Published in final edited form as:

Ann N Y Acad Sci. 2009 April ; 1160: 379–380. doi:10.1111/j.1749-6632.2008.03793.x.

Relaxin/RXFP1 Signaling in Prostate Cancer Progression

Shu Feng^a, Irina U. Agoulnik^b, Zhen Li^a, Hee Dong Han^c, Gabriel Lopez-Berestein^c, Anil Sood^c, Michael M. Ittmann^d, and Alexander I. Agoulnik^a

^aDepartment of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030, USA.

^bDepartment of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA.

^cDepartment of Gynecologic Oncology, MD Anderson Cancer Center, University of Texas, Houston, TX 77030, USA

^dDepartment of Pathology, Baylor College of Medicine, Houston, TX 77030, USA.

Abstract

We have shown that relaxin peptide expression was significantly elevated in recurrent human prostate cancer. Stimulation with relaxin increased migration, invasiveness, proliferation, and adhesion of LNCaP and PC3 cells in vitro. Opposite effects on cellular phenotype were observed after suppression of endogenous relaxin/RXFP1 expression or signaling. We showed an accelerated progression of prostate cancer in TRAMP males with transgenic relaxin overexpression and a longer survival of TRAMP, RXFP1-deficient males. Suppression of RXFP1 expression in PC3 xenografts in nude mice using siRNA intratumoral injections resulted in decrease of tumor size, cell proliferation, and metastasis rate.

Keywords

Prostate cancer; Relaxin; RXFP1; siRNA; TRAMP

We investigated the role of relaxin/RXFP1 signaling in prostate cancer progression. Quantitative RT-PCR analysis showed that while the mRNA expression of relaxin receptor RXFP1 was maintained at the same level in human prostate cancer and normal samples, relaxin mRNA expression was significantly higher in prostate cancer samples compared with normal prostate tissues.¹ Analysis of tissue microarrays confirmed higher relaxin protein expression in recurrent prostate cancer samples; a strong correlation was found between the level of relaxin receptor and two characteristics of aggressive disease - extracapsular extension and seminal vesicle invasion.¹ Stimulation with relaxin increased proliferation, invasiveness, and adhesion of LNCaP and PC3 cells in vitro. Alternatively, the suppression of relaxin/RXFP1 expression via short interfering RNAs decreased prostate carcinoma cell invasiveness by 90–95%, cell growth by 10–25% and increased cell apoptosis from 0.6 to 2.2 times. Similar effects were observed in cells with transient or stable transfection with RXFP1 N-terminus fragments (7BP, full ectodomain,² and LDLa domain³). The RXFP1 expression or signaling suppression in PC3 cells resulted in decreased phosphorylation of protein kinase B (AKT). RNA analysis in PC-3 cells after suppression of RXFP1 by siRNA using Illumina microarrays revealed consistent changes in

Address for correspondence: Alexander I. Agoulnik, Ph.D., Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA, Phone: 713-798-6087; Fax: 713-798-5074; agoulnik@bcm.edu.

the expression of genes associated with ECM remodeling pathways. The effect of activation of the relaxin signaling on prostate cancer progression in autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) was analyzed in mice with transgenic relaxin overexpression and *Rxfp1*-deficient mice. The Tg(Rln1), TRAMP males had shorter median survival time, associated with the decreased apoptosis of tumor cells. Survival curves for *Rxfp1*^{-/-}, *Rxfp1*^{+/-}, and *Rxfp1*^{+/+} TRAMP males that died from prostate cancer were not significantly different; however the median survival times for three groups were somewhat different (194, 182, and 169.5 days). We have shown that RXFP1 deficiency led to the upregulation of probasin expression which might explain the lesser effect of relaxin on tumor progression in the TRAMP model where SV40 T-antigen is under the control of the probasin promoter. There was however a significant difference in the number of TRAMP males surviving until 250 days with no or small primary prostate tumors (<1.5g) between *Rxfp1*-deficient males (8/34) and males with the wild-type allele of relaxin receptor (2/38, Fisher's Exact 2-tail test = 0.018). Finally we analyzed the effect of siRNA targeting RXFP1 expression in the xenograft model *in vivo*. PC3 cell tumors were established in nude male mice by SC injections. Two weeks after transplantation we began 3 week biweekly treatment with siRNA on biodegradable nanoparticles. Ten days after the last treatment the mice were killed and the tumors were analyzed. The treatment resulted in a significant decrease in tumor size, associated with decrease in cell proliferation; there were less metastases in the cervical lymph nodes in the RXFP1 siRNA group. Thus, relaxin/RXFP1 might be a potential therapeutic target in the treatment of prostate cancer.

References

1. Feng S, et al. Relaxin promotes prostate cancer progression. Clin Cancer Res. 2007; 13:1695–1702. [PubMed: 17363522]
2. Hsu SY, et al. Activation of orphan receptors by the hormone relaxin. Science. 2002; 295:671–674. [PubMed: 11809971]
3. Scott DJ, et al. Characterization of novel splice variants of LGR7 and LGR8 reveals that receptor signaling is mediated by their unique low density lipoprotein class A modules. J Biol Chem. 2006; 281:34942–34954. [PubMed: 16963451]