Effect of 5-aza-2′-deoxycytidine on p16INK4a, p14ARF, p15INK4bGenes Expression, Cell Viability, and Apoptosis in PLC/PRF5 and MIA Paca-2 Cell Lines

Masumeh Sanaei MSc[1], Fraidoon Kavoosi PhD[1,*], Maedeh MohammadiMD[2], Maryam KhanezadMSc[3]

AbstractBackground:

Mammalian cell division is regulated by a complex includes cyclin-dependent kinases (Cdks) and cyclins, Cdk/cyclin complex. The activity of the complex is regulated by Cdk inhibitors (CKIs) compressing CDK4 (INK4) and CDK-interacting protein/kinase inhibitory protein (CIP/KIP) family. Hypermethylation of CKIs has been reported in various cancers. DNA methyltransferase inhibitors (DNMTIs), such as decitabine and 5-aza-2′-deoxycytidine (5-aza-CdR) can reactivate hypermethylated genes. The current study aimed to evaluate the effect of 5-aza-CdR on the expression of p16INK4a, p14ARF, p15INK4b genes, cell viability, and apoptosis in HCC PLC/PRF5 and pancreatic cancer MIA Paca-2 cell lines. Materials and Methods: In this laboratory trial, both cell lines were treated with 5-aza-CdR (0, 1, 2.5, 5, 10, 15, and 20 µM) to determine cell viability and then with 3 µM to obtain cell apoptosis and relative gene expression. The cell viability, apoptosis, and genes expression were investigated by 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) assay, flow cytometry, and Real-Time quantitative reverse-transcription polymerase chain reaction (qRT-PCR), respectively.Results: 5-aza-CdR indicated significant inhibitory effect with all used concentrations (P = 0.003). The apoptotic effect of 5-aza-CdR on PLC/PRF5 cells in comparison to pancreatic cancer MIA Paca-2 cells was more significant (P= 0.001). Real-time quantitative PCR analysis revealed that treatment with 5-aza-CdR (3 µM) for 24 and 48h up-regulated p16INK4a, p14ARF, p15INK4b genes expression significantly(P=0.040).Conclusion: Reactivation of p16INK4a, p14ARF, p15INK4b genes by 5-aza-CdR can induce apoptosis and inhibit cell viability in HCC, PLC/PRF5, and pancreatic cancer, MIA Paca-2, cell lines.Keywords: Apoptosis, 5-aza-2'-deoxycytidine-5'-monophosphate, Gene expression, Viability