بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by accumulation of long lived malignant B lymphocytes in blood, bone marrow and peripheral lymphoid organs ***(Hallek et al., 2008).***

The clinical course of CLL is heterogeneous in different patients, some patients die within 2-3 years with refractory disease, whereas others live for decades after diagnosis without need for therapy ***(Martens and Stilgenbauer, 2014).*** To address this heterogeneity and predict the prognosis of patients, several prognostic markers based on genetic phenotyping or molecular characteristic of CLL B cells have been discovered ***(Hendy et al., 2016).*** However, due to limited availability, high cost of markers and methodological complexion, some of these markers cannot be used for routine hematological evaluation ***(Hallek et al., 2008).*** For this reason, researches continue to identify new prognostic factors in order to predict the course of the disease ***(Matuszak et al., 2016).***

The Dipeptidyl peptidase IV (DPPIV/CD26) is a unique multifunctional 110 kDa membrane-bound glycoprotein, that belongs to the serine protease family. It acts as a receptor, binding and proteolytic molecule. It is expressed on a variety of tissue including T- lymphocytes, endothelial and epithelial cells. Although CD26 expression is very low in B-cells, it is

greatly up regulated following activation ***(Bühling et al., 1995).*** It plays an important role in immune regulation, signal transduction and apoptosis ***(Ghannam et al., 2014).***

Studies have suggested that CD26 plays a regulatory role in the neoplastic transformation and progression of various types of tumors, and it may also play a role in tumor migration and metastasis ***(Stremenova et al., 2007).*** However, the importance of CD26 expression on the cells derived from B-lymphocytes is still not completely understood ***(Molica et al., 2009).***

The literature contains several reports on the role of DPPIV/CD26 in the pathogenesis of hematological malignancies, including B-CLL. Many investigators previously suggested that expression of CD26 at B-CLL cells may be a new prognostic marker ***(Molica et al., 2009).***

AIM OF THE WORK

This study aimed to evaluate the prognostic value of CD26 expression in Egyptian patients with B-CLL, and to assess its correlation to other clinical and laboratory parameters with known prognostic significance.

Chapter 1

CHRONIC LYMPHOCYTIC LEUKEMIA

CLL is characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B-cells within the blood, BM, lymph nodes and spleen. Recently, it has been reported that in CLL the capacity to generate clonal B-cells might be acquired at the hematopoietic stem cell (HSC) stage, suggesting that the primary leukemogenic event in CLL might involve multipotent, self-renewing HSCs ***(Hallek, 2019).***

- **Epidemiology:**

CLL is the most prevalent leukemia in the Western hemisphere that accounts for 20 to 30 % of all leukemia in adults ***(Siegel et al., 2017*).** In Egypt, according to NCI hospital-based registry (2002-2010), CLL accounts for 0.5% of all cancers and 3.08% of lympho-hemopoietic malignancies ***(Kamel et al., 2016)***.

The CLL incidence increases by age, with >70% of patients older than 65 years. Also, there is a gender predisposition i.e., men are more frequently affected than women (male: female ratio of 1.5-2.1) ***(Scarfo et al., 2016).***

- **Predisposing Factors:**

1- Occupational factors:

Some investigators linked exposure to agricultural chemicals and herbicides to increase risk of CLL. Other studies suggested that rubber and petroleum industries may be linked to CLL, but such associations remain essentially un-validated. Other occupations that were considered as potential risk sources for CLL include mining, or those with exposure to asbestos and certain chemicals, but again no conclusive etiologic links exist ***(Speedy et al., 2018)***.

2- Infections:

Antibodies (Abs) specific for hepatitis C virus (HCV) and/or viral DNA were identified in some patients, suggesting a pathogenic role. However, some studies failed to verify an association between the development of CLL and HCV infection ***(Lichtman et al., 2011).***

3- Hereditary and genetic Factors:

First-degree relatives of CLL patients have an increased risk of developing CLL. Due to the low incidence of lymphatic neoplasia, the absolute risk of getting the disease amongst family members is still low ***(Wendtner et al., 2014).***

- **Pathogenesis of CLL:**

There are several theories, including:

1- 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 cancer, and represents an important mechanism in clinical resistance to therapies. This is mainly true for CLL ***(Hanahan & Weinberg, 2011).***

The apoptotic machinery comprises 2 main pathways: the tumor necrosis factor (TNF) death receptor (DR) (**extrinsic)** pathway & the mitochondria/cytochrome C (**intrinsic**) pathway that integrates various intracellular signals at the mitochondrial membrane; is regulated by Bcl-2 family. Such family consists of about 20 members that can either promote or inhibit apoptosis. Some bcl-2 family members (bax, bcl-X_S, bak & bad) promote apoptosis, whereas others (bcl-2, bcl-X_L & mcl-1) inhibit apoptosis. In addition, another group (bag-1) can influence the activities of the other family members ***(Billard, 2013)***, as in **Figure (1)**.

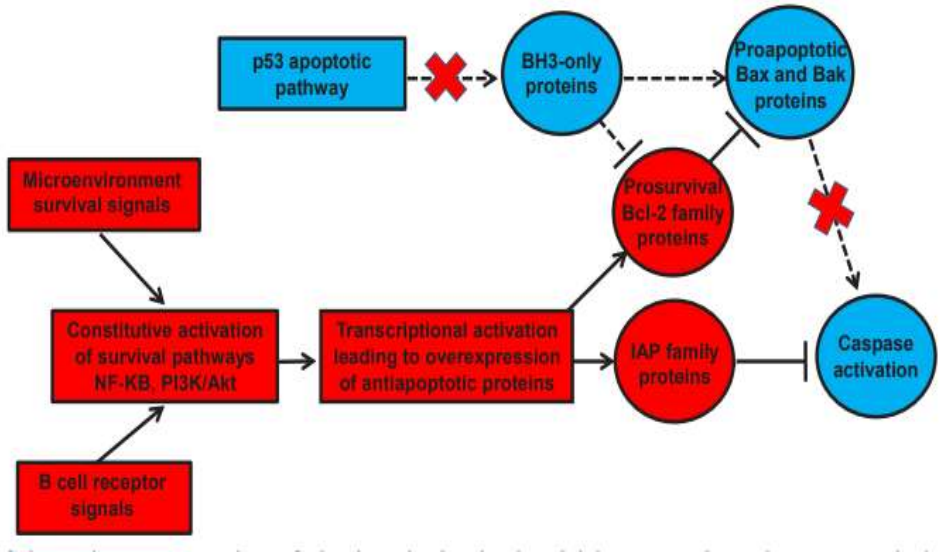

Figure (1): Schematic representation of the impaired mitochondrial caspase-dependent apoptosis in CLL cells ***(Billard, 2013).***

2- Immune dys-regulation:

CLL is characterized by progressive immune dys-regulation, both in the cellular and humoral compartments ***(Riches and Gribben, 2013).***

The leukemic B-cells are responsible for initiating and propagating the immune dysregulation observed in the disease by producing immunosuppressive cytokines like transforming growth factor-β (TGF-β) and IL-4, or by down regulating critical surface molecules required for development of a functional immune system such as CD154 and CD80 ***(Awan and Byrd, 2016).***

Role of the B-cell receptor (BCR) pathway (Figure 2):

CLL cells express on their surface the BCR, where a kay component is represented by surface immunoglobulins (Ig). Surface Ig expression is crucial for survival & functioning of normal B-cells & of many B-cell lympho-proliferative disorders ***(Scarfo et al., 2016).***

The BCR singling plays a crucial role in the pathogenesis of CLL. This signal is transduced through a variety of kinases, including LYN (Lck/Yes novel), PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase), SYK (spleen tyrosine kinase), and BTK (Bruton tyrosine kinase) ***(Stevenson et al., 2011).***

Constitutive activation of the BCR is one of the most important survival signals for the propagation of CLL B-cells ***(Chiorazzi, 2012)*** and result in providing the critical signals that result in leukemic cell-survival and proliferation ***(Awan and Byrd 2016).***

The presence of a distinct B-cell population expressing only one type of Ig light chain (LC) ƙ or ƛ essentially establishes a clonal B-cell process, and support the diagnosis of CLL. The Ig LC restriction by FCM is used to prove clonality in CLL. Clonality was proved in 85% of studied Egyptian CLL patients, using both monoclonal and polyclonal antibodies

simultaneously to increase the sensitivity of FCM- LC detection. Kappa "ƙ" LC restriction was detected in 91% of patients, while lambda"ƛ" LC restriction was detected in 9% of CLL patients ***(Abaza et al., 2015)***.

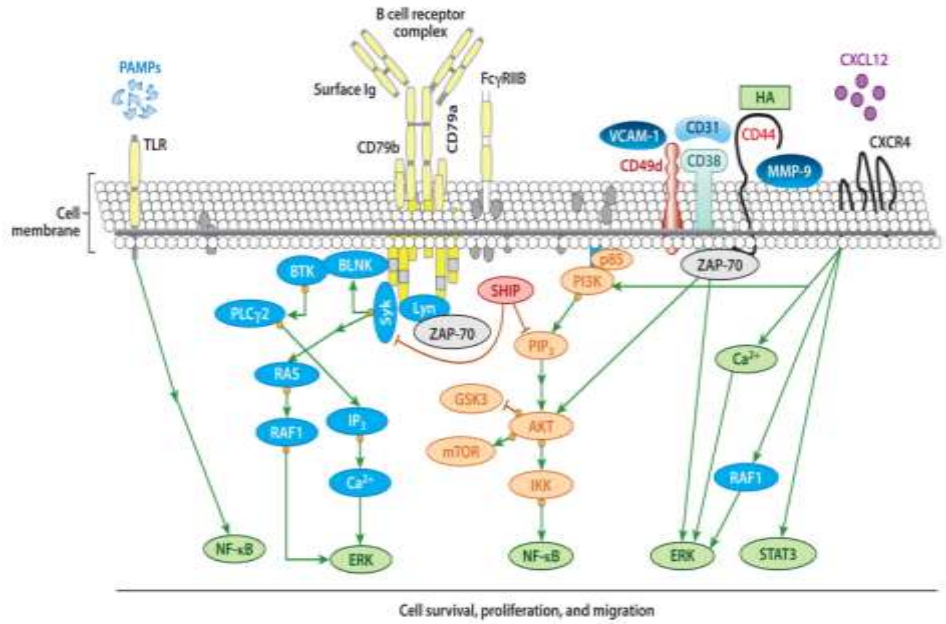

Figure (2): B-cell receptor signaling in CLL. Ligation of B-cell receptor (BCR) by antigen recruits kinases i.e., spleen tyrosine kinase (SYK) & the SRC kinase LYN that phosphorylate immune-receptor tyrosine–based activation motifs (ITAMs) on the cytoplasmic domains of the Ig co-receptors CD79a & CD79b. Such phosphorylation recruits & activates Bruton's tyrosine kinase (BTK) & phosphatidylinositol 3-kinase (PI3K), subsequently activating many downstream targets, including AKT/mTOR (mammalian target of rapamycin), nuclear factor κB (NF-κB) & extracellular signal–regulated kinase (ERK). This signaling can be enhanced by ζ-associated protein of 70 kD (ZAP-70). CD38, CD49d, CD44 & matrix metalloproteinase (MMP)-9 may form a supramolecular complex with ZAP-70. This complex can also recruit ZAP-70 to the plasma membrane, where it can enhance BCR signaling. Following binding to any or all of these receptors by ligands released by accessory cells in the leukemia microenvironment, AKT & ERK undergo enhanced activation. CXCR4 can also directly interact with CXCL12 to induce calcium mobilization, activation of PI3K/AKT, ERK & serine phosphorylation of signal transducer and activator of transcription 3 (STAT3). Activation of Toll-like receptor (TLR) can also enhance or induce activation of NF-κB. Abbreviations: GSK, glycogen synthase kinase; HA, hyaluronic acid; IKK, inhibitor of κB kinase; IP3, inositol triphosphate; PAMP, pathogen-associated molecular pattern; PIP3, phosphatidylinositol (3,4,5) – triphosphate; PLC, phospholipase C; SHIP, SH2-containing inositol phosphatase; VCAM, vascular cell adhesion molecule ***(Billard, 2013).***

3- Genetic aberrations:

Del 13q14 is the most frequent alteration; it occurs in 50%-60% of cases and carries a favorable prognostic value, when isolated. Because this lesion is found at a similar frequency in monoclonal B-cell lymphocytosis and is often detectable as a single lesion, this alteration may represent an early event in the disease. Also, **trisomy 12** occurs in approximately 15% of CLL cases and is associated with a less favorable clinical course ***(Gaidano et al., 2012).***

Del 11q22-q23, in most cases, affects the ataxia telangiectasia mutated (ATM) gene, the deficiency of which causes genomic instability ***(Gaidano et al., 2012).*** In CLL, patients carrying this abnormality often show bulky lymphadenopathies & aggressive clinical course. Also in approximately 5%–10% of untreated CLL patients, del 17p13 disrupts the TP53 tumor suppressor gene and is associated with a dire clinical outcome, being linked to fludarabine-refractoriness, treatment resistance & early disease relapse ***(Scarfo et al., 2016).***

The advent of **next-generation sequencing technologies** coupled with gene copy-number analyses, have identified additional genetic lesions in CLL, e.g., mutations in:

- **The Notch homologue 1, translocation associated (NOTCH1):** It regulates several downstream pathways that induce differentiation of hematopoietic

progenitors into immature T-cells & mature B-cells. NOTCH1 mutations have been detected in ~10% of newly diagnosed cases ***(Billard, 2013)*** and is more frequently associated with unmutated IgHV gene, trisomy 12, aggressive clinical course and increased risk of transformation to Diffuse Large B-Cell lymphoma (so called Richter's transformation) ***(Scarfo et al., 2016).***

- **The splicing factor 3B subunit 1 (SF3B1):** It regulates the alternative splicing program of genes controlling cell-cycle progression and apoptosis. Mutations in SF3B1 may enhance CLL cell proliferation &/or survival. Mutations in SF3B1 were observed in ~10% of newly-diagnosed CLL cases ***(Billard, 2013).*** SF3B1 mutations are associated with dismal clinical course ***(Scarfo et al., 2016).***

- **The baculoviral inhibitor of apoptosis repeat containg 3 (BIRC3):** it is involved in apoptosis inhibition and NFκB regulation. BIRC3 mutations are associated with unfavorable clinical outcome and are mutually exclusive with TP53 mutations ***(Scarfo et al., 2016).*** CLL cells harboring mutations in BIRC3 appear less responsive to conventional chemotherapy ***(Billard, 2013).***

- **Classification of CLL:**

A- French-American-British (FAB) classification:

The **FAB** classification system divides patients into 3 groups, depending on the percentage of abnormal cells

1- **Classical CLL** (80% of patients): more than 90% of lymphocytes are small with clumped chromatin and scanty cytoplasm.

2- **CLL/PLL:** when 11-54% of the cells are prolymphocytes.

3- **Atypical CLL:** when >15% of the lymphocytes are plasmoid or cleaved and <10% are prolymphocytes. ***(O'Brien et al., 2005).***

B- WHO classification:

The WHO classification (2016) update of lymphoid malignancies included several new entities and additional modifications that affect current treatment paradigms and provide a framework for future clinical trials. It is listed in Table (1).