Anti-inflammatory treatment and expression of ccn1 *and* ccn2 *genes*

Glucocorticoids are known to possess strong anti-inflammatory effects and also to be involved in the control of cartilage metabolism [21]. Particularly, the effectiveness of glucocorticoids on RA symptom is so prominent that it is frequently applied clinically, despite its adverse systemic effects. It is already known that *ccn2* gene expression is induced by glucocorticoids in chondrocytic cells as well as in fibroblasts [22,23]. In contrast, molecular interaction between glucocorticoids and *ccn1* gene in chondrocytes has not been investigated. Thus, we performed Northern blot analysis, as well as the real-time RT-PCR quantification to estimate the effects of glucocorticoids on *ccn1* and *ccn2* gene expression. When HCS-2/8 cells were treated with 50 nM dexamethasone for 2.5 – 5 hours and then examined by real-time PCR, *ccn2* mRNA was induced by 1.8 to 2.3 fold, and *ccn1* mRNA was similarly induced by 1.5 to 1.9 fold up to 5 h (Fig. 3A). The results obtained by Northern blot analysis were quite similar to the PCR ones (Fig 3B).

As a control experiment, we carried out similar analysis with 17β-estradiol, which is another steroid hormone and is also one of the regulators to maintain the homeostasis of connective tissue. However, treatment of HCS-2/8 cells with 10 nM estrogen for 1 – 24 hours resulted in no significant change in *ccn1* and *ccn2* mRNA expression levels. In fact, no effects were observed even up to the concentration of 100 nM (data not shown).

Discussion

In this study, we comparatively analyzed the expression profiles of *ccn1* and *ccn2*, while simulating the course of arthritis; i.e., inflammation, tissue regeneration and anti-inflammatory treatment, utilizing a human chondrocytic HCS-2/8. In advance to the evaluation, we established a real-time PCR quantification method by using a LightCycler system. In view of the data provided for sensitivity, linearity, and reproducibility, this assay system accurately allowed the discriminating quantification of these mRNAs from the same gene family. The total reliability of this system was evident, as represented by the facts that every specific primer set produced distinct and specific PCR products (Fig. 1) and quantitative results were comparable to those of Northern blot analysis (Fig. 3).

The involvement of CCN2 in arthritic diseases has been indicated. In fact, expression of *ccn2* gene in clinical cases was reported [3]. Also in an experimental OA model, distinct induction of *ccn2* gene expression was observed. These findings are regarded as a regenerative response of damaged cartilage, since exogenously applied CCN2 was proven to be effective in cartilage regeneration. In rat models, CCN2 captured in collagen hydrogel to allow gradual release of this factor efficiently promoted the regeneration of full-thickness cartilage defect and experimentally induced OA cartilage [2]. Therefore, expression profile of *ccn2* in chondrocytes along the time course of inflammation is thought to represent the proper gene regulation to provide a regenerative molecule during arthritis, and thus itself is worth investigated. More interestingly, *ccn1* gene expression exactly followed the fluctuation pattern of *ccn2* gene expression upon any kind of stimulation tested. These results clearly indicate that not only CCN2, but also CCN1 may be provided as a regenerative molecule in arthritis. This hypothesis is supported by a number of their functional similarities. Indeed, these factors are associated with the ECM, stimulate chemotaxis and promote proliferation of endothelial cells and fibroblasts, and promote neovascularization and chondrogenic differentiation [7-15,24]. Considering such similarities and the concomitant fluctuation of gene expression upon inflammation together, CCN1 is expected to be one of the useful molecular tools to promote cartilage regeneration. In order to examine this hypothesis, it is necessary to evaluate the regenerative potential of CCN1 protein in damaged articular cartilage, as was examined with CCN2. *In vivo* evaluation of the expression of *ccn1* upon OA and RA and the effects of CCN1 protein on cartilage regeneration is currently in progress. Since all of the CCN family members are thought to be mediators of multiple signaling molecules, therapeutic utility of another member, such as CCN3/NOV is also expected and obviously, need to be explored.

Conclusion

In vitro simulation of arthritis with a human chondrocytic cell line revealed the same response pattern of *ccn1* as that of *ccn2*, which is known as a regenerative mediator in cartilage repair. Together with similar functionalities of CCN1 and CCN2 in mesenchymal tissues, these results suggest possible utility of CCN1 in regenerative therapy of damaged mesenchymal tissues.

Methods

Materials

TNF-α and TGF-β1 were purchased from Promega (Madison, WI, USA). Dexamethasone and estrogen (17β-estradiol) were purchased from Sigma (St. Louis, MO,USA).

Cell culture

HCS-2/8 cells, a chondrocytic cell line derived from a well-differentiated type of human chondrosarcoma [19], were maintained in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10 % fetal bovine serum (FBS) under an atmosphere of humidified air containing 5 % CO_2. In the experiments in which the effect of estrogen was studied, the medium was replaced with phenol red-free DMEM and 2 mM glutamine (Nissui