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Review

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The pervasiveness of macropinocytosis in oncological malignancies

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In tumour cells, macropinocytosis functions as an amino acid supply route and supports cancer cell survival and proliferation. Initially demonstrated in oncogenic *KRAS*-driven models of pancreatic cancer, macropinocytosis triggers the internalization of extracellular proteins via discrete endocytic vesicles called macropinosomes. The incoming protein cargo is targeted for lysosome-dependent degradation, causing the intracellular release of amino acids. These protein-derived amino acids support metabolic fitness by contributing to the intracellular amino acid pools, as well as to the biosynthesis of central carbon metabolites. In this way, macropinocytosis represents a novel amino acid supply route that tumour cells use to survive the nutrient-poor conditions of the tumour microenvironment. Macropinocytosis has also emerged as an entry mechanism for a variety of nanomedicines, suggesting that macropinocytosis regulation in the tumour setting can be harnessed for the delivery of anti-cancer therapeutics. A slew of recent studies point to the possibility that macropinocytosis is a pervasive feature of many different tumour types. In this review, we focus on the role of this important uptake mechanism in a variety of cancers and highlight the main molecular drivers of macropinocytosis in these malignancies.

This article is part of the Theo Murphy meeting issue 'Macropinocytosis'.

1. Introduction

A possible link between macropinocytosis and cancer became apparent with the discoveries that proto-oncogenes, such as *RAS*, *SRC* and *EGFR*, have the ability to stimulate this uptake mechanism [1–4]. Although it was clear that activating mutations in these proto-oncogenes leads to macropinocytic induction, a physiological role for this boost in uptake capacity remained uncertain for many years. Taking a clue from the role that macropinocytosis plays in axenic nutrient acquisition in the soil amoeba *Dictyostelium* [5], and inspired by the renaissance of the study of tumour metabolism, we established that macropinocytosis functions as a nutrient delivery pathway in cancer cells [6]. By internalizing extracellular proteins and targeting them for lysosomal degradation, macropinocytosis operates in tumours as an amino acid supply route, providing an endocytic source of protein-derived amino acids that support cancer cell bioenergetics and biosynthesis.

Since our initial studies, which focused on *KRAS*-mutated pancreatic cancer, it has become apparent that cancer-associated macropinocytosis may be more prevalent than we initial surmised (table 1). Recently, studies employing different human *in vitro* systems and *in vivo* animal models have demonstrated that macropinocytosis is a metabolic feature of other solid tumours, including those found in the lung, prostate, bladder and colon. Since macropinocytosis functions as an endocytic gateway for nanomedicines, including nanoparticles, liposomes, nanotubes, DNA nanostructures and protein–drug nanoconjugates [39–41], there are many *in vitro* examples of macropinocytosis in cancer cells that originate from a variety of tissues. The function of macropinocytosis in

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Table 1. Examples of macropinocytosis in different tumour types.

type of cancer		molecular driver	cancer cell lines	*in vivo* models	refs[a]
bladder urothelial carcinoma		*HRAS*, *KRAS*, *PTEN*	T24, J82, UM-UC-3		[6,7]
brain and nervous system	glioma/glioblastoma	?	U251, U87MG, C6, U87		[8–11]
	neuroblastoma	?	SH-SY5Y		[12]
	medulloblastoma	?	Daoy		[13]
breast		?	MDA-MB-231, MCF-7, HTB-20, MDA-MB-157		[14–17]
colorectal adenocarcinoma		*KRAS*?, P13K?, APC	DLD-1, HCT116, HT-29, CT26	colonic epithelium in R26-rtTA TG-Apc.3374 (shAPC) genetically engineered mouse model	[17–21]
haematologic malignancies		?	X63, J558 L, F8, Molt-4		[22–24]
hepatocellular carcinoma		*NRAS*?	HepG2		[25]
human papillomavirus-related cervical adenocarcinoma		?	HeLa, SiHa		[26,27]
lung adenocarcinoma		*KRAS*, integrin $\alpha v\beta 3$, galectin-3	H1792, A549, SKLU1, H1299	subcutaneous patient-derived (PDX) xenograft tumours	[28–30]
osteosarcoma		?	HOS, 6647		[31,32]
ovarian adenocarcinoma		?	OVCAR-3, SKOV-3, OV2008		[33,34]
pancreatic ductal adenocarcinoma		*KRAS*	MIA PaCa-2, PL45, Panc-1	1. subcutaneous xenograft tumours 2. autochthonous tumours in *P48-cre; lsl-Kras*^{G12D}; *Trp53*$^{-/+}$ (KPC) genetically engineered mouse model 3. human tumours from whipple patients	[6,35–37]
prostate		*PTEN*	PC3, LNCaP, DU145, mPCE	subcutaneous allograft tumours	[38]

[a]For each cancer type, the earliest or most extensive studies were selected as representative references.

drug delivery is becoming an important field of research in cancer biology and has been the topic of many other excellent reviews [41–44]. Intriguingly, in precursor cell malignancies affecting the brain and nervous system, such as glioblastoma, neuroblastoma and medulloblastoma, macropinocytosis needs to be finely balanced as amplifying the macropinocytic capacity in these contexts has the propensity to lead to a form of nonapoptotic cell death known as methuosis [45]. Like many examples of macropinocytosis in the oncological context, methuosis is linked to the elevated expression of oncogenic forms of *RAS*. The mechanistic underpinnings of oncogenic Ras-induced macropinocytosis have been reviewed elsewhere, but they entail the induction of downstream effector pathways that regulate actin cytoskeleton remodelling and membrane ruffling behaviour [46].

In this review, we aim to provide a semi-systematic analysis of the pervasiveness of macropinocytosis in cancer by focusing on the role of this important uptake mechanism in different tumour types. We highlight the key molecular drivers of macropinocytosis in these malignancies and provide an overview of similarities between the various pathological settings.

2. Pancreatic cancer

The vast majority of pancreatic ductal adenocarcinoma (PDAC) tumours exhibit oncogenic mutations in *KRAS* [47]. The initial discoveries that macropinocytosis serves as an amino acid supply route in cancer cells were made in cellular and animal models of *KRAS*-driven PDAC [6,35]. These studies demonstrated that PDAC cells that harbour oncogenic *KRAS* mutations display robust levels of macropinocytosis that are dependent on expression of the oncogene. By contrast, PDAC cells that express wild-type *KRAS* have low levels of macropinocytic induction. Importantly, these observations were recapitulated *in vivo* using tumour xenograft models. Additionally, macropinocytic induction was observed in an autochthonous model of PDAC. In this model, mice of the genotype *P48-cre; lsl-Kras*^{G12D}*; Trp53*$^{-/+}$ (KPC) develop early pancreatic intraepithelial neoplasia (PanIN) lesions within four weeks of birth and progress to invasive PDAC between 9 and 13 weeks [48,49]. Using this model, macropinosomes were detected in pancreatic acinoductal cells found in mid- to late-stage PanIN lesions but not in pancreata from wild-type mice [6]. The clinical relevance of macropinocytosis in PDAC was established by scrutinizing human specimens. Although some intratumoral variability was observed, stimulated macropinocytosis was evident in all of the PDAC tumour samples analysed [35]. Altogether, these studies showed that macropinocytosis is an attribute of PDAC tumours and suggested that PDAC cells could take up fluids and their solubilized components from their extracellular environment.

Extracellular physiological fluid is mainly composed of proteins, with serum albumin being the most abundant. Therefore, it was a reasonable assertion that PDAC tumour cells were engaging in macropinocytosis to facilitate the uptake of extracellular serum albumin from the tumour interstitium. This was initially examined *in vitro* with colocalization assays that revealed the macropinocytic uptake of fluorescently labelled bovine serum albumin (BSA) [6]. To serve as a nutrient source, this macropinocytosed BSA would need to be proteolytically degraded into its constituent amino acids. Indeed, using a self-quenched form of BODIPY-dye-conjugated BSA (DQ-BSA) that only emits a bright fluorescent signal upon digestion, it was shown that the incoming BSA is degraded within macropinosomes in a lysosome-dependent manner. The protein-derived amino acids that are produced by macropinocytosis in *KRAS*-mutant cells feed into a multitude of metabolic pathways, including the TCA cycle, glutamine anaplerosis/oxidation, acetyl-coenzyme A metabolism, reductive carboxylation and serine/glycine cycling. Importantly, these protein-derived amino acids have the ability to suppress the deleterious effects of nutrient starvation, leading to albumin-dependent proliferation and growth. Interestingly, catabolism of macropinocytosed serum albumin contributes substantially to PDAC intracellular amino acid pools, even in the absence of amino acid deprivation [35]. An important avenue of further study is determining whether amino acid deprivation can enhance macropinocytic uptake and to what extent macropinocytosis contributes to cellular fitness under nutrient-replete conditions.

The evaluation of the role of macropinocytosis in PDAC tumour growth took advantage of the fact that macropinocytosis is unique in relation to other endocytic pathways, because it is sensitive to 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA) and other amiloride analogues that inhibit Na^+/H^+ exchange [50]. Miniaturized plasma exchange experiments demonstrated *in situ* that live tumours actively engage in macropinocytosis and break down albumin [51]. This albumin catabolism was suppressed by EIPA and resulted in the modulation of amino acid levels within the tumour. Importantly, EIPA administration in animals bearing *KRAS*-mutant xenograft tumours resulted in an attenuation of tumour growth, and in some cases regression, relative to vehicle-only controls [6]. The observed effects of EIPA on PDAC tumours might be specific to highly macropinocytic *KRAS*-mutant tumours because EIPA treatment had no bearing on the growth rate of tumours derived from *KRAS*-wild-type PDAC cells. Altogether, these exciting results implicated macropinocytosis as a potential therapeutic target in PDAC. Further research is required to assess whether EIPA or any other macropinocytosis inhibitors have therapeutic benefit in human PDAC patients.

3. Bladder cancer

While only a subset of bladder carcinomas harbour oncogenic *RAS* mutations, the majority of these genetic perturbations occur in *HRAS* [52]. Similar to PDAC cells, *HRAS*-mutant bladder cancer cells display robust levels of macropinocytic induction that they employ to augment their metabolism through the lysosome-mediated degradation of the extracellular protein [6]. Much knowledge about macropinocytosis in bladder cancer has been gained by studying the internalization of Bacille Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis* that is widely used as an effective intravesical therapy in bladder carcinoma. The mechanisms underlying the anti-tumour effects of BCG are unclear, but likely involve immune processes and/or direct cytotoxic effects on the tumour cells. BCG entry into bladder cancer cells is mediated by macropinocytosis, although not all bladder cancer cells are susceptible to treatment [7]. Sensitivity to BCG was identified to be associated with *PTEN* deletion,

which is a negative regulator of P13K signalling, or oncogenic mutations in *HRAS* or *KRAS*. These BCG-sensitive cells permitted the internalization of BCG via macropinocytosis, which was dependent upon Rho-family GTPases, such as Cdc42 and Rac1, as well as the kinase Pak1. In BCG-resistant bladder cancer cells, either oncogenic *HRAS* or *KRAS* ectopic expression or *PTEN* knockdown was sufficient to enhance BCG uptake. These important findings implicate macropinocytosis not only in supporting bladder tumours metabolically, but also in possibly representing a mode of BCG delivery to bladder tumours that can be dialed-up to optimize effectiveness. Indeed, a recent live-attenuated *Mycobacterium tuberculosis* vaccine that enters bladder cancer cells more readily through macropinocytosis than BCG, has been shown to be more effective in terms of its anti-tumour effects in an orthotopic murine model of bladder cancer [53]. Considering this information, it would be beneficial to explore ways to enhance macropinocytosis in order to improve microbial-based therapies.

The BCG-resistant bladder cancer cell lines, which display very low levels of macropinocytosis and do not harbour *RAS* or *PTEN* mutations, served as a platform to screen for signal transduction pathways that have the capacity to activate macropinocytosis when they are suppressed [18]. This whole-genome gain-of-function screen employed a flow cytometry-based read-out to measure uptake of fluorescent BCG and found that negative regulators of the canonical Wnt signalling pathway could activate macropinocytosis when their expression was suppressed by the shRNA-mediated knockdown. These macropinocytosis modulators, which included *DKK2*, *KREMEN1*, *NKD1*, *SMAD4* and *MAPK9*, might be specific to wild-type Ras cells or to bladder cancer cells because they were not identified as modulators of macropinocytosis in oncogenic *HRAS*-expressing HeLa cells [54]. The activation of the Wnt pathway in various BCG-resistant bladder cancer cell lines was sufficient to support albumin-dependent proliferation under conditions of limiting nutrients, demonstrating that, as was observed in PDAC, macropinocytosis can support metabolic fitness in bladder cancer [18]. Additionally, the Wnt pathway seems to be constitutively activated in BCG-sensitive bladder cancer cells and in other *RAS*-transformed cells, supporting macropinocytosis in these contexts as well. It might be beneficial to establish an animal model of bladder cancer that can be scrutinized for macropinocytic induction *in vivo* and then examine whether Wnt pathway activation can be used to improve BCG therapy.

4. Lung cancer

About a quarter of non-small cell lung cancer (NSCLC) tumours harbour oncogenic *KRAS* mutations [55]. As has been observed in other cancer models, only some of these tumours are dependent on oncogenic *KRAS* expression for their viability—a phenomenon termed oncogene addiction [56]. Recent work exploring the role of macropinocytosis in NSCLC has been in the context of *KRAS* addiction [28]. NSCLC dependence upon oncogenic *KRAS* can be mediated by cell surface receptor integrin $\alpha v\beta 3$, and its modulator galectin-3. Integrin $\alpha v\beta 3$ clusters on the surface of tumour cells and plays a role in anchorage-independent growth, controlling tumour progression and metastasis [57]. Clustered integrin $\alpha v\beta 3$ can potentiate signal transduction, a function influenced by its binding partner galectin-3, and together this interaction has the ability to induce KRas clustering [58]. Interestingly, NSCLC cells that express integrin $\alpha v\beta 3$ are uniquely addicted to oncogenic *KRAS*, and these cells employ macropinocytosis to sustain their cellular fitness, while NSCLC cells that are not addicted to oncogenic *KRAS* exhibit low levels of uptake [28]. Macropinosomes produced in integrin $\alpha v\beta 3$-positive cells drive the internalization of extracellular protein that is targeted for degradation. This process relies on integrin $\alpha v\beta 3$ and its modulator galectin-3, as depletion of either suppresses macropinocytic capacity. A clinically active inhibitor of galectin-3, GCS-100, blocks the integrin $\alpha v\beta 3$/galectin-3 interaction, and as a consequence blocks macropinocytic uptake. GCS-100 has potent anti-tumour effects in integrin $\alpha v\beta 3$-positive mouse models of NSCLC and PDAC; however, the contribution of macropinocytic suppression to this phenotype is unclear because GCS-100 also increases reactive oxygen species levels.

In addition to serum albumin, macropinocytosis may facilitate the internalization of extracellular ATP into lung cancer cells to support viability under conditions of nutrient stress [29]. Uptake of ATP could be physiologically important in the cancer setting because ATP levels can be much higher in tumours than in normal tissues [59]. In an NSCLC xenograft tumour model, the internalization of ATP is mediated by macropinocytosis, but might also involve other endocytic pathways, such as clathrin- and caveolae-mediated endocytosis [30]. Extracellular ATP is known to induce resistance to various chemotherapeutic drugs in cancer cells. Interestingly, drug resistance to sunitinib in NSCLC tumour cells is linked to macropinocytic uptake because it is suppressed by knockdown of *PAK1*, a known regulator of macropinocytosis [60]. The uptake of ATP via macropinocytosis is a prime example that macropinocytic cargo can vary in tumour cells, and depending on the context, identifying novel cargoes could provide clues to the breadth of function for macropinocytosis in cancer [46,61].

5. Prostate cancer

While oncogenic mutations in *RAS* are not associated with prostate cancer, *PTEN*, a negative regulator of P13K signalling, is the most frequently deleted tumour suppressor gene in prostate tumours. It was recently reported that *PTEN*-deficient prostate cancer cells exhibit macropinocytosis and that *PTEN* reconstitution suppresses uptake in these cells [38]. The macropinocytosis in this setting is mediated by AMPK, as a $\beta 1$ subunit-specific AMPK activator, A769662, can transiently enhance macropinocytic capacity and inhibition of AMPK or expression of a dominant negative form of AMPK can suppress uptake. AMPK functions to control energy homeostasis, and its role in cancer has been extensively explored in the context of nutrient stress; however, the macropinocytic induction observed in *PTEN*-deficient prostate cancer cells is nutrient-independent. This might be accounted for by the finding that prostate tumours have high basal AMPK activity relative to normal prostate tissue [62]. Intriguingly, serum albumin uptake in *PTEN*-deficient prostate tumour cells seems to be independent of macropinocytosis, possibly relying on other endocytic pathways for its internalization [38]. Instead, necrotic cell debris is the nutritious macropinocytic cargo that is favoured by prostate

cancer cells. Under conditions of limiting amino acids, the uptake of necrotic cell debris significantly impacted the proliferative capacity of various prostate cancer cells, an activity that was suppressed by *PTEN* reconstitution. Not only does necrotic cell debris provide a source of amino acids, but it also serves to supply lipids that are critical to maintaining cellular membranes and intracellular lipid stores. Importantly, inhibiting macropinocytosis via EIPA administration suppressed prostate tumour growth in a prostate allograft model. Important avenues for further study include exploring to what extent AMPK regulates macropinocytosis in other pathological contexts and whether other tumour types also use necrotic cell debris as a nutrient source to fuel growth.

6. Brain and nervous system cancers

Enhanced activation of the Ras signalling pathway can drive macropinocytic induction in glioblastoma cells [63]. Indeed, this characteristic has been co-opted to study the uptake of potentially therapeutic nanomedicines, especially those that can cross the blood–brain barrier [8]. Macropinocytosis in glioblastoma cells requires fine-tuned regulation because hyperstimulation of the pathway by either a boost in Ras signalling or by small molecules that drive the process can lead to a necrosis-like form of cell death called methuosis. Methuosis was first described as a cellular response to ectopic expression of oncogenic *HRAS* in glioblastoma cells, which induces extreme cytoplasmic vacuolization as a result of hyperactive macropinocytic induction [9]. This peculiar trait might represent a unique vulnerability that can be targeted in glioblastoma. Similar cell death effects were observed when glioblastoma cells were treated with Bacoside A, a natural compound mixture [64]. Although the mechanism driving macropinocytosis in response to bacosides is unclear, it might involve calcium release from the smooth endoplasmic reticulum.

In addition to glioblastoma, macropinocytosis is also evident in neuroblastoma and medulloblastoma. In neuroblastoma cells, two distinct forms of macropinocytosis have been observed, both dependent on nonmuscle myosin II [12]. Insulin-like growth factor-1 induces macropinosomes that are predominantly located in the cell bodies and require P13K signalling for their formation, while phorbol 12-myristate 13-acetate induces macropinocytosis in the neurites that is P13K independent. Similar to glioblastoma, hyperstimulation of macropinocytosis in neuroblastoma cells leads to methuosis, suggesting this might be a common trait in nervous system cancers [65,66]. Indeed, in medulloblastoma cells, nerve growth factor activation of the tropomyosin receptor kinase A (TrkA) puts macropinocytosis in overdrive, stimulating vacuolar accumulation and cell death by methuosis [13,67].

7. Haematologic malignancies

Haematologic malignancies include forms of cancer that originate from cells of the immune system or in blood-forming tissue, such as bone marrow. It is not surprising that macropinocytosis is prevalent in these pathological states since, unlike solid tumours, this uptake mechanism is employed as an aspect of normal function in these cells. For example, dendritic cells are thought to macropinocytose large quantities of exogenous solute as part of their sentinel function [68,69] and macropinocytosis in macrophages is linked to antigen surveillance, as well as foam cell formation through uptake of LDL [70,71]. It is unclear whether the macropinocytosis observed in malignancies such as leukaemia, lymphoma and multiple myeloma supports tumour cell metabolism; however, there are examples of this endocytic pathway being active in these settings, suggesting a possible role in treatment or progression of these diseases. Nanoparticle uptake via macropinocytosis has been demonstrated in an *in vitro* murine model of adult T-cell leukaemia/lymphoma and in myeloma cells [22,23]. Macropinocytosis has also been harnessed to deliver a cell-penetrating peptide with a lymph node-homing motif to leukaemia and lymphoma cells [24]. It is not clear whether macropinocytosis is critical to all haematological malignancies because dendritic cells obtained from chronic myeloid leukaemia patients displayed lower levels of uptake than dendritic cells from healthy individuals, although macropinocytic capacity can still be used in these cells as a functional test [72,73]. It will be important to determine whether the inhibition of macropinocytosis has the ability to modulate the onset or progression of these diseases.

8. Future perspectives

The studies that have focused on the role of macropinocytosis in Ras-driven cancers have been centred on pancreatic, lung and bladder cancers. An important extension of this research is to explore whether macropinocytosis contributes to tumour cell metabolic fitness in other Ras-mutant tumours. For example, *KRAS* mutations are prevalent in colorectal cancer and these aggressive tumours do not respond to established chemotherapies [74]. An attractive hypothesis is that macropinocytosis in these tumours might represent a novel vulnerability that can be exploited as a treatment. Evidence that macropinocytosis is a feature of these tumours both *in vitro* and *in vivo* underscores this idea (table 1). In addition, oncogenic Ras mutations are prevalent in many other malignancies, including multiple myeloma, melanoma, uterine cancer, thyroid cancer, acute myeloid leukaemia and stomach cancer [75]. Future endeavours could be focused on delineating the possible role that macropinocytosis might have in augmenting metabolism in these cancers and evaluating whether macropinocytic inhibition can be harnessed as a therapy in these settings.

In addition to *RAS* mutations, studies have linked the induction of macropinocytosis to other oncogenes, as well as tumour suppressors. EGFR activation, which acts upstream of wild-type Ras, is a classic example of a macropinocytic driver; therefore, tumours with EGFR gain-of-function mutations or EGFR overexpression might present with enhanced macropinocytic capacity. Overexpression of EGFR is an attribute of both triple-negative breast cancer and inflammatory breast cancer [76]. Studies in breast cancer cells have linked EGFR activation in response to growth factor stimuli to enhanced macropinocytic uptake [77], but it is uncertain whether targeting macropinocytosis in breast cancer cells would have deleterious effects on viability. Along the same lines, macropinocytosis might be a robust feature of tumours with oncogenic mutations in other receptor tyrosine kinases, such as PDGFR and HER2/ErbB2 [78]. Another classic

example of oncogene-driven macropinocytosis is uptake caused by Src signal transduction. Activating mutations in c-Src, a non-receptor tyrosine kinase, are not very common in cancer, but enhanced pathway activation driven by elevated protein levels is observed in some colon, breast and prostate tumours [79]. Macropinocytosis depends on the activity of Rac1, which orchestrates the actin cytoskeleton dynamics that lead to membrane ruffling and macropinosome formation. Some sun-exposed melanomas harbour the *RAC1*^{P29S} mutation, which leads to Rac1 protein that exhibits enhanced inherent GDP/GTP nucleotide exchange [80]. Hyperactive Rac1^{P29S} leads to an enhanced macropinocytic response that is dependent on Dock1, a Rac-specific guanine nucleotide exchange factor implicated in a variety of cancers [14]. Interestingly, these studies showed that a Dock1-selective inhibitor suppresses macropinocytosis in these *RAC1*-mutant cells and future work will hopefully determine whether this compound has anti-tumour effects in preclinical models of melanoma. Similar to melanoma cells, some breast cancer cell lines harbour the *RAC1*^{P29S} mutation, potentially making these tumours susceptible to Dock1-inhibition [14].

The best-characterized tumour suppressor that controls macropinocytosis is *PTEN*. PTEN is a negative regulator of P13K signalling, which has a well-established role in controlling macropinocytosis. *PTEN* is one of the most frequently disrupted tumour suppressors in cancer and macropinocytosis induction driven by the loss of *PTEN* has been demonstrated in both bladder and prostate cancer (table 1). As PTEN is a phosphatase that suppresses P13K signalling, a loss in *PTEN* expression unleashes P13K-driven cascades that control actin reorganization, as well as macropinosome closure [81,82]. From this perspective, we would predict that tumours harbouring gain-of-function mutations that enhance P13K signal potentiation are robustly macropinocytic. Oncogenic mutations in *PIK3CA*, which encodes the catalytic subunit of P13K, are quite common in cancer [83]. It might be conceivable that treatment strategies tailored for P13K aberrations are effective, at least in part, owing to their inhibitory properties affecting macropinocytosis induction.

Hyperstimulation of macropinocytosis might not be advantageous in terms of supporting tumour cell viability as it leads to methuosis [63]. This phenomenon has been mainly observed in blastomas that affect immature undifferentiated precursor cells in the brain and nervous system. Cell death is likely attributed to vacuolar expansion that is beyond the trafficking tolerances of these cells and as a result, disrupts endocytic homeostasis. It is not clear whether methuosis is uniquely a feature of blastoma cells; however, evidence that synthetic lethality underlies the mutual exclusivity of oncogenic *KRAS* and *EGFR* mutations in lung cancer suggests that such cell death might occur in other settings [84]. Indeed, forced expression of both oncogenes led to loss of viability that was associated with vacuolarization and increased macropinocytosis [84]. Altogether, these studies highlight that macropinocytosis is highly regulated in tumours and underscore important considerations when designing therapeutic strategies centred on macropinocytosis, especially from the perspective of metabolic interventions and nanomedicines.

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