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

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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.

The data presented here show that a single-chain peptide derivative (β7-33) of human relaxin-2 (hRLX2) is effective in reversing radiation-induced bladder fibrosis and lower urinary tract dysfunction (LUTD) and that β7-33 induces formation of markedly less cAMP than hRLX2, indicating a lower risk of tumorigenic angiogenesis downstream of treatment.

METHODS

We performed assays for the RXFP1-dependent formation of cyclic adenosine monophosphate (cAMP) and compared the effects of β7-33 to the native ligand hRLX2. HEK-293T cells were seeded at a density of 26,000 cells per square centimeter and then transfected with 2.5*10^-13 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-22f (Promega), with three replicates per condition. 24-32 hours after transfection, cells were equilibrated in 2% GloSensor cAMP Reagent (Promega) in 10% FBS in CO_2-Independent Media for two hours before kinetic luminescence measurements were taken for 30 minutes on a SpectraFluor plate reader (Tecan). Cells were treated with forskolin as a positive control. Cells transfected with only pGloSensor-22f showed no response to hRLX2 or β7-33 (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 C57Bl/6 mice were continuously infused with β7-33 (400 µg/kg/day/14 days) or vehicle (saline) *via* subcutaneous osmotic pumps. Mice were evaluated *in vivo* using urine spot analyses, cystometrograms and external urethral sphincter electromyograms; and *in vitro* using length-tension measurements and histology.

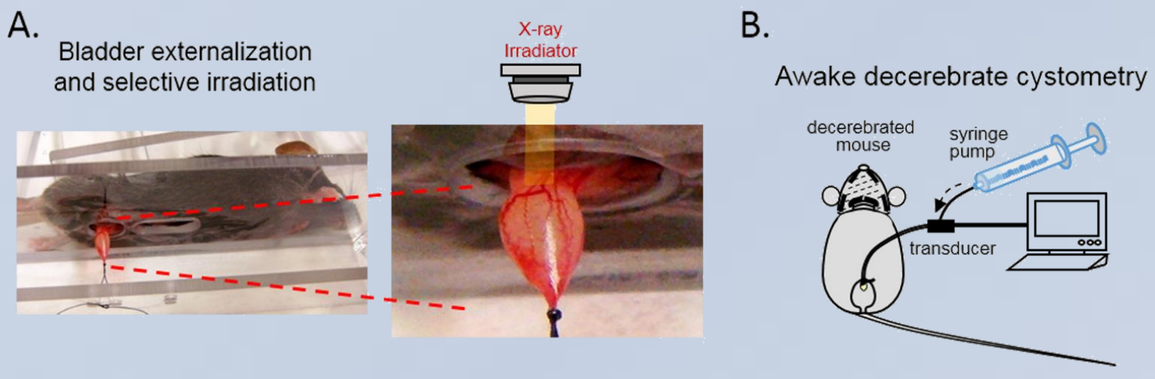

Figure 1. Externalization and selective irradiation of a mouse bladder followed by 6 weeks fibrosis development, then awake decerebrate cystometry. (A) Bladder externalization and selective irradiation. Mice were anesthetized using avertin (2,2,2-tribromoethyl alcohol) and the bladder externalized for 10 Gy x-ray irradiation. Fibrosis develops within 6 weeks. (B) Cystometry performed on decerebrated mice. Saline was infused *via* a syringe pump.

RESULTS

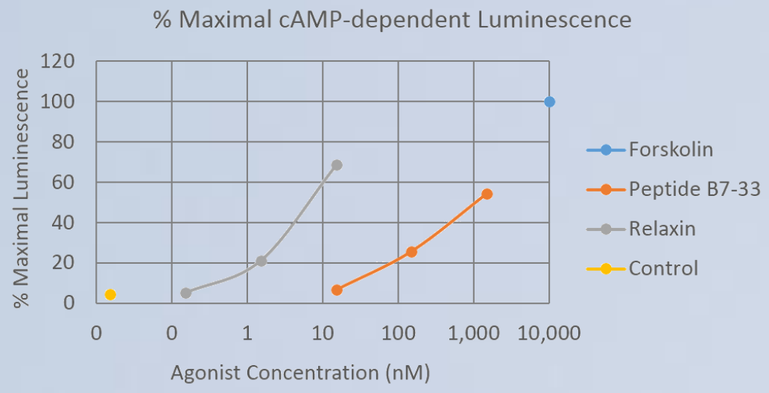

Figure 2. cAMP reporter assay shows β7-33 produces less cAMP at a given dose than hRLX2. Average of three replicates for each data point. HEK-293T cells were transfected with RXFP1 and the cAMP sensor pGloSensor-22f, treated with Forskolin, β7-33, hRLX2, or water. Luminescence was read on a plate reader over a 30 minute time course.

RESULTS

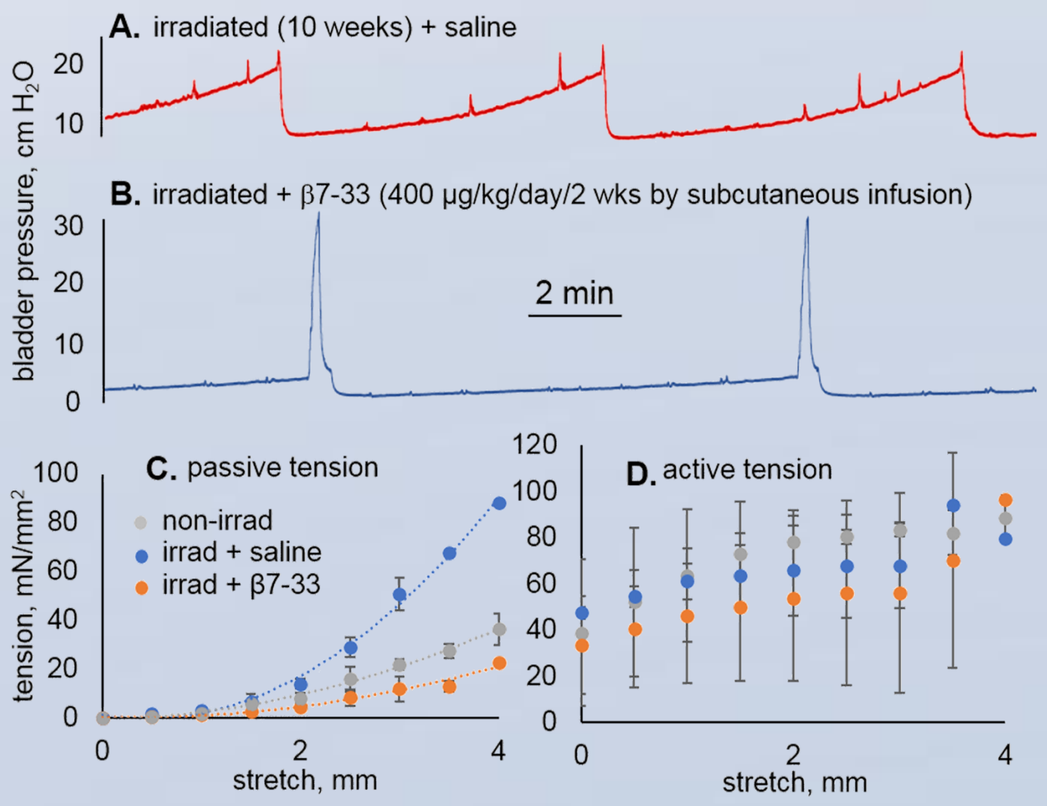

Figure 3. β7-33 rescues mouse bladders with irradiation induced cystitis. Data from mice 9 weeks after bladder irradiation (10 Gy); osmotic pumps were implanted subcutaneously for the last two weeks. β7-33 in pump, n = 4; saline in pump, n = 3; non-irradiated animals, n=3. A) Representative cystometrogram from an irradiated mouse with saline in the pump. B) Representative cystometrogram from an irradiated mouse with β7-33 in the pump. C) Passive tension measurements showed that β7-33 improved the compliance of irradiated bladders. D) Force generation in response to electric field stimulation (EFS) was unchanged by treatment.

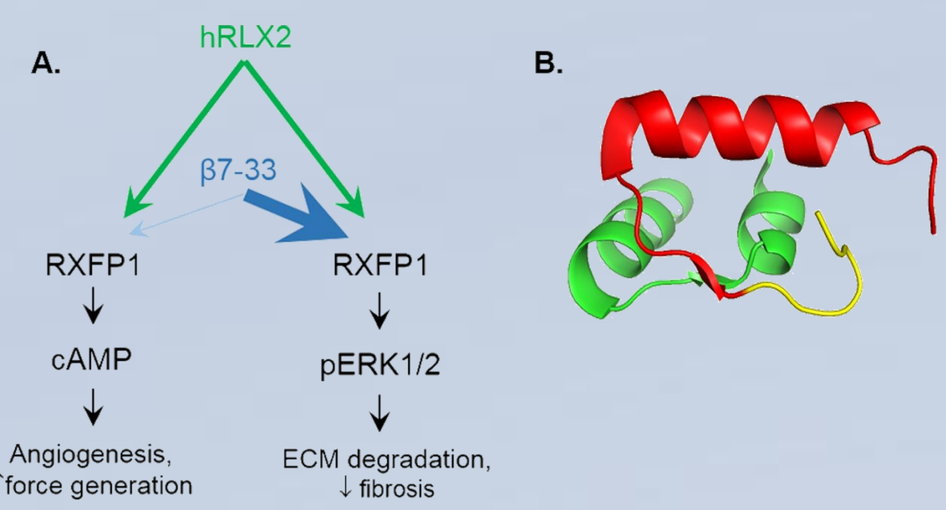

Figure 4. hRLX2 versus β7-33. (A) Simplified signal transduction pathway. β7-33 is a biased agonist that signals more through pERK1/2 than through cAMP, leading to anti-fibrotic activity without angiogenesis or increased bladder smooth muscle force generation. (B) 3-D structure of hRLX2 with β7-33 colored red.

CONCLUSIONS

In HEK-293T cells transfected with RXFP1, β7-33 induced formation of cAMP in a manner comparable to hRLX2, though the peptide required concentrations roughly 100-fold higher for similar effects. In irradiated mouse bladders, β7-33 lowered basal bladder pressure, increased voiding intercontractile interval (ICI), improved voiding efficiency, and increased bladder compliance.

Treatment outcomes were likely caused by the activation of the Relaxin Family Peptide Receptor 1 (RXFP1). In the urinary bladder, these receptors are expressed on the detrusor. While the irradiated, untreated mice show a short ICI with non-voiding contractions, the treated mice show much longer ICI and markedly reduced evidence of non-voiding contractions. The much lower baseline bladder pressure seen in treated mice is strong evidence of a reduction in fibrosis, as is the marked decrease in passive tension in length-tension measurements. Treatment with β7-33 did not increase active tension as treatment with full-length hRLX2 hormone is known to do [1]. That, in combination with the 100-fold lower response in the cAMP assay produced by β7-33, are consistent with previous findings that β7-33 does not promote angiogenesis, which is downstream of elevated cAMP levels in cells with active RXFP1.

β7-33 may be a new therapeutic option for rescuing bladders with chronic radiation cystitis and fibrosis. β7-33 offers a notable advantage over hRLX2 in that β7-33 does not induce angiogenesis as does hRLX2, making it safer to use in patients undergoing radiotherapy for pelvic cancers.

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