TUMOUR CELL DEATH

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The aim of our group is to understand the factors regulating cell viability in cancer. Since it is known that inhibition of cell death mechanisms is a common event in tumour development, this poses problems for many forms of chemotherapy that utilise cell death pathways, leading to drug resistance. We are investigating both known cell death regulators as well as searching for novel proteins and pathways that control cell viability, tumour growth and chemosensitivity. We envisage that the knowledge gained from our studies will be translated and lead to the improvement of existing clinical regimens or new targets for therapeutic intervention.

Mannose affects glucose metabolism and cancer cell growth

A common feature of many cancers is that they exhibit enhanced uptake and a greater dependency on glucose. So marked is this characteristic that the uptake and retention of glucose by tumours is widely used for diagnosis, and particularly for the detection of disseminated disease. As a result, we became interested in how tumour cells may respond to other forms of sugar and found that the monosaccharide mannose can profoundly affect the growth of some, but not all, cancer cell types (Gonzalez *et al.*, Nature 2018; 563: 719–23).

Mannose is very closely related to glucose in terms of molecular structure and it is taken into cells via the same transporters. We therefore reasoned that mannose may be affecting the growth of cancer cells by interfering with glucose uptake. However, in a comparison of glucose uptake in mannose-sensitive versus insensitive cells, there was no correlation with mannose sensitivity. We also considered that the sensitivity to mannose may be related to relative mannose uptake, but again there was no correlation between the uptake and sensitivity to the sugar in different cell lines. In light of these results and previous studies which showed that mannose can inhibit three key enzymes involved in glucose metabolism (Figure 1), we considered instead that mannose may be interfering with the intracellular metabolism of glucose. To test this, we first examined the impact of mannose on glycolysis, which revealed that mannose markedly reduces the amount of glucose converted to lactate in mannose-sensitive cells. Further analysis showed that mannose also impacted other pathways downstream of glucose, including those involved in anabolism such as the pentose phosphate pathway, global transcription and translation and glycan synthesis.

Mannose affects chemotherapeutic responses

Previous studies have shown that glucose deprivation can enhance cell death responses and so we were interested to know if mannose had a similar effect. To test this, cells were treated with cisplatin and doxorubicin – two widely used chemotherapeutic drugs – and cell death was analysed in either the absence or presence of mannose. This revealed that in cells in which mannose impairs cell growth, the levels of cleaved poly-ADP ribose polymerase and cleaved caspase 3 were higher when treated with chemotherapeutic drug and mannose when compared to either mannose or drug alone. This indicated that mannose may be enhancing cell death by caspase-dependent apoptosis and we subsequently found that the entire cell death response could be blocked by treating with the pan-caspase inhibitor zVAD-fmk.

Cell death by apoptosis can proceed by two main pathways – the extrinsic pathway, where initiating signals originate from receptors on the surface of the cell, and the intrinsic pathway, which is controlled by changes in the outer mitochondrial membrane and does not involve cell surface receptors. The extrinsic pathway depends on factors such as caspase-8 and FADD (Fas-associated death domain) and deletion of these factors using CRSPR/Cas9 had no impact on the cell death induced by chemotherapy plus mannose. In contrast, CRISPR/Cas9-mediated deletion of Bax and Bak – key factors in the intrinsic pathway – completely blocked the cell death response.

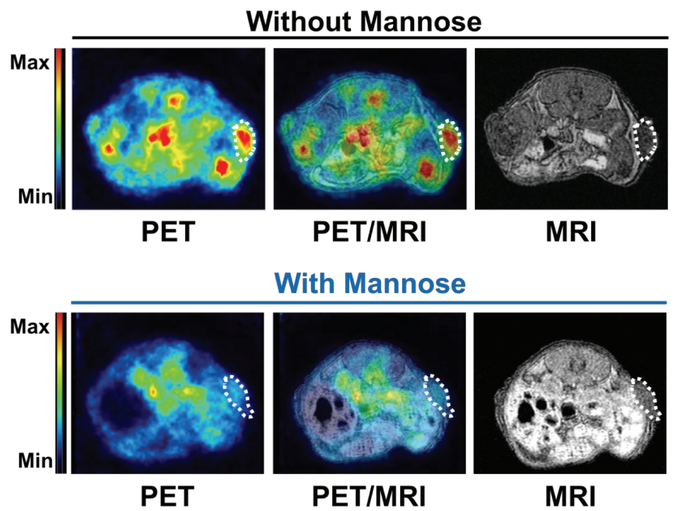
Figure 1
Mannose impairs glucose metabolism
Mannose is taken into cells by the same transporters as glucose and impairs three key enzymes involved in glucose metabolism. Glu, glucose; Man, mannose; HK, hexokinase; Glu-6P, glucose-6-phosphate; Man-6P, mannose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; PGI, phosphoglucose isomerase; Pyr, pyruvate; Lac, lactate.

The intrinsic cell death pathway and permeabilisation of the outer mitochondrial membrane is regulated by members of the Bcl-2 family of proteins. Profiling of these proteins during cell death induced by chemotherapy and mannose showed that Bcl-x_L and Mcl-1 (two anti-apoptotic members) were down-regulated and that Noxa (a pro-apoptotic member) was up-regulated. We were able to show that these changes were causally associated with cell death responses as over-expression of Bcl-x_L or Mcl-1, or CRISPR/Cas9-mediated deletion of Noxa, all impaired the extent of cell death observed.

Mannose affects tumour growth

Inspired by our results *in vitro*, we were keen to understand if mannose could affect tumour growth *in vivo*. Previous studies had shown that mice tolerate the administration of mannose over a period of time, and we also found this to be the case. Tumours were therefore generated in several mouse models and we found that mannose could reduce glucose uptake and retention in these models (Figure 2) and impair tumour growth, without any weight loss or obvious signs of ill health in the mice. Importantly, our *in vitro* studies had shown that sensitivity to mannose was dependent on the levels of phospho-mannose isomerase (PMI) – low levels of PMI indicate sensitivity, and high levels resistance. Building on these findings, we were also able to show *in vivo* that mice bearing tumours with high levels of PMI could be made sensitive to sugar upon PMI knockdown.

Figure 2
Mannose reduces glucose uptake and retention in tumours in mice
Mice were given either a solution of mannose or drinking water as control and injected with ^{18}F-deoxyglucose (FDG). The uptake and retention of FDG by tumours was assessed by positron emission tomography (PET) and magnetic resonance imaging (MRI). Image is abridged from Gonzalez *et al.*, Nature 2018; 563: 719–23



Colorectal cancers are sensitive to mannose

As sensitivity to mannose was connected to the levels of PMI, we examined human tissue microarrays containing samples from breast, ovarian, prostate, kidney and colorectal cancers. Similar to our cell lines, this showed that the levels of PMI vary not only within each tumour type, but also between different tumour types, indicating that the levels of PMI could potentially be used to stratify patients with regard to the likelihood of their tumours responding to the sugar. Furthermore, we found colorectal cancers, when compared to other cancers, often have low levels of PMI, indicating that these tumours may be generally sensitive to mannose treatment. To test this experimentally, we examined two different mouse models of colorectal cancer and in both cases, the administration of mannose in drinking water had a significant impact on tumour formation. Once again, this did not have any negative effects on the weight or overall health of the animals. When taken together, these results show that mannose may be a simple and effective way to improve therapeutic responses in multiple tumour types, and we are currently pressing forward to test this possibility in clinical trials.

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