Article

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Integrated Multi-Cohort Analysis of Whole-Genome and Transcriptome Data Reveals Interplay Between Metabolism and Methylation in Myelodysplastic Syndrome

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Background: The metabolic and methylation interactions among mutated genes in myelodysplastic syndrome (MDS) represent promising avenues for novel anticancer therapies. This study investigates the mutational and transcriptomic landscapes of MDS to identify hallmark gene mutations, deregulated pathways, and their implications for disease pathogenesis and therapeutic strategies.

Methods: A retrospective, cross-sectional analysis was conducted using mutational data from the cBioPortal for Cancer Genomics (UTokyo, Tokyo, Japan; Nature 2011) and multicenter MDS cohorts (Wellcome Trust Sanger Institute, Hinxton, UK; 2020). Transcriptomic data were sourced from three publicly accessible datasets in the Gene Expression Omnibus database: GSE114922 (Wellcome Trust Centre for Human Genetics, Oxford, UK; 2018), GSE63569 (University of Oxford, Oxford, UK; 2014), and GSE183328 (National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland; 2022). Integrated mutational and transcriptomic data analyses were performed to uncover connections between genetic alterations and metabolic deregulation.

Results: We identified a set of genes harboring mutations that form mutually exclusive modules, potentially driving alterations in cellular metabolism and DNA methylation in patients with MDS. Transcriptomic analyses revealed significant upregulation of these mutated genes, implicating them in disease pathogenesis. Pathway enrichment analysis further elucidated dysregulation in key metabolic processes, including oxidative phosphorylation (OXPHOS), glycolysis, and epigenetic regulation via DNA methylation. These findings highlight the molecular heterogeneity of MDS and its intricate interplay with metabolic and epigenetic networks. Moreover, risk stratification models incorporating DNA methyltransferase 3 alpha (*DNMT3A*) and tet methylcytosine dioxygenase 2 (*TET2*) mutations demonstrated robust predictive value for overall survival, reinforcing their clinical relevance and prognostic utility in oncological contexts.

Conclusions: This study offers novel insights into the molecular, metabolic, and DNA methylation mechanisms driving MDS. Integrating mutational signatures with transcriptomic data reveals potential therapeutic targets within key metabolic and DNA methylation pathways. These findings lay the foundation for developing personalized treatment strategies and refining risk stratification models for MDS and related malignancies.

Keywords: MDS; metabolism; DNA methylation; *DNMT3A*; *TET2*

Introduction

Myelodysplastic syndrome (MDS) is caused by a defect in hematopoietic stem cells (HSCs) and increases the likelihood that individuals will develop diverse, undifferentiated hematologic myeloid malignancies [1]. MDS is primarily diagnosed in individuals aged over 65 years, accounting for less than 5% of childhood cancers [2]. While MDS is rare, affecting 40–50 of every 100,000 individuals aged under 70 years—emphasizing its higher frequency in older age groups—its age-standardized incidence ranges from 1.3 to 4.3 per 100,000 person-years and increases steadily with age [3,4]. Recent research suggests that 80%–90% of individuals with MDS exhibit recurrent alterations in multiple genes [5]. Therefore, investigating genetic changes that engender leukemia and lymphoma is crucial to improving disease outcome predictions. Advances in next-generation sequencing (NGS) have deepened our understanding of how genetic changes contribute to dysfunctional blood formation and the prognosis of patients with MDS. For example, MDS often features primary and secondary genetic changes, with around 1500 mutations identified throughout the genome [6]. These genetic aberrations result in complex interactions that may affect survival in certain MDS cases. Identifying these changes can guide the development of tailored treatment approaches for patients with MDS.

The mutations that initiate MDS in HSCs are shaped by clinical features, cellular factors, and genetic makeup [7]. For example, the French–American–British system from 1982 labeled MDS "refractory anemia" and divided it into five types according to cellular morphology and the myeloid blast count [8]. This system predominated for about 20 years. However, the generic divisions of the initial system led the World Health Organization (WHO) to revamp it and emphasize the crucial role of genetic mutations in diagnosing it [9]. In 2001, the WHO presented its first categorization based on HSC mutations that initiate MDS [10]. The ensuing updates from 2008 to 2016 enriched this framework by incorporating the clinical, morphological, immunophenotypic, and genetic aspects of MDS [10]. The WHO further refined the MDS classifications in 2022. The current classifications are based on genetic variations and morphological features, emphasizing genetic disruptions of clonal hematopoiesis. This detailed system supports accurate diagnosis and risk assessment, allowing personalized treatment approaches [7].

One hallmark of cancer is a change in metabolism, which is crucial for cellular processes that instigate carcinogenesis [11]. The foundation of hematopoiesis, HSCs orchestrate various metabolic needs and states when they mature into advanced myeloid and lymphoid cells [3]. HSCs' metabolic versatility is evident in their high energy demand during growth and maturation, allowing them to transition from a glycolysis-focused to mitochondria-focused metabolism [12]. In the 1920s, Warburg [13] identified tumor tissues that exhibited increased aerobic glycolysis and accelerated lactate discharge. This finding, which suggests information about glucose absorption, lactate emission, and oxygen levels, is also pertinent to MDS [3,13].

Conversely, epigenetic regulators represent the most frequently mutated set of genes in patients with MDS. These genetic alterations compromise cellular function, leading to a loss-of-function phenotype in affected cells [14]. Consequently, transcriptional regulation is disrupted, often impairing the inhibition of differentiation pathways while promoting self-renewal mechanisms. DNA hypermethylation, a hallmark of MDS, is also closely associated with poor prognosis in affected patients [15]. Therefore, a novel therapeutic approach for MDS involves identifying metabolic genes and methylation markers that offer predictive value. The complex interactions between these altered genes provide critical insights into their potential role in preventing cancer through regulating metabolism and methylation processes.

To our knowledge, no clinical trials have been specifically designed to target patients with MDS with mutually exclusive mutations. For example, MDS cases have demonstrated synthetic lethality in patients with tet methylcytosine dioxygenase 2 (*TET2*) mutations [16]. In these cases, *TET2*-deficient cells exhibit mutual exclusivity with mutations in isocitrate dehydrogenases ($NADP^+$) 1 (*IDH1*) and 2 (*IDH2*) [17–19]. Therefore, this mutual exclusivity indicates that both metabolic and methylation-related mutated genes might play a role in MDS pathogenesis, and cells cannot tolerate mutations in both pathways simultaneously, suggesting potential functional interactions in MDS pathogenesis.

In this integrated multidisciplinary study, we explored the mutational burdens and transcriptomic profiles of patients with MDS to evaluate the significance of genes and hyperactive molecular pathways associated with MDS metabolism. We identified numerous mutated genes that form modules of mutually exclusive mutations, potentially amplifying cellular metabolism and/or DNA methylation in patients with MDS. By integrating gene mutation data with gene expression profiles, we uncovered metabolism-associated network genes linked to DNA methylation in MDS, offering valuable insights into its underlying biology. Our findings highlight the critical role of metabolic pathways in driving epigenetic modifications, emphasizing their contribution to MDS progression through the interplay of metabolic and methylation dysregulation. Therefore, this understanding paves the way for innovative therapeutic strategies, including developing inhibitors targeting metabolism- and methylation-related mutations.

Materials and Methods

Patient Cohorts

This retrospective cross-sectional study used clinical, mutational, and transcriptomic data for patients with MDS from various datasets and studies obtained from the cBioPortal for Cancer Genomics (cBioPortal henceforth) and Gene Expression Omnibus (GEO) database [20–25]. Genomic mutation data were obtained from cBioPortal, a widely used and extensively curated platform for exploring cancer genomics. Mutation datasets hosted on the cBioPortal undergo standard quality control, normalization, and batch correction before public release. These processes have been validated in previous studies [24,25], supporting the reliability and harmonization of the integrated mutation data.

The NGS-based genomic data used to evaluate changes in potentially relevant genes comprised 4260 patients with MDS in the cBioPortal datasets myelodysplasia (UTokyo, Tokyo, Japan; Nature 2011), reported by a research group at the University of Tokyo, Japan, in 2011, and myelodysplastic (Memorial Sloan Kettering Cancer Center (MSK), New York City, NY, USA; 2020), reported in 2020 by a multinational multicenter study led by the Wellcome Trust Sanger Institute, Hinxton, UK (ClinicalTrials.gov ID NCT00146120) [26–29]. The transcriptomic data were obtained from three independent cohorts in the GEO database: GSE114922 (Wellcome Trust Centre for Human Genetics, Oxford, UK), GSE63569 (University of Oxford, Oxford, UK), and GSE183328 (National Institute for Bioprocessing Research and Training (NIBRT), Dublin, Ireland), made

public in 2018, 2014, and 2022, respectively [20–23,30]. All obtained data reported on the clinical significance of mutations in MDS.

Data Sources and Preprocessing

We analyzed data for 4260 patients with MDS using the cBioPortal platform, where the original sources usually handle batch effects before data deposition. This comprehensive analysis allowed us to identify and examine mutation patterns in this patient group, ensuring that the data were normalized and harmonized across cohorts [24–29].

For the next-generation RNA sequencing (RNA-seq) meta-analysis, we selected three independent cohorts with raw data published in the GEO database and implemented a rigorous preprocessing pipeline. We selected only RNA-seq data from the same biological source (CD34 molecule [CD34]$^+$ HSCs) and technical sequencing platform (Illumina). We retrieved raw RNA-seq datasets for three independent MDS cohorts from the GEO database: GSE114922, GSE63569, and GSE183328. The raw FAST Quality (FASTQ) files were aligned to the Genome Reference Consortium Human Build 38 (GRCh38) (Human Genome version 38 (hg38)) human reference genome using the STAR aligner (v2.7.10a). Polymerase Chain Reaction (PCR) duplicates were marked and removed using Picard (v2.27.1, Broad Institute of MIT and Harvard, Cambridge, MA, USA). Reads were filtered based on mapping quality, retaining only those with a Mapping Quality (MAPQ) score >30 to ensure high-confidence quantification. Gene expression count matrices were then generated using featureCounts (v2.0.3, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia) [31] with a gene annotation Gene Transfer Format (GTF) file downloaded from GENe annotation of the human and mouse genomes (GENCODE) release 44 (GRCh38.p14).

To address batch effects across the three independent cohorts, we used DESeq2 (v1.46.0) in the R statistical software (v4.2.2), incorporating batch as a covariate in the design formula (design = ~batch + condition). Widely accepted in transcriptomic studies, this approach effectively removes technical variation while preserving biological differences [31,32]. These preprocessing steps established a uniform and high-quality dataset for statistical and pathway-level analyses.

Statistical Analysis

We conducted multiple bioinformatics analyses to explore genetic and transcriptomic alterations. Initially, we utilized the cBioPortal to generate a mutual exclusivity table, identifying mutually exclusive mutations across different genes, using Fisher's exact test to determine their statistical significance [24]. We also used data from the Oncology Knowledge Base (OncoKB) database to investigate the clinical relevance of mutations in the DNA methyltransferase 3 alpha (*DNMT3A*) and *TET2* genes, as it is actively maintained and continuously updated with data from large-scale genomic studies, clinical trials, and U.S. Food and Drug Administration (FDA)-approved therapies; thus, it is the most reliable sources for interpreting the oncogenic potential and therapeutic relevance of mutations [33,34].

All statistical analyses were conducted using the R statistical software (v4.2.2) and relevant Bioconductor packages. Differential gene expression analysis was performed using the DESeq2 package (v1.46.0) [32], which applies shrinkage estimators for dispersion and fold-change to improve accuracy and statistical power. Genes with an adjusted p-value (Benjamini–Hochberg method) of <0.05 and an absolute $\log_2$ fold change of ≥ 1 were considered significantly differentially expressed.

We explored the enrichment of metabolic and epigenetic pathways using gene set enrichment analysis (GSEA) with the fgsea package (v1.24.0), utilizing pre-ranked gene lists based on $\log_2$ fold change [35]. Curated gene sets from MSigDB (v2023.1.Hs), including the Kyoto Encyclopedia of Genes and Genomes, Reactome, and Hallmark collections, were used as references. Significance was determined based on a normalized enrichment score with an adjusted p-value of <0.05. Correlations between gene expression levels and pathway scores (e.g., oxidative phosphorylation (OXPHOS), glycolysis, and one-carbon metabolism) were assessed using Spearman's rank correlation coefficient and visualized using the ggpubr package (v0.6.0). Where applicable, mutational enrichment and co-occurrence/mutual exclusivity tests were performed using the cbioportalData and maftools (v2.12.0) packages. Fisher's exact test was used for binary mutation comparisons, and the Benjamini–Hochberg method was used to adjust for multiple comparisons.

Finally, we used the Gene Expression Profiling Interactive Analysis (GEPIA) web platform (Peking University Cancer Hospital & Institute, Beijing, China) to examine the impact of *DNMT3A* and *TET2* gene expression levels on overall survival across multiple independent cohorts from the Cancer Genome Atlas (TCGA) and GTEx Portal for expression-based survival modeling, as cancer cells are hallmarked by metabolic reprogramming and epigenetic changes [36–38]. The log-rank (Mantel–Cox) test was used to assess the significance of differences between the high- and low-expression groups for *DNMT3A* and *TET2* with a median cutoff. Kaplan–Meier plots were generated to visualize survival curves, and hazard ratios were computed with 95% confidence intervals. Heatmaps and hierarchical clustering plots were generated using the ComplexHeatmap package (v2.14.0), and principal component analysis was performed using the PCAtools package (v2.10.0) to assess batch effect correction and biological clustering. All plots were generated using the ggplot2 package (v3.4.0).

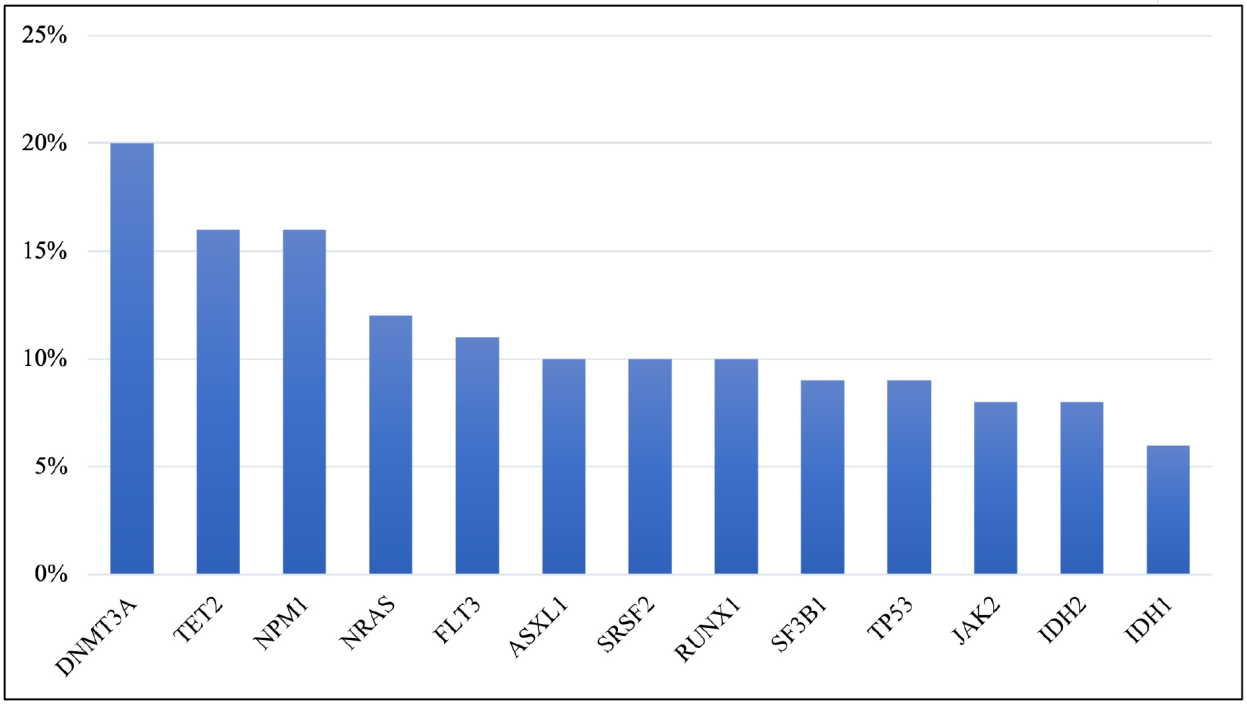

Fig. 1. Percentage of mutated genes in patients with MDS. The most mutated genes among the 4260 patients with MDS were obtained from various datasets and published studies. *DNMT3A*, DNA methyltransferase 3 alpha; *TET2*, tet methylcytosine dioxygenase 2; *NPM1*, nucleophosmin 1; *NRAS*, Neuroblastoma RAS viral oncogene homolog; *FLT3*, Fms-like tyrosine kinase 3; *ASXL1*, ASXL transcriptional regulator 1; *SRSF2*, serine- and arginine-rich splicing factor 2; *RUNX1*, RUNX family transcription factor 1; *SF3B1*, splicing factor 3b subunit 1; *TP53*, tumor protein p53; *JAK2*, Janus kinase 2; *IDH2*, isocitrate dehydrogenases ($NADP^+$) 2; *IDH1*, isocitrate dehydrogenases ($NADP^+$) 1; MDS, myelodysplastic syndrome.

Results

Prevalence and Significance of DNMT3A and TET2 Mutations in MDS

Numerous studies have highlighted biomarkers, especially DNA methylation and mutations in genes such as *IDH1* and *IDH2*, as fundamental indicators of MDS [17,18]. Such genetic indicators can modulate patients' reactions to chemotherapy drugs, including decitabine [39]. Given the complex nature of MDS, recognizing that our comprehension of the pathways through which these mutated genes act remains limited is vital. Our meta-study systematically integrated various genes to reveal the relevant pathways in MDS. Using the cBioPortal, we analyzed data for 4260 patients with MDS drawn from diverse datasets and articles. This analysis highlighted genes with common mutations, including *TET2*, *DNMT3A*, ASXL transcriptional regulator 1 (*ASXL1*), splicing factor 3b subunit 1 (*SF3B1*), serine- and arginine-rich splicing factor 2 (*SRSF2*), RUNX family transcription factor 1 (*RUNX1*), and nucleophosmin 1 (*NPM1*; Fig. 1) [16,24–29,40–45].

Interestingly, *DNMT3A* and *TET2* were among the most mutated genes in patients with MDS (Fig. 1), suggesting a potential interplay between metabolism and DNA methylation. In addition, mutations in *DNMT3A* exhibited mutual exclusivity with those in other vital genes (**Supplementary Table 1**). This highly significant mutual exclusivity suggests that these genes contribute to or manage a common biological function, such as metabolism or DNA methylation. Specifically, these mutations are less likely to co-occur in the same patient (i.e., a potential functional interplay between these genes). Moreover, mutations in *DNMT3A* and *TET2* are well-known drivers of epigenetic dysregulation in MDS. However, their downstream impact on metabolic pathways, such as glycolysis and OXPHOS, remains poorly understood. Further research is needed to link these mutations to specific biological outcomes in disease progression.

As emphasized in **Supplementary Table 1**, there is also mutual exclusivity among patients with MDS, where *DNMT3A* is linked to DNA methylation, *SRSF2* is linked to RNA splicing, *ASXL1* is linked to chromatin modification, Janus kinase 2 (*JAK2*) gene is essential in signal processing, and tumor protein p53 (*TP53*) is fundamental to both tumor inhibition and transcription factor function. Indeed, except for *JAK2*, all these genes correlate with an unfavorable prognosis [7]. Therefore, mutations in genes associated with MDS exhibit mutual exclusivity, suggesting that their simultaneous occurrence could disrupt cellular systems, including metabolism or DNA methylation, to a degree that cells cannot tolerate.

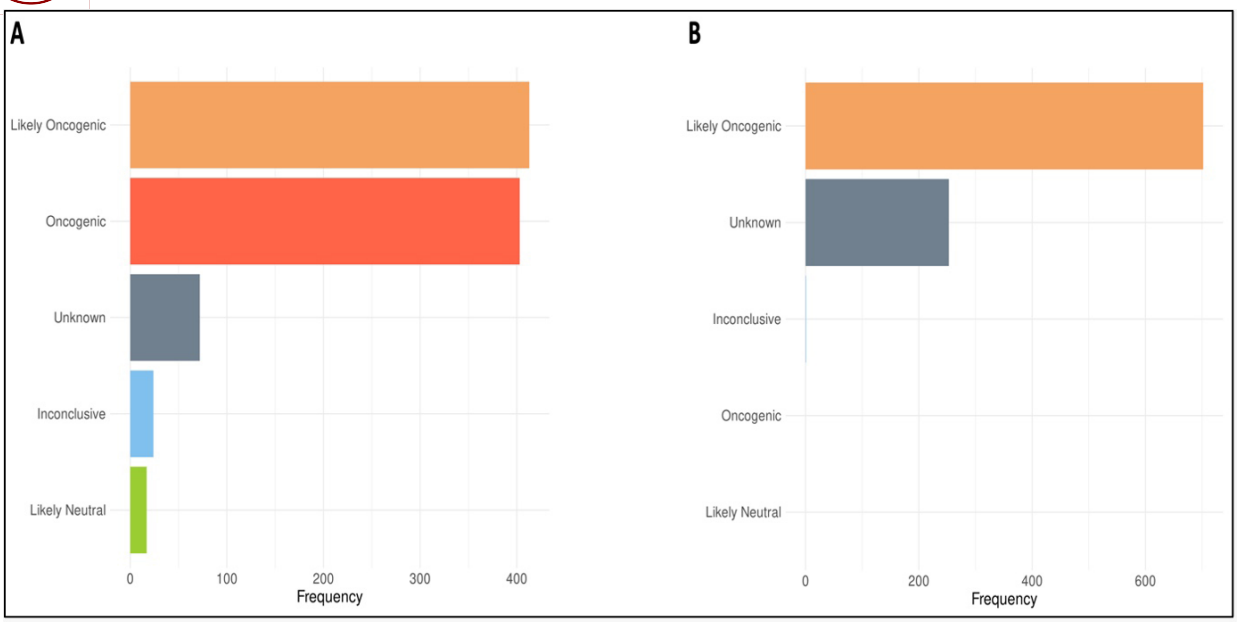

Fig. 2. The role of *DNMT3A* and *TET2* mutations in MDS tumorigenesis. The bar charts show the classification and frequency of (A) *DNMT3A* and (B) *TET2* mutations in the OncoKB database.

The high prevalence of mutations in *DNMT3A* and *TET2* in MDS (Fig. 1) underscores the importance of further investigating their functional impact. Mutations in these genes have also been linked to potential sensitivity to epigenetic therapies, such as hypomethylating agents (HMAs; e.g., azacitidine) [46]; therefore, assessing how the mutation status of these genes can stratify patients or predict therapeutic outcomes offers a pathway to optimizing MDS treatment strategies. Consequently, to gain deeper insights into the clinical implications of these mutations, we extended our analysis to characterize the specific mutation types using data from the OncoKB database. Fig. 2 highlights the gene variant categories with the highest mutation frequencies, revealing that many mutations in *DNMT3A* and *TET2* are associated with oncogenic activity. This observation suggests that the aberrant activity of these genes may contribute significantly to MDS progression. However, it is important to note that not all mutations in *DNMT3A* and *TET2* exhibit clinically relevant effects, emphasizing the need for further research to delineate their precise roles and therapeutic implications in MDS.

Next, we integrated transcriptomic data for additional MDS cohorts to evaluate the effects of *DNMT3A* and *TET2* at the gene expression level, analyzing data from the independent MDS cohorts GSE114922, GSE63569, and GSE183328. This integrative approach helps support mutational findings in Figs. 1,2.

Transcriptomic Analysis Reveals the Heterogeneity of MDS in Diverse Metabolic Pathways

Our findings indicate that *DNMT3A* and *TET2* are among the most frequently mutated genes in MDS. These genes are key regulators of DNA methylation, which interacts with metabolic reprogramming pathways and plays a crucial role in tumorigenesis. This observation highlights the close relationship between metabolic and epigenetic changes in cancer development. To investigate this further, we analyzed transcriptomic profiles associated with key metabolic pathways, focusing on OXPHOS, glycolysis, and DNA methylation. Interestingly, a strong correlation emerged between transcriptional activity and these metabolic pathways, particularly glycolysis, which supplies essential substrates for *DNMT3A* and *TET2* to facilitate DNA methylation regulation [47,48]. These results highlight the interconnected nature of metabolic and epigenetic mechanisms in MDS, emphasizing the need to explore this relationship to uncover potential therapeutic targets.

Our analysis revealed pronounced transcriptomic heterogeneity within key metabolic pathways across MDS patient samples. As illustrated in Fig. 3, **Supplementary Figs. 1,2**, clustering based on metabolic gene expression profiles identified distinct subgroups, which may correlate with specific genetic mutations. These pathways displayed transcriptomic profiles that differentiated patients with MDS from healthy controls, with only a few exceptions. This distinction was marked by a significant increase in metabolic pathway activity, potentially driven by gain-of-function mutations in key genes. These findings suggest that affected cells exhibit a dysregulated metabolic landscape tightly linked to genetic alterations in genes regulating DNA methylation. This association highlights a possible dependency of affected cells on these altered metabolic pathways for survival. This dependency could represent a therapeutic vulnerability, offering a basis for the development of targeted interventions aimed at disrupting these critical metabolic mechanisms.

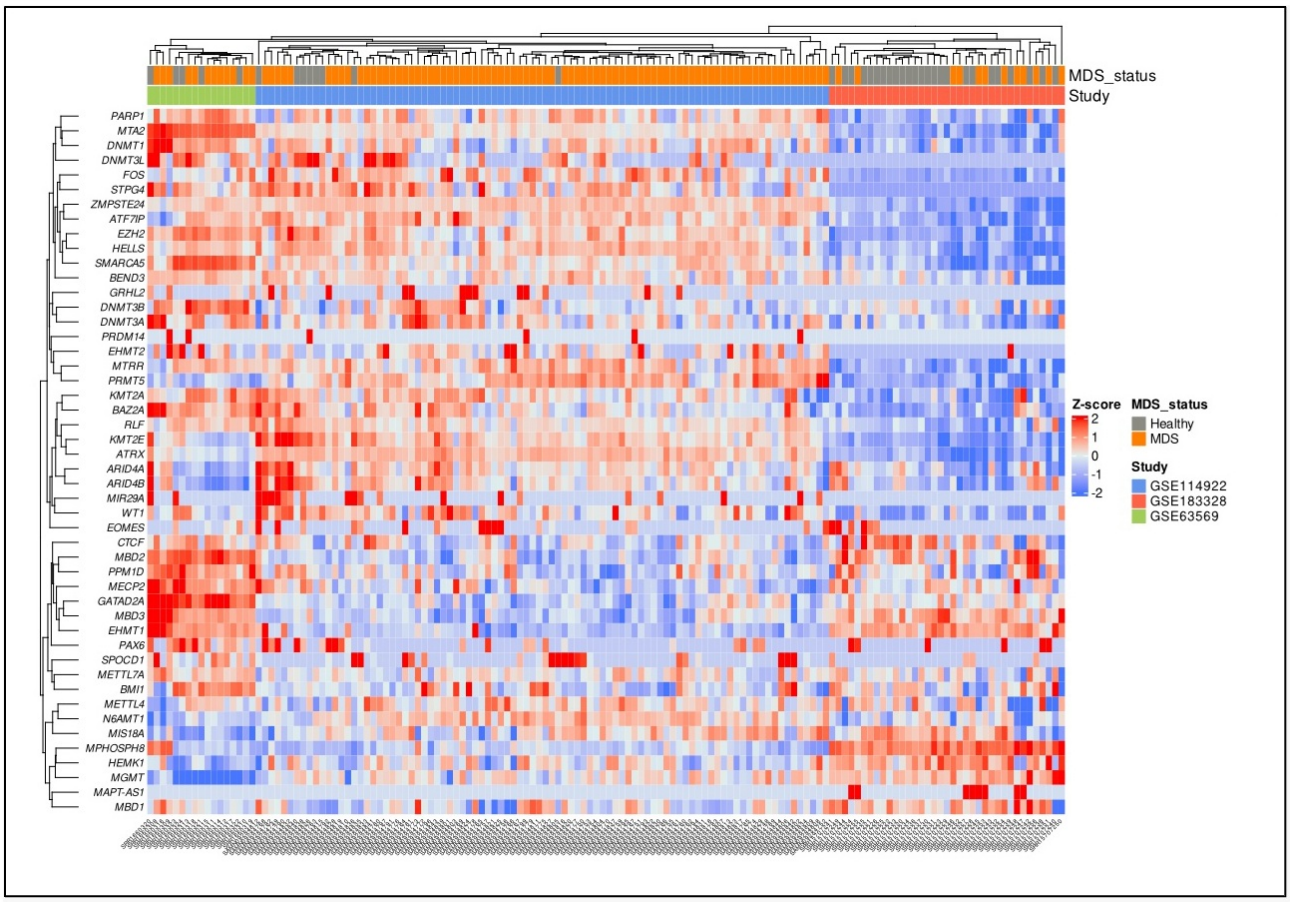

Fig. 3. Identified MDS vs. healthy individuals' gene sets of DNA methylation pathway among independent multiple MDS cohorts. The heatmap shows the discrimination of patients with MDS vs. healthy individuals based on the topmost enriched gene sets in the DNA methylation pathway.

To further understand the gene expression profiles of *DNMT3A* and *TET2*, we analyzed their expression across three MDS cohorts. Despite the observed transcriptomic heterogeneity, *DNMT3A* and *TET2* were significantly upregulated across all cohorts (Fig. 4), consistent with their high mutation frequency (Figs. 1,2). Specifically, *DNMT3A*-mutated samples exhibited markedly higher expression of glycolysis-related genes, pointing to a potential metabolic reprogramming driven by this mutation. These findings suggest that gain-of-function mutations in *DNMT3A* and *TET2* might drive increased gene expression activity, driving disease pathogenesis. Furthermore, the differential expression patterns observed in Fig. 4 suggest that patients with *TET2* mutations may undergo metabolic reprogramming, consistent with existing literature indicating that *TET2* mutations drive a metabolic shift toward mitochondrial respiration [19,49,50], thereby identifying mitochondrial inhibitors as potential therapeutic targets.

Altogether, *DNMT3A* and *TET2* mutations in MDS play a crucial role in tumorigenesis, suggesting a consistent pattern of interplay between metabolic needs, DNA methylation pathways, and regulatory mechanisms in affected cells. Additionally, metabolic gene expression profile clustering emerges as a promising method for stratifying patients with MDS based on metabolic vulnerabilities, potentially informing personalized therapeutic approaches.

Our comprehensive analysis pooled independent RNA-seq datasets from various MDS studies, increasing the sample size and enhancing its statistical power and our ability to identify significant and rare differentially expressed genes in MDS. This approach helped identify novel differentially expressed genes by contrasting the transcriptomes of healthy individuals and patients with MDS. Oxidized low-density lipoprotein receptor 1 (*OLR1*) and adrenoceptor alpha 2B (*ADRA2B*) were among the most characteristically downregulated genes in MDS [51,52]. Notably, erythroferrone (*ERFE*) [53] and sulfotransferase family 4A member 1 (*SULT4A1*) [54] were among the most significantly upregulated genes in affected cells (Fig. 5A), suggesting extensive metabolic programming. The pathway enrichment analysis using the Reactome database revealed that, consistent with our mutational analysis, the DNA methylation pathway was enriched (Fig. 5B,C), suggesting that it plays a key role in gene regulation and epigenetic modifications.

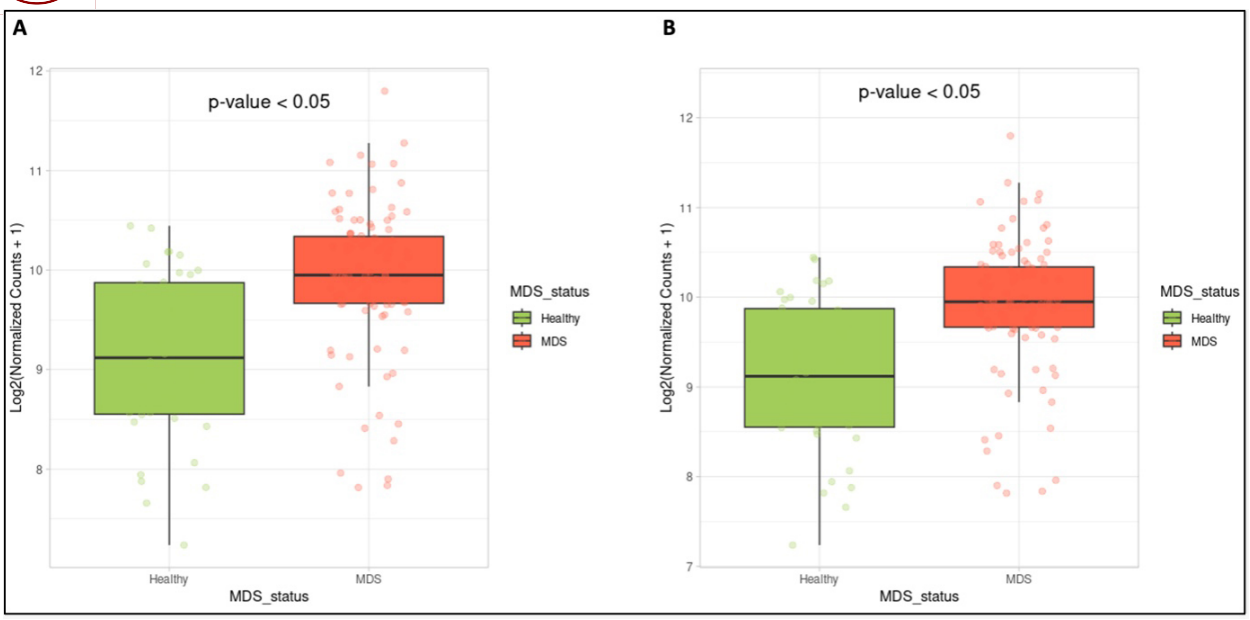

Fig. 4. The gene expression landscape of the *DNMT3A* and *TET2* genes among independent multiple MDS cohorts. The box plots show the upregulation of (A) *DNMT3A* and (B) *TET2* in patients with MDS compared to healthy individuals.

To support this notion, Fig. 5D shows that *ERFE* and *SULT4A1* are novel upregulated genes in the metabolic process, identified by the gene ontology term GO:0008152 [55,56]. ERFE is known to positively regulate glucose imports and fatty acid metabolism, and SULT4A1 participates in mitochondrial biological processes and steroid metabolism [55,56]. Therefore, these results suggest that changes in the DNA methylation landscape may be associated with alterations in the metabolic pathways in MDS. These findings suggest a relationship between increased gene expression activity and mutations in *DNMT3A* and *TET2*, which reshape the MDS metabolome and potentially the epigenetic landscape.

Clinical Implications of Mutational Signatures in MDS

Our study underscores the therapeutic potential of addressing the most commonly mutated genes found in 4260 patients with MDS obtained from diverse published datasets. The specific genes in Fig. 4 could disrupt vital signaling pathways and molecular functions, including metabolic and DNA methylation pathways, that play central roles in the well-being and growth of patients with MDS in parallel with current clinical trials targeting MDS biological pathways (**Supplementary Table 2**). Notably, mutations in *DNMT3A* accounted for a significant fraction (up to 18%) of the mutations found in MDS cases [57], corroborating our extensive multi-cohort findings. Mutated *DNMT3A* is connected to glutathione (GSH) levels that promote chemotherapy resistance. While a definitive association between *DNMT3A* mutations and GSH levels in MDS remains to be determined, GSH plays an essential role as an antioxidant, regulating numerous cell activities [58].

Understanding the effect of *DNMT3A* mutations on metabolic processes in hematologic cancers might clarify the cellular strategies that drive tumor growth, persistence, and chemotherapy resistance. Elevated GSH levels may enhance tumor cells' adaptability and resilience, potentially leading to drug resistance. Therefore, GSH levels might present a potential metabolic weakness in MDS cases with *DNMT3A* mutations. The FDA has sanctioned the use of HMAs to manage acute myeloid leukemia (AML) and MDS [59]. Numerous clinical trials have explored targeted treatments for MDS and myeloid leukemias, such as DNA methyltransferase 1 (DNMT1) inhibitors. Collectively, based on ongoing clinical trials targeting MDS and their biological focuses (**Supplementary Table 2**), we recommend a combination of TET2 and DNMT1 inhibitors as a novel therapeutic regimen for MDS, especially since *TET2* was among the highly mutated genes, accounting for up to 30% of mutations in MDS [60].

Risk Stratification of DNMT3A and TET2 Genes Predicts Overall Survival in Cancer Patients

As we confirmed the interplay between metabolic and DNA methylation represented by *DNMT3A* and *TET2* in patients with MDS, where they were among the highly mutated genes, we next sought to predict patient survival across multiple independent and well-characterized public cohorts based on *DNMT3A* and *TET2* expression. Many malignancies, including MDS, are also characterized by abnormal epigenetic regulation and metabolic changes, and they show promise as targets for cancer treatment [38]. Therefore, we utilized the GEPIA platform to investigate the impact of *DNMT3A* and *TET2* expression on overall survival in multiple independent cohorts [36]. Survival anal-

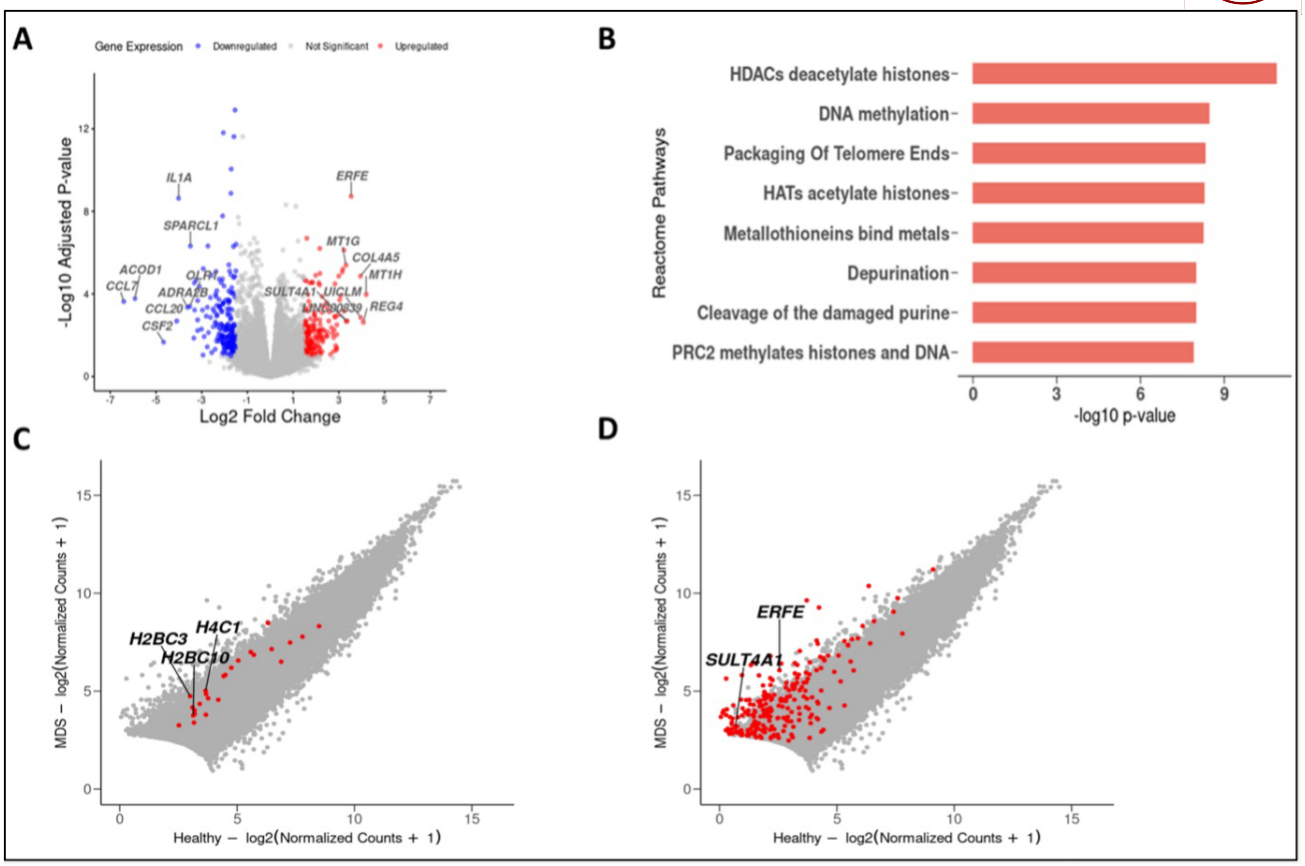

Fig. 5. The gene expression landscapes of patients with MDS vs. healthy individuals. The identified signature genes are involved in DNA methylation and metabolism. (A) Volcano plot showing the gene expression of significantly up- or down-regulated genes in affected cells in MDS. (B) Pathway enrichment analysis using the Reactome database, depicted as a bar plot of the most positively enriched pathways. (C) Scatter plot of gene expression highlighting genes related to DNA methylation (*H4C1*, *H2BC10*, and *H2BC3)* enriched in MDS. (D) Scatter plot of gene expression of metabolism-related genes (*ERFE* and *SULT4A1*) enriched in MDS and inconsistent with the DNA methylation gene set. *ERFE*, erythroferrone; *SULT4A1*, sulfotransferase family 4A member 1.

ysis was conducted to develop prognostic scores across the independent cohorts, utilizing Kaplan–Meier curves.

As illustrated in Fig. 6A–C, *DNMT3A* can significantly predict overall survival for the following cancers: low-grade glioma (LGG), adrenocortical carcinoma (ACC), and mesothelioma (MESO). In patients with LGG, ACC, or MESO with *DNMT3A* mutations, those with high *DNMT3A* expression exhibited significantly worse prognoses than those with low *DNMT3A* expression (Fig. 6A–C). Therefore, these results suggest that the patients with high *DNMT3A* expression have significantly worse prognoses and relative overall survival (<5 years).

Conversely, *TET2* emerged as a significant predictor of overall survival in patients with pheochromocytoma and paraganglioma (PCPG) and thyroid carcinoma (THCA; Fig. 6D,E). Among patients with PCPG and THCA with *TET2* mutations, those with high *TET2* expression exhibited significantly worse prognoses than those with low *TET2* expression. As demonstrated in Fig. 6D,E, patients with high *TET2* expression had significantly shorter overall survival. These findings underscore the potential of *DNMT3A* and *TET2* as critical biomarkers for predicting patient outcomes and guiding therapeutic strategies across various cancers. *DNMT3A* mutations specifically show promise as strong and reliable indicators for risk stratification in patients with MDS. In contrast, the prognostic impact of *TET2* mutations appears more context-dependent, influenced by additional clinical variables.

Our results signal a promising advancement in cancer therapy, particularly for MDS, through integrating these biomarkers into personalized treatment plans, potentially significantly enhancing patient outcomes.

Discussion

Our study examined the genetic mutation patterns and gene expression profiles reported in independent studies on MDS, providing a greater understanding of its biological nature. Specifically, it explored the link between mutations in genes related to MDS metabolism and DNA methylation and the subsequent therapeutic outcomes, drawing on publicly available data from the cBioPortal and the GEO

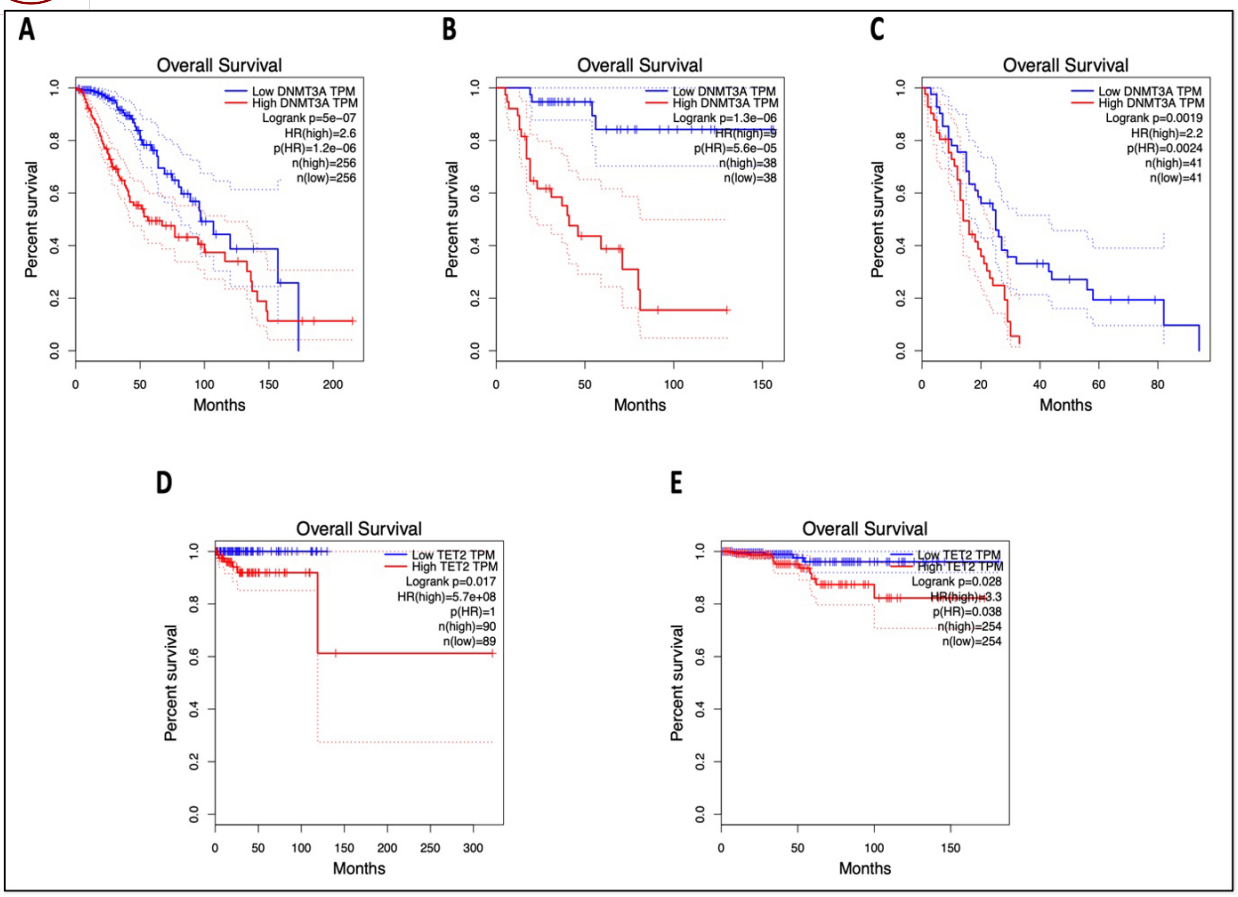

Fig. 6. Prognostic impact of high vs. low expression of *DNMT3A* and *TET2* genes across various independent cohorts. (A) The overall survival plot by Kaplan-Meier analysis shows the difference between the *DNMT3A* high-risk and low-risk patients in LGG. (B) The overall survival plot by Kaplan-Meier analysis shows the difference between the *DNMT3A* high-risk and low-risk patients in ACC. (C) The overall survival plot by Kaplan-Meier analysis shows the difference between the *DNMT3A* high-risk and low-risk patients in MESO. (D) The overall survival plot by Kaplan-Meier analysis shows the difference between the *TET2* high-risk and low-risk patient groups in PCPG. (E) The overall survival plot by Kaplan-Meier analysis shows the difference between the *TET2* high-risk and low-risk patient groups in THCA. The *p*-values were indicated for each cohort. Red represented patients who were classified as high-risk, and blue represented patients classified as low-risk. LGG, low-grade glioma; ACC, adrenocortical carcinoma; MESO, mesothelioma; PCPG, pheochromocytoma and paraganglioma; THCA, thyroid carcinoma.

database. Our analysis found that *DNMT3A* and *TET2* were among the most common genes with somatic mutations in MDS. We further dissected the type of observed mutations, finding that many had oncogenic potential. Therefore, these findings prompted us to explore the transcriptomic landscape of selected MDS cohorts, which showed upregulated *DNMT3A* and *TET2* expression, supporting the MDS mutational data.

DNA methylation is known to influence immune regulation by augmenting tumor immune evasion. Recent studies have shown that alterations in DNA methylation profiles impair immune cell infiltration and function, and MDS is no exception [61]. Our previous study demonstrated upregulated phosphoserine aminotransferase 1 (*PSAT1*) expression, which was associated with rewired immune-related pathways in MDS, leading to poor prognosis [62], emphasizing the heterogeneity of MDS and the importance of our findings. Notably, mutual exclusivity among frequently mutated genes, particularly *DNMT3A* and *TET2*, suggests functional interplay and implies that affected cells might not tolerate both mutations simultaneously, pointing to a possible functional interaction in MDS pathogenesis.

DNM3A encodes an enzyme belonging to a highly conserved family of DNA methyltransferases involved in the 5-methylcytosine (5-mC) methylation reaction. DNA methylation is initiated by DNMT3A and DNA methyltransferase 3 beta (DNMT3B) and then maintained by DNMT1 [63,64]. DNA methylation is an epigenetic phenomenon that impacts X-chromosome inactivation, genomic imprinting, regulation of stem cell differentiation, and disease development and progression [65]. Similarly, *TET2* encodes a member of the ten-eleven translocation (TET) enzyme family, which is impacted by the t(10;11) translocation in infant patients with AML [16]. Indeed, the

TET enzymes are involved in DNA demethylation, where 5-mC is subjected to a hydroxylation reaction to produce 5-hydroxymethylcytosine [66]. Therefore, dysregulated DNA methylation is considered an epigenetic hallmark of cancer.

Given the mutually exclusive nature of *DNMT3A* and *TET2* mutations in MDS [67], it is unclear whether these mutations impact distinct metabolic and methylation pathways, influence the expression of a common set of genes, or lead to distinct gene expression signatures, leading cells to be intolerant to their co-occurrence. Recently, metabolic reprogramming and epigenetic modifications targeting *TET2*'s and *DNMT3A*'s role in regulating DNA methylation have impacted the Wnt signaling pathway, epithelial-mesenchymal transition, and glucose and lipid metabolism, enhancing tumor formation, metastasis, growth, and proliferation, respectively. Therefore, the interaction between these metabolic reprogramming pathways and epigenetic changes is crucial for MDS tumorigenesis [38,68]. In support of this, we observed oncogenic activities associated with *DNMT3A* and *TET2* mutations and extended our investigation to large, independently genotyped MDS cohorts. These findings underscore these mutations' influence on the metabolic landscape and methylation architecture of MDS, suggesting a functional interconnection with other signature genes involved in common biological functions. Moreover, our results imply that the cells of patients with MDS might struggle to accommodate co-mutation mutations based on our mutually exclusive gene list.

Recent advances in MDS treatment underscore the relevance of genetic insights and personalized medicine. Mutations in *TP53* and *SF3B1* are linked to specific disease phenotypes and outcomes, leading to more precise prognostic models and targeted therapies [69]. While many studies have made remarkable efforts to understand the epigenomic landscape, these efforts require insight into the metabolome changes caused by the aberrant *DNMT3A* activity in MDS. Mutation of *DNMT3A* positively impacts malignant cell proliferation and intracellular GSH levels, resulting in chemotherapy resistance [70]. Therefore, it must be noted that the MDS metabolome has increased GSH levels [71], indicating the need for further therapeutic approaches.

In contrast, the impact of *TET2* mutations in the clinical course varies, and dysregulated DNA methylation is a known epigenetic hallmark of cancer. In the MDS context, *TET2* is among the most highly mutated genes, accounting for up to 30% of all mutations [60]. Moreover, a growing body of evidence indicates the involvement of the TET2 enzyme in metabolism. For example, knocking down *TET2* in erythroblasts created a phenotype similar to that seen in MDS, highlighting that *TET2* is indeed a key player in iron/heme metabolism [72]. This connection is further underscored by studies showing that specific MDS subtypes exhibit gene expression profiles strongly linked to heme metabolism, suggesting that metabolic alterations may be a common feature across different mutational backgrounds [73]. In addition, *TET2* deficiency was found to affect lipid metabolism in low-density lipoprotein receptor-deficient mice, accelerating the onset of atherosclerosis [74]. Similarly, *TET2*-deficient hematopoietic cells were associated with metabolic dysfunction in aging mice [75]. Moreover, AMP-activated kinase phosphorylates TET2 at serine 99, and this process is inhibited by high glucose levels, resulting in the dysregulation of both DNA methylation and *TET2* tumor suppressive properties *in vitro* and *in vivo* [50]. These observations emphasize the need for more novel therapeutic strategies.

Therefore, the data highlight the complexity and specificity of genetic interactions in MDS and related conditions, underscoring the extensive efforts in developing targeted therapies. For example, the mutual exclusivity of specific genetic mutations suggests potential pathways for personalized treatment strategies. Additionally, the ongoing clinical trials underscore the focus on targeting metabolic pathways and genetic mutations specific to these diseases, which could lead to more effective and tailored treatment options for patients. Therefore, collaboration between researchers and clinicians is essential to translate these advances into clinical practice, ultimately improving patient outcomes [76]. Consequently, future studies are needed to evaluate the efficacy of dual inhibition of *DNMT3A* and *TET2* in MDS.

Moreover, the high oncogenic activity of *DNMT3A* and *TET2* mutations in MDS highlights the reliance on metabolic pathways to regulate DNA methylation. Therefore, by focusing on the observed phenotype of affected cells in MDS, we monitored the transcriptomic profiles of related metabolic pathways, underscoring the importance of integrating gene expression profiles with metabolism and DNA methylation using GSEA. We observed gene expression changes in the OXPHOS and glycolysis metabolic pathways across multiple independent MDS cohorts. The upregulated *DNMT3A* and *TET2* expression among the independent MDS cohorts linked gain-of-function mutations with increased gene expression in MDS, thereby highlighting the metabolic rewiring driven by *DNMT3A* and *TET2* mutations and supporting the hypothesis that *DNMT3A* and *TET2* are involved in the pathogenesis and could be potential biomarkers or therapeutic targets.

Interestingly, as mentioned previously, *DNMT1* is among the most frequently mutated genes in MDS, which could account for the surge in clinical trials evaluating *DNMT1* inhibitors. However, no registered clinical trial has been designed to address the metabolic changes marked by elevated GSH levels associated with *DNMT1* mutations. Consequently, metabolic dynamics in MDS, especially the potential of GSH as a therapeutic target, remain uncharted territory. Conversely, while *TET2* mutations are common in MDS, no clinical trials targeting them have been registered. Notably, there is significant exclusivity between

mutations in *TET2* and *IDH1/2*. For example, studies have shown that introducing 2-hydroxyglutarate, a byproduct of *IDH1/2* mutations, into *TET2*-deficient cells triggers synthetic lethality [19]. Therefore, patients with MDS with *TET2* mutations might benefit from therapies based on the oncometabolite 2-hydroxyglutarate. Nonetheless, no clinical trials currently target patients with MDS with *TET2* mutations. Therefore, developing *TET2* inhibitors may pave the way for innovative therapeutic interventions. Moreover, *TET2* inhibitors demonstrated a selective effect on TET2 mutant hematopoietic precursor cells *in vitro* and *in vivo* [19]. Overall, these findings emphasize the metabolic influence of *TET2*-mutant cells, thereby suggesting avenues for developing innovative therapeutic approaches. In addition, our analysis across multiple cohorts using the cBioPortal revealed *NPM1* mutations in 16% of patients with MDS. Similarly, metabolic assessments have identified *NPM1*-mutant cases as a unique subset [77]. This discovery underscores a potential metabolic weakness in *NPM1*-mutant cells, offering additional possibilities for therapeutic interventions due to the implied, pronounced metabolic shift in *NPM1*-mutated cells.

Consequently, our gene expression meta-analysis showed that the transcriptome of patients with MDS is associated with the deregulation of pathways related to DNA methylation, consistent with our MDS mutational analysis. In addition, the gene expression meta-analysis highlighted the deregulation of metabolism-related genes (*ERFE* and *SULT4A1*), suggesting a mechanism of metabolic control of the epigenome in MDS. Numerous studies have shown that metabolism profoundly affects DNA methylation in cancer [78–80]. For example, *ERFE*, the primary erythroid regulator of hepcidin, was reportedly involved in alterations in cancer metabolism [81]. Additionally, *SULT4A1* is found within the cytosolic and mitochondrial sub-compartments of the mouse and human brain, suggesting a potential auxiliary function in mitochondrial activity [82]. Indeed, Hossain *et al.* [83] indicated that *SULT4A1* directly influences mitochondrial functionality and redox equilibrium. These observations highlight the roles that *SULT3A1* plays in metabolism. Therefore, exploring the MDS expression profile in the context of metabolic and DNA methylation mutations remains crucial for identifying innovative therapeutic interventions.

Therefore, incorporating our findings into clinical practice will undoubtedly aid in assessing the influence of *DNMT3A* and *TET2* expression on disease MDSs. Our study used the GEPIA platform to examine the effect of *DNMT3A* and *TET2* expression on overall survival across various independent cohorts [36]. The survival analyses revealed that high *DNMT3A* and *TET2* expression correlate with unfavorable clinical outcomes and significantly shorter overall survival in the LGG, ACC, MESO, PCPG, and THCA cohorts, suggesting their involvement in tumor aggressiveness and resistance to therapy. Also, these TCGA cohorts exhibit metabolic reprogramming and epigenetic dysregulation similar to MDS. Some undergo metabolic rewiring, which affects DNA methylation and OXPHOS, while others show significant epigenetic changes, such as histone modifications [84–86]. Additionally, specific cohorts show metabolically driven cancers with strong links to mitochondrial function, epigenetic modifications, and others exhibit metabolic plasticity involving glycolysis, reflecting disruptions in MDS caused by specific mutations in genes such as *DNMT3A* and *TET2* [87,88].

This finding also aligns with our data for MDS, suggesting that the upregulation of these genes significantly affects disease outcomes and plays a crucial role in oncogenesis. Including these TCGA cohorts supports the broader applicability of our findings, suggesting that the interplay between metabolism and epigenetics is not restricted to MDS but extends across multiple malignancies. The consistent impact of *DNMT3A* and *TET2* mutations on survival in these cancers further reinforces that these genes are critical regulators of metabolic and methylation networks across diverse tumor types. By drawing these parallels, our study provides a foundation for exploring common metabolic-epigenetic vulnerabilities in different cancers, paving the way for potential cross-disease therapeutic strategies. Therefore, epigenome-targeted medications encourage the normalization of metabolic reprogramming in cancer cells, which will be a key component of cancer treatment [38]. By leveraging these databases, we could identify differential gene expression patterns and correlate them with clinical outcomes, emphasizing the significance of metabolic reprogramming and DNA methylation in MDS progression.

While our study offers valuable insights into the relationship between metabolism and DNA methylation in MDS, it also has several limitations. Firstly, while we incorporated multiple publicly available transcriptomic and mutational datasets, variations in patient demographics and clinical variables across studies may have introduced inconsistencies, making it difficult to control for factors such as age, sex, disease stage, and treatment history. Therefore, we excluded patients' demographic details to avoid redundancy, as they are already available in the original studies. Secondly, differences between datasets remain a potential limitation despite applying batch effect correction techniques. Thirdly, while our findings highlight strong associations between *DNMT3A* and *TET2* mutations and metabolic changes, they do not establish causality. Further functional studies, such as CRISPR/Cas9-mediated knockdown or overexpression experiments, are needed to clarify the direct link between metabolic pathways and epigenetic regulation in MDS. Fourthly, our analyses relied on gene expression data rather than direct metabolomic measurements, so they do not capture metabolic flux changes. Future research should integrate metabolomics, epigenomics, and immune profiling to offer a more comprehensive view of MDS biology and therapeutic opportunities. Lastly,

while we assessed the prognostic impact of *DNMT3A* and *TET2* mutations using TCGA datasets with diverse disease backgrounds, our findings require independent validation in MDS-specific patient cohorts. Expanding this research to include single-cell transcriptomics and multi-omics approaches will be key to identifying precise metabolic vulnerabilities and advancing targeted therapies for MDS.

Finally, our findings suggest a potential interaction between *DNMT3A* and *TET2* in linking metabolism and methylation processes. Therefore, we recommend targeted therapy for mutations in *DNMT3A* and *TET2* as part of personalized medicine as a novel therapy for patients with MDS. For example, OXPHOS inhibitors such as IACS-010759 have shown efficacy in preclinical myeloid malignancy models with *DNMT3A* and *TET2* mutations due to their mitochondrial dependence [89]. Similarly, combining glycolysis inhibitors such as 2-deoxy-D-glucose with HMAs, one-carbon metabolism-targeting drugs such as methotrexate, or serine hydroxymethyltransferase inhibitors, could disrupt the metabolic and methylation pathways in these mutant clones [90–92]. Moreover, collaboration between researchers and clinicians is crucial to translating these findings into clinical practice, ultimately enhancing patient outcomes [76]. These recent advances underscore the ongoing efforts to improve the understanding and treatment of MDS and to develop more personalized, effective, and less invasive therapies. Further functional studies are needed to clarify the specific roles of *DNMT3A* and *TET2* in MDS tumorigenesis. This evolving landscape offers promising prospects for significantly better management of MDS in the near future.

Conclusion

The inherent complexity and varied nature of MDS continue to obscure its origins, which remain incompletely understood. Our study underscores the dual roles of metabolism and DNA methylation in shaping MDS progression, offering novel insights into its molecular underpinnings. Integrating mutational and transcriptomic data provides a valuable framework for risk stratification, particularly in leveraging *DNMT3A* and *TET2* mutations as predictive biomarkers. Moreover, the oncogenic activities of *DNMT3A* and *TET2* observed in our study pave the way for MDS stratification, highlighting the crosstalk of metabolic processes and DNA methylation. Such insights could further enhance our grasp of the roles of metabolism and DNA methylation in MDS and aid in devising patient-specific treatment strategies after diagnosis. Therefore, our study establishes a foundation for prospective clinical trials and translational investigations to advance precision medicine for patients with MDS.

Availability of Data and Materials

This study utilized publicly available mutational data via the cBioPortal platform from publications in Myelodysplastic (MSK, 2020) and Myelodysplasia (UTokyo, Nature 2011), which can be accessed via the following link: http://www.cbioportal.org/study/summary?id=mds_tokyo_2011%2Cmds_mskcc_2020. The gene expression data utilized in this study were obtained from the public GEO database and can be accessed on the following links: (1) GSE114922 (2018): accessible at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114922. (2) GSE63569 (2014): accessible at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63569. (3) GSE183328 (2022): accessible at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183328.

Author Contributions

Conceptualization: WA and SA. Writing—original draft: WA. Writing—critical revision: SA. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

As this study was retrospective, utilizing published data from the cBioPortal (myelodysplastic [MSK, 2020] and myelodysplasia [UTokyo, Nature 2011]) and publicly available data from three independent cohorts in the GEO database (GSE114922, GSE63569, and GSE183328), the Local Ethics Committee waived the need for additional ethical approval and consent to participate.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537198.116.

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