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Epigenetic Regulation on ASPP Family Members in Choriocarcinoma

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Introduction

Dysregulation of ASPP (apoptosis-stimulating protein of p53) family members has been found in gestational trophoblastic disease (GTD). One of the malignant forms of GTD, choriocarcinoma, exhibited a downregulation of ASPP1and ASPP2 but elevated levels of iASPP when compared to normal trophoblastic tissues. ASPP family members can act as either a stimulator or inhibitor to control p53 mediated apoptosis so that their expressions are essential for the survival of choriocarcinoma cells. Nevertheless, the underlying mechanisms for the dysregulation of their expressions in choriocarcinoma are unclear. Methylation and acetylation are two common epigenetic regulatory mechanisms on gene expression. In this study, such epigenetic regulations on the expression of ASPP family members in choriocarcinoma were investigated. The knowledge on the regulation on ASPPs may be beneficial to understand the pathogenesis of choriocarcinoma.

Objectives

To investigate the epigenetic control on ASPP family members in choriocarcinoma cell lines.

Methodology

Choriocarcinoma cell lines, JEG-3 and JAR, were used to study the gene regulation on ASPP1, ASPP2 and iASPP while normal trophoblastic cell lines, TEV-1 and HTR-8/SVneo cells were used as comparison. 5-azacytidine (5-Aza) was used to study the effect of methylation while Trichostatin A (TSA) was used to study the effect of acetylation on ASPP expressions in trophoblastic cell lines. Bisulfite sequencing was used to detect the CpG methylation of ASPPs' promoter regions. Moreover, chromatin immunoprecipitation (CHIP) was used to examine the acetylation status on their promoter regions. The changes in ASPP expressions were measured by both western blotting and real-time PCR.

Result

Addition of 5-Aza increased both the mRNA and protein expressions of ASPP1 and ASPP2 but not iASPP in JEG-3 and JAR cells. However, most of the native CpG sites sequenced were found to be unmethylated, suggesting that hypermethylation was

unlikely to regulate ASPP1/2 expression directly in choriocarcinoma cell lines. On the other hand, TSA treatment increased the expression of ASPP1 in JEG-3 and JAR cells. CHIP assay also reflected histone hyperacetylation on ASPP1 promoter region after TSA treatment, suggesting that acetylation may be responsible for regulating ASPP1 in choriocarcinoma. ASPP2 and iASPP, nevertheless, seemed to be regulated by different mechanism as no increase in expression was observed through acetylation. Our results indicated that ASPP family members were regulated by different mechanisms and acetylation may act as a modulator on ASPP1 expression in choriocarcinoma.