ONCOMETABOLISM

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The transfer of chemical energy from nutrients into macromolecules is the foundation of cellular and tissue growth. Tumours are no exception to this principle, and their metabolic state ultimately supports anabolism and growth. Our vision is that the tissue of origin influences the biochemical pathways utilised by tumours to grow in two ways. On the one hand by imposing environmental constraints, the tissue of origin exposes metabolic vulnerabilities of the tumour. On the other hand, enzymes normally restricted to a defined population of differentiated cells, and required for tissue physiological functions, can be hijacked by cancer cells to enhance their metabolic fitness.

Glutamine metabolism, liver homeostasis and cancer

The canonical activity of glutamine synthetase catalyses the production of the amino acid glutamine from glutamate and ammonia. This reaction regulates glutamine metabolism from prokaryotes to mammals and is fundamental for processes such as ammonia detoxification and neurotransmission in humans. In the context of cancer, this enzyme is highly upregulated in a subset of liver cancer affecting ~1 in 3 patients which is driven by oncogenic mutations in β-catenin. We are currently studying the role of this enzyme in tumour initiation and progression but a pre-requisite to advance our understanding of its role in cancer was the elucidation of what this enzyme is doing in normal liver.

We demonstrated that a small molecule with uncharacterised biological activity, methylamine, was released by the intestinal microbiome, and it was used by the hepatic glutamine synthetase to produce a glutamine analogue which we identified as N5-methylglutamine.

Technically, the identification of N5-methylglutamine as a novel product of glutamine synthetase demonstrated the discovery potential of state-of-the-art metabolomics when applied to *in vivo* models.

Finally, we showed the translational relevance of our findings in a novel genetically modified mouse model of liver cancer that recapitulates the disease of those patients with β-catenin mutant tumours. We demonstrated that the urine levels of N^5-methylglutamine significantly correlated with liver tumour burden, substantiating the value of this metabolite as a biomarker for patients with this genetically-defined subset of tumours that express high levels of glutamine synthetase.

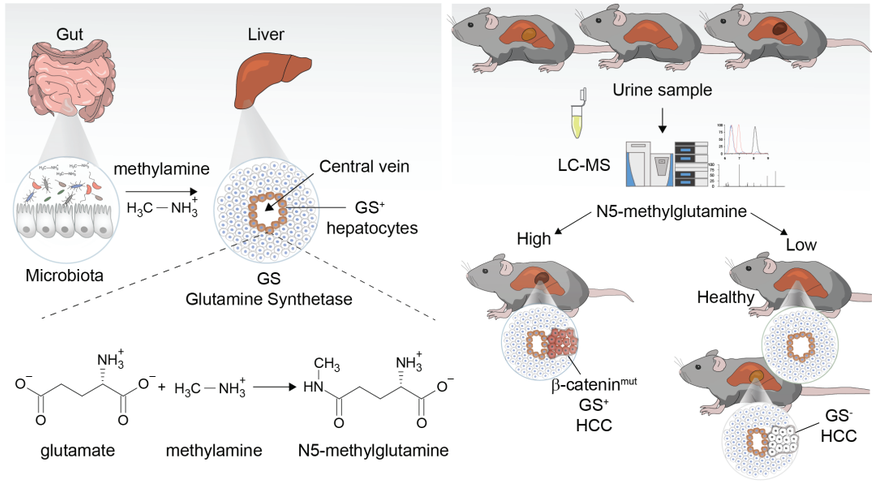

Figure 1
N^5-methylglutamine is the product of a newly identified reaction catalysed by glutamine synthetase
Hepatic glutamine synthetase accepted methylated ammonia to synthesise *N^5*-methylglutamine. In a mouse model of β-catenin mutant hepatocellular carcinoma, the urine levels of this glutamine analogue were predictive of tumour burden. Villar *et al.*, 2022, *Nature Chem Biol.* highlighted in *"A collaborative synthetase"* News & views

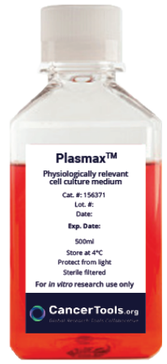

Figure 2
Plasmax™ is a physiological medium based on the levels of nutrients and metabolites found in human plasma that has been developed at the CRUK Beatson Institute. Plasmax™ is available for purchase at https://www.cancertools.org/media/156371.

Identification of metabolic vulnerabilities elicited by glucocorticoids in glioma

Glucocorticoids (e.g. dexamethasone) are part of the mainstay of treatment for glioma patients and are administered to reduce the peritumoral oedema, and to mitigate the adverse side effects of radio- and chemotherapy. As indicated by the name *(glucose + cortex + steroid)* glucocorticoids exert regulatory effects on glucose metabolism. However, the metabolic effects of glucocorticoids are not limited to systemic homeostasis of glucose and may modulate the fitness of glioma cells in the brain environment. While the anti-inflammatory action of glucocorticoids is a mainstay for the clinical management of glioma patients, the metabolic effects of these drugs on the cancer cells could be exploited to improve the prognosis of brain tumour patients. On this basis, glucocorticoids constitute excellent candidates to design novel metabolic combination therapies for the treatment of glioma.

A more physiological cell culture medium improves the relevance to *in vivo* biology

Despite it seeming obvious that the nutrient composition of culture medium affects the phenotypic behaviour of the cells, very little attention has been devoted in perfecting the formulation of historic media.

Indeed, the vast majority of biomedical research employs commercially available growth media, based on the pioneering work done 60 years ago by Harry Eagle. However, these formulations were not designed to reproduce the physiological cellular environment, but rather to enable the continued culture of cells with minimal amount of serum (i.e. Minimal Essential Medium, MEM). Consequently, the standard culture medium known as DMEM is distant from the nutrient levels found in normal human blood. For example, glucose in DMEM is at five-fold the normal glycaemia, and a similar ratio applies to glutamine. Conversely, non-essential amino acids normally circulating in blood are completely missing from DMEM formulation (Ackermann *et al.*, 2019, *Trends in Cancer*). On this basis, we developed Plasmax™ (Figure 2) a cell culture medium with nutrients and metabolites at the concentration normally found in human blood (Vande Voorde et al., 2019, *Science Advances*).

In 2020, Plasmax™ became the first physiological medium to be commercially available.

In 2022, we published a study (Taurino *et al.*, 2022, *Molecular Metabolism*), where we compared the response of primary bone marrow derived human mesenchymal stromal cells to Plasmax™ and DMEM. The results showed that Plasmax™ prevented the nutritional stress imposed by the skewed DMEM formulation, while sustaining mesenchymal stromal cells stemness and proliferation. Further, a panel of donor-derived cell lines were cultured in Plasmax™ at oxygen concentration (1%) relevant to the haemopoietic niche. By integrating transcriptomics, untargeted metabolomics, extracellular flux analysis, and stable isotope tracing we found that mesenchymal stromal cells consistently took up glutamate from the extracellular environment and used its carbons to support citrate synthesis and secretion. We demonstrated that this distinctive metabolic pathway was engaged even when citrate was supplied at concentrations found in human circulation, and oxygen level limits citrate production in the mitochondria (Figure 3).

Overall, our findings demonstrated that distinctive metabolic features of mesenchymal stromal cells were preserved by refined physiological cell culture conditions immediately applicable to the production of cell therapy products.

Publications listed on page 114

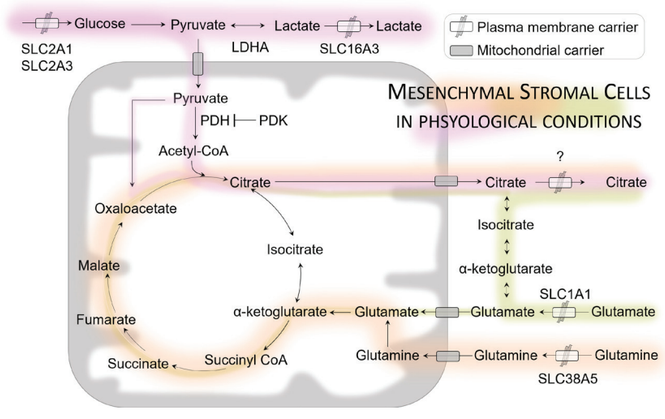

Figure 3
A schematic of the metabolic pathways engaged by human bone marrow-derived primary mesenchymal stromal cells when cultured in Plasmax™. Taurino *et al.*, 2022, Molecular Metabolism