Abstract Topic: 13. Myeloma and other monoclonal gammopathies - Biology & translational research

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MULTIPLE MYELOMA CAUSES CHANGES IN FATTY ACID METABOLISM IN TUMOUR ASSOCIATED MACROPHAGES VIA A FATP2 DEPENDENT MECHANISM

Rosie Taylor*[1], Matthew Markham[1], George Bell[1], Annalisa Altera[1], Rebecca Maynard[1], Katherine Hampton[1], Kristian Bowles[1, 2], Stuart Rushworth[1]

[1]Norwich Medical School, University of East Anglia, Norwich, United Kingdom, [2]Norfolk and Norwich University Hospitals NHS Foundation Trust, Department of Haematology , Norwich, United Kingdom

Background:

Multiple myeloma (MM) is highly dependent on cellular interactions in the bone marrow (BM) microenvironment which promotes survival and progression, suggesting non-malignant cells in the BM microenvironment play a role in MM's pathophysiology (Jibril, A. et al. 2023). Macrophages have a significant role in BM homeostasis but also affect the initiation and progression of MM (Jibril, A. et al. 2023). Macrophages undergo stimuli-specific differentiation into a range of polarisation states between the extremes known as M1 and M2 (Wculek, S. K. et al. 2022). M2 macrophages have a pro-tumoral phenotype (Zaidi, N. E. et al. 2022), which is supported by increased mitochondrial oxidative phosphorylation through fatty acid oxidation. The mechanism of how these changes result in the M2 phenotype is not fully understood and have not been investigated in MM.

Aims:

The aims of this project were to investigate changes in fatty acid oxidation (FAO) in tumour associated macrophages in response to MM and determine the mechanism of these changes.

Methods:

For in vivo assays we used the 5TGM1 KaLwRij mouse model to analyse metabolic changes in BM macrophages. In vitro bone marrow derived macrophages (BMDM) and 5TGM1 cells were co-cultured and analysed at a range of time points to measure the gene expression associated with FAO. Co-cultures were analysed using fluorescently labelled fatty acids to assess macrophage fatty acid uptake. Using the Seahorse metabolic flux assay oxygen consumption in macrophages was measured with and without FAO inhibitors. Cytokine array was used to investigate what 5TGM1 derived soluble factors were causing the metabolic changes. BMDM co-cultures with 5TGM1 cells and a range of inhibitors were used to investigate the mechanisms of these changes.

Results:

Macrophages were isolated from the BM of 5TGM1 injected KaLwRij mice. Data shows upregulation of the fatty acid transporter protein 2 (FATP2) and fatty acid binding proteins 3 and 5 (FABP3/5) with a downregulation of CD36 in F4/80+ BM

macrophages isolated from 5TGM1 injected KaLwRij mice compared to macrophages from control KaLwRij mice. To determine if this was a consequence of direct contact with 5TGM1 cells in vitro trans-well co-cultures were set up. BMDM cultured with 5TGM1 cells showed an upregulation in FATP2 and FABP3/5 and downregulation of CD36. Flow cytometry analysis of the BMDM confirmed changes in protein expression of FATP2 and CD36. Further analysis identified secretory factors derived from 5TGM1 cells inducing these changes. Next, we analysed uptake of fatty acids in BMDM cultured with 5TGM1. BMDM cultured with 5TGM1 had increased uptake of fluorescently labelled long chain fatty acids (LCFA) and medium chain fatty acids (MCFA). The uptake of both LCFA and MCFA was reduced by the FATP2 inhibitor, lipofermata. Seahorse analysis found respiratory capacity was higher in BMDM co-cultured with 5TGM1 cells and this was inhibited by lipofermata. Cytokine arrays were used to determine the cause of the FATP2 increase in BMDM in response to 5TGM1 and identified the soluble factors GM-CSF, IL-6 and Il-1β as potential stimulators of FATP2 upregulation.

Summary/Conclusion:
We have determined that MM changes FAO in BM macrophages through the upregulation of FATP2 which increases uptake of MCFA and LCFA. These changes result in BM macrophages preferentially using FAO in response to MM. These data suggest that MM alters macrophage FAO within the BM microenvironment, potentially contributing to disease progression.

Keywords: Macrophage, Multiple myeloma, Lipid metabolism, Bone marrow microenvironment