عنوان مقاله:

Design and Construction of Expression Vectors for Evaluation of Mutual Interactions between Human Rax and E۲f۱ Transcription Factors

محل انتشار:

بیست و یکمین کنگره پزشکی تولید مثل و شانزدهمین کنگره زیست شناسی و فناوری سلول های بنیادی (سال: 1399)

تعداد صفحات اصل مقاله: 1

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خلاصه مقاله:

Objective: E۲F۱ transcription factor (TF) is a critical regulator of cell cycle and is required for G۱ to S transition. Retina and anterior neural fold homeobox (RAX) play crucial roles in eye development and retinal progenitor cells (RPCs) specification. Because of the restricted rate of in vitro proliferation of RPCs, further studies to understand the molecular mechanisms in-volved in their maintenance are essential. In this study to investigate in vitro interactions between E۲F۱ and RAX, expression vectors harboring their coding sequences and promoters were designed and constructed. These vectors are co-transfected into ۲۹۳T cells to analyze putative reciprocal interactions between these two TFs.Materials and Methods: Based on in silico analysis and litera-ture mining, several putative binding sites for E۲F۱ were pre-dicted within ۳۲۵۸ bp upstream of the human RAX gene. Cod-ing sequences of these two TFs and also E۲F۱ promoter region were amplified from the human genome and cloned into target expression vectors harboring mCherry and EGFP reporters re-spectively. Moreover, single transfection of these vectors into ۲۹۳T cells by LTX lipofectamine was assayed microscopically. Results: The Integrity of the expression vectors was examined by digestion and PCR. The target amplified sequences were also confirmed by sequencing analysis. Results indicated these regions were amplified without mutation, and successfully in-serted into target vectors. Furthermore, transfection of these vectors into ۲۹۳T cells confirmed the successful expression of these target genes and EGFP reporter driven by their putative promoter regions.Conclusion: Considering successful construction and trans-fection of these vectors into ۲۹۳T cells, in vitro evaluation of interactions between E۲F۱ and RAX, as critical modulators of proliferation in .RPCs, might provide better insight into the mechanisms underlying retinal progenitor maintenance

کلمات کلیدی:

E۲F۱, RAX, Retina Progenitor Cells, Proliferation