



# OSTEOARTHRITIS



# Transforming Growth Factor (TGF)-Beta Superfamily in Osteoarthritis and Ovaria: Dependent or Independent Expression

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## Abstract

Among the many different types of arthritis conditions, Osteoarthritis (OA) also known as degenerative arthritis is the most common. Our lack of understanding of the basic mechanisms that initiate and maintain the disease remains a major challenge in the search for an effective and safe cure. Recent studies suggest that TGF-beta/Smad pathway plays a critical role through regulation of articular chondrocyte hypertrophy and maturation during osteoarthritis development. This indicates decreased TGF-beta response might be correlated with increased incidence of OA. Current understanding of TGF-beta suggests that it is essential for cartilage integrity and that it is a powerful tool to prevent or repair cartilage damage. Incidence of OA happens more frequently in women than in men after the age of 50 years. Many data point that estrogens may be involved in the progression of OA, and may have potential protective effects on joint tissues. Members of the TGF-beta superfamily are potent regulators of cell proliferation and differentiation in a number of organ systems, and three members, TGF-beta, Bone Morphogenetic Proteins (BMPs) and SMA and Mother Against Decapentaplegic MAD-related proteins (SMADs) are expressed by the oocyte and may mediate effects attributed to the oocyte. Hence, it might be suggested a correlation between the expression of these growth factors in joints and ovaria. Here, we have aimed to briefly summarize data on that subject.

## Introduction

Osteoarthritis is a disease that causes disability in the older population and is a socioeconomic burden worldwide [1]. The aetiology of OA is multifactorial, including joint injury, obesity, ageing and heredity. Its main signs are cartilage damage, synovial fibrosis, sclerosis of the subchondral bone and osteophyte formation at the joint margins. Clinically, the disease is characterized by joint pain, tenderness and eventually loss of joint function [2,3]. The cause of OA is unknown in most cases. Regardless of the initiating trigger, cartilage damage is the major event in OA attended with a very limited reparative capacity. In normal cartilage there is constant degradation as well as synthesis of cartilage matrix molecules, controlled by the chondrocytes. A high degradation rate does not necessarily implicate OA as long as there is enough compensation by synthesis [4]. Besides catabolic factors OA chondrocytes also express anabolic factors, like insulin-like growth factor-1 and TGF-beta that stim-

ulate extracellular matrix production. This makes it decisive to target cartilage damage at an early stage to prevent further progression [5]. There is no fully efficient remedial treatment for this chronic condition because of its too complicated and multifactorial pathogenesis. Another event simultaneously with the degradation of articular cartilage is the formation of new bone at the joint edges, so-called osteophytes. Important is the role of growth factors such as TGF-beta, BMPs, and others, which do not simply repair the tissue damage induced by catabolic factors, but play an important role in OA pathology.

### Estrogens in osteoarthritis

Recent studies suggest that estrogen may be involved in the progression of OA and have potential protective effects on joint tissues. There is a large body of evidence suggesting that estrogen is primarily involved in bone homeostasis in humans [6,7]. However, the available data show that its role is controversial



and does not have consistently only positive influence on OA. Estrogen could decrease bone turnover and may have potential beneficial effects for early-stage OA. Conversely, the prevention of bone loss may result in effects that are not helpful or are even harmful for late-stage OA. Different animal models are used to study the consequences of hormone depletion by ovariectomy and estrogen treatment on OA. Most studies reported a positive action on the incidence of spontaneous OA after estradiol treatment [8]. The drug inhibited the changes in CTX-II excretion and cartilage erosion in rats. The time of initiation was important, since delayed estrogen therapy was less effective in this model. The loss of proteoglycans seen after combined ovariectomy and meniscectomy was ameliorated by estrogen treatment in sheep [9]. In contrast to the beneficial effects of estrogens, some studies have reported a detrimental result, particularly an increase in degeneration and cell death after estradiol treatment in mice [9]. Estrogen administration after ovariectomy worsened the surgically induced cartilage damage in a rabbit model. A combination treatment with estradiol, progesterone or relaxin alone, or in any combination led to a loss of cartilage collagen [10]. Estrogen receptors alpha and beta, have been found in several tissues of the joint, including cartilage, bone, ligaments, and synovium that indicates that these tissues are responsive to estrogens [11,12]. Also, estrogen is one of the important regulators of the balance between bone formation and bone resorption [13]. Synovial tissue also contains estrogen receptors, and estrogen may modulate the levels and activity of certain growth factors and cytokines produced by synovial cells.

#### **Growth factors ensure crosstalk between OA and ovarian functions**

Cytokines and growth factors play an important role in the pathology of OA [14]. They are produced by the synovial cells, chondrocytes and inflammatory cells, and later, diffuse to the cartilage through the synovial fluid. One of these cytokines, IL-6, can suppress bone resorption by inhibiting the differentiation of osteoclast progenitors and by down-regulating RANK expression on mature osteoclasts [15]. When IL-6 is at a high level, it binds to soluble IL-6R and can directly induce Receptor Activator of Nuclear Factor  $\kappa$ B Ligand (RANKL) expression on osteoblasts and on synovial fibroblasts that favor bone resorption [16]. TGF-beta is a multifunctional growth factor that is produced by many cells in bone. Osteoblasts secrete TGF-beta, which is abundant in bone matrix and can be released and activated by osteoblasts and by osteoclasts during bone resorption [17]. TGF-beta is known to regulate RANKL-induced osteoclast formation and bone resorbing activity exhibiting dual effects. Its inhibitory action is mediated mainly by decreased osteoblast production of RANKL. In contrast, in the absence of osteoblasts, TGF-beta greatly increased osteoclast formation in recombinant RANKL- or TNF-alpha-stimulated cultures [18]. Therefore, although RANKL and TNF-alpha are essential for osteoclast formation, another factor such as TGF-beta may powerfully modify these osteoclastogenic stimuli. TGF-beta increases OPG secretion and also, anti-OPG antibodies partially blocked the inhibitory effects of TGF-beta. Treatment of activated T cells with TGF-beta blocked IL-12-induced tyrosine phosphorylation and activation of both Janus kinase 2 (Jak-2) and Tyk-2 kinase. Furthermore, inhibition of Jaks by TGF-beta was associated with a decrease in tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4 proteins. Abrogation of IL-12-induced Jak-Stat pathway by TGF-beta resulted in decreased T cell proliferation and IFN-gamma production, and increased apoptotic cell death. Its inhibitory action is mediated

mainly by decreased osteoblast production of RANKL. Down-regulated TGF-beta3 and pSmad-2 expression on cartilage cells may contribute to inhibited chondrogenesis, altered chondrocyte terminal differentiation and reduced hypertrophy of chondrocytes [19,20]. BMPs belong to the TGF-beta superfamily and share some of the TGF-beta functions. BMP2 can induce osteophyte formation and is expressed at a late stage of endochondral ossification. Dynamic activation of signaling in collagen-induced arthritis supports their role in joint homeostasis and disease [21]. BMP has been shown to compensate for TGF-beta in Smad3 deficiency. Chondrocytes obtained from Smad3 deficient mice show an absence of TGF-beta signaling, therefore have a high up regulation of BMP-signaling, indicating a compensatory mechanism. BMP2 appears not to be a factor that is present in cartilage under normal conditions. Thus, blocking of BMP2 will not likely interfere with normal cartilage homeostasis although it might play a role as secondary mediator of TGF-beta induced cartilage repair. BMP signaling participates in cartilage and bone remodeling, but this pathway can also influence fibroblast populations in inflamed synovium [22,23]. Overexpression of noggin, a non-specific endogenous BMP antagonist, inhibits both clinical onset and severity of arthritis. Noggin directly binds to BMP members, particularly 2, 4 and 7 and blocks the sites required for interaction with BMP receptors.

TGF-beta superfamily signaling regulates important female reproductive processes and is involved in ovarian development and function. Smad proteins are intracellular transducers of TGF-beta superfamily signaling. BMPs play a critical role in the development of mammalian ovarian follicles [24]. Mutations in the BMP genes or their receptors lead to impaired female fertility such as decreased granulosa cell proliferation, abnormal oocyte growth, and failure of follicle development [25]. The action of BMP2 is attended with enhanced Epidermal Growth Factor Receptor (EGFR) expression and is related to ERBB receptor-mediated MAP kinase signaling. BMP6 is highly expressed in mammalian oocytes and other cell populations [26]. In vivo study using BMP6 deficient mice demonstrated that BMP6 promotes normal fertility and ensures normal oocyte quality [27]. Little data are available to show the exact physiological concentrations of BMP6 in the ovary or serum [28]. Specifically, BMP4 and BMP7 are expressed in theca cells, and their receptors by granulosa cells. These BMPs enhanced and attenuated the stimulatory action of Follicle Stimulating Hormone (FSH) on estradiol production [29]. The group of Smads consists of nine distinct proteins distributed into receptor-regulated SMADs (R-SMADs1/2/3/5/9), a common SMAD (Co-SMAD4), and inhibitory SMADs (I-SMADs6/7/8). For R-SMADs2/3 generally mediate TGF-beta/activin signaling, whereas SMAD1/5/9 transduce BMP signals. I-Smads 6/7 is supposed to antagonize TGF-beta and/or BMP signaling at least in vitro [30]. Special attention should be pointed on I-Smads in the ovary. I-Smad 6 is highly expressed in oocytes of primordial follicles, but weakly expressed in growing oocytes, theca and granulosa cells [31]. So, Smad 6/7 is important for the tight control of TGF-beta signaling in order its hyperactivation in ovaries to be prevented. The results reported by Quezada et al. demonstrated that Smad7 could influence folliculogenesis by inducing apoptosis of ovarian granulosa cells in vitro [32].

Estrogen realized its effects by interaction with at least two specific receptors ER-alpha and ER-beta on classic target cells in reproductive tissues. These receptors, although in lower levels are detected in prostate [33], vascular cells [34] and astrocytes [35]. It is found that in normal bone, in fractures in young men

and women below 25 years of age, in osteophytes, and in secondary hyperparathyroid bone, from older patients. ER-beta is well expressed in osteoclast nuclei. Osteoblasts participating in areas of active bone formation and remodeling, as well as osteocytes, also express ER-beta [11]. These results point on the direct action of estrogen on the processes of osteoclastogenesis. Noggin strongly neutralized BMP2 and BMP4 actions and only slightly reduced BMP6 action. Thus, noggin would represent a physiological modulator of the biological effect of BMP factors on granulosa cells [36].

Interleukin-6 (IL-6) is a cytokine that strongly activates the immune system, enhances inflammatory response and maintains chronic inflammatory states ranging from cardiovascular diseases to infertility [37]. The production of IL-6 in the tissues of the affected joint is usually in response to IL-1beta and TNF-alpha by chondrocytes, osteoblasts, fibroblasts, macrophages, and adipocytes [38]. The increased concentration of IL-6 is present in both the synovial fluid and serum of arthritic patients. Its effect is based on promoting the formation of osteoclasts and thus bone resorption. Interleukin-6 is an autocrine regulator of the mouse cumulus cell-oocyte complex expansion, associated with impaired fertility and probably expresses effect on the oocyte spindle. Therefore, impaired fertility associated with these conditions could be related to the direct effects of abnormally high levels of IL-6 and other potent cytokines that can impair ovarian follicular cell function and oocyte quality [39]. Wang et al. demonstrated a direct link between increasing concentrations of IL-6 and the alteration in morphology of the microtubule and chromosomal alignment in metaphase-II mouse oocytes [40]. It is well established that maintenance of the integrity of the meiotic spindle and the oocyte cytoskeleton is vital for proper cell division and embryo formation. In case of abnormal spindle dynamics mediated by IL-6 elevation we can expect aneuploidy, failure of fertilization or low reproductive ability under certain pathological conditions [41].

Considering the sex-specific incidence and prevalence difference, estrogen is supposed to play a role in OA regulation. Factors influencing endogenous, long-term serum estrogen levels, defined by the fertile period or menopause, may be associated with OA development. Although the importance of meiotic maturation of oocytes and its coordination with the folliculogenesis has long been known, much less is known about the significance of this process in relation to development of OA in females. Oocyte maturation rearrangements are of great significance for the oocyte quality. Any disturbances in these events may lead to chromosomal abnormalities during oogenesis [42]. The follicle growth and development depends on hormonal signaling and on a feedback between pituitary gland and ovaria. Dramatic changes in follicles (antral to preovulatory stage follicle growing) are accompanied with a resumption of meiosis in the oocytes. Meiotic oocyte maturation progresses to the end of first meiotic division and immediately starts the second one. These events require rearrangements of the chromatin, cytoskeletal and other factors depending on protein-kinase activity, motor proteins and gene activity regulators [43,44]. These changes are involved in the organelle redistribution (mitochondria, Golgi complex and endoplasmic reticulum) accompanying the oocyte maturation [45]. The relatively large cytoplasm volume and the control upward of chromatin and ooplasm rearrangements depend on the oocyte environment in the growing follicle: granulosa cells extend thin processes across zona pellucida till the final moments of oocyte maturation in the preovulatory follicle – metaphase II arrest [46]. These fine connec-

tions are part of the follicle and ovarian environmental control of oocyte maturation in vivo and in vitro [47].

Pituitary control of the ovarian function leads to a change of the FSH levels in premenopausal women: the menopause is preceded by a period of rising gonadotropin, predominantly FSH, levels. During this period, ovarian estrogen production appears to be maintained and ovulation continues, but luteal progesterone levels decline [48]. The appearance of high levels of FSH and luteinizing hormone [7] is characteristic of the perimenopause and often precedes the sustained decrease of sex hormone secretion by the ageing ovary. The influence of the elevated pituitary secretion and the ovarian ageing are well known factors of the female transition to menopause. The affected by osteoarthritis individuals are usually elderly and mostly inactive. However, athletes and younger individuals are also susceptible because they use their joints more and the risk of OA incidence is higher [49]. The assumed relationship between the female hormonal aspects and OA was not clearly observed. These relations are perhaps too complex, or other mechanisms that play a role in the increased incidence in women aged over 50 years, yet to be determined. Despite of studies elucidating the influence of different systemic diseases on folliculogenesis and oocyte maturation [50-52], the possible influence of OA on these processes remains still unknown.

Lower estradiol levels in elderly women were associated with increased bone resorption and higher frequency of osteopenia and osteoporosis. The changes in bone density may not clearly exerted but an increase in bone turnover was distinctly apparent in women with severe estradiol deficiency [53]. Interesting results have been reported by Huang et al. [54] which show that low dose estradiol treatment has beneficial effect in postmenopausal women with lower bone mineral density and increased bone turnover. Patients with lower endogenous estradiol concentrations at baseline expressed greater response to ultra-low estradiol therapy evidenced by a reduction in bone turnover markers and greater increase of bone mineral density.

In vitro experiments could give additional insight in the mechanisms of estrogen action on osteoarthritis. In such experiments it has been established that estrogen can increase proteoglycan synthesis [55] and decrease matrix metalloproteinase-1 mRNA levels [56] in and nitric oxide production by chondrocytes [57,58]. The effect from estrogen on bone is probably not the only mechanism involved in the development of OA, since other tissues in the joints are also responsive to estrogen. Cartilage is known to express estrogen receptors. Under physiologic concentrations, neither the mitotic rate of articular chondrocytes nor their proteoglycan metabolism is under the direct action of estradiol [59]. Estrogen receptor-beta is the predominant estrogen receptor subtype detected in normal synovial tissue but not ER-alpha [60].

### Conclusion

TGF-beta signaling pathway is tightly regulated in several cellular levels to ensure its proper physiological function, including ligand, receptor and R-Smad levels. Both chordin and noggin are endogenous TGF-beta antagonists, inhibiting TGF-beta binding to its receptor to activate the signaling pathway. Current understanding of TGF-beta suggests that it is essential for cartilage integrity and that it is a powerful tool to prevent or repair cartilage damage. BMP2 and BMP6 are expressed in arthritic synovium and are strongly up-regulated by proinflammatory cytokines (Figure 1). Although, BMP signaling has been

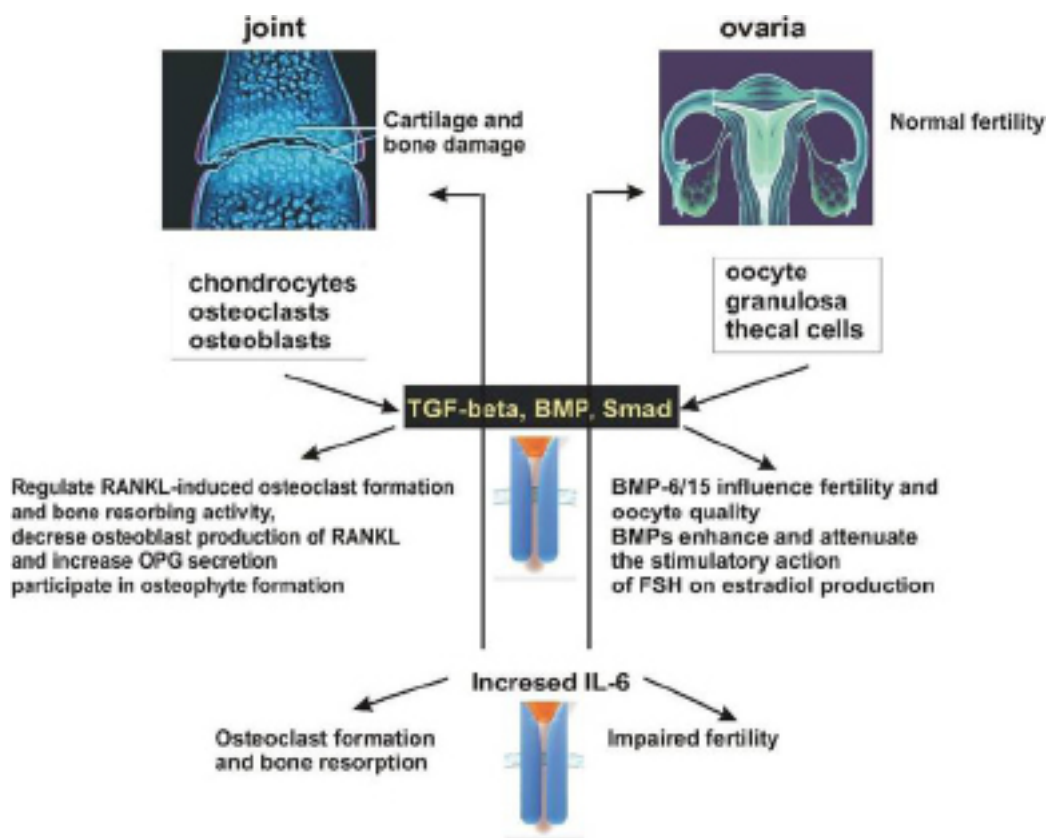
proposed to be involved in cartilage and bone repair in arthritis, this pathway may be equally important in modulating fibroblast like cell populations in inflamed synovium. In arthritis, such strategies are of critical importance in restoring the damaged tissue that causes morbidity and disability, even after control of the inflammation has been achieved. Therefore, there is an emerging interest in embryonic signaling, more specifically in those growth factors and morphogens known to be involved in the embryologic formation of the skeletal tissues and the joint (Figure 1). Animal models give the opportunity to study the disease and for preclinical evaluation of potential anti-arthritic strategies. The administration of estradiol and FSH in preclinical models and the determination of TGF-beta, BMP6 and Smad expression in the joints, simultaneously with the expression in

mouse ovaria, will provide information about their role in the different stages of OA. Gaining new knowledge on the interactions between hormonal levels and OA will help to determine the patients in risk to develop the disease and will contribute for better understanding of OA etiology. Focusing on this wide spread and high cost disease, leading to invalidisation, we expect to support findings of new therapeutic approaches, in regard to prevention and improvement of health care for chronic patients.

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#### Figures



**Figure 1:** TGF-beta superfamily expression in osteoarthritis and ovaria.

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