

吳郭魚第三型肝細胞核因子啟動子片段之功能分析

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摘要

第三型肝細胞核因子 (hepatocyte nuclear factors-3, HNF-3) 家族為富含於肝臟之轉錄因子, 其於胚胎發育、分化、代謝及成體時期器官生成扮演重要之角色。本研究室先前於吳郭魚肝臟及性腺中偵測到第一型及第二型類胰島素生長因子 (insulin-like growth factors-I/II) 基因表現外, 亦有HNF-1、HNF-1及HNF-3之基因表現, 其中以HNF-3之表現較其他因子顯著, 並可受固醇類荷爾蒙 17β -estradiol所調控。因此本研究針對先前所構築之HNF-3啟動子其0.5 kb、1.0 kb、1.5 kb及2.0 kb帶有綠螢光報導基因之四片段進行吳郭魚性腺細胞 (TO-2) 及人類肝癌細胞 (Hep3B) 轉染並顯微注射斑馬魚受精卵與冷光分析。各片段經顯微注射後24小時觀察發現, 於卵黃囊或動物極之體節上有綠螢光之表現, 持續觀察96小時後於幼魚之卵黃囊、體節 (somites)、脊索 (notochord)、底層 (floor plate) 及眼睛等部位均發現綠螢光之表現。0.5 kb片段之啟動子於脊索、卵黃囊、眼睛及頭部之表現率分別為18.3%、1.3%、35.6%及26.0%; 1.0 kb片段之啟動子於脊索、卵黃囊及頭部之表現則分別為約44.4%、44.4%及2.0%; 1.5 kb片段之啟動子其表現於脊索及卵黃囊之表現率分別為33.7%與50.5%, 而2.0 kb片段之啟動子之表現多於脊索、卵黃囊及頭部, 表現率分別為61.2%、26.1%與2.7%, 其表現位置大致與先前之研究結果相似。以西方點墨法分析eGFP表現量與冷光分析之結果顯示TO-2及Hep3B細胞經添加 17β -estradiol後前二數據均有增加之趨勢。因此推測, 位於吳郭魚HNF-3啟動子之estrogen response element (ERE) 結合位置, 可經由固醇類荷爾蒙誘導而促進HNF-3於性腺上表現。

關鍵詞: 吳郭魚、性腺、第三型肝細胞核因子、啟動子、固醇類荷爾蒙

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