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学位論文要旨

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論文題名 The regenerative effects of CCN2 independent modules on chondrocytes in vitro and osteoarthritis models in vivo

論文内容の要旨（2000字程度)

Introduction

Connective tissue growth factor (CCN2/CTGF) promotes both proliferation and differentiation of chondrocytes *in vitro*. In addition, the stimulating effect of CCN2 on chondrocytes differentiation depends on the type of chondrocytes; CCN2 stimulates proteoglycan synthesis and calcification of growth plate chondrocytes, while it stimulates proteoglycan synthesis but not undesired calcification of articular chondrocytes. Moreover, regeneration of articular cartilage and bone can be promoted by exogenously applied CCN2 suggesting its possible therapeutic use. However, large-scale preparation and long-time storage of biologically active CCN2 is difficult, probably because of the fragility of this cysteine-rich protein.

CCN2 is comprised of four modules. These modules are insulin-like growth factor binding protein-like (IGFBP), von Willebrand factor type C repeat (VWC), thrombospondin type 1 repeat (TSP1) and carboxyl terminal cystine knot (CT), each of which was suggested to have its own biological activity. Based on these findings, it is suspected that different combinations of these modules may exert unexpected biological effects through mutual molecular interaction. This study aims to assess the effects of these independent modules, either in single form or different combinations, in order to achieve a more therapeutically usable substitute for CCN2 with a higher regenerative capacity.

Methods

1) The independent modular proteins of human CCN2 were produced and purified through recombinant protein production system with *Brevibacillus choshinensis*. While the full length CCN2 was produced by a mammalian cell culture system, or was purchased.

2) Effect of a single module, or a combination of independent modules was evaluated with human chondrocytic HCS-2/8 cells, where the chondrocytic phenotype was estimated by the gene expression of aggrecan and type II collagen via real time RT-PCR analysis.

3) The evaluation of the effects of the CCN2 modules on proteoglycan synthesis was performed by using [^{35}S]-sulfate incorporation assay on human chondrosarcoma cell line HCS-2/8 after splitting them into different wells and treating each well with either PBS, rCCN2 or the combination of the four modules.

4) Evaluation of the effects of the CCN2 modules on DNA synthesis was performed by using [^{3}H]-thymidine incorporation assays on human chondrosarcoma cell line HCS-2/8 after splitting them into different wells and treating each well with either PBS, rCCN2 or the combination of the four modules.

5) Surface plasmon resonance (SPR) methodology was used to evaluate the inter-modular interaction of CCN2 modules by applying full length CCN2 as a ligand and injecting each of the independent modules as an analyte separately to detect binding.

6) Among the single modules of CCN2 and their combainations, the IGFBP and TSP1 single modules were selected based on the results obtained from our earlier experiment to determine the effectiveness of CCN2 modules *in vitro.* Retention in gelatin hydrogels was also evaluated

Finally, evaluation of cartilage regeneration *in vivo* was performed with two rat modules prepared;

A) Surgically induced osteoarthritis rat module.
B) Chemically induced osteoarthritis rat module.

The regeneration levels were histologically analyzed, followed by statistical analysis.

Results

1) Functional analysis of the independent modules *in vitro* revealed a biological activity comparable to, or even stronger than the full length CCN2 in a few modules. Interestingly, mixed application of all 4 modules almost reconstructed the bioactivity to the level of the full length.
2) Proteoglycan synthesis and DNA synthesis were increased after treatment with either CCN2 or the combination of the four modules put together.
3) Surface plasmon resonance analysis revealed significant interaction of 2 modules and full-length CCN2 indicating partial re-association of separate modules.
4) TSP1 showed higher retention ability than the other active single modules to the hydrogel which lead us to forward it in the next *in vivo* experiment.
5) The result of cartilage regeneration experiments *in vivo* was also consistent with the findings obtained *in vitro* where the single module used (TSP1) and combination of the four modules yielded effects higher than or similar to full length CCN2 regenerative effects.

Conclusion

We are now sure that the 4 modules interact together and reconstruct the effect of full length CCN2. Although the means by which the modules interact together is still unclear yet, it is highly susceptible that through manipulating this inter-modular interaction we can increase the effect of full length CCN2. Not just that, but we are also optimistic about the use of one of CCN2 modules independently to achieve a higher regeneration ratio which give a chance for therapeutic use against osteoarthritis.