# Relaxin and Its Role in the Development and Treatment of Fibrosis

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## Abstract

Relaxin, a peptide hormone of the insulin superfamily, is involved in the promotion of extracellular matrix remodeling. This property is responsible for many of the well-known reproductive functions of relaxin. Recent important findings, including the identification of the relaxin receptor and the development of the relaxin-null mouse, have identified new targets and mechanisms for relaxin's actions, resulting in unprecedented advances in the field. Relaxin has emerged as a natural suppressor of age-related fibrosis in a number of tissues, including the skin, lung, kidney, and heart. Furthermore, relaxin has shown efficacy in the prevention and treatment of a variety of models of experimentally induced fibrosis. The intention of this review is to present a summary of recent advances in relaxin research, with a focus on areas of potential translational research on fibrosis in nonreproductive organs.

Relaxin is a peptide hormone of the insulin superfamily (reviewed in (1)). Like insulin, relaxin is 6 kilodalton protein processed from a prepro-form to the mature hormone containing A and B peptide chains, connected by two interchain disulfide bridges, and one intrachain disulfide within the A chain. Despite their structural similarity, relaxin and insulin bind to distinct and unrelated receptors, and hence have no common cellular effects. The historical role of relaxin has been in reproduction, in which it functions to inhibit uterine contraction and induce growth and softening of the cervix. During the first trimester of pregnancy in humans, circulating relaxin rises from essentially undetectable levels to a maximum of approximately 1ng/mL, and then gradually declines in the period before delivery. In the female, circulating relaxin is produced by the ovarian luteal cells. In the male, relaxin produced by the prostate is found in seminal fluid, but is not generally detected in the circulation. However, recent evidence suggests that relaxin may be produced locally to act in an autocrine or paracrine manner in some tissues.

Recent important findings have resulted in unprecedented interest in relaxin. It is now known that several relaxin-like peptides exist. Two relaxin genes are present in humans, encoding proteins known as H1 and H2 relaxin, but only H2 relaxin is known to circulate. Only humans and great apes are known to possess the H1 relaxin gene; other species have orthologs of the H2 gene, and therefore from this point "relaxin" will refer to H2 relaxin and its orthologs. The Leydig cell-associated insulin-like peptide 3 (InsL3), also known as relaxin-like factor (RLF), was first described in 1993 (2,3). A third relaxin gene was identified in 2002, encoding relaxin-3, known as H3 relaxin in humans (4). Additional relaxin-like peptides have since been

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identified, including insulin-like peptides InsL4, InsL5 and InsL6, but at present little are known about these peptides.

Perhaps the largest influence on the increased interest in relaxin was the identification of the relaxin receptors. Because of its similarity to insulin, most laboratories focused on tyrosine kinase receptors as potential relaxin receptors. However, in 2002, Hsu and colleagues reported that two orphan G-protein receptors, LGR7 and LGR8, were in fact relaxin receptors (5). These receptors are now known as relaxin family peptide receptors RXFP1 and RXFP2, respectively (6). While relaxin will bind and activate both RXFP1 and RXFP2 in studies using *in vitro* cell models, *in vivo* evidence suggests that the cognate receptor for relaxin is RXFP1. The receptor for RLF/InsL3 is now considered to be RXFP2, and together they regulate testicular descent (7). The receptors for relaxin-3 and InsL5 are known as RXFP3 and RXFP4, respectively. The relaxin-3/RXFP3 system is thought to function primarily in the brain, where it stimulates food intake and activation of the hypothalamic-pituitary-gonadal axis (8,9). Little is currently known about the function of InsL5/RXFP4. The emerging data resulting from the study of these ligand-receptor pairs has greatly advanced the understanding of relaxin and its related proteins. However, the primary focus of this review will be on the emerging role of relaxin and RXFP1 in the control and treatment of fibrosis. For further information on relaxin and related peptides and their receptors, the reader is directed to several excellent recent reviews (1,6,10–12).

## Fibrosis, extracellular matrix remodeling, and relaxin

Fibrosis is a condition marked by excessive accumulation of extracellular matrix components, particularly the fibrillar collagens (such as types I and III). This accumulation is primarily due to activation of fibroblasts to a myofibroblastic phenotype characterized by accelerated fibrillar collagen production (13). Furthermore, there is a decrease in the clearance of extracellular matrix due to decreased secretion of the matrix metalloproteinases (MMPs) that degrade collagen, and an increase in their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). This process is part of the normal healing response after tissue injury. But if the process continues unabated, such as with chronic toxin exposure or viral infection, the end result is continued production and decreased clearance of extracellular matrix, resulting in tissue scarring, and disruption of normal organ function. In many types of fibrosis, there are few options for treatment. Therefore, agents that reduce collagen production and increase its clearance are in demand to treat fibrotic diseases. Relaxin is one of these candidate agents.

The first function attributed to relaxin was in lengthening of the pubic ligament. In 1929, Hisaw identified relaxin as a corpus luteum-derived hormone that could experimentally induce relaxation of the pelvic ligament (14). Examination of the relaxed ligament revealed remodeling of the collagen from dense bundles to looser, less structured fibers (15). It was later found that in pregnancy, relaxin is responsible for widespread extracellular matrix remodeling in the cervix, vagina, and in some species, the pubic symphysis (16). Recently, these findings were confirmed using the relaxin-null mouse model (17). Furthermore, in addition to the effects in reproductive organs, the relaxin-null mouse developed multiple fibroses with aging, as evidenced by increased accumulation of interstitial collagen. In many cases, this excess collagen accumulation could be reversed by restoring relaxin levels in these animals (18). Interestingly, in most tissues the fibrosis was particularly evident in male mice, demonstrating that relaxin is relevant in nonreproductive tissues in males as well as females. The property of extracellular remodeling and the role of relaxin in the suppression of age-related fibrosis raises the possibility that relaxin might be used therapeutically to reduce the scarring due to the accumulation of collagen in fibrotic diseases. A summary of recent studies of relaxin treatment of fibrosis follows.

## Scleroderma (Systemic Sclerosis)

The earliest studies of relaxin and fibrosis were on scleroderma, a condition characterized by fibrotic lesions of the skin, lung, gastrointestinal tract, and arteries. Relaxin treatment of normal dermal fibroblasts or scleroderma fibroblasts resulted in a marked decrease in collagen secretion and increased collagen degradation (19,20). Further evidence for a possible role for relaxin in scleroderma came from two *in vivo* models that showed reduced collagen content and fibril density using subcutaneously implanted polyvinyl sponges and capsule formation around osmotic pumps (21). Consistent with this role for relaxin, the relaxin-null mouse developed age-related dermal thickening and fibrillar collagen accumulation, which was reversible by relaxin treatment at early stages of fibrosis, but not when fibrosis became extensive (22). Unfortunately, treatment of scleroderma in humans has produced mixed results. Early studies in the late 1950s and early 1960s generally demonstrated a benefit of partially purified porcine relaxin for many symptoms of scleroderma, but further studies were delayed for many years due to concerns about use of impure relaxin preparations in humans (16,23). More recently, a placebo-controlled trial was performed on human scleroderma patients, using two doses of highly pure recombinant relaxin (24). Relaxin treatment was found to be relatively safe with few reported side effects. The lower dose of relaxin (25 μg/kg/day) induced significant improvement in skin thickness, as well as benefits in other secondary skin and lung parameters. However, at the higher dose of relaxin (100 μg/kg/day), no benefit was seen. In a subsequent phase III trial, these early results were not replicated, and the assessments using lower dose relaxin were not different from placebo (25). A possible reason for the failure may be the advanced degree of scleroderma of the patient population (moderate to severe scleroderma), particularly in light of the finding that relaxin treatment did not reverse advanced stages of fibrosis in the relaxin-null mouse model (22). Additional explanations may include downregulation of relaxin signaling at the doses used, or the development of relaxin autoantibodies, which were detected at both low and high relaxin doses (24). Therefore, although the evidence thus far casts doubt on the utility of relaxin in the treatment of advanced scleroderma, further studies of relaxin in the treatment of less severe scleroderma may be warranted.

## Pulmonary Fibrosis

The lung has emerged as a relaxin target organ. Relaxin treatment of human lung fibroblasts resulted in a reduction in the expression of collagen types I and III and fibronectin in response to transforming growth factor beta (TGFβ), a potent fibrogenic agent, and furthermore promoted extracellular matrix degradation by increasing the levels of MMPs (26). In an *in vivo* model, relaxin treatment dramatically decreased bleomycin-induced collagen content in the lung, alveolar thickening, and improved the overall fibrosis score (26). Recent studies using the relaxin-null mouse model have demonstrated age-associated pulmonary fibrosis in these animals which could be reversed by relaxin treatment (27). Interestingly, the fibrosis was more pronounced in the male mice than in females. A possible explanation for this observation is the presence of estrogen. When the loss of estrogen production by ovariectomy was performed on relaxin-null mice, lung collagen content was increased compared to either sham-operated relaxin-null mice or ovariectomized wild-type mice (28). Additional studies revealed that endogenous relaxin protects against allergic airway disease induced by ovalbumin challenge. The fibrosis induced by chronic ovalbumin treatment in relaxin-null mouse was higher than wild-type controls (29). Furthermore, older relaxin-null mice with established fibrosis showed a very rapid increase in lung collagen in response to ovalbumin (30). Interestingly, some different effects were seen in similar studies performed in RXFP1-null mice. The RXFP1-null mice developed increased peribronchial and perivascular fibrosis more rapidly than the relaxin-null mice (31). However, the range of collagen deposition was limited, and did not extend into the lung parenchyma as seen in the relaxin-null mice (31). When exposed to the ovalbumin

model above, the RXFP1-null mice did not develop more extensive fibrosis than wild-type mice (32). These findings raise the possibility that in the lung, relaxin may not work exclusively through RXFP1, but possibly through additional, as yet unidentified receptors. Taken together, the evidence thus far suggests that relaxin/RXFP1 system is active in the lung, and that relaxin may develop into a clinical treatment for pulmonary fibrosis.

## Renal Fibrosis

Relaxin has antifibrotic effects in experimental models of renal fibrosis. In cultured renal fibroblasts, cortical-epithelial cells and mesangial cells, relaxin decreased TGF-β-induced fibronectin levels, and promoted fibronectin degradation (33). In other studies using renal fibroblast cell lines and primary cortical fibroblasts, relaxin inhibited TGF-β-induced fibroblast-myofibroblast transition, contractility, collagen I and fibronectin secretion, and increased MMP secretion (34,35). In addition, relaxin decreased the phosphorylation and nuclear localization of Smad2, and association of Smad2 with Smad3 (34). Because Smad2 nuclear translocation and association with Smad3 are critical to many of the profibrotic effects of TGF-β, these studies provide a possible mechanism for relaxin's effects on fibrotic pathways triggered by TGF-β. These results were very recently confirmed, and in addition relaxin was shown to act through activation of the nitric oxide/cGMP pathway (36).

Further data in support of a role for relaxin in renal fibrosis have come from several *in vivo* models of renal fibrosis. Using a bromoethylamine model of renal fibrosis *in vivo*, Garber *et al.* showed that relaxin decreased interstitial fibrosis and TGF-β levels, reduced macrophage recruitment, and improved glomerular filtration rate and serum creatinine levels (37). In two models of renal mass reduction, relaxin decreased mesangial collagen levels (38). Similar results were seen with an *in vivo* immune model, in which relaxin decreased interstitial fibrosis, reduced proteinuria, and decreased serum creatinine levels (33). In spontaneously hypertensive rats, relaxin decreased renal interstitial collagen (39). Relaxin treatment of Munich Wister rats, which develop progressive nephrosis, decreased glomerular and tubular collagen content and improved renal function (40).

In addition to the induced models described above, the relaxin-null mouse is itself a model of progressive renal fibrosis. With aging, fibrosis was observed as increased types I and III collagen in the glomeruli and to a lesser extent in the interstitium (41). Similar to the results in the lung from the relaxin-null mice, the renal fibrosis was observed in the male mice only. However, unlike the lung, ovariectomy did not exacerbate fibrosis in the relaxin-null mice (28), suggesting that estrogen is not protective against renal fibrosis in this model. Further evidence of a role for endogenous relaxin in protection against renal fibrosis was reported from studies that combined an induced fibrosis model with the relaxin-null mouse. Unilateral ureteric obstruction of the kidneys resulted in tubulointerstitial fibrosis and a concomitant decrease in the expression of relaxin in the kidney (42). When the obstruction was performed in the relaxin-null mouse, the fibrosis was accelerated, and this effect could be prevented by administration of exogenous relaxin (42). Taken together, these studies make a convincing case for relaxin as a natural protective agent against induced or age-related renal fibrosis. Moreover, relaxin was effective in several different *in* vivo models, suggesting that it may be widely effective in the treatment of renal fibrosis from different causes.

## Cardiac Fibrosis

Relaxin treatment of primary rat atrial and ventricular fibroblasts caused alterations in fibrotic markers, including decreased collagens type I and III, fibroblast to myofibroblast transition and cell proliferation, and increased MMP secretion (43). Male, but not female, relaxin-null mice developed increased left ventricular collagen content and collagen type I expression with aging, which was reversed with exogenous relaxin treatment(43,44). As was seen in the kidney,

there was a gender-specific effect, as the female relaxin-null mice displayed no fibrosis, and estrogen deficiency by ovariectomy failed to induce cardiac fibrosis, although cardiac hypertrophy did develop (28). Relaxin has also shown efficacy in the treatment of experimentally-induced cardiac fibrosis. Isoproterenol-induced cardiac ischemia caused increased interstitial collagen accumulation and cardiac hypertrophy, which were reduced by relaxin treatment (45). In an innovative study, adenoviral-mediated delivery of relaxin was used to treat cardiac fibrosis caused by transgenic overexpression of the β2-adrenergic receptor, resulting in a dramatic decrease in interstitial collagen content in the left ventricle, but not other (nonfibrotic) chambers of the heart (46). In a model of diabetic cardiomyopathy characterized by increased collagen content in the left ventricle myofibroblast activation, relaxin caused decrease in both parameters, and additionally promoted matrix degradation by increasing interstitial MMP content and decreasing the levels of the tissue inhibitors of metalloproteinases (TIMPs), endogenous inhibitors of MMPs (47). However, the protective property of relaxin is not universal, as there was no difference between wild-type and relaxin-null mice in a model of cardiac fibrosis induced by chronic pressure overload (48). Nevertheless, substantial evidence exists suggesting the possible use of relaxin in the treatment of cardiac fibrosis.

## Hepatic Fibrosis

The role of relaxin in fibrosis of the liver has been less studied than that in other organs. The major collagen-producing cell in hepatic fibrosis is the hepatic stellate cell (HSCs), a perisinusoidal lipid-storing cell that transdifferentiates into a myofibroblastic cell with liver injury, with increased smooth muscle actin expression, collagen expression and secretion, contractility, and TIMP expression, and decreased MMP expression (49). Relaxin treatment of male rats caused reduced contractility of sinusoidal cells of the liver, although the identity of these cells is unclear (50). Treatment of primary rat myofibroblastic HSCs resulted in decreased collagen synthesis and secretion, and decreased secretion of TIMPs (51). In similar studies, relaxin decreased several fibroblastic markers of activated HSCs, including smooth muscle actin levels, collagen synthesis and deposition, and secretion of TIMPs, and increased expression and secretion of MMP13 (52). Expression of RXFP1 is low in the normal liver and quiescent HSC, but increases with hepatic fibrosis and HSC activation (53). Male relaxin-null mice had increased liver weight compared to wild-type (44). In an *in vivo* model of fibrosis prevention in rats, relaxin reduced hepatic collagen accumulation induced by carbon tetrachloride (51). A similar fibrosis prevention study in mice also showed reduced hepatic collagen content, as well as reduced myofibroblast activation and improved serum levels of liver enzymes (54). However, when applied to advanced established fibrosis with persistent carbon tetrachloride administration, relaxin transiently slowed the hepatic collagen accumulation and reduced myofibroblast activation, but the benefit was lost at later time points (54). More studies are needed to determine if relaxin can improve less severe hepatic fibrosis, or fibrosis induced by less toxic means. Nevertheless, the above evidence suggests that the liver is a relaxin target organ. Intriguingly, transgenic overexpression of relaxin in unchallenged (nonfibrotic) mice resulted in an increase in hepatic collagen with time, perhaps suggesting a biphasic effect with increasing exposure to high levels of relaxin (55).

## Summary

Studies conducted over the past ten years have produced remarkable advances in the area of relaxin research. New relaxin family peptides have been discovered, and their receptors have been identified. The number of physiological functions of relaxin has grown to include many nonreproductive actions. The development of the relaxin-null mouse has provided particularly strong evidence that one of these functions is in the protection against fibrosis in a number of organs. The lack of relaxin results in age-related fibrosis in the skin, lung, kidney, and heart. Some considerable challenges remain, including the mechanism for the gender-specific

responses observed in a number of tissues, and the ineffectiveness of relaxin in the treatment of some models of extensive fibrosis. Nevertheless, relaxin treatment has improved fibrosis in a variety of *in vivo* models, suggesting that this may be a valuable area for translational research into human studies.

## Acknowledgments

## Abbreviations

**InsL**

insulin-like peptide

**RLF**

relaxin-like factor

**RXFP**

relaxin family peptide receptor

**MMP**

matrix metalloproteinase

**TIMP**

tissue inhibitor of metalloproteinases

**TGFβ**

transforming growth factor β

**HSC**

hepatic stellate cell

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