Effect of Zebularine on p16INK4a, p14ARF, p15INK4b, and DNA Methyltransferase 1 Gene Expression, Cell Growth Inhibition, and Apoptosis Induction in Human Hepatocellular Carcinoma PLC/PRF5 and Pancreatic Cancer PA-TU-8902 Cell Lines

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Abstract

Tumorigenesis must be understood as a summary of altered genetic and genomic changes resulting in the inactivation of tumor suppressor genes (TSGs). One of the characterizations of epigenetic alterations is DNA methylation. Epigenetic alteration of the p16INK4a, p14ARF, p15INK4b, and DNA methyltransferase 1 gene (DNMT1) expression occurs in hepatocellular carcinoma (HCC) and pancreatic cancer frequently. DNA methyltransferase inhibitors (DNMTIs), such as zebularine, play a significant effect on the demethylation and reactivation of TSGs. This study aimed to investigate the effect of zebularine on p16INK4a, p14ARF, p15INK4b, and DNA methyltransferase 1 gene expression, cell growth inhibition, and apoptosis induction in HCC PLC/PRF5 and pancreatic cancer PA-TU-8902 cell lines. Both cell lines were cultured and treated with zebularine at different times. The MTT assay, real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR), and flow cytometry were used to determine cell viability, gene expression, and apoptotic cells, respectively. The result indicated that zebularine inhibited cell growth of both cell lines significantly as time-and dose-dependent manner ($P < 0.007$). The agent induced significant down-regulation of DNMT1 and up-regulation of p16INK4a, p14ARF, p15INK4b ($P < 0.028$). Besides, it had a significant apoptosis effect on both cell lines ($P < 0.001$). This compound had a strong significant effect on PLC/PRF5 in comparison to PA-TU-8902 cells. Concluding, zebularine inhibited PLC/PRF5 and PA-TU-8902 cell growth and induced apoptosis in these cell lines. The most likely mechanism underlying the zebularine played its role involves down-regulation of DNMT1 and up-regulation of p16INK4a, p14ARF, and p15INK4b genes.

Keywords

Author Keywords: INK4 CDKI; DNA-methyltransferase 1; Zebularine Cancer

KeyWords Plus: PROMOTER METHYLATION; CDK INHIBITORS; VALPROIC ACID; P53 MUTATIONS; P16 GENE; P16(INK4A); P14(ARF); 5-AZA-2'-DEOXYCYTIDINE; P15(INK4B); PROLIFERATION