生長休止基因八與精蟲生成作用

Growth arrest-specific gene 8 and spermatogenesis

中文摘要

生長休止基因（Growth-arrest specific genes, Gas genes）是生長休止期(G0 期)細胞內才大量表現的一群基因，此種細胞生長休止可能是因高細胞密度之接觸抑制或生長因子缺乏所造成。本研究室利用反轉錄病毒之基因誘捕策略，選殖並確認兩個新的生長休止基因，並命名爲生長休止基因七(growth arrest-specific gene 7,)與生長休止基因八（growth arrest-specific gene 8, Gas8）。先前之研究已發現 Gas7 基因之產物，爲形成神經突所需要的一種肌動蛋白絲結合蛋白。Gas8 位於小鼠第八對染色體長臂的末端，且在人類、大鼠、斑馬魚、果蠅、瘧蚊、單胞藻、Bruce 氏錐蟲等物種體內，都有相似性很高的基因存在。因爲多種組織之 Northern 與 Western 墨點染色研究中，發現睪丸具有此二者 mRNA 及蛋白之大量表現，尤其是生長休止基因八（Gas8），於是進一步探討此生長休止基因於精蟲生成作用中所扮演的角色。Gas8 基因於睪丸的表現受青春期發育之調控：在新生幼鼠至性發育的早期於睪丸中只存有微量的 Gas8 mRNA，隨著青春期發育的進行（生殖細胞減數分裂完成後）睪丸中 Gas8 mRNA 的含量快速增加，到了性成熟的睪丸內有最高量的 mRNA。睪丸切片中發現 Gas8 蛋白主要位於早期精細胞的細胞質及晚期精細胞（包含成熟精子）的鞭毛內。這些結果暗示 Gas8 基因可能與精蟲之分化作用或活動力有關。我們同時發現呼吸道與輸卵管內的纖毛內也具有 Gas8 蛋白；另一方面，在一種分子生物學上用來研究鞭毛的單胞藻內，存在一種與鞭毛運動有關的 PF2 蛋白，與 Gas8 具有高度的相似性；此發現可進一步支持我們的假設。最近的研究發現某些纖毛蛋白的異常，是造成多囊腎、原發性纖毛運動不良症、Bardet-Biedl 症候群、Kartagener 症候群的原因，因此 Gas8 在這些疾病中的角色值得進一步探討。Gas8 基因在人類睪丸內也有相似的表現，且某些因生殖細胞無發育（Sertoli cell only syndrome）的不孕症病人的睪丸中以免疫組織化學染色法檢測不到 Gas8 蛋白，此發現再一次確定 Gas8 蛋白主要表現於生殖細胞內，且可能與精蟲的活動力不良，或某些男性不孕症有關。

英文摘要

Growth-arrest-specific (Gas) genes are expressed during serum starvation or contact inhibition of cells grown in culture. Here we report the isolation and characterization of Gas8, a novel gene identified on the basis of its growth-arrest-specific expression in murine fibroblasts. Gas8 is located at the terminus of mouse chromosome 8. Recently, Gas8 homologues were found in Homo sapiens, Rattus norvegicus, Drosophila melanogaster, Anopheles gambiae, Danio

rerio, Chlamydomonas reinhardi, and Trypanosoma brucei. We show that production of Gas8 mRNA and protein occurs in adult mice predominantly in the testes, where expression is regulated during post-meiotic development of male gametocytes. Whereas a low level of Gas8 mRNA was detected by Northern blotting in testes of murine male neonates and young adolescents, Gas8 mRNA increased rapidly post-meiotically. In adult males, both Gas8 mRNA and protein reached steady state levels in testes that were 10 fold higher than in other tissues. Immunohistochemical analyses showed that Gas8 protein accumulates in gametocytes as they approach the lumen of seminiferous tubules and was localized to the cytoplasm of round spermatids, the tails of elongating spermatids, and mature spermatid tail bundles protruding into the lumen; in epididymal spermatozoa Gas8 protein was present in the flagella. However, pre-meiotic murine gametocytes lacked detectable Gas8 protein, as did seminiferous tubules in biopsy specimens from seven human males having cytological evidence of non-obstructive azoospermia secondary to Sertoli cell only syndrome. Our findings, which associate Gas8 production developmentally with the later stages of spermatogenesis and spatially with the sperm motility apparatus, collectively suggest that this growth-arrest-specific gene product may have a role in sperm motility and male fertility. This postulated role for Gas8 is supported by our observation that highly localized production of Gas8 protein occurs also in the cilia of epithelial cells lining pulmonary bronchi and fallopian tubes, and by the flagellar association and motility function of a Chlamydomonas reinhardi ortholog (PF2) of Gas8. Because new studies on several fronts have revealed that mutations in cilia/basal body proteins are responsible for cilia-related human disorders such as polycystic kidney disease, primary ciliary dyskinesia, Kartagener syndrome, and Bardet-Biedl syndrome, the role of Gas8 in these human disorder deserves further investigation.