利用甲基化核糖核酸多型分析法找尋印痕基因

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摘 要

DNA甲基化是一種epigenetic的機制，在哺乳類動物中它主要是利用甲基化轉換? (Methyltransferase)，將甲基共價鍵結在5，-CG-3，序列上胞嘧啶環上的第5個碳的位置。DNA甲基化這種特殊的DNA印痕和調控基因的表現與組織專一性基因之表達有密切的關係，並且也調節一些細胞功能的表現，如染色體印痕基因的表現、DNA的突變及癌症的形成。 儘管DNA甲基化是那麼重要，但是即將定序完成的基因序列卻不能代表任何DNA甲基化的訊息，又加上過去的甲基化研究都只限於已知的基因。有鑑於此，利用10組AP引子進行甲基化敏感性PCR (AP-PCR) 的方法，能夠廣泛地在基因組中來尋找組織專一性的甲基化片段和胚胎組織中的甲基化片段。在此，我們將基因組DNA以Hind III限制酵素作用，再分別以甲基化敏感性限制酵素Hpa II及甲基化非敏感性限制酵素Msp I處理，以AP引子做PCR反應，利用這種技術可於組織中尋找甲基化差異片段，以AP-1引子作用所得到差異片段，分別為AP101、 AP102、AP103、AP104、AP105，而AP104片段順利的選殖並定序，但經比對並無相似片段；以AP-2引子作用得到AP201片段，經選殖後定序，比對後於DNA序列中未有DNA甲基化Hpa II (CCGG) 酵素之切位，因此沒有進一步分析；而以AP-3及AP-8引子PCR得到的差異片段AP301及AP803，選殖後定序之結果，DNA序列與NCBI基因庫小鼠基因組序列比對相似度高達98 % 及99 %，並且序列 中具有Hpa II酵素切位，表示此二片段DNA序列中確實為甲基化差異性片段，因此，將此二片段由放射線標定後進一步以南方點墨雜合分析法分析甲基化差異片段之相似度。

關鍵詞 : 甲基化 ; 印痕基因 ; 基因組DNA ; Arbitrary Primed PCR

目錄

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