促性腺激素對體外培養吳郭魚性腺蛋白質表現之二維電泳分析 = The Analysis of Protein Expression Profiles in the Gonads of Ti

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摘 要

硬骨魚類（teleosts）之繁殖內分泌作用與哺乳類相似，皆由 下丘腦-腦垂體-性腺軸（hypothalamus-pituitary-gonadal axis）所調 控。下丘腦釋放出促性腺激素釋放素（ gonadotropin-releasing hormone, GnRH）誘導腦垂體分泌促性腺激素（gonadotropins, GTHs）並經由循環系統運送至性腺中，除促進性腺發育、配子生 成（ gametogenesis ） 與成熟及第二性徵（ secondary sex characteristics ） 之表現外， 亦促進固醇類荷爾蒙之生成（ steroidogenesis）。吳郭魚（tilapia, Oreochromis mossambicus）為 台灣養殖漁業中之重要經濟魚種，然目前對其繁殖過程之內分泌 蛋白質調控機制研究仍偏少。本試驗將以蛋白質體學（proteomics ）方式分析經體外培養吳郭魚性腺受促性腺激素誘導處理後其蛋 白質之表現並探討其與繁殖之相關性。性成熟之雌雄吳郭魚性腺 經8小時預培養（Pre-Culture）後添加人類絨毛膜性腺激素（ human chorionic gonadotropin, hCG）、孕馬血清促性腺激素（ pregnant mare serum gonadotropin, PMSG）及兩者共同添加（ hCG+PMSG）等處理2小時，並以未添加者（新鮮組織 [Fresh]、 Pre-Culture及Control）為對照組，續以二維電泳分析比較其蛋白質 之表現。結果顯示精巢經hCG＋PMSG處理後其蛋白質之表現數顯 著高於Pre-Culture組者（444 ?b 8 vs. 309 ?b 44, P＜0.05），但於其他 處理組則否（444 ?b 8 vs. 420 ?b 14, 377 ?b 67, 389 ?b 68, and 374 ?b 62, P＞0.05），濾泡蛋白之Control組則顯著低於其他處理組（101 ?b 4 vs. 150 ?b 15, 139 ?b 20, 139 ?b 2, and 151 ?b 35, P＜0.05），但與hCG -iv- 組相較則否（101 ?b 4 vs. 112 ?b 14, P＞0.05）。而經去卵黃（ deyolk）處理後之濾泡其蛋白質表現量均顯著高於未去卵黃者（ undeyolk）（101~151 vs. 260~467, P＜0.05），且其PMSG處理組 表現量顯著高於其他組（467 ?b 10 vs. 297 ?b 5, 362 ?b 4, 260 ?b 4, 307 ?b 29, and 367 ?b 15, P＜0.05）。將具差異之蛋白質點經MALDITOF 進行鑑定分析後，於精巢成功鑑定出13個蛋白質，於濾泡6個 ，而於去卵黃後之濾泡僅3個，且多與細胞骨架及結構之形成（ keratin, type II cytoskeletal 1、keratin type II cytoskeletal 2 epidermal 、keratin-1、actin A3、actin cytoplasmic 1、67 kDa cytokeratin、 actin、fibrinogen beta B 1-118 、fibrinopeptide B、myosin regulatory light chain 2、myosin regulatory light polypeptide 9及Profilin-1）、 脂質結合運輸（fatty acid binding protein 7及annexin A5）、氧化還 原（quinone reductase）、代謝（metallothionein B及alpha-enolase ）及卵黃蛋白前質（vitellogenins）相關，另有多個功能未明者（ conserved hypothetical protein、hypothetical protein CBG 10348及 hypothetical protein Bamb_3203）。目前結果證實吳郭魚性腺蛋白 質於體外確實可受促性腺激素而影響其表現，此研究結果將有助 於釐清魚類繁殖過程之內分泌蛋白質調控機制，並對繁殖相關研 究及漁業之發展極具助益。

關鍵詞 : 促性腺激素、吳郭魚、性腺、蛋白質體學、二維電泳

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