EBioMedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Low Density Lipoproteins Amplify Cytokine-signaling in Chronic Lymphocytic Leukemia Cells

Lindsay McCaw [a,c], Yonghong Shi [a], Guizhi Wang [a], You-Jun Li [a,g], David E. Spaner [a,b,d,e,f,*]

[a] Biology Platform, Sunnybrook Research Institute, Toronto, ON M4N 3M5, Canada
[b] Department of Immunology, University of Toronto, Toronto, ON M5S 1A8, Canada
[c] Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada
[d] Department of Medicine, University of Toronto, Toronto, ON M5G 2C4, Canada
[e] Department of Medical Biophysics, University of Toronto, Toronto, ON M5G 2M9, Canada
[f] Sunnybrook Odette Cancer Center, Toronto, ON M4N 3M5, Canada
[g] Department of Human Anatomy, College of Basic Medical Sciences, Jilin University, Changchun, Jilin 130021, China

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 26 November 2016
Accepted 28 November 2016
Available online xxxx

Keywords:
Chronic lymphocytic leukemia
Janus kinases
STAT3
Ruxolitinib
Cholesterol
Lipoproteins
Lysosomal lipase
HMGCR
Nuclear receptors

ABSTRACT

Recent studies suggest there is a high incidence of elevated low-density lipoprotein (LDL) levels in Chronic Lymphocytic Leukemia (CLL) patients and a survival benefit from cholesterol-lowering statin drugs. The mechanisms of these observations and the kinds of patients they apply to are unclear. Using an *in vitro* model of the pseudofollicles where CLL cells originate, LDLs were found to increase plasma membrane cholesterol, signaling molecules such as tyrosine-phosphorylated STAT3, and activated CLL cell numbers. The signaling effects of LDLs were not seen in normal lymphocytes or glycolytic lymphoma cell-lines but were restored by transduction with the nuclear receptor PPARδ, which mediates metabolic activity in CLL cells. Breakdown of LDLs in lysosomes was required for the amplification effect, which correlated with down-regulation of *HMGCR* expression and long lymphocyte doubling times (LDTs) of 53.6 ± 10.4 months. Cholesterol content of circulating CLL cells correlated directly with blood LDL levels in a subgroup of patients. These observations suggest LDLs may enhance proliferative responses of CLL cells to inflammatory signals. Prospective clinical trials are needed to confirm the therapeutic potential of lowering LDL concentrations in CLL, particularly in patients with indolent disease in the "watch-and-wait" phase of management.

© 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Initiating and promoting events are required for the development of cancer. Initiation involves genetic lesions that transform normal cells while promoters stimulate transformed cells to proliferate and acquire more DNA changes to cause increasingly malignant behavior (Foulds, 1954). There is much interest in the idea that obesity and hyperlipidemia are tumor promoters (Goodwin and Stambolic, 2015).

Obesity is associated with a higher risk of developing many cancers, perhaps from increased levels of inflammatory cytokines and bioactive lipids that stimulate proliferation of cancer cells. Obesity is also a risk factor for dyslipidemias such as hypercholesterolemia. Low-density lipoproteins (LDLs) are the major carriers of cholesterol and have been studied mainly as cardiovascular risk factors but are increasingly recognized to play a role in cancer. LDLs promote proliferation, survival and migration of breast cancer cells (Nelson et al., 2013; dos Santos et al., 2014; Kitahara et al., 2011) and high LDL levels are associated with increased risk of prostate (Moses et al., 2009) and colorectal cancer (Holtzman et al., 1987). Statins lower LDL levels by blocking 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), the rate-limiting enzyme of cholesterol synthesis, and have anti-cancer properties (Mucci and Stampfer, 2014; Chae et al., 2014; Gronich and Rennert, 2013). Explanations for these observations are unclear.

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the developed world. It is a heterogeneous disease with some patients never needing treatment in their life-time while others exhibit a rapidly progressive course that can be fatal (Fabbri and Dalla-Favera, 2016). We found that LDL levels are elevated in up to 75% of CLL patients attending a specialized clinic at a single center and that statins delayed the need for chemotherapy in these patients by nearly 3 years (Chow et al., 2016) We also recently used administrative databases in a population-based case-control study involving 2124 CLL patients and 7935 controls to demonstrate a significantly higher incidence of hypercholesterolemia before a diagnosis of CLL and a survival benefit of 3.7 years for patients taking statins (Mozessohn et al., 2016).

* Corresponding author at.: David Spaner, Biology Platform, S-116A, Research Building, Sunnybrook Research Institute, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada.
E-mail address: spanerd@sri.utoronto.ca (D.E. Spaner).

http://dx.doi.org/10.1016/j.ebiom.2016.11.033
2352-3964/© 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article as: McCaw, L., et al., Low Density Lipoproteins Amplify Cytokine-signaling in Chronic Lymphocytic Leukemia Cells, EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.11.033

ARTICLE IN PRESS

Progression of CLL takes place in lymphoid organ microenvironments called pseudofollicles where leukemic cells are stimulated to proliferate by signals from B cell receptor (BCR) and toll-like receptor (TLR) ligands, TNF-family members, cytokines, and chemokines (Li et al., 2015; Herishanu et al., 2011). CLL cells with aggressive clinical behavior exhibit greater proliferative responses to microenvironmental signals (Tomic et al., 2011). Circulating CLL cells are readily obtained but studies of pseudofollicles require the use of *in vitro* models. We have found that much of the biology of pseudofollicles is captured by culturing circulating CLL cells with IL2, to represent T cell activity, along with the TLR7-agonist Resiquimod (Oppermann et al., 2016). The studies in this manuscript were designed to try to understand why hypercholesterolemia is apparently a tumor promoter for CLL by using this *in vitro* pseudofollicle model to observe how LDLs affect the biology of proliferating CLL cells.

2. Materials and Methods

2.1. Antibodies and Reagents

Fluorescent human CD19 and CD5 antibodies were from Pharmingen (San Francisco, CA). IL10-receptor (CD210) antibodies were from eBioscience (San Diego, CA) while IL10, IL10-receptor blocking antibodies, and low-density lipoprotein receptor antibodies were from R&D Systems, Inc. (Minneapolis, MN). The IL6-receptor blocking antibody Actemra (Roche Canada, Mississauga, ON), IL2 (Chiron, Corp., San Francisco, CA), and IFNα2b (Schering Canada Inc., Pointe-Claire, QC) were purchased from the Sunnybrook Cancer Centre pharmacy. 7-aminoactinomycin D (7-AAD) was obtained from Biolegend (San Diego, CA). Fatty acid–free bovine serum albumin, Nile Red, α-tocopherol, methyl-β-cyclodextrin, resiquimod, water-soluble cholesterol, oleic acid, phorbol dibutyrate (PDB), and β-actin antibodies were from Sigma-Aldrich (St. Louis, MO). Phospho-(Y705) STAT3 (Cat. No 9131), total STAT3, phospho-p44/42 MAPK(Erk1/2)(Thr202/Tyr204) (Cat. No 9102), phospho-(Ser17) SRC (Cat. No 5473), phospho-(Thr308)AKT (Cat. No 9275), and secondary horseradish peroxidase–conjugated anti-rabbit and anti-mouse antibodies (Cat. Nos. 7074 and 7076, respectively) were from Cell Signaling Technology (Beverly, MA). Low-, high-and very low-density lipoproteins were from EMD Chemicals (San Diego, CA). Lalistat (Hamilton et al., 2012) was a generous gift from Synageva BioPharma (Lexington, MA, USA). Perfringolysin O (PFO), a cytolysin from *Clostridium perfringens* that binds cholesterol in target membranes, was a gift from Alejandro Heuck (University of Massachusetts, Amherst, MA, USA). Ruxolitinib and Ibrutinib were from SelleckChem (Houston, TX, USA). Goat anti-human IgM Fc-specific antibodies were from Jackson ImmunoResearch Labs (West Grove, PA, USA). The Amplex® Red Assay Kit was from Invitrogen™. RPMI 1640 cell culture media was from Wisent Bioproducts (Quebec, Canada). The chemically defined CD lipid extract was from Thermo-Fisher Scientific (Mississauga, ON, Canada).

2.2. Purification of CLL Cells and Normal Lymphocytes

CLL cells were isolated as before by negative selection from the blood of consenting patients (Tomic et al., 2011), diagnosed with CLL by a persistent monoclonal expansion of $CD19^{+} CD5^{+} IgM^{lo}$ lymphocytes. The cells were used directly for experiments. Patients had not been treated for CLL for at least 6 months prior to blood collection. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over ficoll gradients as before (Spaner et al., 2006). Protocols were approved by the Sunnybrook Research Ethics Board (PIN 222-2014).

2.3. Cell Culture

Unless specified otherwise, purified CLL cells and PBMCs were cultured at 1×10^6 cells/ml in RPMI-1640 medium supplemented with transferrin and 0.25% fatty-acid free albumin in 6-, 12-or 24-well plates (BD Labware) at 37 °C in 5% CO_2 for the times indicated in the figure legends.

2.4. Flow Cytometry

Viable cells were indicated by 7AAD-exclusion and reactive oxygen species (ROS) by 2′7′-dichlorofluorescin diacetate (DCFH2-DA; Molecular Probes) as before (Tomic et al., 2011; Tung et al., 2013). Nile Red and PFO were used to indicate lipoprotein-uptake by activated CLL cells. Nile Red reflects lipid droplets (Listenberger and Brown, 2007) that form in the presence of increased intracellular fatty acids and cholesterol. PFO was conjugated to Alexa Fluro 488 fluorochrome and used to measure plasma membrane cholesterol (Johnson et al., 2012). One million cells were stained with 3 μl 7AAD for 10 min, 3 μM Nile Red for 20 min, or 5 μl PFO for 15 min at room temperature or with 10 μM DCFH2 for 30 min at 37 °C. Cells were then washed in PBS and 10,000 viable events collected with a FACScan flow cytometer using Cellquest software (Becton Dickinson). Data was analyzed using FLOWJO software (Ashland, OR, USA). DCFH2 oxidation was measured as "green" (FL1) fluorescence on a log-scale.

2.5. $PPARD^{hi}$ Daudi Cells

Human *PPARD* full-length cDNA was obtained from Addgene (Cambridge, MA, USA) and sub-cloned into the *Xho*I and *Eco*RI sites of retroviral MSCV2.2 plasmids or into the *Xho*I and *Not*I sites of lentiviral pLemiR plasmids. Construct sequences were confirmed before transfection. Replication-defective viruses were made by transfecting the MSCV-PPARD viral plasmid into the helper-free packaging cell line GP + A (B8), as described before (Wang et al., 2016). $CD5^{+}$-Daudi cells (Spaner et al., 2013) at 2×10^6 cells/ml were infected with supernatants from the virus-producing cells. Stably transfected clones were obtained by limiting dilution and selection in G418 (Multicell). Transfection was conducted with Lipofectamine 3000 according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Cells infected with retroviruses containing the empty vectors but otherwise handled in the same way were used as controls.

2.6. Immunoblotting

Protein extraction and immunoblotting were performed as before (Tomic et al., 2011). Proteins were resolved in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membranes (Millipore Corp., Billerica, MA). Western blot analysis was performed according to the manufacturers' protocol for each antibody. Chemiluminescent signals were created with SupersignalWest Pico Luminal Enhancer and Stable Peroxide Solution (Pierce, Rockford, IL) and detected with a Syngene InGenius system (Syngene, Cambridge, United Kingdom). For additional signal, blots were stripped for 60 min at 37 °C in Restore Western Blot stripping buffer (Pierce), washed twice in Tris-buffered saline plus 0.05% Tween-20 at room temperature, and reprobed as required. Densitometry was performed using Image J software. The densitometry value for each sample was normalized against the value for β-actin to obtain the intensities for phosphorylated-STAT3, -ERK, -AKT or -SRC reported in the figures.

2.7. Sample Preparation for Amplex® Red Assay Kit

The detergent/surfactant-free lysis/extraction method recommended by the manufacturer was used to remove chemical or cellular components that might interfere with the activity of the enzymes or dye. CLL cells (1×10^6 cells) were purified, homogenized immediately in 200 μl chloroform-methanol (2:1 ratio), and centrifuged for 10 min at 14,000 rpm at room temperature in a microcentrifuge. The organic phase was collected and vacuum dried for 20 min using a DNA Speed

Please cite this article as: McCaw, L., et al., Low Density Lipoproteins Amplify Cytokine-signaling in Chronic Lymphocytic Leukemia Cells, EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.11.033

Download English Version:

https://daneshyari.com/en/article/8438649

Download Persian Version:

https://daneshyari.com/article/8438649

Daneshyari.com