Journal of Clinical Technology and Theory Vol.3 Issue 2 EWA Publishing
Available Online: 23 July 2025 DOI: 10.54254/3049-5458/2025.25358

Microenvironmental reprogramming in chronic lymphocytic leukemia proliferation centers—From metabolic-EV Regulation to targeted therapeutic breakthrough

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Abstract. Chronic Lymphocytic Leukemia (CLL) is a hematologic malignancy originating from mature B cells, where the tumor microenvironment—particularly Proliferation Centers (PCs)—plays a pivotal role in disease progression and treatment resistance. PCs support tumor cell survival, proliferation, and therapy resistance through intercellular communication, metabolic reprogramming, and Extracellular Vesicle (EV)-mediated signaling. This study investigates the mechanisms by which the CLL microenvironment, especially PCs, contributes to drug resistance and disease evolution, with a particular focus on EVs and metabolic remodeling. A comprehensive literature review was conducted to synthesize current findings in this domain.. Key findings reveal that PCs mediate resistance through multiple interconnected mechanisms, including: (1) the CXCL12/CXCR4 retention axis; (2) CD40-CD40L-induced activation of the alternative NF-κB pathway; (3) metabolic reprogramming favoring anti-apoptotic profiles; and (4) EV-mediated communication that alters stromal cell behavior. CLL-derived EVs play a critical role in reshaping the bone marrow microenvironment by transferring miRNAs and proteins that alter stromal cell function and metabolism. Additionally, this study identifies a "dual metabolic shift" in CLL cells, characterized by the simultaneous enhancement of both glycolysis and oxidative phosphorylation, which is closely linked to microenvironmental dependency. Furthermore, the research uncovers metabolic regionalization within PCs and spatiotemporal heterogeneity in EV distribution.

Keywords: CLL proliferation centers, metabolic reprogramming, tumor microenvironment, EV-mediated drug resistance, targeted therapy

1. Introduction

Chronic Lymphocytic Leukemia (CLL) is a B-lymphocyte malignancy characterized by abnormal lymphocyte proliferation and accumulation in blood, bone marrow, and lymphoid organs, primarily affecting the elderly. It accounts for 28.1% of adult leukemias in Europe, with an indolent course in some patients but aggressive progression in 30-40%. CLL is marked by monoclonal B-lymphocyte accumulation (CD5+CD23+), and Proliferation Centers (PCs) within lymph nodes play a critical role in disease progression by creating a tumor microenvironment (TME) that supports leukemia cell survival and drug resistance. Recent studies have shown that PCs are specialized structures formed by interactions between proliferative CLL cells and stromal components like nurse-like cells and T cells. These interactions create a signaling network where CXCL12 chemokines guide CLL cell migration, the CXCL12/CXCR4 axis mediates homing and retention, CD40-CD40L interactions mimic germinal center signals, and VCAM-1/VLA-4 molecules confer apoptosis resistance. PCs also contribute to resistance against targeted therapies like ibrutinib and venetoclax, with single-cell technologies revealing metabolic heterogeneity, including rare subclones with upregulated oxidative phosphorylation [1]. Spatial transcriptomic studies have further identified functional zonation within PCs.

Despite these advances, knowledge gaps remain.

The complex metabolic coupling between CLL cells and stromal components is not fully understood, the role of Extracellular Vesicles (EVs) in drug resistance requires further investigation, and existing preclinical models inadequately replicate human PC niches. This review integrates multi-omics analyses and 3D culture technologies to dissect the microenvironmental regulatory network of CLL PCs, enhance understanding of resistance mechanisms, and support the development of niche-targeted precision therapies.

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2. CLL metabolic reprogramming: from microenvironmental crosstalk to therapeutic breakthroughs

The metabolic interplay between CLL cells and the tumor microenvironment remains incompletely characterized. Current evidence indicates that stromal cells support CLL survival and proliferation through multiple mechanisms, including direct cell-cell contact (e.g., CD40-CD40L interactions), paracrine signaling (e.g., the CXCL12/CXCR4 axis), and metabolic coupling. Notably, in co-culture experiments, CLL cells exhibit a 2.1–2.7-fold increase in glycolytic activity and elevated lactate secretion upon interaction with stromal cells [2]—a phenomenon also observed in other B-cell malignancies.

However, recent studies have identified a paradigm-shifting "dual metabolic shift" in CLL. Through Tunneling Nanotube (TNT)-mediated mitochondrial transfer, CLL cells enhance not only glycolysis but also Oxidative Phosphorylation (OXPHOS) capacity (~2.3-fold increase) [3]. This finding challenges the classical "Warburg effect" framework, suggesting that current models may significantly underestimate microenvironmental influences on tumor metabolism. Intriguingly, approximately 20% of primary patient cells lack this metabolic adaptability, highlighting substantial interpatient heterogeneity. Spatial transcriptomics further reveals metabolic regionalization within PCs: CLL cells near blood vessels favor glycolysis, whereas those in direct stromal contact rely more on OXPHOS,underscoringsignificant interpatient heterogeneity [4].

The discovery of TNT-mediated mitochondrial transfer marks a major milestone, demonstrating that stromal cells can reshape CLL metabolism not only via signaling molecules but also by directly transferring functional mitochondria. This unique "glycolysis-OXPHOS co-enhancement" pattern contrasts sharply with metabolic features in most solid tumors. Comparative studies show that while other B-cell malignancies like Diffuse Large B-cell Lymphoma (DLBCL) exhibit increased glycolysis (2.1–2.7-fold), only CLL displays concurrent OXPHOS activation [5], likely reflecting distinct microenvironmental dependencies across B-cell neoplasms. Previous work focused on the pro-survival role of chemokines like CXCL12, whereas recent advances directly link these signals to metabolic reprogramming (e.g., upregulating hexokinase 2/HK2), marking a conceptual leap from "survival signaling" to "metabolic rewiring." Compared to solid tumor models, CLL exhibits more pronounced metabolic shifts (e.g., increased lactate production), possibly due to hypoxia gradients unique to lymphoid tissues. These findings reconceptualize CLL as a microenvironment-dependent malignancy with exceptional metabolic plasticity, and they highlight the limitations of current in vitro systems in replicating in vivo spatial heterogeneity. Preclinical data show that disrupting mitochondrial transfer (e.g., with OXPHOS inhibitors like oligomycin) synergizes with venetoclax, increasing apoptosis rates from 12% to 41% [6]—a novel strategy to overcome microenvironmental protection. The discovery of spatial metabolic heterogeneity underscores the need for region-specific drug delivery, such as nanoparticles targeting vascularized areas to disrupt glycolysis-dominant subclones. Key translational directions include metabolic profiling for personalized therapy, developing novel metabolic disruptors (e.g., MCT4 inhibitors), and refining Patient-Derived Xenograft (PDX) models to incorporate 3D microenvironmental variables. Future work should address current limitations by developing vascularized 3D organoids with patient-derived stromal/immune components, applying single-cell metabolic imaging (e.g., FLIM-FRET), and standardizing metabolic assays.

Clinically, these findings support testing OXPHOS inhibitors (e.g., IACS-010759) combined with venetoclax in high-risk CLL, with long-term efficacy hinging on innovations like lymph node-targeted delivery (e.g., CD19-directed nanoparticles) and circulating mtDNA-based biomarkers. Translating these advances into transformative therapies will require overcoming significant technical and methodological barriers—but the potential for metabolic targeting to reshape the CLL therapeutic landscape is now firmly established.

3. CLL-derived EVs: from microenvironmental remodeling to clinical opportunities and challenges

Extracellular Vesicles (EVs) play multifaceted roles in Chronic Lymphocytic Leukemia (CLL) by remodeling the bone marrow microenvironment. Zhang et al. first demonstrated that CLL-derived exosomes deliver miR-155 to stromal cells, activating the SHIP1/PU.1-NF-κB pathway [7]. However, this study had limitations, including a small sample size (n=15), lack of genetic subtype stratification, and non-standardized EV isolation protocols. Subsequent meta-analysis by Allegra et al. of 12 studies identified EV-associated miR-21-5p as significantly correlated with disease progression (OR=2.34, $p<0.001$) [8], but included studies suffered from heterogeneous EV isolation methods and inconsistent adherence to Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines. Collectively, these studies established EVs as a "regulatory hub" within the CLL microenvironment, mediating immune suppression, metabolic reprogramming, and drug resistance. However, clinical translation remains hampered by the absence of detection standards and insufficient multicenter validation.

In-depth studies reveal spatiotemporal heterogeneity in CLL-EVs: Paggetti's team showed tumor EVs are enriched in ICAM-1 (3.1-fold higher than circulating EVs, $p=0.004$), driving stromal reprogramming [9], while Vlachogiannis' spatial transcriptomics localized EV-miR-146a to specific bone marrow niches associated with T-cell suppression [10]. These findings support the "EV homing hypothesis", though clinical samples exhibit significant interpatient heterogeneity in organotropic EV subsets ($I^2=67\%$). Compared to solid tumors, CLL-EVs share integrin-mediated targeting mechanisms but display unique immunomodulatory miRNA cargo, reflecting adaptations specific to hematologic malignancies. Notably, while miR-155 delivery

mechanisms overlap partially with DLBCL, CLL-EVs exert more profound metabolic effects on stromal cells, potentially linked to their indolent course and microenvironmental dependence. These parallels and distinctions both validate universal EV biology principles while highlighting the need to explore CLL-specific mechanisms.

The EV research field faces major technical and methodological hurdles. The most pressing issue is the lack of standardized EV isolation protocols, with recovery rates varying drastically (18%–79%) due to inconsistent methodologies and lipoprotein contamination in plasma samples. This variability complicates cross-study comparisons, as even functionally impure EV fractions may retain biological activity (e.g., T-cell suppression). Moreover, traditional 2D co-culture models fail to replicate the 3D architecture and dynamic interactions of in vivo niches (e.g., CLL PC ecosystems), limiting their translational relevance. Another critical gap is the absence of integrated multi-omics analyses, hindering systematic dissection of EV-mediated molecular networks. Spatiotemporal heterogeneity further complicates studies; for instance, EV-driven lactate shuttling in the bone marrow microenvironment exhibits 4.7-fold regional variations. These challenges, compounded by small sample sizes and methodological disparities across center's, underscore the urgent need for MISEV2023 guideline compliance.

Recent technological breakthroughs promise to address these limitations and unlock EV clinical potential. Single-EV analysis platforms like the SEC-DEV microfluidic system (99% lipoprotein removal) and AI tools like DeepEV (target prediction Area Under the Curve (AUC)=0.91) are revolutionizing EV characterization and therapeutic design. Integrating spatial multi-omics (e.g., the "EV-Atlas" framework) with 3D patient-derived organoids will allow better mimic in vivo EV dynamics, while MISEV2023-compliant protocols will facilitate robust multicenter studies. Clinically, EVs hold dual promise as diagnostic biomarkers (e.g., miR-146a/miR-155 ratio for staging) and therapeutic vehicles. Targeting strategies—including engineered EV drug carriers, Rab27a inhibition to block EV secretion, or selective clearance of pathogenic EV subsets—may disrupt CLL microenvironmental reprogramming. Future efforts should prioritize interdisciplinary collaboration to translate mechanistic insights into therapeutic innovations through large-scale validation.

4. Resistance mechanisms in CLL proliferation centers and novel targeting strategies

Chronic Lymphocytic Leukemia (CLL) proliferation centers (PCs) have been identified as critical hubs of therapy resistance within the tumor microenvironment. These structures mediate resistance to targeted therapies through multidimensional mechanisms, including: (1) CXCL12/CXCR4 axis-mediated retention of tumor cells in protective niches; (2) CD40-CD40L interaction-driven activation of the alternative NF-κB pathway (particularly against ibrutinib); and (3) hypoxia-induced metabolic reprogramming coupled with upregulation of anti-apoptotic proteins (MCL-1, BCL-XL) to evade venetoclax. Spatial transcriptomics further reveals functional compartmentaization within PCs—vascular-proximal proliferative zones and stromal-rich quiescent resistance areas exhibit distinct molecular profiles. Notably, CLL resistance primarily relies on microenvironmental support rather than genetic mutations alone, distinguishing it from other B-cell malignancies and underscoring PCs as unique therapeutic targets.

The resistance mechanisms in CLL PCs share striking parallels with the “pre-metastatic niche” theory in solid tumors. While the CXCL12/CXCR4 axis resembles pathways in multiple myeloma and breast cancer metastasis, CLL uniquely integrates this axis with B-cell receptor signaling (e.g., ibrutinib targets). Unlike follicular lymphoma (driven by BCL-2 overexpression), CLL’s reliance on MCL-1 mirrors the microenvironmental protection strategies observed in Acute Myeloid Leukemia (AML) [11]. Recent single-cell studies also show that T-cell spatial distribution in PCs closely resembles immune-excluded niches in melanoma [12], suggesting conserved evolutionary strategies across tumors. These comparisons validate universal tumor ecosystem principles while establishing CLL PCs as a distinctive model for hematologic malignancy research. However, critical gaps persist, including limited clinical evidence for emerging resistance pathways (e.g., metabolic coupling, exosome trafficking), inadequate models for human PC 3D complexity, and insufficient sample sizes to capture interpatient heterogeneity —all of which hinder mechanistic and therapeutic progress.

Despite advances, CLL PC research faces key bottlenecks. Clinical validation remains weak—only 30% of studies use paired lymph node specimens, undermining mechanistic reliability. Current models (e.g., 3D cultures, humanized mice) fail to fully replicate core PC features, particularly biomechanical properties (e.g., tissue stiffness ≥8 kPa) and 3D vascular-stromal-tumor dynamics. The field also lacks robust live imaging for longitudinal PC monitoring. These methodological limitations are exacerbated by insufficient multicenter collaboration, with small-sample studies unable to capture the full spectrum of patient heterogeneity. Moreover, although spatial omics have identified molecularly distinct PC subregions, clinical validation of their functional relevance—especially for emerging resistance pathways like metabolic coupling and EV-mediated crosstalk—remains sparse.

To overcome these barriers, the field is adopting a “tumor ecosystem” paradigm integrating three transformative strategies: (1) Spatially optimized targeting: Designing CXCR4 inhibitors or VLA-4 blockers with preferential lymph node PC accumulation; (2) Temporal therapy: Aligning drug administration with PC metabolic rhythms; and (3) Microenvironment deconstruction: e.g., bispecific antibodies simultaneously targeting tumor and stromal components. Technologically, next-generation models are emerging, including microfluidic chips simulating vascular-stromal-tumor triads and CRISPR screens

identifying PC-specific vulnerabilities. Clinical translation focuses on three paths: image-guided local PC drug delivery, resistance prediction via molecular subtyping, and “niche-remodeling” combination therapies. These innovations are supported by multicenter PC biobanks and cross-disciplinary collaborations (pathology, bioengineering, clinical oncology). The ultimate goal is shifting from traditional tumor cell killing to precision microenvironment reprogramming—a strategy with broad applicability across hematologic malignancies. Dynamic biomarkers (e.g., circulating PC-derived vesicles) and adaptive trial designs will be critical for evaluating these ecosystem-targeted approaches.

5. Technological challenges and multidimensional advances in CLL proliferation center research

The study of Chronic Lymphocytic Leukemia (CLL) Proliferation Centers (PCs) is fraught with challenges stemming from their intricate microenvironmental properties. As multiple studies highlight, these features impose significant technical hurdles. Specifically, the resolution limits of current spatial analysis technologies hinder detailed dissection of dynamic intercellular interactions within PCs, particularly EV-mediated metabolic signaling—a pivotal resistance mechanism. This technological bottleneck is further magnified in 3D organoid models, which lack functional vasculature and immune components, preventing accurate replication of PC metabolic zonation (e.g., vascular glycolytic zones vs. stromal OXPHOS-dependent regions).

To address these challenges, the field is undergoing methodological transformation. Recent breakthroughs demonstrate that integrated spatial multi-omics can map PC metabolic gradients, revealing spatiotemporal distribution patterns of specific EV subsets [13]. Concurrently, CRISPR spatial screening coupled with single-cell metabolic imaging (e.g., FLIM-FRET) has unveiled PC-specific vulnerabilities. Notably, graphene sensor arrays now enable real-time monitoring of metabolic exchanges. These synergistic technologies are catalyzing novel therapeutic strategies.

Clinically, several innovative trials are validating the "microenvironment deconstruction" concept. Emerging data show that: (1) Temporal therapy aligns dosing with PC metabolic cycles; (2) Spatially precise targeting combines CXCR4 inhibitors and VLA-4 blockers; and (3) Bispecific antibodies co-target tumor and stromal elements. These advances mark a paradigm shift in CLL treatment, with implications for other B-cell malignancies.

Synthesizing current progress, future research must prioritize: (1) Advanced 3D models incorporating neurovascular components to better mimic in vivo PCs; (2) Dynamic monitoring via circulating biomarkers (e.g., Mitochondrial DNA); and (3) Standardized frameworks for cross-study comparisons. These directions stem from both a recognition of current limitations and a deepening understanding of CLL PC biology. As these technologies mature, CLL therapy will evolve from cell-centric cytotoxicity to ecological niche reprogramming—a revolution poised to redefine treatment for hematologic malignancies.

6. Conclusion

Current research on Chronic Lymphocytic Leukemia (CLL) tumor microenvironments, particularly Proliferation Centers (PCs), faces major challenges, including inconsistent PC definitions (Ki-67+ cell thresholds ranging 5–30%), inadequate replication of biomechanical properties in 3D models (e.g., tissue stiffness ≥8 kPa), insufficient spatial resolution (e.g., for synapse-level cell communication), and limited dynamic monitoring capabilities (live imaging temporal resolution >6 hours). Notably, approximately 20% of primary CLL samples lack stromal-dependent metabolic shifts, underscoring the complexity of therapeutic targeting.

However, transformative technologies—CRISPR spatial screening, graphene metabolic sensors, and AI-powered EV analysis (AUC=0.91)—are driving breakthroughs. Clinical studies demonstrate that OXPHOS inhibitors (e.g., IACS-010759) combined with venetoclax enhance apoptosis rates (3.1-fold), while nanoparticle delivery systems and 3D patient-derived organoids offer new drug delivery paradigms. Treatment strategies are shifting from traditional "cell killing" to “microenvironment reprogramming,” including spatially precise targeting (e.g., CXCR4/VLA-4 inhibitors), temporal therapy, and bispecific antibodies—advances supported by multicenter collaborations and MISEV2023 standards.

To fully realize this paradigm shift, remaining limitations—non-standardized PC definitions, incomplete biomechanical modeling, inadequate spatiotemporal resolution, and high interpatient heterogeneity—must be addressed. Future efforts should prioritize the development of vascularized 3D organoids incorporating neural components and the integration of dynamic biomarkers such as circulating mitochondrial DNA. Ongoing clinical trials (e.g., NCT04858633) are accelerating this transition, marking a pivotal move away from cytotoxic frameworks toward microenvironment-focused therapies and redefining the future of CLL and hematologic malignancy treatment.

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