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ONCOMETABOLISM

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At the foundation of cellular and tissue growth stands the transfer of chemical energy from nutrients into macromolecules. Tumours are no exception to this principle, and unavoidably seek metabolic states that support 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 and glutamate metabolism in brain and liver cancer

Glutamine and glutamate are instrumental to physiological processes, such as neurotransmission in the brain and ammonia homeostasis in the liver. At the same time, they are obligate substrates for anabolism of tumours originating in these organs, such as glioma and hepatocellular carcinoma. Glutamine synthetase (GS) catalyses the ligation of glutamate and ammonia and is the only known enzyme able to synthesise glutamine in mammalian cells (Figure 1). We previously showed that GS-derived glutamine provides the nitrogen required for nucleotide biosynthesis in glutamine-restricted glioblastoma, the most aggressive type of glioma. Currently, we are assessing the effects of GS interference on the metabolism and growth of human primary glioblastoma cells and xenografts.

The liver is an ammonia-detoxifying organ and maintains homeostatic levels of circulating ammonia and glutamine. The functional unit of the liver constitutes an elegant example of metabolic zonation. In fact, the periportal zone, where hepatocytes express the urea cycle enzymes, converts the majority of ammonia into urea. The ammonia escaping this metabolic zone is captured by the hepatocytes surrounding the central vein, which express GS. This enzyme has a high affinity for ammonia, and fixes it into the non-toxic glutamine, that can be returned to blood circulation.

In liver tumours this metabolic zonation is disrupted. Liver tumours with an overactive WNT/β-catenin signalling pathway show a widespread and sustained GS expression.

By means of HPLC–mass-spectrometry–based metabolomics and cell biology approaches, we are studying the carbon and nitrogen metabolism of liver tumours with high GS expression.

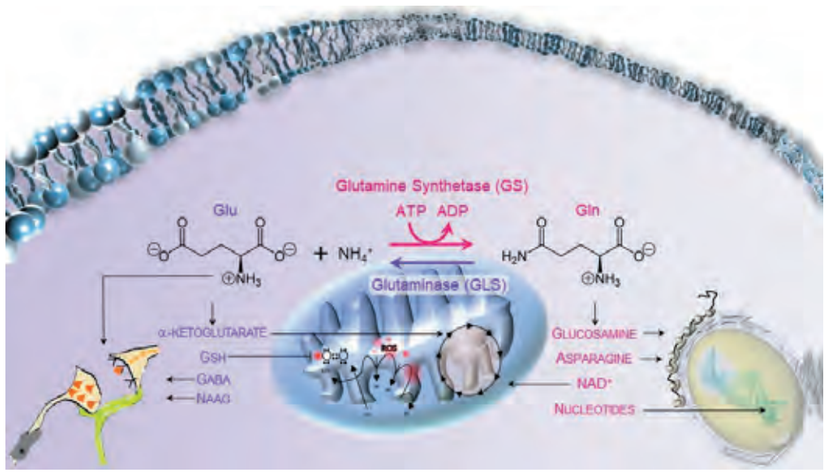

Figure 1
A schematic of the reactions catalysed by the enzymes Glutaminase and Glutamine Synthetase. These two enzymes catalyse opposed reactions essential to maintain glutamine and glutamate at homeostatic levels in cells, tissues and organisms. More than fifty metabolic reactions utilise or produce these two amino acids that are key for a multitude of cellular processes and tissue functions, some of which are selected by cancer cells, supporting their growth and survival.

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. It is available for biomedical research at Ximbio.

Identification of the 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 peritumoural 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, unavoidable collateral effects of these drugs could be exploited to improve the prognosis of brain tumour patients. On these bases, glucocorticoids constitute excellent candidates to design novel metabolic combination therapies for the treatment of glioma.

More physiological cell culture media to obtain results more relevant to human tumour 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, a standard culture medium known as DMEM is distant from the nutrient levels found in normal human blood and it profoundly skews the metabolism of cancer cells in culture (Vande Voorde J *et al.* Sci Adv. 2019, Ackermann T, *et al.* Trends Cancer. 2019). For example, glucose in DMEM is at five-fold the normal glycaemia. A similar ratio applies to glutamine, the most abundant amino acid in circulation. Conversely, non-essential proteinogenic amino acids normally circulating in blood are missing from DMEM.

On this basis, we developed Plasmax™ (Figure 2), a cell culture medium with nutrients and metabolites at the concentration normally found in human blood. The newly formulated medium allows the culture of mammalian cells with reduced supplementation of foetal bovine serum (Figure 3). We are currently testing Plasmax™ in a variety of cell culture systems, including murine normal, stem and cancer cells, as well as in established primary human cells derived from different tissues.

In 2020, Plasmax™ became the first physiological medium to be commercially available (Ximbio.com). We are confident that the availability of a physiologically relevant cell culture medium will further reduce the inconsistencies between *in vitro* and *in vivo* results, thus favouring more translational biomedical research.

Publications listed on page 111

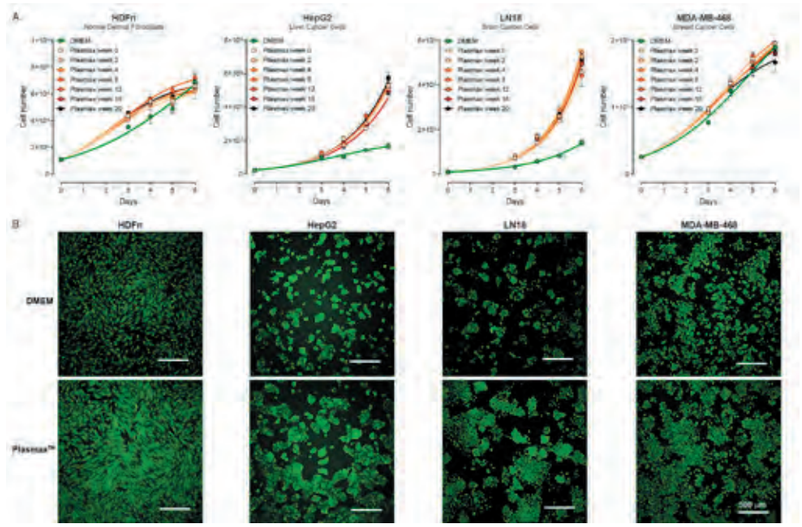

Figure 3
Plasmax™ sustains proliferations of normal and cancer cells comparably or better than DMEM even when stored at 4°C for up to 20 weeks.
(A) Normal Human Dermal Fibroblasts (HDFn), HepG2, LN18 and MD-MB-468 cells were seeded in DMEM in 24 wells plates, at 1x104, 2x104, 1x104, 2x104 cells/well respectively. The day after seeding (day 0) medium was replaced with 2ml/well of DMEM or Plasmax™, both supplemented with 2.5% dialysed FBS. Different Plasmax™ media were used for the experiment: either prepared on day0 from frozen stock components (Plasmax week 0) or left at 4°C for up to 20 weeks (Plasmax week 2–20). To prevent nutrient exhaustion, all media were replaced at days 4 and 5. Cells were trypsinysed and counted with a CASY cell counter. Values are mean ± SD obtained from 4 wells. Cell numbers were interpolated with a logistic growth curve (Graph Pad Prism 8.3). (B) Representative images of cells cultured in DMEM or PlasmaxTM at day 4 of the experiment described above. Cells were stained with Calcein AM 10 μM) for 20 minutes, and images aquired with a Zeiss Axiovert 25 microscope, objective magnification 2.5x.