生物物理学报 2011, 27(5) 417-423 DOI: 10.3724/SP.J.1260.2011.00417 ISSN: 1000-6737 CN: 11-1992/Q

研究论文

人甘丙肽2型受体基因启动子的克隆与初步功能分析

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摘要：

人甘丙肽2型受体（GalR2）作为G蛋白偶联受体，其功能已被广泛研究，但*GalR2*基因的转录调控机制尚不明确。我们利用聚合酶链式反应（polymerase chain reaction，PCR）方法扩增人*GalR2*基因上游1121 bp的片段，再通过PCR方法将其缺失成3 段长度不等的片段，然后分别将其克隆到荧光素酶报告基因载体pGL3-Basic中，并将相应的质粒命名为pGL3-B-1121、pGL3-B-922、pGL3-B-521和pGL3-B-200；通过荧光素酶活性分析检测不同长度的*GalR2*基因启动子在人神经母细胞瘤细胞(SK-N-SH)中的活性，发现*GalR2*基因上游1121 bp区段具有明显的转录活性，缺失－1121到－922 bp区域引起启动子活性的明显增高，暗示该区域存在负调控元件；利用TESS在线软件分析*GalR2*基因上游序列，发现该基因启动子含有Sp1、AP-1、AP-2、NF-1、YY1 和ERα等多种顺式作用元件。因此，本实验初步确定了人*GalR2*基因启动子的转录调节区，为进一步研究该基因转录调控机制奠定了基础。

关键词： *GalR2*基因 启动子 转录调节

Cloning and Preliminary Functional Analysis of Human Galanin Receptor 2 Promoter

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Abstract:

As a G protein-coupled receptor, the role of galanin receptor 2 (GalR2) has been studied extensively, but its transcriptional regulation mechanism is still unknown. In this study, 5'- upstream promoter sequence (spanned from －1121 to －1 bp) of human *GalR2* gene and its three deletion segments were isolated and cloned into the pGL3-Basic vector. Corresponding plasmids were named as pGL3-B-1121, pGL3- B-922, pGL3-B-521 and pGL3-B-200, respectively. Those four reporter plasmids were transfected into SK-N- SH cells, and luciferase activities were measured. The results showed that deletion of the region between -1121 to -922 bp resulted in a significantly increased activity compared with pGL3-B-1121, indicating the presence of negative elements in this region. Moreover, it was also found that the *GalR2* promoter had many putative transcription factor binding sites by using online software TESS, such as Sp1, AP-1, AP-2, NF-1, YY1 and ERα, suggested that those elements might be involved in transcriptional regulation. In conclusion, this study provides important clues for elucidating the transcriptional regulation mechanisms of the *GalR2* gene.

Keywords: *GalR2* gene Promoter Transcriptional regulation

收稿日期 2011-02-25 **修回日期** 2011-04-27 **网络版发布日期**

DOI: 10.3724/SP.J.1260.2011.00417

基金项目:

国家自然科学基金项目(30870815)，北京市自然科学基金重点项目(09G0013)，人事部留学人员科技活动择优资助项目，Swedish Research Council (3106)，北京市人才强教深化高层次人才计划(PHR20100510)

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