The Proton Channel HVCN1 Inhibits Leukaemia Progression In The CLL Mouse Model Eμ-TCL1 And Regulates Sialylation of T antigen In MM

Ala'a Alyahyawi[1], Elayne Hondares[1], Mark Brown[1], Fabienne McClanahan[2], Shehrazed Lounis[3], Nathalia Zorze Rossetto[3], Sarah Spear[3], John Gribben[2] and Melania Capasso[1]
[1,3]Centre for Cancer and Inflammation, Barts Cancer Institute, Queen Mary University of London, UK
[2]Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, UK

Bckground:
HVCN1 (hydrogen voltage-gated channel 1) is the only mammalian gene that codes for a voltage-gated proton channel[1]. HVCN1 is highly expressed in immune cells. In B cells, HVCN1 modulates B cell receptor (BCR) signalling while supporting ROS production by the NADPH oxidase activity[2]. Human HVCN1 expression is increased in a number of mature B cell malignancies such as lymphoma and chronic lymphocytic leukaemia (CLL). CLL cells in particular show an upregulation of a shorter isoform of HVCN1, which we showed mediates stronger proton currents[3].
Given the increase in HVCN1 expression in CLL cells and their reliance on BCR signalling for survival and proliferation, we hypothesized HVCN1 loss or inhibition would counteract leukaemia progression. As a comparison, we also investigated HVCN1 role in BCR negative B cell tumours such as multiple myeloma.

Methods
We generated a transgenic TCL1/HVCN1$^{-/-}$ mouse by crossing HVCN1 knock-out (KO) mice (HVCN1$^{-/-}$) with the Em-TCL1, a well- known and extensively studied mouse model of CLL[4]. We also knocked down HVCN1 in a multiple myeloma cell line (EJM) to study the role HVCN1 in BCR deficient cells.

Results
We monitored a cohort of TCL1/HVCN1 KO (n=15) and TCL1 wild-type mice (n=19) for the development of CLL. Unexpectedly, TCL1/HVCN1 KO mice had significantly shorter survival than controls. Interestingly, we noticed an increase in CD3+ T cells in the spleen of TCL1/HVCN1 KO mice. However, HVCN1 KO leukaemic cells showed diminished BCR signalling compared to wild-type controls, as expected.
On the other hand, in multiple myeloma cells lines, which do not express a BCR, downregulation of HVCN1 by shRNA showed no significant changes in proliferation, survival and energy metabolism compared to control cells. However, analysis of the cell glycomics profile illustrated a significant difference in the sialylation of the O-glycan core 1 (T antigen) in HVCN1 knock-down cells compared to scrambled control.

Conclusions
The proton channel HVCN1 plays a role in CLL, surprisingly this appears to be inhibiting tumour growth. We are currently extending our mouse studies as well as assessing human CLL cells in order to establish if, contrary to what happens in normal B cells, proton channels support a novel mechanism that results in tumour inhibition in CLL cells. Furthermore, we are investigating the functional consequences of increased T antigen sialylation in multiple myeloma on tumour spreading.

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