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DNA甲基化程度在大腸直腸癌扮演的角色

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摘要

目前在大腸直腸癌中研究最透徹的表觀基因修飾（epigenetic modification）包括兩種：第一是「基因起動子（gene promoter）」之CpG島（CpG islands）的「高度甲基化（hypermethylation）」；第二是基因體的「全面低度甲基化（global hypomethylation）」。DNA的高度甲基化會造成抑瘤基因的靜止及基因體的不穩定；而DNA的低度甲基化會造成致癌基因的活化及染色體的不穩定。兩者都與大腸直腸癌的發生有關，且有特定的臨床現象。（生醫2014;7(1):40-44）

關鍵字：大腸直腸癌、高度甲基化（hypermethylation）、低度甲基化（hypomethylation）、CpG島（CpG islands）

前言

「表觀基因學（epigenetics）」指的是任何有關基因的調控機轉但不涉及改變核苷酸序列者，譬如某基因之胞嘧啶（cytosine）的甲基化（methylation）可影響基因的表達，但此並未改變DNA序列。目前認為表觀基因修飾（epigenetic modification）是癌化過程中改變基因表現的重要過程。在癌細胞裡表觀基因修飾比基因突變更為常見[1]。目前已知DNA之高度甲基化（hypermethylation）和低度甲基化（hypomethylation）都在大腸直腸癌的發生上皆扮演一定的角色。本文就此作一回顧。

高度甲基化之致癌機轉

脊椎動物之基因體中約20%之序列為5'-GC-3'（簡稱CpG）之雙核苷酸（dinucleotide）。然而有些長約1至2kb之序列段，其CpG含量高達60%，這

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種序列謂之「CpG島（CpG islands）」。據估計，人類基因體中約有45,000個CpG島，其中約10,000個和基因調控有關。據估計，大約60%的基因，其上游可見CpG島[2]。

不論那種組織細胞，起動子（promoter）之CpG島皆可發生甲基化[3]，只是程度的不同而已[4,5]。CpG島的低度甲基化會促進基因轉錄，而高度甲基化則使基因靜止。自從Issa等人[6] 於1994年在大腸直腸癌中發現，起動子之CpG島甲基化會抑制「雌激素受體（estrogen receptor; ER）」基因的活性後，研究者已發現，大腸直腸癌有上百個基因也可同樣地因甲基化而使其表達受到抑制[7,8]。目前認為有35%的大腸直腸癌，其起動子之CpG島甲基化在癌化過程中扮演重要角色[9]。在此過程中，抑瘤基因和涉及凋亡、DNA修復及調控細胞週期的基因，會因DNA甲基化而停止表達[10,11]。目前已知，與此有關的抑瘤基因包括*p16*、*p14*、*MGMT* 和 *hMLH1*[10]。

高度甲基化的起動子會使基因靜止，其機轉之一是與「甲基化CpG結合域（methyl CpG-binding domain protein; MBD）」蛋白有關。MBD蛋白一旦與甲基化的DNA結合後，會阻礙轉錄因子結合到起動子，因而轉錄作用無從開始。此外，也能招引其它的抑制因子到起動子，而干擾轉錄作用[12]。MBD蛋白是一個大家族，包括11個成員：MeCP2、MBD1、MBD2、MBD3、MBD4、MBD5、MBD6、SETDB1、SETDB2、BAZ2A和BAZ2B[13]。現已有研究指出，在大腸直腸癌中MeCP2[14]、MBD1[12,15]、MBD2[15]和 MBD4[16-21]可抑制基因的表現。

「多齒群（polycomb group; PcG）」蛋白也與基因靜止有關[22]。這是因為「多齒抑制複合體（Polycomb Repressive Complex; PRC）」之主要成員PRC1和PRC2可與甲基轉移酶（methyltransferase）作用[23-25]，而CpG之胞嘧啶的甲基化需要甲基轉移酶之參與。此外，也有研究發現大腸直腸癌可過度表達EZH2蛋白，而此蛋白為PRC之次單元，它可調控PcG介導之轉錄作用[26]。

高度甲基化之大腸直腸癌

「CpG島甲基者外表型（CpG island methylator phenotype; CIMP）」這個名詞最早是由Toyota等人於1999年提出，來描述某些有高度早基化的大腸直腸癌[27]。因此，CIMP陽性腫瘤的特徵就是，廣泛的甲基化導致許多基因的靜止[1]。

若將CIMP陽性分成高度（CIMP1）和低度（CIMP2）[28,29]，那麼可見CIMP1較常為「微衛星不穩定（microsatellite instable; MSI）」者（80%），且有較高的比率出現*BRAF*突變（53%），以及偏向女性患者。此外，CIMP1之大腸直腸癌較常源於「齒狀腺瘤（serrated adenoma）」[30,31]。所謂齒狀腺瘤即「隱窩腔面呈鋸齒狀」的息肉[32]。這種息肉的發生與凋亡受抑制及RAS-RAF-MEK-ERK訊息途徑活性太強有關[32]。這種息肉常被稱為「微小泡性增生息肉（microvesicular hyperplastic polyp）」。據估計，發生較久的齒狀息肉中，30%有高度的CIMP[33]。相反地，CIMP2較常有*KRAS*突變（92%），而少見*BRAF*或*TP53*突變，以及偏向男性患者。此外，CIMP2之大腸直腸癌較常源於管絨狀腺瘤（tubulovillous

adenoma）[28,31]。

DNA高度甲基化亦可導致微衛星不穩定

DNA甲基化可導致「錯配修復（mismatch repair; MMR）」系統不全，此會使突變率上升100倍。錯配修復系統中研究最多的就是*MLH1*基因。偶發型之大腸直腸癌而有微衛星不穩定（microsatellite instability; MSI）者，75%是因*MLH1*基因之高度甲基化所造成的[27]。CIMP型大腸直腸癌與MSI型大腸直腸癌之共同處是，腫瘤易發生在大腸近側端且分化較差。

雖然CIMP型大腸直腸癌常出現*MLH1*基因的甲基化，但是一半以上的CIMP型大腸直腸癌卻是「微衛星穩定（microsatellite stable; MSS）」者。與MSI型大腸直腸癌不同的是，CIMP型大腸直腸癌通常預後不佳[10]且常有*KRAS*或*BRAF*突變[1]，但若同時伴隨著「微衛星不穩定」者，則預後較好[31]。

低度甲基化與大腸直腸癌的關係

三、四十年前已有研究者觀察到，腫瘤基因體之5-甲基胞嘧啶（5-methylcytosine）有全面降低的傾向[34-37]，此現象謂之DNA低度甲基化（DNA hypomethylation）。

DNA低度甲基化可出現於良性及惡性的腫瘤[38]。不例外地，大腸直腸癌也會出現「全面性的DNA低度甲基化（global DNA hypomethylation）」[39]。此低度甲基化現象也可出現於腺瘤性息肉（adenomatous polyps）[40]及增生性息肉（hyperplastic polyps）[41]。

低度甲基化可能與致癌基因的活化有關[42]。譬如，40%的大腸直腸癌有IGF2的低度甲基化[43]；此可造成IGF2/H19區失去印跡（loss of imprinting）[44]，而導致癌細胞的增殖。

CpG雙核苷酸全面性的低度甲基化主要發生在特定的序列，例如重複序列（repetitive sequences）像是衛星（satellite）和LINE重複段（LINE repeats）、致癌基因等等[45]。而此也因易造成染色體的斷裂而與染色體的不穩定有關[46-48]。關於此現象，我們可從LINE-1略窺一二。人類基因體內17%是LINE-1，這是一種反轉錄轉座子（retrotransposon）。在正常情況下，LINE-1的CpG是高度甲基化的，因此能抑制轉座子之活性而維持基因體的穩定性[49]。相反地，當LINE-1出現低度甲基化時，轉座子會活化造成染色體的不穩定。有研究指出，75%至80%的早發型（年齡<60歲）大腸直腸癌有LINE-1的低度甲基化。此種大腸直腸癌預後不佳[50-53]。

結語

就臨床實用性而言，CIMP陽性之大腸直腸癌常出現於於高齡、有家族史及抽煙之婦女；腫瘤較常出現於近端大腸、具有黏液細胞分化形態、且較有*KRAS*或*BRAF*突變[31,54,55]。至於CIMP陰性之大腸直腸癌，則有較高的比率有*TP53*突變（71%）、中等程度的*KRAS*突變（33%）、而且較常源於「管狀腺瘤（tubular adenoma）」[33]。就預防性醫學而言，由於環境因素可改變DNA的甲基化程度，而DNA的高度或

低度甲基化可分別抑制抑瘤基因和活化致癌基因，甚至也可影響基因體的穩定性而導致突變。因此，大腸直腸癌提供一個很好的模型，來解釋環境因子的致癌機轉。這值得我們深入學習。

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