EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Distinct Activities of Glycolytic Enzymes Identify Chronic Lymphocytic Leukemia Patients with a more Aggressive Course and Resistance to Chemo-Immunotherapy

Georg Gdynia [a], Tadeusz Robak [b], Jürgen Kopitz [a], Anette Heller [a], Svetlana Grekova [a], Katarina Duglova [a], Gloria Laukemper [a], Monika Heinzel-Gutenbrunner [c], Cornelius Gutenbrunner [d], Wilfried Roth [e], Anthony D. Ho [f], Peter Schirmacher [a], Michael Schmitt [f,g], Peter Dreger [f,g], Leopold Sellner [f,g,*]

[a] Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany
[b] Medical University of Lodz, Copernicus Memorial Hospital, Lodz, Poland
[c] Department of Child and Adolescent Psychiatry, University Hospital Marburg, Marburg, Germany
[d] MH Statistical Consulting, Marburg, Germany
[e] Institute of Pathology, University Medical Center of the Johannes Gutenberg University Mainz, 55131 Mainz, Germany
[f] Department of Medicine V, University Hospital Heidelberg, Heidelberg, Germany
[g] National Center for Tumor Diseases (NCT), German Cancer Consortium (DKTK), Heidelberg, Germany

A R T I C L E   I N F O

Article history:
Received 15 January 2018
Received in revised form 24 May 2018
Accepted 24 May 2018
Available online xxxx

Keywords:
CLL
Metabolism
PK M2
LDH
High-risk

A B S T R A C T

A higher capacity to grow under hypoxic conditions can lead to a more aggressive behavior of tumor cells. Determining tumor activity under hypoxia may identify chronic lymphocytic leukemia (CLL) with aggressive clinical course and predict response to chemo-immunotherapy (CIT). A metabolic score was generated by determining pyruvate kinase and lactate dehydrogenase, key enzymes of glycolysis, ex vivo in primary CLL samples under normoxic and hypoxic conditions. This score was further correlated with clinical endpoints and response to CIT in 96 CLL patients. 45 patients were classified as metabolic high risk (HR), 51 as low risk (LR). Treatment-free survival (TFS) was significantly shorter in HR patients (median 394 vs 723 days, $p = .021$). 15 HR patients and 14 LR patients received CIT after sample acquisition. HR patients had a significantly shorter progression-free survival after treatment compared to LR patients (median 216 days vs not reached, $p = .008$). Multivariate analysis evaluating age, IGHV, *TP53* deletion or mutation and 11q22–23 deletion besides the capacity of tumor cells to grow under severe hypoxic conditions identified the metabolic profile as the strongest independent risk factor for shorter TFS (hazard ratio 2.37, $p = .011$). The metabolic risk can provide prognostic and predictive information complementary to genetic biomarkers and identify patients who might benefit from alternative treatment approaches.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Different malignancies contain subpopulations of tumor cells that can grow in hypoxic (severely oxygen deficient) microenvironment [1,2]. These cancer cells can be responsible for poor outcome and resistance to radiation and chemotherapy [3]. Cancer cells growing under hypoxia produce energy and building blocks for macromolecules by anaerobic glycolysis, independent from mitochondrial (oxygen dependent) respiration [4]. Increased activities of the pyruvate kinase (PK) isoform M2 and of lactate dehydrogenase (LDH) allow faster incorporation of glucose metabolites into biomass facilitating cancer cell growth without oxygen [5,6]. Previously we have shown that oxygen-independent growth renders cancer cells resistant to Natural Killer (NK) cells. Cancer withstanding metabolic killing by the innate immune system use both, PK (M2) and LDH, to up-regulate anabolism and energy supply via glycolysis [7,8]. In summary, the capacity of cancer cells to grow fast under hypoxia correlates with resistance to therapy and elimination by immune cells. However, the ability of glycolysis in tumor cells is still unexploited for stratification and treatment of cancer patients. Here we provide a tool that could be easily implemented in clinical diagnostics. Employment of LDH and PK M2 under hypoxia (compared to normoxia) indicates fast hypoxic (anaerobic) cellular growth [5,9]. We developed an assay mimicking the hypoxic cancer microenvironment ex vivo by cultivation of primary chronic lymphocytic leukemia (CLL) cells from peripheral blood under hypoxia with PK M2 and LDH activity as readout.

Intensive chemo-immunotherapy (CIT) with fludarabine, cyclophosphamide and rituximab (FCR) [10] or bendamustine and rituximab (BR) [11] has dramatically improved the outcome of CLL with potential for long-term remissions. However, substantial variability can be

* Corresponding author at: Department of Medicine V, Heidelberg University Hospital, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.
E-mail address: leopold.sellner@med.uni-heidelberg.de (L. Sellner).

https://doi.org/10.1016/j.ebiom.2018.05.030
2352-3964/© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article as: Gdynia G, et al, Distinct Activities of Glycolytic Enzymes Identify Chronic Lymphocytic Leukemia Patients with a more Aggressive Course and Resista..., EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.05.030

observed in the course of CLL. Some patients are asymptomatic at time of diagnosis and do not need treatment for a long period. On the other hand, subgroups of patients develop rapid disease progression and early therapy resistance. A major issue in CLL is the identification of these patients who do not benefit from intensive CIT. So far, only *TP53* disruption (del17p13 and *TP53* mutation) is an established predictive marker for CIT refractoriness. These patients rather benefit from novel treatment approaches in CLL such as inhibitors of the B-cell receptor pathway (BCRi), e.g. the BTK inhibitor ibrutinib [12] and the PI3Kδ inhibitor idelalisib [13], or antiapoptotic proteins, e.g. the Bcl-2 inhibitor venetoclax [14]. However, a large proportion of CIT refractory patients do not harbor a disruption in *TP53*. Despite intense efforts, no reliable markers are available to predict response towards CIT in *TP53* wild-type patients [15]. The aim of the current study was to assess feasibility as well as prognostic and predictive value of PK M2 and LDH activity after cultivation of leukemia cells under hypoxia for the identification of CLL patients with aggressive clinical courses and resistance to CIT.

2. Patients and methods

2.1. Sample extraction and clinicopathologic data

The study sample consisted of consecutive 96 patients diagnosed with CLL who presented at the University Hospital Heidelberg between 2013 and 2014. Peripheral blood mononuclear cells (PBMCs) were isolated by a Ficoll gradient. The research was approved by the Ethics Committee of the University of Heidelberg (S-356/2013 and S-254/2016). Informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

2.2. Genetic aberrations

Chromosomal aberrations by fluorescence in situ hybridization (FISH) were obtained from medical reports and were available for del [11](q22.3) ($n = 92$), trisomy 12 ($n = 91$), del [13](q14) ($n = 92$) and del [17](p13) ($n = 91$). Targeted sequencing for genetic aberrations in *NOTCH1*, *SF3B1*, and *TP53* was performed on a GS Junior benchtop sequencer (Roche, Penzberg, Germany) as described before [16].

2.3. Cell lines

The CLL cell line Mec-1 was obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; RRID: CVCL_1870) and cultured in RPMI 1640 (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 2 mM L-glutamine (Thermo Fisher Scientific) and 10% heat-inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific) at 37 °C.

2.4. Cytotoxicity assay

Cytotoxicity measurements were performed under very low oxygen conditions in 96-well plates using the ATP-based CellTiter Glo assay (Promega, Madison, WI, USA). Cells were cultured for 24 h with or without fludarabine (Sigma-Aldrich, St. Louis, MI, USA). In addition, PK M2 activity was modulated by PM2-tide (GGAVDDDpYAQFANGG; Enzo Life Sciences, Farmingdale, NY, USA; 10 μM) or DASA (1-(2,6-Difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine; Merck Millipore, Burlington, MA, USA; 10 μM). The number of viable cells was calculated as % of the untreated control.

2.5. Glucose flux and lactate efflux

Glycolysis was measured by monitoring the conversion of 5- ^{3}H-Glucose to ^3H_2O as described by Liang et al. [17]. In brief, cells were washed in PBS and resuspended in 1 ml Krebs buffer containing 10 mM glucose, and spiked with 370 MBq 5-^{3}H-Glucose (Hartmann Analytic, Braunschweig, Germany). Following incubation for 1 h at 37 °C diffusion through a PCR vial was used to separate ^3H_2O formed by glycolysis. Radioactivity was determined in a liquid scintillation counter (TRICARB 2900, PerkinElmer, Waltham, USA). Lactate efflux was quantified by spectrophotometric assay as described by Brandt et al. [18].

2.6. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed with either 1:5 or 1:10 diluted cDNA and analyzed in triplicates using the StepOne Plus thermo cycler (Applied Biosystems, Foster City, CA, USA). The cycling program was performed as follows: 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Gene expression was normalized to two variants of the housekeeping gene 18S rRNA and data were quantified by StepOne Software v2.1. Fold change of expression was determined by the ΔΔCt method as described by Schmittgen and Livak et al. [19] The primer pairs used are listed in the supplementary methods.

2.7. Phosphofructokinase and hexokinase activity

Phosphofructokinase and hexokinase activity were assayed as described in Teslaa et al. [20] using homogenates from 10 [6] Mec-1 cells.

2.8. Immunoblot analysis and protein preparation

Immunoblotting was performed according to standard procedures by SDS–polyacrylamide gel electrophoresis. Cells were lysed in lysis buffer P (20 mM Tris-HCl (pH 7.4), 137 mM NaCl, 10% (*v*/v) glycerine, 1% Triton X-100, 2 μM EDTA, 100 mM phenylmethylsulfonyl fluoride and protease inhibitors (Complete mini from Roche). Lysates were centrifuged at 14,000 *g* (10 min) at 4 °C. Total protein was measured by the Bradford (Bio-Rad, Hercules, CA, USA) method. Soluble protein was resolved by SDS–polyacrylamide gel electrophoresis, blotted onto nitrocellulose and incubated with one of the following antibodies: rabbit polyclonal anti PKM2 (1:1000, Cell Signaling, Danvers, MA, USA; 4053S; RRID: AB_1904096), rabbit polyclonal anti LDHA (1:1000, Cell Signaling; 2012S; RRID: AB_2137173), rabbit polyclonal anti GAPDH (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA; sc-365,062; RRID: AB_10847862) and HIF1 alpha (1:1000, StressMarq Biosciences, Victoria, Canada; SMC-184; RRID: AB_2570396). Appropriate secondary antibodies (1:3000, horse-radish peroxidase-conjugated, #170–6515 (goat anti rabbit IgG; RRID: AB_11125142) and #170–6516 (goat anti mouse IgG; RRID: AB_11125547)) were from Bio-Rad. Visualization was done by enhanced chemiluminescence technique (GE-Healthcare, Little Chalfont, UK). Uncropped versions of the membranes are shown in Supplementary Fig. S1/2.

2.9. Metabolic score

2.9.1. Preanalytic

Two 3 cm petri dishes per patient were filled with 3 ml RPMI 1640 (Life Technologies, Paisely, UK) and $1*10^7$ cells. One was wrapped with an oxygen impermeable shell (GasPak™ EZ, Becton Dickinson, New Jersey, USA) to generate anaerobic conditions. Both dishes were incubated overnight (16-24 h) at 37 °C and 5% CO_2 (hypoxic (anaerobic) (Hx) and normoxic (Nx) sample). There was no significant difference in the total number of viable CLL cells between hypoxic and normoxic conditions after overnight culture. After incubation, cells were washed with PBS and resolved in 500 μl buffer solution (50 mmol/l KCl, 5 mmol/l $MgCl_2$, Tris 20 mmol/l, 250 mmol/l sucrose in ddH_2O, pH 7.4). To extract cytosolic proteins, the cell suspension was homogenized by ultrasonication (Diagenode Bioruptor® Sonication System, Diagenode, Seraing, Belgium). The enzyme activity of the three enzymes PK-la, PK-ha, and LDH was analyzed in the supernatant.

Please cite this article as: Gdynia G, et al, Distinct Activities of Glycolytic Enzymes Identify Chronic Lymphocytic Leukemia Patients with a more Aggressive Course and Resista..., EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.05.030

Download English Version:

https://daneshyari.com/en/article/8437118

Download Persian Version:

https://daneshyari.com/article/8437118

Daneshyari.com