吳郭魚第三型肝細胞核因子啟動子之選殖與調控分析

陳惠玲、黃尉東

E-mail: 9501990@mail.dyu.edu.tw

摘 要

第三型肝細胞核因子(hepatocyte nuclear factor-3, HNF-3)家族為富含於肝臟之轉錄因子，並於胚胎發育、細胞分化及成體時期之器官生成與代謝調控上扮演重要之角色。本研究室先前於吳郭魚之肝臟及性腺中偵測到第一型類胰島素生長因子(insulin-like growth factor-I, IGF-I)、第二型類胰島素生長因子(IGF-II)與HNF-1a、HNF-1b及HNF-3b等之表現，其中以HNF-3b於性腺中之表現量較其他因子顯著，且其可受固醇類荷爾蒙17b-estradiol所調節。故本研究除進行HNF-3b基因之啟動子之篩選及定序外，亦將分析其活性區域，期可為後續探討吳郭魚HNFs與其性腺發育及配子形成之相關研究奠定基礎，進而應用於生物醫學如肝癌、糖尿病之發生與治療及發育生物學之胚層與肝臟等器官生成之相關研究。吳郭魚HNF-3b之啟動子序列目前完成定序2364 bp，預測其上有373個可能之轉錄因子結合位置，其中包含HNF家族(HNF-1, -3, -4及-6)、CCAAT/enhancer binding protein(C/EBP)、cAMP responsive element binding protein (CREB)、固醇類荷爾蒙之受體結合區域、訊息傳導活化子(signal transducer and activator of transcription, STAT)及GATA等數種調控因子，而此等因子亦於性腺發育及相關功能上扮演重要角色。另並構築其2 kb、1.5 kb、1 kb及0.5 kb等片段於含有綠螢光報導基因(green fluorescent protein, GFP)之無啟動子之載體上，進行細胞株(吳郭魚卵巢細胞-2 [TO-2]及人類肝癌細胞株[Hep3B])轉染(transfection)及顯微注射斑馬魚卵以分析其活化功能區域。經顯微注射上述四片段10小時後發現於卵黃囊(yolk)及體節(somites)均有綠螢光之表現，於72小時後於幼魚之體節、脊索(notochord)、底層(floor plate)等部位亦均有螢光之表現，而0.5 kb啟動子之片段多數表現於頭部及眼睛，且2 kb及1.5 kb組之總體表現率似優於1 kb及0.5 kb者(p

關鍵詞 : 吳郭魚、第三型肝細胞核因子、性腺、類胰島素生長因子、固醇類荷爾蒙

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