Nutrient scavenging in cancer

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Abstract | While cancer cell proliferation depends on access to extracellular nutrients, inadequate tumour perfusion means that glucose, amino acids and lipids are often in short supply. To overcome this obstacle to growth, cancer cells utilize multiple scavenging strategies, obtaining macromolecules from the microenvironment and breaking them down in the lysosome to produce substrates for ATP generation and anabolism. Recent studies have revealed four scavenging pathways that support cancer cell proliferation in low-nutrient environments: scavenging of extracellular matrix proteins via integrins, receptor-mediated albumin uptake and catabolism, macropinocytic consumption of multiple components of the tumour microenvironment and the engulfment and degradation of entire live cells via entosis. New evidence suggests that blocking these pathways alone or in combination could provide substantial benefits to patients with incurable solid tumours. Both US Food and Drug Administration (FDA)-approved drugs and several agents in preclinical or clinical development shut down individual or multiple scavenging pathways. These therapies may increase the extent and durability of tumour growth inhibition and/or prevent the development of resistance when used in combination with existing treatments. This Review summarizes the evidence suggesting that scavenging pathways drive tumour growth, highlights recent advances that define the oncogenic signal transduction pathways that regulate scavenging and considers the benefits and detriments of therapeutic strategies targeting scavenging that are currently under development.

Desmoplasia
A process by which dense stromal cells extensively deposit extracellular matrix proteins, increasing interstitial pressure and decreasing vascular perfusion.

Anabolism
The biosynthetic processes that assembles nutrients into macromolecules that contribute to cellular biomass.

Nutrient scavenging
The removal and breakdown of macromolecules from the microenvironment into components that can be used for ATP production and/or anabolism.

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https://doi.org/10.1038/s41568-018-0048-x

Cancer cells must take up glucose, amino acids and lipids at an accelerated rate to support their non-homeostatic proliferation[1]. Cell surface transporters for amino acids, glucose, monocarboxylates, fatty acids and lipoproteins are upregulated across cancer types; elevated expression of various nutrient transporters correlates with aggressive, late-stage disease and a poor prognosis. The nutrients that these proteins transport are delivered by the vasculature. However, tumour blood vessels are dilated, leaky and tortuous[2]. Abnormal vasculature combined with the high intratumoural interstitial fluid pressure produced by desmoplasia can severely compromise nutrient delivery to tumour cells[3]. Amino acids and glucose are depleted in pancreatic, breast, colon and prostate tumours relative to normal tissue, particularly in poorly vascularized areas[4–7]. Increased nutrient demand and uptake by tumour cells may further reduce extracellular nutrient levels. While increasing nutrient transporter expression can help cancer cells harvest scarce blood-borne nutrients, it is now appreciated that cancer cells also bypass the bloodstream and acquire nutrients by scavenging macromolecules from the tumour microenvironment (TME).

By definition, scavengers search for and collect items that can be repurposed into useful material. In the context of tumour metabolism, we define scavenging as removing macromolecules synthesized by other cells from the TME and breaking them down into components that can be used for ATP production and/or anabolism. This definition of nutrient scavenging excludes autophagy, a process by which cells recycle their own components after sequestering them in double-membrane-bound vesicles that fuse with lysosomes. Autophagy is akin to a starving animal catabolizing muscle and fat to redirect autologous carbon and nitrogen to tissues that are essential for organismal viability. A hungry animal deprived of its normal food source might also turn to scavenging, consuming less desirable items in the environment that were discarded or produced by other organisms. Discriminating between processes that catabolize intrinsic and extrinsic macromolecules is not a trivial, semantic distinction; the origin of the repurposed material has important ramifications for cancer cell biology. When a cell reallocates its own resources through recycling, there is no opportunity for growth; autophagy is a zero-sum game. While blocking autophagy often reduces both tumour volume and the number of viable, proliferating cells[8–10], it is impossible for autophagy to fuel cell-autonomous growth. By the law of conservation of mass, autophagy cannot produce growth; autophagic cells atrophy[11]. Autophagy is necessary for maximal growth of the macroscopic tumour

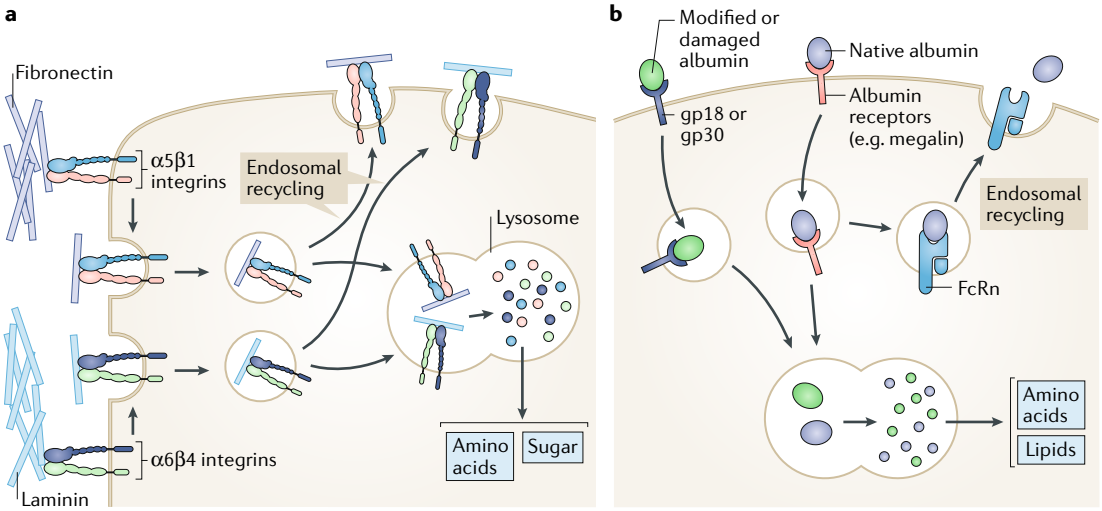

Fig. 1 | **Receptor-mediated scavenging of albumin and extracellular matrix proteins.** Cells utilize receptor-mediated scavenging to fuel growth and proliferation. Receptor-mediated scavenging includes integrin-mediated endocytosis of extracellular matrix (ECM) components or receptor-mediated endocytosis of albumin. **a** | Fibrillar α5β1 integrin adhesions are endocytosed and either recycled back to the cell surface, where they are exocytosed or degraded in the lysosome along with bound fibronectin[31]. Laminin-bound α6β4 integrins are similarly endocytosed and degraded in response to calorie restriction or serum and growth factor deprivation[25]. ECM scavenging yields amino acids and possibly sugars that sustain proliferation under nutrient stress. **b** | Native albumin is endocytosed following binding to megalin or other cell surface scavenger receptors[37,44]. Endocytosed albumin is either recycled by exocytosis if it binds to neonatal Fc receptor (FcRn) in endosomes[40] or sent to the lysosome for degradation[35,37]. Tumour cells generally express low levels of FcRn, and forced overexpression of FcRn limits tumour growth by undermining cancer cell scavenging[41,42]. Modified or damaged albumin (for example, ischaemia-modified albumin) is internally bound to the scavenger receptors albumin-binding protein gp18 and albumin-binding protein gp30 (REFS[46–48]). Degradation of albumin yields amino acids and possibly bound fatty acids and/or lipids.

because it promotes cell survival; these viable cells can proliferate only if they are able to acquire extrinsic nutrients or macromolecules from the TME. In contrast to autophagy, scavenging can both maintain survival and promote growth because the nutrient source is cell-extrinsic. The critical role autophagy plays in tumour biology is indisputable[12–17]. While scavenging has the potential to have an even greater impact on tumour growth, it has received substantially less attention. Here, we highlight exciting, recent studies defining the scavenging strategies employed by cancer cells, the molecular signalling pathways that control these processes and the potential therapeutic value of targeting scavenging alone or as part of combination therapies.

Receptor-mediated protein scavenging

Integrin-mediated scavenging

The TME contains dense arrays of extracellular matrix (ECM) proteins produced by fibroblasts. In pancreatic ductal adenocarcinoma (PDAC) and breast cancer, abundant stromal cells overproduce ECM proteins, causing extensive fibrosis termed desmoplasia[3,18]. By increasing the interstitial pressure, desmoplasia reduces tumour perfusion, leading to hypoxia and nutrient limitation. In this environment, scavenging becomes essential to build biomass for proliferation, and the dense meshwork of collagen, laminin and fibronectin laid out by fibroblasts offers a protein-rich feast. Given that ECM proteins are heavily glycosylated[19–23], sugars may also be liberated by ECM scavenging. Cells interact with ECM proteins using integrins, heterodimeric cell surface receptors that link ECM components to the cytoskeleton[24]. As ligand engagement of integrins regulates cell migration, proliferation and survival, it is not surprising that integrin expression levels and activation states are altered in many cancers. Recent reports suggest a novel, protumorigenic role for integrins: fuelling tumour anabolism by mediating ECM scavenging (FIG. 1a).

Normal cells and cancer cells survive periods of nutrient limitation by scavenging. Restricting calorie intake in mice by 40% triggers laminin scavenging by mammary epithelial cells via α6β4 integrins; similar results were obtained in vitro in non-transformed MCF10A mammary epithelial cells deprived of serum and growth factors[25]. Although nutrients are abundant in serum-deficient and growth factor-deficient culture medium, loss of growth factor signalling triggers transcriptional and post-transcriptional nutrient transporter downregulation, thereby limiting nutrient access[26–28]. Indeed, uptake of glucose and glutamine was reduced in MCF10A cells when serum and growth factors were withdrawn[25]. However, supplementing the medium with laminin restored both intracellular essential amino acid pools and cell proliferation, suggesting that laminin scavenging provides fuel for anabolism. β4 integrin expression was necessary for laminin-driven proliferation under nutrient stress[25]. Although this study did not evaluate whether breast cancer cells scavenge ECM using integrins, β4 integrin is overexpressed in basal-like breast cancers and in breast

Recycling
The catabolism of a cell's own macromolecules into subunits that are used to fuel ATP production or to synthesize new polymers; autophagy is a recycling process.

Cell-autonomous growth
Cellular growth (both biosynthesis and proliferation) that does not depend on building blocks produced by other cells.

Collagen
The most abundant structural protein in the ECM.

Laminin
A high molecular weight heterotrimeric glycoprotein that forms the basement membrane that facilitates cell adhesion and tissue structural maintenance.

Fibronectin
A high-molecular-mass protein dimer that binds cell membrane integrin receptors and neighbouring extracellular matrix proteins like collagen to facilitate cell adhesion.

Box 1 | Scavenging and metastasis

The molecular pathways that promote scavenging also regulate cell motility and metastasis. If the rates of macropinocytosis and cell migration are inversely correlated, as some studies suggest[168], inhibiting macropinocytosis might be contraindicated if it tips the balance towards invasion and metastasis. The bulk of the evidence, however, favours a model in which blocking macropinocytosis and other forms of scavenging would limit tumour invasion and metastasis. Anoikis activates 5′-AMP activated protein kinase (AMPK) and downregulates nutrient transporter expression and nutrient uptake[169–171]. Scavenging pathways may allow the survival and proliferation of detached cells, facilitating the acquisition of a metastatic phenotype and fuelling the energy-dependent process of cell migration. Consistent with this idea, treatment with the lysosome inhibitors chloroquine or bafilomycin A1 sensitizes anoikis-resistant, invasive ovarian cancer cells to death[170]. A recent study points towards the carbohydrate-binding protein galectin 3 as a therapeutic target that could simultaneously block macropinocytosis and metastasis. Lung tumours with activating mutations in *KRAS* require galectin 3 to scavenge albumin by macropinocytosis when grown in 3D[70]. The galectin 3 inhibitor GCS-100 blocks macropinocytosis and dramatically reduces the growth of patient-derived non-small-cell lung cancer xenografts (TABLE 1). Considering that galectin 3 drives anchorage-independent growth, GCS-100 might limit metastasis. Invasion might also be facilitated by extracellular matrix (ECM) removal via scavenging. Indeed, ECM scavenging by integrins compromises the integrity of the basement membrane of the mammary epithelium[25]. In macropinocytic cells, massive plasma membrane internalization could lead to the loss of cell surface cadherins[85,172] or other proteins that mediate cell–cell adhesion, favouring an invasive phenotype. If this is the case, blocking macropinosome formation might restore adhesion and reduce invasive potential. Exosomes promote metastasis[173]. Given that macropinocytosis is the predominant pathway for exosome uptake[83,94], macropinocytic cells may become primed for metastasis through exosome ingestion. Clearly, the relationship between scavenging and tumour cell invasion and metastasis merits further investigation once more selective genetic and chemical tools become available.

cancer stem cells, and elevated β4 integrin expression correlates with reduced 5-year relapse-free survival in patients with triple-negative breast cancer[29,30]. Thus, while experimental evidence is currently lacking, it will be interesting to evaluate whether β4 integrin-mediated laminin scavenging protects breast cancer cells from nutrient deprivation. Other integrins have been shown to promote ECM scavenging by cancer cells. For example, α5β1 integrin-bound fibronectin is endocytosed and degraded in the lysosome of ovarian cancer cells[31]. Similar to what has been observed with laminin supplementation in mammary epithelial cells[25], fibronectin supplementation activates the amino acid-sensitive mTOR complex 1 (mTORC1) in ovarian cancer cells in a manner dependent on both integrins and lysosomal catabolism, suggesting that amino acids are released from the degraded ECM molecule[31]. ECM scavenging might also liberate sugars. Laminin is a heterotrimer with between 11 and 14 oligosaccharide chains on each subunit[19–21], while fibronectin dimers contain at least 5 chains per subunit[22,23]. Scavenged monosaccharides could be reused for glycoprotein synthesis, potentially sparing the glucose and glutamine normally consumed by glycosylation for use in other metabolic pathways[32]. Many other ECM proteins, including vitronectin and collagen, are internalized via receptor-mediated endocytosis and directed to the lysosome[33,34]. Whether receptor-mediated endocytosis of these other ECM proteins also supports anabolism has not yet been assessed. It will be critical to evaluate whether internalizing ECM proteins promotes intravasation and metastasis by compromising the integrity of the basement membrane (BOX 1).

Mechanistic details of ECM scavenging via integrins are currently limited. While α5β1 integrin-mediated scavenging requires tensins, ARF4 and the SCAR–WAVE complex for fibronectin internalization[31], it remains unclear whether these proteins also regulate laminin, vitronectin and/or collagen scavenging by integrins. It will be necessary to identify the proteins and the molecular signals that control whether ECM-bound integrins are internalized, recycled or degraded in the lysosome in order to define the functional importance of integrin-mediated scavenging in tumours and to develop therapeutics targeting this pathway if such treatments are warranted.

Receptor-mediated scavenging of albumin

Albumin, produced primarily by the liver, is the most abundant plasma protein[35]. Many nutrients and signalling molecules that are poorly soluble in aqueous solutions are transported in the blood bound to albumin. Receptor-mediated transcytosis across endothelia delivers albumin to tissues and prevents its loss in urine. Leaky tumour vasculature and insufficient lymphatic drainage, often referred to as the enhanced permeability and retention effect, result in the accumulation of macromolecules above 40 kDa in the tumour interstitium[36]. At approximately 66 kDa, albumin is trapped in the TME, where it is accessible to scavengers[35]. Albumin has up to seven fatty acid-binding sites[37] and thus may provide cancer cells with fatty acids as well as amino acids when digested. Cancer cells can scavenge albumin by macropinocytosis[6,38,39], which we describe further below. However, many cell surface receptors for albumin, such as megalin (also known as LRP2) and cubilin, have been identified (reviewed in REFS[35,37]). Recent evidence suggests that receptor-mediated albumin scavenging also contributes to cancer cell anabolism (FIG. 1b).

Consistent with the idea that the main function of albumin is as a carrier molecule, and not a fuel source, albumin is generally recycled after its receptor-mediated endocytosis[40]. The intracellular neonatal Fc receptor (FcRn) inhibits lysosomal degradation of albumin by binding with high affinity to albumin in acidic endosomes and promoting its recycling back to the extracellular space (FIG. 1b). FcRn thus undermines albumin scavenging. Indeed, FcRn expression is significantly reduced in non-small-cell lung cancer (NSCLC) relative to normal tissue, and low FcRn expression is strongly correlated with a poor prognosis[41]. Many breast and prostate cancer cell lines also have low or undetectable levels of FcRn[42], a state that is favourable for receptor-mediated albumin scavenging. Multiple prostate cancer cell lines, including DU145, exhibit robust receptor-mediated, dynamin-dependent albumin uptake that is insensitive to the macropinocytosis inhibitor 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA)[43]. Strikingly, forced overexpression of FcRn in DU145 cells is sufficient to prevent albumin scavenging, reduce intracellular glutamate levels and slow xenograft growth[42]. Conversely, knockdown of endogenous FcRn in HCC1419 breast cancer cells increases intracellular glutamate levels and dramatically accelerates tumour growth[42]. Glutamic acid makes up 10% of the amino acid residues in albumin;

Integrins
Heterodimeric receptors that facilitate cell adhesion to extracellular matrix (ECM) and coordinate diverse signalling processes. Internalization of integrins allows scavenging of ECM components.

Anoikis
A form of programmed cell death induced by detachment of anchorage-dependent cells from the extracellular matrix; metastatic tumour cells escape death by anoikis and become anchorage-independent.

Albumin
The most abundant serum protein; albumin facilitates transport of solutes (fatty acids, vitamins, metal ions, and so on) throughout the body.

Macropinocytosis
A non-selective form of endocytosis by which cells assimilate both extracellular fluid and macromolecules by generating large, uncoated endocytic vesicles (macropinosomes) that range in diameter from 0.2 to 5.0 μm.

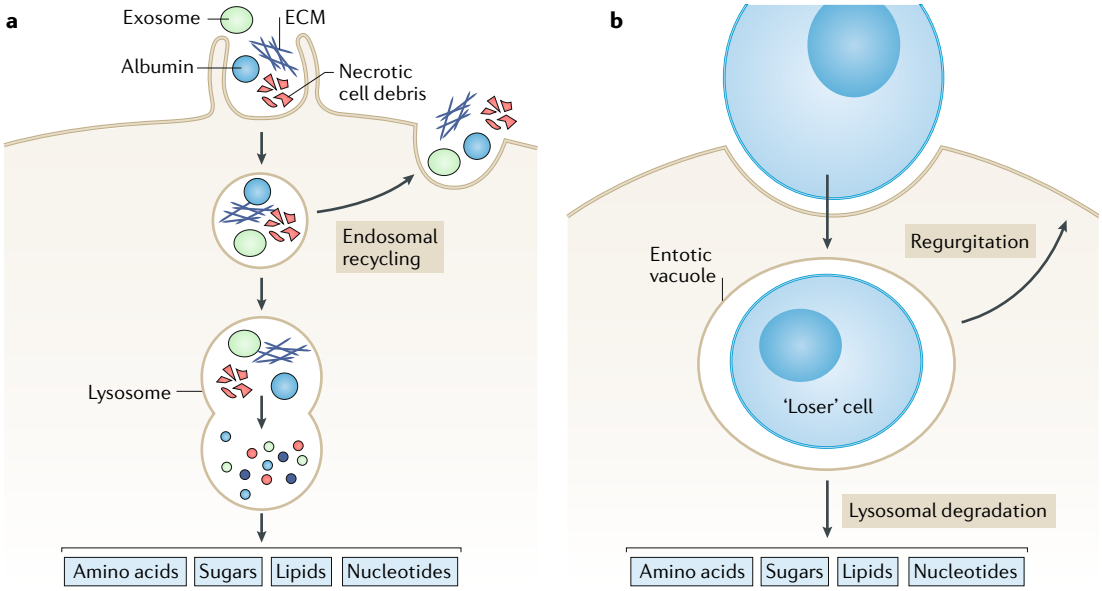

Fig. 2 | **Scavenging through macropinocytosis and entosis. a** | Macropinocytosis is a non-selective, bulk uptake process that provides access to both extracellular fluid and small particles, including albumin[6,39], extracellular matrix (ECM) proteins[38,73], necrotic cellular debris[43] and exosomes[83,94,95]. Macropinosomes can also be recycled back to the plasma membrane, where they fuse and release their contents back into the extracellular space, limiting nutrient generation. **b** | While too large to enter via macropinocytosis[43], intact live cells invade neighbouring cells through a process called entosis[96]. 'Loser' cells in entotic vacuoles can be released back into the tumour microenvironment upon fusion of the entotic vacuole with the plasma membrane or killed and degraded following fusion of the vacuole with lysosomes[96–99]. Entosed cells and macropinocytosed necrotic cell debris or exosomes have the potential to yield all the nutrients necessary for proliferation, including amino acids, sugars, lipids and nucleotides.

scavenged albumin should increase intracellular levels of glutamate and other amino acids. These studies suggest that FcRn functions as a tumour suppressor by limiting albumin scavenging. Other albumin receptors also regulate tumour progression but have not yet been directly linked to nutrient scavenging. For example, megalin, a cell surface protein that mediates endocytosis of albumin and other ligands[44], is overexpressed in melanomas and is necessary for melanoma cell proliferation and survival[45]. Together, these studies suggest that receptor-mediated albumin scavenging fuels the growth of multiple tumour types.

While endocytosed albumin is usually recycled, some modified forms of albumin are endocytosed and directed to the lysosome for degradation[35]. The cell surface glycoproteins gp18 and gp30 preferentially bind chemically modified albumin (for example, formaldehyde-treated or maleic anhydride-treated albumin) and are important for the clearance of damaged albumin[46,47] (FIG. 1b). These scavenger receptors are broadly expressed in both normal and transformed cell lines[46–48]. During hypoxia, increased reactive oxygen species (ROS) generation results in the modification of the amino terminus of albumin. This modified form, called ischaemia-modified albumin[49–51], is abundant in hypoxic areas of prostate and gastric tumours[52,53], providing a potential source of amino acids for poorly perfused tumour cells that express the receptors necessary to capture it. The major obstacle limiting our ability to assess the importance of receptor-mediated scavenging of modified and native albumin by tumours is the fact that albumin-scavenging receptors have not been well characterized; gp18 and gp30 are not cloned, and the ligands bound by this family of receptors are incompletely catalogued. Given that albumin-bound paclitaxel (also known as nab-paclitaxel) is US Food and Drug Administration (FDA)-approved and used for the treatment of metastatic breast, lung and pancreatic cancer, there is a doubly strong rationale for understanding how albumin enters and is processed in tumour cells.

Scavenging via macropinocytosis

RAS-transformed cancer cells overcome amino acid limitation by scavenging extracellular proteins via macropinocytosis[6,38,39]. Macropinocytosis is a non-selective form of endocytosis through which cells assimilate both extracellular fluid and macromolecules by generating large, uncoated endocytic vesicles (macropinosomes) that range in diameter from 0.2 to 5.0 μm (REF.[54]) (FIG. 2a). Macropinocytosis thus enables the uptake of a range of macromolecules (including but not limited to albumin, ECM proteins, exosomes and necrotic cell debris, as discussed further below). The building blocks that make up these macromolecules can be released after lysosomal degradation and used for biosynthesis or ATP generation. Macropinocytosis begins with the activation of small GTPases like RAC1 and cell division control protein 42 homologue (CDC42)[55–58] that produce actin-rich, sheet-like membrane protrusions or ruffles that form circular cups[59]. Closure of these cups into macropinosomes depends on both phosphatidylinositol (3,4,5)-triphosphate (PIP_3) production[57,60,61] and RAC1

inactivation[55]. Nascent macropinosomes can fuse with the plasma membrane, releasing their contents back into the extracellular space[62,63], or traffic to the lysosome[64] for degradation. The signals controlling this choice are incompletely defined but important to elucidate, as macropinosomes can fuel growth only if their cargo is degraded in the lysosome and the products are exported to the cytosol (FIG. 2). Macropinocytosis has been extensively studied in *Dictyostelium discoideum* and in mammalian macrophages, dendritic cells, growth factor-stimulated fibroblasts and A-431 carcinoma cells in a variety of different contexts, including viral entry, antigen processing and metabolism. The molecular details of macropinosome biogenesis and trafficking that have been gleaned from these studies are comprehensively covered in several excellent reviews[54,59,65,66]. While the signals and machinery regulating macropinocytic flux are likely to exhibit extensive overlap in non-transformed and transformed cells, some features may be cell type-dependent or context-dependent. Here, we focus on the literature defining a role for macropinocytosis in cancer cell anabolism.

RAS-driven cancers

RAS activation is sufficient to induce the signalling events necessary for macropinosome formation[67]. Cancers of the pancreas, urinary bladder, colon and lung with activating mutations in *HRAS*, *NRAS* or *KRAS* exhibit constitutive macropinocytosis[39,43,68–70]. Glutamine-addicted, *KRAS*-transformed PDAC cells continue to proliferate when glutamine or essential amino acids are limiting by catabolizing macropinocytosed proteins into amino acids, pyruvate, lactate and tricarboxylic acid (TCA) cycle intermediates[6,39,71]. Degradation of macropinocytosed albumin in mouse embryonic fibroblasts (MEFs) with the activating $Kras^{G12D}$ mutation maintains mTORC1 activity despite amino acid deprivation[72], suggesting that amino acids are released from the catabolized albumin protein. In another study, 1 week of macropinocytosis inhibition with the Na^+/H^+ exchanger (NHE) inhibitor EIPA was sufficient to inhibit macropinocytosis (as measured by intratumoural injection of 70 kD fluorescein isothiocyanate (FITC)–dextran) and to slow the growth of macropinocytic, $KRAS^{G12D}$ but not non-macropinocytic, *KRAS*-wild-type pancreatic tumour xenografts, suggesting that macropinocytosis promotes tumour growth in vivo[39] (TABLE 1). More recent studies using imaging mass spectrometry, which allows for identification, quantification and localization of small molecules and metabolites within tissue sections ex vivo, have confirmed that macropinocytosed extracellular protein maintains amino acid pools in both xenograft and autochthonous $KRAS^{G12D}$ pancreatic tumours[38]. Collagen scavenging also provides proline to support PDAC cell proliferation under nutrient stress[73]. However, collagen can be scavenged by both EIPA-sensitive and EIPA-insensitive mechanisms[73], suggesting that both macropinocytic and receptor-mediated collagen scavenging occurs in tumours.

KRAS activation may not be sufficient to predict macropinocytic capacity in all tumour types. Preliminary, in vitro findings show that $Kras^{G12D/+}Trp53$-null cancer cells derived from the lung degrade less albumin than isogenic cancer cell lines derived from the pancreas[69], raising the possibility that epigenetic changes characteristic of the tissue of origin also control scavenging in cells with identical genomes. This result is intriguing because it suggests that tissue context impacts flux through scavenging pathways, though this study has some important caveats (BOXES 2,3). For example, macropinocytosis in these lung and pancreatic cancer cells was measured ex vivo by monitoring by DQ-bovine serum albumin (BSA) fluorescence. In addition to the limitations of using DQ-BSA to measure flux (BOX 2), in vitro conditions may not reflect how these cells behave within tissues. Additionally, an inherent limitation of genetically engineered mouse models is that they fail to capture the genetic diversity of real human tumours. The state of stress-sensitive signalling pathways that modulate macropinocytic flux (for example, 5′-AMP activated protein kinase (AMPK)) may be affected by both the TME and the mutational burden. Both human A549 NSCLC cells (KRAS-G12S) and PANC-1 PDAC cells (KRAS-G12D) are robustly macropinocytic in vitro[43], exhibiting equivalent macropinocytic indices in our hands (B.T.F., V.J. and A.L.E., unpublished observations). Additional studies utilizing a wider array of human cell lines, patient-derived xenografts and orthotopic tumours will be required to conclusively determine whether the same mutation in KRAS produces different amounts of macropinocytic flux in different tissue types. Whether different mutations in KRAS stimulate macropinocytosis to different extents is also unknown. In summary, macropinocytosis has been shown to be an important source of amino acids in KRAS-mutant pancreatic tumours in vitro and in vivo, and other cancer types bearing these mutations are also macropinocytic in vitro.

Flux through the macropinocytic pathway determines its nutritional value, and the efficiency of macropinosome–lysosome fusion may vary between cell types and tissue contexts. Somewhat paradoxically, mTORC1 inhibition drives proliferation when MEFs with activating mutations in *Kras* are nutrient deprived[71,72]. Reducing mTORC1 activity promotes proliferation in part by increasing macropinocytic flux: amino acid limitation or direct mTORC1 inhibition increases macropinosome–lysosome colocalization and BSA degradation[43,71,72]. As macropinosome–lysosome fusion appears very efficient in prostate cancer cells despite elevated mTORC1 activity[43], multiple signals are likely integrated at the step in which macropinosomes are committed to the degradative pathway. Because mTOR inhibition reduces the rate of protein synthesis, it may also increase proliferation by slowing anabolism, thereby preventing a lethal bioenergetic crisis when resources are limiting[71]. However, the export of hydrophobic, essential amino acids from the lysosome is mTORC1-dependent[74], and if mTORC1 activity is too low, efficient lysosomal degradation (as measured by increased DQ-BSA fluorescence and free amino acid levels) may not equate to increased export of amino acids to the cytosol. Given that mTORC1

Macropinocytic flux
The rate at which macropinocytosed macromolecules are converted into nutrients that are exported to the cytosol; variables contributing to the rate of flux include uptake, evasion of endocytic recycling, catabolism to monomers in lysosomes and release into the cytosol.

Na^+/H^+ exchanger
(NHE). Plasma membrane protein that promotes exchange of protons for sodium ions; NHE proteins play a key role in maintaining cellular pH.

Table 1 | **Scavenging inhibitors with potential value in cancer therapy**

Compound	Target	Pathway targeted	Step blocked	Tumour type where effective	Refs
EIPA	NHE1 and/or NHE3	MP	Uptake	• MIA PaCa-2 xenograft PDAC tumours • PTEN-null and p53-null prostate tumour isografts	39,43
Cariporide	NHE1	MP	Uptake	Not evaluated in vivo tumour models; clinical trials suggest favourable safety profile	125–127
PI3K inhibitor (pan)[a]	Class I PI3K	MP	Uptake	• BKM120 in clinical trials; progressed to phase III in some cancer types • Many other PI3K inhibitors (for example, ZSTK474) in clinical trials with encouraging results	179–181
EHT1864	RAC1	MP	Uptake	• BT-474 breast cancer xenografts • Fulvestrant-resistant MCF7 breast cancer xenografts in combination with fulvestrant	129
EHop-016	RAC1 and/or RAC3	MP	Uptake	• Orthotopic MDA-MB-453 breast cancer tumours • MXF8000 myxofibrosarcoma xenografts	182,183
TBOPP	DOCK1	MP	Uptake	• Metastatic ex-3LL Lewis lung carcinoma xenograft • DLD-1 colorectal adenocarcinoma xenografts	68
FRAX597	PAK (group I)	MP	Uptake	• SC4 NF2-null orthotopic Schwannoma tumours • Murine Pan02 orthotopic PDAC tumours in combination with gemcitabine	184,185
GCS-100	αvβ3 integrin and galectin 3 binding	MP	Uptake	KRAS-mutant, αvβ3 integrin-positive lung and PDAC PDX models	70
Compound C	AMPK[b]	MP, integrin and entosis	• Uptake • Trafficking • Lysosome biogenesis and/or function	• Single agent in A549 and SMMC-7721 xenograft tumours • In combination with cisplatin in HCT116 xenograft tumours	186,187
Apilimod	PIKfyve	All	Lysosomal fusion	B cell non-Hodgkin lymphoma	188
CQ and/or HCQ	Lysosomal acidification	All	• Lysosomal function • Export	Many tumour models; combinations with chemotherapy successful in clinical trials	15–17
DQ661	Lysosomal acidification and PPT1	All	• Lysosomal function • Nutrient export from lysosome	• Melanoma (BRAF-V600E-mutant xenograft) • Colon cancer (HT29 xenograft) • Gemcitabine-resistant PDAC (isograft derived from KPC GEMM for PDAC)	159
Bafilomycin A1	v-ATPase	All	• Uptake • Lysosomal function • Nutrient export from lysosome	• BEL7402 and HepG2 hepatocellular carcinoma xenograft tumours • Capan-1 PDAC xenograft tumours • HIF-wild-type fibrosarcoma xenograft tumours	189–192
SH-BC-893	PP2A	All	Lysosomal fusion	Autochthonous and isograft PTEN-null and p53-null prostate tumours and SW620 colorectal cancer xenografts	123,193

AMPK, 5′-AMP activated protein kinase; CQ, chloroquine; DOCK1, dedicator of cytokinesis protein 1; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride; HCQ, hydroxychloroquine; HIF, hypoxia-inducible factor; KPC GEMM, genetically engineered mouse model (GEMM) for PDAC (*Kras*$^{G12D/+}$;LSL-*Trp53*$^{R172H/+}$;*Pdx1-Cre*$^{tg/+}$); MP, macropinocytosis; NF2, neurofibromin 2; NHE, Na^+/H^+ exchanger; PAK, p21-activated kinase; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenograft; PIKfyve, also known as phosphatidylinositol 3-phosphate 5-kinase; PP2A, protein phosphatase 2A; PPT1, palmitoyl-protein thioesterase 1. [a]While isoform-specific inhibitors may be effective in tumours with *PIK3CA* activating mutations, dual inhibition of PI3Kα and PI3Kβ was required to block macropinocytosis in PTEN-deficient cells[43]. [b]Compound C inhibits AMPK but also has many off-target effects[194,195].

also promotes growth in nutrient-limiting conditions by stimulating the adaptive upregulation of cell surface nutrient transporters[75], it is clear that mTORC1 activity must be carefully titrated to balance supply and demand in nutrient-limited, proliferating cells. A similar 'Goldilocks effect' has been described for ROS[76] and is proposed below for AMPK. When interpreting the results of these studies, it is also important to consider that the nutrient stress conditions employed (for example, whether individual amino acids, groups of amino acids or all amino acids are eliminated or present at limiting concentrations) can substantially impact experimental outcomes.

Non-RAS-driven cancers

Activating mutations in SRC kinases, which are common in colon, breast and other cancers[77], also drive constitutive macropinocytosis[63,78,79]. Protein kinase C (PKC), which is activated in many cancer types, is another potent driver of macropinocytosis[60,80,81]. Finally, stimulation of epidermal growth factor receptor (EGFR) or platelet-derived growth factor receptor (PDGFR) by EGF or PDGF, respectively, transiently stimulates macropinocytosis in various transformed and non-transformed cell lines[82–85]. Cancers that overexpress or have activating mutations in EGFR or PDGFR[86,87] may exhibit constitutive, or contextual, macropinocytosis.

Box 2 | **Practical considerations when studying scavenging**

Unravelling the roles that scavenging plays in tumour initiation and progression will require rigorous experimental design and careful interpretation of experimental results. Data from the literature and personal experience reveal several important points to consider when studying scavenging.

- Lysosomal function inhibitors (for example, bafilomycin A1 or chloroquine and its derivatives) will inhibit all forms of scavenging and autophagy (FIG. 3). Although these agents may be valuable therapeutics, they do not discriminate between individual scavenging pathways or between autophagy and scavenging pathways.
- 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) is relatively selective for macropinocytosis among endocytic processes but has broad effects on cells that likely follow from changes in intracellular pH upon Na^+/H^+ exchanger 1 (NHE1) and/or NHE3 inhibition. EIPA is a valuable tool in the study of macropinocytosis and can distinguish between receptor-mediated and macropinocytic scavenging pathways, but experiments must be carefully designed and controlled to confirm that the effects of EIPA can be attributed to its ability to block macropinocytosis. Moreover, the dose of EIPA required to completely arrest macropinocytosis varies and must be determined in each cell line; care must also be taken to ensure that EIPA is selective for macropinocytosis at the required dose.
- Kinetic analysis is necessary. The time it takes to reach steady state after dextran addition varies in different macropinocytic cells; similarly, the time required for newly formed endocytic structures to fuse with the lysosome varies between cells lines. Experiments conducted at and before steady state may both be necessary.
- We have observed that, consistent with the critical role played by 5′-AMP activated protein kinase (AMPK), passage number and culture conditions (for example, cell density, serum lot and frequency of passage) can have substantial effects on experimental outcomes. These variables should be carefully controlled.
- Particular care should be taken to monitor cell lines for *Mycoplasma* spp. *Mycoplasma* spp. enter cells via macropinocytosis and stimulate this process[174,175]. As *Mycoplasma* spp.-containing endosomes fail to recruit Ras-related protein Rab-7a (RAB7) and fuse with the lysosome[176], *Mycoplasma* spp. contamination may alter flux through scavenging pathways.

However, the anabolic contributions of macropinocytosis have not been assessed in cancers with these mutations. Evaluating macropinocytic flux will be important (BOX 3), as growth factor signalling (for example, via EGF[88]) may regulate the rate of macropinosome degradation. To gauge the potential therapeutic impact of macropinocytosis inhibitors in these different tumour types, it will be important to assess whether macropinocytosis supports survival and proliferation in nutrient-replete and/or nutrient-deficient media or in vivo.

Recent studies in prostate cancers with mutation or deletion of the tumour suppressor *PTEN* have met these standards. PIP_3 produced by class I PI3Ks is essential for macropinosome closure and can, in some cell types, drive RAC1 activation and membrane ruffling[57,60,61]. Inactivation or loss of *PTEN* elevates PIP_3 levels and is common in prostate, breast, endometrial and lung cancers as well as in melanoma[89,90]. Deleting or inhibiting *Pten* in murine fibroblasts stimulates macropinocytic uptake of 70 kD fluorescent dextran, which is dramatically increased by glucose deprivation or direct AMPK activation[43]. When PTEN-null MEFs are also deprived of amino acids, macropinocytic flux is further increased, consistent with a role for mTORC1 in promoting lysosomal degradation. PTEN inhibition allows fibroblasts to grow in nutrient-deficient medium in a manner that depends on macropinocytosis but not autophagy. Deletion of *Pak1* eliminated both macropinocytosis and proliferation under nutrient stress without affecting autophagy. By contrast, loss of the essential protein autophagy protein 5 (ATG5) only minimally impacted proliferation under the same conditions. PTEN-deficient prostate cancer cell lines, patient-derived prostate cancer organoids and tumours and autochthonous prostate tumours exhibit robust macropinocytosis in vitro and in vivo. Similar to results obtained with macropinocytic PDAC tumours[39], systemic administration of EIPA significantly inhibits prostate tumour growth, even triggering some regressions[43] (TABLE 1). Whether other tumour types with reduced PTEN activity or elevated PIP_3 levels use macropinocytosis to support growth and proliferation requires further investigation.

Macropinocytosis pleases every palate

Albumin is the only cargo provided in most in vitro studies evaluating the contribution of macropinocytosis to tumour anabolism. However, macropinocytosis is a non-selective, bulk uptake process; many components of the TME are likely to be engulfed in macropinosomes, not just extracellular proteins[38,73].

On the menu: necrotic cell debris. Decaying cell corpses are another component of the microenvironment that is ripe for scavenging. Macropinosomes are large enough to accommodate necrotic cell fragments but not intact apoptotic or live cells[43]. Necrotic cell debris robustly stimulates proliferation in amino acid-deprived and glucose-deprived macropinocytic *KRAS*-mutant or *PTEN*-mutant PDAC or prostate cancer cells, respectively, while non-macropinocytic cells do not benefit or even die when supplemented with necrotic material. Macropinocytic scavenging of necrotic cell debris not only represents a previously unappreciated tumour–microenvironment interaction that can fuel growth and survival but also affords novel opportunities for metabolic tracing studies.

Studies using isotopically labelled necrotic cell debris (BOX 3) demonstrated that macropinocytosed necrotic cell proteins contribute 35–71% of the amino acids for protein synthesis under nutrient stress in prostate cancer cells[43]. Even in complete growth medium, amino acids derived from macropinocytosis contributed 15–25% of the amino acids present in prostate cancer cell proteins, suggesting that scavenging supports cancer anabolism even when extracellular nutrients are abundant. While these macropinocytic flux studies were confined to proteins, scavenging cell debris likely provides lipids, sugars and nucleotides as well as amino acids (FIG. 2a). Indeed, macropinocytosed necrotic cell debris, but not fatty acid-free albumin, maintained lipid droplets in nutrient-deprived prostate cancer cells[43]. Many late-stage and aggressive tumours contain necrotic regions, and tumour necrosis is negatively correlated with prognosis in many cancer types. It is tempting to speculate that macropinocytic cells on the borders of poorly perfused necrotic regions or that survive cytotoxic therapies use the corpses of their deceased brethren to fuel survival and growth. Indeed, it has long been known that the cancer cells that survive radiation or chemotherapy proliferate at an accelerated rate[91,92]. Apoptotic death leads to the caspase-dependent production of prostaglandin E_2 (PGE_2), which

Necrotic cell debris
Physical remnants of cells that have died from a metabolic crisis or fragmented following apoptosis (secondary necrosis).

Box 3 | **Measuring macropinocytic flux**

Flux analysis is essential to accurately measure the contribution of scavenging pathways to tumour anabolism. Internalized extracellular material does not afford nutritional benefits until it is successfully trafficked to the lysosome, degraded and the products released into the cytosol. Studies measuring flux through scavenging pathways should be designed such that the successful completion of all of these steps is accounted for. Some groups indirectly measure macropinocytic flux with DQ-BSA[39,71,72]. DQ-BSA fluorescence indicates that albumin is at least partially degraded, but does not show whether amino acids are liberated or released from the lysosome. Labelling studies can more accurately measure flux. Early stable isotope labelling studies[39] fed cancer cells ^{13}C-labelled protein extract derived from yeast grown in medium containing ^{13}C-labelled glucose and no amino acids. Yeast proteins are a non-physiologic fuel source that may impact growth and survival through macropinocytosis-independent mechanisms[177]. Macropinocytic flux has also been measured by growing cells in medium in which all amino acids and glucose were replaced with uniformly ^{13}C-labelled and ^{15}N-labelled forms and then tracking the incorporation of unlabelled amino acids from albumin[6]. Other subsequent, more cost-effective studies were successfully conducted with only a subset of ^{13}C-labelled amino acids[71]. Isotopic labelling of recombinant albumin is another option[38]. This approach requires the purification of labelled albumin from yeast cultures; producing sufficient quantities of labelled protein may not be cost effective. Finally, when labelled free amino acids are measured[6,38,71], the transport of amino acids from the lysosome into the cytosol, their incorporation into nascent proteins and/or their conversion into metabolites are not accounted for. Using necrotic cell debris as the labelled macropinocytic cargo overcomes each of these obstacles. Growing cells in medium containing ^{13}C-labelled and ^{15}N-labelled arginine and lysine before the induction of necrosis and monitoring the transfer of isotopically labelled amino acids from necrotic cell proteins to the macropinocytic cell proteome provides an accurate assessment of macropinocytic flux with a simple and cost-effective labelling protocol[43]. Necrotic cell debris is a physiologic fuel source present in the tumour microenvironment (TME), and relatively modest amounts of material (for example, 0.1% protein) are sufficient to support the growth of macropinocytic cells[43]. Additionally, unlike albumin, necrotic cell debris is taken up solely by macropinocytosis. Notably, supplementation with necrotic debris reduces the viability of non-macropinocytic cells[43], most likely due to signalling molecules and damage-associated molecular patterns present in necrotic cell debris. However, as these bioactive molecules are likely to be present in the TME[178], their effects are at least likely to be physiologically relevant.

stimulates proliferation in viable tumour cells[91,92]; secondary necrosis may provide the fuel for this PGE_2-driven growth. Thus, macropinocytosis inhibitors may increase the effectiveness of cytotoxic cancer therapies.

On the menu: exosomes. Exosomes, which are approximately 100 nm vesicles released into the microenvironment by both normal and transformed cells, are another TME element that is consumed via macropinocytosis[83,93,94] (FIG. 2a). Cancer-associated fibroblasts release exosomes that, like necrotic cell debris, contain proteins, lipids, nucleotides and even metabolic intermediates that can fuel prostate cancer growth[95]. While the survival advantage conferred by exosomes was reversed by the addition of EIPA, even non-macropinocytic BxPC3 cells benefited from exosome supplementation, suggesting that exosomes can be taken up by macropinocytosis-independent mechanisms or stimulate growth through cell surface interactions.

In summary, cancer cells are surrounded by a veritable banquet of TME components (for example, proteins, dead cells and exosomes) that contain all of the nutrients necessary for proliferation (FIGS 1,2). Most cancer cells are likely to carry mutations that drive macropinocytosis and render these alternative fuels accessible.

Exosomes
Small cell-derived vesicles released into the microenvironment that can contain metabolic intermediates, sugars, RNAs (for example, microRNAs), DNA and intact proteins.

Entosis
The invasion of a living cell into another cell; engulfed 'loser' cells can either escape back to the microenvironment or be degraded and provide nutrients to the 'winner' cell.

Cannibalism and murder: entosis

While macropinocytic cells can feed on decaying corpses, some cancer cells take things a step further, consuming their intact, and still viable, neighbours. The process of entosis, studied primarily in breast cancers but also observed in melanomas and lung, cervical, colon and stomach cancers, produces a 'winner' cancer cell that contains a living, internalized 'loser' cell in a single-membrane vacuole[96,97] (FIG. 2b). During entosis, RHOA and RHO-associated protein kinase (ROCK) activation in the loser cell promotes myosin-driven invasion into the winner cell. This process is distinct from macropinocytosis for several reasons: the internalized loser cell controls both the initial and subsequent steps of entosis; ROCK activation is required; and whole living cells are found in entotic vacuoles. Some loser cells are eventually released by the winner cell, but recruitment of autophagy-related protein LC3 and other autophagic machinery to the entotic vacuole promotes lysosomal fusion and the cannibalization of the loser cell, providing amino acids and likely other nutrients to support the survival and proliferation of starving winner cells[98,99]. The signalling pathways that control the fate of the entosed loser cell require further definition. Additionally, the degree to which catabolism of entosed loser cells supports cancer cell growth in vivo requires investigation. Entosis may also support cancer cell anabolism indirectly by selecting for cancer cells capable of other forms of scavenging. Because activating mutations in *KRAS* or *RAC1* confer winner cell status[100], entosis may push a tumour cell population towards a macropinocytic phenotype by eliminating cells without these mutations. Determining whether entosis contributes to cancer cell anabolism and affects tumour heterogeneity will require the identification of proteins that selectively control this process, rendering the complete molecular dissection of this process a high priority in the field.

Scavenging control: AMPK and mTORC1

Two kinases, AMPK and mTOR, play critical roles in coordinating the cellular response to nutrient stress. AMPK is a heterotrimeric enzyme activated by glucose deprivation, a reduction in cellular ATP levels and oxidative stress[101,102]. As a component of the amino acid-sensitive complex mTORC1, the mTOR kinase coordinates diverse cellular processes to promote growth and survival[103]. mTORC1 is inactivated when amino acids are limiting. Because AMPK negatively regulates mTORC1 by phosphorylating the mTORC1 component RAPTOR (regulatory-associated protein of mTOR) and the RHEB (RAS homologue enriched in brain) GTPase-activating protein TSC2 (tuberous sclerosis 2 protein)[104,105], low ATP or glucose levels also reduce mTORC1 activity. Amino acid restriction, however, selectively inactivates mTORC1 without activating AMPK. Together, AMPK activation and mTORC1 inactivation coordinate an adaptive response to nutrient stress, limiting energy expenditure and substrate consumption by reducing biosynthesis while simultaneously stimulating catabolic processes like autophagy[103,106]. During tumour initiation, AMPK appears

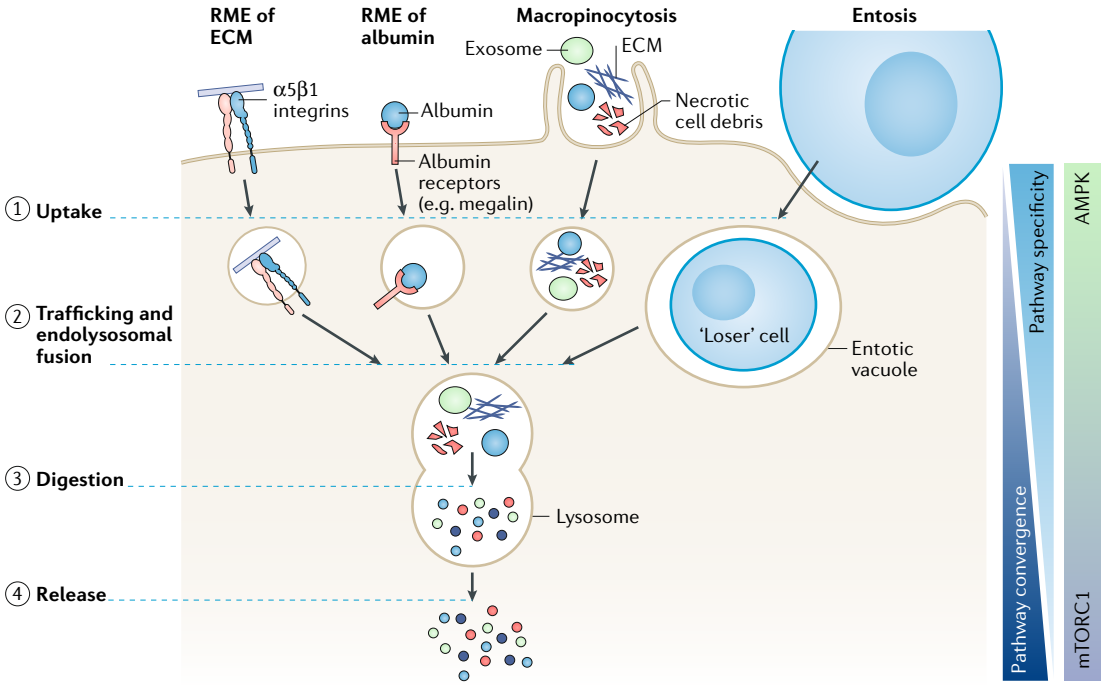

Fig. 3 | **Pathway specificity in nutrient scavenging.** Successful scavenging requires four distinct steps: (1) uptake of macromolecules, (2) trafficking and fusion of endocytic intermediates with lysosomes, (3) catabolism of macromolecules in lysosomes and (4) release of the liberated nutrients into the cytosol. Each of these steps is regulated by multiple signalling inputs. The first and second steps of scavenging require pathway-specific molecular components to control both internalization and trafficking of endosomes towards the lysosomal pathway. However, all scavenging pathways converge at the lysosome. Lysosomal enzymes (for example, lysosomal proteases, nucleases, glycosidases and lipases) digest scavenged material, and lysosomal nutrient transporters (for example, solute carrier family 38 member 7 (SLC38A7), SLC38A9, SLC17A5, SLC36A1 and SLC29A3) release nutrients into the cytoplasm. While the proteins required for the internalization (1) and trafficking (2) steps are generally pathway-specific, 5′-AMP activated protein kinase (AMPK) likely promotes these early steps in each scavenging pathway[43,98,111–113]. Because of its effects on lysosomal biogenesis and function, mTOR complex 1 (mTORC1) likely limits all nutrient scavenging; mTORC1 does, however, promote the export of amino acids from lysosomes and the import of extracellular amino acids through plasma membrane transporters[43,71,72]. Given the multiple, parallel nutrient-generating pathways that are active in cancer cells, molecules that target shared features will likely have the greatest therapeutic impact. ECM, extracellular matrix; RME, receptor-mediated endocytosis.

to function as a tumour suppressor and mTORC1 as an oncogene[103,107–110]. However, recent work suggests that these roles are reversed in established tumours; when nutrients are limiting, AMPK activation and mTORC1 suppression drive tumour cell proliferation by promoting scavenging[31,43,71,72,98,111–113], suggesting that the roles of AMPK and mTORC1 in cancer progression are more nuanced.

AMPK activation promotes scavenging

With the exception of receptor-mediated albumin uptake, AMPK activation has been shown to stimulate all forms of nutrient scavenging (FIG. 3). Glucose depletion, dietary restriction (which lowers glucose and activates AMPK) and the allosteric AMPK activator A769662 all promote integrin-mediated ECM scavenging[25,31,113]. By phosphorylating sorting nexin 17 (SNX17), AMPK also inhibits integrin recycling back to the cell surface, thereby increasing lysosomal degradation of ligand-bound integrins[111,112,114]. AMPK activation promotes entosis. Glucose deprivation increases the formation of entotic structures by approximately 15-fold, increasing the fraction of MCF7 cells containing loser cells from 2% to 30%; AMPK activation was necessary and sufficient to account for this increase[98]. Glucose deprivation also increases the likelihood that the internalized loser cell will be killed and digested rather than released from 40% to 80%. While the fact that AMPK is activated in the loser cell somewhat complicates the interpretation of this result, the outcome of AMPK-driven entosis is the survival and proliferation of winner cells. Finally, it was recently revealed that AMPK activation is necessary for macropinosome formation in KRAS-mutant lung and PTEN-deficient prostate cancer cells and in MEFs with these oncogenic mutations; AMPK likely promotes macropinocytosis by activating RAC1[43]. This same study provides a dramatic illustration of the protumorigenic potential of AMPK. *Pten*-deficient MEFs die when all amino acids are reduced to 1% of normal levels but proliferate when also deprived of glucose or stimulated with the allosteric AMPK activator A769662. In this context, AMPK activation promotes survival and proliferation by driving macropinocytosis. By extension, AMPK activation may drive cell-autonomous proliferation in nutrient-stressed cancer cells by stimulating multiple forms of

scavenging. These studies add to the growing body of evidence suggesting that inhibiting AMPK may prove to be a more effective chemotherapeutic strategy than activating AMPK, particularly in late-stage, established tumours (discussed further below)[108,115–117,157].

mTORC1 limits scavenging

mTORC1 activity can limit nutrient generation from macropinocytosis-mediated and integrin-mediated scavenging by compromising lysosomal catabolism[31,72]; as all scavenging pathways intersect at the lysosome, mTORC1 can have a globally repressive effect on scavenging (FIG. 3). Like AMPK activators, mTORC1 inhibitors may be contextually oncogenic, driving proliferation under nutrient-limiting conditions selectively in scavenging-dependent cells[71,72]. mTORC1 inactivation increases lysosomal biogenesis, and thus degradative capacity, by promoting the nuclear localization of MiT/TFE factors (transcription factor EB (TFEB), microphthalmia-associated transcription factor (MITF) and transcription factor E3 (TFE3)), which drive expression of lysosomal hydrolases and structural proteins[118,119]. In fact, nuclear localization of TFE3 is necessary to maintain intracellular amino acid pools in pancreatic cancer cells that depend on both autophagy and macropinocytosis for their growth and survival[120]. Conversely, overexpression of MITF in *Kras*$^{G12D/+}$ cells that normally produce low-grade pancreatic intraepithelial neoplasia lesions dramatically increases tumour growth and invasive potential[120]. While reports that increasing degradative capacity drives tumour growth seem paradoxical, these results make sense in light of the fact that scavenged macromolecules must be broken down to release the nutrients they contain. mTORC1 inhibition might also stimulate lysosomal fusion and nutrient generation through post-transcriptional mechanisms. For example, while a role for mTORC1 has not been explicitly defined, growth factor withdrawal activates Ras-related protein Rab-7a (RAB7), a small GTPase that promotes lysosomal fusion reactions[121,122]. In summary, observations that AMPK activation and mTORC1 inhibition stimulate tumour progression may be attributed in part to the opposing roles these kinases play in scavenging processes (FIG. 3).

Therapeutic targeting of scavenging

The oncogenic mutations that drive cancer initiation and progression engender a dependence on continuous nutrient influx to support constitutive anabolism[1]. Substrate limitation should not only stop cancer cell growth but also kill cancer cells given their limited ability to adapt to starvation[123]. Cancer cells may be vulnerable to drugs that block scavenging, particularly when they are stressed by other therapeutic agents.

Inhibiting macropinocytosis

Small molecule inhibitors of macropinocytosis already exist, but each has pleiotropic effects on tumour cells. Because class I PI3Ks are necessary for macropinosome formation (FIG. 3), PI3K inhibitors could be deployed as macropinocytosis inhibitors even in cancers without PI3K pathway mutations (TABLE 1). Systemic dosing of mice with the NHE inhibitors amiloride and EIPA limits tumour growth in multiple model systems[43,124] (TABLE 1). Phase I/II trials with amiloride and the NHE1 (also known as SLC9A1) inhibitor cariporide in patients with cardiovascular disease were halted primarily owing to lack of efficacy rather than pharmacologic liabilities or toxicities[125–127]. EIPA has macropinocytosis-independent antineoplastic effects that likely contribute to its effectiveness in cancer models[124], and whether these pleiotropic actions could benefit patients with cancer deserves further investigation. EIPA blocks macropinocytosis by inhibiting RAC GTPases[128]. Although clinically useful direct inhibitors of RAC GTPases are not yet available, the small molecule EHT1864 inhibits macropinocytosis[43] and tumour growth[129,130] (TABLE 1). p21-activated kinase (PAK) inhibitors have activity in a variety of cancer classes, including pancreatic cancer; a block in macropinocytosis may contribute to their activity. PAK catalytic activity is required for macropinocytosis downstream from RAC1 (REFS[43,84]), and small molecule PAK inhibitors (for example, FRAX597) exhibit selective toxicity to macropinocytic *Pten*-null MEFs under nutrient stress[43] (TABLE 1). In summary, several signalling inhibitors with pleiotropic effects could be deployed against macropinocytic tumours, in some cases pending optimization of their pharmacological properties. Specific inhibitors of macropinocytosis would have fewer toxicities but would also likely be less effective therapeutics.

Inhibiting receptor-mediated scavenging

While targeting receptor-mediated albumin scavenging will depend on elucidation of the molecules and signalling pathways involved, agents that interfere with integrin-dependent scavenging are already available (TABLE 1 and below). Almost all existing integrin-directed therapeutics limit ligand binding[131,132] and would therefore interfere with receptor-mediated ECM scavenging (FIG. 1). Volociximab, a monoclonal antibody that recognizes the α5β1 integrin heterodimer, reduces tumour growth and angiogenesis in mice, though it lacked efficacy in clinical trials[133–136]. ATN-161, a non-arginine, glycine, aspartic acid (RGD) integrin based peptide inhibitor of α5β1 integrin, blocks tumour growth and metastasis in mice, improves chemotherapy and was well tolerated in clinical trials[137–139]. While ATN-161 and volociximab are no longer in clinical development, they might be more efficacious when predictive biomarkers have been identified and/or when combined with scavenging-sensitizing drugs.

Simultaneous blockade of multiple pathways

Clinical experience indicates that tumours quickly adapt to the loss of individual pathways, either owing to the outgrowth of pre-existing resistant clones present in heterogeneous tumours before the onset of therapy or by upregulating compensatory pathways that eliminate dependence on the targeted molecule[140–143]. Thus, therapies that simultaneously block parallel nutrient access pathways are more likely to starve cancer cells to death and produce durable tumour regression than agents that block individual pathways[1].

Inhibiting AMPK. AMPK confers resistance to nutrient stress by promoting multiple forms of scavenging, autophagy and glucose uptake[108,115,117,144] (FIG. 3). While AMPK activators have been proposed as anticancer agents that would exploit the tumour suppressive functions of AMPK, studies supporting this therapeutic strategy have substantial caveats. Pharmacologic AMPK activators applied to established tumours often activate AMPK indirectly. Metformin, an inhibitor of complex I of the electron transport chain[145–149], reduces the growth of scavenging-dependent PDAC tumours[149–151]. However, this effect may be independent of, or even in spite of, AMPK activation, given that limiting oxidative phosphorylation would itself be generally tumour suppressive[152]. Indeed, there is some evidence that the antineoplastic actions of metformin are independent of AMPK[147,153]. It is also important to consider that, similar to autophagy, AMPK likely plays opposing roles in tumour initiation and progression[14,154,155]. Constitutive deletion of AMPK catalytic subunit α1 accelerates growth in nutrient-replete culture media and promotes MYC-driven lymphomagenesis[107]. AMPK inhibits acetyl-CoA carboxylase 1 (ACC1) and ACC2, and expression of ACC mutants that cannot be phosphorylated by AMPK reduces xenograft growth[156]. Both these studies demonstrate that AMPK suppresses tumour initiation. On the other hand, inducible deletion of AMPK in mouse models of acute myeloid leukemia[117] and glioblastoma[157] suggest that AMPK inhibition is the appropriate therapeutic approach to controlling established cancers. Systemic deletion of AMPK in adult mice is well tolerated[157], suggesting that AMPK inhibitors would not be toxic. Additional studies in which AMPK is silenced in established solid tumours will be required to test the hypothesis that reducing AMPK activity would halt tumour growth by simultaneously limiting glucose import, ECM scavenging, macropinocytosis, entosis, and autophagy.

Inhibiting lysosomal degradation. Blocking lysosomal degradation is currently the primary strategy for inhibiting autophagy in tumours[15–17]. However, agents that target the lysosome will also inhibit scavenging; the ability of these agents to suppress scavenging may be essential for their antineoplastic actions, given that scavenging, but not autophagy, can drive cell-autonomous growth[43]. Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ), which are lysosomotropic agents that disrupt lysosomal function by increasing luminal pH, exhibit antineoplastic activity alone or in combination with other therapies (reviewed in REF.[16]). While most groups ascribe the actions of CQ to autophagy inhibition, CQ and its derivatives may inhibit tumour growth through effects on autophagy, scavenging or both. Because HCQ does not have ideal pharmacological properties, alternative inhibitors of lysosomal function are under development; several are already FDA-approved. The lysosomotropic agent quinacrine (QN) inhibits lysosomal acidification with a 60-fold higher potency than CQ[158]. Dimerizing QN further increases potency[159]. The most potent dimeric QN analogue, DQ661, depends on polypharmacology for its exceptional activity (TABLE 1). DQ661 not only limits lysosomal acidification but also reduces mTORC1 activation by inhibiting palmitoyl-protein thioesterase 1 (PPT1)[159], a lysosomal enzyme required for proteolysis of palmitoylated proteins. DQ661 limits the growth of *BRAF* mutant, HCQ-resistant melanomas as well as PDAC and colorectal tumours in vivo[159], suggesting that it would be an effective anti-scavenging therapeutic. Some lysosomotropic agents such as QN and unmethylated dimeric QN analogues intercalate and damage DNA[159], an effect that could contribute to their antineoplastic actions. However, DQ661 is methylated and inhibits lysosomal acidification without damaging DNA. In summary, lysosomal inhibitors may prove to be the most effective scavenging inhibitors owing to their dual actions on scavenging pathways and autophagy.

Inhibiting parallel pathways. Other antineoplastic agents with even more comprehensive effects on nutrient-generating pathways could also be deployed against tumours that depend on scavenging. Tumour cells might compensate for the loss of lysosomally derived nutrients by increasing nutrient import via transcriptional upregulation of plasma membrane nutrient transporters and receptors. One novel, sphingolipid-inspired small molecule, SH-BC-893, not only blocks the lysosomal degradation of autophagosomes, macropinosomes and low-density lipoprotein (LDL)-bearing endosomes, it also reduces access to monomeric extracellular nutrients by downregulating cell surface transporters for glucose, monocarboxylates, amino acids and LDL[123]. SH-BC-893 limits the growth of autochthonous prostate tumours and induces regressions in a related isograft model without toxicity to normal tissues. Testing SH-BC-893 in additional tumour model systems, particularly those dependent on scavenging such as PDAC, is clearly warranted.

Scavenging as a resistance mechanism

Autophagy confers resistance to a range of chemotherapeutic agents[160]. It is very likely that scavenging pathways provide similar protection, particularly given that many studies evaluating the contribution of autophagy to drug resistance employ CQ analogues that will simultaneously disrupt both autophagy and scavenging pathways. As one example, integrin-mediated ECM scavenging may confer resistance to PI3K and/or mTOR inhibition. Combined inhibition of β1 integrin, β4 integrin, integrin-linked protein kinase (ILK) and focal adhesion kinase (FAK) resensitizes resistant ovarian and breast cancer cells to the dual PI3K–mTOR inhibitor BEZ235 (REF.[161]). β1 integrin is a major player in drug resistance, as overexpression confers resistance to radiotherapy in head and neck cancer[162], resistance to trastuzumab and lapatinib in breast cancer[163] and resistance to erlotinib in lung cancer[164]. Whether increased integrin-mediated ECM scavenging[31] or increased macropinosome–lysosome fusion following mTORC1 inhibition[72] contributes to drug resistance merits further investigation. It is also possible that macropinocytosis of necrotic cell debris produced following radiation, chemotherapy or the administration of any agent that induces metabolic

stress or cell death could limit therapeutic efficacy[91,92]. Blocking scavenging with AMPK inhibitors or lysosomal function inhibitors or by more globally disrupting nutrient access with SH-BC-893 might improve tumour cell killing by targeted or cytotoxic therapeutics, leading to deeper and more durable patient responses.

Conclusions

Autophagy has an uncontested role in promoting tumour progression and drug resistance, and the potential therapeutic value of autophagy inhibitors is widely recognized. While scavenging cannot remove damaged organelles or orchestrate cellular remodelling, in regard to dealing with nutrient stress, scavenging is the superior adaptive strategy. Because it provides substrates from cell-extrinsic rather than cell-intrinsic sources, scavenging can fuel cell-autonomous growth, while autophagy can only stave off death. Because autophagy in non-transformed cells supports tumour cell growth in a non-cell-autonomous manner[165,166], therapeutic agents that undermine both autophagy and scavenging will likely have the greatest clinical impact. Ongoing preclinical and clinical studies with agents that target scavenging are exciting, as they could lead to therapeutic advances in solid tumours in which the available treatments provide limited survival benefits. Patients with KRAS-driven tumours are obvious potential beneficiaries, but recent work implies that tumours with many different oncogenic mutations would be responsive to scavenging inhibitors. Whether targeting scavenging might limit drug resistance is a question with clear translational relevance that should be explored.

As the scavenging field matures, it will be important to apply the lessons learned from studying autophagy and cancer metabolism in order to ensure a high degree of scientific rigour (BOX 2). It is our deep, molecular understanding of autophagy that has provided the foundation for the genetic experiments that accurately measure the contribution of autophagy to tumour initiation, progression and metastasis and that can separate the role autophagy plays in tumour and non-tumour tissues. A similar level of molecular understanding would dramatically stimulate research in the scavenging field. Labelling studies will also be important to elucidate whether sugars, nucleotides and lipids can in fact be salvaged through scavenging. When designing experiments to test these ideas, it will be important to take into account that tissue context influences metabolic wiring and nutrient dependence[69,167]; in vivo studies in autochthonous and orthotopic tumour models are most likely to recapitulate the biology of patient tumours.

Although much remains to be done, there is sufficient evidence to establish that scavenging is a key component of cancer's anabolic supply chain and that therapies limiting nutrient access have great promise. Starving cancer cells should be both safe and effective, given that the synthetic sphingolipid SH-BC-893 simultaneously inhibits all nutrient access pathways and significantly reduces prostate and colorectal tumour growth without toxicity[123]. While we strive to develop more selective inhibitors of scavenging, it is also important to recognize that selectively blocking scavenging is, by itself, unlikely to induce profound cancer cell starvation or lead to stable tumour regressions. Drugs like SH-BC-893 and inhibitors of AMPK or lysosomal function that affect multiple pathways are much more likely to produce clinically meaningful substrate limitation in cancer cells, although therapies that limit individual scavenging pathways may make a substantial impact as components of drug combination regimens. With so many important open questions, this emergent subdivision of the cancer metabolism field will no doubt continue to produce highly impactful studies that shift our thinking about cancer growth and treatment for many years to come.

Published online 10 August 2018

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Acknowledgements
A.L.E. was supported by grants from the Congressionally Directed Medical Research Programs (CDMRP) (W81XWH-15-1-0010), the University of California Cancer Research Coordinating Committee (CRR-17-426826), University of California Irvine (UCI) Applied Innovation and the UCI Chao Family Comprehensive Cancer Center Anti-Cancer Challenge. The authors thank the referees for the peer review of this work.

Author contributions
All authors contributed to substantial discussions, research and collection of references and the writing and editing of the article. B.T.F. and A.L.E. organized and planned the display figures and boxes.

Competing interests
A.L.E. is listed as an inventor on a patent covering the synthesis of SH-BC-893 and its use as a treatment for cancer and other diseases.

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Reviewer information
Nature Reviews Cancer thanks J. Swanson, A. Thorburn and the anonymous reviewer(s) for their contribution to the peer review of this work.