A Single-Chain Peptide Derived from Relaxin-2 is a Biased RXFP1 Agonist that Rescues Mouse Bladders with Irradiation-Induced Cystitis

Samuel Getchell, Irina Zabbarova, Youko Ikeda, Mark Kozlowski, Lori Birder, Anthony Kanai
University of Pittsburgh School of Medicine

ABSTRACT

Radiation cystitis is a consequence of radiotherapy for pelvic malignancies. Acutely, irradiation leads to reactive oxygen/nitrogen species in urothelial cells, apoptosis, barrier disruption, and inflammation. Chronically, this results in collagen deposition, bladder fibrosis, and attenuated storage and voiding functions. In severe cases, cystectomies are performed as current therapies do not reverse fibrosis.

Our previous work demonstrated that treatment with human relaxin-2 (HRLX2) reverses radiation-induced fibrosis and restores bladder function in mice [1]. However, HRLX2 is known to promote angiogenesis and to support the growth of tumors [2], suggesting the need for a therapeutic approach without this potential complication.

We performed assays for the RXFP1-dependent formation of cyclic adenosine monophosphate (cAMP) and compared the effects of H7-33 to the native ligand HRLX. HEK-293T cells were seeded at a density of 26,000 cells per square centimeter and then transfected with 2.5×10^13.9 g DNA per cell per plasmid using Lipofectamine 3000 (Thermo Fisher Scientific) according to manufacturer instructions. Cells were transfected with pcDNA3.1-RXFP1-GFP and pGloSensor-221 (Promega), with three replicates per condition. 24-32 hours after transfection, cells were equilibrated in 2% GloSensor cAMP Reagent (Promega) in 10% FBS in CO2-Independent Media for two hours before kinetic luminescence measurements were taken for 30 minutes on a SpectraFluor plate reader (Tecan). Cells were treated with H7-33 as a positive control. Cells transfected with only pGloSensor-221 showed no response to HRLX or H7-33 (data not shown).

We previously developed a mouse model for selective bladder irradiation (10 Gray; 1 Gy = 100 rads) that results in chronic fibrosis within 6 weeks, with decreased bladder compliance, contractility, and overflow incontinence (Fig. 1). Seven weeks post-irradiation, female C57/Bl6 mice were continuously infused with H7-33 (400 μg/kg/day/14 days) or vehicle (saline) via subcutaneous osmotic pumps. Mice were evaluated in vivo using uroflow analyses, cystometrograms and external urethral sphincter electromyograms; and in vitro using length-tension measurements and histology.