Introduction: The incidence of joint injuries and joint diseases are more prevalent in women than in men. Differences observed in the rate of injury to ligaments in males and females have been attributed to the response of tissues to sex hormones such as estrogen and its receptors (ER-alpha and ER-beta) (Seneviratne et al. 2004 and Yu et al. 1999). Ligament tissues such as the anterior cruciate ligament (ACL) and medial collateral ligament (MCL) exhibit the presence of estrogen receptors at both the mRNA and protein levels (Sciore et al. 1998 and Yu et al. 1998). The matrix metalloproteinase (MMP) family of enzymes are well recognized for their role in the repair of ligament tissue following injury and in the growth of tissues towards their mature functions. Elevated expression levels of MMP-13, a member of the MMP family, have been observed following injuries to knee structures (Hollo Le Graverand, 2000). Remodeling of connective tissue is an essential process following injury and MMP-13 is responsible for initiating the degradation of interstitial collagens during wound healing. Abnormally, abnormal expression of these proteases has been attributed to pathological conditions such as rheumatoid arthritis (Murphy and Hempby, 1992) and osteoarthritis (Wang et al. 2004).

Purpose: Although several studies have demonstrated that both MMP-13 and ER are present in the connective tissues, it is not clear if there is a relationship between the transcription levels of ER-alpha and MMP-13 in these tissues. If a relationship is established, it will be important to determine whether the relationship is direct and to define a role for estrogen in the relationship. The present studies were aimed at understanding the potential molecular mechanisms involved in the regulation of MMP-13 by ER-alpha (+/-hormones).

Methods: For in vitro studies, a rabbit synoviocyte cell line (HIG-82) was transiently transfected with expression constructs for ER-alpha and varying lengths of MMP-13 promoter/reporter constructs. Transfection was performed using the FuGene6 transfection reagent (Roche) as directed by the manufacturer. Following transfection, the cells were either left untreated or they were treated with ligands for estrogen receptor alpha – tamoxifen, raloxifene and 17-β estradiol. Activity levels were determined using luciferase assays. Luciferase activity was determined using a Turner TD-20 illuminometer and Dual Luciferase® Reporter Assay kit (Promega). To further clarify the role of the individual promoter elements of the MMP-13 promoter, site-directed point mutations were used in the promoter elements of rabbit MMP-13. Prior to using the HIG-82 cell line for transfections, the cells was analyzed for the presence or absence of endogenous ER using immunohistochemistry and rabbit uterus was used as a positive control for ER expression. In addition to immunohistochemistry, western blot were used on cells transfected with estrogen receptor alpha to determine if ER protein was being expressed in transfected cells.

Results: The HIG-82 cells were found to be negative for endogenous ER using both western blot analysis and immunohistochemistry. The HIG-82 cells originally determined to be ER negative became ER positive after transfection with the ER-α plasmid construct. The in vitro findings indicate that expression of the MMP-13 promoter constructs was influenced by co-transfection with ER-α. Further, the ligands of ER-α exhibit differential effects on the activity of MMP-13 in the presence of estrogen receptor alpha (See Figure 1). Several deletion constructs of the MMP-13 promoter were generated and used in transient co-transfection experiments along with ER-alpha. These transfection studies identified the promoter elements of MMP-13 gene that could potentially be involved in its regulation by ER-alpha in the presence and absence of estrogen receptor alpha ligand, 17-β Estradiol (See Figure 2). Site-directed mutagenesis was used to introduce point mutations at the key regulatory sites of the MMP-13 promoter and these were also used for transfections with ER-alpha. The results from these studies suggest that AP-1 regulatory site in the MMP-13 promoter is vital for regulation of its expression by ER-alpha in the presence and absence of ER-alpha agonist, 17-β Estradiol.

Discussion: It is well known that the binding of steroid hormones to their specific intracellular receptors modulate gene expression within hormone-responsive tissue. The results from these studies suggest that MMP-13 expression is influenced differently by ER-alpha depending upon the presence or absence of the hormone estrogen. Specific regulatory sites such as the AP-1 site present in the MMP-13 promoter are essential for the regulation of expression by ER-alpha. Further this influence can be modulated by the presence and absence of ER-alpha agonists and antagonists. These findings may have relevance to the functioning of knee components during different phases of the menstrual cycle and following menopause.

Figure 1. Effect of equivalent doses of agonists and antagonists of estrogen receptor alpha on the activity of rabbit MMP-13 promoter in HIG-82 cells.

Figure 2. Functional characterization of the promoter elements of rabbit MMP-13 in response to estrogen receptor alpha and its agonist, 17-β Estradiol. The AP-1 site in the rabbit MMP-13 promoter is critical for estrogen receptor alpha modulation of rabbit MMP-13 activity and its inhibition by 17-β Estradiol.

Acknowledgements: These studies were supported by the CIHR Institute for Gender and Health and TAS.