TUMOUR METABOLISM

Feeding the TCA cycle *in vivo*

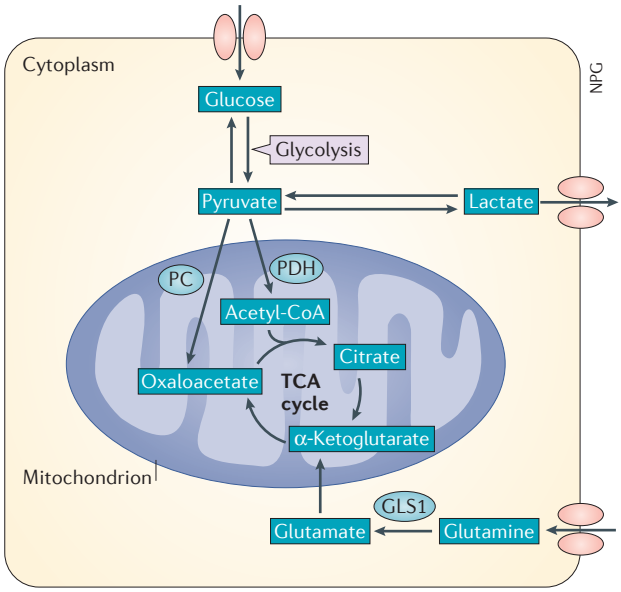
Two papers assess the role of glucose in lung cancer metabolism *in vivo*.

Because conflicting metabolomics data are often derived from cell culture experiments, Davidson *et al.* used mouse models of $Kras^{G12D}$-driven lung tumorigenesis to examine the role of glucose metabolism *in vivo*. The LA2 model ($Kras^{LA2/+}$) develops mostly low-grade lung adenomas, $Kras^{LSL\text{-}G12D/+}Trp53^{loxP/loxP}$ (KP) mice develop higher-grade metastatic lung tumours and KPS mice develop even more aggressive lung tumours as they are formed from transplantation and outgrowth of KP tumour cells. Mice were infused with [U-^{13}C]glucose and metabolites from lung tumours and normal lung tissue were analysed by gas chromatography–mass spectrometry (GC–MS). The levels of ^{13}C-labelled lactate and pyruvate were similar in the tumours and normal lung tissue from all models, although lactate pools were higher in the KP and KPS tumours, suggesting that these more aggressive tumours may be more glycolytic.

Increased ^{13}C-labelling of citrate in LA2 and KPS tumours (but not in KP tumours) indicated that glucose-derived carbon entered the tricarboxylic acid (TCA) cycle via pyruvate dehydrogenase (PDH) activity (see figure). [1-^{13}C]pyruvate infusion of KP mice also revealed increased activity of pyruvate carboxylase (PC) in tumours compared with lung tissue. Therefore, in lung cancers *in vivo* glucose is metabolized to produce lactate and it contributes carbon to the TCA cycle through PDH and PC. Next, the contribution of glutamine to TCA cycle carbon was investigated using [U-^{13}C]glutamine infusion. TCA cycle intermediates were not substantially labelled with ^{13}C in any model. However, [U-^{13}C]glutamine substantially labelled TCA cycle intermediates when KP lung cancer cells were cultured in 2D or 3D. Moreover, these cell lines depended on glutamine for survival, as determined by treatment with the glutaminase (GLS1) inhibitor CB-839 and by deletion of *Gls1*. Conversely, neither treatment of KP mice with CB-839 nor *Gls1* deletion affected tumour growth *in vivo*.

Interestingly, deletion of *Pc* or *Pdha* (which encodes a subunit of PDH) in KP cells had no effect in cultured cells, but prevented growth when these cells were transplanted into the flanks or lungs of syngeneic mice. Therefore, *in vivo*, $Kras^{G12D}$-driven lung tumours depend on glucose-derived carbon, whereas *in vitro* $Kras^{G12D}$-driven lung tumour cells depend on glutamine-derived carbon. The authors speculate that the differences between *in vivo* and *in vitro* glucose and glutamine metabolism may result from the environment, although it is unclear how.

Also assessing metabolism *in vivo*, Hensley *et al.* carried out metabolic profiling of nine patients with different grades of non-small-cell lung cancer of diverse histology, grade and genetics. Tumours that were due for surgical excision were assessed by FDG-PET as well as multiparametric MRI (mpMRI) to elucidate anatomy, diffusion and perfusion. On the day of surgery, patients received [U-^{13}C]glucose infusion and tissue sections taken during surgery were analysed by GC–MS and NMR. They found that all normal lung and lung tumour tissue exhibited ^{13}C-labelling of glycolysis and TCA cycle intermediates, although heterogeneity in the extent of the labelling was detected between patients and, to a lesser degree, within the same tumour. Lactate ^{13}C-labelling was substantially increased in the tumours, indicating that lung tumours convert glucose to lactate more than normal lung tissue. NMR showed increased ^{13}C-labelling of TCA cycle intermediates derived from PDH activity and increased levels of acetyl-CoA in the lung tumours. PC activity and levels of ^{13}C-labelled oxaloacetate were also increased in the lung tumours, but PDH activity was dominant. Moreover, all of the tumours consumed other nutrients in addition to glucose, with some tumours scavenging lactate from the circulation and using it as a carbon source.



> tissue context and tumour microenvironment regulate the choice of metabolic pathways in tumour cells

Finally, they found that regions of tumour or individual tumours that were poorly perfused exhibited the highest levels of flux through PDH; there was no difference in flux through PC according to perfusion. Therefore, metabolic heterogeneity within a tumour and between tumours may be determined by factors in the tumour microenvironment, potentially including the availability of nutrients.

Together these papers provide pause for thought about how metabolomics experiments are conducted, as well as how the tissue context and tumour microenvironment regulate the choice of metabolic pathways in tumour cells.

Gemma K. Alderton

ORIGINAL ARTICLES Davidson, S. M. *et al.* Environment impacts the metabolic dependencies of Ras-driven non-small cell lung cancer. *Cell Metabolism* **23**, 517–528 (2016) | Hensley, C. T. *et al.* Metabolic heterogeneity in human lung tumors. *Cell* **164**, 681–694 (2016)