Epigenetic Study in Waldenstrom's Macroglobulinemia

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The deleted in liver cancer-1 (DLC-1) gene encodes a Rho GTPase activating protein (RhoGAP) with potential tumor-suppressor activity. Hypermethylation in the DLC-1 promoter is an epigenetic modification associated with transcriptional silencing of DLC-1 in various types of human cancers. This study was designed to investigate if the DLC-1 gene expression is regulated by DNA methylation in Waldenström's macroglobulinemia (WM). We found that the CpG islands in the DLC-1 promoter region were methylated in the two WM cell lines BCWM.1 and WM-WSU. The DLC-1 promoter was also methylated in the multiple myeloma cell lines RPMI, U266 and INA6, but not in MM1S and MM1R. The methylation status was highly correlated with DLC-1 mRNA expression in the cell lines. Of the 20 WM patients examined, 14 patients had methylation in the DLC-1 promoter region (70%). In contrast, no methylation was seen in the normal control subjects. DLC-1 mRNA expression was significantly lower in WM patients compared to the normal controls ($p = 0.014$). Treatment with the demethylation agent azacytidine resulted in significant up-regulation of DLC-1 in the WM cell lines. The combination of azacytidine and the HDAC inhibitor SAHA resulted in synergistic induction of DLC-1 mRNA. These results suggest that methylation in the DLC-1 promoter region is a frequent epigenetic modification and DLC-1 is a potential tumor-suppressor gene in WM.

Azacytidine is a potent demethylation agent and has been approved by the FDA for treatment of myelodysplastic syndrome. We found that azacytidine exhibited significant dose-dependent cytotoxicity against the WM cell lines. Treatment of BCWM.1 WM cells with 2 uM of azacytidine rapidly induced cell cycle arrest at G1 and induced apoptosis in 48 hours. Azacytidine also induced significant apoptosis in WM patient samples as opposed to healthy donor PBMCs. Cleavage of caspase 3, 7, 8 and 9 along with PARP-1 were associated with the azacytidine induced apoptosis, suggesting mitochondrial and death receptor apoptotic pathway involvement. While Bcl-2 and pBcl-2 were unchanged following azacytidine, BH3 proteins were significantly induced, suggesting that azacytidine induced apoptosis was mediated by induction of BH3 proteins which subsequently interacted with survivin. Azacytidine did not affect PTEN, Akt or mTOR but did significantly down regulate Raptor resulting in the inactivation of mTORC1. Furthermore, the combination of azacytidine and SAHA induced synergistic cytotoxicity against the WM cell lines and patient samples as opposed to healthy donor PBMCs. These studies provide the framework for clinical trials examining azacytidine alone and in combination with SAHA or mTOR inhibitors in the treatment of WM.