ELSEVIER

Blood 142 (2023) 3265–3266

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

The Deubiquitinase CYLD Acts As an Oncogene in a Cellular Model of Chronic Lymphocytic Leukemia

Marina Gerousi [1], Georgios Gavriilidis [1], Sofoklis Keisaris [1], Artemis Kourouni [1], Aspasia Orfanou [1], Anastasia Iatrou [1], Athanasios Pseftogkas [2,3], George Mosialos [4], Eleni Theodosiou [1], Anastasia Chatzidimitriou [1], Fotis Psomopoulos [1], Paolo Ghia, MDPhD [3,2], Kostas Stamatopoulos [1], Konstantinos Xanthopoulos [1,5]

[1] Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thessaloniki, Greece
[2] Vita-Salute San Raffaele University, Milan, Italy
[3] Division of Experimental Oncology, B cell neoplasia, IRCCS Ospedale San Raffaele, Milan, Italy
[4] School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[5] School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece

The cylindromatosis protein (CYLD) is a functional deubiquitinase that regulates critical signaling pathways, e.g. NF-κB and Wnt, thus modulating several cellular functions. *CYLD* acts as a tumor suppressor gene in solid tumors, and is also involved in the pathogenesis of hematological malignancies, including B cell lymphomas, in as yet unclear ways. In chronic lymphocytic leukemia (CLL), preliminary evidence suggests that reduced expression of CYLD correlates with a worse clinical prognosis, which appears to be in line with its postulated role as a tumor suppressor. Here we sought to gain insight into the function of CYLD in CLL through genetic engineering, molecular characterization and bioenergetic profiling. To this end, we used CRISPR/Cas9 technology and a *CYLD*-targeting or an unrelated gRNA to generate stable *CYLD*-knockout (*CYLD* ^{ko}) and control (*CYLD* ^{wt}) MEC1 cells. Phenotypic characterization of *CYLD* ^{ko}versus *CYLD* ^{wt} MEC1 cells by flow cytometry showed (i) significantly reduced viability, assessed by Annexin V (fold difference, FD=1.2, $p<0.05$); (ii) lower cell proliferation rate, assessed by Ki67 expression (FD=1.4, $p<0.05$); (iii) increased apoptosis, determined by measuring active caspase 3 expression levels (FD=6.1, $p<0.01$); and, (iv) reduced expression of CD86 (FD=4.3, $p<0.001$) and CD40 (FD=4.1, $p=0.05$). Western blotting analysis of *CYLD* ^{ko} versus *CYLD* ^{wt}MEC1 cells revealed down-regulation of the NF-κB pathway evidenced by diminished expression of IKKβ (FD=2.4, $p<0.01$), phospho-IκBα (FD=3.4, $p<0.01$) and phospho-p105 (FD=2.5, $p=0.2$); and, the Wnt pathway evidenced by reduced β-catenin levels (FD=2, $p<0.05$). Transcriptome profiling by RNA-seq gave concordant results, in documenting increased apoptosis and decreased NF-κB signaling in *CYLD* ^{ko}versus *CYLD* ^{wt}MEC1 cells. The former also showed downregulation of calcium, BcR and PI3K/AKT/mTOR signaling pathways and, in contrast, upregulation of the glutathione and KEAP1-NFE2L2 pathways, which contribute to antioxidant defense and nutrient metabolism, as well as, regulation of redox balance and cellular metabolism, respectively. To evaluate the impact of *CYLD* deletion on CLL bioenergetics, we assessed ATP production rate, as a marker of active cellular metabolism, using the Seahorse XF Real-Time ATP Rate assay. *CYLD* ^{ko}MEC1 cells exhibited impaired ATP production reflected in decreased OCR (oxygen consumption rate) and ECAR (extracellular acidification rate), that are proportional to OXPHOS (oxidative phosphorylation) and glycolysis, respectively. Regarding the metabolic phenotype, *CYLD* ^{wt}MEC1 cells displayed a shift towards mitochondrial OXPHOS, whereas, in *CYLD* ^{ko}MEC1 cells ATP production was mostly based on glycolysis. High-performance liquid chromatography was performed using culture supernatants obtained at sequential time points to quantify glucose uptake and lactate secretion rates. The analysis revealed that *CYLD* ^{wt} MEC1 cells primarily directed glucose carbon toward biomass biosynthetic pathways, as reflected in both higher cell number and proliferation rate achieved, while CYLD deletion redirected carbon flux enhancing lactate formation. In both cases, once glucose was consumed (day 4), the secreted lactate was re-used, yet this was more pronounced in *CYLD* ^{ko} MEC1 cells. Finally, we explored whether *CYLD* knockout might impact MEC1 sensitivity to targeted agents i.e. the BTK inhibitor ibrutinib and the BCL2 inhibitor venetoclax. We found that *CYLD* ^{ko}MEC1 cells presented increased apoptosis compared to their *CYLD* ^{wt}counterparts when cultured in the presence of either drug. Moreover, treatment with ibrutinib or venetoclax led to reduced ATP production rates in both *CYLD* ^{ko}and *CYLD* ^{wt}MEC1 cells, albeit the reduction was more pronounced in the former (FD=4.5 for ibrutinib; FD=10 for venetoclax compared to the respective *CYLD* ^{wt} MEC1 treated cells). Taken together, we demonstrate for the first time that *CYLD* can also act as an oncogene, at least in the context of CLL and in particular in the MEC1 cell line model of CLL, since its elimination leads to (i) lower proliferation and increased apoptosis rates coupled with diminished signaling capacity; (ii) metabolic rewiring toward enhanced lactate formation; and, (iii) augmented

sensitivity to CLL therapeutic agents. It remains to be elucidated under which condition this could also occur in the patients with CLL or other B lymphoproliferative disorders.

Disclosures Chatzidimitriou: *Novartis:* Other; *Jansenn:* Other. **Ghia:** *Roche:* Consultancy, Honoraria, Research Funding; *MSD:* Consultancy, Honoraria, Research Funding; *Janssen:* Consultancy, Honoraria, Research Funding; *Lilly/Loxo Oncology:* Consultancy, Honoraria, Research Funding; *BMS:* Consultancy, Honoraria, Research Funding; *BeiGene:* Consultancy, Honoraria, Research Funding; *AstraZeneca:* Consultancy, Honoraria, Research Funding; *AbbVie:* Consultancy, Honoraria, Research Funding.

https://doi.org/10.1182/blood-2023-188983