Effects of Alterations on SAM/Met Pathway on Normal HSPCs

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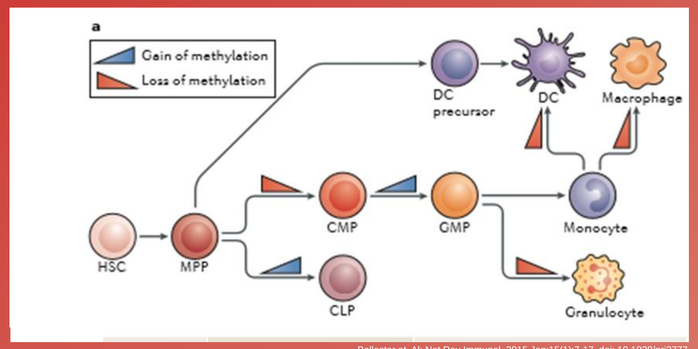
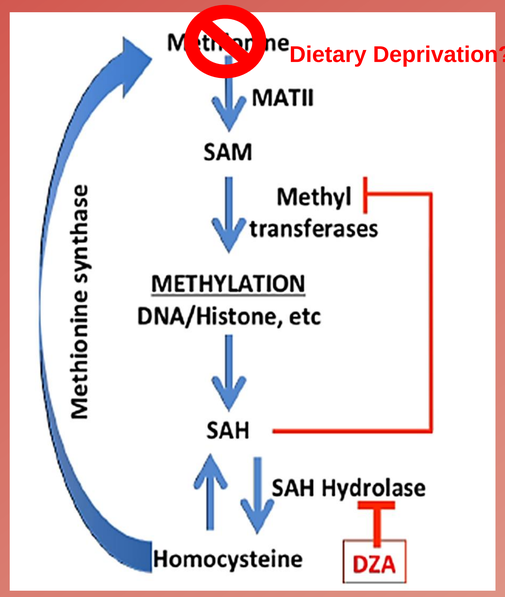
Abstract

The highly heterogenous clonal disorder acute myeloid leukemia (AML) is is characterized by rapid proliferation of abnormal myeloblastic progenitors, normal differentiation being blocked, and blast accumulation disrupting normal hematopoiesis. The key point being leukemogenesis is the dysregulation of normal hematopoiesis. AML is the most prevalent form of adult leukemia and the second most common childhood leukemia. Modern chemotherapeutic treatment of AML results in approximately 60-70% initial remission rates, but unfortunately the overall 5-year survival remains poor at only 26% due largely to refractory relapse. Recently, epigenetic dysregulation has been found to play an important role in controlling hematopoiesis, specifically DNA & histone methylation, therefore acting as a driving mechanism for the induction and maintenance of AML. Seeing how normal hematopoiesis is important, this dysregulation was further studied by targeting the methionine/SAM biosynthetic pathway. In this pathway, Methionine is converted by MATII into SAM. SAM, as a key methyl donor, forms S-adenosylhomocystine (SAH) upon donation. SAH is then converted by SAH hydrolase into Homocysteine, which can then be recycled into methionine to start the cycle over again. Importantly, accumulation of SAH shifts cellular potential away from methylation and globally inhibits Methyltransferase enzymes through feedback inhibition. Thus, it was hypothesized that by blocking SAMe production (via Methionine deprivation) or blocking SAH breakdown (via inhibition of SAH hydrolase by using 3-deazaadenosine or DZA) causes a decrease in methyltransferase activity, and may provide a novel treatment for AML by diminishing cellular methylation. Through testing the effects of SAHH inhibition using DZA on several human leukemia cell lines (MV411, RS411) *in vitro*, we found that it resulted in apoptotic cell death and the induction of cellular markers of apoptosis including caspase-3 and PARP-1 cleavage. Thus, we chose to focus on investigating the molecular mechanisms underlying death induced by inhibition of SAHH, in the MV-4-11 and RS-4-11 cell lines. Protein analysis revealed that inhibition of SAM metabolism induced several distinct anti-proliferative/death pathways in MV-4-11 and RS-4-11 cells namely: 1.) ER stress 2.) Oxidative stress and 3.) DNA damage. Consistent with our initial hypothesis, inhibition of SAM metabolism also resulted in epigenetic changes to the histone methylome and induced multiple death pathways. Given the effect of the pathway on AML and epigenetics, we decided to further look into the effect the pathway had on normal HSPCs. FVB bone marrow or human PBMCs were not as affected by methionine deprivation as dramatically as cancer cells, where in as little as 2 days 60% death was observed. To examine this further, we lineage depleted FVB mice, and cultured them with our four conditions (+M, +M+DZA, -M, -M+DZA) and then analyzed them with various antibodies. An increase in GRI and CD8 was observed in our double positive cells, as well as a similar increase with SCA and C-kit. Through analyzing this data and the cells, it was evident that cells were proliferating. Hypothesizing these were NK cells, we found they were positive for CD3 and LY6C, markers that are also present on NK cells. Taken together, these findings indicate that inhibition of SAM metabolism and subsequent epigenetic outcomes may provide a unique adjuvant treatment strategy for AML, where HSPCs could be used to then kill cancer cells.

Rationale

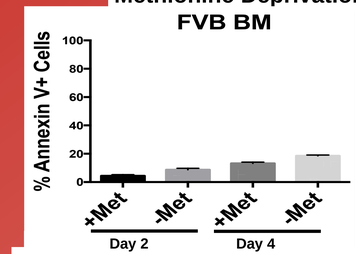


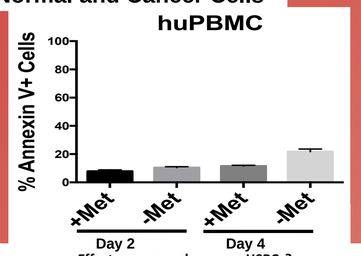
Ballestar et. Al; Nat Rev Immunol. 2015 Jan;15(1):7-17. doi: 10.1038/nri3777.

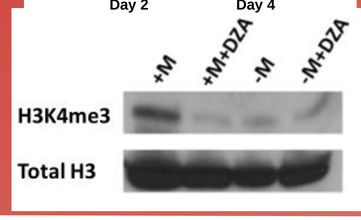


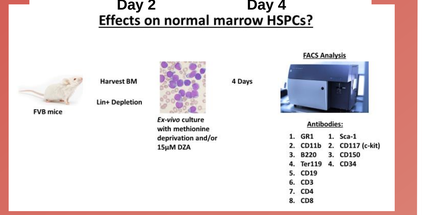
Results

Methionine Deprivation on Normal and Cancer Cells

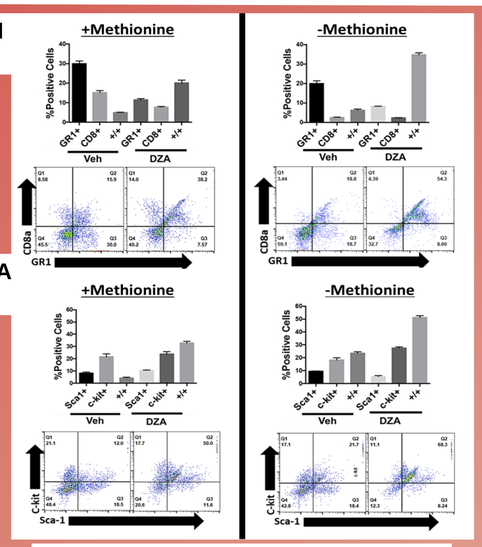




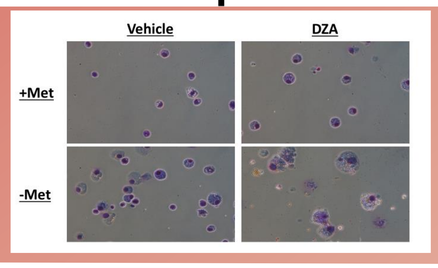




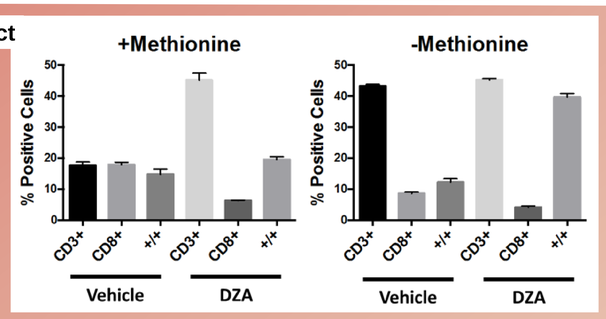
Effect on GRI and CD8



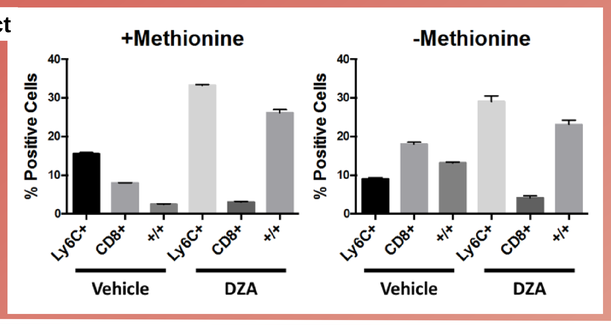
Effect on SCA and C-Kit



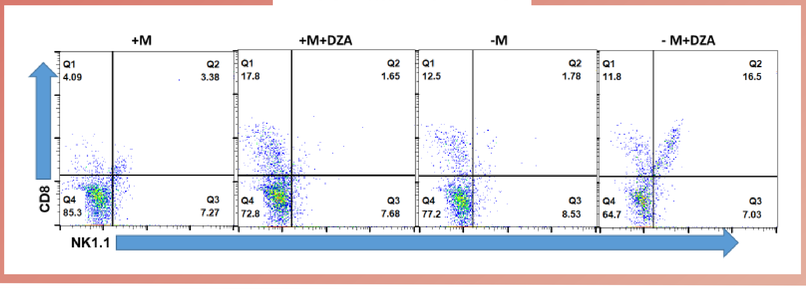
CD3 Effect

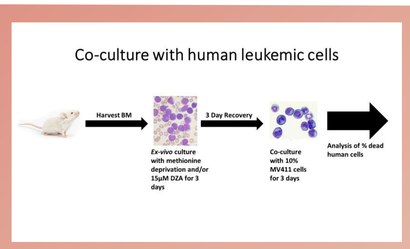


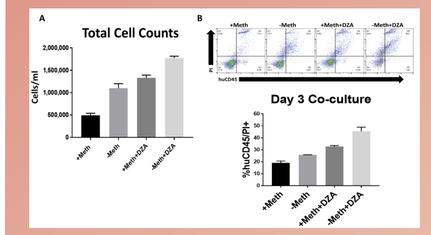
Ly6C Effect

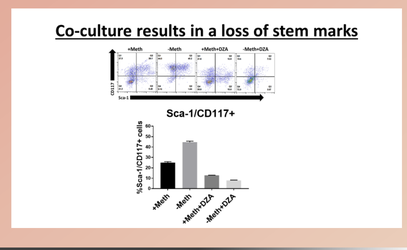


FACS Data with NK1.1









Conclusions

Blocking SAMe production (via Methionine deprivation) or blocking SAH breakdown (via inhibition of SAH hydrolase by using 3-deazaadenosine or DZA) causes a decrease in methyltransferase activity, and may provide a novel treatment for AML by diminishing cellular methylation.
Upcoming studies: chip profiling of known leukemogenic genes for both cell lines and co-culture & stimulation experiments to further study effects on differentiated HSPCs

Acknowledgements

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