Suppression of Notch Signaling for Rheumatoid Arthritis Therapy Using Polymerized siRNA/Thiolated Glycol Chitosan Nanoparticles

Min Ju Kim1,2, Jong-Sung Park3, So Jin Lee2, Dong-Gyu Jo3, Sang Yoon Kim4, In-San Kim5, Ick Chan Kwon1,2, and Kwangmeyung Kim1,2*

1Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, South Korea, 2KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul, South Korea, 3School of Pharmacy, Sungkyunkwan University, Suwon, South Korea, 4Department of Otolaryngology, Asan Medical Center, College of Medicine, University of Ulsan, Seoul, South Korea, 5Department of Biochemistry and Cell Biology, School of Medicine, Cell and Matrix Research Institute, Kyungpook National University, Daegu, South Korea.

113375@kist.re.kr

ABSTRACT SUMMARY
Notch signaling is involved in the pathogenesis of rheumatoid arthritis and inhibition of Notch signaling can be considered as a therapeutic strategy of rheumatoid arthritis. Here we designed poly-siRNA delivery system to silence the expression of Notch-1, and the knockdown of Notch-1 led to the attenuation of RA progression in animal model.

INTRODUCTION
Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by inflammation and bone erosion of synovial joints. Many studies have demonstrated the involvement of Notch signaling in the pathogenesis of RA through cytokine release or angiogenesis. Thus, Notch signaling can be considered as a therapeutic target for RA. Recently, it has been reported that the inhibition of Notch signaling using a γ-secretase inhibitor reduced the proinflammatory cytokine levels and attenuated the severity of inflammation in mouse model of RA.1

Gene silencing can also be concerned as a blockade strategy for Notch signaling. Since RNA interference mechanism was discovered in 1998, small interfering RNA (siRNA) have received a great attention as a powerful tool to specifically silence a target gene. However, there are critical hurdles in applying siRNA for therapy, such as poor stability in serum and low delivery efficiency.

To overcome these drawbacks, we developed a poly-siRNA delivery system, which consisted of poly-siRNA (psi) and thiolated glycol chitosan (tGC). psi is a polymerized structure of siRNA, connected by disulfide bond formations of 5’-thiol modified siRNA. Glycol chitosan, a biocompatible and biodegradable polymer, was modified with thiol groups to become tGC to form into a stable delivery complex with psi. The tGC/psi nanocomplex system exhibited an improved siRNA stability and efficient delivery to the target region in vivo.

Herein, tGC/psi nanosystem was applied to RA therapy. psi targeting Notch-1 (psi-Notch-1) formed a nanocomplex with tGC through electrostatic interactions and self-crosslinking via disulfide bond. According to our results, tGC/psi-Notch-1 successfully accumulated at the inflammation joints and exhibited therapeutic effects for RA in collagen-induced arthritic (CIA) mouse model.

EXPERIMENTAL METHODS
Poly-siRNA and thiolated glycol chitosan were synthesized as previously described.2 To form a stable tGC/psi-Notch-1 nanocomplex, psi solution (in 10 mM HEPES, pH 8.0) was slowly added tGC solution (in 10 mM HEPES with 1 mM EDTA, pH 8.0), and incubated for 1 h at 37 °C. The tGC/psi-Notch-1 consists of tGC and psi ratio of 10:1 (wt/wt).

All animal experiments were performed in accordance with guidelines of the Institutional Animal Care and Use Committee of KIST. The murine CIA model was generated using the published protocol by Chondrex. The limbs of CIA model were scored within the range from 0 of no redness or swelling to 4 of severe swelling of ankle, foot, and digits, or ankylosis of the
limb. The in vivo accumulation of nanoparticle at the arthritis joints were verified using the Cy5.5-conjugated psi. The molecular imaging of the fluorescence was monitored using spectral am X (Tucson, AZ, USA). The in vivo therapeutic efficacy of tGC/psi-Notch-1 in CIA model was evaluated by average limb scores. The tGC/psi-Notch-1 (1.5 mg/kg) was intravenously injected into CIA mouse when the average limb score of mice reached 4 out of 16. The tGC/psi-Notch-1 nanoparticle was administered every 72 h.

RESULTS AND DISCUSSION

Successful formation of TGC/psi-Notch-1 nanoparticle was verified using 8% poly-acrylamide gel (data not shown). tGC/psi-Notch-1 was systemically delivered in CIA model, and the in vivo distribution of tGC/psi-Notch-1 was monitored by fluorescence imaging at 10 h point after injection. As shown in Figure 1, high fluorescence was observed at arthritic joint of tGC/psi-Notch-1-treated mouse, compared to that of free psi treated mouse. In quantitative analysis of fluorescence intensity, tGC/psi-Notch-1 treated group exhibited approximately 4-fold higher fluorescence intensity than the intensity of free psi treated group. Figure 1 indicates that tGC protected psi-Notch-1 were able to target the arthritic joints when systemically delivered.

CONCLUSION

tGC/psi-Notch-1 nanocomplex system was developed for efficient RA therapy. The nanocomplex was successfully delivered to the arthritic joints, and the gene silencing of Notch-1 reduced the inflammation of CIA mouse model. Thus our tGC/psi-Notch-1 system offers possibility of clinical application for rheumatoid arthritis gene therapy.

REFERENCES


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