以二維電泳分析固醇類荷爾蒙對體外培養吳郭魚性腺組織蛋白質表現之影響 = The effect of Steroid Hormones on the Protein ...

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摘 要

內分泌系統於生物體內調控如個體之生長及發育、細胞之功能、免疫（immunity）及代謝（metabolism）與繁殖等多種之生理功能。而脊椎動物（vertebrates）透過下視丘腦垂體性腺之主軸（hypothalamus-pituitary-gonadal axis, HPG axis）調控其性腺發育、配子生成（gametogenesis）與成熟、性徵及性轉換（sex reversion）等繁殖之功能。現已知硬骨魚類（teleost）HPG軸之作用乃由性腺固醇類荷爾蒙（gonadal steroids）所調控，然除傳統內分泌軸外，硬骨魚類之性腺受固醇類荷爾蒙所影響之基因或相關蛋白質目前仍未明瞭。本研究以蛋白質體學（proteomics）之方式，分析經固醇類荷爾蒙誘導處理後之吳郭魚性腺，其蛋白質之表現並探討其與繁殖之相關性。經體外培養並分別添加10 nM之固醇類荷爾蒙－雌二醇（-estradiol）、腎上腺皮質素（hydrocortisone）、助孕酮（progesterone）及睪固酮（testosterone）等處理後之吳郭魚精巢（testes）及去卵黃（deyolking）前後之卵巢濾泡（ovarian follicles）蛋白質，先以二維電泳（two-dimensional gel electrophoresis）之pH 3~10範圍分析性腺蛋白質之表現型態顯示，二性別之蛋白質表現均多集中於pH微酸至中性區域，續以pH 4~7範圍分析。結果顯示，精巢內蛋白質經pH 4~7範圍分析之175~305個點較pH 3~10範圍之130~230者為多；然於卵巢中，pH 4~7與pH 3~10範圍分析之蛋白質點分別為40~90個與35~60個點，相較其表現量後不具明顯增加之現象，但於此二範圍均可觀察出6個明顯之蛋白質點。濾泡蛋白質經去卵黃及蛋白質點統計之結果顯示，去卵黃之蛋白質點為110~250個，明顯較未去卵黃之40~90個為多。性腺蛋白質經MALDI-TOF/TOF-MS進行鑑定分析後，於精巢成功鑑定出7種內標蛋白質（actin、tropomyosin、cathepsin D、14-3-3 protein、myosin、unnamed protein product及Cu/Zn-superoxide dismutase），然於卵巢僅4種（actin、vitellogenin、tropomyosin及quinone reductase），並證實濾泡中大量表現之蛋白質點皆為卵黃前質蛋白（vitellogenins）。而於雄性所鑑定出具差異性之蛋白質為8種，並分別為與壓力（heat shock protein 70、heat shock 60 kDa protein 1及glutathione S-transferase）、代謝（enoyl coenzyme A hydratase及enolase 1 alpha-like）、免疫（78 kDa glucose-regulated protein）、結構（keratin）及發育或繁殖（cofilin-2）相關之蛋白質。上述結果證實，吳郭魚性腺蛋白質多集中於pH 4~7範圍內，並可受荷爾蒙或去卵黃而影響其表現，然具差異之蛋白質似亦與繁殖具直接或間接之相關性。

關鍵詞 : 下視丘腦垂體性腺軸、固醇類荷爾蒙、蛋白質體學、二維電泳、性腺、吳郭魚

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