CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S1728

Project Title

Expression and Functional Analysis of Human Mutated Relaxin-2

Abstract

Objectives/Goals

In this project we attempt to design and obtain in Pichia pastoris a functional recombinant human Relaxin-2 with mutations reducing the binding specificity toward RXFP2, but preserving the binding to RXFP1, for pharmaceutical purposes.

Methods/Materials

Pichia pastoris was induced by 3% Methanol, with cell density at 30~35 OD600nm, and for 24 hours. Proteins in the supernatant were separated by SDS-PAGE was detected on the membrane using a specific anti-His antibody. The protein was purified through a TALON column. The purified protein was then cleaved by thrombin to produce the mature mutated human Relaxin-2 protein. The CHO cells were transfected with empty vector or the vectors carrying RXFP1 or RXFP2 gene by lipofectamine. Recombinant mutated human Relaxin-2 was added to the cells with a final concentration of 1nM. The cAMP was detected by ELISA.

Results

1. Recombinant mutated human Relaxin-2 can be expressed and secreted in the Pichia pastoris system.
2. The purity of recombinant mutated human Relaxin-2 is over 98% after affinity purification.
3. Under the physiological concentration, 1mM recombinant mutated human Relaxin-2 can significantly activate RXFP1 receptor resulting in an accumulation of cAMP. However, there is not obvious difference of the RXFP2 expressed cells and Control cells in cAMP level.

Conclusions/Discussion

In this study, we successfully expressed and purified a mutated human Relaxin-2 from Pichia pastoris. This mutated human Relaxin-2 can significantly activate the cells with expression of RXFP1 receptor, but not RXFP2, suggesting selective activation of RXFP1 by the mutated Relaxin-2. However, our study does not provide direct ligand-receptor binding evidence thus we cannot exclude the binding possibility between the mutated human Relaxin-2 and RXFP2. Addressing this question and examining the cardioprotective functions of the mutated human Relaxin-2 in animal models will be our next focus.

Summary Statement

It have designed and produced a mutated human Relaxin-2 that preferrally activates RXFP1

Help Received

Used lab at Newcca corporation under the supervision of Dr. Wenbin Tan