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Investigation of Leukemogenic Mechanisms using Metabolomic Approaches

Daniel Alex Anand [1,*], Kamisetty Venkata Pranava Kavya [1], Antony V Samrot [2,*], S Elizabeth Amudhini Stephen [3], Shimri Fernando [4], Jane Cypriyana Pedinedi Jerald [5]

1 Department of Bioinformatics and the Centre for Molecular Data Science and Systems Biology, Sathyabama Institute of Science and Technology, Chennai – 600119, India; danielalexanand@gmail.com (D.A.A.);
2 School of Bioscience, Faculty of Medicine, Bioscience & Nursing, Jalan SP 2, Bandar Saujana Putra, Jenjarom, 42610, Selangor, Malaysia; antonysamrot@gmail.com (A.V.S.);
3 School of Science and Humanities, Karunya Institute of Technology and Sciences, Coimbatore -641114, India
4 Department of Bioinformatics, Stella Maris College, Chennai -600086, India
5 Department of Biotechnology, School of Bio and chemical engineering, Sathyabama Institute of Science and Technology, Chennai – 600119, India
* Correspondence: danielalexanand@gmail.com;

Scopus Author ID 56270163200
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Abstract: In the bone marrow, the rapid distribution of white blood cells, which agglomerate and ultimately associate with normal blood cells, characterizes leukemogenic processes. Leukemogenic system AML is a complex neoplastic disorder characterized by naive myeloid cell upsurge and bone marrow incompetence. AML is the most common form of leukemia that affects the elderly. One of the prognostic characteristics of this response is the predominance of triggering the FMS-like tyrosine kinase 3 mutations (FLT3), in particular the probability of internal tandem duplication (FLT3-ITD). AML has a poor prognosis of the FLT3-ITD mutation. Metabolic profiling is the ability to understand metabolite modality. In this study, we attempted to understand AML with FLT3 from a metabolomic perspective. It has identified all the metabolites involved in Acute Myeloid Leukemia (AML) and their pathways. AML is a hematologic disease arising from the proliferation and intensification of malignant myeloid cells. In the past, fewer metabolites, such as inborn metabolism defects, were used to diagnose complex metabolic diseases and monogenic disorders. The results of this study have helped to create metabolic pathways for patients FLT3 / ITD, and we have further investigated how these metabolites are relevant from a network biology perspective. With Cytoscape and its plug-ins, such as Metscape, we studied molecular networks. Metabolisms of arachidonic acid and purine in cell metabolites during glycolysis and gluconeogenesis in plasma metabolites are pathways with the greatest impact of AML FLT3 / ITD, urea cycle, and metabolism of arginine, proline, glutamate, aspartate, and asparagine metabolism.

Keywords: leukemia; mutations; FLT3 ITD.

1. Introduction

1.1. Metabolomics.

Metabolism is a sequence of chemical reactions in life-sustaining living organism cells. Cellular regulation cycle metabolites end products. The metabolomics technique analyses all metabolites in cells, bio-liquids, tissues, or organisms that are cultivated or studied under specified conditions.

The extraction and analysis of the information in a biological sense from the large amount of data provided by high throughput analyzers is another challenge in the metabolomics. The key methods for analyzing a large number of metabolites simultaneously are mass spectrometry, combined with a variety of chromatographic separation techniques, including liquid or gas chromatography or NMR. Metabolomics is the scientific study of metabolite chemical processes, the molecular substrates, medium, and metabolism products [1,2].

There is a normal karyotype in a wide number of patients (roughly 45%) diagnosed with AML. The intermediate clinical prognosis is graded because these patients have no clinical reference markers. The biological origin of AML is still unclear. KIT, FLT3, NPM1, CEBPA, RAS, WT1, BAALC, ERG, MN1, DNMT, TET2, IDH, ASXL1, PTPN11, and CBL are now the genes identified. FLT3 in cells of leukemia is a gene alteration or mutation. This mutation occurs among 20 to 30 percent of people with AML. The FLT3 gene is the code that allows the white blood cells to produce a protein called FLT3. The development of too many abnormal leukemia cells is empowered by a gene mutation [3,4].

AML is a heterogeneous, malignant disorder that ensures a need to develop our understanding of AML biology. AML is a heterogeneous, malignant disorder that ensures a need to develop our understanding of AML biology. AML is Acute Myeloid Leukaemia. A prognostic feature associated with a reduced response is the presence of FMS-like tyrosine kinase 3 (FLT3) activating mutations, especially the incidence of internal tandem duplication (FLT3-ITD). Even if poorly understood, differential metabolic and signaling pathways to FLT3-ITD can lead to poor prognosis [5-7].

FLT3 is a tyrosine kinase receptor with essential functions in hematopoietic stem and frontal cell survival and proliferation. It mutates in approximately one-third of patients with acute myeloid leukemia (AML) either by internal tandem duplication (ITDs) of the juxtamembrane domain or by point mutations normally involving a kinase domain (KD). The two types of mutations trigger FLT3. Several studies have shown that due to recurrence, AML patients with FLT3 / ITD mutations are partly treated. This led to the development of a number of small inhibitors of FLT3-activated tyrosine kinase (TKI) molecules. FLT3 in cells of leukemia is a gene alteration or mutation. This mutation occurs among 20 to 30 percent of people with AML. The FLT3 gene is the code that allows the white blood cells to produce a protein called FLT3. A gene mutation causes the development of so many anomalous leukemia cells. The type of internal tandem FMS tyrosine kinase-3 is one of the most frequently found mutations in patients with a prevalence of 20-30 percent in acute myeloid leukaemia (AML) (FLM3-ITD). The ITDs are very different in dimension. The FLT3-ITD consists of duplicate sequence frames, most (70%) in the juxtamembrane domain (JMD), and all others in tyrosine kinase domain 1 (TKD1). This mutation is associated with an aggressive disease form and carries an increased chance of relapse in about 25-30 percent of patients with AML [8-10].

The effective targeted development of cancer depends on the detection of diseases associated with driver mutation that is responsible for the pathogenesis of ’‘passenger' mutation malignancies that are dispensable for cancer initiation or maintenance. Clinically successful clinical trials will undoubtedly discriminate against passenger accident driving powers and provide useful insights into human cancer biology [11,12]. Activating internal tandem duplication (ITD) mutations in FLT3 (FLT3-ITD) are found in around 20 percent of patients with acute myeloid (AML) and are related to poor prognosis [13,14]. A tyrosine kinase receptor that regulates the proliferation and differentiation of hematopoietic stem cells is coded by the

FLT 3 gene. Internal tandem duplication of the acute myelogenous leukemia (AML) FLT3 gene (FLT3/ ITD) has been reported and may be associated with poor prognosis. [15-17].

2. Materials and Methods

2.1. Human metabolome database (HMDB).

This analysis was carried out using metabolomic data obtained from HMDB Version 4.0 [18] using twenty-one known metabolites of plasma, thirty-three known metabolites of FLT3 leukemia [19-24]. Biologically essential pathways trace these metabolic properties. The metabolites concerned cancer functions, cell growth, purine and metabolism involvement, cysteine/methionine metabolism, tryptophan metabolism, carnitine-mediated fatty acid oxidation, and lysophospholipid metabolism [25-28]

2.2. Metaboanalyst.

With Metaboanalyst software, the characteristics separating FLT3-ITD from FLT3-WT AML were identified, and 21 plasma and 33 cellular metabolites were annotated for recognized metabolites and differentiated from FLT3 status. Metabolites with a greater abundance of FLT3-ITD were to be analyzed here. These included (1) organic acids from previous studies: 3-methyl-2-oxovaleric acid associated with the metabolism of isoleucine, pyridine-2,3-dicarboxylate, 6-carboxyhexanoate (also commonly referred to as pimelic acid, which has been reported to be higher in uremic serum patients) and methyl indole-3-acetate; (2) amino acids and intermediates such as guanine, N-acetyl arginine, N-alpha-acetyl-L-Lysine, N-acetyl-DL-glutamic acid, L-carnitine, N-acetyl glycine, GABA, N-acetyl-amine, cysteine-S sulfate, and threonine/homoserine; (3) phosphocholine. Metabolites L-cysteic acid and asparagine were considered to be less abundant in FLT3-ITD vs. FLT3-WT patients.

List of 21 plasma metabolites:

- Guanine
- Pyridine-2,3-Dicarboxylate
- N-Alpha-Acetyl-L-Lysine
- N-Acetyl glycine
- GABA
- N-Acetyl-L-Alanine
- Phosphocholine
- Diphenylamine
- 3-Methyl-2-Oxovaleric Acid
- L-Carnitine
- Cysteine-S-sulfate
- 6-Carboxyhexanoate
- Methyl Indole-3-Acetate
- Threonine/Homoserine
- N-Acetyl-DL-Glutamic Acid
- N-Acetyl-Arginine
- Asparagine
- L-Cysteic Acid
- 4-Acetamidobutanoate
- 3-Hydroxydecanoic acid
- Betaine

List of 33 cellular metabolites:

- Xylenesulfonate
- Succinate
- Disaccharide-6C/6 C
- L-Acetylcarnitine
- Glyceraldehyde/Lactate
- Glucose/Fructose
- Inosine
- Adenosine 5′-Monophosphate
- Allopurinol
- Guanosine
- Hypoxanthine
- Adenosine
- LysoPE (p-526.2933–12.92; 22:6)
- Tryptophan
- LysoPE (n-500.2768-12.71; 20:4)
- Benzoate

- Formyl-5-hydroxykynurenamine
- LysoPE (n-452.2772-13.35; 16:0)
- C6H12O6-HEXOSE/KETOSE/INOSITOL
- L-Carnitine
- LL-2,6-Diaminoheptanedioate
- L-leucyl-L-proline
- LysoPE (p-502.2908-12.90: 20:4)
- Arachidonic Acid (20:4)
- 4-oxoproline
- Glycodeoxycholic acid
- Palmitoleic acid
- Citramalate
- LysoPE (n-480.3097-14.59: 18:0)
- Guanine
- L-Methionine
- Leucine
- 2-Alpha-D-glucosyl-D-glucose

MetaboAnalyst is an online resource collection established for metabolomic data analysis and interpretation by Wishart Research Group members of the University of Alberta [30]. MetaboAnalyst offers a broad range of data input types commonly produced by metabolomic studies, including raw spectral GC / LC-MS, MS / NMR peak lists, maximum intensity chart NMR / MS, NMR, and MS tables and metabolic concentrations [29-31].

A PDF report is developed by MetaboAnalyst [31], which documents in writing each step of the study and shows graphical and table results. Users can access data files and PNG image files that have already been processed. This includes the Human Metabolome Database (HMDB) as well as the Small-Molecule Pathway Database and the Drug Bank and Toxin / Target Databases. MetaboAnalyst is one of a number of databases on metabolomics. The HMDB contains more than 7,900 human metabolites and around 7,200 associated DNA and protein sequences associated with these metabolites. While 6,707 drug targets and 4,228 non-redundant drug targets are included in the Drug Bank, the T3DB contains over 2900 specific environmental pollutants and toxins. Information on medicine is also supported by T3DB. SMPDB completes the suite of pathway charts for over 350 human pathways of metabolism and disease [32-34].

2.3. Cytoscape, metscape, and cytohubba.

For the visualization of molecular interaction networks and integration with gene expression profiles and other state data, Cytoscape was used. Additional functionality is available as plug-ins. Network and molecular profiling analysis plug-ins, new templates, additional file format support, and database connexion and broad network search are available. Metscape is a Cytoscape plug-in used for visualizing and analyzing metabolomics information in the context of human metabolic networks. Metscape allowed us to trace the links between metabolites and genes by querying this database, to visualize compound networks, and to view compound structures as well as reaction, enzyme, gene, and pathway information. We could construct subnetworks that consist of compounds and responses from a given pathway by applying the pathway philter. Metscape was used for uploading experimental data and visualizing and exploring compound networks over time or conditions of the experiment. These complex shifts are visualized using the color and scale of the nodes. Metscape showed the entire metabolic network or some of the path-specific networks in the database [36]. Cytohubba given the eleven topological analysis methods consisting of degree, Edge Percolated Component, Maximum Neighborhood Component, Maximum Neighborhood Component Density, Maximal Clique Centrality, and six centralities (Bottleneck, Excentricity, Closeness, Radiality, Betweenness, and Stress) centered on the shortest paths. It uses ranking features to rank various nodes in a network, and genes are recorded based on their values.

2.4. KEGG.

The list of genomes, biological methods, diseases, medications, and chemical compounds is KEGG. KEGG is known as the Encyclopedia of Genes and Genomes of Kyoto. The KEGG is used for bioinformatics research and education, including data processing for genomics, metagenomics, metabolomics, and other omics studies, modeling and simulation of system biology, and translation analysis for drug discovery [37].

The KEGG PATHWAY database (the cable diagram database) is the core of the KEGG tool. The pathway map involves different entities, including genes, proteins, RNAs, chemical compounds, glycans and chemical reactions, and genes and therapeutic targets of diseases that are used as individual data entries in the other KEGG databases.

3. Results and Discussion

To perform Pathway Assessment, MetaboAnalyst software was used. Sixteen significantly affected metabolites of interest, which were significantly affected by FLT3-state metabolism and pyruvate metabolism, were reported in the cell metabolome pathway analysis and were metabolites involved in purine or biosynthesis pathways. In both patient samples, essential pathways linked to disease progression include purine and cysteine, and methionine metabolism.

Table 1 shows the full results of the pathway analysis. Since we evaluated many pathways simultaneously, statistical p values for the enrichment study are also suitable for several experiments. In particular, the total value of the compounds in the path is that of the total number of compounds; the sum of the actual hits is the corresponding value of the loaded user data; the value of the raw p is the original p-value from the enrichment analysis; the value of the holm p is the p-value of the Holm-Bonferroni method; the value of the FDR p is the p-value changed from the false discovery.

Table 1. Pathways affected by 33 cellular metabolites.

Pathway	Total	Expected	Hits	Log P	Negative Log P	Holm adjust	FDR	Impact
Purine metabolism	65	1.0903	6	0.000513	7.5757	0.043	0.04	0.09707
Neomycin, kanamycin and gentamicin biosynthesis	2	0.03355	1	0.033278	3.4029	1	1	0
Aminoacyl-tRNA biosynthesis	48	0.80516	3	0.04397	3.1243	1	1	0
Galactose metabolism	27	0.4529	2	0.073486	2.6107	1	1	0.08787
Valine, leucine and isoleucine biosynthesis	8	0.13419	1	0.12684	2.0648	1	1	0
Tryptophan metabolism	41	0.68774	2	0.14906	1.9034	1	1	0.14305
Butanoate metabolism	15	0.25161	1	0.22501	1.4916	1	1	0
Starch and sucrose metabolism	18	0.30194	1	0.26376	1.3327	1	1	0.4207
Citrate cycle (TCA cycle)	20	0.33548	1	0.28854	1.2429	1	1	0.03273

Pathway	Total	Expected	Hits	Log P	Negative Log P	Holm adjust	FDR	Impact
Propanoate metabolism	23	0.38581	1	0.32422	1.1263	1	1	0
Alanine, aspartate and glutamate metabolism	28	0.46968	1	0.3799	0.96785	1	1	0
Cysteine and methionine metabolism	33	0.55355	1	0.43115	0.8413	1	1	0.10446
Biosynthesis of unsaturated fatty acids	36	0.60387	1	0.45992	0.77671	1	1	0
Arachidonic acid metabolism	36	0.60387	1	0.45992	0.77671	1	1	0.3135
Amino sugar and nucleotide sugar metabolism	37	0.62065	1	0.46919	0.75674	1	1	0
Valine, leucine and isoleucine degradation	40	0.67097	1	0.49611	0.70096	1	1	0

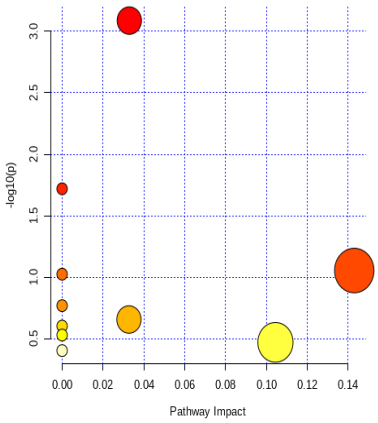
The findings are shown both graphically and in a comprehensive table. The results are summarized. To allow data exploration, an interactive visualization framework has been implemented. There are three levels of view in the graphic output-metabolomic view, path view, and composite view. Figure 1 shows the pathway analysis purine metabolism showing maximum impact.

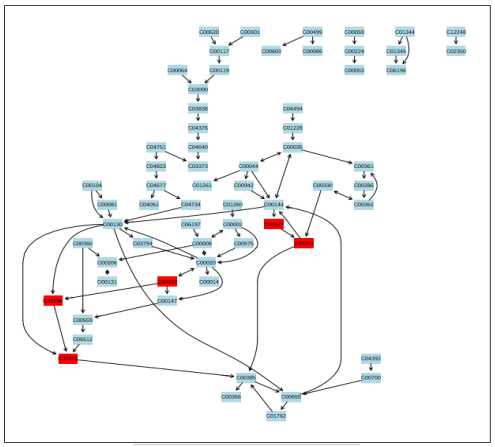
Figure 1. (a) shows the pathway analysis of 33 cellular metabolites, which are differently abundant. Each of that dot represents one of the 16 pathways. Figure 1. (b) shows the pathway of purine metabolism, which has a high impact (the red dot in figure 1. (a) is purine metabolism).

The study of the plasma metabolome pathway identified 12 significantly affected metabolic pathways involving pathways such as cysteine, which involves metabolism of methionine, purine, and biosynthesis, as well as several metabolism pathways of amino acids.

Table 2 displays the findings of the pathway study. Since we search many directions at the same time, statistical p values for enrichment analysis are also suitable for several experiments. In particular, the total number of compounds on the track is the total number of the database; the hits are the actual numbers from the data submitted by the user; the raw p is the original p-value calculated by the analysis of the enriching process; the holm p-value is the p-value changed by the Holm-Bonferroni method; the FDR p is a revised p-value using the False Discovery Rate.

The pathway analysis of 21 different plasma metabolites is shown in Figure 2. Each dot is one of the twelve pathways. Figure 2 demonstrates the pathway with high impact metabolism of alanine, aspartate, and glutamate. Table 3 lists the metabolites not recognized by metaboanalyst.



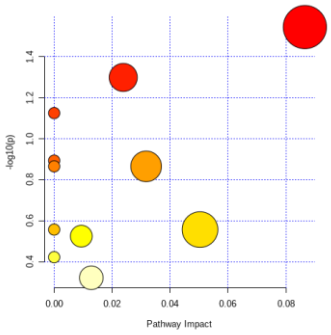


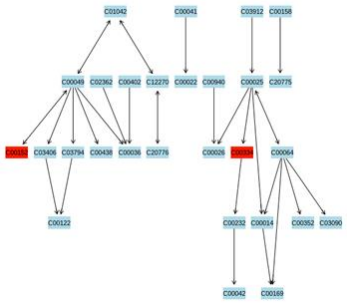
(a) (b)

Figure 1. (a) Overview of pathway analysis purine metabolism showing the maximum impact (b) Schematic representation of the pathway.

Table 2. Pathways effected by the 21 plasma metabolites.

Pathway	Total	Expected	Hits	Raw P	Negative Log P	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	28	0.28903	2	0.032306	3.4325	1	1	0.08654
Glycine, serine and threonine metabolism	33	0.34065	2	0.043793	3.1283	1	1	0.05034
Arginine and proline metabolism	38	0.39226	2	0.056589	2.8719	1	1	0.02385
Valine, leucine and isoleucine biosynthesis	8	0.082581	1	0.079832	2.5278	1	1	0
Taurine and hypotaurine metabolism	8	0.082581	1	0.079832	2.5278	1	1	0
Aminoacyl-tRNA biosynthesis	48	0.49548	2	0.085538	2.4588	1	1	0
Arginine biosynthesis	14	0.14452	1	0.13574	1.997	1	1	0
Nicotinate and nicotinamide metabolism	15	0.15484	1	0.14474	1.9328	1	1	0
Butanoate metabolism	15	0.15484	1	0.14474	1.9328	1	1	0.03175
Cysteine and methionine metabolism	33	0.34065	1	0.2925	1.2293	1	1	0
Glycerophospholipid metabolism	36	0.37161	1	0.31467	1.1562	1	1	0.00937
Purine metabolism	65	0.67097	1	0.49785	0.69746	1	1	0.01281





(a) (b)

Figure 2. (a) Overview of pathway analysis alanine, aspartate, and glutamate metabolism showing the maximum impact (b) Schematic representation of the pathway.

Table 3. List of metabolites that are not recognized by metaboanalyst and the used synonyms.

Unrecognized Metabolites	Synonyms from HMDB And Pubchem
LysoPE(n-452.2772-13.35; 16:0)	5Z,8Z,11Z,14Z-Eicosatetraenoic acid
LysoPE(n-480.3097-14.59: 18:0)	Octadecanoyl-lysophosphatidylethanolamine
LysoPE(n-500.2768-12.71; 20:4)	1-hydroxy-2-palmitoyl-sn-glycero-3-phosphoethanolamine
LysoPE(p-502.2908-12.90: 20:4)	Lysophosphatidylethanolamine(20:4/0:0)
LysoPE(p-526.2933–12.92; 22:6)	Lysophosphatidylethanolamine(0:0/22:6)
Arachidonic Acid (20:4)	1-Arachidonoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine

Cytoscape network analysis was performed for all 33 cellular and 21 plasma metabolites from FLT3 / ITD. The KEGG (Kyoto encyclopedia of genes and genomes) gathers ID' S on these metabolites. The KEGG ids were obtained and tabled in Table 4 and Table 5 for these cellular and plasma metabolites.

Table 4. List of plasma metabolites and their KEGG IDs.

PLASMA METABOLITES	KEGGID
N-Acetylglycine	C00033
N-Acetyl-L-Alanine	C04341
Methyl Indole-3-Acetate	C01926
3-Hydroxydecanoic acid	C02774
Asparagine	C00152
Threonine	C00188
Guanine	C00242
L-Carnitine	C00318
GABA	C00334
L-Cysteic Acid	C00506
Phosphocholine	C00588
N-Acetyl-DL-Glutamic Acid	C00624
Betaine	C00719
6-Carboxyhexanoate	C02656
4-Acetamidobutanoate	C02946
3-Methyl-2-Oxovaleric Acid	C03465
Pyridine-2,3-Dicarboxylate	C03722
Cysteine-S-sulfate	C05824
Diphenylamine	C11016
N-Alpha-Acetyl-L-Lysine	C12989
N-Acetyl-Arginine	C00624

Table 5. List of cellular metabolites and their KEGG IDs.

Cellular Metabolites	KEGG ID
Allopurinol	C00695
Lysophosphatidylethanolamine(0:0/22:6)	C05973
1-Arachidonoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	C11695
1-hydroxy-2-palmitoyl-sn-glycero-3-phosphoethanolamine	C00416
L-leucyl-L-proline	C00993
Lysophosphatidylethanolamine(20:4/0:0)	C04438
Octadecanoyl-lysophosphatidylethanolamine	C04438
Adenosine Monophosphate	C00020
Glucose	C00031
Succinate	C00042
L-Methionine	C00073
Tryptophan	C00078
Leucine	C00123
Adenosine	C00212
5Z,8Z,11Z,14Z-Eicosatetraenoic acid	C00219
Guanine	C00242
Hypoxanthine	C00262
Inosine	C00294
L-Carnitine	C00318
Guanosine	C00387
Benzoate	C00539
LL-2,6-Diaminoheptanedioate	C00666
Citramalate	C00815

Cellular Metabolites	KEGG ID
HEXOSE	C00984
4-oxoproline	C01877
Glyceraldehyde	C02154
L-Acetylcarnitine	C02571
Xylenesulfonate	C02824
Disaccharide	C04932
Glycodeoxycholic acid	C05464
Formyl-5-hydroxykynurenamine	C05647
Palmitoleic acid	C08362
2-alpha-D-glucosyl-D-glucose	C19632

The cellular metabolites were analyzed with the use of cytoscape, and from a total of only 23 metabolites, the remaining metabolites were identified, and the recognizable metabolites were analyzed and constructed in the network, with reactions, enzymes, genes, and compounds related to these metabolites.

Table 6. List of cellular metabolites not recognized by cytoscape.

Cellular Metabolites	KEGG ID
L-leucyl-L-proline	C00993
Benzoate	C00539
LL-2,6-Diaminoheptanedioate	C00666
Citramalate	C00815
4-oxoproline	C01877
Glyceraldehyde	C02154
Xylenesulfonate	C02824
Glycodeoxycholic acid	C05464
Palmitoleic acid	C08362
2-alpha-D-glucosyl-D-glucose	C19632

Table 7. List of plasma metabolites not recognized by cytoscape.

Plasma Metabolites	KEGGID
N-Acetyl-L-Alanine	C04341
Methyl Indole-3-Acetate	C01926
3-Hydroxydecanoic acid	C02774
6-Carboxyhexanoate	C02656
Cysteine-S-sulfate	C05824
Diphenylamine	C11016
N-Alpha-Acetyl-L-Lysine	C12989

Build a metabolite network in Metscape and perform a compound-reaction-enzyme-gene analysis. The drug, reaction, enzyme, and genes associated with the query metabolites are visualized, and the connectivity is obtained in this particular study. Tables 6 and 7 lists the cellular and plasma metabolites not recognized by cytoscape.

Table 8. Plasma metabolites in cytoscape.

SUID	CanonicalName	Category	Enzyme.ecnum	Enzyme.name
62	Choline dehydrogenase	Enzyme	1.1.99.1	Choline dehydrogenase
63	Aminobutyraldehyde dehydrogenase	Enzyme	1.2.1.19	Aminobutyraldehyde dehydrogenase
64	Aldehyde dehydrogenase (NAD(+))	Enzyme	1.2.1.3	Aldehyde dehydrogenase (NAD(+))
65	Aldehyde dehydrogenase (NAD(P)(+))	Enzyme	1.2.1.5	Aldehyde dehydrogenase (NAD(P)(+))
66	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)	Enzyme	1.2.4.4	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)
67	L-amino-acid oxidase	Enzyme	1.4.3.2	L-amino-acid oxidase
81	Betaine--homocysteine S-methyltransferase	Enzyme	2.1.1.5	Betaine--homocysteine S-methyltransferase
82	Glycine amidinotransferase	Enzyme	2.1.4.1	Glycine amidinotransferase

SUID	CanonicalName	Category	Enzyme.ecnum	Enzyme.name
83	Amino-acid N-acetyltransferase	Enzyme	2.3.1.1	Amino-acid N-acetyltransferase
84	Carnitine O-octanoyltransferase	Enzyme	2.3.1.137	Carnitine O-octanoyltransferase
85	Carnitine O-palmitoyltransferase	Enzyme	2.3.1.21	Carnitine O-palmitoyltransferase
86	Purine-nucleoside phosphorylase	Enzyme	2.4.2.1	Purine-nucleoside phosphorylase
87	Nicotinate-nucleotide diphosphorylase (carboxylating)	Enzyme	2.4.2.19	Nicotinate-nucleotide diphosphorylase (carboxylating)
88	tRNA-guanine transglycosylase	Enzyme	2.4.2.29	tRNA-guanine transglycosylase
89	Thymidine phosphorylase	Enzyme	2.4.2.4	Thymidine phosphorylase
90	Adenine phosphoribosyltransferase	Enzyme	2.4.2.7	Adenine phosphoribosyltransferase
91	Hypoxanthine phosphoribosyltransferase	Enzyme	2.4.2.8	Hypoxanthine phosphoribosyltransferase
92	Aspartate transaminase	Enzyme	2.6.1.1	Aspartate transaminase
93	4-aminobutyrate transaminase	Enzyme	2.6.1.19	4-aminobutyrate transaminase
94	Branched-chain-amino-acid transaminase	Enzyme	2.6.1.42	Branched-chain-amino-acid transaminase

Table 8 shows the plasma metabolites in Cytoscape, the canonical names, and the EC numbering of the related enzymes.

There are about 50 compounds, 40 reactions, 39 Enzymes, and 68 genes involved in these 13 recognized metabolites. The network in fig shows 198 other compounds and 9 pathways involved in these 13 metabolites mechanism. These pathways are - Glycerophospholipid metabolism; Glycine, serine, alanine Schematic representation of the pathway, and threonine metabolism; Methionine and cysteine metabolism; Purine metabolism; Tryptophan metabolism; Urea cycle and metabolism of arginine, proline, glutamate, aspartate, and asparagine; Vitamin B3 (nicotinate and nicotinamide) metabolism.

4. Conclusions

Acute myeloid leukemia (AML) arises inside the bone marrow (the soft internal portion of certain bones that contain fresh blood cells), but it spreads more commonly into the blood. This can also spread to other parts of the body, including lymph nodes, liver, spleen, cord brain, and spinal cord, and testes. A striking reaction to the need to develop our awareness of the biology of AML. The presence of FMS-like tyrosine kinase 3 (FLT3) activating mutations, in particular internal tandem duplication (FLT3-ITD), is one of the prognoses associated with decreased response [38].

FLT3 is a tyrosine kinase receptor that plays a crucial role in the hematopoietic stem/progenitor cell survival and proliferation. It is mutated in around 1/3 of acute myeloid leukemia (AML) patients by an internal duplication of the juxtamembrane domain (ITD), or point mutations that normally affect the kinase domain (KD). Both forms of mutation constitutively activate the FLT3. Ultra-high-performance liquid-chromatography metabolomic profile spectrometry showed that the plasma status of 21 plasma metabolites and 33 FLT3 metabolites found in leukemic cells differed in abundance. We concentrated on these cell and plasma metabolites separately. Using a metaboanalyst, Cytoscape plug-ins, we performed pathway analysis and defined the most significant and important paths in this system. Using Cytoscape's plug-ins as Metscape and CytoHubba, we concluded that the main pathways for the synthesis of arachidonic acid synthesis and purine in the cellular metabolites Glycoliosis

and Gluconeogenesis and Urea cycles are arginine, proline, glutamate, aspartate, and asparagine metabolism.

In this research, the intermediate pathological markers were used to explain new leukemogenic mechanisms. From the Metaboanalyst analysis, the FLT3 status had considerable influences on purine metabolism and pyruvate metabolism. Important pathways related to disease progression include purine and cysteine and the metabolism of methionines in both patient samples. In AML with FLT3 / ITD, proline, glutamate, aspartate, and asparagine are essential to Cytoscape metabolism of arachidonic acid and purine in cellular metabolites, glycolyses and gluconeogenic metabolism and the urea cycle and of arginine. In the last decade, glutamine has been the most common nutrient studied in the field of metabolism of cancer cells other than glucose. Glutamine has an important role to play in many bio-proliferation processes, including biosyntheses and bioenergy, anti-oxidant defense, modification/gene transcription of chromatin, fast transport of certain amino acids through the plasma membrane, and cell signaling regulations. Both the tissue and the oncogenic background will determine the relative effects of glutamine as well as glutamine-derived metabolites.

In normal proliferating cells and in the context of tumorigenesis, glutamine metabolism is essential for survival, growth, differentiation, and resilience. Finding new intersections between metabolism and disease, revealing new therapeutic opportunities for intervention, can lead to further exploration of ways cellular glutamine affects these different processes and to a study of strategies that cells may adapt to withstand glutamine limitations. Apart from the already established glutamine metabolomics, the other identified key amino acid pathways need elucidation in the context of AML with FLT3 and general hematopoiesis.

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Conflicts of Interest

The authors declare no conflict of interest.

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