Analysis of Small Molecule Antagonist Binding with Relaxin Receptor

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Increased relaxin (RLN) peptide expression has been found in prostrate, breast, and endometrial cancers. The proliferative and anti-apoptotic effects of relaxin contribute to cancer growth, and thus a small-molecule antagonist of relaxin signaling may inhibit these cancer-promoting effects. We have identified several antagonists, which suppress activation of the G protein-coupled relaxin receptor, relaxin/insulin like family peptide receptor 1 (RXFP1), and are completely or partially ineffective against the closely related receptor RXFP2. The goal of this study is to define the region of relaxin receptor responsible for antagonist response. Comparison of amino acid sequences between RXFP1 and RXFP2 revealed 14 differences in this region of the proteins from which we created point mutants to analyze the differential response of the two receptors. The activation of the receptors transiently transfected in HEK293T cells was quantified by measuring cyclic AMP (cAMP) production, which is induced when RXFP1 is stimulated by RLN or when RXFP2 is activated by its cognate ligand, INSL3. It was determined that a singular amino acid change in RXFP1 is not sufficient to affect antagonistic response. Then, we tested the efficacy of the antagonists against a series of mutant receptors containing different portions of RXFP1 and RXFP2. The cDNA fragments of the two receptors were produced using high-fidelity PCR and combined by an infusion protocol. All chimeric receptors were tested for their cAMP response to RLN and INSL3. First, two chimeric receptors were analyzed: RXFP1/2 and RXFP2/1, containing the ectodomain of one receptor and seven transmembrane domain (7TM) of the second. An additional 5 chimeras were created with the C-terminal parts of the RXFP1 or RXFP2 7TM. The substitution of the 7TM in RXFP2 with the RXFP1 domain rendered the chimeric receptor more responsive to antagonists. Furthermore, another two chimeras were created to assess the significance of the individual C-terminal parts for RXFP1 and RXFP2 on overall antagonism. Our data provides insight on the nature of small molecule binding to the receptor, which will facilitate further modifications of the antagonists to increase their activity and efficacy as a potential agent to suppress tumorigenesis.