Tie-2 Expressing Monocytes in The Pathogenesis of Chronic Lymphocytic Leukemia

Amira Raafat Elsheikh 1, Gehan Abd Elkader 2, Ahmad Sallam Soliman 1, Noran Salah Eldin Elsabbagh 1
Departments of 1 Clinical Pathology and 2 Internal Medicine, Faculty of Medicine, Zagazig University, Egypt.
Corresponding Author: Noran Salah Eldin Elsabbagh
Email: elsabbaghnoran@gmail.com

Abstract

Background: Chronic lymphocytic leukemia (CLL) is the most common hematologic malignancy in the Western Countries. Despite several and important recent therapeutic advances, CLL is still an incurable disease. CLL is characterized by several clinical complications related to alterations in the immune system, including hypogammaglobulinemia, predisposition to infections and increased incidence of autoimmune disorders. CLL is a lymphoproliferative disorder characterized by the clonal proliferation and progressive accumulation of morphologically mature, monomorph B lymphocytes in the blood, bone marrow, and lymphatic tissues. Morphologically, these leukemic cells appear as small, mature lymphocytes that may be found admixed with occasional larger or atypical cells, or prolymphocytes. Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains 2 (Tie-2) expressing monocytes are subpopulation of monocytes expressing Tie-2 as a representative surface marker.

Keywords: Chronic Lymphocytic Leukemia, Tie-2 Expressing Monocytes.

Background

Chronic lymphocytic leukemia (CLL), the most frequent type of leukemia in adults, is a lymphoproliferative disorder that is characterized by the expansion of monoclonal, mature CD5+CD23+ B cells in the peripheral blood, secondary lymphoid tissues and bone marrow with the morphology of small mature lymphocytes (1).

The heterogeneity of the clinical course of CLL ranges from an indolent course, where patients do not require therapy for many years, to a very aggressive disease, where treatment is required soon after diagnosis and relapses may occur early (2).

CLL is the most common leukemia in Western countries, representing approximately 22% to 30% of all leukemias worldwide with more men affected than women. The incidence of CLL is approximately 4.2 cases per 100,000 (3).

Pathogenesis of CLL

Chronic lymphocytic leukemia (CLL) is characterized by the clonal expansion of CD5+CD23+ B cells in blood, marrow, and secondary lymphoid tissues. Although CLL cells in the blood appear to be predominantly resting lympho the lymphoid tissue and are presumably where leukemia cel lcytes in G0, focal aggregates of different-sized lymphocytes are scattered throughout is proliferate (4).
There are several theories regarding the pathogenesis of CLL including:

**a- Defective Apoptosis:**

Apoptosis is a physiological cell suicide program that is essential for the regulation of development, the maintenance of homeostasis and the prevention of tumorigenesis. Evading the apoptotic program is one of the hallmarks of CLL (6).

The apoptotic machinery comprises two main activation pathways (the extrinsic and the intrinsic pathways) and an execution phase mediated by proteases of the caspase family. The extrinsic pathway (also known as the death receptor pathway) is triggered by ligation of death receptors (TNF-R, Fas, DR4) by their respective ligands (TNF, Fas L, TRAIL) and recruitment of adapter molecules, which activates the initiator caspase-8. The intrinsic pathway (also known as the mitochondrial pathway) integrates various intracellular signals at the mitochondrial membrane and is regulated by Bcl-2 family proteins (that share at least one of the four Bcl-2 homology domains, BH1 to 4) (5).

**Figure (1):** The two pathways of apoptosis: death receptor (extrinsic) and mitochondrial (intrinsic) pathways (5).

**b- Genetic Aberrations:**

Another theory in the pathogenesis of CLL is that CLL cells commonly harbor deletions at 13q14, 11q22–q23, or 17p13 or may have an extra copy of chromosome 12 (trisomy 12); such genetic alterations are significantly associated with clinical outcome. The advent of next-generation sequencing technologies, coupled with gene copy-number analyses, have identified additional genetic lesions in CLL, such as mutations in NOTCH1, SF3B1, and BIRC3 (4).

Genomic studies have identified recurrent mutations in genes regulating tumor cell-microenvironment interactions, which are already required for tumor cell growth. Thus, NOTCH1 mutations are dependent on the presence of Notch ligands in the microenvironment and activate processes such as cell migration,
invasion and angiogenesis. BCR and NOTCH1 pathways are functionally linked, mutually enhancing their activation (7).

c- Cytokines:
Individual, specific cytokines and chemokines have been reported to be elevated in the sera, plasma, or both of CLL patients and to correlate with clinical course and outcome. For example, high serum levels of IL-10, a cytokine that regulates inflammation, correlates with shorter survival. In addition, plasma levels of CCL3 and CCL4, 2 inflammatory chemokines that regulate cell recruitment and activation, are elevated in CLL and correlate with time-to-first treatment (TTFT); these chemokines are secreted by nurse-like cells and by CLL cells in response to B-cell receptor (BCR) engagement, and their secretion by leukemic cells can be down-regulated by small-molecule inhibitors of BCR signaling, linking chemokines with another environmental influence on CLL–antigen stimulation (8).

d- Micro RNA:
MicroRNAs are small non-coding RNAs involved in several cellular processes and expressed in a tissue-specific manner. Recently, researchers have focused on the molecular impact of deregulation of microRNA expression in CLL. MiR-15/16 cluster, miR-34b/c, miR-29, miR-181b, miR-17/92, miR-150, and miR-155 family members, the most deregulated microRNAs in CLL, were found to regulate important genes, helping to clarify molecular steps of disease onset/progression (9).

**Tie-2 Expressing Monocytes (TEMs):**
Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains 2 (Tie-2) expressing monocytes are subpopulation of monocytes expressing Tie-2 as a representative surface marker. They have been found in various human tumors to form tumor blood vessels and promote tumor angiogenesis and growth by paracrine secretion of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), and matrix metalloproteinase-9 (MMP-9) (10).

TEMs account for 2% to 7% of blood mononuclear cells in healthy donors and are distinct from rare circulating endothelial cells (ECs) and progenitors are distinct from classic inflammatory monocytes, expressing functional Tie-2 receptor, and directly respond to Angiopoietin 2 (Ang-2) which is up-regulated in activated and angiogenic blood vessels. Interestingly, human TEMs are preferentially recruited to tumors by Ang-2, where they constitute the pre-eminent monocyte population, amplifying the production of pro-angiogenic factors. The Ang-2/Tie-2 axis, mediates cell-to-cell interactions between TEMs and tumor endothelial cells; further, it initiates angiogenesis by destabilizing existing blood vessels (11).

TEMs have now been detected in multiple solid and hematological human tumors and represent the main population of TAMs in breast cancer, renal cell carcinoma and hepatocellular carcinoma (HCC). In most of cancers, TEMs have been reported to display an inherent vascular growth-promoting activity, which is largely associated with tumor vascularization (12). The tumor signals and the corresponding TEMs pathways controlling Tie-2 expression, TEMs frequency and TEMs angiogenic activity remain still poorly understood. Nevertheless, Ang-2 has emerged as tumor factors critically controlling TEMs pro-angiogenic activity and in parallel Tie-2 and VEGFR signaling axes arose as attractive therapeutic target. Most importantly, besides their pro-angiogenic activity, TEMs infiltrating breast cancer endow a lymphangiogenic, immune suppressive and pro-metastatic activity. They also represent a circulating reservoir of cells committed to a pro-angiogenic function, which in some cancers, not all, function as a
diagnostic marker. Thus, TEMs represent attractive therapeutic and diagnostic targets, but further studies are needed to elucidate their pro-tumoral functions (10).

**Tie-2 Receptor**

Tie-1 and Tie-2 are receptor tyrosine kinases (RTKs), so named because they mediate cell signals by inducing the phosphorylation of key tyrosines, thus initiating cell signaling. Tie-2 receptor is also referred to as TEK, CD202b, Mucocutaneous Venous Malformation (VMCM), Tunica Interna Endothelial Cell Kinase and human TIE2 (hTIE2) (13).

Angiogenesis is the physiological process of neovascular formation from pre-existing blood vessels, which can occur during embryogenesis, adult tissue homeostasis and carcinogenesis. It is a hallmark of cancer, which provides oxygen and nutrition for tumor growth while removing deposits and wastes from the tumor microenvironment. There are many angiogenesis stimulators, among which vascular endothelial growth factor (VEGF) is the most well-known. VEGF has three tyrosine kinase receptors, which, following VEGF binding, initiate proliferation, invasion, migration, and angiogenesis of endothelial cells in the tumor environment (Figure 1). (14).

**Tie-2 gene:**

Tie-2 gene (Figure 2) lies on the short arm of chromosome 9 (9p21). It codes for a protein that contains 1124 amino acids (aa). It belongs to the protein kinase superfamily, Tyrosine protein kinase family, Tie subfamily. The Deoxyribo nucleic acid (DNA) contains 120887 base pairs (bp) encoding 23 coding exons and the messenger Ribonucleic acid (mRNA) contains 4619 bp transcribed. The mRNA contains a long (372 bp) 5'-Untranslated region (UTR) with 5 upstream open reading frames (ORFs) and one internal ribosomal entry sites (IRES) that allows RNA to be translated under hypoxic conditions (15).

**Tie-2 expression:**

Expression of high level of Tie-2 is found in placenta, lung, spleen and heart tissues. Tie-2 is expressed by ECs, hematopoietic stem cells, pericytes, monocytes and certain tumor cells. It is mostly explored at the level of hemopoietic progenitors. Approximately 10%–20% of adult bone marrow or cord blood hemopoietic progenitor cells expressed Tie-2 on their surface. However, circulating endothelial cells and endothelial progenitor cells expressing Tie-2 are detected at very low frequency in peripheral blood (16).

**Tie-2 ligands:**

The angiopoietins are proteins that regulate angiogenesis. In humans, three angiopoietins have been identified: Ang-1, Ang-2 and Ang-4 (Ang-3 is the mouse ortholog of human Ang-4). Ang-1 and Ang-4 function as agonistic or activating ligands for Tie-2, whereas Ang-2 and Ang-3 behave as competitive antagonists. They function by binding their physiologic receptors, Tie-1 and Tie-2 regulating EC survival and vascular maturation (17).

It was initially reported that Ang-2 binds, but does not activate Tie-2, but instead acts as a competitive Ang-1 antagonist on endothelial cells. A subsequent study demonstrated that at high concentrations Ang-2 can induce phosphorylation of the Tie-2 receptor, leading to its activation, finally promoting cell survival and proliferation. These effects, however, were not observed at lower concentrations (18).
Angiopoietin structure:
The angiopoietins consist of an N-terminal super-clustering domain (SCD), a central coiled-coil domain (CCD) responsible for ligand homo-oligomerization, a linker region and a C-terminal fibrinogen-related domain (FReD) required for binding to the Tie-2 receptor (Figure 5). In addition, Ang-1 and Ang-2 form dimers, trimers and tetramers, Ang-1 further assembles into higher order multimers via its SCD. Only tetrameric or higher multimeric forms of Ang-1 activate Tie-2, while oligomeric Ang-2 is a weak context dependent agonist of Tie-2, and may even antagonize Ang-1-mediated Tie-2 activation (19).

Figure (2): Molecular structure of Tie receptors and angiopoietin (20).

Angiopoietin Production:
Angiopoietin-1 is produced by many cell types such as pericytes, smooth muscle cells, fibroblasts and also by several tumor cell lines. Ang-1 acts in paracrine manner on ECs. Ang-2 is almost exclusively produced by ECs. Ang-2 is stored in endothelial Weibel-Palade bodies from where it can be rapidly released upon stimulation. Ang-2 acts in autocrine fashion on ECs. Ang-1 functions as a stabilizing signal for mature vasculature, and Ang-2 can be regarded as a regulator of vessel plasticity (18).

Angiopoietin Actions:
Tie receptors, Tie-1 and Tie-2, and their secreted angiopoietin ligands, Ang-1 and Ang-2, have been identified as the main factors of the Ang/Tie system. The Ang/Tie system is one of the most important vascular-tissue specific signaling pathways, essential during embryonic vascular development and maturation. Ang-1 is considered as key regulator of adult homeostasis. (21).
Tie-1 and Tie-2 are both expressed by EC and share high similarities in their overall domain structure. Ang-1 is primarily produced by perivascular cells and acts in a paracrine fashion as strong Tie-2 agonist that mediates ECs survival and maturation signals. Ang-2, expressed and stored in ECs, functions primarily as an antagonist of Tie-2 and promotes vascular destabilization. Nevertheless, it can act as a partial agonist of Tie-2 in a context-dependent manner (22).
In the presence of VEGF-A, Ang-2 promotes vascular sprouting and destabilizes blood vessels by disrupting interactions between ECs and peri-ECs, thus enhancing VEGF-A stimulation. On the other hand, in the absence of VEGF-A, Ang-2 acts as a suppressor that accelerates vessel regression (17). It is believed that the quantitative balance between Ang-1 and Ang-2 determines the grade of endothelial Tie-2 phosphorylation. However, experimental data suggest that this balance is influenced by a soluble form of the Tie-2 receptor (sTie-2) which results from cleavage of the extracellular domain of the membrane bound full length Tie-2 receptor by matrix metalloproteases upon VEGF or basic fibroblast growth factor (bFGF) stimulation. Hence, enhanced sTie-2 production (i.e., cleavage of the full length Tie2 receptor) by matrix metalloproteases may further shift the Ang-2/Ang-1 ratio in favor of Ang-2 by binding Ang-1 with ~20-fold higher affinity than Ang-2 (23).

Ang-2 acts as a Tie-2 agonist in non-pathological conditions, whereas in the setting of inflammation, it functions as a Tie-2 antagonist and promotes vascular dysfunction. Inflammation promotes cleavage of the Tie receptors ECD which corresponds with the switch of Ang-2 from a Tie-2 agonist to an antagonist (22).

### Tie-2 regulation:

Regulation of the kinase activity of Tie-2 is a complex process. Conformational changes in the nucleotide binding loop, activation loop and the C-terminal tail are required for ATP and substrate binding (24).

### Tie-2 autoinhibition:

Several potential autoinhibition mechanisms are present, these include:

- Blockade of the ATP binding site by residues within the nucleotide binding loop.
- Impairment of ATP binding by residues within the A-loop.
- Obstruction of the substrate binding site by the C-terminal tail.
- Phosphorylation of the N-terminal domain may serve as a negative regulatory mechanism by either preventing dimerization and autophosphorylation of the intracellular catalytic domains or recruiting a phosphatase (24).

### Tie-2 activation and signal transduction:

The collective interactions between angiopoietins, RTKs, vascular endothelial growth factors and their receptors form the two signaling pathways Tie-1 and Tie-2. The two receptor pathways are named as a result of their role in mediating cell signals by inducing the phosphorylation of specific tyrosines. This in turn initiates the binding and activation of downstream intracellular enzymes, a process known as cell signaling. Tie-1 heterodimerizes with Tie-2 to enhance and modulate signal transduction of Tie-2 for vascular development and maturation (19).

RTKs activation and signalling are initiated by ligand-mediated receptor dimerization, which results in cross-phosphorylation of each member of the dimer pair on specific tyrosine residues (19). Receptor cross-phosphorylation has a dual effect on receptor function: First, enhances the receptor’s kinase activity, Second, to provide binding sites for signalling molecules possessing phosphotyrosine binding domains. several signaling proteins that are recruited to the activated, autophosphorylated Tie-2 kinase domain. Two of these molecules, Src homology 2 (SH2)- containing protein tyrosine phosphatase (SHP2) and growth factor receptor- bound protein 2 (GRB2), are part of the pathway upstream of mitogen-activated protein kinase (MAPK) activation which is a pathway that may be responsible for morphogenetic effects of Tie-2 on ECs. Another signaling molecule, p85, is responsible for recruitment of phosphatidylinositol-3 kinase (PI3-K) and activation of the Akt/PI3-K pathway.
Akt/PI3-K is considered a critical pathway downstream of Tie-2 that is necessary for cell survival effects as well as for chemotaxis, activation of endothelial nitric oxide synthase, and perhaps for anti-inflammatory effects of Tie-2 activation. Therefore, the Tie-2 pathway has important functions in adult tissues, in both quiescent vasculature and during angiogenesis. (19).

**Tie-2 functions:**

**A-Role in vascular development and maintenance:**

The formation of the vascular system is one of the earliest and most important events during organogenesis in the developing embryo because the growing organism needs a transportation system to supply oxygen and nutrients and to remove waste products. Two distinct processes termed vasculogenesis and angiogenesis lead to a complex vasculature covering the entire body (26). Recently, it was reported that Tie-1 deficiency was shown to result in abnormal lymphangiogenesis during embryogenesis, the primary lymphatic network became disorganized with a significant increase in the number of abnormal lymphatic connections. Furthermore, its deficiency leads to the failure of lymphatic remodeling to form collecting vessels during embryogenesis (27).
Hereditary cutaneomucosal venous malformation is a rare condition which is inherited in an autosomal dominant fashion due to abnormalities in the Tie-2 receptor gene. This causes small and usually asymptomatic venous malformations mostly in the mucous membranes and face and much less common involving the internal organs (28).

Ang-1/Tie-2 system in normal adult blood vessels is important in maintaining the integrity of nonproliferating ECs by strongly inducing EC survival against insult-mediated damage. Blood vessels remain in a stable state when Tie-2 receptor is constitutively engaged with Ang-1 by stabilizing blood vessels through interactions with perivascular cells and the extracellular matrix. When Ang-2 expression is up-regulated, the interaction between Tie-2 and Ang-1 is disrupted, and the vessel is destabilized. (29).

B-Role in haematopoiesis:
It has been demonstrated that Tie-2 positive cells in the aorta-gonad-mesonephros region contain hemangioblasts capable of differentiating into both hematopoietic and endothelial lineages. Targeted deletion of the Tie-2 gene results in early embryonic lethality because of vascular abnormalities. (30).

Tie-2 effect on adult hematopoiesis is exerted at both stem cell and progenitor levels (Figure 7):

a) At the stem cell level, Tie-2/Ang-1 promotes the adhesion of stem cells to bone and maintain the quiescence of these cells.

b) At the progenitor level, Ang-1 can promote the adhesion of Tie-2-expressing cells to fibronectin present on the surface of ECs, enhancing proliferation of hemopoietic progenitor cells.

c) At the stem cell and progenitor levels, Tie-2 and Ang-1 have been implicated in recruitment and mobilization of these cells from bone marrow.

The expression and activation of Tie-2 contribute to the interplay between regenerating bone marrow, neovessels hematopoietic progenitors, leading to the rapid reconstitution of hematopoiesis after myelosuppression (31).

Figure (4): Role of Ang-1/Tie-2 in the bone marrow niche (32).

C-Role in pathological angiogenesis:
Angiopoietin and its cellular receptor Tie-2, together with VEGF and its receptor VEGF-R2, have been implicated as the main endothelial pathways required for tumor vascularization. During tumor development, the Ang-1/Ang-2 balance shifts in favor of Ang-2, with ECs in a high proportion of tumor vessels expressing Ang-2 (33).
Strong correlation between circulating levels of Ang-1, Ang-2, sTie-2 and Ang-2/sTie ratio and overall survival in Acute myeloid leukemia patients. It was reported that patient with high Ang-2 displayed significantly worse overall survival than with low levels (34).

Tie-2 seem to be significant prognostic factors in primary epithelial ovarian cancer. Their expression levels are also increased in metastatic lesions in comparison with primary tumors (34).

the percentage of TEMs in peripheral blood monocytes of HCC patients was significantly increased compared with that in healthy donors. In addition to the identification values, the elevated circulating TEMs may also play a crucial role in predicting prognosis of HCC patients (33).

sTie2 increased serum concentrations were measured in cardiovascular diseases, diabetic retinopathy and systemic sclerosis (35).

Methods of Tie-2 detection:

I- Immunological methods:

- **Flowcytometry**
  Flow cytometry measures multiple physical characteristics of a single cell such as size and granularity simultaneously as the cell flows in suspension through a measuring device. Its working depends on the light scattering features of the cells under investigation, which may be derived from dyes or monoclonal antibodies targeting either extracellular molecules located on the surface or intracellular molecules inside the cell (36).

- **Enzyme linked immunosorbet assay (ELISA).**
  ELISA method uses an enzyme to detect the binding of antigen (Ag) antibody (Ab). The enzyme converts a colorless substrate (chromogen) to a colored product, indicating the presence of Ag-Ab binding. An ELISA can be used to detect either the presence of Ags or Abs in a sample, depending on how the test is designed (37).

- **Immunohistochemistry.**
  Immunohistochemical (IHC) staining is a sensitive, and effective method to detect the presence and localization of proteins in the cellular compartment in tissues. The basic concept of IHC is detecting the antigen in tissues by means of specific antibody binding, which is then demonstrated with a colored histochemical reaction that can be observed under a light microscope (38).

II- Molecular methods:

- **Western blot**
  Western blot is often used in research to separate and identify proteins. In this technique, a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies specific to the protein of interest (39).

- **Real time-Polymerase Chain Reaction (PCR)**
  Real-time quantitative PCR is the reliable detection and measurement of products generated during each cycle of the PCR process, which are directly proportional to the amount of template prior to the start of the PCR process (40).
Tie-2 inhibitors as therapeutic target

Inhibition of the angiopoietin/Tie-2 axis induces immunogenic modulation, which sensitizes human tumor cells to immune attack, the angiopoietin/Tie-2 pathway is an attractive target for cancer therapy (Figure 8) due to its well-known role in regulating angiogenesis (41).

BAY-826 is the first specific Tie-2 kinase inhibitor that is selective versus other angiogenic RTKs such as vascular endothelial and fibroblast growth factor receptors. Pre-clinical and clinical development of specific inhibitors of the Ang/Tie axis center around biologics specifically targeting Ang-2, for instance, the Ang-2-specific antibody REGN-910 or trebananib (AMG-386), a highly potent Ang-2 peptibody which also binds Ang-1 (41). Harney et al. (42) reported that Rebastinib is a promising therapy for achieving Tie-2 inhibition in cancer patients especially solid carcinomas, characterized by prominent Tie2+ macrophage presence in the tumor microenvironment.

![Figure 5: Therapeutic approaches targeting human TEM](image)

**Figure (5):** Therapeutic approaches targeting human TEM. On the left in red, strategies reported in clinical trials, while proposed strategies are shown on the right in blue. (10).

Tie-2 expressing monocytes (TEMs) in chronic lymphocytic leukemia

B lymphocytes from patients with CLL are resistant to apoptosis and accumulate in lymphoid tissues in which the microenvironment provides long-term protection and allows cancer progression. Hence, the microenvironment has been assigned a critical role in CLL-pathophysiology (43). CLL cells establish a tight and intimate interaction with many cell types in the tissue, such as monocyte/macrophage population assuming their role in the maintenance and progression of CLL cells (44).

Tumor-associated macrophages (TAM) play an important role in tumor cell invasion, proliferation and survival. An increase in the number of TAM has been associated with shortened survival and drug-resistance in patients with hematologic malignancies. Macrophages are also involved in the ingestion of rituximab-opsonized cells via the Fcγ receptor and may have a role in rituximab resistance (45).
In the tumoral micro-environment of CLL, nurse-like cells (NLC) are Tie-2 expressing monocytes (TEMs) with high proangiogenic and immune suppressive activity, which play a critical role in the survival and chemoresistance of tumoral cells (46).

Tie2+ NLCs are detected in CLL-infiltrated lymph nodes, mainly in a peri-vascular distribution, suggesting that leukemic cells secreting Ang-2 in infiltrated-tissue recruit them into tissue from the TEM subpopulation. The altered composition and function of blood monocytes in CLL patients could derive from a specific CLL-mediated education of immune cells including an establishment of a skewed phenotype in the monocyte/macrophage population. (45).

Numerous studies have shown enhanced angiogenesis in many hematological malignancies. It has been demonstrated that elevated markers of angiogenesis correlate with unfavorable prognosis of CLL. Angiopoietin-2 its receptor Tie2, play an important role in disruption of old blood vessels, thus facilitating their remodeling and subsequent sprouting of new vasculature. Increased Ang-2 plasma concentrations were reported in patients with unmutated sequence for variable region of immunoglobulin heavy chain (IgVH), high expression of ZAP-70 and CD38 as well as in patients with intermediate and high cytogenetic risk and at advanced Binet stages. In addition, elevated mRNA expression of Ang-2 in CLL cells was associated with unmutated IgVH genes and shorter progression free survival (47).

References


