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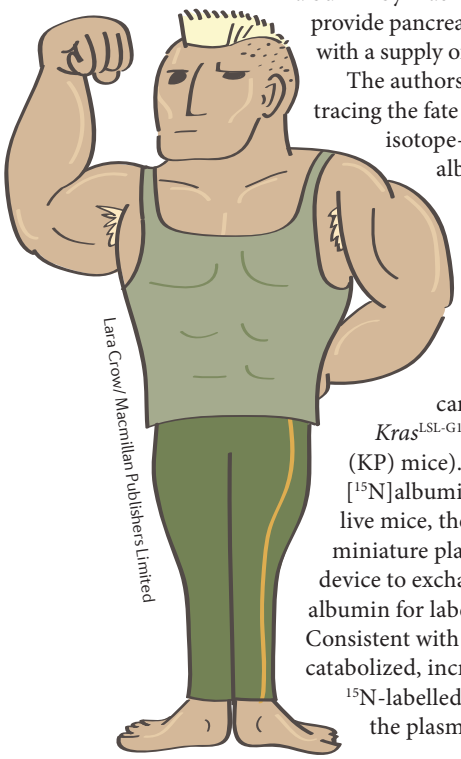
TUMOUR METABOLISM

Packed full of protein!

> tumour cells within MIA PaCa-2 xenografts can take up and degrade labelled albumin by macropinocytosis



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Altered metabolism promotes the uncontrolled growth of tumours. An important mechanism by which pancreatic cancer cells might achieve this is through the uptake and breakdown of extracellular protein, via macropinocytosis, as a source of amino acids for metabolism. Yet, the degree to which protein catabolism takes place in the tumour itself *in vivo* and whether macropinocytosis is required to sustain amino acid levels within pancreatic tumours was unknown. To address these questions, Davidson *et al.* used two different approaches to directly show *in situ* in live mice that catabolism of albumin by macropinocytosis can provide pancreatic cancer cells with a supply of amino acids.

The authors began by tracing the fate of nitrogen isotope-labelled albumin ([^{15}N]albumin) in a mouse model of spontaneous *Kras*^{G12D}-driven pancreatic ductal adenocarcinoma (PDAC; *Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{loxP/loxP}$ (KP) mice). To deliver [^{15}N]albumin to tissues in live mice, they generated a miniature plasmapheresis device to exchange endogenous albumin for labelled albumin. Consistent with albumin being catabolized, increased levels of ^{15}N-labelled amino acids in the plasma of KP mice as compared with wild-type (WT) control mice were detected following plasma exchange. Moreover, higher levels of both ^{15}N-labelled albumin peptides and ^{15}N-labelled free amino acids were observed in tumour tissues than in normal pancreas tissue, indicating that the catabolism of labelled albumin produces amino acids that are specifically abundant within tumours. Perhaps unexpectedly, the authors also found increased amounts of ^{15}N-labelled free amino acids in the livers and lungs of KP mice as compared with WT mice, but with equivalent levels of labelled albumin peptides, suggesting that free labelled amino acids generated by distal non-tumour tissues could also be taken up by pancreatic tumour tissues and contribute to the free amino acid supply in tumours.

To establish whether the protein catabolism of pancreatic tumours is cancer cell-autonomous, Davidson *et al.* used another microdevice — a modified small cylinder with reservoirs for controlled small and large molecule release into solid tissues — which they implanted into xenografts derived from human PDAC cell lines (*KRAS*^{G12C}-expressing MIA PaCa-2 and WT *KRAS*-expressing BxPC-3 cells) and monitored by intravital microscopy. Using this system to simultaneously deliver fluorescently labelled high molecular mass dextran (a marker of macropinocytosis) and albumin (which becomes fluorescent upon proteolytic cleavage in lysosomes) intratumourally, the authors found that tumour cells within MIA PaCa-2 xenografts can take up and degrade labelled albumin by macropinocytosis, in contrast to the mimimal albumin degradation observed in BxPC-3 cells. Implantation of the same device into KP tumours confirmed the finding in MIA PaCa-2 xenografts and was also used to show that fluorescently labelled fibronectin, a constituent of extracellular matrix, is taken up by pancreatic tumours but not by normal pancreatic tissue.

To look at the therapeutic effect of inhibiting macropinocytosis *in situ*, the authors used the implantable microdevice to release hydroxychloroquine or 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA), inhibitors of lysosomes and macropinosomes, respectively, in tumours of KP mice. Hydroxychloroquine exposure led to reduced degradation of fluorescently labelled albumin compared with vehicle delivery alone while tumour tissue regions exposed to EIPA were depleted of amino acids — an effect mediated by the inhibitory action of EIPA on macropinocytosis and not through the decreased uptake or exchange of free amino acids.

Importantly, this study demonstrates *in vivo* that pancreatic tumour cells with oncogenic KRAS can consume protein in their surrounding environment and highlights the potential applications of implantable microdevices to advance preclinical research.

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ORIGINAL ARTICLE Davidson, S. M. *et al.* Direct evidence for cancer-cell-autonomous extracellular protein catabolism in pancreatic tumors. *Nat. Med.* http://dx.doi.org/10.1038/nm.4256 (2016)