Involvement of lipogenic pathway in 5-azacytidine-induced cytotoxicity in Waldenstrom's Macroglobulinemia.

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5-azacytidine and 5-aza-2'-deoxycytidine are demethylation agents used for the treatment of myelodysplastic syndrome (MDS). Despite a putative role for the reactivation of epigenetically silenced tumor-suppressor genes, the mechanism of action for these agents remains to be clarified. Recent studies suggest that 5-azacytidine may also act at the post-transcriptional level by insertion into RNA transcripts. As part of these efforts, we investigated the therapeutic potential of these agents in Waldenstrom's Macroglobulinemia (WM), and characterized molecular changes associated with treatment. We observed that 5-azacytidine was more potent than 5-aza-2'-deoxycytidine in inducing dose-dependent cytotoxicity against BCWM.1 WM cells and primary WM lymphoplasmacytic cells. 5-azacytidine-induced cell cycle arrest at G1 was accompanied by up-regulation of the cyclin-dependent kinase inhibitor p21 (Cip1/WAF1) through a p53-independent pathway. Activation of caspase-3, 7, 8, and 9, and PARP was also observed indicating involvement of both mitochondrial and death receptor pathways for 5-azacytidine-induced apoptosis. The BH3 domain proteins Bim and Puma were increased in WM cells treated with 5-azacytidine, and siRNA-mediated knockdown of Bim and Puma provided moderate protection from 5-azacytidine-induced apoptosis. In contrast, PI3K/AKT/mTOR and NF-κB activities were not significantly altered by 5-azacytidine. To further characterize the molecular changes with 5-azacytidine, we profiled mRNA expression in the WM cells associated with 5-azacytidine treatment. The most strikingly down-regulated genes by 5-azacytidine include FASN and SCD1, which encode enzymes essential for lipogenesis. Importantly, by quantitative RT-PCR, FASN mRNA expression levels were significantly higher in WM patients versus healthy donors ($p=0.001$). WM patients who expressed higher levels of FASN mRNA showed greater bone marrow involvement ($p=0.003$). Importantly, siRNA-mediated knockdown of FASN or SCD1 induced significant growth arrest and apoptosis in WM cells. siRNA-mediated knockdown of the sterol regulatory element binding protein 1 (SREBP-1), a transcriptional activator for FASN and SCD1 was associated with reduced transcription of FASN and SCD1, along with increased cytotoxicity in WM cells. Inhibition of FASN activity by Cerulenin also induced significant apoptosis, and the combination of 5-azacytidine and Cerulenin resulted in a synergistic induction of apoptosis in WM cells. In conclusion, the results of this study support an important role for the lipogenic genes in the 5-azacytidine-induced cytotoxicity in WM cells, and highlight a novel mechanistic pathway for this agent, as well as novel targets for drug therapy in WM.