REVIEW ARTICLE

Mutational Landscape and Potential Metabolic Associations in Relapsed/Refractory RUNX1::RUNX1T1 Acute Myeloid Leukemia: Analysis and Literature Review

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ABSTRACT

RUNX1::RUNX1T1 requires cooperating mutations to develop full-blown RUNX1::RUNX1T1 acute myeloid leukaemia (AML). Leukaemic cells undergo metabolic reprogramming to survive chemotherapy and form drug-resistant clones. Approximately 50% of RUNX1::RUNX1T1 AML patients relapse, and there are no specific treatments for relapsed or refractory (R/R) RUNX1::RUNX1T1 AML patients. Two studies from the Beat AML programme conducted in 2018 and 2022 were reviewed using cBioPortal and Vizome to classify oncogenic mutations in R/R RUNX1::RUNX1T1 patients. Mutations and their potential links to metabolism were explored via a literature review. Eight R/R RUNX1::RUNX1T1 AML patients' data was reviewed to exhibit a cumulative total of 14 oncogenic mutations. Literature review showed potential connections with metabolism which could explain some of the clinical findings from the studies. Specifically, $EZH2^{F445Lfs*3}$, $EZH2^{E745Pfs*19}$, $EZH2^{I744Sfs*19}$, $NRAS^{G12D}$, $TET2^{N752Kfs*60}$, $TET2^{Q1664*}$ and $JAK-3^{M511I}$ showed metabolic implications on essential metabolic pathways, including lipid metabolism and glycolysis. This review suggests that secondary mutations may have the potential to cause development and therapy-resistance of RUNX1::RUNX1T1 AML leading to metabolic reprogramming. Further metabolomic studies are necessary to elucidate the mutations' precise role in metabolic reprogramming.

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Keywords: Acute Myeloid Leukaemia, Metabolic Reprogramming, Mutations, RUNX1::RUNX1T1, t(8;21) AML

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INTRODUCTION

RUNX1::RUNX1T1 acute myeloid leukaemia (AML), also known as t(8;21) AML, is a subtype of leukaemia caused by the fusion of the RUNX1 gene on chromosome 21 to the RUNX1T1 gene on chromosome 8. Despite favourable outcomes, approximately 50% of RUNX1::RUNX1T1-AML patients eventually relapse or develop refractory AML (1). Additionally, RUNX1::RUNX1T1-AML is genetically heterogeneous, increasing the disease's complexity. Furthermore, currently, there is no established treatment for relapsed or refractory (R/R) RUNX1::RUNX1T1-AML (2).

One way cancer cells develop chemotherapy-resistance is through metabolic reprogramming to satisfy high metabolic demands for leukaemic transformation, growth, and proliferation, thus gaining a survival advantage. However, expression of RUNX1::RUNX1T1 alone is insufficient for RUNX1::RUNX1T1-AML; it requires cooperating mutations (3) which are present in two-thirds of AML cases (4). In this study, we explored potential links between oncogenic mutations in this subtype of patients and resistance mechanisms via metabolic reprogramming. Identifying these associations may facilitate the identification of metabolic weaknesses that could function as prospective treatment targets. Furthermore, the characterization of secondary

mutations in R/R AML may enhance diagnostic accuracy and provide individualized treatment strategies.

METHODOLOGY

Sixteen myeloid cancer studies were explored on cBioPortal (5) using the keywords "t(8;21)", "RUNX1/ETO" and "AML1/ETO". Two studies reviewed in this paper; BeatAML 2018 study (6) included RUNX1::RUNX1T1-AML patient data from waves 1+2 of the study while the BeatAML 2.0 2022 study (7) combined waves 1-4. Data from both studies is made publicly accessible on BeatAML data viewer, Vizome; hence this resource along with cbioportal.com were used to review relevant data. The patients with a "refractory" response to induction and/or with cumulative treatment regimens including "salvage" chemotherapy were considered as R/R RUNX1::RUNX1T1-AML patients. Patient data from BeatAML study updated in the BeatAML 2.0 study was manually aggregated to observe changes in clinical data across the two studies. Eight patients fulfilled the criteria for R/R RUNX1::RUNX1T1-AML patients from the BeatAML 2018 and 2022 studies (Table I).

To acquire data about specific genetic mutations, each R/R RUNX1::RUNX1T1-AML patient's data was accessed. OncoKB™ (8), an in-built precision oncology knowledge base feature on cBioPortal, was used to review the specific mutations' potential oncogenicity and gain or loss of function as a result of the mutation (Table II). A minimum allele frequency criterion of 5% was utilized to exclude low-confidence variants. Furthermore, we employed Vizome's variant filter to verify mutational data and evaluate the functional consequences of each variant. A subsequent literature search was conducted to elucidate connections between patient clinical data, mutation data, and the possible roles of these mutations in metabolic reprogramming. This was done via a combination of multiple keywords, including but not limited to, the gene names (e.g. NRAS) and/or the specific genetic mutation (e.g. $NRAS^{G12D}$) and keywords related to metabolism (e.g. glucose metabolism) or "loss-of-function mutation". The summary of the methodology is provided in Figure I.

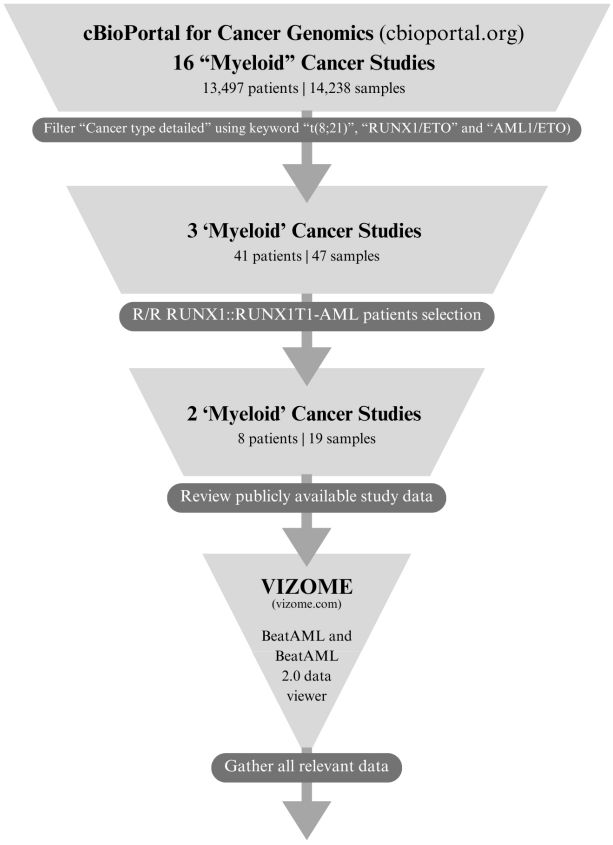

Figure 1: Brief Overview of the Methodology

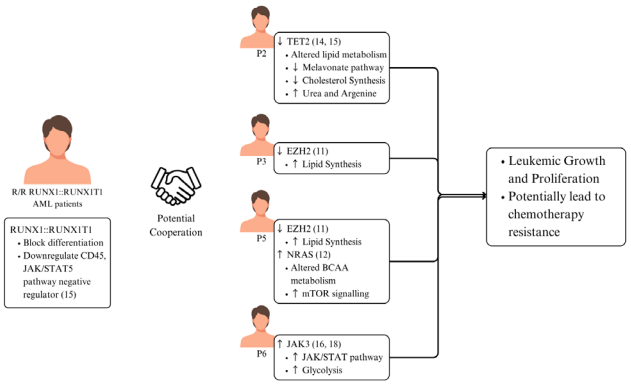

Figure 2: Potential Synergistic Interaction Between RUNX1::RUNX1T1 and Oncogenic Mutations in Metabolic Reprogramming. This figure illustrates the potential cooperation between RUNX1::RUNX1T1 and oncogenic mutations in driving metabolic reprogramming. Key mutations, including TET2, EZH2, NRAS, and JAK3 may contribute to altered metabolic pathways, influencing leukemic cell survival and proliferation

DISCUSSION

This section reviews oncogenic mutations significantly linked to metabolic reprogramming, aiding in the understanding of condition and prognosis in certain R/R RUNX1::RUNX1T1-AML patients. For example, despite the well documented metabolic effects of $TP53^{R175H}$ and $KRAS^{G12V}$ in AML (9), we will not discuss these mutations as patient 7 (P-07) responded well to matched unrelated donor (MUD) allogeneic stem cell transplant (allo-SCT) and has been in remission for eight years (Table I).

Enhancer of zeste homolog 2 (EZH2)

EZH2 possessed the highest mutation frequency (25%) with all three EZH2 frame-shift insertions being loss-of-function (LOF) mutations. In AML patients, LOF EZH2 mutations have demonstrated resistance to cytarabine, utilized commonly in "7+3" induction regimen or subsequent treatment phases (10). Loss of

Table I : Clinical data of R/R RUNX1::RUNX1T1-AML patients

Patient No.	Study Patient ID	Whole exome sequencing/targeted sequencing	Induction Response	Cumulative Treatment Stages	Dead/Alive	Overall Survival (Months)
P-01	aml_ohsu_2018_2694	Whole-exome	Refractory	Consolidation	Induction	Alive	13.95
	aml_ohsu_2022_2085					
P-02	aml_ohsu_2018_1094	Whole-exome	Complete Response	Consolidation	Induction	Re-induction	Allogeneic - Matched Unrelated Donor	Alive	45.95
	aml_ohsu_2022_2249		Complete Response	Consolidation	Salvage	Induction	Re-induction	Allogeneic - Matched Unrelated Donor	Dead-Disease	49.22
P-03	aml_ohsu_2018_1763	Whole-exome	Refractory	Salvage	Consolidation	Induction	Allogeneic - Matched Unrelated Donor	Alive	18.62
	aml_ohsu_2022_2050		Refractory	Salvage	Consolidation	Induction	Allogeneic - Matched Unrelated Donor	Alive	44.32
P-04	aml_ohsu_2022_2668	-	Refractory	Consolidation	Salvage	Induction	Re-induction	Dead-Disease	40.11
P-05	aml_ohsu_2018_2333	Whole-exome	Complete Response	Allogeneic - Sibling	Induction	Alive	8.29
	aml_ohsu_2022_2411		Complete Response	Allogeneic – Sibling	Salvage	Induction	Re-induction	Alive	68.48
P-06	aml_ohsu_2022_2784	-	Complete Response	Consolidation	Salvage	Induction	Dead-Unknown	15.03
P-07	aml_ohsu_2022_2635	-	Refractory	Consolidation	Induction	Re-induction	Allogeneic - Matched Unrelated Donor	Alive	96.07
P-08	aml_ohsu_2018_2305	Whole-exome	Refractory	Consolidation	Induction	Alive	7.01
	aml_ohsu_2022_2356		Complete Response	Consolidation	Induction	Alive	18.15

"-" = data not provided

Table II: Mutation data of R/R RUNX1::RUNX1T1-AML patients

Gene	Mutation frequency (%)	Patient No.	Oncogenic/Likely Oncogenic/Resistance Mutations	Type of Mutation	(Gain/Loss) of function	Allele frequency
EZH2	25	P-03	F445Lfs*3	Frame-shift insertion	Loss	0.17
		P-05	E745Pfs*19	Frame-shift insertion	Loss	0.3
			I744Sfs*19	Frame-shift insertion	Loss	0.43
TET2	12.5	P-02	N752Kfs*60	Frame-shift deletion	Loss	0.42
			Q1664*	Nonsense	Loss	0.48
KIT	12.5	P-05	D816Y	Missense	Gain	0.45
NRAS	12.5	P-05	G12D	Missense	Gain	0.45
FLT3	12.5	P-06	E611_F612insLDFREYEYDLKWEFPRENLE	Frame-shift insertion	Gain	NA
JAK3	12.5	P-06	M511I	Missense	Gain	0.23
TP53	12.5	P-07	R175H	Missense	Loss	0.42
EPHA3	12.5	P-04	R684*	Nonsense	Loss	0.29
RAD21	12.5	P-06	X125_splice	Splice	Loss	0.42
NPM1	12.5	P-06	W288Cfs*12	Frame-shift insertion	Loss	NA
KRAS	12.5	P-07	G12V	Missense	Gain	0.35

EZH2 causes overexpression of its target genes, granting leukaemic cells a selective growth advantage, mediating chemotherapy resistance (10).

Zhang and colleagues showed widespread effects on glucose, amino acid, and lipid metabolism in multiple cancer cell types when subjected to EZH2 inhibition (11). The most significant effects can be seen in lipid synthesis, where due to the upregulation of SCD1 and ELOVL2, there is an abundance of unsaturated fatty acids, which could affect leukaemic growth and proliferation (11).

Neuroblastoma RAS (NRAS)

Interestingly, P-05 exhibited the $NRAS^{G12D}$ mutation in addition to LOF EZH2 mutations with high allele frequencies (>30%) (Table I). These two mutated genes cooperatively lead to reprogramming in the branched-chain amino acids (BCAA) metabolism and increased mTOR signaling in Kasumi-1, a RUNX1::RUNX1T1-positive AML cell line, along with mouse models and human samples (12). This was achieved via aberrant activation of BCAT1 due to EZH2 inactivation and NRAS cooperation (12).

Tet methylcytosine dioxygenase 2 (TET2)

P-02 exhibited two TET2 mutations: N752Kfs*60 frame-shift deletion and nonsense Q1664* mutation. The patient previously underwent re-induction therapy and MUD allo-SCT but succumbed four months later due to disease progression and unresponsiveness to salvage chemotherapy. Loss of TET2 has been shown to speed up leukemogenesis and enhance leukaemia stem cell self-renewal in the bone marrow (13). Regarding its role in metabolic reprogramming, TET2 directly modulates the expression of HMG-CoA synthase (HMGCS1), which is crucial for cholesterol synthesis; thus, the loss of TET2 results in decreased HMGCS1 expression, impacting the mevalonate pathway and altering lipid metabolism in AML, potentially leading to metabolic reprogramming that generates chemotherapy-resistant TET2-deficient leukemic cells (14). Furthermore, TET2 plays a key role as a negative regulator of the urea cycle and arginine production which leads to mTORC1 suppression. Hence loss of TET2 leads to promotion of cell growth and inhibition of autophagy (15). Due to the high reliance of TET-deficient leukemic cells on metabolic alterations, these types of leukemic cells may be more sensitive to lipid metabolism inhibitors such as statins.

Janus Kinase 3 (JAK3)

Despite undergoing three different treatment regimens, including salvage chemotherapy, P-06 had the lowest overall survival (15 months) compared to other R/R RUNX1::RUNX1T1-AML patients (Table I). The treatment resistance and accelerated leukaemic development may be ascribed to the metabolic effects induced by the $JAK3^{M511I}$ mutation in this patient. $JAK3^{M511I}$ is a gain-of-function (GOF) mutation, causing the JAK/STAT signalling pathway to be upregulated. Additionally, RUNX1::RUNX1T1 down-regulates CD45, a negative regulator of the JAK/STAT pathway (16), resulting in a more significant hyperactivation of the JAK/STAT pathway. This may have resulted in severely adverse outcomes for the patient as STAT proteins have numerous roles in reprogramming the metabolic processes to regulate tumour progression and drug resistance (17). For example, JAK3 activation is a crucial precursor for STAT5 recruitment; hence, its hyperactivation results in STAT5 hyperactivation, which has been demonstrated to enhance glycolysis in AML cells by activating the promoters of glycolytic genes (18).

CONCLUSION

Certain mutations that alter metabolism could enable pre-leukaemic cells to induce RUNX1::RUNX1T1 AML as well as, adapt and resist treatment. EZH2, NRAS, TET2, and JAK3 have substantial non-canonical functions in regulating metabolic activity; thus, mutations in these genes and their implications for patients can be explored in line with clinical data. Further metabolomic studies are needed to characterize the role of these mutations, specifically in RUNX1::RUNX1T1-AML, and the mechanism of resistance in response to specific treatments. Subsequent research should focus on functional validation by metabolomic profiling, CRISPR-mediated gene editing to evaluate metabolic dependencies, and therapeutic screening targeting modified metabolic pathways in relapsed or refractory AML patients.

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