

Exploring the metabolic requirements for CLL proliferation

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Introduction

Glucose and glutamine are essential fuels for *de novo* synthesis of nucleotides in proliferating cells. In our previous work, we deciphered the metabolic dependencies of CLL resistance to the Bcl-2 inhibitor venetoclax (VEN). We showed that VEN resistance was attenuated by inhibition of the glutamine transporter SLC1A5, while blocking glucose metabolism did not affect the sensitivity to the drug (Chen, Blood, 2022; Chen, Haematologica, 2023). In this study, we aim to dissect the requirements for CLL proliferation in lymph nodes, which are still unknown.

In parallel, this study aims to assess the potential of a new glutamine-based tracer for positron emission tomography (PET) to overcome the suboptimal detection of lymph node activity in CLL when using conventional [¹⁸F]-FDG PET.

Methods

We used a 3D model mimicking the lymph node microenvironment previously developed by our group (Haselager, HemaSphere, 2023), in combination with different metabolic inhibitors to assess proliferation and spheroid growth of CLL cells from treatment-naive patients. In parallel, we developed a ¹⁸F-labelled (2S,2R)-4-fluoro-glutamine (4-[¹⁸F]FGln) PET tracer and tested it in the TCL1 adoptive transfer model. PET imaging was performed in TCL1 tumor bearing (n=3) and wild-type (n=1) C56BL/6 mice. Mice were imaged with 4-[¹⁸F]FGln followed by second scan with [¹⁸F]FDG with 48h in between. A second mouse study is currently ongoing.

Results

Proliferation and spheroid growth were found to be dependent on the availability of both glucose and glutamine. The glucose competitor 2-deoxy-glucose impeded

spheroid formation completely, while inhibition of the glutamine transporter SLC1A5 by V9302 allowed growth but reduced the size of the spheroids by half compared to controls. This was due to decreased CLL proliferation. Inhibition of the alternative glutamine transporters SLC38A1/2 did not affect spheroid growth, confirming that SLC1A5 is the main glutamine transporter in CLL. Remarkably, mTORC1/2 inhibitors had a similar outcome as V9302, and combination of V9302 with mTOR inhibition resulted in an additive effect, indicating that the impact of amino acid starvation on CLL proliferation is beyond inhibition of mTOR.

With regards to our mouse PET studies, 4-[18F]FGln showed significantly higher uptake in the spleens from mice bearing CLL tumors compared to control mice, while [18F]FDG demonstrated minimal spleen uptake compared to control, indicating higher sensitivity of the glutamine-based PET.

Conclusion

These data show that glucose and glutamine are essential for CLL proliferation in the lymph node microenvironment, and constitute proof of concept for the use of 4-[18F]FGln PET tracer as new diagnostic tool for CLL.