DNA methyltransferase 1 促進非小細胞肺癌轉移之探討

Over-expressed DNA Methyltransferase 1 Protein Promotes the Metastatic Capacity of Non-Small Cell Lung Cancer

中文摘要

轉錄抑制因子 DNA methyltransferase 1（DNMT1）屬於 DNMTs 家族的成員之一，在生物體中主要於基因序列上游啓動子位置造成甲基化的情形，並且藉由甲基化某些特定基因啓動子以達調控這些基因之基因表現。然而，目前的研究中，有關非基因序列層次改變在癌症惡化過程中所扮演的角色仍有許多處於未知狀態。於是，我們的研究主題著重在探討癌症轉移過程時 Dnmt 基因扮演之角色。在肺癌病患體內，過量的 DNMT1 表現與癌症惡化息息相關。首先，利用 cDNA library 雜合法篩選出包含 DNMT1 等幾個與肺癌症轉移有關的基因；利用免疫組織染色法分析肺癌及其相對應的正常組織，發現 DNMT1 在癌細胞中高量表現。接著，我們利用一對具有代表性的細胞株作爲體外試驗的模式：CL1-0（具低侵襲力）及 CL1-5F4（具高侵襲力），發現無論在 mRNA 以及蛋白質層次，具高侵襲利之 CL1-5F4 細胞株其 DNMT1 之表現量皆顯著地高於低侵襲力之 CL1-0 細胞倲。若對 CL1-5F4 細胞處理 DNMT1 特異性抑制劑－5-aza-2’-deoxycytidine（5-azaCdR）及 zebularine（ZEB）－發現不但可降低細胞中 DNMT1 蛋白表現量，亦可明顯地下降 CL1-5F4 細胞之爬行其侵襲能力；且處理 5-azaCdR 及 ZEB 所造成的細胞侵襲力下降並非由於增生能力下降所造成。利用 RNA 干擾技術將 CL1-5F4 中 DNMT1 下調亦會使這些細胞爬行及侵襲力下降約三倍。相反的，若我們將 DNMT1 構築送入低侵襲力的 CL1-0 細胞中表現亦可有意義地增加其爬行之能力。綜合以上的實驗，我們的結果顯示 DNMT1 在癌症細胞中因其轉譯後修飾作用造成人類非小細胞肺癌惡性轉移而扮演了一個極爲重要的角色。

英文摘要

DNA methyltransferase 1（DNMT1）is one of DNMTs family known for playing an important role in DNA replication by methylation of certain gene promotes to regulate corresponding gene expression in a cell. However, it is not clear if epigenetic modification can play a role on tumor malignancy. The study investigates the functions of DNMT1 on tumor metastasis. Overexpression of DNMT1 is associated with tumor malignancy of patients with lung cancer. DNMT1 is originally one of cloned genes identified from a cDNA library by subtracting metastatic tumors from primary tumors. Immunohistochemical study from patients with non-small-cell lung cancer shows higher expression of DNMT1 on the tumor parts than that of tumor-adjacent normal counterparts. Using a pair of cell lines, CL1-0 (low invasive cells) and CL1-5F4 (high invasive cells) as an in vitro tumor model shows DNMT1 mRNA and protein expression were higher in invasive CL1-5F4 cells than CL1-0 cells. Treatment of specific DNMT1 inhibitors, 5-aza-deoxycytidine and zebularine, significantly suppress cell migration and invasion ability of CL1-5F4 cells, and the reduced capability of cell migration and invasion in CL1-5F4 cells was not due to decrease in their cell growth which was measured by MTT assay. Furthermore, knockdown of DNMT1 by shRNA technique suppress cell migration and invasion ability about 3-fold in CL1-5F4 cells. On the other hands, overexpressed DNMT1 in low-invasive CL1-0 cells raise the cell migration ability. In conclusion, these results suggest that DNMT1 may participate in tumor cell invasion and metastasis.