Enhancement of Regnase-1 expression with stem loop-targeting antisense oligonucleotides alleviates inflammatory diseases(Abstract_要旨)

AUTHOR(S):

Tse, Ka Man Carman

ISSUE DATE:

2022-09-26

URL:

https://doi.org/10.14989/doctor.k24192

京都大学	博士（　　医学　　）	氏　名	Tse Ka Man Carman
論文題目	Enhancement of Regnase-1 expression with stem loop-targeting antisense oligonucleotides alleviates inflammatory diseases (mRNA ステムループ構造標的アンチセンスオリゴ核酸を用いた Regnase-1 発現増強による炎症抑制法の開発)		

（論文内容の要旨）

Post-transcriptional regulation of gene expression plays important roles in fine-tuning inflammatory responses by regulating the stability of cytokine transcripts. Regnase-1 (also known as Zc3h12a and MCPIP1) is an RNase that restricts inflammations by degrading inflammation-related mRNAs through recognition of the stem-loop (SL) structural motifs at the 3' untranslated regions (UTRs). Accumulating evidence suggest that reduced Regnase-1 level is associated with the pathogenesis of a number of inflammatory diseases, however, strategies aiming to specifically increase Regnase-1 availability is lacking.

The goal of this study is to develop a therapeutic means to augment Regnase-1 expression through the control of its mRNA expression, and blocking Regnase-1 self-regulation pathway comes into focus. To this end, screening of the Regnase-1 3' UTR by luciferase assay allowed the identification of two SL motifs responsible for Regnase-1 self-regulation. Consistently, introduction of antisense phosphorodiamidate morpholino oligonucleotides (MOs) targeting the right-arm of the two SL structures abrogated the binding interaction between Regnase-1 and its mRNA, thereby increasing Regnase-1 mRNA stability and protein availability. Further *in vitro* analyses showed that the enhanced Regnase-1 abundance greatly reduced the expression of inflammatory transcripts targeted by this RNase, highlighting the potential usage of Regnase-1-targeting MOs (Reg1-MOs) in mitigating inflammation.

Encouraged by these findings, further experiments were performed to examine the therapeutic potentials of Reg1-MOs *in vivo* using mouse models. In a LPS-induced lung injury model, Reg1-MOs ameliorated the detrimental effects of LPS by limiting neutrophil entry to the lung through repressing the cytokine and chemokine expressions from the alveolar macrophages. In addition, in a chronic inflammation model where bleomycin was used to induce pulmonary fibrosis, repeated treatment of Reg1-MOs effectively decreased the fibrotic lesions in lungs collected from Reg1-MO group. Quantitative analyses of the lung tissue corroborated that Reg1-MO treatment decreased the level of transcripts involved in lung fibrosis development. Lastly, the experimental autoimmune encephalomyelitis (EAE) model, a mouse model of human multiple sclerosis (MS), was used to investigate if increased Regnase-1 availability is beneficial for the treatment of autoimmune diseases. Local treatment of Reg1-MOs resolved the EAE disease severity and demyelination, partly resulted from a decreased level of inflammatory transcripts in the central nervous system (CNS).

In summary, this study has deepened the understanding of the potential of Regnase-1 enhancing therapies in mitigating inflammations. The acquired evidence suggested that Reg1-MOs not only enhanced Regnase-1 availability, but also effectively reduced the expression of proinflammatory transcripts in both *in vitro* and *in vivo* systems. To the best of its knowledge, this is the first study showing that targeting the self-regulatory elements in the mRNA untranslated regions with antisense MOs could be beneficial to alter the mRNA stability and expression of target mRNAs.

（論文審査の結果の要旨）

転写後制御はサイトカイン mRNA 等の分解を通じて炎症制御に重要な役割を果たしている。Regnase-1 (Reg1)は mRNA のステムループ構造を認識して分解する RNA 分解酵素として機能し、様々な炎症性疾患の抑制に関与する。

本研究では、Reg1 が自身の mRNA を分解する自己制御機構を利用し Reg1 の発現を増強させ、炎症を抑制する方法の開発を試みた。まず Reg1 mRNA に自己制御に必要なステムループ構造が 2 か所存在する事を見出した。この構造を標的としたアンチセンスモルフォリノオリゴ（MO）核酸をマクロファージに導入したところ、Reg1 の自己 mRNA への結合を抑制し、Reg1 mRNA を安定化することでその発現を増強し、サイトカイン産生を抑制した。さらに、Reg1 標的 MO 核酸をマウスに経気道的に投与したところ、リポ多糖により誘導される肺の急性炎症モデルや、肺線維症モデルに対し改善効果を示した。また、多発性硬化症マウスモデルに対し Reg1 標的 MO 核酸を脳室内投与したところ、免疫細胞浸潤が抑制され脱髄や臨床症状が緩和したことから、Reg1 標的 MO 核酸は様々な炎症関連疾患モデルに効果を示すことを見出した。さらに、ヒト Reg1 標的 MO 核酸もヒト免疫細胞において炎症抑制効果を示すことを明らかにした。

以上の研究は mRNA 分解を標的とした核酸医薬による新たな炎症制御法の開発に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　４年７月１５日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　令和　　年　　月　　日　以降