

一新穎膜型鳥苷酸環化酶受體 G 於人類精子所扮演角色之功能性分析

Functional Characterization of a Novel Guanylate Cyclase G in Human Spermatozoa

中文摘要

鳥苷酸環化酶(guanylate cyclase, GC)存在兩種類型：水溶性蛋白型鳥苷酸環化酶(soluble guanylate cyclase, sGC)與膜蛋白型鳥苷酸環化酶(particulate guanylate cyclase, pGC)。本實驗室先前的研究成果顯示，GC-G 表現於小鼠體內的睪丸組織以及影響成熟精子的生理活性；本論文主題承接先前的研究工作，進一步探討膜蛋白型鳥苷酸環化酶中一新穎孤兒受體 G (GC-G) 於人類精子所扮演的角色。

利用與小鼠鳥苷酸環化酶受體 G 基因 (mGC-G) 的同源相似性，我們在人類 cDNA library 中，發現一段位於第十對染色體的基因，與 mGC-G 具有高度的相似度，將之命名為 human GC-G (hGC-G)。利用 Q-PCR 與南方墨點法分析結果顯示，hGC-G 高度特異地表現於生殖器官中的睪丸。進一步利用預測之 hGC-G 的 extracellular domain 中胺基酸序列的一段短肽鏈作為抗原，對兔子進行免疫反應，取得血清進行 anti-hGC-G IgG 之純化，並利用抗體的專一性來進行辨識。實驗結果顯示，hGC-G 蛋白表現於人類成熟精子當中；進一步利用共軛焦顯微鏡及流式細胞儀分析，證實 hGC-G 蛋白為一表現在精子之頂體與尾部中段的膜上型受體；至於功能性的評估方面，我們發現專一性辨識 hGC-G 的抗體，並未抑止精子獲能相關的酪胺酸磷酸化現象；而在 hGC-G extracellular domain 中的 RGD 序列，有效的中和 HEK293T 細胞膜外的 integrin 且顯著降低與 fibronectin 結合的能力。顯示 hGC-G 的功能可能不參與精子獲能作用之訊息傳遞，而是精卵進行受精作用時，扮演分子之間辨識的角色。

英文摘要

Guanylyl cyclase (GC) is known to transmit signaling by synthesizing of intracellular cyclic GMP. We have previously described an orphan GC receptor on mouse sperm (mouse GC-G) which is able to regulate sperm motility and capacitation-associated protein tyrosine phosphorylation. Here, we reported the identification and functional characterization of its apparent homologue in humans (hGC-G). By the comparative genomic approach, hGC-G gene is composed of 21 exons, spanning a minimum of 48 kb on chromosome 10q25. When compared with the mouse orthologue, hGC-G showed in the presence of deletions, incomplete splicing of introns, or in-frame termination codons. The real-time quantitative reverse transcriptase (RT)-PCR analysis demonstrated that hGC-G is expressed in human testis, followed by a low

expression in placenta, but with no expression in other tissues. Western blotting revealed that hGC-G in sperm composed three isoforms ranged in 30 ~ 45 kDa, and was expressed on the cell surface of spermatozoa by utilizing flow cytometry and confocal immunofluorescent analysis. Furthermore, with hGC-G-RGD peptide and anti-hGC-G antibody neutralizing assay, we found that hGC-G may involve in molecular recognition of sperm (RGD motif of hGC-G on sperm) and oocyte (integrin on oocyte), but not in receptor-transmitted capacitation signalings.