Weekend Educational Course

Preclinical Cancer Imaging

The basics

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Highlights

- Solid tumours are heterogeneous masses composed of variable proportions of proliferating and non-proliferating cells.
- The metabolism of glucose, glutamine and lipids is different between proliferating and non proliferating (tumour) cells.
- In proliferating tumour cells glucose consumption mostly provides carbon (C) and reducing potential (NADPH) for anabolic purposes, glycolysis may be truncated at the pyruvate kinase (PK, PKM2 isoform inhibited) level, glutaminolysis strongly contributes to ATP production at the TCA cycle level and to continuing glycolysis by providing oxidized NAD^+. Lipid biosynthesis requires both glycolytic 3C precursors (DHAP) and fatty acids derived from glutamine carbon.
- In non proliferating tumour cells glucose consumption provides ATP and carbon for TCA cycle use, glycolysis is not truncated at PK level (active PKM1 isoform) and glutaminolysis is down. Lipid biosynthesis is reduced although plasma membrane lipid turnover may be maintained.
- Poorly perfused/hypoxic tumour episodes further impact in tumour metabolism. Tumour cells switch from a proliferative to a non-proliferative phenotype upon hypoxia, adapting their metabolic pathways accordingly.

Tumour Metabolism: Glucose, Glutamine & Lipids

Target audience: Basic scientist and translational cancer researcher not familiar with the changes in the interpretation of tumour metabolism differential characteristics appearing in the past decade.

Objectives: To emphasize the need for considering the heterogeneity in the proliferation rate of tumour regions when interpreting changes in metabolism or biomarkers related to those changes.

Purpose: There is a large amount of literature dealing with tumour metabolism, mostly in established cell lines but also from intact tumours. Sometimes results obtained may seem in conflict for studies on cells or tumours, for example, for their glutamine consumption. Results from the last decade seem to reconcile some of those apparent discrepancies.

Summary of some of the reviewed literature data to be considered during the presentation:

It is well accepted that biopsies from solid tumours display a large range of average percentage of proliferating cells (<5% - 90%) [1 and references cited therein]. A single tumour also shows regional heterogeneity with respect to proliferation [2 and references cited therein, 3-4]. This in turn is caused, among other factors, by the goodness of their regional perfusion, leading to locally diverse supply of oxygen and other substances from blood. Tumours adapt to this trying to improve perfusion through neoangiogenesis and also modulate their metabolism while this is being achieved.

Cancer cells display the Warburg effect, namely, fast glucose consumption by glycolysis, with concomitant lactic acid production, even under normal oxygen saturation. This is not restricted to cancer cells but is also observed when normal cells proliferate actively [5-6]. The most accepted cause of the Warburg effect at present is the need by proliferating cells of precursors for biosynthetic pathways that only glucose can produce (i.e. ribose for nucleotide synthesis), as compared to other sources of carbon and energy, like fatty acids [7, 5]. Furthermore, proliferating cells, including proliferating tumour cells, preserve for anabolic use the carbon pool originating from glucose by limiting the possible consumption of pyruvate by the mitochondria. This is performed by expressing the M2 isoform of pyruvate kinase (PKM2) which can be inhibited under varying conditions of growth (metabolic modulators, growth factors) in proliferating cells [8-10]. For example, lack of serine, a positive modulator of PKM2 [9] reduces flow of carbon to pyruvate and the TCA cycle, sparing carbon for serine biosynthesis from 3-phosphoglycerate in the glycolytic pathway. This could in turn compromise bulk glycolytic flow in case NAD^+ becomes limiting, because of reduced pyruvate availability for lactate DH (LDH) activity. Proliferating cells apparently have two possible solutions to this problem. The most recently proposed one would be the alternative glycolytic pathway described by Vander Heiden and collaborators in proliferating cells [11] in which Phophoenolpyruvate bypasses PKM2 inhibition by reacting with phosphoglycerate mutase 1 (PGAM1), another glycolytic enzyme, releasing pyruvate, which is then fully accessible for LDH activity and NAD^+ regeneration. Still, this may have more use as a pseudo futile cycle for reducing excess ATP produced by glycolysis [12] than to spare carbon for anaplerosis. Another more widely evaluated option for NAD^+ regeneration is the glutaminolysis, that is, carbon contribution from blood originated glutamine to a truncated version of the TCA cycle with entry point at alfa-ketoglutarate level and exit points at either malate or citrate [7]. In both exit conditions, lactate and reducing power production at the cytosol may take place. Additionally, glutamine carbon will contribute carbon to fatty acid synthesis in the form of cytosolic Acetyl CoA.

The contribution of glutamine to in vivo tumour metabolism has been challenged by some on the grounds that most studies showing this Gln role were carried out in cell culture. Still, recent work on the heterogeneity of PKM2 expression in an intact orthologous breast cancer tumour model [10] could shed some light into the apparent controversy in relation to glutamine contribution to intact tumour anaplerosis. Authors in [10] nicely demonstrated that PKM2 null (KO) tumours accelerated tumour progression in a spontaneous model of Brca1-loss-driven model of breast cancer in mice. Moreover, PKM2 null tumours displayed heterogeneous PKM1, the classical wt PK isoform, expression. Thus, PKM1 was only found in

non-proliferating tumour cells, while no detectable pyruvate kinase was detected in proliferating cells. We would then expect this to be a good model to investigate glutamine contribution to the glycolytic pathway functionality, due to the absence of PK activity in part of the tumour cell population. In this respect, glutamine consumption for glutaminolysis has been evaluated in theoretical simulations, which predict that "the lactate excretion switch in proliferating cells coincides with activation of glutaminolysis" [6]. Accordingly, whenever glutamine consumption and lactate production derived from glutamine is investigated, we should consider the percentage of proliferating cells in the investigated tumour. Then, Israelsen and collaborators [10] using uniformly ^{13}C labelled glucose concluded that the percentage labelling of lactate in allograft mammary tumours subcutaneously growing in nude mice was between 70-90%, depending of cell line and PKM2 status investigated. Interestingly enough, the percentage of PCNA positive (proliferating) cells in those tumours was reported to be by the same authors between 21-26% This would be in agreement with the lactate label dilution (10-30%) originating in the proliferating cells (21-26%) lacking PK activity, being contributed by sources other than glucose and classical glycolysis, potentially from glutamine (pyruvate from the PGAM1 shunt described in [11] would also be labelled from ^{13}C enriched glucose and could not contribute to the measured label dilution). In short, whenever a heterogeneous tumour composed of proliferating and non-proliferating cells is considered for glucose and glutamine consumption, we would expect the canonical Warburg effect only at the proliferating fraction of cells, while non-proliferating cells would be expected to display a standard glycolytic or even oxidative metabolism depending of their perfusion status.

Lipids are also required for tumour cell duplication. Fatty acids in phospholipids are incorporated from endogenous origin (anabolic pathways), the major source [13], but also from exogenous sources (circulating lipoproteins and albumin bound fatty acids), especially in proliferating tumour cells in culture [14]. The uptake of other essential components like choline or ethanolamine is also increased in proliferating tumour cells compared to growth arrested cells [15]. This is facilitated by reprogramming of the uptake and retention mechanisms of those precursors in proliferating cells [16]. Additionally, membrane turnover in proliferating cells may require changes in the circulation and the dynamics of intracellular lipid bodies (lipid droplets) with respect to growth arrested cells [17 and references cited therein,18-19].

Conclusion: Proliferating cells in tumours avidly consume glucose and glutamine as carbon sources for tissue mass duplication, producing lactic acid, which is exported (canonical Warburg effect). Energy consumption in this situation is mostly for maintenance and can be fed by basal aerobic metabolism in well perfused regions of tumours. Non-proliferating cells in tumours will display reduced glucose consumption and produce varying amounts of lactate depending of their level of perfusion (glycolysis upon hypoxia, OXPHOS at normoxia). Evaluating tumour biomarkers without considering the heterogeneity in the percentage and zonation of the proliferating population of a tumour could lead to erroneous interpretations of the obtained results.

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