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

吳依芬、黃尉東

E-mail: 9801339@mail.dyu.edu.tw

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（luteinzing 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、促濾泡素受體、促黃體素受體、cAMP

目錄

參考文獻

徐永年。（2006）。斑馬魚發育初期促性腺素受體基因失活之研究。大葉大學分子生物科技學系碩士論文。 Abrahams, M. V. and Sutterlin, A. (1999). The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. Anim Behav 58, 933-942. Angelova, K. and Puett, D. (2002). Differential responses of an invariant region in the ectodomain of three glycoprotein hormone receptors to mutagenesis and assay conditions. Endocrine 19, 147-54. Apaja, P. M., Tuusa, J. T., Pietila, E. M., Rajaniemi, H. J. and Petaja-Repo, U. E.(2006). Luteinizing hormone receptor ectodomain splice variant misroutes the full-length receptor into a subcompartment of the endoplasmic reticulum. Mol Biol Cell 17, 2243-55. Bluhm, A. P., Toledo, R. A., Mesquita, F. M., Pimenta, M. T., Fernandes, F. M.,Ribela, M. T. and Lazari, M. F. (2004). Molecular cloning, sequence analysis and expression of the snake follicle-stimulating hormone receptor. Gen Comp Endocrinol 137, 300-11. Burns, F. J., Chen, S., Xu, G., Wu, F. and Tang, M. S. (2002). The action of a dietary retinoid on gene expression and cancer induction in electron-irradiated rat skin. J Radiat Res (Tokyo) 43 Suppl, S229-32. Cepni, I., Erkan, S., Ocal, P. and Ozturk, E. (2006). Spontaneous ovarian hyperstimulation syndrome presenting with acute abdomen. J Postgrad Med 52,154-5. De Leener, A., Caltabiano, G., Erkan, S., Idil, M., Vassart, G., Pardo, L. and Costagliola, S. (2008). Identification of the first germline mutation in the extracellular domain of the follitropin receptor responsible for spontaneous

ovarian hyperstimulation syndrome. Hum Mutat 29, 91-8. De Leener, A., Montanelli, L., Van Durme, J., Chae, H., Smits, G., Vassart, G.and Costagliola, S. (2006). Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. J Clin Endocrinol Metab 91,555-62. Dierich, A., Sairam, M. R., Monaco, L., Fimia, G. M., Gansmuller, A., LeMeur,M. and Sassone-Corsi, P. (1998). Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc Natl Acad Sci U S A 95, 13612-7. Dorsam, R. T. and Gutkind, J. S. (2007). G-protein-coupled receptors and cancer.Nat Rev Cancer 7, 79-94. Dufau, M. L. (1998). The luteinizing hormone receptor. Annu Rev Physiol 60,461-96. Dufau, M. L., Baukal, A. J. and Catt, K. J. (1980). Hormone-induced guanyl nucleotide binding and activation of adenylate cyclase in the Leydig cell. Proc Natl Acad Sci U S A 77, 5837-41. Fredriksson, R., Hoglund, P. J., Gloriam, D. E., Lagerstrom, M. C. and Schioth,H. B. (2003). Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. FEBS Lett 554, 381-8. Heckert, L. L. and Griswold, M. D. (2002). The expression of the follicle-stimulating hormone receptor in spermatogenesis. Recent Prog Horm Res 57, 129-48. Hillier, S. G. (2001). Gonadotropic control of ovarian follicular growth and development. Mol Cell Endocrinol 179, 39-46. Huhtaniemi, I. (2006). Mutations along the pituitary-gonadal axis affecting sexual maturation: novel information from transgenic and knockout mice. Mol Cell Endocrinol 254-255, 84-90. Kawate, N. and Menon, K. M. (1994). Palmitoylation of luteinizing hormone/human choriogonadotropin receptors in transfected cells. Abolition of palmitoylation by mutation of Cys-621 and Cys-622 residues in the cytoplasmic tail increases ligand-induced internalization of the receptor. J Biol Chem 269,30651-8. Klabunde, T. and Hessler, G. (2002). Drug design strategies for targeting G-protein-coupled receptors. Chembiochem 3, 928-44. Kumar, R. S., Ijiri, S. and Trant, J. M. (2001). Molecular biology of channel catfish gonadotropin receptors: 1. Cloning of a functional luteinizing hormone receptor and preovulatory induction of gene expression. Biol Reprod 64,1010-8. Kumar, T. R., Palapattu, G., Wang, P., Woodruff, T. K., Boime, I., Byrne, M. C.and Matzuk, M. M. (1999). Transgenic models to study gonadotropin function:the role of follicle-stimulating hormone in gonadal growth and tumorigenesis.Mol Endocrinol 13, 851-65. Kwok, H. F., So, W. K., Wang, Y. and Ge, W. (2005). Zebrafish gonadotropins and their receptors: I. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone receptors--evidence for their distinct functions in follicle development. Biol Reprod 72, 1370-81. Laan, M., Richmond, H., He, C. and Campbell, R. K. (2002). Zebrafish as a model for vertebrate reproduction: characterization of the first functional zebrafish (Danio rerio) gonadotropin receptor. Gen Comp Endocrinol 125,349-64. Latronico, A. C., Chai, Y., Arnhold, I. J., Liu, X., Mendonca, B. B. and Segaloff,D. L. (1998). A homozygous microdeletion in helix 7 of the luteinizing hormone receptor associated with familial testicular and ovarian resistance is due to both decreased cell surface expression and impaired effector activation by the cell surface receptor. Mol Endocrinol 12, 442-50. Latronico, A. C. and Segaloff, D. L. (2007). Insights learned from L457(3.43)R, an activating mutant of the human lutropin receptor. Mol Cell Endocrinol 260-262,287-93. Lei, H. Q. and Xu, Y. X. (1993). Effects of gossypol acetic acid on the motility of rat oviduct smooth muscle. Yao Xue Xue Bao 28, 6-10. Lei, Z. M., Mishra, S., Zou, W., Xu, B., Foltz, M., Li, X. and Rao, C. V. (2001).Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. Mol Endocrinol 15, 184-200. Logan, K. A., Juengel, J. L. and McNatty, K. P. (2002). Onset of steroidogenic enzyme gene expression during ovarian follicular development in sheep. Biol Reprod 66, 906-16. Lunenfeld, B. and Insler, V. (1993). Follicular development and its control. Gynecol Endocrinol 7, 285-91. Mertani, H. C., Pechoux, C., Garcia-Caballero, T., Waters, M. J. and Morel, G.(1995). Cellular localization of the growth hormone receptor/binding protein in the human anterior pituitary gland. J Clin Endocrinol Metab 80, 3361-7. Miwa, S., Yan, L. and Swanson, P. (1994). Localization of two gonadotropin receptors in the salmon gonad by in vitro ligand autoradiography. Biol Reprod 50, 629-42. Munshi, U. M., Clouser, C. L., Peegel, H. and Menon, K. M. (2005). Evidence that palmitoylation of carboxyl terminus cysteine residues of the human luteinizing hormone receptor regulates postendocytic processing. Mol Endocrinol 19, 749-58. Nakayama, Y., Yamamoto, T., Oba, Y., Nagahama, Y. and Abe, S. (2000).Molecular cloning, functional characterization, and gene expression of a follicle-stimulating hormone receptor in the testis of newt Cynops pyrrhogaster.Biochem Biophys Res Commun 275, 121-8. Oba, Y., Hirai, T., Yoshiura, Y., Yoshikuni, M., Kawauchi, H. and Nagahama, Y.(1999). Cloning, functional characterization, and expression of a gonadotropin receptor cDNA in the ovary and testis of amago salmon (Oncorhynchus rhodurus). Biochem Biophys Res Commun 263, 584-90. Schubert, R. L. and Puett, D. (2003). Single-chain human chorionic gonadotropin analogs containing the determinant loop of the beta-subunit linked to the alpha-subunit. J Mol Endocrinol 31, 157-68. So, W. K., Kwok, H. F. and Ge, W. (2005). Zebrafish gonadotropins and their receptors: II. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone subunits--their spatial-temporal expression patterns and receptor specificity. Biol Reprod 72, 1382-96. Takeda, J. and Inada, E. (2002). Next generation of drugs used in anesthesia. Masui 51 Suppl, S183-8. Themmen, A. P. N. and Huhtaniemi, I. T. (2000). Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev 21, 551-83. Timossi, C., Maldonado, D., Vizcaino, A., Lindau-Shepard, B., Conn, P. M. and Ulloa-Aguirre, A. (2002). Structural determinants in the second intracellular loop of the human follicle-stimulating hormone receptor are involved in G(s)protein activation. In Mol Cell Endocrinol 189, 157-68. Ulloa-Aguirre, A., Maldonado, A., Damian-Matsumura, P. and Timossi, C.(2001). Endocrine regulation of gonadotropin glycosylation. Arch Med Res 32,520-32. Ulloa-Aguirre, A., Uribe, A., Zarinan, T., Bustos-Jaimes, I., Perez-Solis, M. A.and Dias, J. A. (2007). Role of the intracellular domains of the human FSH receptor in G(alphaS) protein coupling and receptor expression. Mol Cell Endocrinol 260-262, 153-62. Uribe, A., Zarinan, T., Perez-Solis, M. A., Gutierrez-Sagal, R., Jardon-Valadez,E., Pineiro, A., Dias, J. A. and Ulloa-Aguirre, A. (2008). Functional and structural roles of conserved cysteine residues in the carboxyl-terminal domain of the follicle-stimulating hormone receptor in human embryonic kidney 293 cells. Biol Reprod 78, 869-82. Vassart, G., Pardo, L. and Costagliola, S. (2004). A molecular dissection of the glycoprotein hormone receptors. Trends Biochem Sci 29,

119-26. Wang, Y. and Ge, W. (2003). Gonadotropin regulation of follistatin expression in the cultured ovarian follicle cells of zebrafish, Danio rerio. Gen Comp Endocrinol 134, 308-15. Weiss, J., Axelrod, L., Whitcomb, R. W., Harris, P. E., Crowley, W. F. and Jameson, J. L. (1992). Hypogonadism caused by a single amino acid substitution in the beta subunit of luteinizing hormone. N Engl J Med 326,179-83. Wood, A. J. (2002). Variability in beta-adrenergic receptor response in the vasculature: Role of receptor polymorphism. J Allergy Clin Immunol 110,S318-21. Yamashita, S., Nakamura, K., Omori, Y., Tsunekawa, K., Murakami, M. andMinegishi, T. (2005). Association of human follitropin (FSH) receptor with splicing variant of human lutropin/choriogonadotropin receptor negatively controls the expression of human FSH receptor. Mol Endocrinol 19, 2099-111. Yan, L., Swanson, P. and Dickhoff, W. W. (1992). A two-receptor model for salmon gonadotropins (GTH I and GTH II). Biol Reprod 47, 418-27. Zhang, F. P., Kero, J. and Huhtaniemi, I. (1998). The unique exon 10 of the human luteinizing hormone receptor is necessary for expression of the receptor protein at the plasma membrane in the human luteinizing hormone receptor, but deleterious when inserted into the human follicle-stimulating hormone receptor.Mol Cell Endocrinol 142, 165-74. Zhang, F. P., Poutanen, M., Wilbertz, J. and Huhtaniemi, I. (2001). Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. Mol Endocrinol 15, 172-83.