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Tumor Suppressor Gene Inactivation during Cadmium-Induced Malignant Transformation of Human Prostate Cells Correlates with Overexpression of *de Novo* DNA Methyltransferase

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BACKGROUND: Aberrant DNA methylation is common in carcinogenesis. The typical pattern appears to involve reduced expression of maintenance DNA methyltransferase, DNMT1, inducing genomic hypomethylation, whereas increased expression of *de novo* DNMT3a or 3b causes gene-specific hypermethylation.

OBJECTIVES: During cadmium-induced malignant transformation, an unusual pattern of genomic hypermethylation occurred that we studied to provide insight into the roles of specific DNMTs in oncogenesis.

METHODS: Gene expression and DNA methylation were assessed in control and chronic cadmium-transformed prostate epithelial cells (CTPE) using reverse transcription-polymerase chain reaction (RT-PCR), Western blot analysis, methylation-specific PCR, and methyl-acceptance assay.

RESULTS: During the 10-weeks of cadmium exposure that induced malignant transformation, progressive increases in generalized DNMT enzymatic activity occurred that were associated with overexpression of DNMT3b without changes in DNMT1 expression. Increased DNMT3b expression preceded increased DNMT enzymatic activity. Procainamide, a specific DNMT1 inhibitor, reversed cadmium-induced genomic DNA hypermethylation. Reduced expression of the tumor suppressor genes, RASSF1A and p16, began about the time DNMT3b overexpression first occurred and progressively decreased thereafter. RASSF1A and p16 promoter regions were heavily methylated in CTPE cells, indicating silencing by hypermethylation, while the DNA demethylating agent, 5-aza-2'-deoxycytidine, reversed this silencing. DNMT1 inhibition only modestly increased RASSF1A and p16 expression in CTPE cells and did not completely reverse silencing.

CONCLUSIONS: These data indicate that DNMT3b overexpression can result in generalized DNA hypermethylation and gene silencing but that DNMT1 is required to maintain these effects. The pattern of genomic DNA hypermethylation together with up-regulation of DNMT3b may provide a unique set of biomarkers to specifically identify cadmium-induced human prostate cancers.

KEY WORDS: cadmium, carcinogenesis, DNA methylation, DNMT3b, p16, prostate, RASSF1A.

镉诱发人类前列腺细胞恶性转化过程中肿瘤抑制基因失活与从头DNA甲基转移酶过表达的关系

背景: 癌发生过程中常可见 DNA 甲基化的紊乱。其典型的模式包括持续 DNA 甲基转移酶 DNMT1 的表达降低导致基因组的低甲基化, 从而从头 DNMT3a 或 3b 的表达增加导致基因特异的超甲基化。

目的: 在镉诱发恶性转化过程中, 我们研究发现一种不平常的基因组超甲基化模式, 这种模式为特定的 DNA 甲基转移酶在肿瘤发生过程中的作用提供了新的见解。

方法: 用 RT-PCR, Western blot, 甲基特异性 PCR 以及甲基容受性试验来评价对照细胞和慢性镉转移前列腺上皮细胞 (CTPE) 中的基因表达以及 DNA 甲基化。

结果: 10 周镉暴露导致恶性转化过程中, DNMT 酶活性整体进行性增加与 DNMT3b 的过表达和 DNMT1 表达未改变有关。DNMT3b 表达增加发生于 DNMT 酶活性增加之前。普鲁卡因胺 (一种特异的 DNMT1 抑制剂) 逆转了镉诱发的基因组 DNA 超甲基化。肿瘤抑制基因 RASSF1A 和 p16 的表达降低开始于 DNMT3b 过表达首次发生时并随后渐渐降低。CTPE 细胞的 RASSF1A 和 p16 启动子区域严重甲基化, 且由于超甲基化而沉默。DNA 去甲基化物——脱氧氮杂胞苷逆转了这种沉默。而 DNMT1 的抑制剂仅是稍增加了 CTPE 细胞中 RASSF1A 和 p16 的表达而并未完全逆转这种沉默。

结论: 这些数据表示 DNMT3b 的过表达可导致广泛的 DNA 超甲基化以及基因沉默, 而 DNMT1 是保持这些效应所必需的。这种基因组 DNA 超甲基化与 DNMT3b 的上调协同的模式可为特异性鉴别镉诱发的人类前列腺癌提供一系列独特的生物标志物。(张勇燕译)