NEOPLASIA
www.neoplasia.com

Volume 19 Number xx Month 2017 pp. 762–771 762

The Stromal Microenvironment Modulates Mitochondrial Oxidative Phosphorylation in Chronic Lymphocytic Leukemia Cells[1,2]

Hima V. Vangapandu[*,§], Mary L. Ayres[*], Christopher A. Bristow[‡], William G. Wierda[†], Michael J. Keating[†], Kumudha Balakrishnan[*,§], Christine M. Stellrecht[*,§] and Varsha Gandhi[*,†,§]

[*]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA; [†]Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA; [‡]Applied Cancer Science Institute, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA; [§]The University of Texas MD Anderson Cancer Center UT Health Graduate School of Biomedical Sciences, 1515 Holcombe Blvd., Houston, TX 77030, USA

Abstract

Peripheral blood chronic lymphocytic leukemia (CLL) cells are replicationally quiescent mature B-cells. In short-term cultures, supporting stromal cells provide a survival advantage to CLL cells by inducing transcription and translation without promoting proliferation. We hypothesized that the stromal microenvironment augments malignant B cells' metabolism to enable the cells to cope with their energy demands for transcription and translation. We used extracellular flux analysis to assess the two major energy-generating pathways, mitochondrial oxidative phosphorylation (OxPhos) and glycolysis, in primary CLL cells in the presence of three different stromal cell lines. OxPhos, measured as the basal oxygen consumption rate (OCR) and maximum respiration capacity, was significantly higher in 28 patients' CLL cells cocultured with bone marrow–derived NK.Tert stromal cells than in CLL cells cultured alone ($P = .004$ and $<.0001$, respectively). Similar OCR induction was observed in CLL cells cocultured with M2-10B4 and HS-5 stromal lines. In contrast, heterogeneous changes in the extracellular acidification rate (a measure of glycolysis) were observed in CLL cells cocultured with stromal cells. Ingenuity Pathway Analysis of CLL cells' metabolomics profile indicated stroma-mediated stimulation of nucleotide synthesis. Quantitation of ribonucleotide pools showed a significant two-fold increase in CLL cells cocultured with stromal cells, indicating that the stroma may induce CLL cellular bioenergy and the RNA building blocks necessary for the transcriptional requirement of a prosurvival phenotype. The stroma did not impact the proliferation index (Ki-67 staining) of CLL cells. Collectively, these data suggest that short-term interaction (≤24 hours) with stroma increases OxPhos and bioenergy in replicationally quiescent CLL cells.

Neoplasia (2017) 19, 762–771

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of quiescent mature B cells. CLL is a compartmentalized disease; malignant cells reside in bone marrow, spleen, lymph nodes, and peripheral blood. Therefore, CLL cells inhabit diverse microenvironments, and in general, the CLL cell population is either slowly proliferating or quiescent [1].

Several investigations have focused on the crosstalk between CLL cells and the cellular components of the microenvironment. In one study, CLL cells from the bone marrow, peripheral blood, or lymph nodes of the same patients showed different mRNA signatures depending on the microenvironment with which they were

Abbreviations: BCR, B-cell receptor; CLL, chronic lymphocytic leukemia; ECAR, extracellular acidification rate; ETC, electron transport chain; MM, mitochondrial mass; MOMP, mitochondrial outer membrane potential; MRC, maximum respiration capacity; mtDNA, mitochondrial DNA; mTOR, mechanistic target of rapamycin; NTP, ribonucleoside triphosphate; OCR, oxygen consumption rate; OxPhos, mitochondrial oxidative phosphorylation; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; XF, extracellular flux.

Address all correspondence to: Varsha Gandhi, Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Unit 1950, 1901 East Road, Houston, TX 77054.
E-mail: vgandhi@mdanderson.org

[1] Conflict of interest disclosure: The authors do not have a conflict of interest.

[2] Funding: This work was supported by a CLL Global Research Foundation Alliance grant.
Received 25 May 2017; Revised 20 July 2017; Accepted 24 July 2017

1476-5586/17
http://dx.doi.org/10.1016/j.neo.2017.07.004

interacting [2]. The lymph nodes provided the niche for proliferation, and CLL cells from the lymph nodes showed the distinct signature for growth, proliferation, and DNA replication. The lymphoid tissue has been found to harbor nascent CLL cells [3]. In short, at least a portion of CLL cells that reside in the lymph nodes are proliferating; this change in biology is due to signals from the non-CLL cells of this microenvironment.

The microenvironment of the bone marrow has been shown to provide anti-apoptotic signals to resident CLL cells. In vitro investigations clearly demonstrate that stromal cells provide a survival advantage (but no proliferation advantage) to CLL cells by protecting them from apoptosis [4–6]. Mechanistic studies have further elucidated that stromal interaction induces the anti-apoptotic members of the BCL-2 family at the transcript and protein levels; after coculture with stromal cells, the levels of MCL-1, BCL-2A1, and BCL-XL proteins in the CLL cells are increased [6–10].

The stroma-mediated induction of anti-apoptotic proteins in CLL cells also results in the resistance of these primary cells to a variety of chemotherapeutic agents [11–14]. The coculture of CLL cells with stromal cells induces a cascade of events, such as the phosphorylation of Ser2 and Ser5 in the C-terminal domain of RNA polymerase II; the elevation of the global RNA synthesis rate; the amplification of pro-survival transcripts; and the up-regulation of protein synthesis. This expansion in macromolecule synthesis is without an increase in CLL cell replication, which remains inert [8]. Collectively, these findings indicate that stroma-induced apoptosis resistance may be mediated through transcriptional and translational changes of the signaling proteins that regulate the expression of prosurvival proteins.

The B-cell receptor (BCR) and CD19 pathways, both pivotal to the survival and maintenance of CLL B cells, are also activated by microenvironmental factors [8,15,16]. Microenvironmental factors have also been found to stimulate CLL cells, resulting in the activation of the BCR pathway. Stroma-derived factors such as SDF-1 activate BCR and its downstream targets in CLL cells [4]. At the apex of this pathway are transcription factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells, which induce transcription in CLL lymphocytes.

Specific microenvironmental events, such as the stimulation of CpG sites and activation of toll-like receptors, are associated with an increase in the proliferation index of CLL cells. The stimulation of these pathways and the BCR axis increases thymidine incorporation, indicating the replication of CLL cells. Various chemokines and cytokines in this microenvironment also influence CLL cell behavior [17–19].

Collectively, these data suggest that bone marrow stromal cells, through direct contact and signaling, generate a microenvironment that provides survival and anti-apoptotic signals without affecting cellular replication. Despite the absence of DNA synthesis, CLL cells that are exposed to stromal cells have augmented RNA and protein production; these processes require a bioenergy reservoir and consumption.

We hypothesized that stromal cells have an active role in modulating the energy metabolism of malignant B cells that enables them to cope with their energy demands. We assessed the two major ATP-generating pathways, mitochondrial oxidative phosphorylation (OxPhos) and glycolysis, in primary CLL cells in the presence of three different adherent stromal cell lines. Our data demonstrate that stromal cell interaction augmented CLL cells' biosynthesis of not only ATP but also other nucleotides. Furthermore, stromal cells mediated a modulation of the OxPhos pathway without changing the glycolysis route of ATP generation in CLL cells.

Patients, Materials, and Methods

Patient Sample Collection

Peripheral blood samples from 58 CLL patients (Supplemental Table 1) were collected in green-top tubes containing sodium heparin. All patients had given written informed consent in accordance with the Declaration of Helsinki and under a protocol approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center.

Cell Isolation and Coculture

Peripheral blood mononuclear cells were separated by Ficoll–Hypaque (Atlanta Biologicals, Flowery Branch, GA) density gradient centrifugation. CLL cells were cultured in RPMI-1640 medium with L-glutamine plus 10% human serum. All experiments were performed using freshly isolated CLL cells; the purity of this cell population was ≥95%. Depending on the experiment, CLL cells were cocultured with stromal cells (NK.Tert, M2-10B4, or HS-5) for 2, 24, or 48 hours as indicated in the figure legends. CLL and stroma cells were plated at a ratio of 100:1 as described previously [14]. The culture conditions for the stromal cell lines are given in Supplemental Table 2.

Cytotoxicity Assays

For cytotoxicity assays, CLL cells (1×10^6) were washed with 1× phosphate-buffered saline (PBS) and resuspended in 100 μL of 1× Annexin V binding buffer with 5 μL of Annexin V–fluorescein isothiocyanate as described previously [20]. Samples were incubated in the dark for 15 minutes at room temperature, and then 400 μL of 1× propidium iodide buffer (Annexin V binding buffer, 0.625 μg/mL propidium iodide) was added to the reaction. Cells were analyzed with a BD Accuri flow cytometer (BD Biosciences, Franklin Lanes, NJ). Ten thousand events were recorded per sample.

Metabolite Mass Spectrometry

CLL cells were cocultured with NK.Tert cells for 24 hours, washed twice with ice-cold 1× PBS, counted with a Coulter counter, and then saved as pellets at −80°C. Cellular metabolites extracted from 5×10^6 cells using 80% methanol were assessed at the mass spectroscopy facility at Beth Israel Deaconness Medical Center for levels of polar metabolites (e.g., nucleobases, nucleotides, sugars, amino acids, polyamines) [21]. The changes in CLL cell metabolites after coculture were expressed as the ratios of values obtained for each metabolite after coculture versus suspension culture. The metabolites were then grouped into pathways using Kyoto Encyclopedia of Genes and Genomes identifiers. Pathway analysis was performed using Ingenuity Pathway Analysis (Qiagen Bioinformatics, Redwood, CA).

Extracellular Flux Assays

Extracellular flux (XF) assays (Seahorse Bioscience, Chicopee, MA) were used to measure the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of CLL cells in suspension culture or after co-culture with stromal cells for 2 or 24 hours as indicated in the figure legends. For OCR both the basal OCR and maximum respiration capacity were assessed. For ECAR, both the glycolytic flux and glycolytic capacity were evaluated. Stromal cells