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基于共转染的植物雌激素活性成分筛选方法的建立与应用

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中文摘要:目的: 建立一种以共转染为基础的、高效灵敏的植物雌激素活性成分的细胞筛选方法,应用此方法研究鹰嘴豆提取部位的雌激素效应。 方法 :RT-PCR方法扩增人雌激素受体α(hERα)cDNA,并构建哺乳细胞表达载体pERα。将此载体与含有雌激素应答序列(3×ERE)的Luc报告基因载体(pERE-Luc)以不同比例共转染MCF-7细胞,比较不同比例下Luc的活力,确定最佳共转染比例。用芒柄花素、鹰嘴豆豆芽素A和染料木素等植物雌激素验证了该模型的灵敏性,并进一步测定了鹰嘴豆不同提取部位的Luc活力。结果 :将pERα与pERE-Luc共转染MCF-7细胞,与pERE-Luc单转染相比,显著提高Luc的活力,且在10 ： 1(pERE-Luc ： pERα)时活力最高,Luc活力提高了5倍。用此转染比例测定芒柄花素、鹰嘴豆豆芽素A和染料木素等植物雌激素Luc活力测定结果表明,共转染能诱导Luc的表达,且ER特异性抑制剂ICI 182,780能抑制其活性。利用此模型发现鹰嘴豆的70%乙醇总提取物、乙酸乙酯部位和石油醚部位均具有大量的雌激素活性物质。ICI 182,780能有效抑制其雌激素效应。 结论 :成功建立了一种共转染为基础的植物雌激素活性成分的筛选方法,该方法具有较高的特异性和灵敏性,可用于植物雌激素活性成分的筛选。

中文关键词:植物雌激素 共转染 虫荧光素酶 人雌激素受体α 鹰嘴豆

Establishment and application of co-transfection screening method for phytoestrogen active constituents

Abstract: Objective : To establish a highly sensitive screening method for phytoestrogen active constituents and to primarily screen the phytoestrogenic active constituents from the chickpea extractions by the method. Method : Human ER*α* cDNA was cloned using MCF-7 total RNA as the template by RT-PCR and then was constructed into a pcDNA3 and named as pER*α*. The cell line MCF-7 was co-transfected with pER*α* and the reporter plasmid pERE-Luc which carrying the estrogen response element (ERE) plus the luciferase reporter gene. The luciferase activity was then assayed. The model was optimized by changing the ratio of two plasmids. The feasibility of the optimized model was further proved by the several known phytoestrogen compounds including fermononetin, biochanin A and genistein, et al. As an application of the model, the phytoestrogen activity of the extracts of the chickpea was assayed. Result : The recombinant plasmid (pER*α*) can enhance luciferase activities of pERE-Luc transfected MCF-7 cells. The highest transfection efficiency and luciferase activity were found at the ratio of 10 ： 1 (pERE-Luc ： pER*α*), the luciferase activity was improved five times as high as the unique pERE-Luc transfection. The co-transfection screening model also indicated that fermononetin, biochanin A and genistein could induce ERE-driven luciferase activity and ICI 182,780 suppressed the induced transcription. As the application of the model, the results showed that the ethanol (70%) total extraction, the ethyl acetate extraction and the ligarine extraction of the chickpea can induce ERE-driven luciferase activity. Concurrent treatment with ICI 182,780 abolished the induced luciferase activity. Conclusion : A phytoestrogen active constituent screening mode have been established based on co-transfection method. It is sensitive to assay the phytoestrogen active constituents and can be applied to screen the active component of phytoestrogens.

keywords:co-transfection phytoestrogen luciferase human estrogen receptor *α* *Cicer arietinum*

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