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Regulatory network analysis of microRNAs and genes in Imatinib-resistant chronic myeloid leukemia

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Regulatory network analysis of microRNAs and genes in Imatinib-resistant chronic myeloid leukemia

Ismael Soltani [1], Hanen Gharbi[1], Islem Ben Hassine[1], Ghada Bouguerra[1], Kais Douzi[1], Mouheb Teber [1], Salem Abbes[1] and Samia Menif [1]

[1]Molecular and Cellular Hematology Laboratory, Institut Pasteur de Tunis, Université Tunis El Manar, Tunis, Tunisia.

Corresponding author:

Soltani Ismael, Molecular and Cellular Hematology Laboratory, Institut Pasteur de Tunis, Université Tunis El Manar, Tunis, Tunisia. 13 Place Pasteur, BP 74, 1002 Belvédère, Tunisia, Tel: (+216) 71 84 26 09,

E-mail: soltaniismael@yahoo.fr

Abbreviation : miRNA (microRNA); TFs (transcription factors); IM (Imatinibmesylate); CML (Chronicmyeloidleukemia); National Center for Biotechnology Information (NCBI); Transcription factor binding sites (TFBSs).

Abstract:

Targeted therapy in the form of selective breakpoint cluster region-abelson (BCR/ABL) tyrosine kinase inhibitor (Imatinib mesylate) has successfully been introduced in the treatment of the chronic myeloid leukemia (CML). However, acquired resistance against Imatinib (IM) has been reported in nearly half of patients and has been recognized as major issue in clinical practice. Multiple resistance genes and microRNAs (miRNAs) are thought to be involved in the IM resistance process. These Resistance genes and miRNAs tend to interact with each other's through a regulatory network. Therefore, it is crucial to study the impact of these interactions in the IM resistance process. The present study focused on miRNA and gene network analysis in order to elucidate the role of interacting elements and to understand their functional contribution in therapeutic failure. Unlike previous studies which were centered only on genes or miRNAs, the prime focus of the present study was on relationships. To this end, three regulatory networks including differentially expressed, related and global networks were constructed and analyzed in search of similarities and differences. Regulatory associations between miRNAs and their target genes, transcription factors and miRNAs, as well as miRNAs and their host genes were also macroscopically investigated. Certain key pathways in the three networks, especially in the differentially expressed network, were featured. The differentially expressed network emerged as a fault map of IM-resistant CML. Theoretically, the IM resistance process could be prevented by correcting the included errors. The present network based approach to study resistance miRNAs and genes might help in understanding the molecular mechanisms of IM resistance in CML as well as in the improvement of CML therapy.

Key words: Chronic myeloid leukemia, Imatinib mesylate, Resistance, MicroRNA, Network, Transcription factor.

Introduction

Chronic myeloid leukemia (CML) is a type of cancer initiated in the blood-forming cells of the bone marrow, which gradually invades the peripheral blood. CML is characterized by the generation of the Philadelphia chromosome, a direct result of the t(9;22) (q34;q11) balanced reciprocal translocation–that creates an oncogene designated "breakpoint cluster region - Abelson murine leukemia viral oncogene homolog 1 (BCR - ABL)" encoding a chimeric BCR - ABL protein characterized by a constitutive tyrosine kinase activity (Sun et al. 2013).

The treatment of CML has been revolutionized by the development of imatinib mesylate (IM) (trade name Gleevec®), the first specific inhibitor of the BCR-ABL protein (Alikian et al. 2012). IM is a small molecule that specifically recognizes, binds and inactivates the tyrosine kinase activity of the BCR-ABL oncoprotein, thus inhibiting leukemogenesis. Treatment of CML patients with IM generated excellent responses, in terms of symptom control and hematological indices. However, due to continual IM presence, primary and secondary resistance to IM as well as molecular evidence of persistent malignancy have been observed in a significant number of CML patients (A et al. 2010, Esposito et al. 2011).In several cases, point mutations in the BCR-ABL kinase domain have been incriminated in the development of IM resistance, secondary resistance particularly, eventually leading to treatment failure (Weisberg et al. 2007). However, more than half of IM-resistant CML patients had no mutations in the BCR-ABL oncogene (Mohamad Ashari et al. 2014). Therefore, the basis of BCR-ABL-independent IM resistance remains to be elucidated.

Previous studies have demonstrated that the exploration of differentially expressed genes is a crucial step in the definition of possible targets for therapeutic intervention. Differential gene expression is controlled by two classes of regulators: Transcription factors (TFs) and microRNAs (miRNAs). While TFs bind to cis-regulatory DNA elements that are often located in or near their target genes, miRNAs bind to cis-regulatory RNA elements that are mainly located in the 3' UTR region of their target mRNAs (Martinez and Walhout 2009).Moreover, miRNAs could be located within several genes referred to as "miRNA host genes". In a pathological context, miRNAs could be co-expressed with their host genes (Baskerville and Bartel 2005),and could participate, together with the host gene, in molecular signaling (Cao et al. 2010).What is more, abundant evidence have established that the spatiotemporal expression of miRNAs is in part regulated by TFs and could result in phenotypic variations (Hobert 2008).

Current research is focused on the factors that contribute to BCR-ABL independent IM resistance, i.e. the genetic background (random drug-induced mutational events), the epigenetic mechanisms (drug-induced non-mutational alterations of gene function) and the drug induced karyotypic changes (Schoch et al. 2003).

Recently, increasing evidence indicated that aberrant miRNAs expression is strongly implicated in anticancer drug resistance phenotype (Li and Yang 2014, Zheng et al. 2010), an argument further supported by reports validating miRNAs' involvement in the tumor-cell response to chemotherapeutic agents (Zheng, Wang, Chen and Liu 2010). What is more, expression profiling and in vitro studies have predicted the involvement of differentially expressed genes in IM resistance and disease progression (Liu et al. 2012, Mosakhani et al. 2013).

So far, studies mostly focused on a single element (i.e. genes or miRNAs) making it challenging to analyze the IM resistance mechanisms due to the fact that complex physiological processes may rarely

be ascribed to a single molecule (Barabasi and Oltvai 2004). In addition, resistance genes and miRNAs have a tendency to interact with each other. Therefore, it becomes necessary to understand the impact of these interactions on the IM resistance mechanisms.

In the current study, a system-based networks' approach was used to examine the key regulatory associations between miRNAs, their target genes, their host genes and TFs in IM-resistant CML patients.

Experimentally validated data from databases and literature was collected and three networks were constructed. In order to improve the understanding of how resistance against IM is acquired in patients with CML, the regulatory associations between all the elements in the constructed networks were explored, with a focus on certain miRNAs and genes.

Materials and Methods

Data Collection and standardization

In order to construct the global network, three data sets (A1, A2 and A3) were collected.

Set A1, encompassing experimentally validated datasets of human miRNAs and their target genes, was collected from two databases: miRecords, version 4 (http://mirecords.biolead.org/), and miRtarbase (http://mirtarbase.mbc.nctu.edu.tw/). While miRecords is an integrated resource which hosts collections of animal miRNA-target interactions from both low-throughput and high-throughput experiments (Xiao et al. 2009), miRTarBase is a database of experimentally-validated miRNAs and targets interactions (Hsu et al. 2011).

Set A2 comprised of human miRNA and TF regulatory associations, was collected from TransmiR, an assembly of TF - miRNA regulations, based on experimental evidence only (http://www.cuilab.cn/transmir) (Wang et al. 2010).

Set A3, consisted of the association-mapping between miRNAs and their host genes based on data from miRBase (http://www.mirbase.org/), and NCBI. MiRBase provides a collection of confirmed miRNAs sequences and annotation(Kozomara and Griffiths-Jones 2011), and NCBI was used to establish the official symbols and official host gene IDs.

Set A4 compiled differentially expressed genes and a set of related genes both involved in IM-resistant CML.First, multiple types of variations were collected, including genetically mutated genes, abnormally expressed protein genes, copy number variants, single nucleotide polymorphisms (SNPs), and downregulated and upregulated genes. To this end, genome-wide gene expression change studies in response to IM were included in the data set through a search of PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and HuGE Navigator Phenopedia (https://phgkb.cdc.gov/HuGENavigator/startPagePhenoPedia.do/) (Yu et al. 2010) using a combination of key words including '*chronic myeloid leukemia*', '*IM resistance*', '*gene mutations*', '*gene profiling*' and '*gene expression*'. Afterwards, genes were selected from filtered papers, based on the criteria that their pharmacological roles in IM resistance have been determined genetically, biochemically or epidemiologically. Finally, the obtained genes were mapped to Entrez gene symbols. Additionally, numerous CML IM resistance related genes were collected from pertinent literature, such as those that have been demonstrated to serve a role in the IM pharmacological processes, including genes associated with the reduced uptake of IM, metabolic alterations, increased energy-dependent efflux and reduced apoptosis. Furthermore, a list of TFs predicted by the P-match method

(Chekmenev et al. 2005) were included in the related genes, with a focus on the TFs that appear in transmiR database. The P-match method combines pattern matching and weight matrix approaches in order to identify TF binding sites (TFBSs) in 1,000 nt promoter region sequences of the differentially expressed miRNA target genes, which were downloaded from the Ensembl database (Cunningham et al. 2015), and maps the TFBSs onto the promoter region of the targets. Furthermore, the matrix library of P-match consists of sets of known TFBSs collected from TRANSFAC (http://www.gene-regulation.com/cgi-bin/pub/databases/transfac/search.cgi/), thereby providing the ability to search for a large variety of different TFBSs. Finally, in order to reduce false-positive validation using P-match, the'high quality vertebrate matrices only' option was selected as default.

The last data set (A5) included differentially expressed miRNAs associated with the IM resistant phenotype. The miR2Disease (Jiang et al. 2009), PhenomiR (Ruepp et al. 2012) and HMDD (Lu et al. 2008) databases were searched for relevant articles using the keywords '*chronic myeloid leukemia*', in addition to a search of PubMed using the keywords '*chronic myeloid leukemia AND microRNA AND imatinib resistance*.' Subsequently, each title and abstract was manually checked for relevance. The full text was reviewed in all cases where the abstract indicated that the article reported associations between miRNA expression and IM resistance in CML. Consequently, 19 up/downregulated miRNAs along with descriptions of the miRNA-malignancy relationship, miRNA expression patterns in the diseased state and detection methods for miRNA expression, were compiled. Assessment of relevant studies was necessary as a complementary resource to collect miRNAs associated with IM resistance.

Seeing that different databases use varying symbols to label miRNAs and genes, the symbolic representation was standardized using the official symbols from the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/gene/).

Three networks construction

The following method was used to construct three level networks: The differentially expressed network, the related network and the global network. The regulatory relationships between TFs, miRNAs, target genes and host genes were extracted from sets A1, A2 and A3, from which the associations were combined and the global network was derived. The global network was the source of both the differentially expressed network and the related network.

Network models could be globally analyzed. However, they may provide more local information through the characterization of smaller sub-networks, such as the differentially expressed network and the related network. Therefore, the associations between the differentially expressed genes (A4) and miRNAs (A5) were combined and mapped onto the global network in order to derive the differentially expressed network. If an association between a differentially expressed gene and differentially expressed miRNA was observed in the global network, the elements and the associations were incorporated into the differentially expressed network. All single nodes that did not have a regulatory association with miRNAs were omitted. The related network was constructed by using the same selected method to generate the differentially expressed network.

Results

Differentially expressed network of imatinib-resistant chronic myeloid leukemia

Using the aforementioned datasets and methods, a differentially expressed network was constructed that defined the regulation in the complex process of IM-resistant CML. This network showed interactions between 6 TFs, 21 target genes, 19 miRNAs and their host genes. All other nodes

were differentially expressed, with the exception of the host genes. Fig. 1 presents significant regulatory pathways. The sub-network centering on MYC was the key component of the system and revealed several regulatory pathways. MYC is targeted by hsa-miR-451a, hsa-miR-26a-5p and hsa-miR-17-5p, which regulate four miRNAs including hsa-miR-23a-3p, hsa-miR-29a-3p, hsa-miR-29c-3p and hsa-miR-17-5p. Hsa-miR-17-5p seemed to have targeted its regulators, i.e. MYC and E2F1, forming two separate feedback loops. Taking into consideration that MYC and E2F1 activate each other's transcription (Matsumura et al. 2003), this result suggests the existence of a double feed forward loop between the three nodes, thereby bringing forward a balanced mechanism. Such a loop, which has been defined as a Type-I circuit, exhibits bi-instability, with hsa-miR-17-5p playing a critical role in regulating the position of the ON-OFF switch at the E2F1/MYC gene product level.

On a different note, it has been observed that BCL2L11 is an experimentally validated target gene of hsa-miR-17-5p. By targeting MYC, both hsa-miR-451a and hsa-miR-26a-5p, which regulates hsa-miR-17-5p, might additionally affect the expression of the BCL2L11 gene towards the end of the pathway. The miR-17 family, located within the miR-17-92 cluster, has been described as having crucial oncogenic activities in different types of cancer, including CML (Machova Polakova et al. 2011, Venturini et al. 2007). According to the organized data, it was reported that a silent SNP, c465C.T (rs724710), located in the BCL2L11 gene, was significantly associated with a longer delay to achieve a major molecular response, leading to more frequent mutations in the kinase domain of BCR-ABL and eventually to IM resistance (Augis et al. 2013). At the same time, MYC, hsa-miR-29a and hsa-miR-451a bind together as an ordered control chain. It has been observed that hsa-miR-451a is directly involved in activating the expression of ABCB1 and BCL2 gene products, and to regulate hsa-miR-29a together with MYC, with a resulting signal flow to ABL1 and RAN. RAN and ABCB1 are a nucleocytoplasmic transport protein and an energy dependent efflux pump, respectively, that are both responsible for reduced IM accumulation, therefore limiting its intracellular concentration.

As presented in Fig. 1, certain miRNAs were downregulated in CML IM resistance including hsa-miR-451a, hsa-miR-7-5p and hsa-miR-181c-5p, while others are upregulated, such as hsa-miR-191-5p. To sum up, the network demonstrates a way through which cells might acquire IM resistance.

Imatinib-resistant CML associated network

The IM-resistant CML related network contained a large number of additional pathways. In the present study, the differentially expressed elements were included in the IM-resistant CML associated elements, which indicated that the differentially expressed regulatory network is a partial network of the IM-resistant CML-related network. These elements included 6 differentially expressed TFs, 10 additional TFs, 29 miRNAs and several target genes. Fig. 2 presents the numerous additional pathways that might potentially affect the process of IM-resistant CML. Certain factors were prominent, such as the related gene NFKB1, which regulates hsa-miR-17-5p, hsa-miR-155-5p, hsa-miR-224-5p, hsa-miR-29a-3p, hsa-miR-199a-5p and hsa-miR-29c-3p. The 6 miRNAs target 26 relevant genes including BCL2L11, ABL1, RAN and MYC, which are all involved in the differentially expressed network.

It was also demonstrated that additional miRNAs in the related network could be able to regulate differentially expressed genes. For example, hsa-miR-30a-5p targets two differentially expressed genes, ABL1 and RUNX2, and three related genes including GMFB, BAX and IT6A6. Differentially

expressed miRNA might additionally regulate related genes. For example, hsa-miR-7-5p is a differentially expressed miRNA which targets BCL2, ABCC1, SOCS2, RYK and CFLAR in the differentially expressed network. In the related network it targets the related genes CASP9, RELA and CDC37. Several feedback loops were also identified, including NFKB1 and hsa-miR-155-5p, MYB and hsa-miR-155-5p, MYC and hsa-miR-17-5p, E2F1 and hsa-miR-17-5p, and ZEB1 and hsa-miR-141-3p. The related network sheds a new on the mechanisms of IM resistant CML through the presentation of additional pathways that might be future research targets when it comes to overcoming IM resistance.

The Global network

The global network was constructed from the regulatory associations that were collected in the first three steps. It harbors the experimentally validated signaling transmissions and potential pathways in the human body. Therefore, it includes the differentially expressed network and the related network.

Host genes and miRNAs in Imatinib-resistant CML related network

Despite the fact that the differentially expressed genes do not include the miRNA host genes, the latter and their differentially expressed miRNAs might provide insights into the mechanisms of differential gene expression at a systems' level. Several pathways of host genes and their respective miRNAs are presented in Fig. 3. MIR155HG contains hsa-miR-155-5p, which regulates 11 target genes including ETS1, NEUROG1, TOMM34, DDB2, RREB1, DRAP1, RUNX2, AXL, MYB, KRAS and NFKB1. Conversely, hsa-miR-155 is regulated by BCR, IRF1, TGFB1, with NFKB1 and MYB in two separate feedback loops. A single miRNA can be located within several host genes. For example, hsa-miR-10a-5p might be located in HOXB-AS3-012, HOXB3 as well as within HOXB4. This result suggests that exploring the associations between host genes and their miRNAs, in particular when their miRNAs are differentially expressed, might help understand anticancer drug resistance, especially the IM resistance mechanism.

Transcriptional network of predicted TFs

Based on the hypothesis that the complexity of the eukaryotic transcriptional regulation machinery reflects a multitude of responses, and that regulatory circuits involving miRNAs and TFs are not isolated instances, the predicted TFs obtained by the P-match method were incorporated along with the differentially expressed miRNAs in a transcriptional network. Predicted TFs might be involved in the transcriptional process of the development of IM resistance, as they may lead to similar results with differentially expressed TFs. A total of five TFs including ZEB1, NFKB1, YY1, NKX2-5 and E2F1 along with their regulatory interactions with differentially expressed miRNAs and their targetgenes are presented in Fig. 4. Notably, TFs and miRNAs might form regulatory circuits consisting of a self-adapting loop, meaning that TFs can modulate their own expression through miRNAs. For example, ZEB1 and E2F1 indirectly regulate their expression through hsa-miR-141-3p and hsa-miR-17-5p, respectively. A single TF could regulate many miRNAs simultaneously. For example, hsa-miR-17-5p, hsa-miR-29c-3p, hsa-miR-199a-5p, hsa-miR-29a-3p and hsa-miR-224-5p were co-regulated by NFKB1. Certain TFs can cooperate with other TFs in order to regulate the same miRNA, thereby influencing the same genes. For example, NFKB1 and YY1 both regulate hsa-miR-29a-3p, which targets PTEN, RAN, ABL1 and BCL2. In addition, TFs may indirectly influence other genes via miRNAs. For example, YY1 regulates hsa-miR-29c-3p, which in turn targets BCL2,

IGFBP1 and VEGFA. Furthermore, TFs can cooperate with other TFs regulating different miRNAs, therefore influencing the same genes. For example, NFKB1 regulates hsa-miR-29a-3p, NKX2-5 regulates hsa-miR-17-5p, and the two miRNAs simultaneously target PTEN. A single miRNA can indirectly alter other miRNAs via TFs. For example, hsa-miR-26a-5p indirectly controls hsa-miR-17-5p via NKX2-5. The network of predicted TFs and miRNAs indicates the possible transcriptional regulation in the CML IM resistance phenomenon.

Regulatory pathways of differentially expressed genes

A prerequisite for understanding CML progression and overcoming treatment failure is acquiring knowledge of its component interactions. Thus, in order to improve the understanding of the associations within the three networks as well as the upstream and downstream elements of the differentially expressed genes, differentially expressed miRNAs and TFs were extracted and compared. Based on the data drawn from the three topology networks, adjacent nodes of each differentially expressed gene were selected at a level and then categorized based on the organization of the regulatory associations into successor and predecessor nodes in order to facilitate the comparison and analysis of the components' interactions in the systems' network architecture.

Using MYC as an example, the predecessor and successor nodes of the MYC gene are grouped in Table 1with regards to their regulatory associations in the differentially expressed, related and global networks. MYC, a key factor in the CML IM resistance process,was deregulated in nearly half of human tumors, and was frequently associated with tumor progression (Vita and Henriksson 2006). Porro et al (Porro et al. 2011) demonstrated that MYC is able to transcriptionally upregulate the ATP-binding cassette transporter genes in CML CD34+ progenitors cells, which is consistent with the hypothesis that leukemia stem cells are resistant to the therapy. The current study indicates that MYC has numerous adjacent nodes in IM resistant networks. A total of three miRNAs (hsa-miR-451a, hsa-miR-26a-5p and hsa-miR-17-5p) appear to target MYC, and four miRNAs (hsa-miR-23a-3p, hsa-miR-29a-3p, hsa-miR-17-5p and hsa-miR-29c-3p) were regulated by MYC in the differentially expressed and related networks. Concerning the global network, 26 miRNAs were identified as targeting MYC, and in turn, MYC regulates 28 miRNAs. An attached dependency between the upstream and downstream elements was observed in the three networks. The upstream region of MYC seems to be indirectly affected by events taking place in its downstream region. Therefore, both regions might be affected by their predecessors and successors.

Regulatory pathways of differentially expressed miRNAs

Similarly to differentially expressed genes, regulatory pathways of differentially expressed miRNAs were compared and analyzed using the same method. Hsa-miR-17-5p was closely examined due to its presence in several potential nodes. A summary of predecessors and successors of hsa-miR-17-5p in the three networks is presented in Table 2 including 6 genes (MYC, E2F1, MYCN, MIR17HG, C13orf25 and STAT5) regulating hsa-miR-17-5p, which in turn targets 8 genes in the differentially expressed network. In the related network, two further genes (NFKB1 and NKX2-5) regulate hsa-miR-17-5p in order to alter the expression of a further single gene (TRAP1). MYC, E2F1 and hsa-miR-17-5p seem to form self-adaptation associations in the three networks. Additionally, certain miRNAs seem to indirectly affect hsa-miR-17-5p through TFs, e.g. hsa-miR-126-3p targeting E2F1, which regulates hsa-miR-17-5p. In the global network, hsa-miR-17-5p has a greater number of

associations with targets and TFs, and has many adjacent nodes, suggesting that it has a crucial role in IM-resistant CML.

Regulatory pathways of transcription factors

The successors and predecessors of the predicted TFs by the P-match method were thoroughly studied. TFs that have upstream and downstream associations in the differentially expressed and the related networks were of particular interest, such as E2F1 (Table 3). E2F1 was differentially expressed in the IM resistance phenotype.E2F1, alone, regulates hsa-miR-17-5p in both the differentially expressed and therelated networks. Hsa-miR-17-5p was targeted by two miRNAs (hsa-miR-17-5p and hsa-miR-126-3p) in the differentially expressed network and by three miRNAs (hsa-miR-17-5p, hsa-miR-126-3p and hsa-miR-10a-5p) in the related network. A total of 22 miRNAs targeted E2F1, which regulates 21 miRNAs in the global network indicating that through a TF, upstream miRNAs could affect the level of expression of downstream miRNAs, which could potentially have a profound effect on the activity of said miRNAs. Furthermore, E2F1 could be considered as a bridge, connecting predecessors and successors, and affecting miRNAs expression in response to environmental signals.

Discussion

Studying the mechanisms of resistance to IM has not been thoroughly examined on a larger scale. Therefore, many cases of CML remain refractory to chemotherapy. One of the contributors to IM resistance in patients with CML is suggested to be associated with the differential expression of certain genes and miRNAs. However, the interactions between deregulated elements remain to be fully elucidated. Understanding the network topology through the investigation of regulatory circuits involving miRNAs and genes rather than the traditional single element approach facilitates the rational drug conception process, and could aid in overcoming therapeutic failure.

In the present study, the regulatory associations of differentially expressed genes, differentially expressed miRNAs and predicted TFs in the development of patients with IM-resistant CML, were investigated. Additionally, the interactions between resistance genes and miRNAs were presented. Using the collected data, a global network, consisting of experimentally validated signaling transmissions and potential pathways was constructed. Subsequently, two further networks, the differentially expressed network and the related network, were derived from said global network, were characterized in order to provide more local information. Several significant pathways were revealed in the three network levels. In addition, the existence of reciprocal regulation between miRNAs and TFs was demonstrated, together coordinating and controlling their targets, thereby affecting gene expression.

In the differentially expressed network, there are some critical data linkages showing notable features. As a potential TF, MYC was observed to directly or indirectly regulate ~1,000 genes, and to bind to 15% of genomic loci (Dang et al. 2006). Liu et al (Liu, Wang, Chen, Wu, Zhang, Huang, Zhang, Xiao, Wang and Liang 2012) showed that MYC overexpression in CML IM resistant cells corelated with the downregulation of hsa-miR-451a, and that MYC knockdown sensitizes IM resistant cells to apoptosis. Alternatively, Virgili et al (Virgili et al. 2011) postulated that the loss of the expression of ABL1 kinase might contribute to IM resistance in patients with CML-chronic phase, who do not achieve complete cytogenetic response during 12 months on IM and eventually progress to the CML-blast phase. In the differentially expressed network, a connection between ABL1, ABCB1, RAN and BCL2L11 along with MYC was observed. Additionally, the network indicated that the

MYC gene could affect the expression of the aforementioned genes through its association with hsa-miR-451a and hsa-miR-17-5p thereby regulating hsa-miR-29a which in turn regulates cellular processes, including proliferation, apoptosis and IM transport potentially affecting the process of IM-resistant CML.

The related network is an extension of the differentially expressed network. The related factors affect the IM-resistant CML process by interacting with differentially expressed factors. For example, constitutive NFKB1 activity has been observed in several hematological malignancies, through its anti-apoptotic role. Lounnas et al revealed that inhibition of NFKB1 activation could be a way to increase apoptotic sensitivity to IM treatment (Lounnas et al. 2009), an argument part supported by data from the related network of the current study.

Although they remain to be validated by scientific experiments in association with IM-resistant CML, the studied pathways provide theoretical foundations for the improvement of CML therapy. Our results could help shed light on how gene expression is associated with the development of anticancer drug resistance.

In conclusion, in order to clearly explain how drug resistance is acquired, it will be advantageous to integrate miRNA and TF-containing regulatory networks with additional comprehensive and functional datasets, such as the interaction of proteins in addition to other types of interactions that remain largely uncharacterized.

Conflict of interest statement

The authors declare that they have no conflict of interest

References

A J, Qian S, Wang G, Yan B, Zhang S, Huang Q, Ni L, Zha W, Liu L, Cao B, Hong M, Wu H, Lu H, Shi J, Li M, Li J (2010) Chronic myeloid leukemia patients sensitive and resistant to imatinib treatment show different metabolic responses. PLoS One 5:e13186. DOI 10.1371/journal.pone.0013186

Alikian M, Gerrard G, Subramanian PG, Mudge K, Foskett P, Khorashad JS, Lim AC, Marin D, Milojkovic D, Reid A, Rezvani K, Goldman J, Apperley J, Foroni L (2012) BCR-ABL1 kinase domain mutations: methodology and clinical evaluation. Am J Hematol 87:298-304. DOI 10.1002/ajh.22272

Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F (2013) A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One 8:e78582. DOI 10.1371/journal.pone.0078582

Barabasi AL, Oltvai ZN (2004) Network biology: understanding the cell's functional organization. Nat Rev Genet 5:101-113. DOI 10.1038/nrg1272

Baskerville S, Bartel DP (2005) Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 11:241-247. DOI 10.1261/rna.7240905

Cao G, Huang B, Liu Z, Zhang J, Xu H, Xia W, Li J, Li S, Chen L, Ding H, Zhao Q, Fan M, Shen B, Shao N (2010) Intronic miR-301 feedback regulates its host gene, ska2, in A549 cells by targeting MEOX2 to affect ERK/CREB pathways. Biochem Biophys Res Commun 396:978-982. DOI 10.1016/j.bbrc.2010.05.037

Chekmenev DS, Haid C, Kel AE (2005) P-Match: transcription factor binding site search by combining patterns and weight matrices. Nucleic Acids Res 33:W432-437. DOI 10.1093/nar/gki441

Cunningham F, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Giron CG, Gordon L, Hourlier T, Hunt SE, Janacek SH, Johnson N, Juettemann T, Kahari AK, Keenan S, Martin FJ, Maurel T, McLaren W, Murphy DN, Nag R, Overduin B, Parker A, Patricio M, Perry E, Pignatelli M, Riat HS, Sheppard D, Taylor K, Thormann A, Vullo A, Wilder SP, Zadissa A, Aken BL, Birney E, Harrow J, Kinsella R, Muffato M, Ruffier M, Searle SM, Spudich G, Trevanion SJ, Yates A, Zerbino DR, Flicek P (2015) Ensembl 2015. Nucleic Acids Res 43:D662-669. DOI 10.1093/nar/gku1010

Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F (2006) The c-Myc target gene network. Semin Cancer Biol 16:253-264. DOI 10.1016/j.semcancer.2006.07.014

Esposito N, Colavita I, Quintarelli C, Sica AR, Peluso AL, Luciano L, Picardi M, Del Vecchio L, Buonomo T, Hughes TP, White D, Radich JP, Russo D, Branford S, Saglio G, Melo JV, Martinelli R, Ruoppolo M, Kalebic T, Martinelli G, Pane F (2011) SHP-1 expression accounts for resistance to imatinib treatment in Philadelphia chromosome-positive cells derived from patients with chronic myeloid leukemia. Blood 118:3634-3644. DOI 10.1182/blood-2011-03-341073

Hobert O (2008) Gene regulation by transcription factors and microRNAs. Science 319:1785-1786. DOI 10.1126/science.1151651

Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, Chien CH, Wu MC, Huang CY, Tsou AP, Huang HD (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 39:D163-169. DOI 10.1093/nar/gkq1107

Jiang Q, Wang Y, Hao Y, Juan L, Teng M, Zhang X, Li M, Wang G, Liu Y (2009) miR2Disease: a manually curated database for microRNA deregulation in human disease. Nucleic Acids Res 37:D98-104. DOI 10.1093/nar/gkn714

Kozomara A, Griffiths-Jones S (2011) miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 39:D152-157. DOI 10.1093/nar/gkq1027

Li H, Yang BB (2014) MicroRNA-in drug resistance. Oncoscience 1:3-4

Liu L, Wang S, Chen R, Wu Y, Zhang B, Huang S, Zhang J, Xiao F, Wang M, Liang Y (2012) Myc induced miR-144/451 contributes to the acquired imatinib resistance in chronic myelogenous leukemia cell K562. Biochem Biophys Res Commun 425:368-373. DOI 10.1016/j.bbrc.2012.07.098

Lounnas N, Frelin C, Gonthier N, Colosetti P, Sirvent A, Cassuto JP, Berthier F, Sirvent N, Rousselot P, Dreano M, Peyron JF, Imbert V (2009) NF-kappaB inhibition triggers death of imatinib-sensitive and imatinib-resistant chronic myeloid leukemia cells including T315I Bcr-Abl mutants. Int J Cancer 125:308-317. DOI 10.1002/ijc.24294

Lu M, Zhang Q, Deng M, Miao J, Guo Y, Gao W, Cui Q (2008) An analysis of human microRNA and disease associations. PLoS One 3:e3420. DOI 10.1371/journal.pone.0003420

Machova Polakova K, Lopotova T, Klamova H, Burda P, Trneny M, Stopka T, Moravcova J (2011) Expression patterns of microRNAs associated with CML phases and their disease related targets. Mol Cancer 10:41. DOI 10.1186/1476-4598-10-41

Martinez NJ, Walhout AJ (2009) The interplay between transcription factors and microRNAs in genome-scale regulatory networks. Bioessays 31:435-445. DOI 10.1002/bies.200800212

Matsumura I, Tanaka H, Kanakura Y (2003) E2F1 and c-Myc in cell growth and death. Cell Cycle 2:333-338

Mohamad Ashari ZS, Sulong S, Hassan R, Husin A, Sim GA, Abdul Wahid SF (2014) Low level of TERC gene amplification between chronic myeloid leukaemia patients resistant and respond to imatinib mesylate treatment. Asian Pac J Cancer Prev 15:1863-1869

Mosakhani N, Mustjoki S, Knuutila S (2013) Down-regulation of miR-181c in imatinib-resistant chronic myeloid leukemia. Mol Cytogenet 6:27. DOI 10.1186/1755-8166-6-27

Porro A, Iraci N, Soverini S, Diolaiti D, Gherardi S, Terragna C, Durante S, Valli E, Kalebic T, Bernardoni R, Perrod C, Haber M, Norris MD, Baccarani M, Martinelli G, Perini G (2011) c-MYC oncoprotein dictates transcriptional profiles of ATP-binding cassette transporter genes in chronic myelogenous leukemia CD34+ hematopoietic progenitor cells. Mol Cancer Res 9:1054-1066. DOI 10.1158/1541-7786.MCR-10-0510

Ruepp A, Kowarsch A, Theis F (2012) PhenomiR: microRNAs in human diseases and biological processes. Methods Mol Biol 822:249-260. DOI 10.1007/978-1-61779-427-8_17

Schoch C, Haferlach T, Kern W, Schnittger S, Berger U, Hehlmann R, Hiddemann W, Hochhaus A (2003) Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia 17:461-463. DOI 10.1038/sj.leu.2402813

Sun QC, Liu MB, Shen HJ, Jiang Z, Xu L, Gao LP, Ni JL, Wu SL (2013) Inhibition by imatinib of expression of O-glycan-related glycosyltransferases and tumor-associated carbohydrate antigens in the K562 human leukemia cell line. Asian Pac J Cancer Prev 14:2447-2451

Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, Muckenthaler MU, Ganser A, Eder M, Scherr M (2007) Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood 109:4399-4405. DOI 10.1182/blood-2006-09-045104

Virgili A, Koptyra M, Dasgupta Y, Glodkowska-Mrowka E, Stoklosa T, Nacheva EP, Skorski T (2011) Imatinib sensitivity in BCR-ABL1-positive chronic myeloid leukemia cells is regulated by the remaining normal ABL1 allele. Cancer Res 71:5381-5386. DOI 10.1158/0008-5472.CAN-11-0068

Vita M, Henriksson M (2006) The Myc oncoprotein as a therapeutic target for human cancer. Semin Cancer Biol 16:318-330. DOI 10.1016/j.semcancer.2006.07.015

Wang J, Lu M, Qiu C, Cui Q (2010) TransmiR: a transcription factor-microRNA regulation database. Nucleic Acids Res 38:D119-122. DOI 10.1093/nar/gkp803

Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD (2007) Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. Nat Rev Cancer 7:345-356. DOI 10.1038/nrc2126

Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T (2009) miRecords: an integrated resource for microRNA-target interactions. Nucleic Acids Res 37:D105-110. DOI 10.1093/nar/gkn851

Yu W, Clyne M, Khoury MJ, Gwinn M (2010) Phenopedia and Genopedia: disease-centered and gene-centered views of the evolving knowledge of human genetic associations. Bioinformatics 26:145-146. DOI 10.1093/bioinformatics/btp618

Zheng T, Wang J, Chen X, Liu L (2010) Role of microRNA in anticancer drug resistance. Int J Cancer 126:2-10. DOI 10.1002/ijc.24782

Fig 1. Differentially expressed network of imatinib-resistant chronic myeloid leukemia

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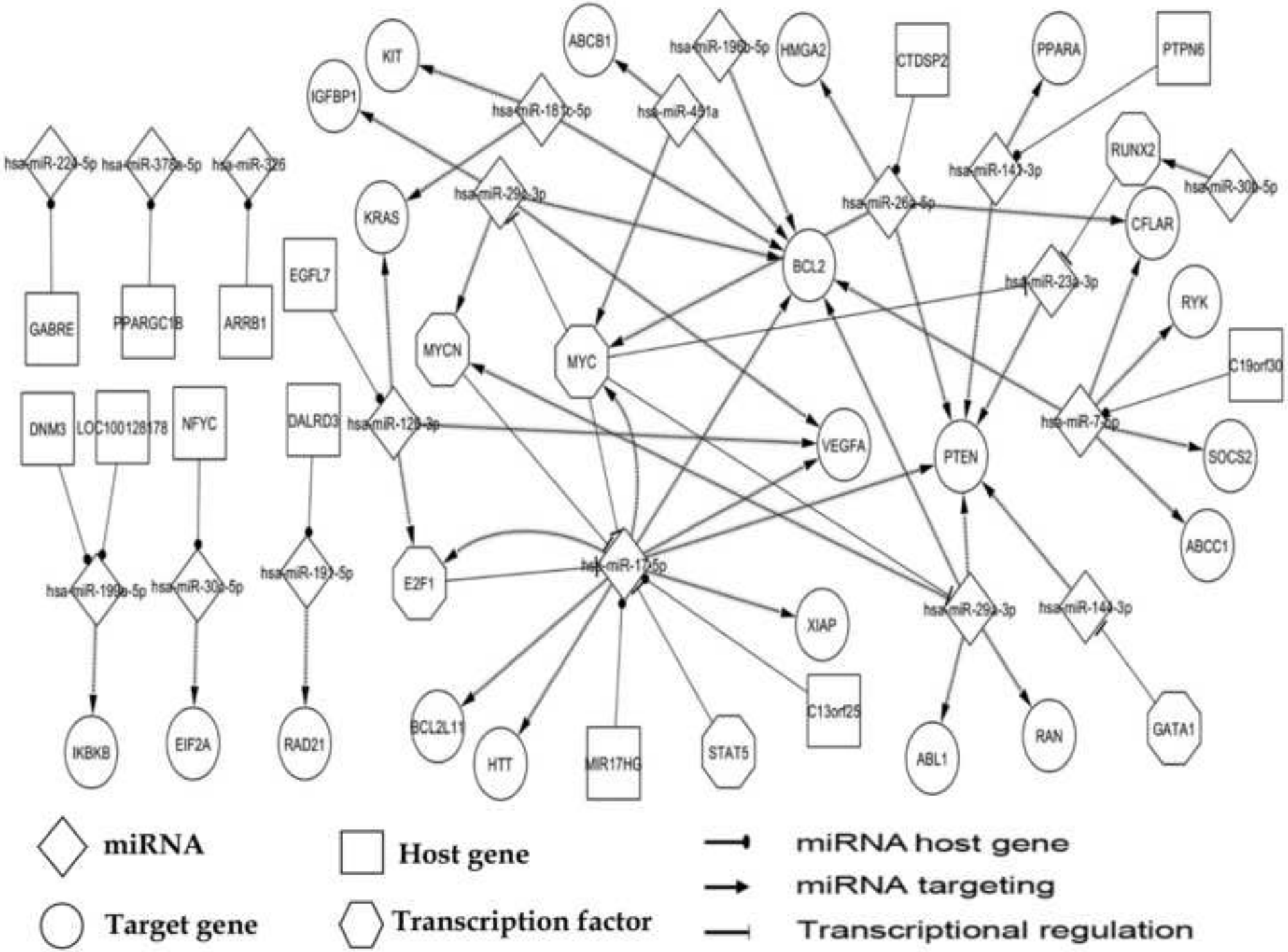

Fig 2. Related network of imatinib-resistant chronic myeloid leukemia.

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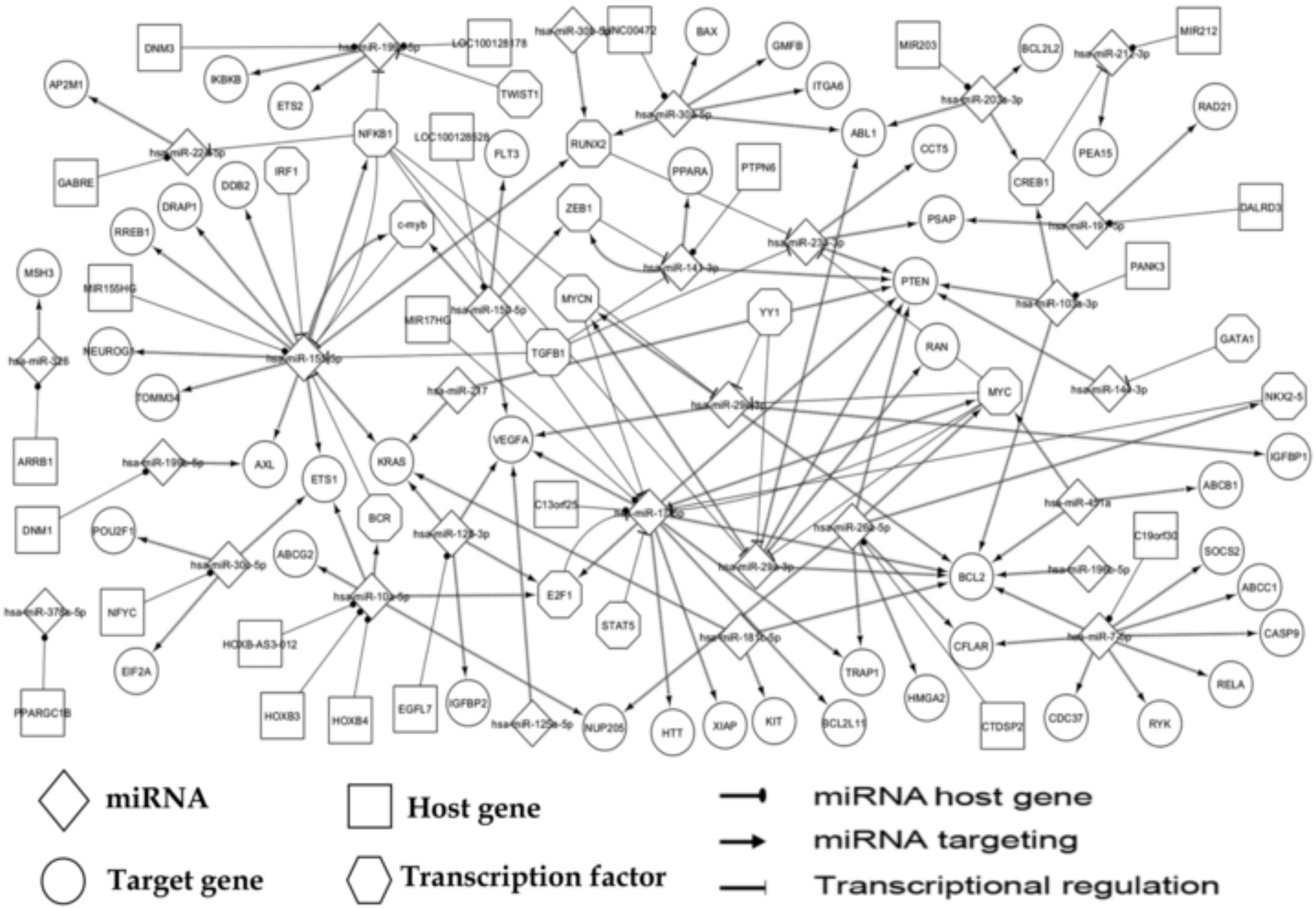

Fig 3. Host genes and microRNAs in the imatinib-resistant chronic myeloid leukemia related network.

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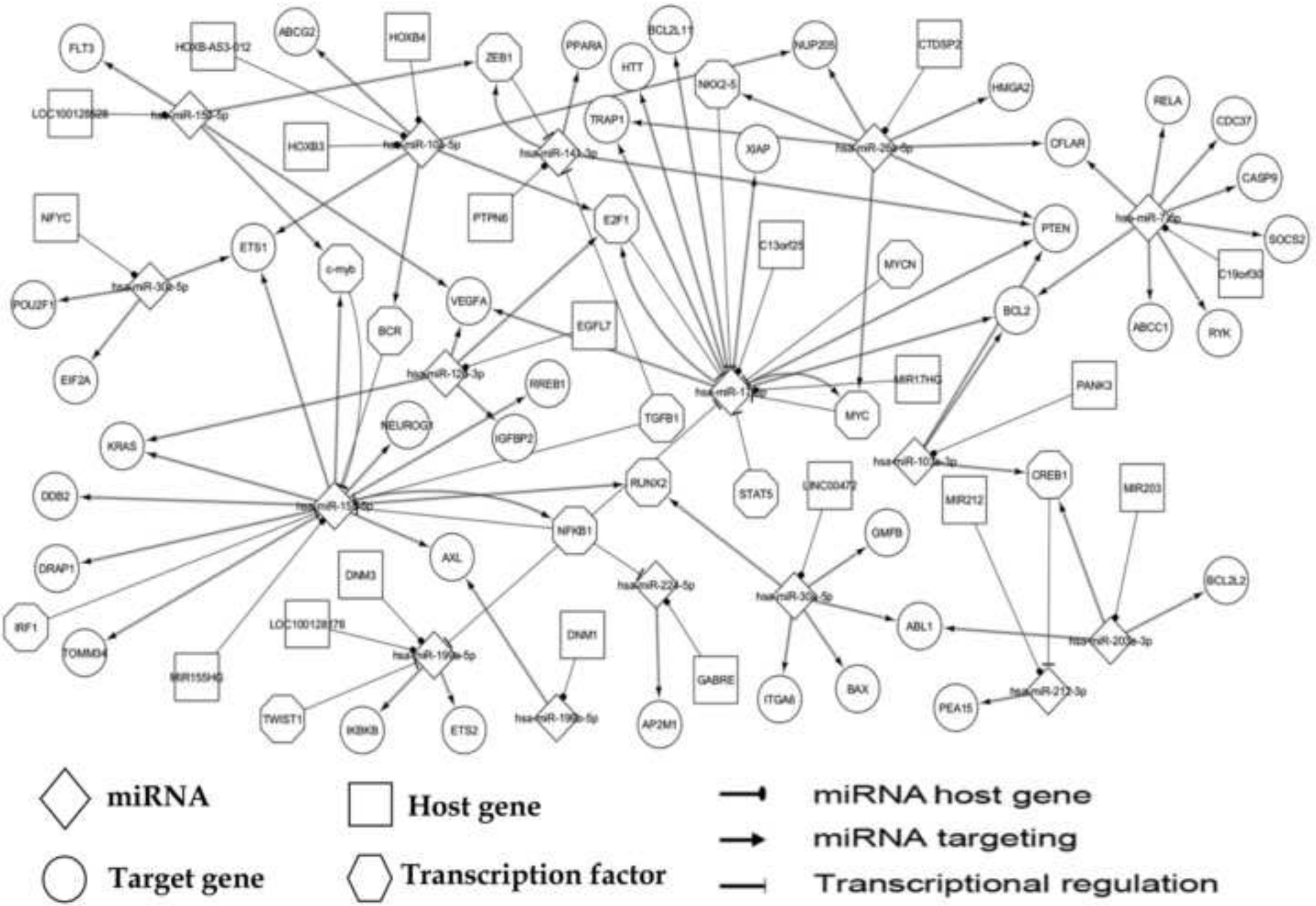

Fig 4. Transcriptional network of predicted transcription factors.

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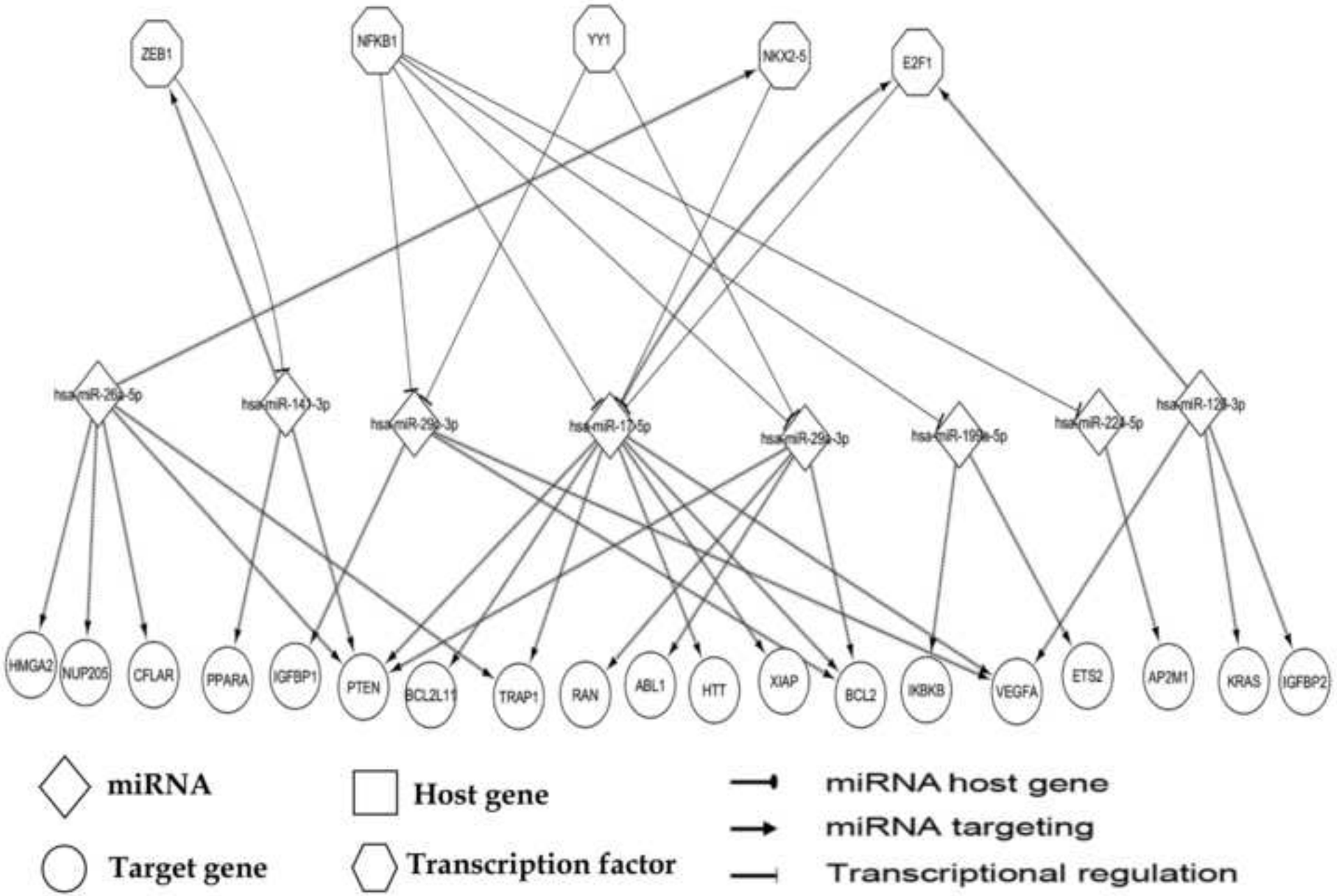

Table 1. Upstream and downstream informations of MY C in the three networks of CML imatinib resistant

Table 1: Upstream and downstream informations of MYC in the three networks of CML imatinib resistant

Upstream (miRNAs that target gene)			Gene	Downstream (miRNA that is regulated by gene)		
Differentially expressed network	Related network	Global network		Differentially expressed network	Related network	Global network
hsa-miR-451a	hsa-miR-451a	hsa-miR-320a	MYC	hsa-miR-17-5p	hsa-miR-17-5p	hsa-let-7f-2-3p
hsa-miR-26a-5p	hsa-miR-26a-5p	hsa-miR-744-5p		hsa-miR-23a-3p	hsa-miR-23a-3p	hsa-miR-195-5p
hsa-miR-17-5p	hsa-miR-17-5p	hsa-miR-335-5p		hsa-miR-29c-3p	hsa-miR-29c-3p	hsa-miR-19b-3p
		hsa-miR-34b-3p		hsa-miR-29a-3p	hsa-miR-29a-3p	hsa-miR-26a-1-3p
		hsa-miR-24-3p				hsa-miR-34a-5p
		hsa-miR-423-5p				hsa-let-7i-5p
		hsa-miR-34a-5p				hsa-let-7g-5p
		hsa-miR-451a				hsa-let-7a-3p
		hsa-let-7g-5p				hsa-miR-221-5p
		hsa-miR-429				hsa-miR-29c-5p
		hsa-miR-34c-5p				hsa-let-7f-1-3p
		hsa-miR-34b-5p				hsa-miR-20a-5p
		hsa-miR-449c-5p				hsa-miR-29b-1-5p
		hsa-miR-378a-3p				hsa-miR-22-5p
		hsa-miR-20a-5p				hsa-miR-23a-5p
		hsa-miR-21-5p				hsa-miR-18a-5p
		hsa-miR-135a-5p				hsa-miR-19a-5p
		hsa-miR-98-5p				hsa-miR-26b-5p
		hsa-miR-26a-5p				hsa-miR-23b-5p
		hsa-let-7a-5p				hsa-miR-29a-5p
		hsa-let-7f-5p				hsa-miR-92a-3p
		hsa-miR-17-5p				hsa-miR-15a-5p
		hsa-miR-320b				hsa-miR-17-5p

		hsa-miR-145-5p				hsa-let-7c-5p
		hsa-let-7c-5p				hsa-let-7b-5p
		hsa-let-7b-5p				hsa-let-7e-5p
						hsa-miR-106a-5p
						hsa-let-7d-5p

Table 2. Upstream and Downstream of hsa-miR-17-5p i n Three Networks of CML imatinib resistant

Table 2: Upstream and Downstream of hsa-miR-17-5p in Three Networks of CML imatinib resistant

Differentially expressed network	Related network	Global network		Differentially expressed network	Related network	Global network			
MYCN	MYCN	MYCN	Hsa-miR-17-5p	BCL2	BCL2	BCL2	ELMO2	ATF3	SYNDIG1
C13orf25	C13orf25	C13orf25		PTEN	PTEN	PTEN	SIRPA	ASNS	CPE
MIR1HG	MIR1HG	MIR1HG		HTT	HTT	HTT	ZBED3	PDPK1	POGK
STAT5	STAT5	STAT5		BCL2L11	BCL2L11	BCL2L11	EFHC1	IMMT	CLPTM1
E2F1	E2F1	E2F1		E2F1	E2F1	E2F1	SOX4	ZNFX1	APEX1
MYC	MYC	MYC		VEGFA	VEGFA	VEGFA	RUNX1	MED13	ARL9
	NFKB1	NFKB1		XIAP	XIAP	XIAP	RAB5B	ACOT2	QARS
	NKX2-5	NKX2-5		MYC	MYC	MYC	POGZ	ZSWIM3	COA1
		SPI1			TRAP1	TRAP1	IRAK1	CHD4	ELP2
		CCND1				PRPF8	LSM14A	MLL	ARHGAP5
		ERS1				TRUB1	SURF4	NOTCH2	ERLIN1
		TLX1				TMED10	RBL2	TBC1D15	CPSF1
		TLX3				UBE3C	AMD1	RAB23	MTF1
						UBE4A	AZIN1	PTPRO	PDLIM5
						TRIM11	IGFBP5	DEPDC1	CCDC47
						FBXO28	ZNF689	USP38	TMSB10
						DCTPP1	ATP6	NPAS2	CBL
						TMEM165	BMPR2	MEF2D	SUOX
						DHX33	ND4	FAM8A1	KDM4A
						MFHAS1	EHMT2	STK11	STIL
						ASH1L	MEN1	ZC3H18	SLC25A3
						PCGF5	GAPDH	YES1	WAC
						EPB41L2	NABP1	PRICKLE1	C15orf41
						SDHA	COX2	CHTF8	GNB1
						CNEP1R1	NPAT	WDR82	PGAM1

						SH3GLB2	HBP1	40787	NUFIP2
						TAF9B	HIST2H2AA3	DCUN1D4	TNPO3
						NUCKS1	CAP1	TMEM9	MRPS6
						SLC35E2B	CCL1	CTSA	BAZ2A
						COX7B	MAP3K8	NONO	MUC21
						KIF5C	NAPEPLD	GPI	CCND2
						PBXIP1	PRKD2	GDAP1	ARHGAP10
						PPP1R15A	FER	THBS1	CCT6A
						LCOR	RBL1	ABI2	RLF
						HDAC10	PRKD1	TSC2	CHST14
						MAP3K12	C11orf52	UQCRFS1	RTN3
						MDK	TSG101	RB1	SLC16A2
						MNT	MAPK9	ADARB1	TNFRSF10B
						DNAJC27	GAB1	TEFM	ANKRD27
						NOC2L	MOB1B	SMAD3	TRRAP
						CANX	EDG1	PLAG1	RND3
						HDHD1	WEE1	GPM6A	EEF1A1
						EIF4G3	PLS1	TNFSF13	BTN3A1
						SLC25A28	APP	EIF4G2	GLO1
						HNRNPU	IL-8	TCF3	CETN2
						MMP-2	ATP5B	GDF11	TRIM8
						MTMR3	Peli1	RTCD1	COPS3
						RPL7	RPL21	LAPTM4A	RUNDC1
						EARS2	NCOA3	SON	MGEA5
						PCNX	MUC17	MBNL1	RYR2
						HUWE1	UBA52	TRIM44	UBE2S
						ENPP5	TRIM71	SMIM15	ZBTB5

						TMEM19	ND2	TMTC4	RALGAPA1
						NFAT5	AGO1	LARP1	NAP1L1
						ARPC2	JAK1	DNMBP	TGFBR2
						SLC25A37	PKD2	OPTN	ZFYVE26
						RMDN1	BACE1	KAT2A	FXR2
						HN1	EPB41L5	KIF1A	MED12
						UBE2C	NAGK	NBR1	CPS1
						PLXNA1	Ppp1ca	VIM	NAT8L
						ARIH1	E2F3	HIST1H4A	ZIK1
						HEXIM1	TUT1	ATXN7	HSP90AA1
						HIST2H3A	MTRF1L	DPYSL2	SELE
						RABGEF1	ZNF598	TXLNA	MKI67
						RNF145	RBM5	CCND1	PSD3
						TCEA2	ZNF507	CDKN1A	RNF146
						RBM12B	PIK3CA	RPSA	REEP5
						WASL	PAIP1	MORF4L2	ATRX
						LAS1L	PER1	FBXO31	FASN
						KIAA1919	ILF3	KANSL1	RPL37
						PLEKHM1	TUBB4B	FOPNL	LPIN1
						DAPK3	TNFAIP1	VPRBP	PTBP1
						42249	HIST3H2A	MRPL40	GANAB
						MYCBP2	OFD1	SFRS10	AK1
						MAP3K9	SIPA1L3	TJP1	PTTG1
						GNAS	BLVRA	ICAM1	RTTN
						FTH1	PPP2R1A	PIGS	GPR137B
						SMAD4	DCBLD2	ERCC2	TOB1
						HIST1H2AL	ARHGEF7	RPS15A	FANCA

Table 3: Upstream and downstream informations of E2F1 in the three networks of CML imatinib resistant

Upstream (miRNAs that target TF)			TF	Downstream (miRNA that is regulated by TF)		
Differentially expressed network	Related network	Global network		Differentially expressed network	Related network	Global network
hsa-miR-17-5p	hsa-miR-17-5p	hsa-miR-17-5p	E2F1	hsa-miR-17-5p	hsa-miR-17-5p	hsa-miR-17-5p
hsa-miR-126-3p	hsa-miR-126-3p	hsa-miR-126-3p				hsa-miR-92a-3p
	hsa-miR-10a-5p	hsa-miR-10a-5p				hsa-miR-15b-5p
		hsa-miR-23b-3p				hsa-miR-106b-5p
		hsa-miR-149-3p				hsa-miR-25-5p
		hsa-miR-181b-5p				hsa-miR-20b-5p
		hsa-miR-24-3p				hsa-miR-18b-5p
		hsa-miR-93-5p				hsa-miR-449a
		hsa-let-7a-5p				hsa-miR-92a-2-5p
		hsa-miR-34a-5p				hsa-miR-93-5p
		hsa-miR-98-5p				hsa-miR-15a-5p
		hsa-miR-330-3p				hsa-miR-363-5p
		hsa-miR-223-3p				hsa-miR-18a-5p
		hsa-miR-205-5p				hsa-miR-19a-5p
		hsa-miR-106b-5p				hsa-miR-16-5p
		hsa-miR-20a-5p				hsa-miR-19b-3p
		hsa-miR-106a-5p				hsa-miR-106a-5p
		hsa-miR-21-5p				hsa-miR-20a-5p
		hsa-miR-331-3p				hsa-miR-449c-5p
		hsa-miR-130b-3p				hsa-miR-449b-5p
		hsa-miR-193b-3p				hsa-miR-19b-2-5p
		hsa-miR-362-3p				