INTRODUCTION

CHRONIC MYELOID LEUKEMIA

Definition:

Chronic myeloid leukemia (CML) is a clonal malignant myeloproliferative disorder believed to originate in a single abnormal hematopoietic stem cell. It involves neoplastic overproduction of myeloid, monocytic, erythroid, megakaryocytic, B-lymphoid, and occasionally T-lymphoid lineages.[1]

Epidemiology:

The disease accounts for 15% of adult leukemias. The incidence of CML is greatest between ages of 40 and 60 years, with slightly more men affected by the disease compared to women.[1] However, CML occurs in all age groups according to Surveillance, Epidemiology, and End Results (SEER) statistics.[2]

Pathophysiology:

At the molecular level, CML is the first human disease in which a specific abnormality of the karyotype could be linked to pathogenesis. Thus, CML is the best-characterized leukemia at a molecular level, and many studies had helped to further define the molecular events involved in its initiation and progression in order to relate these events to clinical manifestations, the course of the disease, and therapeutic interventions.[3]

The reciprocal translocation between chromosomes 9 and 22 is the hallmark of the disease, which results in creation of an elongated chromosome 9, and a truncated chromosome 22 known as the Philadelphia chromosome (Ph). The result of this translocation is the juxtapositioning of the breakpoint cluster region (BCR) gene on chromosome 22 at band q11 and the Abelson murine leukemia (ABL) gene located on chromosome 9 at band q34.This chromosomal translocation is therefore designated as t(9;22)(q34;q11). The presence of this translocation is a highly sensitive test for CML, since 95% of people with CML have this abnormality.[3] (Figure. 1)

This abnormal fusion gene leads to the generation of a protein known as p210 owing to its molecular weight being 210 kilo daltons (kDa). This BCR-ABL fusion gene product is a tyrosine kinase because ABL carries a tyrosine kinase domain.[4] The action of the BCR-ABL protein p210 had been established as the pathophysiologic cause of CML. BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and increasing cellular susceptibility for developing further genetic abnormalities.[5]

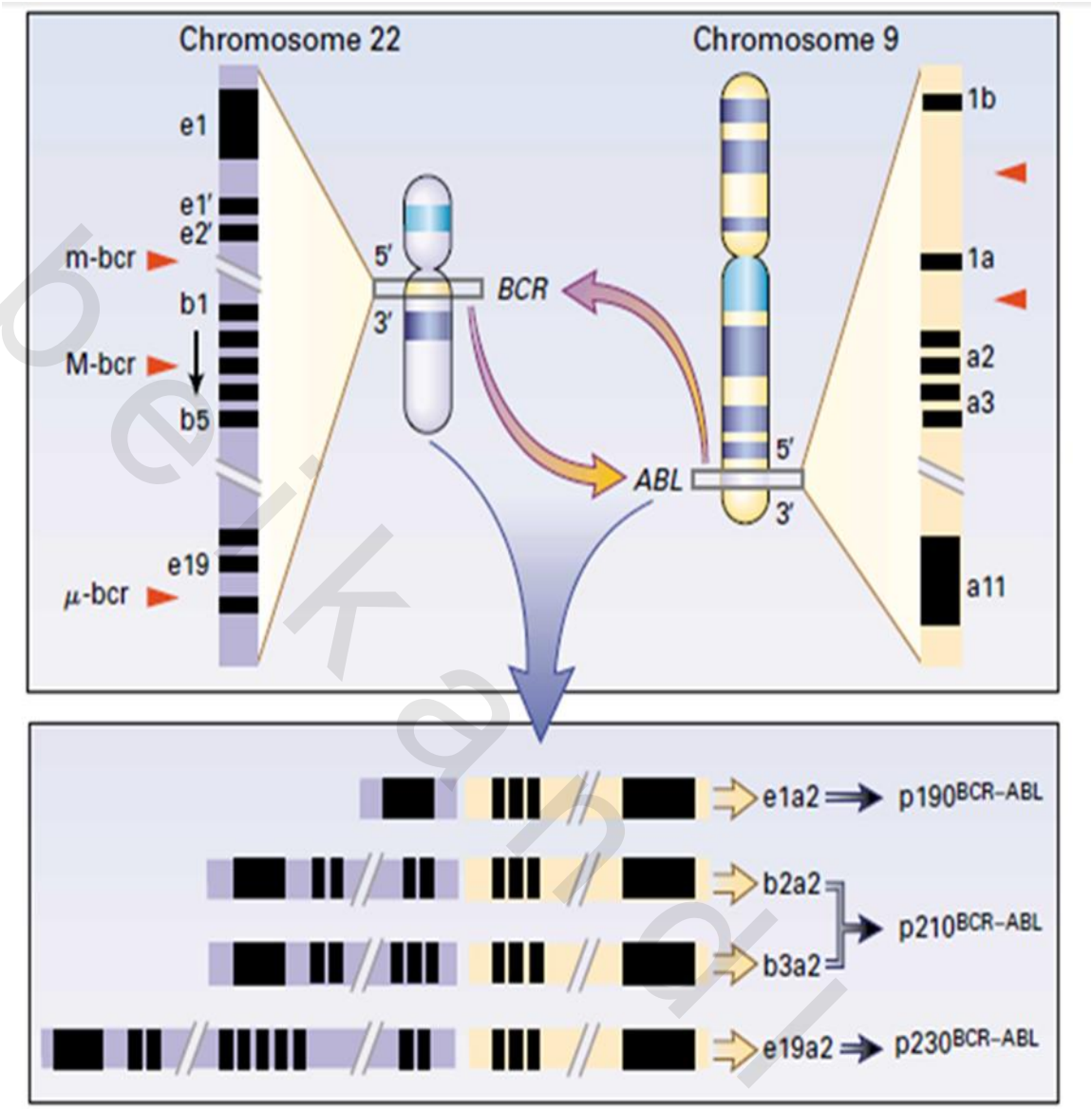

Fig. 1. **The Philadelphia chromosome (Ph).** Philadelphia chromosome (Ph) is a shortened chromosome 22 that results from the translocation of 3' (toward the telomere) ABL segments on chromosome 9 to 5' BCR segments on chromosome 22. Breakpoints (arrowheads) on the ABL gene are located 5' (toward the centromere) of exon a2 in most cases. Various breakpoint locations have been identified along the BCR gene on chromosome 22. Depending on which breakpoints are involved, different-sized segments from BCR are fused with the 3' sequences of the ABL gene. This results in fusion messenger RNA molecules (e1a2, b2a2, b3a2, and e19a2) of different lengths that are transcribed into chimeric protein products (p190, p210, and p230). The protein product associated with CML is p210. The abbreviation m-bcr denotes minor breakpoint cluster region, M-bcr major breakpoint cluster region, and μ-bcr a third breakpoint location in the BCR gene that is downstream from the M-bcr region between exons e19 and e20.[3]

ABL proteins are tyrosine kinases that play important roles in signal transduction and the regulation of cell growth.[6] The N-terminal segment of ABL includes two Sarcoma (SRC) homology domains (SH2 and SH3), which regulate the tyrosine kinase function of ABL, the catalytic domain, and a myristoylation sequence that connects ABL to proteins of the plasma membrane.[6,7,8] The C-terminal part of ABL contains a DNA-binding domain, nuclear localization signals, and a binding site for actin.[9](Figure. 2)

The N-terminal coiled-coil motif of BCR increases the tyrosine kinase activity of ABL and enables binding of F-actin.[10] The serine–threonine (ser-thr) kinase domain of BCR activates signaling pathways mediated by ABL tyrosine kinase.[11] BCR interferes with the adjacent SH3 kinase regulatory domain, which in turn causes ABL to become constitutively active as a tyrosine phosphokinase.[12] (Figure. 2)

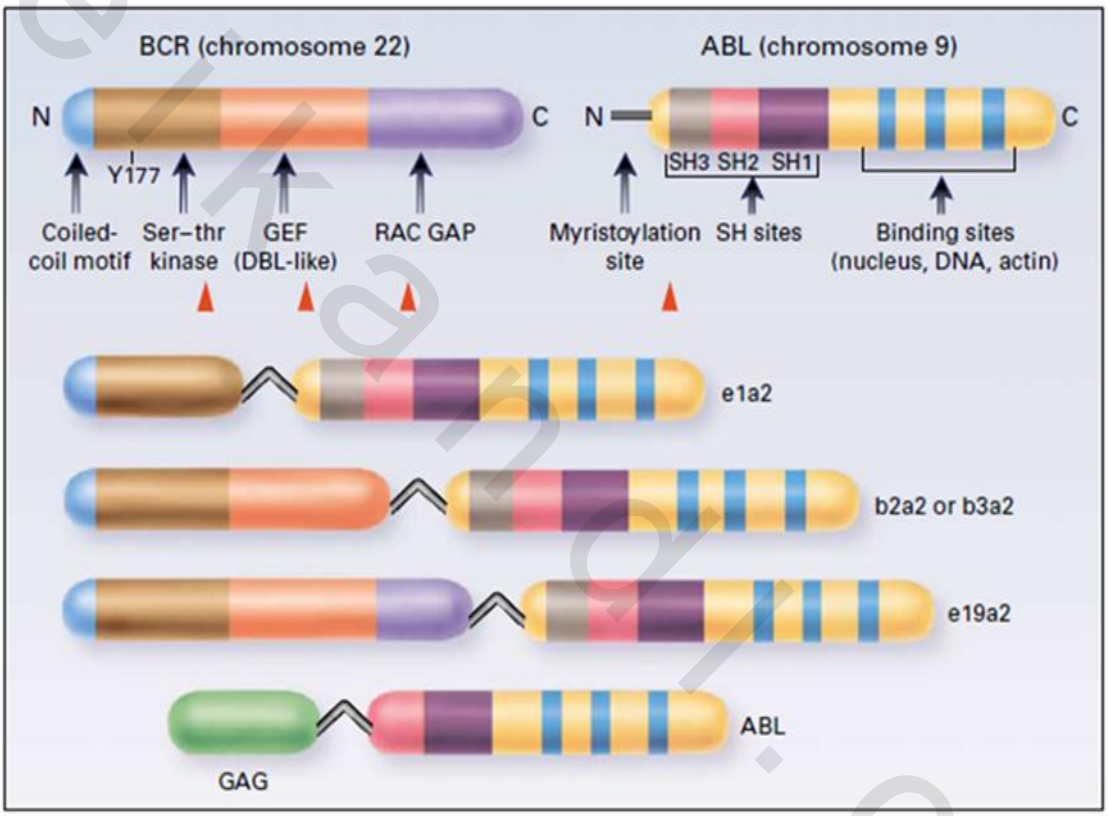

Fig. 2. **Functional Domains of BCR–ABL.** Important functional domains of the BCR and ABL gene products as well as of the different fusion-protein products are shown. Breakpoints are indicated by arrowheads. N denotes amino-terminal amino acid, C carboxyl-terminal amino acid, Ser–thr serine–threonine, GDP guanosine diphosphate, GTP guanosine triphosphate, GEF GDP–GTP exchange factor, DBL diffuse B-cell lymphoma oncogene, RAC a RAS-like GTPase, GAP guanosine triphosphatase activating protein, and SH denotes SRC homology domain.[3]

The structure of p210 BCR–ABL allows multiple protein-protein interactions and suggests the involvement of diverse intracellular signaling pathways. Several BCR domains serve to bind adapter proteins such as growth factor receptor bound protein 2

(GRB2), CRK oncogene like protein (CRKL), casitas B-lineage lymphoma protein (CBL), and SRC homology 2 containing protein (SHC).[13](figure.3)

The SH2 domain of GRB2 binds to a conserved tyrosine residue (Y177) of BCR in p210 BCR–ABL. This links p210 BCR–ABL to RAS, a guanosine triphosphate–binding protein involved in the regulation of cell proliferation and differentiation and at the core of the most prominent signaling pathway in the pathogenesis of CML.[14] (figure.3)

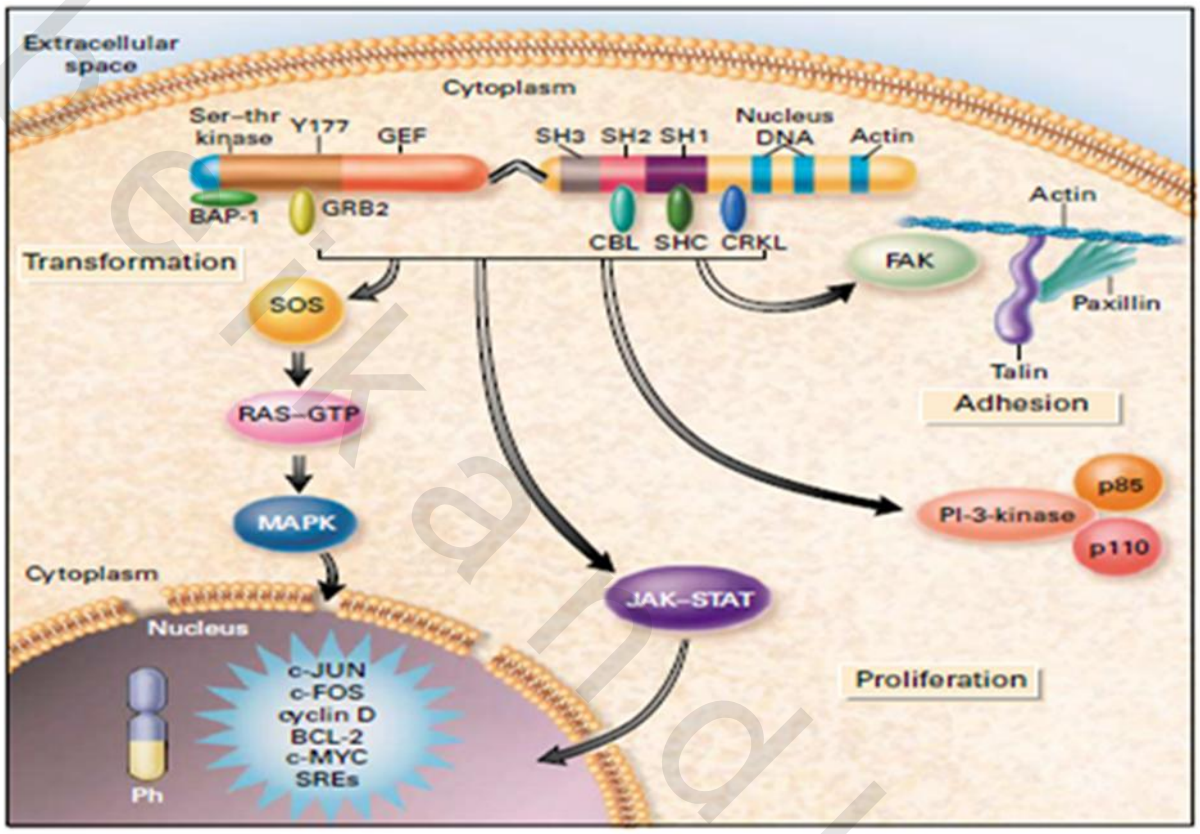

Fig. 3. **Signaling Pathways of p210 BCR–ABL.** Several regions of BCR–ABL serve as important control elements for RAS, which is at the center of the most prominent signaling pathways in CML. Activation of RAS is mediated through a series of adapter proteins, such as GRB2, CBL, SHC, and CRKL. Adapter proteins also connect p210 BCR–ABL to focal adhesion complexes, PI-3 kinase, and other messenger systems such as JAK–STAT kinases. Signaling events downstream of RAS appear to involve mainly mitogen-activated protein kinases (MAPKs), preferably the JUN kinase (JNK) pathway. BAP-1 denotes BCR-associated protein 1, GRB2 growth factor receptor–bound protein 2, CBL casitas B-lineage lymphoma protein, SHC SRC homology 2–containing protein, CRKL CRK-oncogene–like protein, JAK–STAT Janus kinase–signal transducers and activators of transcription, FAK focal adhesion kinase, SOS son-of-sevenless, GDP guanosine diphosphate, GTP guanosine triphosphate, SRE stimulated response element, Ser–thr serine–threonine, Y177 a conserved tyrosine residue, GEF GDP–GTP exchange factor, and SH SRC homology domain.[3]

Signaling events downstream of RAS involve mitogen-activated protein kinases (MAPKs), such as the JUN kinase (JNK) pathway.[15] Signaling cascades of p210 BCR–ABL not involving RAS, such as cyto-myelocytomatosis (c-Myc), have been identified, but their role in the pathogenesis of CML is unclear.[16]

Diagnosis:

The disease is often suspected on the basis of a complete blood count (CBC), which shows increased granulocytes, basophils and eosinophils.[17] However, diagnosis is based on detecting the Ph chromosome by fluorescent in situ hybridization (FISH), or by polymerase chain reaction (PCR) for the BCR-ABL fusion gene.[18]

Stages:

Based on clinical characteristics and laboratory findings, CML is often divided into three phases. In the absence of intervention, CML typically begins in the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. Drug treatment will usually stop this progression if started early. Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed.[19]

I- Chronic phase:

Approximately 85% of patients with CML are in the chronic phase (CP-CML) at the time of diagnosis. During this phase, patients are usually asymptomatic or have only mild symptoms of fatigue, left side pain, joint pain, or abdominal fullness.[19]

The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase.[19]

II- Accelerated phase:

Criteria for diagnosing transition into the accelerated phase (AP-CML) are somewhat variable. The world health organization (WHO) criteria are the most widely used, and define the AP-CML by any of the following.

- 10–19% myeloblasts in the peripheral blood or bone marrow.
- >20% basophils in the blood or bone marrow.
- Platelet count <100,000/mm^3, unrelated to therapy.
- Platelet count >1,000,000/mm^3, unresponsive to therapy
- Cytogenetic evolution with new abnormalities in addition to the Ph chromosome.
- Increasing splenomegaly or white blood cell count, unresponsive to therapy.[20]

The AP-CML is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages.[21]

III- Blast crisis:

Blast crisis (BP-CML) is the final phase in the evolution of CML, and behaves like an acute leukemia, with rapid progression and short survival. According to WHO Criteria Blast crisis is diagnosed if any of the following are present in a patient with CML:

- Blasts forming 20% of peripheral white blood cells or of nucleated bone marrow cells
- Extramedullary blast proliferation
- Large foci or clusters of blasts in the bone marrow biopsy.[21]

Prognostic scoring systems:

Sokal and Hasford are the two prognostic scoring systems available for the risk stratification of patients with CML.[21] The Sokal score is the most commonly used and is based on the patient's age, spleen size, platelet count, and percentage of blasts in the peripheral blood.[22] The Hasford model includes eosinophils and basophils in the peripheral blood in addition to the same clinical variables used in the Sokal model.[23] (Table.1)

Table. 1 Prognostic scoring systems of chronic myeloid leukemia[21]

Study	**Risk definition by calculation**
Sokal	Low < 0.8 Intermediate 0.8 – 1.2 High > 1.2
Hasford	Low ≤ 780 Intermediate 781-1,480 High > 1,480

Imatinib mesylate

Since the 2000s, BCR-ABL tyrosine-kinase inhibitors (TKIs), have become the gold standard treatment for newly diagnosed cases of CML.[24] They act by specifically targeting, the constitutively activated tyrosine kinase fusion protein caused by the Ph translocation.[25](figure 4)

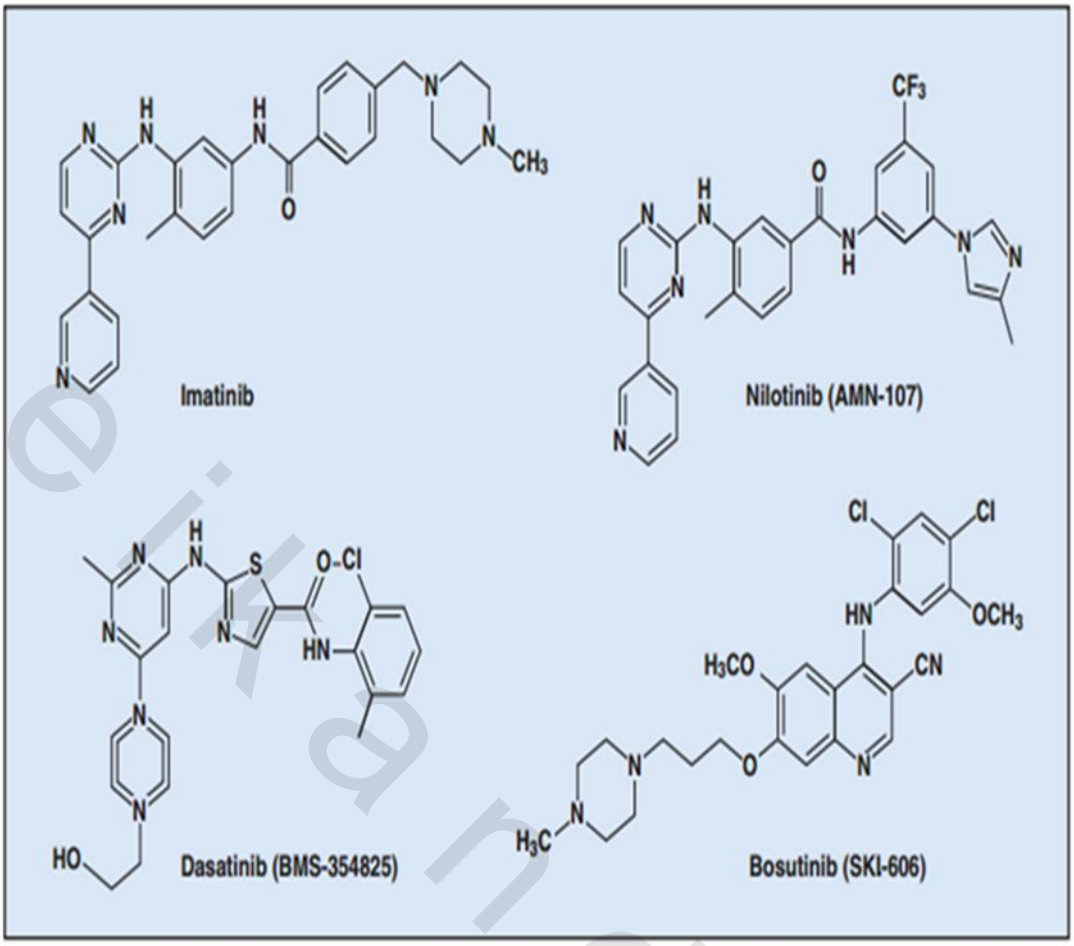

Fig.4. Chemical structures of imatinib mesylate and second generation tyrosine kinase inhibitors.[26]

Imatinib mesylate (IM), marketed as Gleevec or Glivec, and sometimes referred to by its investigational name STI-571, is the first TKI to be introduced for treatment of CML. It is a phenyl amino pyrimidine compound approved by the Unites States Food and Drug Administration (FDA) in 2001.[27]International Randomized Study of Interferon Vs STI571 (IRIS), an international study carried out on 2004 that compared interferon/cytarabine combination and IM, demonstrated the clear superiority of tyrosine-kinase-targeted inhibition over other treatments.[28]

The drug acts by blocking BCR-ABL tyrosine kinase activity. Because the BCR-ABL tyrosine kinase enzyme exists only in cancer cells and not in healthy cells, IM works as a form of targeted therapy i.e. only cancer cells are killed through the drug's action. [28]

The development of IM, had a major impact on patients with CML.[28] Treatment with IM induced a complete hematological and cytogenetic response in more than 90% of newly diagnosed patients and 80% with chronic phase CML patients, which has established IM as the standard therapy for CML.[29]

According to the National Comprehensive Cancer Network (NCCN) guidelines version 2014 for CML, the criteria for hematologic, cytogenetic, molecular response and relapse are defined as follows:

Complete hematologic response

- Complete normalization of peripheral blood counts with leukocyte count <10 cells x 10^9 /L
- Platelet count <450 cells x 10^9 /L
- No immature cells, such as myelocytes, promyelocytes, or blasts in peripheral blood
- No signs and symptoms of disease with disappearance of palpable splenomegaly.(30)

Cytogenetic response

- Complete cytogenetic response (CCyR): No Ph-positive metaphases
- Partial: 1% - 35% Ph-positive metaphases
- Major: 0% - 35% Ph-positive metaphases (complete + partial)
- Minor: >35% Ph-positive metaphases.(30)

Molecular response

- Complete molecular response: no detectable BCR-ABL mRNA by real time polymerase chain reaction (qPCR).
- Major molecular response (MMR): BCR-ABL transcripts 0.1% by qPCR.(30)

Resistance

- Any sign of loss of response (defined as hematologic or cytogenetic relapse)
- 1 log increase in BCR-ABL transcript levels with loss of MMR should prompt bone marrow evaluation for loss of CCyR but is not itself defined as relapse.(30)

Despite the clinical success obtained with the use of IM, primary resistance to IM and molecular evidence of persistent disease had been observed in 20-25% of IM treated patients. Different mechanisms of resistance had been described, including the presence of point mutations in the tyrosine kinase domain of BCR-ABL, amplification and overexpression of BCR-ABL, over-expression of IM efflux transporters and under-expression of its uptake transporters.(31)

It had also been shown that IM does not inactivate all BCR-ABL activated signaling pathways that are essential for CML cell survival. This implies that persistent malignant progenitors can be a potential source of relapse in CML patients and that there is a need to improve our understanding of the biology of CML to provide new targets for therapy.(32)

MICRO-RIBONUCLEIC ACIDS

Definition:

Micro-ribonucleic acids, usually referred to as micro-RNAs (miRNA, miR), are short noncoding ribonucleic acids (RNAs). These 21-24 nucleotide (nt) RNAs are involved in diverse biological functions through the regulation of target genes during normal development and various pathological responses.[33]

History:

In the early 1990s, the first miRNA was characterized during a study of the gene lin-4 in Caenorhabditis elegans (C. elegans) development. This work provided the initial example for the mode of miRNA action showing that lin-4 gene encoded a short RNA product which functioned to regulate LIN-14 protein. The study showed that a 61-nt precursor from the lin-4 gene matured to a 22-nt RNA that contained sequences partially complementary to multiple sequences in the 3' untranslated region (3' UTR) of the lin-14 messenger RNA (mRNA).[34]

Several other studies aiming at a better understanding of miRNAs followed. However, miRNAs were not recognized as a distinct class of biological regulators with conserved functions until the early 2000s. Since then, miRNA research had evolved greatly revealing the multiple roles of miRNAs as negative regulators of genes by influencing translational suppression or transcript degradation and sequestering. Moreover, the possible role of miRNAs in the positive gene regulation by transcriptional and translational activation was also studied. Therefore, miRNAs are likely to influence most biological processes by affecting gene regulation.[35]

There is increasing evidence suggesting that miRNAs are implicated in the regulation of several biological processes, such as cell cycle, apoptosis, cellular signaling network, tissue differentiation, and embryonic development.[36,37,38] As such, mutation of miRNAs, dysfunction of miRNA biogenesis and dysregulation of miRNAs and their targets may result in various diseases. Having profound implications in medicine, today miRNAs present a new and exciting field of molecular biology which continues to advance.[39]

Biogenesis:

1) Transcription:

The coding sequences of miRNAs can be found in introns or exons of a protein-coding gene or in the intergenic regions. It is quite common that several miRNA genes are clustered along the genome, sharing the same promoter, they can also be present individually.[40]

Genes encoding miRNAs are usually transcribed by RNA polymerase II (RNA pol II) generating a long primary transcript known as primary miRNA (pri-miRNA), the resulting transcript is capped with 7-methylguanylate cap at the 5' end, polyadenylated with multiple adenosines acquiring a poly A tail and spliced.[41] The emerging pri-miRNA transcripts in the nucleus are in the form of specific ''hairpin shaped'' secondary

structures.[42] These hairpin loop structures are composed of about 70 nts each.[43] (Figure 5)

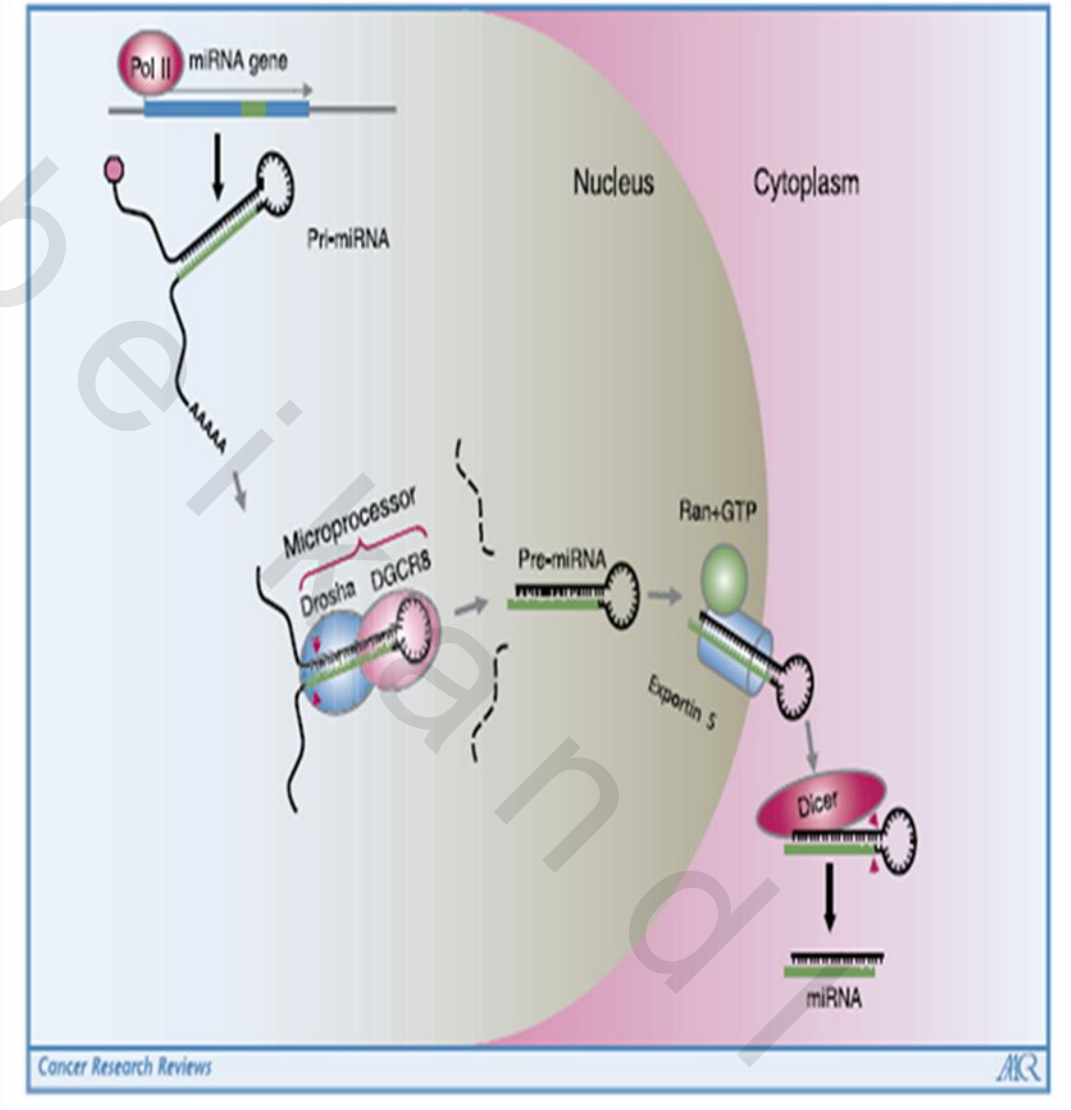

Fig. 5. Model of miRNA biogenesis.[43]

2) Stepwise Maturation:

Following transcription by RNA pol II, the pri-miRNA transcript undergoes conversion into a mature 21 to 24 nt miRNA through two successive processing steps. The first processing step is nuclear followed by export of the processed molecules to the cytoplasm where the second processing step occurs.[44] (Figure 5)

a. Nuclear processing:

It was established that processing of pri-miRNAs in the nucleus is mediated by the "Microprocessor" complex. This complex is formed by the association of the ribonuclease III (RNase III) named Drosha with the nuclear protein known as DiGeorge Syndrome Critical Region 8 (DGCR8), named for its association with DiGeorge Syndrome (A syndrome due to deletion 22q11 and is also associated with truncus arteriosus and tetralogy

of Fallot). First, the double-stranded RNA structure of the pri-miRNA is recognized by DGCR8 which then associates with Drosha.[44]

In this microprocessor complex, DGCR8 functions to orient the catalytic RNase III domain of Drosha allowing it to cleave RNA about eleven nucleotides from the hairpin base liberating hairpins from pri-miRNAs. The product resulting from microprocessor complex action is often termed as a precursor miRNA (Pre-miRNA). Pre-miRNA has a 2 nt overhang at its 3'end.[45]

Precursor miRNA hairpins are exported from the nucleus in a process involving the nucleocytoplasmic shuttle known as Exportin-5 (XPO5). Exportin 5 is a protein that recognizes the two-nucleotide overhang left by the RNase III enzyme Drosha at the 3' end of the pre-miRNA hairpins. Exportin 5 mediated transport to the cytoplasm is an energy dependent process, using GTP bound to the RAs-related Nuclear (Ran) protein.[46]

b. Cytoplasmic processing:

In the cytoplasm, the pre-miRNA is cleaved by a second RNase III enzyme known as Dicer. This endoribonuclease enzyme interacts with the 3' end of the hairpin and cuts away the loop joining the 3' and 5' arms, yielding a 21-24 nt mature double-stranded miRNA. The final product of dicer processing is often referred to as miRNA: miRNA* duplex with phosphate groups at the 5'end and a 2 nt overhang at its 3'end.[47] Dicer processing of the pre-miRNA is thought to be coupled with unwinding of the duplex.[48]

Mechanism of action

Critical changes in gene expression programs are elicited by miRNAs through the regulation of target genes, which have been reported to underlie diverse aspects of biology, including developmental timing, differentiation, proliferation, cell death, and metabolism.[49]

Mature miRNA regulation of genes occurs by promoting the association of a large protein complex known as RNA-induced silencing complex (RISC), with specific regions in the 3′UTR of the target genes.[50] RISC is also known as a microRNA ribonucleoprotein complex (miRNP). RISC with incorporated miRNA is sometimes referred to as "miRISC."[51] (Figure 6)

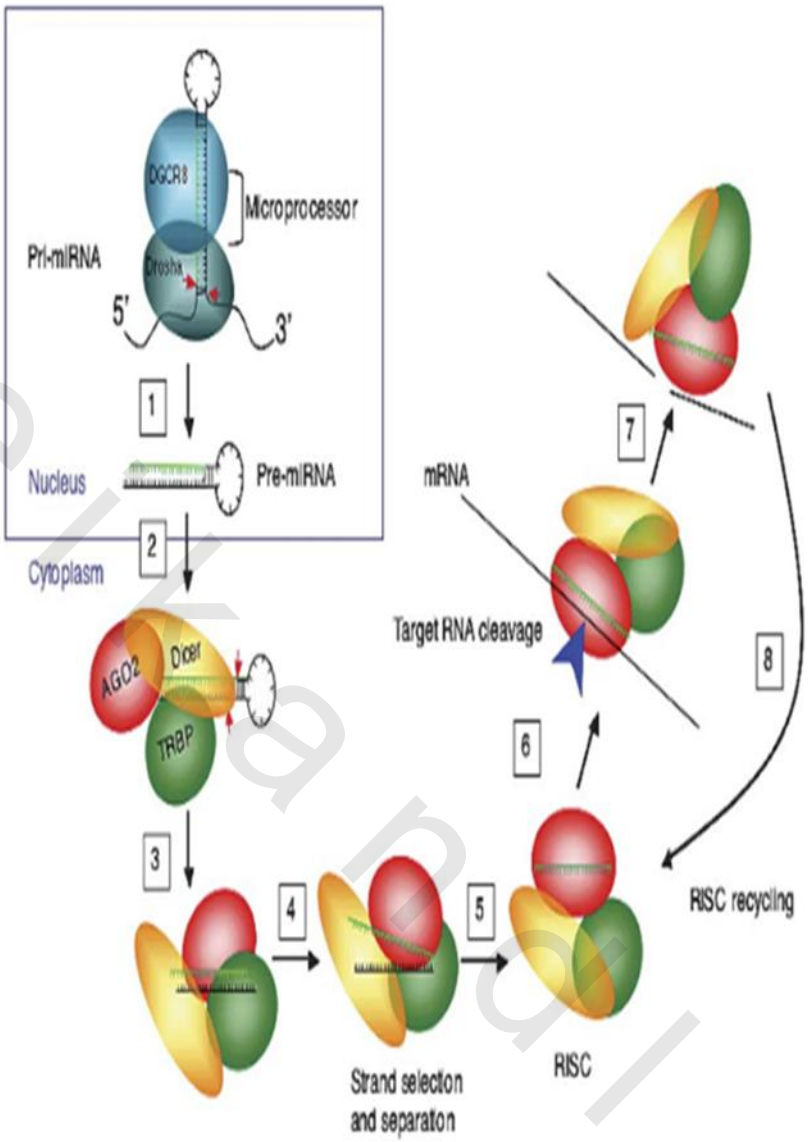

Fig.6. Ribonucleic acid-induced silencing complex (RISC) Assembly and Function.[52] The figure shows the steps RISC Assembly and Function. AGO2 denotes Argonaute 2 protein, TRBP double-stranded RNA binding protein and RISC RNA induced silencing complex

One strand from the mature double-stranded miRNA: miRNA* duplex is incorporated into RISC guiding this complex to target mRNA sequences. The strand incorporated into RISC, is known as the guide strand and is selected by the Argonaute 2 protein (AGO2), the catalytically active RNase in the RISC, on basis of thermodynamic stability of the 5' end.[53] The guide strand is the strand with a less thermodynamically stable 5' end.[54] The remaining strand, known as the anti-guide or passenger strand, due to its lower levels in the steady state, is denoted with an asterisk (*) and is normally degraded as a RISC complex substrate.[52]

The guide strand of the miRNA remains associated with AGO2 in the active RISC complex. Ribonucleic acid induced silencing complex recognizes target mRNAs based on complementarity between the guide miRNA strand and the mRNA transcript. AGO2 specifically cleaves the target mRNA for posttranscriptional gene silencing. The cleaved product is released, enabling RISC to catalyze the destruction of another target RNA.(52)

Importantly, all steps of RISC activity requires no energy derived from ATP hydrolysis.(54) There are also data that support Dicer as a potential RISC component in addition to AGO2 and double-stranded RNA binding protein (TRBP). In some cases, both strands of the miRNA duplex are viable and become functional miRNA that target different mRNA populations.(52)

Association of the miRISC with a target mRNA results in the repression of the target gene by promoting mRNA degradation and/or translational inhibition.(55) Selection of miRNA targets is mediated by imperfect base pairing between the miRNA and miRNA binding site present in the 3'UTR of the target mRNA. This imperfect nature of the miRNA: mRNA interaction means that a single miRNA can target tens to hundreds of mRNAs.(56)

The latest miRBase release contains 1872 precursor miRNAs processed to produce 2578 mature human miRNA products.(57) Since a single miRNA is said to be able to target several mRNAs, aberrant miRNA expression is capable of disrupting the expression of several mRNAs and proteins.(58)

Micro-RNAs in human diseases and cancer

Understanding the significance of miRNAs in the pathogenesis of human diseases represents an important dimension in miRNA research today as it may lead to the development of miRNA-based novel therapeutic strategies or diagnostic/ prognostic biomarkers.(59) Among human diseases, it has been shown that miRNAs are aberrantly expressed or mutated in cancer, suggesting that they may play a role as a novel class of oncogenes or tumor suppressor genes.(60) (figure. 7)

Through many mechanisms, the deregulation of expression of miRNAs has been shown to contribute to cancer development. Examples of such mechanisms include; deletions, amplifications, or mutations involving miRNA loci, epigenetic silencing or the dysregulation of transcription factors that target specific miRNAs.(61) Moreover, it was found that more than half of miRNA genes are located at cancer susceptibility loci.(62) However, since then many more miRNAs have been identified and the relationship between site fragility and miRNA density seems far more complex than previously thought.(63)

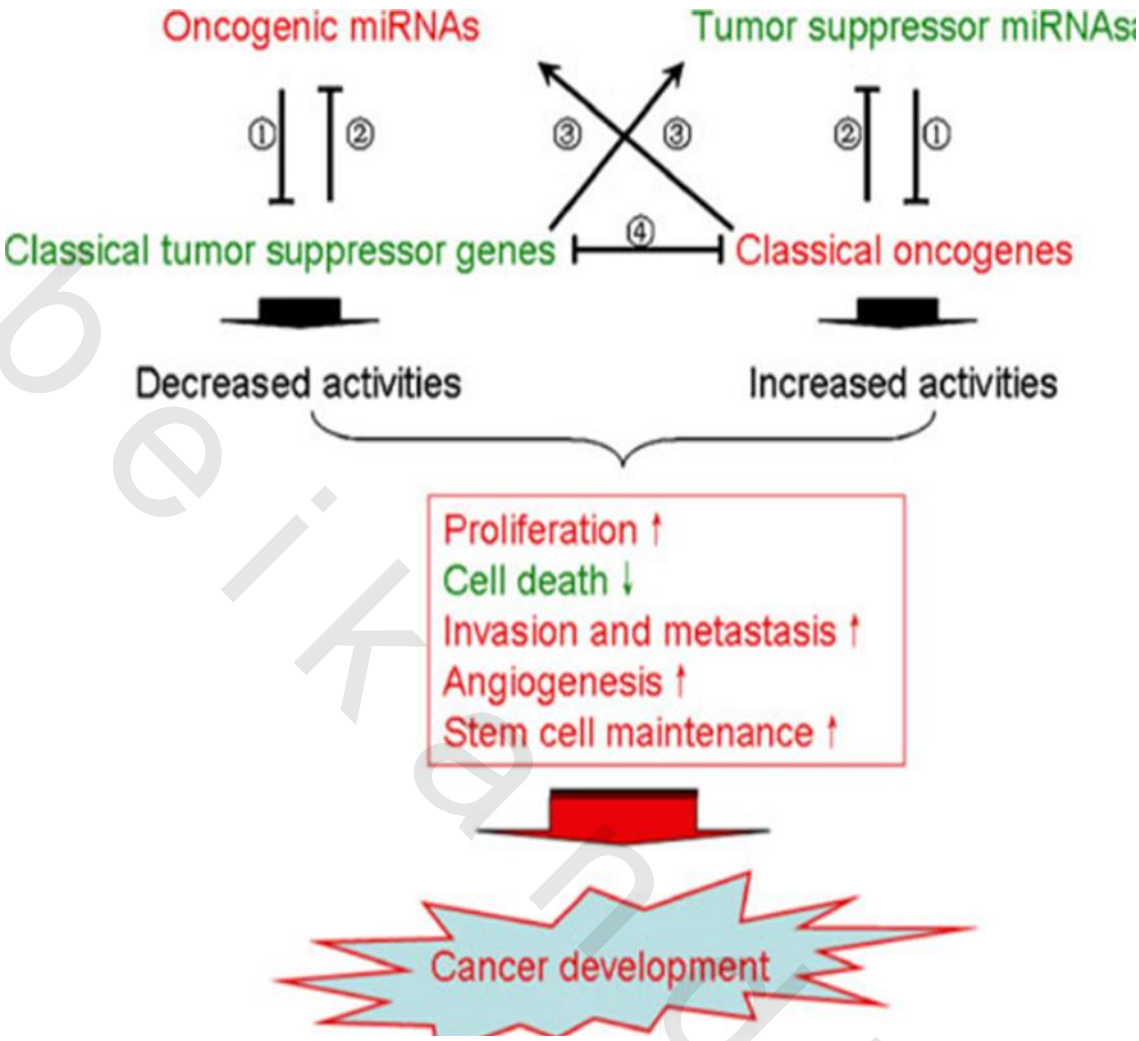

Fig. 7. Functions of micro-ribonucleic acid in cancer development and progression. Possible interactions among oncogenic and tumor suppressor miRNAs and classical genes and consequences in cancer development are illustrated. Green color, down-regulated or attenuated. Red color, up-regulated or enhanced. (1)Translation inhibition/mRNA degradation; (2) Transcriptional inhibition (direct or indirect); (3) Transcriptional activation (direct or indirect); (4)Transcriptional inhibition/ activity inhibition.[40]

The involvement of miRNAs in human cancer was first evidenced in chronic lymphocytic leukemia (CLL), in which deletion of a chromosomal region (13q14) was associated with down-regulation of miR-15a in the majority of chronic lymphocytic leukemia patients.[64]

Following this initial finding, more and more miRNAs have been found to be aberrantly expressed in various types of cancer cell lines and clinical tumor specimens. In addition to the identified abnormal levels of specific miRNAs in certain types of human cancers, biological evidence that suggests an important role of miRNAs in cancer development and progression was also experimentally demonstrated in animal models.[65]

In some types of cancers, global deregulation of miRNAs has been found, indicating that miRNAs have a general potential to target genes involved in cell proliferation, apoptosis, differentiation, invasiveness, and motility that are critical for development or progression of human cancers.[66]

MicroRNA expression deregulation in human cancer had been described in numerous cancers, such as lung cancer,[65] breast cancer,[67]glioblastoma,[68] hepatocellular carcinoma,[69] endocrine pancreatic tumors,[70] papillary thyroid carcinoma,[71] testicular germ cell tumors,[72] prostate cancer,[73] urothelial carcinomas,[74] melanoma,[75] nasopharyngeal carcinomas,[76] colorectal cancers, etc.[77]

Moreover, miRNAs belong among important regulators of aberrant hemopoiesis of leukemias,[78] and are involved in various hematologic malignancies, such as acute promyelocytic leukemia,[79]chronic lymphocytic leukemia,[80] acute lymphoblastic leukemia,[81] CML,[82] and lymphomas.[83] (Table. 2)

Table 2. MicroRNAs Aberrantly Expressed in Cancer.[40]

Cancer type	Upregulated	Downregulated
Breast cancer	miR-10b, miR-21, miR-22, miR-27a, miR-155, miR-210, miR-221, miR-222, miR-328, miR-373, miR-520c	let-7, miR-7, miR-9-1, miR-17/miR-20, miR-31, miR-125a, miR-125b, miR-146, miR-200 family miR-205, miR-206, miR-335
CLL	miR-21,miR-155	miR-15, miR-16, miR-29b, miR-29c, miR-34a, miR-143, miR-145, miR-181b, miR-223
Lung cancer	miR-17-92 cluster, miR-21, miR-106a, miR-155	miR-1, let-7 family, miR-7, miR-15a/miR-16, miR-29 family
Lymphoma	miR-17-92 cluster, miR-155	miR-143, miR-145
Prostate cancer	miR-221, miR-222	miR-15a-miR-16-1 cluster, miR-101, miR-127, miR-449a
Glioblastoma	miR-21, miR-221, miR-222	miR-7
Hepatocellular carcinoma	miR-17-92 cluster, miR-21, miR-143, miR-224	miR-1, miR-101, miR-122a
Colorectal cancer	miR-17-92 cluster, miR-21	miR-34a, miR-34b/c, miR-127, miR-143, miR-145, miR-342
Gastric cancer	miR-21, miR-27a	miR-143, miR-145
Ovarian cancer	miR-214	miR-34b/c, miR-200 family
Melanoma	miR-221, miR-222	let-7a, miR-34a
Head and neck squamous	miR-21	let-7d, miR-138, miR-205

Early transcriptional profiling data evidenced that miRNA expression profiling may successfully classify different tumour types and do so more reliably than mRNA profiling, suggesting that the miRNA repertoire is a stable and unique feature of different cell types and differentiation stages.[84,85] Since then, an extensive bulk of literature had reported specific miRNA signatures for individual cancers and cancer stages.[86,87]

Because of their stability in formalin fixed tissues and the relative ease by which they can be routinely quantified, miRNAs are quickly entering clinical laboratories as important tools for diagnostics and prognostics. Importantly, the cell and cancer-type specificity of miRNA expression profiles also holds a promise for the efficient identification of metastatic cancers of unknown primary origin.[88]

Moreover, the discovery of miRNAs in body fluids, such as serum, urine and colostrum,[89,90,91] has intensified investigations into the use of miRNAs as non-invasive biomarkers of disease and therapeutic response in a range of cancers including lung cancer,[92] ovarian cancer,[93] colorectal cancer,[94] renal cancer[95] and glioblastoma.

In CML, several works had demonstrated aberrant miRNA expression. For example, the enhanced expression of the miR-17-92 cluster (onkomir-1) in CML CD34+ cells,[97] the aberrant expression miRNAs in mononuclear and CD34+ cells separated from bone marrow of CML patients at diagnosis and in mononuclear cells, [78] the epigenetic silencing of miR-203 in human leukemic Philadelphia chromosome-positive (Ph+) cell lines,[98] and derivative 9q+ chromosome deletions carrying miR-199b that occurred in some CML patients and were found to be associated with miR-199b decrease.[99]

Furthermore, other works contributed to the knowledge about expression change in specific miRNAs associated with resistance to or responsiveness to IM after the treatment initiation in CML patients.[100,101]

MicroRNA 451

MicroRNA 451 (miR-451) is also known as MIR451A, homo sapiens mir451 (hsa-mir-451) and hsa-mir-451a. The sequence of mature miR-451 is AAACCGUUACCAUUACUGAGUU.[58] In the human genome, the miR-451 gene is located on chromosome 17 at 17q11.2 (Gene ID: 574411), 100 bp downstream of the miR-144 gene.[102] The miR-144/451 loci reside in intergenic regions adjacent to the protein-coding gene Era G-protein-like 1 (ERAL1), which is transcribed in a direction opposite to that of miR-144/451.[103] (figure. 8)

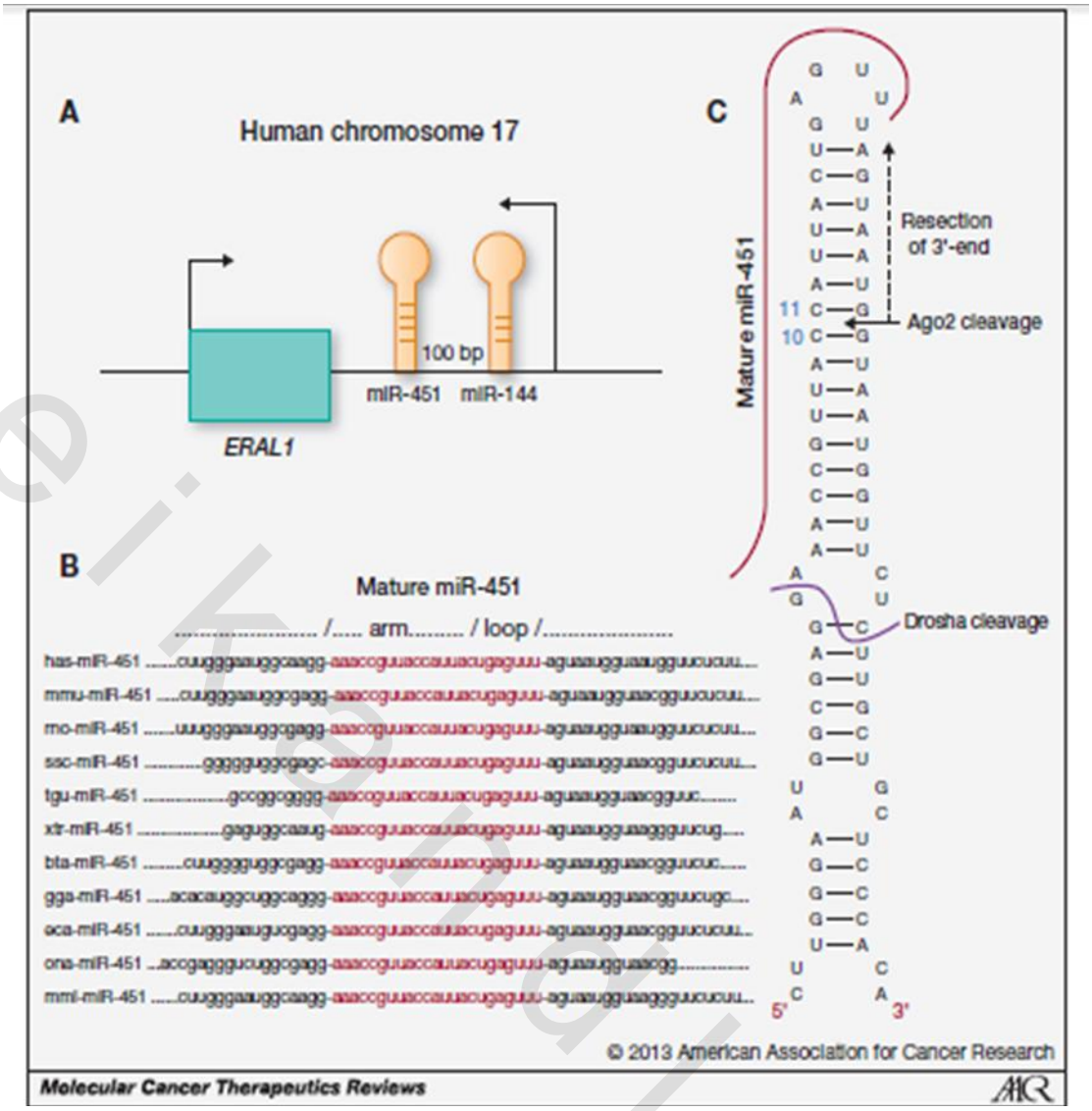

Fig. 8. **Micro-ribonucleic acid 451 gene locus in human genome and the non-canonical biogenesis pathway.** A, features of the miR-144/451 locus and the adjacent Era G-protein-like 1 (ERAL1) gene. B, atypical conservation of miR-451 among vertebrates. C, model for miR-451 processing.[102]

Micro-ribonucleic acid 451 is an exception to the canonical mode of processing described earlier and is processed through a non-canonical pathway. Several studies have identified miRNA classes that bypass Drosha-mediated processing, including short hairpin introns (miRtrons), miRNAs derived from transfer RNAs, and small nucleolar RNA(snoRNAs).[104,105,106] Interestingly, mature miR-451 includes nucleotides from both sides of the pre-miRNA hairpin loop. As annotated, the 6 terminal nucleotides of mature miR-451 (23 nt) span the loop region and extend into the complementary strand of the hairpin precursor, which has not been shown to occur in other known miRNA so far.[107]

The 42-nt Drosha-cleaved miR-451 hairpin with a 17-nt stem does not possess a sufficiently long duplex to be a Dicer substrate (>19 nt), suggesting that its maturation might bypass a requirement for Dicer.[108] In addition, 3 studies evidenced that unlike other

miRNAs, the mir-451 hairpin does not mature via a Dicer dependent pathway as maturation to miR-451 seemed refractory to loss-of-function mutations in Dicer.[109-111] Furthermore, whereas most other miRNA hairpins contain multiple unpaired nucleotides within the stem, mir 451 showed an unusual perfect base-pairing in pre-miR-451 providing another reason that miR-451 differs from other canonical miRNAs.[112] (figure. 8)

Several studies involving miR-451were conducted in the past few years providing evidence regarding its various biological and pathological functions.[112] For example, a study detected approximately 35-fold increase in miR-451 expression in late normoblasts and some reticulocytes as compared with the miR-451 levels in immature early erythroid progenitors indicating level of miR-451 expression rapidly increases during erythroid maturation in humans and establishing that miR-451 is essential for maintenance and late-stage maturation of committed erythroid precursors.[113]

In addition, results obtained from several other studies implied that miR-451 is required for the development and maintenance of normal tissues and might be down-regulated during the transition to cancer, providing evidence that miR-451 plays crucial roles in development, diagnosis and treatment of human cancer.[112]

Many works confirmed that circulating miR-451 may serve as a new, powerful, and noninvasive biomarker for human malignancies. For example, combined detection of miR-378 and miR-451 in serum enabled the identification of renal cell carcinoma.[114] miR-451 and miR-373 were dramatically down-regulated in childhood B-cell precursor-acute lymphoblastic leukemia (pre-B-ALL) compared with samples from healthy individuals.[115] In addition, miR-451 was found to be significantly down-regulated in 3 glioblastoma cell lines, functioning as a tumor suppressor in human gliomas.[116] These data indicated that the expression of miR-451 was associated with disease states and was cell-specific, supporting the clinical value of miR-451 as a diagnostic biomarker in human cancers.[112](Table. 3)

Table. 3. Micro-ribonucleic acid 451 as a diagnostic biomarker of human cancer. Microribonucleic acid has been established as a diagnostic biomarker in several human cancers. Pre-B-ALL denotes B-cell precursor-acute lymphoblastic leukemia and NSCLC denotes Non- small cell lung cancer.**[112]**

Cancer type	miR-451 expression
Gastric cancer	Downregulation
	Upregulation
Colorectal cancer	Downregulation
NSCLC	Downregulation
Renal cell carcinoma	Downregulation
Pre-B-ALL	Downregulation
Glioblastoma multiforme	Upregulation
Glioblastoma	Downregulation

Moreover, previous studies have shown that ectopic overexpression of miR-451 inhibits proliferation and triggers apoptosis of non-small cell lung cancer (NSCLC) cells by directly inhibiting its target, ras-related protein 14 (RAB14).[117] Up-regulation of miR-451 could also sensitize NSCLC cells to cisplatin partly through inactivation of the Akt signaling pathway.[118] Likewise, in CML, c-myc reduced the expression of miR-144/451 in IM resistant K562 (K562R) cells, and restoration of miR-144/451 might reverse the resistance of K562R cells to IM therapy.[119]

In addition, another study revealed that miR-451 overexpression reduced cell proliferation and sensitizes gastric and colorectal cancer cells to radiotherapy.[120] The above evidence shows that miR-451 could reverse the resistance to chemotherapy or radiotherapy in many types of cancers. However, further studies evaluating their molecular mechanisms in the drug-resistant signal transduction network are still demanded.[112]

MicroRNA 451 was also reported to be associated with the clinical outcome of human patients with cancer,[112] such as in colorectal cancer,[121] osteosarcoma,[122] glioblastoma multiforme,[123,124] lung cancer,[125] hepatocellular cancer,[126] esophageal squamous cancer,[127] gastric cancer[120], NSCLC[117], and leukemia.[80] Therefore, miR-451 can serve as a candidate prognostic indicator in human malignancies.[112]

Moreover, recent works suggested the role of miR-451 in the maintenance of the leukemic state of CML cells and that its down regulation miR-451 might related to an increased BCR-ABL kinase activity in CML cells.[128,129]

The present study was conducted with the aim to validate the value of micro RNA 451 in CML as an early predictor for IM resistance in Egyptian patients.