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Article

A network-guided approach for unraveling potential pharmacological role of JAK2 and analysis of the frequency the mutations in myeloproliferative neoplasm Saudi Patients

Rowaid M. Qahwaji [1], Ibraheem Ashankyty [1,*], Eman F. Bahakeem [1], Mohammed H. Qari [2], Majed Almashjary, Mohammad Mobashir [3,*]

[1] Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 22233, Saudi Arabia.
[2] Hematology Department, College of Medicine, King Abdulaziz University, Jeddah 22233, Saudi Arabia.
[3] Department of Biomedical Laboratory Science, Norwegian University of Science and Technology, Trondheim - 7491 Norway.
* Correspondence: mohammad.mobashir@ntnu.no (M.M.) and ishankyty@kau.edu.sa (I.M.A.)

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Abstract: JAK2 is a non-receptor tyrosine kinase that regulates cellular proliferation and growth. JAK2 is frequently the target of mutations in cancer, particularly in myeloid leukemia, lymphoid leukemia, and myeloproliferative neoplasms (MPN), because of its important function in hematopoiesis. There is a lot of work being done to discover therapeutic medications for the affected people because these mutations are widespread among various ethnicities throughout the world. Constitutive kinase activation is the most frequent outcome of JAK2 mutations, whether they are point, deletion, or gene fusion mutations. A drug's mechanism of action can frequently be divided into positive (activator, potentiator, agonist, etc.) and negative (inhibitor, blocker, antagonist, etc.) categories. The combined mechanisms of action of various medications on the group of shared targets can be examined using the signed edges of a drug-target network. Herein, our objective was to identify the mutations detected in a previous study by whole genome sequencing using the Mis-eq Illumina Platform. Also, it will help us to find the availability and the frequency of JAK2, CARL and MPL mutations in Saudi CML and MPN patients. Finally, we applied a network-guided approach to predict potential herbal drugs for JAK2. Based on our results, we conclude that JAK2 has significant mutation in in case of MPN and Allicin, Curcumin, and Gingerol could be the potential herbal drugs against targeting MPN.

Keywords: JAK2; mutations; myeloproliferative neoplasm; pharmacogenomics; herbal drugs; putative biomarkers; network medicine

1. Introduction

In cancer, abnormal body cells divide and proliferate in an uncontrolled, unpredictable, irreversible, and monoclonal manner. These abnormal cells can infiltrate nearby tissues and travel to other parts of the body via the lymphatic and circulatory systems.[1-3]. The more than 200 types of cancer impact more than 60 different body organs. Cancer is an intricate illness. The development of cancer is influenced by genetic, biochemical, and environmental variables. the genetic, which includes mutations; the biological, which includes getting viruses that cause cancer; and the environmental, which includes exposure to chemicals and radiation. Cancer can be divided into four basic groups according to its location, and within each group are other types of cancer. Sarcoma, cancer, lymphoma, leukemia, and myeloma[4-11].

Leukemia, lymphoma, and myeloma are examples of hematological cancers. Before 187 years had gone by, Thomas Hodgkin identified the first hematological malignancy; since then, further hematological cancers have been identified, described, and categorized. Hematological malignancies are a large category of disorders that are mostly classified into kinds according to the cells they include in secondary lymphoid organs like the lymphatic system or main lymphoid organs like the bone marrow (BM). Hematological malignancies differ in their biochemical and clinical features. Clinical heterogeneity is the distinction between some patients' responses to treatment and those that do not, between those that respond to treatment and those who do not, and between those that have short half-lives and those that have long half-lives. The unique genetic and biological characteristics that cells from different individuals exhibit are known as heterogonies in biology. Myeloid and lymphoid neoplasms are the two main types of hematological malignancies.

Based on the affected lineage, the World Health Organization (WHO) classified hematological neoplasms. Each lineage divides into different diseases. The three main types of hematological malignancies are leukemia, lymphoma, and plasma cell neoplasms[12-18].

Tyrosine Kinase 2 (TK2), JAK1, JAK2, and JAK3 (TYK2) are members of the JAK gene family. JAK genes encode intracellular, non-receptor proteins called human tyrosine kinases (TKs). Therefore, many of the functions of the cell are controlled by intracellular enzymes that use the phosphorylation process to speed up a range of actions. The human tyrosine kinase protein signals a variety of growth factors, such as cytokines I and II, interleukin-4, IL-10, and IL-2. Each of these growth factors is essential for controlling the proliferation, differentiation, and maturation of hematopoietic progenitor cells. The JAK/STAT signaling system also includes the JAK protein. This pathway facilitates the transfer of information to the nucleus for gene expression. It is also essential for the development of malignancies, immunity, and cell division and death. Normally, the JAK/STAT signaling system is activated by cytokine signals such as interleukin, interferon, and certain growth factors. As soon as the cytokine signal binds to its receptors, the JAK molecules start to function. Once the JAK molecules have drawn the STAT molecules to the receptor, they can be activated. After the activated STAT molecules reach the nucleus, which is the last stage, the transcription of several genes essential for cell growth and survival will begin. Numerous tumors have been connected to the dysregulation of this system, especially hematological malignancies[19-28].

The first formal demonstration of the Philadelphia chromosomes Ph was accomplished earlier. When it was found that the Ph gene causes cancer, imatinib became the first medication to effectively treat this specific type of cancer. Chromosomes 22q11 and 9q34 rearrange to generate Ph, which subsequently served as a cytogenetic marker for CML. The BCR/ABL chimeric oncogene, which codes for an active BCR/ABL fusion tyrosine kinase (FTK), is produced as a result of this translocation. It is currently unclear what cellular or molecular mechanisms led to the creation of BCR/ABL FTK and the onset of leu-kemogenesis in CML, despite the fact that it is increasingly recognized as a critical CML signal. Massive myeloid cell clonal evolution is one of the genetic traits associated with CML. At first, these cells are still capable of differentiating. However, differentiation potential is gradually lost as the disease worsens into a blast crisis and new mutations that give imatinib resistance emerge. The T315I mutation is distinct from all other BCR-ABL domain mutations in patients who are resistant to dasatinib and imatinib. In contrast, one-third of individuals who experience a blast crisis get lymphoblasts, and two-thirds have mye-loblasts. According to a Japanese study on atomic bomb survivors, radiation exposure is thought to be one of the primary causes of sickness[12, 18, 29-40].

By causing hematopoietic cells to enter the cell cycle without the aid of growth factor stimulation, BCR-ABL1 exhibits primary mitogenic activity. In addition to being a strong activator of the JAK/STAT pathway, BCR-ABL1 also has significant effects on pathways associated with uncontrolled proliferation. In order to form complexes with BCR-ABL1, Janus kinases (JAKs) attach to the C-terminus of ABL1. The SRC kinase LYN is activated by the resultant complex. However, it was discovered that neither JAK2 nor the JAK2 binding site on BCR-ABL1 were active in mice with CML-like leukemia[14, 41-47].

JAK2's primary function is to control cytokine signaling, which affects immune response, cell division, proliferation, and survival. Deregulated JAK signaling is strongly associated with solid tumors, immune-inflammatory disorders, and hematological malignancies. Most MPNs with BCR/ABL1 deficiency had the JAK2 V617F mutation. Consequently, JAK proteins were discovered to represent a possible target for treatment. Targeting JAK2 V617F led to the development of several medications, including the JAK inhibitor ruxolitinib. Although it has been shown that medications that target JAK2 kinase delay or stop BCR-ABL1-expressing cells from multiplying and surviving in culture, JAK2-deficient cells are nonetheless susceptible to these medications. Patients with BCR-ABL-negative myeloproliferative neoplasms (MPNs) frequently have the JAK2 V617F mutation[21, 27, 29, 48-52]. While these mutations are less common in patients with myelofibrosis (MF), they are more common in patients with polycythemia vera (PV) than in 50–60% of patients with essential thrombocythemia (ET). Both national and international standards advise using this mutation because it is frequently used to identify MPN. It is still difficult to distinguish ET without a JAK2 mutation from reactive thrombocytosis in a clinical setting.

In ET and MF patients, exon 9 of the CARL gene was shown to have the second-highest number of INDEL (insertions and deletions) somatic mutations, after JAK2. Because of the discovery, the diagnosis of ET and MF is reinforced. The MPL gene has been discovered to have a third mutation that is absent in PV sufferers. As a result, in ET and PMF, CARL and MPL mutations are considered crucial diagnostic genetic markers. The JAK2 gene (Jak2V617f or JAK2 exon 12 mutation), the MPL gene, and the CALR gene are currently thought to be important components needed to meet WHO criteria for an MPN diagnosis. JAK2V617f or JAK2 exon 12 mutations were present in over 95% of individuals with PV, ET, and primary PMF, but not CML. However, mutations in JAK2, CARL, and MPL were discovered in nearly 60%, 20%, and 5% of ET and PMF patients, respectively. Gene changes in specific diseases can sometimes be linked to different test results. For example, compared to ET patients with the JAK2V617F mutation, those with the CALR mutation were younger, had a lower WBC count, a lower hemoglobin level, and a higher platelet count. Many of the

same characteristics were present in PMF patients. The CARL mutation is also linked to a higher percentage of men, a decreased risk of thrombosis, and an enhanced overall survival rate[21, 27, 48-53].

It is commonly known that dietary factors may play a role in the development and management of a variety of human disorders. Many dietary supplements and medicinal plants have been used as a kind of medical panacea for many years and are believed to be beneficial to human health. Three medicinal plants—Curcuma longa (turmeric), Zingiber officinale (ginger), and Allium sativum (garlic)—have been selected because they have been shown to have numerous biological and therapeutic benefits, including the ability to treat respiratory conditions. It is challenging to separate the many plants that contain anti-cancer chemicals from the many that may be utilized to treat pathogen-infected individuals, particularly in a short amount of time. The Zingiberaceae family includes the ginger plant, Curcuma longa. By targeting different cellular pathways or directly inhibiting the viral replication machinery, curcumin, the primary bioactive component of curcumin, has been demonstrated to have a range of pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, and anti-cancer properties against various viruses. Zingiber officinale has been used as a spice for a long time. It is rich in a variety of chemical components, including phenolic chemicals, organic acids, and terpenes. Ginger's phenolic compounds are believed to have health advantages. Gingerols, the main polyphenolic components of ginger, have been connected to possible uses in the treatment of a number of cancers. Allium sativum is one of the most widely used herbs in human history. Garlic's bioactive ingredient, allicin, has been demonstrated to have antiviral properties in living things[19, 54-62].

We are concentrating on Saudi MPN patients in our study because we are certain that they have distinct genetic correlates with distinct clinical features compared to those from other ethnic groups. Because all of the studies and data that were made public had been conducted on Caucasian populations, with the exception of one study from China, Korea, Japan, and Saudi Arabia. We compared the genetic findings with the other clinical and laboratory data and looked at the changes in the JAK, CALR, and MPL genes. Due to the lack of epidemiological data for the Saudi population, it is difficult to allocate patient cases to a specific WHO-MPN group. Here, we used sanger sequencing to confirm our findings based on data generated by the Miseq Illumina Platform and the frequency of such mutations in Saudi CML patients. Additionally, this work integrates data from many pertinent databases and proposes a novel network-guided strategy for the possible involvement of JAK2 in MPN.

2. Methods

4.1. Study design and subjects

The King Abdulaziz University Hospital's (KAUH) ethical committee gave the study authorization to gather samples from patients who were seen in the hematology clinic. Each participant has been given comprehensive information about the study's purpose and requested to sign a permission form that has been authorized by the KAUH ethics committee. Fourteen CML patients and one JMML patient were chosen for this study at the direction of the treating physician (Table 1). These patients have already received a diagnosis and confirmation that they have CML and are undergoing treatment. One healthy person was chosen to serve as a control, and his blood was used. This subject was a healthy person who came to KAUH to donate blood. We ensure that he had no family members with CML and that he was not coming to donate to any of the patients chosen for the trial.

We examined ten CML patients who are now receiving therapy at the King Abdelaziz University Hospital (KAUH) in Jeddah and who have already been diagnosed and confirmed as such. This project requires ethical approval, which will be submitted for as soon as the Deanship of Research approves the application. Since samples will be extracted from the EDTA vial collected for routine CBC at the Hematology lab, patients won't have to undergo any additional procedures. Patients will also be told about the test and asked to sign a consent form, either from their parents if they are under the age of 18 or from themselves if they are above the age of 18.

This study comprised 15 individuals, 14 of whom were found to have CML, while the remaining patient was confirmed to have juvenile myelomonocytic leukemia (JMML), also known as chronic myelomonocytic leukemia (CMML). With an average age of 52 years, nine of the participants were female and the remaining six were male. They came from a variety of ethnic backgrounds. With a mean of the blood counts of 232 Thousand per cubic milliliter (K/ul), 3 Million per microliter (M/ul), 9 Gram per deciliter (g/dl), and 338 K/ul, respectively, their initial complete blood count (CBC) results at the time of diagnosis were very similar to one another: an unexplained increase in the WBCs count, a decrease in the RBCs and Hb, and a normal platelets (PLTs) count.

Since BCR-ABL expression is thought to be the primary criterion in the diagnosis of CML, it was examined using qualitative PCR.All of the participants had positive BCR-ABL results at the time of diagnosis. Seven of the 15 patients had BCR-ABL negative results during treatment, but they continued to follow the plan in order to reach a state of complete deep remission.

Nine individuals were in the chronic phase of the condition, while the remaining six were in the accelerated phase, according to the disease information. The bomb incident did not affect any of the patients. Since ten of the patients do not report being resistant to a particular medicine, five of the patients did. Consequently, the treatment that is given

varies. Table 1 shows that six of the patients were on imatinib, four were taking dasatinib, four were taking nilotinib, and the final patient was taking simply hydroxyurea.

Table 1. Disease information:

Patient	Current clinical stage	Current treatment	Transplantation Plane	Supportive medication if available	Treatment-resistant if available	Family history	Vital status
1	Accelerated phase	Dasatinib	No	Hydroxyurea	Imatinib	No	Alive
2	Accelerated phase	Dasatinib	No	No	Imatinib	No	Deceased
3	Chronic phase	Nilotinib	No	Hydroxyurea	Imatinib	No	Alive
4	Accelerated phase	Imatinib	No	Hydroxyurea	No	No	Alive
5	Accelerated phase	Imatinib	No	Hydroxyurea	No	No	Deceased
6	Chronic phase	Nilotinib	No	No	No	No	Alive
7	Accelerated phase	Imatinib	No	Hydroxyurea	No	No	Deceased
8	Chronic phase	Nilotinib	No	Hydroxyurea	Imatinib	No	Alive
9	Chronic phase	Nilotinib	No	No	No	No	Alive
10	Accelerated phase	Dasatinib	No	Hydroxyurea	Imatinib	No	Alive
11	Chronic phase	Hydroxyurea	No	Tranexamic acid	No	No	Alive
12	Chronic phase	Imatinib	No	No	No	No	Alive
13	Chronic phase	Imatinib	No	No	No	No	Alive
14	Chronic phase	Imatinib	No	No	No	No	Alive
15	Chronic phase	Dasatinib	No	No	No	No	Alive

4.2. Blood collection and DNA extraction

Patients who are on an EDTA tube will have a venous blood sample taken. The Qiampl DNA blood Mini Kit, a commercially available kit, will be used to extract the total genomic DNA and mRNA from the peripheral blood leukocytes. The Qubit® fluorometer or the nanodrop spectrophotometer will be used to measure the yield of DNA and mRNA. Then, using these kits (QIAGEN OneStep RT-PCR Kit), (RNeasy Mini Kit), (QIAshredder), and (QuantiFast Mutiolex PCR Kit), the individual JAK2 gene will be amplified using polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR) techniques.

4.3. DNA concentration assessment

The Nanodrop 2000 spectrophotometer (Thermo Scientific) at the King Fahad Medical Research Center (KFMRC) core facility was used to evaluate the isolated DNA.

4.4. DNA amplification

Using the Top Taq DNA Polymerase Kit, Qiagen, and a set of primers (JAK1 Exon 6, 11, 14, and 15) from SynBio Technologies, the extracted DNA was amplified by Polymerase Chain Reaction (PCR).

4.5. GEL electrophoresis

PCR products were visualized and the PCR product size was determined by running the PCR on 1% agarose gel.

4.6. PCR products purification

Following the manufacturer's instructions, the PCR Fragment Purification Kit (Dongsheng Biotech, DSBIOTM) was used to purify and clean the PCR product.

4.7. Sanger sequencing

The JAK2 gene sequence was obtained by DNA sequencing. The nucleotide sequence of DNA was ascertained using Sanger sequencing in order to search for potential mutations in the JAK2 gene. The Applied Biosystems™ Sanger Sequencing Kit was used to carry out the sequencing. The same primer that was used in the PCR reactions was used to do the sequencing on the PCR product. Two reactions were carried out using the PCR products as a DNA template: one using the forward primer and the other using the reverse primer.

The Sanger sequencing method was employed to search for mutations in the JAk2 gene. Additionally, the same primer used in the PCR reactions will be utilized for the DNA sequence analysis utilizing the Applied Biosystems TM Sanger Sequencing Kit. The sequences will be analyzed and compared to the reference JAK2 gene sequence using the BLAST program.4.8. Data analysis

The sequences were examined and contrasted with the reference gene sequence using BioEdit. Mutation Taster was used to determine which mutations were statistically significant.

4.9. Bioinformatics analysis details

In order to find possible targets and herbal medications that can interfere with the active signaling pathways, we planned to look at the involvement of JAK1 in CML. The FunCoup PPI network database was utilized to prepare the JAK1 network. The GECIP toolbox and KEGG database were used for the enriched pathway analysis of JAK1 genes.
Throughout the entire experiment, JAK1 networks have been generated using FunCoup2.0 and displayed using Cytoscape. Most of our programming and computations have been done in MATLAB. FunCoup predicts four forms of functional coupling or linkages, including metabolic processes, signaling pathways, protein complexes, and physical interactions between proteins[10, 63-66].

4.10. Docking approach:

The UniProt database (www.uniprot.org) provided the protein sequences. The 3D structures of herbal medications were obtained in SDF format from PubChem. PyMol was used to visualize protein and ligand structures. The aforementioned proteins were homologously modeled using the Swiss Model website (www.swissmodel.org). The model structures have been selected based on the findings of the GMQE, QMEANDisCo, and QMEAN Z-score investigations. The GMQE (Global Model Quality Estimate) and QMEANDisCo global scales for overall model quality range from 0 to 1, with greater values denoting higher predicted quality. Using the Swiss PDB Viewer with all parameters set to default, the energy of proteins' three-dimensional structures was decreased after hydrogen atoms were added.

A search engine was used to find the total number of active sites, together with details on their amino acid sequence, cavity locations, and cavity average volume. Therefore, using the default probe radius (1.4 Å), the binding pocket of each of the proteins indicated above was predicted using the Discovery Studio and CASTp servers.

The molecular docking experiment was conducted using PyRx (AutoDock Vina). The atomic coordinates of the protein and its ligand were transformed into pdbqt files in preparation for docking. The binding pocket was created using AutoDock Vina and grid box dimensions with predetermined spacing and size pointing in the x, y, and z directions. The default parameters were used in the docking studies. Based on the number of hydrogen bonds, other hydrophobic interactions, and the lowest binding free energy (delta G), the compounds with the most beneficial binding configurations were selected. Hydrogen bonds, carbon-hydrogen bonds, van der Waals contacts, pi-sigma, pi-sulfur, alkyl, pi-alkyl, pi-pi T-shaped, and halogen links were among the several interactions that Discovery Studio and PyMol investigated[67-69].

3. Results

3.1. Clinical relevance of JAK2: As an initial step, we have analyzed the expression patterns of JAK2 of clinical samples in different organs both in terms of mRNA and protein expressions by using Protein Atlas database (Figure 1a, 1b, and 1c). In Figure 1a, we observe that JAK2 display very strong expression (mRNA and protein) in case of bone marrow and lymphoid tissues followed by respiratory system and liver and gall bladder. Similar pattern could be seen in protein expression overview in Figure 1b. Analysis of JAK2 expression for HPA dataset shows that heart muscle as maximum expression level and then bone marrow and lymphatic tissues (Figure 1c). After analyzing Figure 1, we could say that bone marrow and lymphatic tissues are JAK2 enriched which means that JAK2 might have potential role in the diseases associated with bone marrow and lymphatic tissues. Among the mRNA and protein expression in different types

human tissues/organs, JAK2 is dominantly expressed in terms of mRNA and protein (Figure 1a) and similar observation could be seen in protein expression overview (Figure 1b) and HPA dataset (Figure 1c).

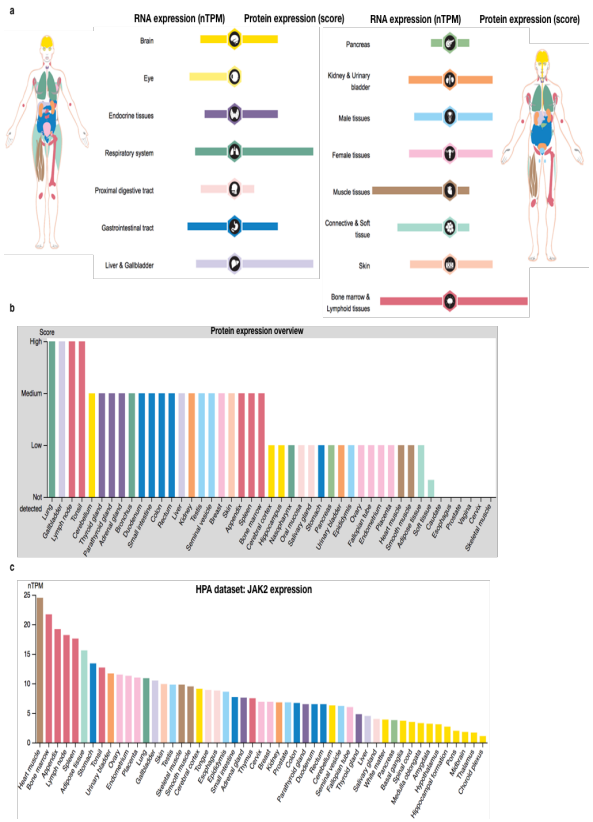

Figure 1. Clinical relevance of JAK2. (a) mRNA and protein expression in different types human tissues/organs. (b) JAK2 Protein expression overview in descending order in different human organs. (c) JAK2 expression profiling for different organs based on HPA dataset in descending order.

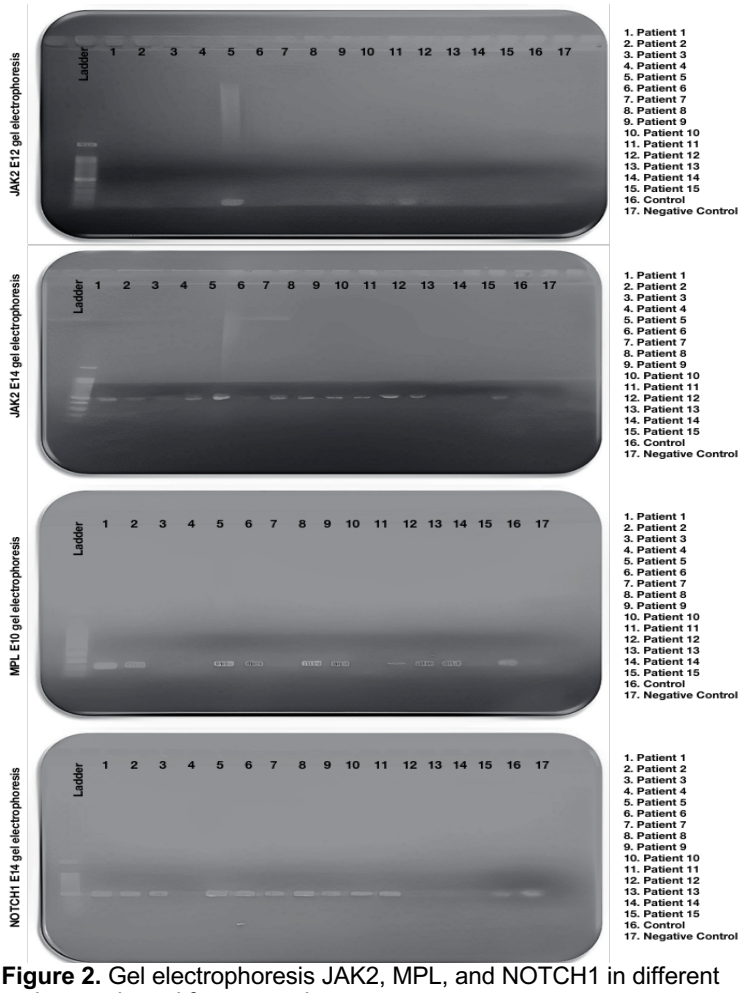

Figure 2. Gel electrophoresis JAK2, MPL, and NOTCH1 in different patients selected for our study.

After performing clinical relevance of JAK2 in terms of expression, we also fetched the overall known clinical relevance of JAK2 mutations in terms of different human disease for which we used www.uniprot.org. In this portal, human JAK2 was fetched and from the disease section, all the known identifiers have been

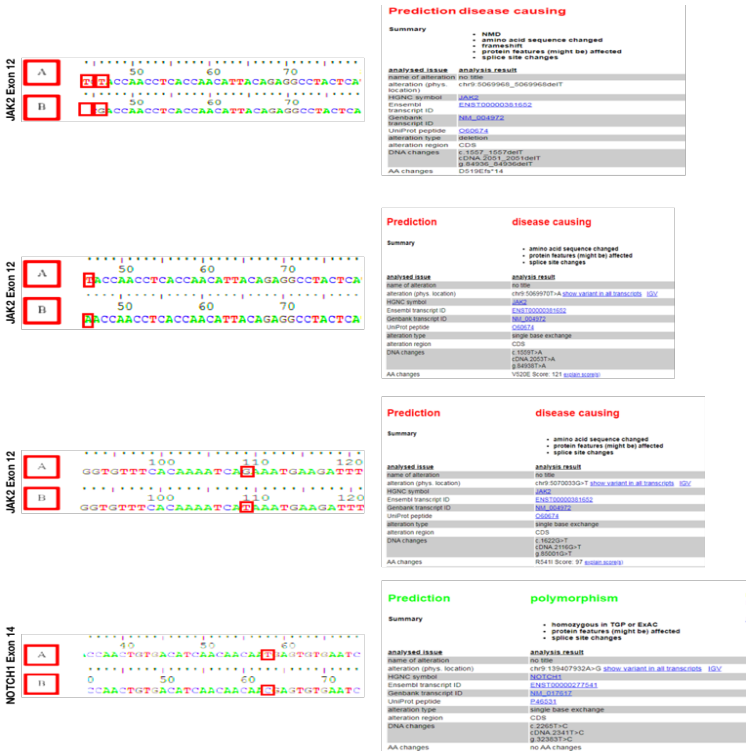

Figure 3. Sanger sequencing of the selected exons of JAK2 and NOTCH1 and disease prediction. DNA sequencing of the JAK2 gene (Exon 12) in CML patient. JAK2 gene (Exon 12) reference sequence and JAK2 gene (Exon 12) patient No. 6 sequence shows a nucleotide change at 1557 (c.1557_1557delT) followed by Mutation Taster report of JAK2 (Exon 12) mutation. JAK2 gene (Exon 12) reference sequence and JAK2 gene (Exon 12) patient No. 5 and 7 sequence shows a nucleotide change at 1559 (c.1559T>A) followed by Mutation Taster report of JAK2 (Exon 12) mutation. JAK2 gene (Exon 12) reference sequence (B) JAK2 gene (Exon 12) patient No. 9 sequence shows a nucleotide change at 1622 (c.1622G>T) followed by Mutation Taster report of JAK2 (Exon 12) mutation. DNA sequencing of the NOTCH1 gene (Exon 14) in CML patient. NOTCH1 gene (Exon 14) reference sequence and NOTCH1 gene (Exon 14) patient No. 14 sequence shows a nucleotide change at 2265 (c.2265T>C) followed by Mutation Taster report of NOTCH1 (Exon 14) mutation.

fetched and presented in Table 2. Here, 13 natural variants were seen which are well-established to be associated with JAK2 mutations and majority of these mutations infer to human cancer including leukemia. Thus, it motivates for further identification of the mutations mainly in CML.

3.2. Gel elctrophoresis shows the different exons of JAK2, MPL, and NOTCH1 in CML patients: We found four genetic alterations, three of them in exon 12 of the JAK2 gene and the last one in exon 14 of the NOTCH1 gene (Table 3). By direct sequencing we found one deletion mutation result in frame shift in exon 12 of JAK2 gene (c.1557_1557delT) (Figure 2). Two more mutation have been found in exon 12 of JAK2 gene both of them are single nucleotide polymorphism (SNPs) (c.1559T>A, c.1622G>T) (Figure 2). Last reported mutation has been found in exon 14 of NOTCH1 gene which is silent mutation result in no amino acid changed (c.2265T>C) (Figure 2).

After performing the basic analysis of JAK2, we have performed the mutational analysis of genes in case of CML for which TCGA database was utilized by using cBioPortal (Figure 3). From mutational analysis (Figure 3a), we observe that SF3B1, ATM, NOTCH1, TP53, CHD2, and POT1 as the extremely high mutated genes and the JAK1 and JAK2 seems to be quite less mutated which have added after selecting top 50 mutated genes because our study focuses on JAK1. Based on the network of genes and the associated/inferred pathway network (Figure 3b) analysis, ATM, JAK1, TP53, KRAS, and BRAF appear to be the most critical genes because they comparatively control critical biological pathways and the genes which we have processed for possible herbal drug-target predicion. Furthermore, we also presented the exon sequence details and the summary of reported mutations in Tables 4 and 5.

Table 2. Known natural variants of JAK2 associated with human diseases.

Type	ID	Position(s)	Description
Natural variant	VAR_041716	127	in dbSNP:rs56118985
Natural variant	VAR_041717	191	in an ovarian serous carcinoma sample; somatic mutation
Natural variant	VAR_041718	346	in dbSNP:rs55667734
Natural variant	VAR_041719	377	in dbSNP:rs55953208
Natural variant	VAR_041720	393	in dbSNP:rs2230723
Natural variant	VAR_032693	537-539	in myeloproliferative disorder with erythrocytosis
Natural variant	VAR_032694	538-539	in myeloproliferative disorder with erythrocytosis
Natural variant	VAR_032695	539	in myeloproliferative disorder with erythrocytosis; requires 2 nucleotide substitutions; dbSNP:rs121912473
Natural variant	VAR_043129	584	in dbSNP:rs17490221
Natural variant	VAR_032696	607	in AML; dbSNP:rs121912472
Natural variant	VAR_032697	617	in PV, THCYT3 and AML; associated with susceptibility to Budd-Chiari syndrome; somatic mutation in a high percentage of patients with essential thrombocythemia or myelofibrosis; leads to constitutive tyrosine phosphorylation activity that promotes cytokine hypersensitivity; dbSNP:rs77375493
Natural variant	VAR_067534	617	in THCYT3; dbSNP:rs77375493
Natural variant	VAR_041721	1063	in dbSNP:rs41316003

Table 3. Gel electrophoresis.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Control 1	Neg. control
JAK2 E12	+	+	+	+	++ + Smear	+	+	+	+	+	++	++	+	+	+	+	-

JAK2 E14	+++	++	+	++	+++ Smear	+	++	++	++	++	++	++	+	+	++	+	-
MPL E10	+++	++	+	+	+++	++	+	++	++	+	++	++	++	+	++	+	-
NOTCH1 E14	+++	++	++	+	+++	++	++	++	++	++	++	+	+	+	++	++	-

Table 4. Exon sequence details.

JAK2 (EXON 12)	F	ACCAACCTC ACCAACATTA CAGAG
	R	AAAAGGACAAAAAAGACAGTAATGAGTATC
JAK2 (EXON 14)	F	GGGTTTC CTCAGAACGT TGA
	R	TCATTGCTTTCCTTTTTCACAA
MPL (EXON 10)	F	TGGGCCGAAGTCTGACCCTTT
	R	ACAGAGCGAACCAAGAATGCCTGT
NOTCH1 (EXON 14)	F	TACAAGTGCGACTGTGACCC
	R	ATACACGTGCCCTGGTTCAG

Table 5. Summary of reported mutations and their outcomes

Gene	Exon No.	Patient No.	DNA change	Amino acid (AA) change	Type of mutation	Effect of the mutation on the DNA level	Effect of the mutation
JAK2	12	6	c.1557_1557delT	D519Efs*14	Deletion (Frameshift)	NMD Amino acid sequence changed Frameshift Protein features might be affected Splice site changes	Disease causing
JAK2	12	5, 7	c.1559T>A	V520E	Single base exchange	Amino acid sequence changed Protein features might be affected Splice site changes	Disease causing
JAK2	12	9	c.1622G>T	R541I	Single base exchange	Amino acid sequence changed Protein features might be affected Splice site changes	Disease causing

NOTCH1	14	14	c.2265T>C	no AA changes	Single base exchange	Homozygous in TGP or ExAC Protein features (might be) affected Splice site changes	Polymor-phism

We analyzed all the previously reported mutational exons of JAK1 by the Sanger sequencing method. From this, we found two genetic alterations in exon 6 and 15 of the JAK1 gene. Direct sequencing resulted in two silent mutations in exon 6 and exon 15: (c.579T>C and c.2049C>T). The single nucleotide polymorphism (SNPs) are T to C transition at nucleotide 579 (c.579T>C) in exon 6 and a C to T transition at nucleotide 2049 (c.2049C>T) (as shown in Figure 1a, 1b, and 1c) in exon 15. The details of all the sequences as chromatogram are available on request.

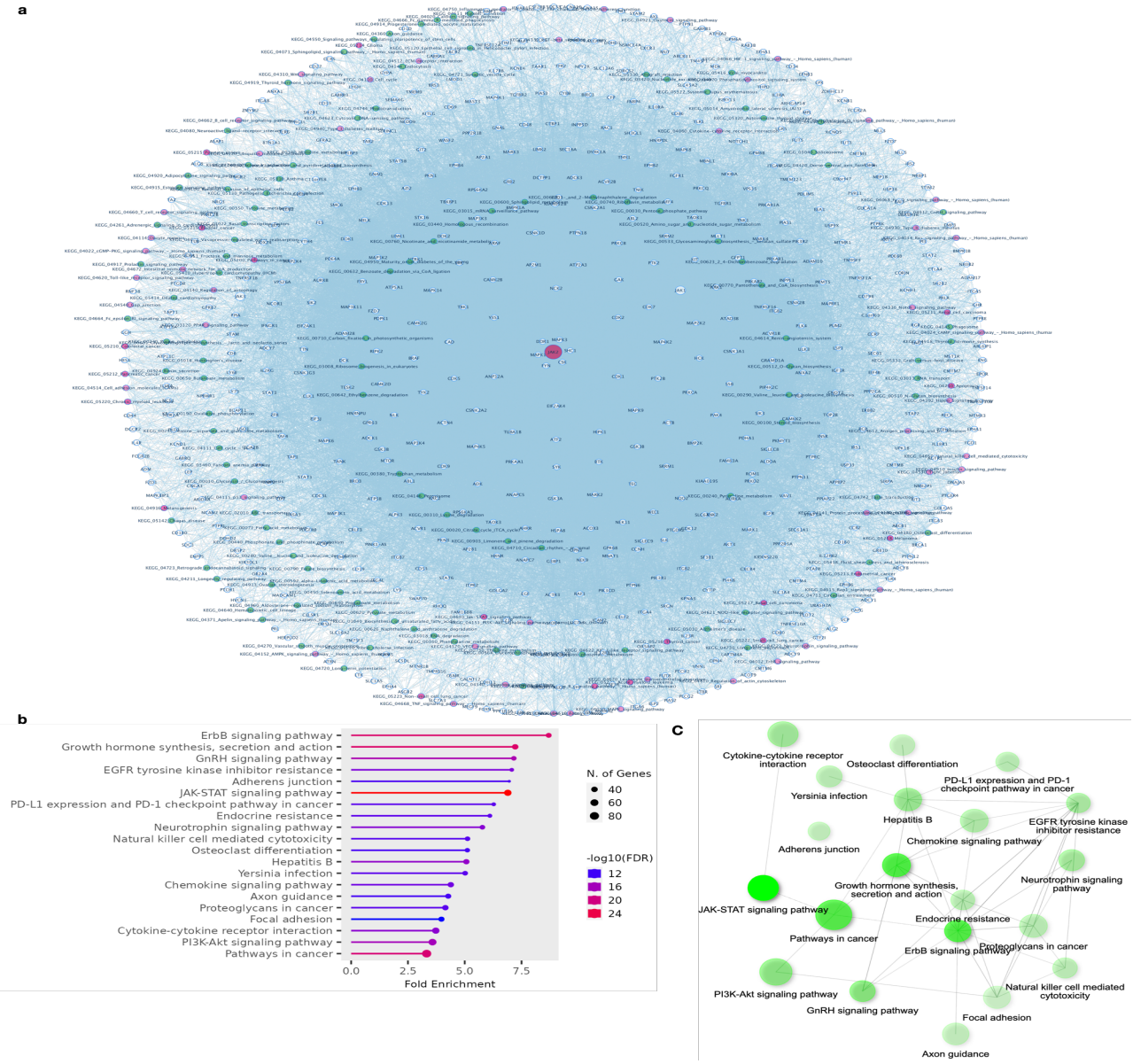

Figure 4. JAK2 network and its functional analysis. (a) JAK2 network, (b) Enriched pathways, and (c) network of pathway-pathway association.

3.3. Prediction of putative herbal drug-targets in case of CML: To explore the potentials of JAK2 we carried out screening of the genes/proteins directly associated with it and predicted the associated biological functions and the

pathways (Figure 4a). For this, FunCoup network database was used and the associated genes were processed for functional analysis in ShinyGO 0.77. In the network of JAK2, we could clearly see the critical pathways are associated with JAK2 and its interactors. Similar to the network analysis, pathway enrichement analysis display that among the top 20 pathways, ErbB signaling, EGFR signaling, JAK-STAT signaling, Cytokine signaling, and NK signaling were present which are known to be associated with CML.

3.4. Prediction of putative herbal drug-targets in case of CML: After analyzing the network-level understanding of JAK1, associated genes, and the pathways, we performed drug-target anlysis for which we have used herbal drugs to target the selective JAK2 which appear highly connected with more genes and the pathways.

The binding affinity (delta G) were presented for the respective docking outcomes. Here, we observe that JAK2 binds with Allicin with -4.7 kcal/mol binding affinity, with Curcumin -9.0 kcal/mol, and with Gingerol -7.7 kcal/mol. Thus, we could say that Curcumin could be the potential drug to target JAK2 compared to Gingerol and Allicin. We presented the structural view for the complex of JAK2 and the drug followed by the respective binding sites (Figure 5). Gingerol and Curcumin displayed higher number of binding sites compared to Allicin which displayed only few binding sites. In terms of binding site location, the three drugs displayed different binding cavity.

After analyzing the binding sites, we also analyzed the target proteins for these three drugs (Figure 6) by using swisstarget prediction server. In case of Allicin, enzymes (>46%) were among the highest number of target proteins. Enzymes and oxidoreductase were 20% among all the targets of Curcumin followed by kinase (13.3%). In case of Gingerol, enzymes were 26.7%, nuclear receptors were 20%, and kinases were also 20%. Based on target protein classification, we could clearly see that the three drugs target different classes of proteins and thus their binding sites also vary. While the networks of the target proteins showed different classes of proteins while the proteins are functionally highly relevant in terms of their known relevance with different human diseases including human cancers.

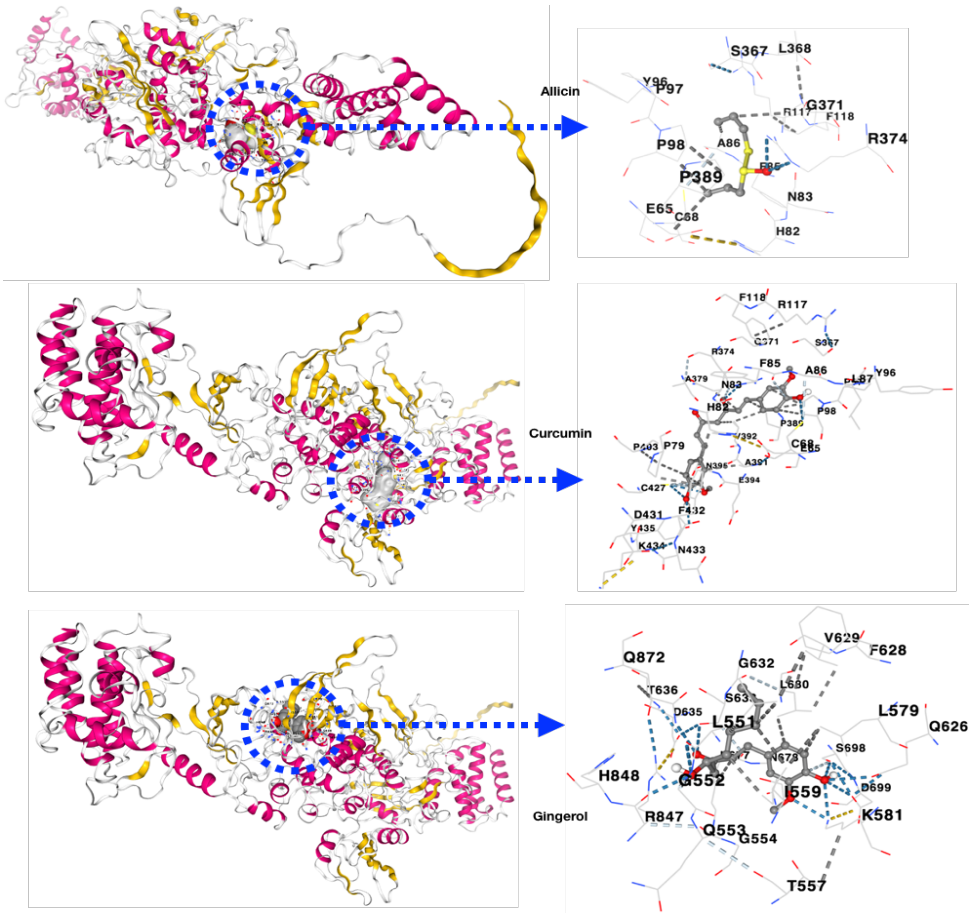

Figure 5. The overall significant molecular docking result of JAK2 protein with Allicin, Curcumin, and Gingerol represented as a pictorial presentation. The protein-ligand complexes are shown as helical ribbon structure in presence of respective drugs. The 3D H-bonds surface representation as donor and acceptor region of proteins and binding state of drugs in their respective predicted binding pockets. 2D representation of drug interactions within the predicted catalytic pocket forming different types of covalent and non-covalent bonds with multiple residues.

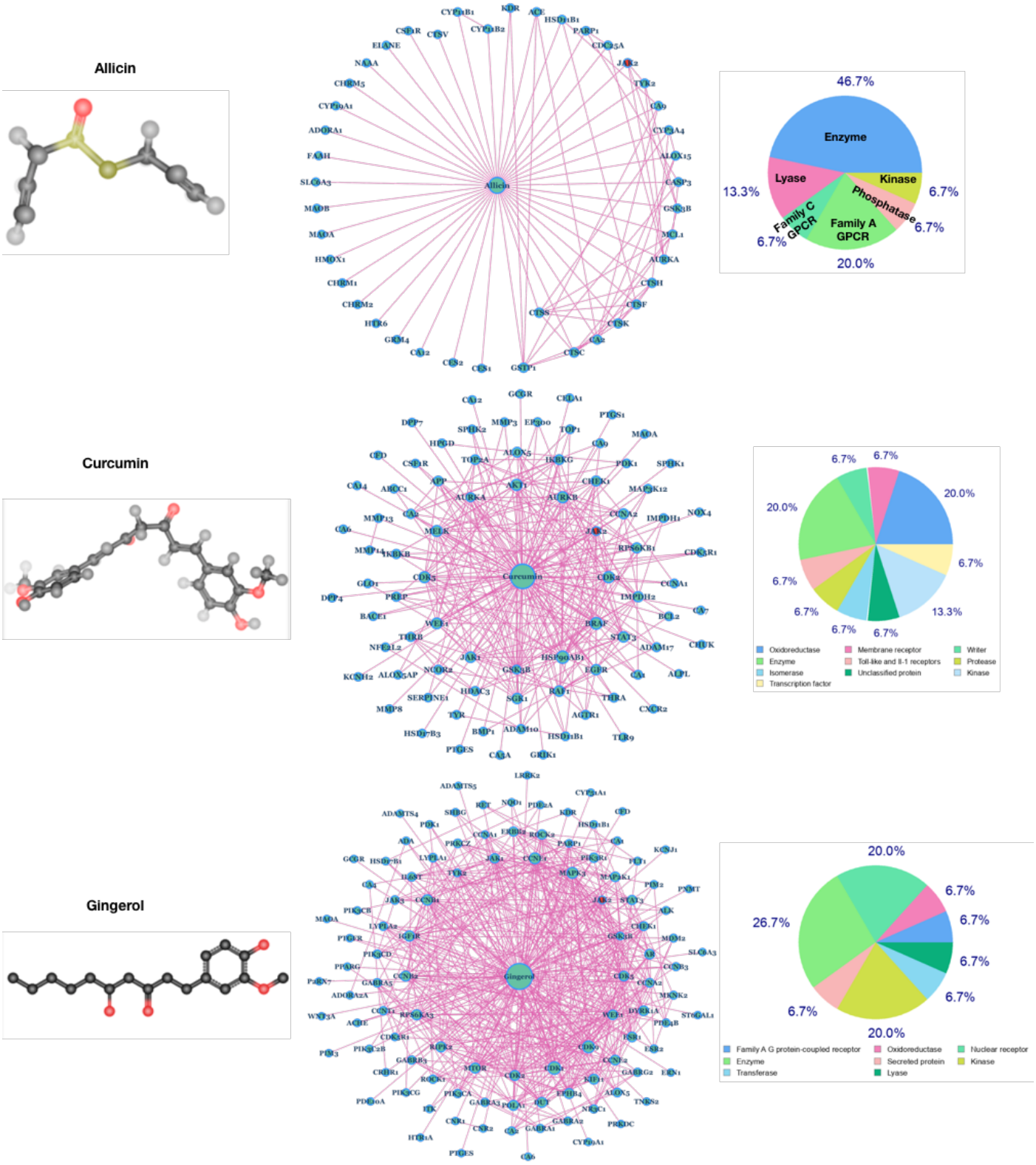

Figure 6. Structure, targets, and the classes of the target proteins of Allicin, Curcumin, and Gingerol.

4. Discussion

We may have focused much of our emphasis on JAK2 mutations in CML patients in our paper. We examined its connections to potential genes and important biological processes using an in-silico methodology. JAK2 may serve as a master regulator of essential biological processes and the components that comprise them, and our results suggest that the selected patients may differ from one another in terms of sequencing. Additionally, the combined method

showed that ATM, JAK1, TP53, KRAS, and BRAF are the most significant genes, and that the highest binding affinities were found for KRAS and TP53.

The genetic makeup of CML patients is not well understood, especially in Saudi Arabia, where 1.2% of patients have CML (BCR-ABL Positive) and 13.0% have CML not otherwise specified (NOS), which indicates that the BCR-ABL status is unknown. The significance of these research is reflected in the leukemia type. The purpose of this study was to advance scientific understanding of the JAK1 gene's existence and relationship to the Ph. Ch. in CML illness. At the outset, the average age of CML infection is 52 years old, which is quite similar to the mean age of the previous studies, which focused on senior individuals. The CML patients' initial blood counts, leukocytosis, and anemia are likewise fairly comparable to those from the previous studies. However, there is diversity in the PLTs count; in our investigations, most patients have normal PLTs counts, whereas in other studies, patients have thrombocytosis. Similar to earlier research, BCR-ABL positivity is regarded as a diagnostic criteria for CML and is present in all patients. Several cytokines can readily activate the JAK/STAT signaling system; once JAK is activated, the STAT transcription factors will remain activated indefinitely[52, 70-79]. The significance of examining the function of the JAK/STAT signaling system in hematological malignancies is reflected in its involvement in our cells' survival, proliferation, and differentiation. Despite the fact that a number of malignancies exhibit constitutive STAT activation, no activating mutations in JAK or STAT have been found. According to mutational research, mutations in exon 11 of the JAK1 gene potentially indicate a molecular target for new treatments because they are associated with a poor prognosis for patients with T-ALL. Exon 14 mutations reported by JAK1 have been linked to a poor response to leukemia treatment, frequent disease recurrence, and a lower overall survival rate in individuals with B-ALL and T-ALL. In addition to their responsiveness to JAK inhibitor treatment to suppress the JAK/STAT signaling system in T-cell lymphoma, other mutational investigations have demonstrated that mutations in exon 14 of the JAK1 gene may result in an increase in protein function[27, 49, 50, 52, 80-82].

For numerous research pertaining to distinct cancer kinds, a variety of methodologies have been employed, and many of these have investigated putative biomarkers.

Two silent mutations (c.579T>C and c.2049C>T) in JAK1 were discovered in CML patients in this investigation. These two silent mutations (c.2049C>T; p.S683S) and (c.579T>C; p.A193A) have been previously identified in patients with hemangioblastomas who do not have any amino acid alterations (Shankar et al., 2014). Finally, we present here two silent mutations in six CML patients in exons 6 and 15 of the JAK1 gene. Despite the low incidence of mutation, we propose that mutation of the JAK1 gene may change the activation of the JAK-STAT signaling system, which in turn may contribute to the formation and progression of CML and CMML. Our knowledge of the JAK1 gene's function in CML and CMML will be expanded by more mutational analysis and full exon sequencing.

Additionally, this area is progressively crossing the "translational interface" and into the clinical setting, where it will be integrated into the development of novel drugs and subject to regulatory oversight. The article will discuss the history of pharmacogenetics and pharmacogenomics, scientific developments that have facilitated the development of this field, the use of transcriptomic and metabolomic data in attempts to understand and predict variation in drug response phenotypes, and difficulties surrounding the "translation" of this significant area of biomedical science into the clinic.

5. Conclusions: Our study's limitations, which need to be addressed in future research, include the small number of patients we included and the ethnic group variation. In conclusion, this study is one of the first in Saudi Arabia to investigate the possible connection between the JAK2 gene and CML patients. There is currently no research on the connection between the JAK2 gene and CML. This endeavor will benefit the patient and scientific communities in many ways. It will increase our knowledge of the relationship between the JAK2 gene and CML, which may help with early detection and diagnosis of the condition or perhaps be used as a tactic to limit it. These 20 enriched pathways included ErbB signaling, EGFR signaling, JAK-STAT signaling, Cytokine signaling, and NK signaling. Docking research suggests that, in contrast to gingerol and allicin, curcumin may be a medication that targets JAK2. We displayed the structural view of the drug-JAK2 complex, followed by the corresponding binding sites. While allicin showed a small number of binding sites, gingerol and curcumin showed a greater number. The three medications showed distinct binding cavities in terms of binding site positions. It was evident from the target protein classification that the three medications target distinct protein classes, and as a result, their binding sites differ. Although the target protein networks displayed a variety of protein classes, the proteins themselves are highly important in terms of their functional significance in relation to several human illnesses, including cancer.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Unit of Biomedical Ethics and Research Committee, King Abdulaziz University, KSA (protocol code 699-19 and 27/11/2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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review and editing, I.M.A. and M.M.; visualization, M.M.; supervision, I.M.A. and M.M.; project administration, I.M.A. and M.M.; funding acquisition, I.M.A. All authors have read and agreed to the published version of the manuscript.

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