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# A study of the correlation between the serum Latexin levels and the mTORC subunits Raptor and Rictor in the molecular pathogenesis of chronic lymphocytic leukemia

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**Abstract**

Hematopoietic stem cells (HSCs) ensure the lifelong production of blood cells throughout a lifetime. Latexin (Lxn) is thought to have a tumor suppressor role and endogenously down-regulate the number of HSCs via increased apoptosis. Therewithal, Raptor, and Rictor are components of the mammalian target of rapamycin complex-1, and 2 (mTORC), which are the regulatory structures for cell growth. However, Lxn, Raptor, and Rictor-associated molecular mechanisms underlying leukemia-induced HSCs proliferation are largely unknown. Nowadays, chronic lymphocytic leukemia (CLL) remains the most common leukemia type in adults. Therefore, we investigated the serum levels of Lxn, Raptor, and Rictor in CLL patients. We randomized 40 patients with newly diagnosed, untreated CLL. Serum levels of Lxn, Raptor, and Rictor were examined using ELISA assay. The results showed that serum Lxn levels reduced in patients with CLL. Moreover, the Rictor level increased in association with the up-regulation of leukocytosis. Although there was a tendency for an increase of the Raptor levels, the differences did not reach statistical significance. The up-regulated Raptor and Rictor levels in CLL suggested that it was associated with cancer pathogenesis. However, decreased Lxn levels raised the question of whether the disease is secondary to epigenetic features or if it is caused by pathology related to Lxn. The negative correlation between Lxn and Raptor/Rictor levels can provide new methods for the treatment of CLL, which are likely to increase the quality of life and improve the prognosis of the disease. In conclusion, further clinical studies are needed to elucidate the role of Lxn and Raptor/Rictor with the newly defined molecular properties in hematological malignancies and the clinical implications of their use.

**Keywords:** Latexin; Raptor; Rictor; mTOR; chronic lymphocytic leukemia

## Introduction

The Chronic lymphocytic leukemia (CLL), the most common leukemia type in adults, is a lymphoproliferative disorder characterized by the clonal expansion of CD5-expressing B cells in peripheral blood, bone marrow, and secondary lymphoid tissues [1]. It is thought that many factors including genetic susceptibility, environmental factors, and antigen/autoantigen that promote the division and clonal evolution of precursor cells play a role in the etiology of the disease [2,3]. When assessed in terms of molecular changes, constitutive or mutational activation of phosphatidylinositol-3 kinase (PI3K) appears to be involved in the pathology of many cancer models, including CLL [4].

The mammalian target of rapamycin complex (mTORC) is one of the main sub-kinases of the PI3K/Akt signaling pathway, which regulates cell proliferation and apoptosis involved in cancer etiology. While mTORC takes place in the cell as two interrelated complexes known as mTORC1 and mTORC2, Raptor is the main component of mTORC1 and Rictor is the basic component of mTORC2. Activation of mTORC1 occurs by inducing the translation of p70-S6 kinase, S6 ribosome protein, and eukaryotic initiation factor 4E binding protein-1 by phosphorylated Akt. These target molecules both regulate mitochondrial biogenesis and autophagy and control cell growth and proliferation. Activated mTORC2 mediates the continuation of cell viability by providing

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Akt phosphorylation [5,6]. Based on these properties, in a study in which rapamycin inhibited mTORC1, it was reported that apoptosis could not be induced in cycling or quiescent cells [7]. However, mTORC1-mTORC2 dual inhibition exhibited stronger activity against cancer growth in a wide variety of malignancies [8]. As supported by similar studies, inhibition of mTORC1 by mTORC2 over Akt prevents translation and cell growth [9]. Hematopoietic stem cells (HSCs) produce blood cells throughout a lifetime. Natural genetic diversity offers an important reservoir for deciphering the regulatory mechanisms of HSCs and hematopoiesis [10]. One of the structures discovered at this stage is Latexin (Lxn). Lxn is the only carboxypeptidase inhibitor known to naturally occur in mammals. First discovered in different regions of the developing rat brain, Lxn is known to reduce the number of HSCs by increasing apoptosis and preventing their self-renewal [11]. In addition, Lxn expression is induced by lipopolysaccharide in mast cells, indicating the potential role of Lxn in inflammation [12]. Increased survival of HSCs has been shown in Lxn knock-out mice [13]. Several additional studies have shown that Lxn expression is associated with a large number of human malignancies, such as leukemia [14], melanoma [15], hepatocellular carcinoma [16] and pancreatic ductal adenocarcinoma [17]. Lxn has been found to make leukemic cells more susceptible to gamma-irradiation therapy through the protein ribosomal protein subunit 3 (Rps3) pathways, which is a binding protein [18]. The decrease in Lxn expression increases the self-renewal and survival of HSCs but does not affect their reproduction and cycle [10]. In a similar study on samples obtained from patients diagnosed with pancreatic ductal adenocarcinoma, Lxn levels were found to be significantly decreased and CD133 levels increased [19]. In some studies, changes in Lxn expression have been associated with inflammatory responses in the pancreas and lung [20]. A review of the literature revealed no study evaluating the Lxn and PI3K/mTORC pathway and the relationship between Ltx and CLL. Therefore, in our study, we aimed to investigate the correlation between Lxn expression and Lxn/Raptor/Rictor levels in serum samples of CLL patients. (Therefore, in this study, we aimed to investigate the expression of Lxn and the correlation between Lxn/Raptor/Rictor levels in serum samples of patients with CLL.)

## Material and Methods

### Subjects

The patient group consisted of 40 Turkish patients diagnosed with CLL according to World Health Organization (WHO) at Muğla Education and Research Hospital of Muğla Sıtkı Koçman University; forty healthy volunteers were enrolled as a control group. All individuals gave informed consent to participate in this study. The CLL patients included 20 males and 20 females, with an age range of 50-71 years. Healthy controls were 20 males and 20 females, with an age range of 54-71 years. Detailed characteristics of the study subjects were summarized in (Table 1.)

**Table 1.** The routine clinical and laboratory findings including complete blood count (CBC). liver, and kidney function tests in the healthy volunteers and the CLL patients. The Results are expressed as mean±SEM; * $p<0.05$ denotes a statistically significant difference from the controls.

| | Control | | | CLL | | |
|---|---|---|---|---|---|---|
| | **Male (n=20)** | **Female (n=20)** | **Total (n=40)** | **Male (n=40)** | **Female (n=40)** | **Total (n=80)** |
| **Age** | 65.9±1.9 | 66±1.7 | 65.7±1.2 | 66.7±1 | 64.9±1.3 | 65.8±1 |
| **Hemoglobin (g/dl)** | 15±0.2 | 13.4±0.2 | 14.2±0.2 | 14.2±0.4 | 13.8±0.2 | 14±0.5 |
| **Leukocyte (µl)** | 7.120±321 | 7.214±424 | 7.167±263 | 28.905±177 * | 18.252±247 * | 23.351±391 * |
| **Platelet (µl)** | 261.750±10.930 | 266.600±12.824 | 264.175±8.325 | 259.200±133 | 261.897±10.827 | 260.401±11.373 |
| **Sedimentation rate (mm/h)** | 13.5±1.6 | 21.8±2.3 | 17.7±1.5 | 15.3±3.9 | 21.4±2.2 | 18.6±2.4 |
| **TSH (mU/L)** | 1.17±0.1 | 1.15±0.2 | 1.16±0.1 | 1.17±0.08 | 1.16±0.2 | 1.16±0.1 |
| **Ferritin (ng/ml)** | 105±15 | 55.5±8.5 | 80±9.4 | 109.5±11 | 54.3±6.6 | 80±16.4 |
| **Vitamin B12 (pg/ml)** | 294±21 | 343±33.4 | 323±20 | 478±20 * | 419.6±36 * | 443±58 * |
| **Iron (µg/dl)** | 118±7.4 | 91±7 | 105±5.5 | 112±3.5 | 91.6±6 | 91±6.2 |
| **Iron binding capacity (µg/dl)** | 319±7 | 274±7.8 | 297±6.3 | 312±3 | 289±4 | 301±11 |
| **Glucose (mg/dl)** | 94.7±1.1 | 91.5±1.6 | 93±1 | 113±8 | 103±6 | 108±6 |
| **Urea (mg/dl)** | 32.1±2 | 31±1.6 | 31.6±1.3 | 31.3±3.5 | 30.2±1.3 | 30.8±2.6 |
| **Creatinine (mg/dl)** | 0.86±0.03 | 0.7±0.03 | 0.8±0.03 | 0.84±0.1 | 0.63±0.02 | 0.73±0.1 |
| **Albumin (g/L)** | 4.6±0.1 | 4.4±0.06 | 4.5±0.04 | 4.5±0.1 | 4.4±0.04 | 4.4±0.6 |
| **Lactate Dehydrogenase (U/l)** | 202±8.6 | 200±11.2 | 201±7 | 207±3.6 | 208±8 | 207±8.5 |
| **AST (U/L)** | 19±1.1 | 16.9±0.9 | 18.2±0.8 | 18.8±1.4 | 16.6±0.6 | 18±1 |
| **ALT (U/L)** | 7.2±1.6 | 14.6±1.5 | 11.3±1.1 | 5.2±0.8 | 15.5±1.1 | 11±1.3 |
| CRP (mg/L) | 1.9±0.4 | 3±0.4 | 2.5±0.3 | 7.4±1.7 * | 4.5±0.5 * | 6.4±1.3 * |

Patients with a history of systemic disease, regular drug use, and individuals with cytopenia due to nutritional or other reasons were excluded from our study's patient and control groups. Our study was approved by the Muğla Sıtkı Koçman University Local Clinical Research Ethics Committee (28/11/2019, 17-I).

### Sample collection and laboratory investigations

Whole blood samples were collected into 10 ml sodium heparin tubes and immediately centrifuged at +4 °C and 3000 g for 10 min. Samples were stored at −85 °C until analysis. The serum samples were tested for routine clinical and laboratory investigations including complete blood count (CBC), liver, and kidney function tests.

### Measurement of serum Latexin, Raptor, and Rictor levels

Serum Lxn, Raptor, and Rictor levels were assessed by using ELISA kits (Shanghai YL Biotech Co.Ltd, China) according to the manufacturer's instructions. The Serum Lxn, Raptor, and Rictor concentrations of the samples were calculated by measuring their absorbance at 450 nm wavelength and using from a standard curve, with the concentrations being expressed in nanograms per milliliter (ng/L).

### Statistical analysis

Statistical analysis of the study data was carried out by using GraphPad Prism 3.0 statistical software (GraphPad Software Inc, San Diego, CA, USA). A comparison between the mean value of the two groups was analyzed using Student's t-test and Mann Whitney-U test for parametric and non-parametric variables, respectively. A P value of less than 0.05 was considered statistically significant

## Results

### Laboratory findings

There was no difference in the serum hemoglobin, platelet, sedimentation, thyroid stimulating hormone (TSH), ferritin, iron, iron-binding capacity, glucose, urea, creatinine, albumin, lactate dehydrogenase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels, and age of the CLL patients compared to healthy subjects (Table 1). Serum lymphocyte count must be over 5000/mm3 in order to diagnose CLL according to the WHO criteria. In this study, serum leukocyte levels were found to be high in CLL patients. Additionally, serum vitamin B12 and CRP levels of the CLL patients were higher compared to those of the healthy volunteers (Table-1).

### Serum Lxn, Raptor, and Rictor levels

As shown in (Figure 1). Lxn, a key protein for the activation of the apoptosis mechanism in the HSCs, was dramatically reduced in the patients with CLL compared to the healthy subjects (p= 0,0396). The expression of serum Raptor, a protein kinase that functions as a transcription, autophagy, and protein synthesis control factor, showed a tendency for an increase in the CLL patients compared to the controls although the difference was statistically non-significant (Fig. 2; p= 0.1559). Another protein, Rictor, which is formed by serine/threonine kinase that regulates cell migration, proliferation, and survival, was significantly increased in the CLL-patients compared to the control subjects (Figure 3; p= 0.0133).

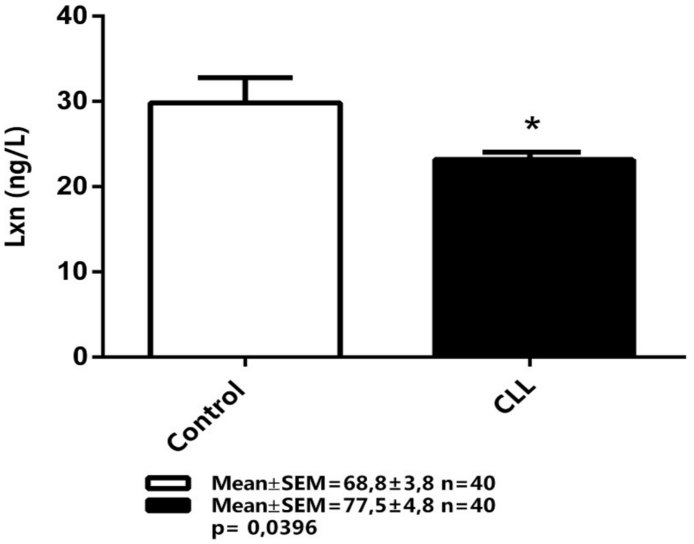


**Figure 1.** The serum Lxn level in healthy volunteers and CLL patients. The results are expressed as mean±SEM; * p< 0.05 denotes a statistically significant difference from the controls

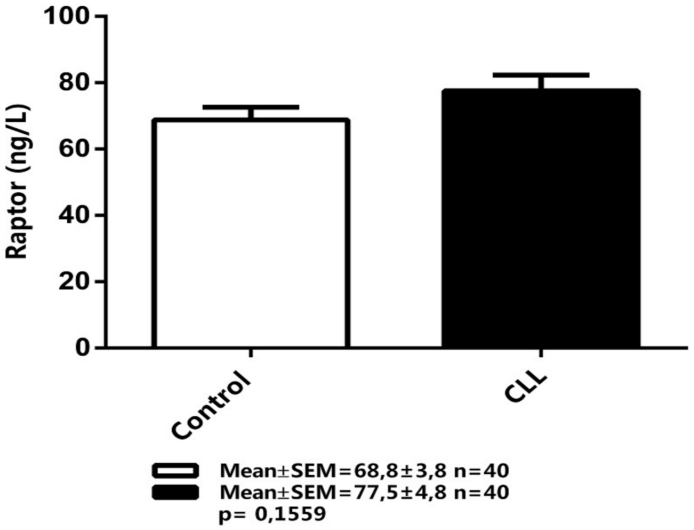


**Figure 2.** The serum Raptor level in healthy volunteers and CLL patients. The results are expressed as mean±SEM; *p<0.05 denotes a statistically significant difference from the controls

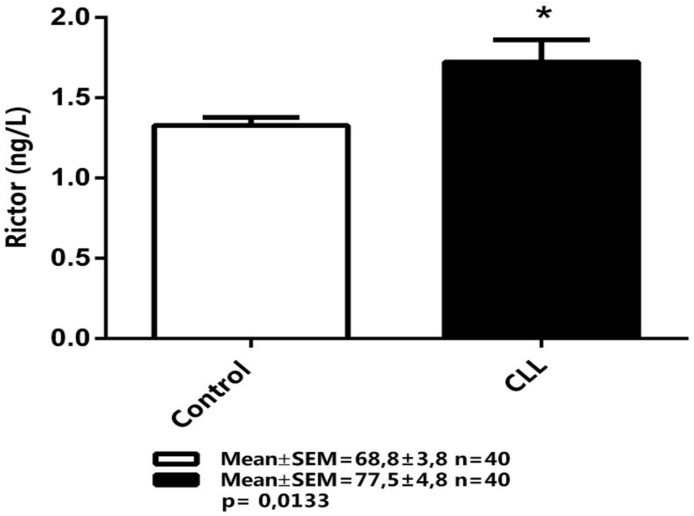


**Figure 3.** The serum Rictor level in healthy volunteers and CLL patients. The results are expressed as mean±SEM; *p<0.05 denotes a statistically significant difference from the controls.

## Discussion

TChronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults, with a frequency of 4.2/100.000/year [21]. The vast majority of leukemias are sporadic, and research suggests that leukemia is often caused by exposure to certain risk factors such as genetic abnormalities, immunosuppression, radiation, carcinogenic chemicals, and oncogenic viruses [22–24]. Metabolism has endogenous regulators for the body to react to these undesirable risk factors. One of them is the recently discovered Lxn, which negatively regulates the population size of HSCs by promoting apoptosis while suppressing proliferation and regeneration. However, the main mechanisms underlying this phenotype are unknown [10]. Here, we examined Lxn level and Lxn/Raptor/Rictor correlation in serum samples of CLL patients to investigate one of the underlying causes.

As there is an insufficient number of studies in the literature to prove the variability of Lxn with age, gender, hemogram, and other biochemical parameters, we ensured that these parameters belonging to the volunteers in the patient and control groups were homogeneous and standardized. Accordingly, when we evaluate the results of age, gender, serum iron, kidney, and liver functions in our study, it is noted that the healthy and patient population presents a homogeneous picture (Table 1). On the other hand, we noted that the levels of white blood cells (WBC), vitamin B12, and CRP significantly increased in both genders (Table 1). From the perspective of the pathophysiology of CLL disease, these results are known to be the expected changes in CLL patients [25].

HSCs are attached in bone marrow gaps called niche by means of several proteins such as N-Cadherin, Tie2, and Robo4 [26]. It has been shown by a study that Lxn provides up-regulation of such structures involved in the uptake of HSCs [27]. Thus, Lxn plays a key role in HSCs to continuously produce mature and functional blood cells in physiological hematopoiesis [28]. In a study similar to our study, it was found that Lxn expression in human leukemia/lymphoma cells was significantly reduced [26]. However, Lxn expression has been shown to be reduced in different malignancies such as lymphoma, leukemia, gastric carcinoma, and thyroid carcinoma [14,29,30]. In a recent study, it was shown that NftB/Akt activation reduced Lxn transcription in breast tumors [31]. compared comparison of the expression levels of Lxn between the healthy volunteers and the CLL patients revealed that Lxn was significantly suppressed in the serum samples from the CLL patients. This indicates that Lxn protects HSCs' homeostasis by preventing HSCs' self-renewal, with the Lxn level decreasing with the development of CLL.

Inhibition of the PI3K/Akt/mTOR pathway for the purpose of reducing the number of HSCs or tumor suppression is considered a promising therapeutic target. PI3K induction occurs when growth and/or proliferation factors activate receptor tyrosine kinases in the cell membrane. Activation of TORC1/TORC2 occurs when activated PI3K approximates Akt towards the cell membrane and phosphorylates it. This message cascade has certain consequences such as cell proliferation, increased survival, and angiogenesis, in addition to the regulation of various cellular functions related to oncogenic phenotypes [32]. In clinical practice, mTOR and PI3K inhibitors are used to activate the apoptosis mechanism by disrupting this axis. However, clinical trials of some recently discovered Akt inhibitors are ongoing. On the other hand, mTORC1 and mTORC2 are known to induce cell proliferation and increase survival by causing Akt activation [33,34]. In this study, it was found that Rictor levels significantly increased in CLL patients compared to healthy individuals while Raptor levels tended to increase. It can be suggested that the findings obtained from the currently limited studies show an opposite correlation between Lxn and Raptor/Rictor on Akt. When evaluated as a whole, it is possible to argue that the findings provided by our study are in the same direction. Our study results indicate an Akt/Lxn/mTORC relationship, excluding the PI3K/Akt/mTOR axis. Based on these findings, it can be argued that Lxn activation by inhibition of these protein structures can also induce apoptosis. On the other hand, it can be suggested that Lxn deficiency may be the underlying cause of the development of hematological malignancies. Therefore, it can be emphasized that exogenous Lxn application along with PI3K/Akt/mTOR inhibitors for leukemia treatment may be an alternative treatment model and provide more favorable treatment outcomes. Of course, more in vitro and in vivo studies are needed both to explain the mechanism of action of Lxn and to elucidate its role in treatment.

**Conflict of interests**
*The authors state that they have no interests that might be perceived as posing a conflict or bias.*

**Financial Disclosure**
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**Ethical approval**
*This study was approved by the Muğla Sıtkı Koçman University Local Clinical Research Ethics Committee (28/11/2019, 17-I).*

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