3OST3B與CDH3基因於台灣乳癌檢體組織之甲基化變異及基因功能分析

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摘 要

台灣女性乳癌死亡率逐年攀升，殊值關注。形成乳癌的危險因子繁多，除了家族遺傳、內分泌因子與外在環境的致癌因子外，腫瘤抑制基因及生長調節相關基因之基因啟動子區域的異常甲基化，所導致之基因表現異常亦與乳癌生成息息相關。本論文遂以研究基因啟動子區域的異常甲基化和乳癌生成的關係為主軸。針對76個乳癌病患檢體組織之細胞膜外的黏附因子胎盤神經鈣黏蛋白基因CDH3及調控膜外蛋白之硫酸乙醯肝素糖胺聚糖硫基轉移?基因3OST3B，做基因甲基化異常修飾及其功能分析分析。首先利用甲基化特異性聚合?連鎖反應（MS-PCR）、結合亞硫酸鈉化學修飾與限制?分析（COBRA）等技術分析CDH3及3OST3B啟動子區域的甲基化狀態。由於在CDH3之甲基化分析中無法觀察到甲基化現象，故僅選擇3OST3B 進行後續實驗，採用亞硫酸鈉核酸化學修飾定序分析(bisulfite-sequencing)、反轉錄聚合?連鎖反應(RT-PCR)、免疫組織化學染色（IHC）等技術探討3OST3B於臨床乳癌檢體中甲基化CpG小島分佈及mRNA與蛋白質表現情形。試驗結果可將76位乳癌病患分為低度（27位）、中度（20位）及高度（29位）甲基化三族群，且在3OST3B基因啟動子區域內可搜尋到主要作用之轉錄因子，包括了NF-κB、E2F及n-MYC，這些轉錄因子的結合區皆包含了CpG雙核?酸，一旦發生甲基化之現象可能會影響上述轉錄因子結合而影響3OST3B基因的表現。並由IHC觀察到3OST3B高度甲基化之病理切片中腫瘤組織之3OST3B的表現量較低，而高度甲基化乳癌細胞株（MDA-MB-231）經去甲基化藥劑5-azadC處理後，結果顯示可降低3OST3B甲基化程度並回復3OST3B mRNA及蛋白質的表現。在臨床病理資料的統計中可觀察到，當3OST3B為中、高度甲基化時，與不具遠端轉移（M0）及p53表現較高的乳癌病患族群，具有統計顯著之相關性，因此，3OST3B可能是一個有意義的基因，可作為乳癌風險評估的一個指標。

關鍵詞 : 胎盤神經鈣黏蛋白基因 ; 硫酸乙醯肝素糖胺聚糖硫基轉移?基因 ; 結合亞硫酸鈉化學修飾與限制?分析 ; DNA甲基化 ; 乳癌

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