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Article

Integrated Multi-Cohort Analysis of Whole-Genome and Transcriptome Data Reveals Interplay between Metabolism and Methylation in Myelodysplastic Syndrome (MDS)

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Abstract: Objectives: Metabolic interactions amongst mutated genes in myelodysplastic syndrome (MDS) offer promising avenues for novel anticancer treatments. Our comprehensive study delves into these mutational and transcriptomic landscapes, pinpointing hallmark gene mutations and deregulated gene expression that could influence MDS patients' metabolomes. **Methods:** The study utilized a retrospective, cross-sectional approach, employing mutational data on the cBio Cancer Genomics Portal conducted and reported by the University of Tokyo in 2011 and multi-center MDS cohorts regulated by Wellcome Trust Sanger Institute, United Kingdom, all in 2020. For transcriptomic data, we selected three publicly accessible independent cohorts on the Gene Expression Omnibus (GEO) database (GSE114922 in Wellcome Trust Centre for Human Genetics, United Kingdome, GSE63569 in University of Oxford, United Kingdome, and GSE183328 in CIMA, Spain) held in 2015, 2018, and 2022, respectively. To compile clinical, mutational, and transcriptomic data on MDS patients from multiple datasets and studies. This meta-analysis included genomic data derived from cellular genomics sources to assess mutations in specific genes, alongside an examination of transcriptomic data from three separate datasets that have been previously published. **Results:** *DNMT3A* presented a 20% mutation frequency, playing a pivotal role in MDS metabolomics. The *DNMT3A* gene mutations displayed significant mutual exclusivity with the *SRSF2*, *ASXL1*, *JAK2*, and *TP53* genes. The mutational analysis also showed that the gene expression landscape in MDS is associated with alterations to DNA methylation pathways. **Conclusion:** This analysis suggests a potential therapeutic niche. Identifying signature genes in MDS that have metabolic and methylation affiliations could illuminate the disease's intricate biology and inspire novel treatments.

Keywords: Myelodysplastic syndrome (MDS); Metabolism; DNA Methylation; *DNMT3A*; *TET2*

1. Introduction

Myelodysplastic syndrome (MDS) increases the likelihood that individuals will develop diverse undifferentiated hematologic malignancies [1]. While MDS is primarily found in people over 65, it accounts for less than 5% of childhood cancers [2]. Despite the fact that out of every 100,000 individuals under 70, 40–50 have MDS, emphasizing its higher frequency in older age groups, the age-standardized incidence of MDS, a rare illness, ranges from 1.3 to 4.3 per 100,000 person-years and rises steadily with age [3,4]. The current research indicates that 80–90% of individuals with MDS present repetitive changes in multiple genes [5]. Therefore, investigating hereditary changes that engender leukemia and lymphoma is crucial to improving disease outcome predictions. Advances in next-generation sequencing (NGS) have deepened our understanding of the role that genetic changes

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play in malfunctioning blood formation and the prognosis for MDS patients. For instance, MDS often features primary and secondary genetic changes, with around 1,500 mutations throughout the genome [6]. These genetic changes result in complex interactions that may affect survival rates in certain MDS cases. Identifying these inherent changes can guide the development of tailored treatment approaches for MDS patients.

One of cancer's hallmarks is a change in metabolism, which is crucial for cellular processes that instigate carcinogenesis [7]. Hematopoietic stem cells (HSCs), the foundation of hematopoiesis, 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 metabolism to a mitochondria-centered one [8]. In the 1920s, Warburg *et al.* 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 [3,9]. A transformative approach to MDS entails identifying metabolic gene markers that are prognostically valuable. The nuanced relationships between frequently altered genes and metabolic systems provide a lens into how these genes may counteract cancer development by effectively adjusting various aspects of metabolism.

The mutations that initiate MDS in HSCs are shaped by clinical features, cellular factors, and genetic makeup [10]. For example, the French-American-British (FAB) system from 1982 labeled MDS "refractory anemia" and segmented it into five types according to cellular morphology and the myeloid blast count [11]. This system was predominant 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 the condition [12]. In 2001, the WHO presented its first categorization based on HSC mutations that initiate MDS [13]. The ensuing updates from 2008 to 2016 enriched this framework by incorporating clinical, morphological, immunophenotypic, and genetic aspects of the condition [13]. By 2022, the WHO had further refined the MDS classifications. The current divisions hinge on genetic variations and morphological features, emphasizing genetic disruptions of clonal hematopoiesis. This detailed system supports accurate diagnosis and risk assessment, allowing for personalized treatment approaches [10].

In our integrated multidisciplinary study, we emphasized the mutational burden and the transcriptomic profile landscape found in MDS patients to evaluate the relevance of the genes and overly active molecular pathways that are linked to MDS metabolism. We pinpointed numerous genes with mutations that are recognized as modules of mutually exclusive mutations, which could amplify cellular metabolism in individuals with MDS. Ultimately, we combined gene mutational data with gene expression data to identify metabolism-associated signature genes related to DNA methylation in MDS, providing potential insights into the biology of the condition.

2. Methodology

This work followed a retrospective, cross-sectional study design using the cBio Cancer Genomics Portal tool and the Gene Expression Omnibus (GSE) to gather clinical, mutational, and transcriptomic information on MDS patients from various datasets and studies [14–19]. The genomic data for evaluating changes in potentially useful genes came from 4,260 MDS samples from joint studies that reported cellular genomics data in 2011 held at the University of Tokyo and in Wellcome Trust Sanger Institute and others in ClinicalTrials.gov number NCT00146120, comparative study in the United States, and Wellcome Trust Sanger Institute, Hinxton, United Kingdom all in 2020 [20–23]. The transcriptomic data found on the GSE database consists of three independent cohorts (GSE114922 in Wellcome Trust Centre for Human Genetics, United Kingdome, GSE63569 in University of Oxford, United Kingdome, and GSE183328 in CIMA, Spain) held in 2015, 2018, and 2022, respectively [14–17]. All obtained data were parallel with mutations in MDS of clinical significance.

We analyzed these 4,260 MDS patient samples with the cBio platform. This comprehensive analysis allowed us to identify and examine mutation patterns in this group of patients [18–23]. For the RNA-seq meta-analysis, we selected three independent cohorts with raw data published in the

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GSE. We selected only MDS RNA-seq data from the same biological source (CD34+ hematopoietic stem cells) and technical sequencing platform (Illumina). Raw sequencing reads were downloaded from the GSE database with the SRA toolkit. These reads were then mapped to the human genome (*hg38*). The resulting BAM files were filtered to remove duplicates, leaving only primary aligned reads. Gene expression count matrices were then generated with a gene annotation GTF file downloaded from Gencode release 44 (GRCh38.p14). The DESEQ2 package was used for data normalization and differential gene expression was reviewed afterward. To identify differentially expressed genes, we established comparisons by contrasting MDS samples with healthy samples. Genes were labeled significant if they had an adjusted *p*-value of less than 0.1 and a log2 fold change of more than 1.5. Upregulated genes were then subjected to pathway enrichment analysis using the reactome database. Scatter plots were generated using the ggplot package.

3. Results

In numerous studies, biomarkers, especially DNA methylation, and mutations in genes such as isocitrate dehydrogenase (NADP(+)) 1 (*IDH1*) and isocitrate dehydrogenase (NADP(+)) 2 (*IDH2*) have been spotlighted as fundamental indicators of MDS [24,25]. Such genetic indicators can modulate patients' reactions to chemotherapy drugs, including decitabine [26]. Considering the complex nature of MDS, recognizing that our comprehension of the routes that these notably mutated genes define remains limited is vital. Our meta-study systematically integrated various genes to reveal the pathways that are relevant to MDS patients. Using the cBio cancer genomics portal tool, we assessed 4,260 MDS patient samples drawn from diverse datasets and articles. This examination highlighted genes with prevalent mutations, such as tet methylcytosine dioxygenase 2 (*TET2*), DNA methyltransferase 3 alpha (*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*) (Figure 1) [18–23,27–33].

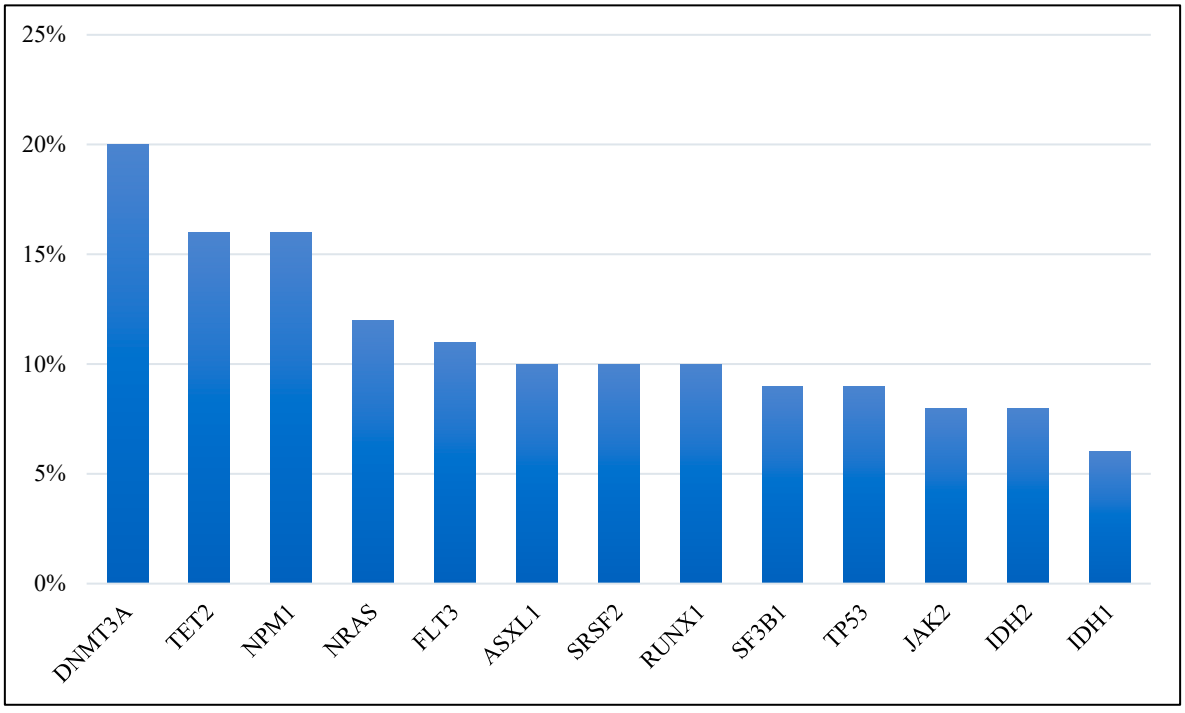

Figure 1. Percentage of Mutated Genes in MDS Patients. The most significantly altered genes among 4,260 MDS patient samples from various datasets and published studies.

Interestingly, we found that the *DNMT3A* gene mutations displayed mutual exclusivity with multiple other vital genes, as Table 1 shows. This mutual exclusivity pattern suggests that these genes contribute to or manage a common biological function, possibly metabolism. Therefore, concurrent

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mutations in multiple genes could harm cell systems. Further analysis showed that the *DNMT3A* gene is linked to DNA methylation, the *SRSF2* gene is involved in RNA splicing, the *ASXL1* gene contributes to chromatin modification, the *JAK2* gene is essential for signal processing, and the *TP53* gene is fundamental to both tumor inhibition and transcription factor function. Significantly, except for *JAK2*, all of these genes are correlated with an unfavorable prognosis [10]. Given their critical roles, many of these genes will likely be integrated into the standard clinical assessment of MDS patients.

Table 1. Mutual exclusivity observed among MDS patients with specific genetic alterations.

A	B	Neither	A Not B	B Not A	Both	p-Value	Tendency
SRSF2	*DNMT3A*	3026	379	816	39	<0.001	Mutual exclusivity
ASXL1	*DNMT3A*	3014	391	810	45	<0.001	Mutual exclusivity
JAK2	*DNMT3A*	3110	295	814	41	<0.001	Mutual exclusivity
TP53	*DNMT3A*	3063	342	796	59	0.002	Mutual exclusivity

Our comprehensive meta-analysis, which pooled independent RNA-seq datasets from various MDS studies, amplified the sample size, thereby enhancing its statistical power and our capacity to pinpoint genes that are differentially expressed and unique to MDS. Using this approach helped us identify novel differentially expressed genes by contrasting the transcriptomes of healthy individuals and those with MDS. The data showed that the interleukin 1 alpha (*IL1A*), oxidized low-density lipoprotein receptor 1(*OLR1*), and adrenoceptor alpha 2B (*ADRA2B*) genes in [34–36] were among the most characteristically downregulated genes in the MDS genotype. Notably, the erythroferrone (*ERFE*) [37] and sulfotransferase family 4A member 1 (*SULT4A1*) [38] are the most upregulated genes in MDS cells (Figure 2A), which suggests extensive metabolic programming. The pathways enrichment analysis using the Reactome database revealed that, as our mutational analysis showed, the DNA methylation pathway was enriched, as Figure 2B and C show, suggesting that it plays a massive role in gene regulation and epigenetic modifications.

Moreover, Figure 2D shows the *ERFE* and *SULT4A1* genes as novel upregulated genes in the metabolic process, identified with the GO term GO:0008152. According to this analysis, the *ERFE* gene positively regulates glucose imports and the fatty acid metabolic process, and the *SULT4A1* gene contributes to mitochondria-involving biological processes and steroid metabolic processes. Therefore, these results suggest extensive changes to the DNA methylation pathways, which can result from a defective metabolism in MDS.

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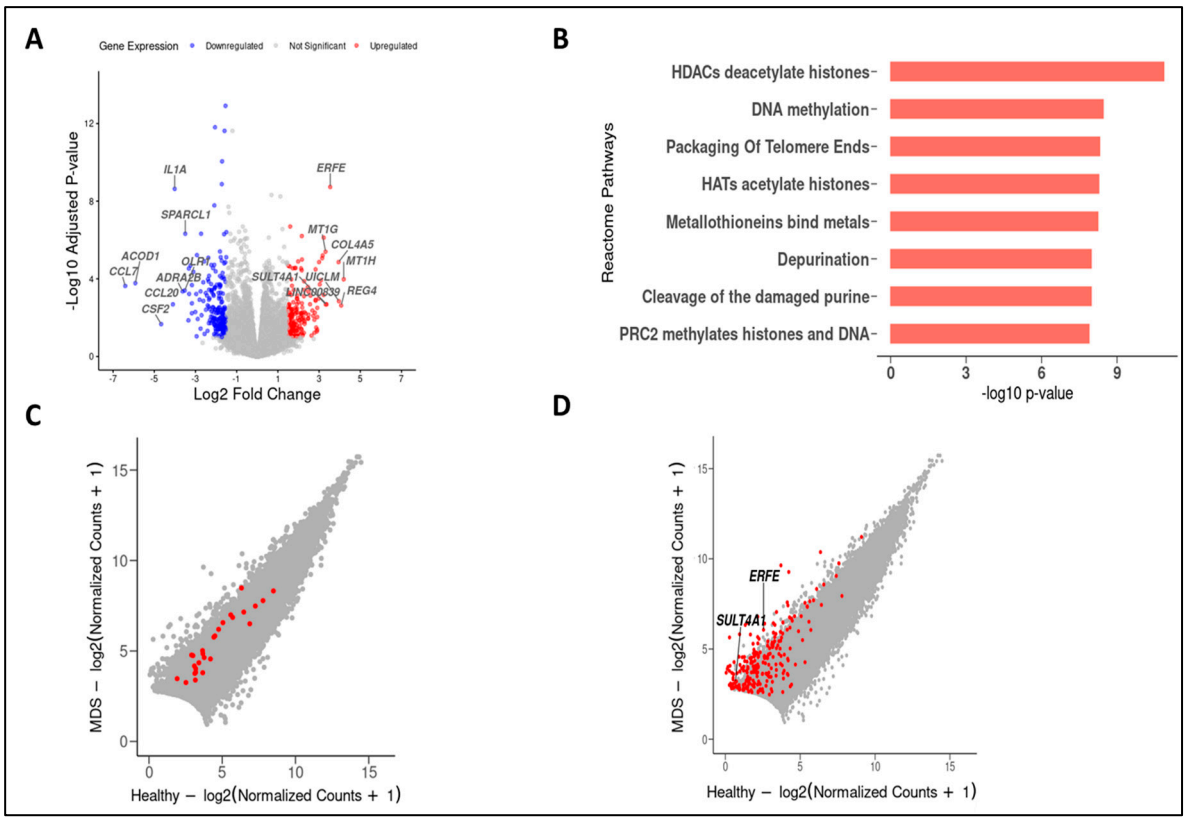

Figure 2. The expression landscape of individuals with MDS vs. healthy individuals. The identified signature genes that are involved in the DNA methylation and metabolic processes in MDS cases. (A) Volcano plot showing the gene expression of significantly up- or down-regulated genes in MDS cells. (B) 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 in MDS. (D) Scatter plot of gene expression of metabolism-related genes (*ERFE* and *SULT4A1*) that are enriched in MDS and inconsistent with the DNA methylation gene set.

This study underscores the therapeutic potential of addressing the most commonly mutated genes that were found in 4,260 MDS patient samples gathered from diverse datasets and academic articles. These specific genes could disrupt vital signaling pathways and molecular functions, including metabolism, that play central roles in the well-being and growth of individuals with MDS. Notably, mutations in the *DNMT3A* gene accounted for a significant fraction (as much as 18%) of the mutations found in MDS cases [39], corroborating our extensive multicohort findings. Mutated *DNMT3A* has a connection with GSH levels that oppose chemotherapy. A definitive association between *DNMT3A* mutations and GSH levels in MDS remains to be determined, but GSH plays an essential role as an antioxidant regulating numerous cell activities [40].

Understanding the effect of *DNMT3A* mutations on metabolic processes in hematologic cancers might offer clarity regarding the cellular strategies that drive tumor growth, persistence, and resistance to chemotherapy. Elevated GSH levels may enhance tumor cells' adaptability and resilience, potentially leading to drug resistance. Therefore, GSH concentration might present a potential metabolic weakness in MDS cases featuring *DNMT3A* mutations. The U.S. Food and Drug Administration (FDA) has sanctioned the use of hypomethylating agents (HMAs) to manage acute myeloid leukemia (AML) and MDS [41]. Ongoing clinical trials targeting MDS and their biological focuses are presented in Table 2.

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Table 2. The current ongoing clinical trials targeting metabolic pathways associated with mutated genes in MDS and myeloid leukemias. The table summarizes the list of recruiting clinical trials focused on targeted biological processes (according to https://clinicaltrials.gov, retrieved on the 14th of August, 2023).

NCT Number	Study Status	Conditions	Targeted Biological Process	Interventions/Drugs /Procedure
NCT04493164	Recruiting	MDS/AML	Mutant *IDH1* Inhibitor	Ivosidenib/Liposome -encapsulated Daunorubicin-Cytarabine
NCT03503409	Recruiting	MDS/AML	Mutant *IDH1* Inhibitor	AG-120
NCT03744390	Recruiting	MDS/AML	Mutant *IDH2* Inhibitor	AG-221
NCT04827719	Recruiting	MDS/AML	High-dose cytarabine delivery	BST-236
NCT04279847	Recruiting	MDS	*BET* inhibitor and *JAK* inhibitor	INCB057643/Ruxolitinib
NCT04140487	Recruiting	MDS/AML	*FLT3* tyrosine kinase inhibitors and DNA Methylation and synthesis Inhibitor	Azacitidine/Gilteritinib/Venetoclax
NCT05010122	Recruiting	MDS/AML	*FLT3* tyrosine kinase inhibitor and DNMT1 Inhibitor	Decitabine and Cedazuridine/Gilteritinib/Venetoclax
NCT03661307	Recruiting	MDS/AML	*DNMT1* Inhibitor and *FLT3* tyrosine kinase inhibitor	Decitabine/Quizartinib/Venetoclax
NCT03683433	Recruiting	MDS/AML/CML	DNA Methylation and synthesis Inhibitor and Mutant *IDH* Inhibitor	Azacitidine/Enasidenib Mesylate
NCT05636514	Recruiting	MDS/AML/CML	*FLT3* tyrosine kinase inhibitor and *FAK* inhibitor	Decitabine-Cedazuridine 35 Mg-100 Mg ORAL TABLET/Defactinib
NCT04493138	Recruiting	MDS/CML	*DNMT1* Inhibitor and *FLT3* tyrosine kinase inhibitor	Azacitidine/Quizartinib
NCT05817955	Recruiting	MDS	DNA Methylation and synthesis Inhibitor and *JAK* Inhibitor	Azacitidine (AZA) with Ruxolitinib
NCT04803721	Recruiting	MDS		
NCT04250051	Recruiting	MDS/AML	Mutant *IDH1* Inhibitor	Cytarabine/Filgrastim/Fludarabine/Fludarabine Phosphate/Ivosidenib
NCT04167917	Recruiting	MDS/AML/CML	DNA methyltransferase 1 (*DNMT1*) inhibition	NTX-301
NCT04187703	Recruiting	MDS	DNA methyltransferase (*DNMT*) inhibition	5-azacytidine/Decitabine
NCT05282459	Recruiting	MDS	Mutant *IDH2* Inhibitor	Enasidenib mesylat dose escalation
NCT04741945	Recruiting	MDS	Antihyperglycemic	Metformin
NCT04477291	Recruiting	MDS/AML	*FLT3* Inhibitor	CG-806
NCT03839771	Recruiting	MDS/AML	Mutant *IDH1* Inhibitor and Mutant *IDH2* Inhibitor	AG-120/Placebo for AG-120/AG-

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				221/Placebo for AG-221
NCT05030675	Recruiting	MDS/CML	Tyrosine kinase inhibitor	Fostamatinib
NCT03953898	Recruiting	MDS/AML	poly (ADP-ribose) polymerase (PARP) inhibitor	Biospecimen Collection/Bone Marrow Aspiration/Olaparib
NCT03999723	Recruiting	MDS/AML/CML		Vitamin C/Placebo
NCT04764474	Recruiting	Hematological Malignancies With *IDH* mutations	Mutant *IDH1/2* Inhibitor	HMPL-306

4. Discussion

We delved into the genetic mutation patterns and gene expression profiles reported in independent MDS studies. This approach provided a greater understanding of the biological nature of MDS. Our investigation explored the link between mutations in genes related to MDS metabolism and the subsequent therapeutic outcomes, drawing on data from cBioPortal (MSK, 2020) and Myelodysplasia (UTokyo, Nature 2011). This approach differentiates our work from previous studies focusing on singular genes or a confined group of MDS genes. For example, the patterns of mutual exclusivity in specific genes, as presented in Table 1, underscore their pivotal roles in MDS. The *DNMT3A* gene, which registered the highest mutation frequency (Figure 1), also indicates potential interplay with other genes, elaborated in Table 1. Such observations illuminated the significant effect that the *DNMT3A* gene has on the MDS metabolic landscape, possibly including interlinkage with the enumerated genes in a common biological function. Moreover, our results imply that the cells of individuals with MDS might struggle to accommodate concurrent mutations in the genes specified in Table 1.

Interestingly, as mentioned previously, *DNMT1* mutations feature prominently among the most frequent MDS mutations. This could account for the surge in clinical trials evaluating *DNMT1* inhibitors. However, no recognized clinical trial has been designed to address the metabolic changes marked by elevated GSH levels associated with *DNMT1* mutations. The metabolic dynamics of MDS, especially the potential therapeutic target presented by GSH, remain uncharted territory. Conversely, while *TET2* gene modifications are prevalent in MDS, no clinical trials targeting these alterations have been registered. There is notable exclusivity between mutations in *TET2* and *IDH1/2*. Studies have shown that introducing 2-hydroxyglutarate, a byproduct of *IDH1/2* mutations, into *TET2*-deficient cells can trigger synthetic lethality [42]. This suggests that MDS patients with *TET2* mutations might benefit from therapies based on the oncometabolite 2-hydroxyglutarate. Currently, no clinical trials are specifically aimed at MDS patients with *TET2* mutations. Therefore, the development of inhibitors for *TET2* mutations may pave the way for innovative therapeutic interventions.

Our study underscores the mutual exclusivity of *TET2* and *IDH1/2* mutations, hinting at a potential therapeutic target within the myeloid neoplasia dependency pathway. Recent research has begun to elucidate this relationship, revealing that the 2-hydroxyglutarate produced by *IDH1/2* mutations can be lethal to *TET*-deficient cells. Furthermore, *TET2* inhibitors demonstrate a selective effect on *TET2* mutant hematopoietic precursor cells *in vitro* and *in vivo* [42]. These findings emphasize the metabolic influence of *TET2*-mutant cells, suggesting avenues for developing innovative therapeutic approaches. In addition, our analysis across multiple cohorts using the cBioportal database revealed *NPM1* mutations in 16% of MDS patients. Metabolic assessments have also identified *NPM1*-mutant cases as a unique subset [43]. 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.

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Our gene expression meta-analysis showed that MDS patients' transcriptome is associated with deregulation of DNA methylation, as our MDS mutational analysis suggested. In addition, the gene expression meta-analysis highlighted the deregulation of metabolism-related genes (*ERFE* and *SULT4A1*). This suggests a mechanism of metabolic control of the epigenome in MDS. Numerous studies have shown that metabolism has a profound effect on DNA methylation in cancer [44–46]. The *ERFE* gene, the primary erythroid regulator of hepcidin, was reportedly involved in alterations in cancer metabolism [47]. *SULT4A1* is found within the cytosolic and mitochondrial sub-compartments of both mouse and human brains, hinting at a potential auxiliary function in mitochondrial activity [48]. A study by Hossain et al. indicated that *SULT4A1* directly influences mitochondrial functionality and redox equilibrium [49]. These observations highlight the roles that *SULT3A1* plays in metabolism. Exploring the MDS profile in the context of metabolic and DNA methylation mutations remains crucial for pinpointing innovative therapeutic interventions.

5. Conclusion

The inherent complexity and varied nature of MDS continue to obscure its origins, which remain to be fully understood. Moreover, exploring MDS patients' mutation load, especially concerning metabolic processes and methylation, could illuminate their cellular molecular makeup. Such insights could further enhance our grasp on the roles of metabolism and DNA methylation in MDS and aid in devising patient-specific treatment strategies after diagnosis. In summary, this study offers a deeper understanding of how to identify novel MDS-associated metabolic genes and DNA methylation, the potential clinical applications and the possibility of improving therapeutic avenues for MDS patients, and a foundation for prospective clinical trials and investigations.

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Competing Interests: The authors declare that they have no potential competing interests.

Ethics approval: Given the nature of this design, there is no need for additional ethical approval. Thus, the ethical approval was waived by the Local Ethics Committee. The current study investigated the publicly available data using the cBioPortal, publications from Myelodysplastic (MSK, 2020) and Myelodysplasia (UTokyo, Nature 2011), and data from three independent cohorts (GSE114922 in 2015, GSE63569 in 2018, and GSE183328 in 2022).

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