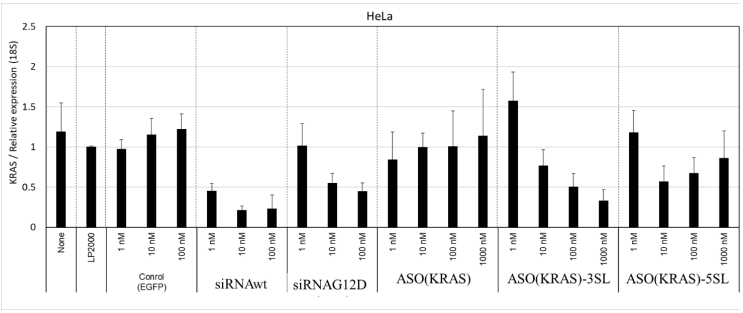

Figure 4. Suppression of *KRAS* mRNA by ASO-3SL and ASO-5SL

ASO-3SL suppressed $KRAS^{wt}$ mRNA in HeLa cells in 49.2% at 100nM. 66.8% at 1μM, while ASO-5SL did not affect *KRAS* mRNA level at all. The results indicate Regnase-1 recruited onto SL motif and cleaved mRNA at 3'-side of the SL. On the other hand, ASO-3SL suppressed $KRAS^{G12D}$ mRNA in PK-45H cells very slightly. ASO din not suppress $KRAS^{wt}$ mRNA at all at 1 μM and siRNAwt suppressed $KRAS^{wt}$ mRNA in 55.6% at 1 nM, 78.3% at 10 nM and 76.6% at 100 nM. Silencing efficiency of ASO-3SL was stronger than ASO but weaker than siRNA. The results indicate that ASO-3SL suppressed the target mRNA moderately but not too much because Regnase-1 can also regulate its own mRNA to keep the immune activity properly. This characteristic feature of ASO-3SL will be important to target immunity associated mRNAs.

Conclusion

Here we successfully report a novel regulation method of mRNA using RNA stem-loop motif. It is to be pointed out that ASO-SL could target ORF of cancer gene and that this regulation method is a universal method targeting any position of any types of mRNA. Moreover, ASO-SL is very selective and sensitive to single base mutation of cancer genes and will provide a great benefit in cancer immunotherapy such as CAR-T cell therapy and immune check point inhibitors.

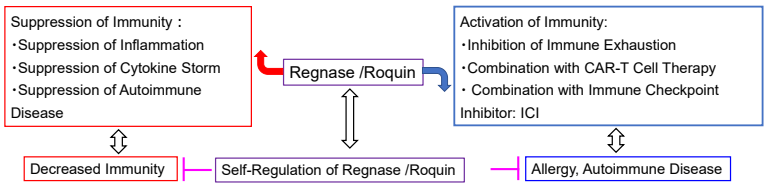

Figure 5. Immunity can be suppressed or activated by regulation of Regnase /Roquin.

COI: We have no conflict of interest to disclose for this presentation.