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Topic: *AS04-MDS Biology and Pathogenesis/AS04f-Gene expression profiling*

THE COMBINATION OF A RAS SIGNALING MODULATOR WITH AZACITIDINE IMPROVES HEMATOPOIESIS IN VIVO AND HAS IN VITRO EFFECTS ON METABOLIC/DIFFERENTIATION PATHWAYS AND INNATE IMMUNE SIGNALING

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Background and Aims: Although Azacitidine (AZA) is the mainstay treatment for higher-risk MDS patients, almost all patients become resistant to treatment. The addition of a novel Ras mimetic that inhibits Ras/Raf signaling, Rigosertib (RIGO), yields a response rate of 54% of HMA failures and results in significant improvement in hematopoiesis overcoming the epigenetic clinical resistance phenotype but the mechanism is still elusive.
Methods: We investigated the protein expression in response to different treatment (AZA, RIGO and sequential combinations (SC) RIGO/AZA) *in vitro* in MDS-L cell line by protein-array and further performed functional and immunophenotyping assays.
Results: We previously demonstrated that RIGO/AZA upregulates RIG-I, Wnt/B-catenin and hematopoiesis signaling pathways. In this study we report downregulation of PIK3R1, AKT1, mTOR, p38 MAPK, PTEN, RPS6KA1 and upregulation of mitochondrial and oxidative phosphorylation related gene in MDS-L cells treated with RIGO/AZA. Additionally, we found that AZA increases the percentage of CD34+CD38+ cells, indicative of differentiation, whereas RIGO alone increased the percentage of CD34+CD38- cells, representing a primitive stem cell population (PSCP). RIGO alone, and RIGO/AZA SC, impacts different progenitors. Moreover, we found a remarkable reduction in colony forming unit number in response to RIGO (83%) and RIGO/AZA (90%).
Conclusions: Altogether results indicate RIGO appears to promote maintenance of a PSCP, while the RIGO/AZA SC appears to push the cells toward a cycling stage with increased expression of genes associated with OXPHOS. In comparison, when treated with RIGO, cells remain in a less differentiated stage. Studies are underway to determine the linkage of these pathways with hematopoiesis and the immune landscape.

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Topic: *AS04-MDS Biology and Pathogenesis/AS04f-Gene expression profiling*

STUDY OF ANGIOGENESIS GENE EXPRESSION AND BIOMARKERS IN MYELODYSPLASTIC SYNDROME AND CYTOPENIAS

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Background and Aims: The microenvironment of bone marrow can influence disease profile in Myelodysplastic Syndrome (MDS). Aim: to evaluate the expression of the genes and cytokines in patients with MDS, Idiopathic Cytopenia of Undetermined Significance (ICUS), Clonal Cytopenia of Undetermined Significance (CCUS) and Cytopenia Non-Neoplastic Cause (CNN).
Methods: Analysis were performed by RT PCR for gene expression e and enzimaimmunoassay for bone marrow plasma biomarkers.
Results: 72 patients was enrolled: MDS (n = 34), ICUS/CCUS (n = 31) and CNN (n = 7) HIF1-α gene was more expressed in CNN group (p = 0.019). IFNγ levels were also higher in CNN group (p = 0.017). Patients with fibrosis had higher levels of TNFα (p = 0.031) and IL-6 (p = 0.001). In MDS group, patients with advanced disease presented higher concentrations of TNFα (p = 0.045). IFNγ was higher in the initial stages (p = 0.003). There was a positive correlation between VEGFA and HIF1α gene (R = 0.834; p < 0.001); HIF1α and TP53 gene (R = 0.595; p < 0.001); VEGFA and TP53 gene (R = 0.551; p < 0.001); HIF1α gene and IFN cytokine (R = 0.628; p < 0.001); HIF1α gene and VEGFA cytokine (R = 0.502; p < 0.001) for all groups. In MDS group the correlation was bettween VEGFA and HIF1α genes (R = 0.685; p < 0.001).
Conclusions: Patients from CNN presented higher gene expression and cytokines levels. The reduced levels of IFNγ in the most advanced stage of SMD (RAEB-1 and RAEB-2) may suggest a deficient or insufficient production of functionally competent NK cells, which contributes to the progression of the disease through immune escape.

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Topic: *AS04-MDS Biology and Pathogenesis/AS04g-Epigenetic deregulation*

2-HG ENANTIOMERS IN MDS CLINICAL COURSE AND PROGNOSIS

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Background and Aims: An untargeted metabolomic analysis performed on the maturing myeloid lineage from MDS patient, unraveled two distinct metabolic groups depending on bone marrow (BM) blast percentage. Apart from their overall differences the two metabotypes featured pronounced discrepancies in redox potential and mitochondrial (dys) function while sharing increased 2-Hydroxyglutarate (2-HG) levels. Given the distinct origins and properties of the 2-HG enantiomers herein, we aim to shed light on the link of D-2-HG and/or L-2-HG levels with MDS metabotypes and gain clinical insights.
Methods: Isolated bone marrow differentiating myeloid lineage (>95% purity, <1% blast contamination) from MDS patients with <5% (No.11) and >5% (No.4) intra-BM blasts and aged matched controls (No.7) were subjected to untargeted mass spectrometry-based metabolomics analysis. D-2-HG and L-2-HG levels were thereafter differentially determined.
Results: Both groups shared increased 2-HG levels. Metabolomic analysis of all samples featured a strong link between 2-HG levels, both enantiomers, and mitochondrial Complex IV abnormal function. D-2-HG has a known Complex IV inhibitory action while L-2-HG is supposed to act as a source of cellular reducing equivalents. Accordingly, the <5% intra BM blast group showed dominant mitochondrial dysfunction compatible with the D-2-HG inhibitory action on complex IV while the >5% group presented improved redox and respiratory potential despite the phenomenal electrontransport chain blockage both compatible to a primarily L-2-HG driven profile.
Conclusions: Our findings underscore an etiologic relationship between the 2-HG enantiomer, mitochondrial (dys)function and redox state while justifying further research on this oncometabolite as a prognostic MDS biomarker.

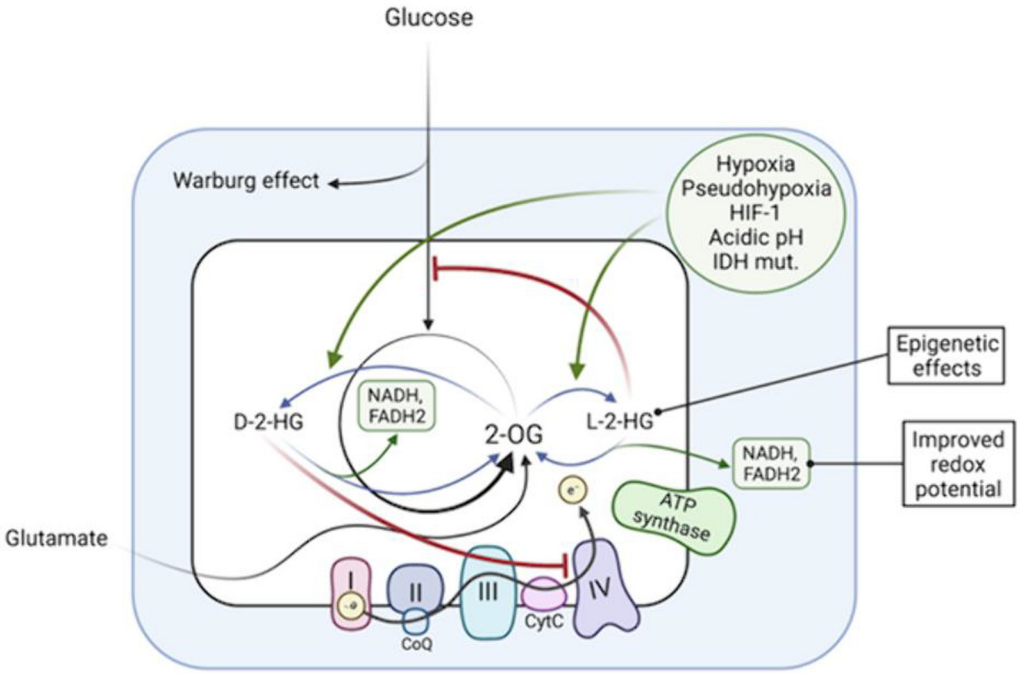

Figure: (abstract: P28)

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Topic: *AS04-MDS Biology and Pathogenesis/AS04g-Epigenetic deregulation*

MECHANISMS OF RESISTANCE TO THE HYPOMETHYLATING AGENT AZACITIDINE IN MYELODYSPLASTIC SYNDROMES: FOCUS ON METHYLATION

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Background and Aims: Hypomethylating agents (HMA) are standard of care for Myelodysplastic syndromes (MDS) but, still only 50% of patients have a clinical response and all of them will lose response. In this scenario, we aimed to investigate how and why some patients are primary resistant to azacitidine (AZA) therapy while others initially respond and then relapse.
Methods: We evaluated 22 cases of high-risk MDS, treated with AZA (75 mg/m^2/d for 7 days every 28 days). Bone marrow aspirates were collected before and after treatment with the drug. For some cases, samples at baseline, at remission and at relapse were available. DNA methylation in CD34+ cells were investigated by ERRBS.
Results: Complete methylation analysis is available for three sets of paired samples. 25·538 (Baseline vs Post treatment), 4·010 (Post treatment vs relapse), 127 (Baseline vs relapse) differentially methylated regions (DMRs) were identified. The majority of DMRs localize in intergenic and intronic regions. After treatment with AZA, global genome DNA methylation decreased due to the widespread hypomethylating effect of the drug, while at relapse methylation increased in specific genomic regions. Some DMRs gained methylation at relapse and were different from the baseline ones indicating a reprogramming of methylation in CD34+ cells. Gene Ontology annotation of DMR-related genes reveals an enrichment in biological process related to neutrophil and granulocyte pathways.
Conclusions: This finding is important because for the first time a methylation analysis has identified in relapsed cases that loss of response to AZA could be caused by emerging reprogrammed clones.

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Topic: *AS04-MDS Biology and Pathogenesis/AS04h-Immune deregulation*

RELATION BETWEEN INMUNE CELL POPULATIONS AND MALIGNANT CLONE MUTATIONAL STATUS IN MYELODYSPLASTIC SYNDROMES

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Background and Aims: The role of immune dysregulations and somatic gene mutations are known prognostics factors in myelodysplastic syndromes (MDS). However, the importance of the interaction between them in the disease course is not entirely understood. This study aimed to characterize how the microenvironment regulates the malignant clone advantage.
Methods: We prospectively studied 40 MDS patients, 12 idiopathic cytopenia of unknown significance (ICUS), and 4 healthy donors (HD). We evaluated in bone marrow, by flow cytometry the following populations: natural killers (NK) (CD3CD56+CD16+/CD56+CD16-/CD56-CD16+); myeloid-derived suppressor cells (MDSC), granulocytic (Gr-MDSC) (CD11b$^+$CD33$^+$HLA-DR$^-$CD15$^+$CD14$^-$) and monocytic (Mo-MDSC) (CD11b$^+$CD33$^+$HLA-DR$^-$CD15$^+$CD14$^+$). T cells subpopulations were studied in peripheral blood (CD3/CD4/CD8/CCR7/CD45RA/CD27/CD28/CD279/