斑馬魚發育初期促性腺素受體基因失活之研究

徐永年、黃尉東

E-mail: 9510679@mail.dyu.edu.tw

摘 要

隨生物科技之進步及基因體計畫之執行，眾多之基因轉殖或複製動、植物已被或將被產製，因此，生物安全(biosafety)之重要性亦愈獲重視。促濾泡素受體 (follicle stimulating hormone receptor, FSHR) 及促黃體素受體 (luteinizing hormone receptor, LHR) 為促性腺素(gonadotropins) 調控生殖功能之重要之促性腺素受體，當二者於生物個體中表現異常時，將影響生殖系統之正常功能。本研究以morpholino及siRNA等方式，探討FSHR及LHR於斑馬魚發育初期基因失活 (gene knockdown) 後，對其性腺形成相關調控基因之影響，藉以提供建立基改生物 (genetically modified organisms, GMO)安全評估平台技術之方法。由基因選殖之結果發現一長度為662胺基酸之促性腺素受體基因 (LHR)，其似為一dominant negative form，可能具調控荷爾蒙與受體之結合能力或訊號傳遞之磷酸化作用 (phosphorylation)。以反轉錄?-聚合?鏈鎖反應 (reverse transcriptase-ploymerase chain reaction, RT-PCR)、PCR雜合反應 (PCR hybridization)、北方墨點法 (Northern blot) 及全覆式原位雜合法 (whole mount in situ hybridization) 等方式證實促性腺素受體基因FSHR及LHR二者於1-2-cell、512-cell、germ ring、12 hpf、24 hpf、48 hpf及72 hpf等各發育階段皆有表現，並與VASA、SDF1及CXCR4b之表現相關，另LHR基因於12 hpf、24 hpf、48 hpf及72 hpf階段有兩種剪切形式 (splicing form) 之表現。當以morpholino或siRNA等方式使LHR或FSHR基因失活後，將直接影響LHR、FSHR、LH、FSH、CEBP、STAR、VASA、NANOS1、SDF1及CXCR4b等基因之表現，證實以質體為模式，經由利用RNA polymerase III (RNA pol III) promoter驅動siRNA之方式可成功應用於斑馬魚，且FSHR及LHR於胚胎發育過程中之表現似可參與類固醇生成 (steroidogenesis) 之調控。另由全覆式原位雜合法證實FSHR及LHR基因失活後除將影響始基生殖細胞 (primordial germ cells, PGCs) 之正確移行外，亦將導致PGCs之死亡。

關鍵詞 : 促濾泡素受體 ; 促黃體素受體 ; 始基生殖細胞 ; 核糖核酸干擾術 ; 生殖內分泌 ; 生物安全

目錄

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