CHAPTER IV

DISCUSSIONS AND CONCLUSIONS

Transforming growth factor-β family (TGF-β) plays a major role in cartilage development. It is commonly used to induce chondrogenesis in embryonic (5) and adult MSCs to increase cartilage ECM synthesis (6). TGF-β consists of three isoforms, TGF-β1, 2 and 3. Among them, TGF-β1 is the major isoform (27, 9) and plays role as stimulator of synthesis extracellular matrix biomolecules such as proteoglycans and type II collagen (77). Tanimoto *et al*., demonstrated that this growth factor increase HA synthesis by induction of the HAS2 gene in rabbit synovial fibroblasts (15). In present study, HAS2 gene in equine chondrocytes was activated by TGF-β1. It was concomitant with an increase in HA level, both in monolayer and 3-D cultures. The TGF-β1 used throughout the experiment was human recombinant TGF-β1 protein. It performed very well on equine HAS2 gene expression and HA synthesis. This agreed with previous reports which using human recombinant TGF-β1 protein in various species (9, 42, 78). It has been reported that the equine TGF-β1 amino acid sequence is unique to the horse, with a difference of two amino acids in comparison to other mammalian species (79). Watts, E.J. has demonstrated that the structure of TGF-βRI and TGFβRII are, respectively, 98% and 100% homologous between the human and equine proteins. Therefore, the close homology between the receptors of the two species suggests that equine TGF-β receptors should recognize human TGF-β1 (79).

There were reported that expression of HAS2 and HAS3, but not HAS1, are found in many mammalian chondrocytes (42). Expression of HAS2 gene appears to be constitutive and plays the most important role in synthesis of high molecular weight HA (15, 42). However, HAS3 expression differently responses to cytokines and growth factors (42). It was found that HAS3 expression of rabbit synovial fibroblasts is suppressed by TGF-β1 (15). These suggested that TGF-β1 effects on HA synthesis by selective up-regulation of HAS-2 expression.

In the present study, an increase in HAS2 mRNA level was highest at 3 hours, then, decreased after 15 hours of TGF-1β stimulation. It has been reported in rabbit synovial fibroblasts that HAS2 mRNA level is highest at 6 hours which decreases after 12 hours of TGF-1β stimulation (15). These indicated that TGF-β1 responsiveness was different on various cell types.

Our report showed that cell proliferation in the monolayer culture was gradually increased with time, however, various concentrations of TGF-β1 did not significantly effect on the proliferation. In 3-D culture, cell proliferations in untreated group and all TGF-β1 treated groups were increased only the second week of culture then stable until the third week. Various concentration of TGF-β1 showed ineffectiveness on cell proliferation. Surprisingly, the proliferation in all TGF-β1 treated groups was found significantly lower compared to the untreated group. These may suggest that effect of TGF-β1 on cell proliferation depend on culture condition. It has been reported that 3D system facilitates the reversion of dedifferentiated cell back to chondrocyte phenotype (80). Therefore, the decline of cell proliferation in all TGF-β1 treated groups may result from influent of this growth factor on dedifferentiation

rather than proliferation when cultured in 3D system. These were concomitant with the better appearance of the TGF-β1 treated construct compared to the non-treated construct when investigated by histological and immunohistochemical analysis. An increase in HA and uronic acid content in 3D culture treated with TGF-β1 indicated the up-regulation of synthesis of ECM biomolecules by TGF-β1. The expression of other cartilage ECM biomolecules such as type II collagen and aggrecan will be further investigated in order to reinsure these results. Our results agreed with previous report that has shown lower production of GAGs in high proliferated chondrocytes compared to the lower proliferated chondrocytes (73).

It has been reported that TGF-β1 regulates cell proliferation in various cell types including articular chondrocytes (38, 81, 82). However, the results were controversial depending on culture condition, such as the present of serum in the medium, type of culture and physiological origin of the sample (38) or differentiation state of cells. de Haart WJ *et al*., demonstrated an enhancing effect of TGF-β1 on proliferation of bovine articular chondrocyte in monolayer culture (83). On the contrary, the stimulatory effect of TGF-β on cell proliferation is declined during chondrocytes subcultures, especially in late passage (84). These indicate many parameters influence chondrocyte responsiveness to TGF-β1.

In order to complete the present study, it is interesting to further investigate effect of TGF-β1 on construction of cartilage tissue when HAS2 mRNA is destroyed. In addition, HA product results from activation of TGF-β1 will also be investigated for identifying sizes of the product. Signaling pathway involves in TGF-β1induced HAS2 gene expression in chondrocytes is interesting to further study.

Conclusion

These results suggested that HA synthesis of equine chondrocytes in monolayer culture was induced by TGF-β1 via up-regulation of HAS2 mRNA expression. In 3-dimensional culture TGF-β1 increased HA amount in conditioned media and significantly enhanced HA synthesis and proteoglycans in constructs. These information was an important for encourage tissue engineering applications.