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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Novel Role of Mitochondrial Folate Metabolism in Cell Fate Decisions and Leukaemogenesis

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Folate-mediated one carbon (1C) metabolism sustains proliferation of rapidly dividing cells through its direct contribution to nucleotide biosynthesis. In brief, new 1C units are donated from the amino acid serine (the primary source of 1C units), while folate molecules function as 1C unit carriers. Accordingly, antifolates have been used in the treatment of several malignancies, particularly in the field of haemato-oncology. Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder that arises in a single haematopoietic stem cell with the introduction of the BCR::ABL1 oncoprotein. Although it is generally accepted that CML is driven by leukaemic stem cells (LSCs) that are insensitive to standard therapy due to their quiescent/slow-cycling status, the role of folate metabolism in LSCs remains undescribed.

Transcriptomic analysis of patient-derived CML LSCs ($CD34^{+}38^{-}$) revealed a significant upregulation of folate metabolism genes, including the mitochondrial hydroxymethyltransferase (SHMT2; $p \leq 0.05$), when compared with normal counterparts. Furthermore, CML stem/progenitor ($CD34^{+}$) cells displayed a significant increase in the exchange rate of formate (folate intermediate necessary for nucleotide synthesis) compared to the secretome of normal $CD34^{+}$ cells ($p < 0.05$), suggesting an upregulation of 1C metabolism in therapy resistant CML cells.

Following SHMT2 knockout (KO), we observed a strong antiproliferative effect and significantly impaired tumour formation in CML cell line xenograft models ($p < 0.01$), verifying that mitochondrial 1C metabolism is not dispensable for CML cells. Metabolic characterisation using liquid chromatography-mass spectrometry revealed that both genetic and pharmacological inhibition (using the SHMT1/2 inhibitor, SHIN1) of 1C metabolism results in a significant decrease in *de novo* purine synthesis. Furthermore, we uncovered activation of AMPK signalling and suppression of mTORC1 activity following mitochondrial 1C metabolism inhibition. Notably, formate supplementation or reconstitution of purine levels was sufficient to reverse the effect on AMPK and mTORC1.

Phenotypically, SHIN1 treatment induced the expression of erythropoiesis markers CD71 and Glycophorin A in CML cells, including patient derived $CD34^{+}$ cells. AMPKα1/α2 KO revealed that the increased expression of these markers was independent of AMPK activity. Mechanistically, we discovered that reconstitution of purine levels (adenine) and mTORC1 activity prevented erythroid differentiation following SHMT1/2 inhibition, highlighting that depletion of 1C units drives differentiation of leukaemic cells through mTORC1-mediated purine sensing.

Of clinical relevance, combination of SHIN1 with imatinib, a frontline treatment for CML patients, decreased the number of therapy-resistant CML LSCs in a patient-derived xenograft model ($p < 0.05$). Lastly, stable isotope-assisted metabolomics using $^{13}C_3{}^{15}N_1$-serine revealed that imatinib reduced labelling into purine nucleotides, but it maintained labelling into glutathione (GSH), the most abundant antioxidant in the cell, and its oxidised derivate (GSSG). Through generation of glycine, folate metabolism directly contributes to the GSH pool. These data suggest that standard therapy does not affect the incorporation of serine into GSH, implying that the synergistic effect with folate metabolism inhibition might be related to impeded antioxidant defence.

Overall, our findings highlight a novel role for mitochondrial folate metabolism in stem cell fate decisions and leukaemogenesis.

This work is available as a preprint: https://www.biorxiv.org/content/10.1101/2022.12.21.521404v1

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