

斑馬魚促性腺素受體基因突變型於人類293T細胞cAMP產生之分析 = Analysis of the cAMP production in human 293T cells transfect

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摘要

現今基因轉殖動物已為因應人類廣大需求而被產製，然此種動物最大之隱憂，乃在於如其流入自然生態中，將引起生態之失衡，因此生物防治已成重要課題，故本試驗以斑馬魚基因進行生殖調控研究。於生殖內分泌中，下視丘-腦下腺-性腺軸為調控繁殖之重要核心，而促濾泡素受體 (follicle-stimulating hormone receptor, FSHR) 與促黃體素受體 (luteinizing hormone receptor, LHR) 均為G蛋白耦合性受體 (G protein-coupled receptors, GPCRs) 家族成員之一。此受體中之第二及三細胞質內區域 (intracellular loop 2 and 3, IL2 and IL3) 被認定可間接調控G protein之作用，因此本試驗分別構築一段zfFSHR及zfLHR之IL-2序列，另將野生型 (wild type) 之zfFSHR及zfLHR (zfFSHR-WT和zfLHR-WT) 上具保守區進行定點突變 (point mutation) 而突變後之基因分別為zfFSHR-dominantnegative mutant (DNM) 及zfLHR-DNM，並將構築之載體 (zfFSHR-WT、zfLHR-WT、zfFSHR-DNM及zfLHR-DNM) 轉染於人類胚胎腎臟細胞 (human embryonic kidney 293 cells) 後，藉此等受體經促性腺素 (孕馬血清激素及人類絨毛膜促性腺素，PMSG及hCG) 誘導後其cAMP濃度之變化，以評估細胞訊號傳遞之效率，而顯著負相突變 (DNM) 為野生型基因與突變型基因於生物體內所生之競爭現象，此試驗之目的為分別探討野生型及突變型受體經轉染於細胞後所造成之效果。於轉染zfFSHR-WT及zfLHR-WT之細胞內cAMP濃度顯示，PMSG僅能誘導轉染zfFSHR-WT之細胞株，然轉染zfLHR-WT者則可經PMSG及hCG誘導，此似因與對應之促性腺素受體具相似結構使然，亦顯示使用物種相異之荷爾蒙雖仍可被誘導，但表現量仍不及同物種間高。於大多突變轉染細胞之cAMP濃度於各組間似無顯著變化，而轉染zfFSHR-DNM基因者經hCG誘導後，其之cAMP濃度顯著高於轉染zfFSHR-WT所產生者，另轉染zfLHR-DNM基因之細胞經hCG誘導後，其產生之cAMP濃度較zfLHR-WT者顯著為低。而zfFSHR-R448H與zfLHR-R474A突變株之cAMP濃度經PMSG誘導後均顯著升高，且分別與負回饋及正回饋作用相似，皆有可能造成不孕之傾向，然此仍有待續之活體 (in vivo) 試驗證明。期應用此結果調控基因轉殖及複製動物之繁殖生理，以達生物防治之效。

關鍵詞：斑馬魚、顯著負向突變、點突變、G蛋白耦合性受體、促濤泡素受體、促黃體素受體、cAMP

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