ESTROGEN RECEPTOR- BETA ISOFORM MODULATES MMP-13 PROMOTER ACTIVITY DIFFERENTLY FROM ER-ALPHA IN RABBIT SYNOVIOCYTES.

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Introduction: Remodelling of the connective tissue occurs throughout the growth and maturation phases of life and is subject to hormonal influences. The presence of estrogen receptors in the ligaments is well established and it suggests that female sex hormone, estrogen, may have a role to play in the proper functioning of the ligament tissue. Gender-related factors have been attributed to observed differences in the rate of injury to ligaments (e.g., anterior cruciate ligament) between male and female subjects. One of the molecules implicated in tissue remodelling following ligament injury is MMP-13, a matrix metalloproteinase that is highly regulated in connective tissues. It is well known that the biological effects of sex hormone, estrogen, are mediated through its intra-cellular receptors ER-alpha and ER-beta (reviewed by Nilsson et al.2001). Parallel studies have indicated that constructs of estrogen receptor (ER)-alpha can influence the expression of MMP-13 constructs in vitro. The alpha and beta isoforms of estrogen receptors are members of the super-family of nuclear receptors and they share a common modular structure. In spite of their structural similarity, a range of molecular evidence from in vitro studies and animal models point towards a distinct expression and functionality for ER-beta from that of ER-alpha (Omato et al. 2003; Barkhem et al. 1998). A major difference between ER-alpha and ER-beta lies in their binding affinities for the different ligands such as tamoxifen, 17-β estradiol, ICI 182,780 and raloxifene. Differences in the NH2-terminal regions of ER-alpha and ER-beta have been attributed to their response to various ligands (McDonnell et al.1995). Ligand bound estrogen receptors can regulate the transcription of a subset of genes.

Purpose: The present study was based on the hypothesis that relative expression levels of ER-alpha and ER-beta in ligament tissues and the influence of estrogen agonists and antagonists on the two isoforms will be key determinants of their regulatory role on a subset of genes such as MMP-13. The aim of the present study was to identify the key transcriptional regulatory element in MMP-13, which is required to mediate the effects of ER-beta in the presence and absence of estrogen agonists or antagonists.

Methods: ER-beta (a gift from K.Dechering, Organon) and ER-alpha (a gift from P.Chambon, Strasbourg) were first sub-cloned into the same expression vector. These constructs were then used to co-transfect a rabbit synoviocyte cell line (HIG-82, purchased from ATCC) along with a series of deletion or mutated MMP-13 promoter-luciferase promoter constructs using 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 receptors – 17-β estradiol, tamoxifen and raloxifene. Differences in the NH2-terminal regions of ER-alpha and ER-beta have been attributed to their response to various ligands (McDonnell et al.1995). Ligand bound estrogen receptors can regulate the transcription of a subset of genes.

Results: Comparison of the effects of two isoforms of estrogen receptors revealed ER-beta to be a more potent modulator of MMP-13 expression in the rabbit synoviocyte cell line (see Figure 1). The results also indicate that specific transcription factor binding sites of MMP-13 are involved in modulating the influence of ER-beta in rabbit synoviocyte cells (see Figure 2). The pattern of results obtained with the serial deletions or site directed mutational analysis of the MMP-13 promoter revealed that different regulatory sites act in conjunction to mediate the effects of ER-beta. Additionally, the impact of estrogen agonists and antagonists on the influence of ER-beta on MMP-13 was also different from that observed when ER-alpha was used.

Discussion: Our results provide evidence that ER-beta is a more potent activator of the MMP-13 promoter than ER-alpha. Furthermore, the activation of the MMP-13 promoter constructs by ER-beta was influenced by estrogen agonists and antagonists, and the pattern of responsiveness differed from that observed when ER-alpha was used. Therefore, if both ER-alpha and ER-beta are expressed in normal connective tissues, their impact on MMP-13 may depend on the relative ratio of the two receptors in specific tissues, and the availability of ligands. An increased understanding of the response of MMP-13 to estrogen receptor isoforms in connective tissue of the knee may lead to an explanation for the increased incidence of joint injuries observed in women. As the androgen receptor did not appear to impact the expression of the MMP-13 constructs (Lu, Achari and Hart, unpublished), MMP-13 expression in females may be regulated quite differently than in males.

Figure 1. ER-β is a more potent regulator of rabbit MMP-13 promoter activity than ER-α. Rabbit synoviocytes, HIG-82, were transiently co-transfected with A) expression vector for estrogen receptor β (pSG5-ERβ) or B) expression vector for estrogen receptor α (pSG5-ERα) along with the rabbit MMP-13 DNA (p370-Luc), containing the first 370 proximal bp of the rabbit MMP-13 promoter fused to the pGL2 Basic reporter gene. Values represent the mean +/- S.E. for three determinations and are expressed as percentage of p370-Luc luciferase activity without treatment with 10-8M 17β Estradiol.

Figure 2. Functional characterization of the promoter elements of rabbit MMP-13 in response to ER-β and its agonist,17β Estradiol.

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