Review Article

Macropinocytosis: mechanism and targeted therapy in cancers

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Received September 14, 2020; Accepted November 16, 2020; Epub January 1, 2021; Published January 15, 2021

Abstract: Macropinocytosis is a form of endocytosis which provides an effective way for non-selective uptakes of extracellular proteins, liquids, and particles. The endocytic process is initiated by the activation of the growth factors signaling pathways. After activation of the biochemical signal, the cell starts internalizing extracellular solutes and nutrients into the irregular endocytic vesicles, known as macropinosomes that deliver them into the lysosomes for degradation. Macropinocytosis plays an important role in the nutritional supply of cancer cells. Due to the rapid expansion of cancer cells and the abnormal vascular microenvironment, cancer cells are usually deprived of oxygen and nutrients. Therefore, they must transform their metabolism to survive and grow in this harsh microenvironment. To satisfy their energy needs, cancer cells enhance the activity of macropinocytosis. Therefore, this metabolic adaptation that is used by cancer cells can be exploited to develop new targeted cancer therapies. In this review, we discuss the molecular mechanism that actuates the process of macropinocytosis in a variety of cancers, and the novel anti-cancer therapeutics in targeting macropinocytosis.

Keywords: Macropinocytosis, growth factors, cancer mechanism, targeted cancer therapy

Introduction

Cancer macropinocytosis is an endocytic uptake process by which cancer cells internalize extracellular proteins or necrotic cell debris and deliver them to lysosomes for further degradation [1-4]. The decomposition of these macropinocytic cargos contributes to the supply of the desperately needed amino acids that support cancer cells' survival and growth. As shown in Figure 1, the molecular mechanism of macropinocytosis in cancer cells is quite complicated. The Ras and PI3K signaling pathways are the most common in cancer macropinocytosis (Table 1). The macropinocytic induction is associated with the activation of oncogenes (e.g., RAS or EGFR) or deactivation of tumor suppressor genes (e.g., PTEN) in cancer cells [5-7]. Macropinocytosis is closely related to actin cytoskeleton remodeling and the macropinosomes’ generation process includes plasma membrane ruffling, macropinocytic cup formation and closure, and detachment from the plasma membrane. Several pivotal regulators of actin polymerization, such as small GTPases (e.g., Ras, Rac, Cdc42, Arf6, and Rab5), p21-activated kinase 1 (Pak1), and phosphoinositide 3-kinase (PI3K), have been linked to the formation of plasma membrane protrusions and macropinocytic activity [8]. Interestingly, RAS and PI3K activation is related to the stimulation of receptor tyrosine kinases (RTK), such as platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR) [1, 9]. Certainly, there are also negative-regulatory factors that weaken macropinocytosis. For instance, the PTEN phosphatase blocks the PI3K signaling pathway by converting PI (3, 4, 5) P3 to PI (4, 5) P2. Importantly, PI (3, 4, 5) P3 is necessary for plasma membranes’ ruffling and macropinosomes’ closures [7]. In addition, one particular study demonstrated that mTORC1 could suppress the lysosomal catabolism of extracellular proteins [2]. Of course, there are
also other specific pathways, such as the Wnt signaling pathway, that play an important role in micropinocytosis in colorectal and bladder cancers. Therefore, with the identification of the molecular mechanism of micropinocytosis, novel therapeutic approaches can be developed to treat cancers. At present, the major therapeutic methods in targeting micropinocytosis include small molecules and antibodies. Intriguingly, nucleic acids, as an emerging therapeutic drug, might become the third modality in cancer treatments using micropinocytosis [10]. Here, we aim to summarize the role of macropinocytosis in different type of cancers, including pancreatic, lung, colorectal, bladder, prostate, brain and nervous system cancers. Meanwhile, we discuss three major therapeutic modalities, including chemotherapy, immunotherapy, and nucleic acid therapy that are used for targeting macropinocytosis in cancers.

**Macropinocytosis in different types of cancer**

**Pancreatic cancer**

Approximately 95% of pancreatic ductal adenocarcinomas (PDAC) have poor prognosis and are associated with KRAS mutation [11]. Using mouse models [12, 13] and human specimens [14] of KRAS-driven PDAC, previous studies have shown that macropinocytosis acts as a nutrient supply pathway. It is interesting to note that macropinocytic inductions can be detected in PDAC autochthonous models. $Kras^{LSL-}$
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The initiation of RAS-driven macropinocytosis is closely associated with several signaling pathways, such as the RAS and PI3K pathways (Table 1). Firstly, growth factors that induce macropinocytosis, specifically bind to RTKs (e.g., EGFR, PDGFR), which stimulate membrane ruffling through the activation of Ras. Rac1 and Cdc42 activations by Ras are crucial for membrane ruffling and macropinocytic cups formation and the stimulation of Pak1 leads to induce actin polymerization. In addition, PI (3, 4, 5) P3 that can coordinate actin remodeling, is generated with the increase of PI3K. Therefore, both Pak1 and PI (3, 4, 5) P3 are important for the macropinocytic cups closure [1, 9]. However, there are other factors that affect the process of macropinocytosis. For instance, Rac1 and Cdc42 require activation under appropriate submembranous alkaline pH that is maintained by Na\(^+\)/H\(^+\) exchanger (NHE) and vacuolar H\(^+\)-ATPase (v-ATPase) (Figure 1) [1, 5, 18]. Furthermore, Rac1 activity was markedly restrained in KRAS-driven PDAC cells with low syndecan 1 (SDC1, a protein mediator of macropinocytosis) expression [19]. The mechanistic targeting of rapamycin (mTOR) is associated with the presence of two distinct multiprotein complexes: mTORC1 and mTORC2 [20]. As a critical regulator of macropinocytosis, mTORC1

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Molecular driver</th>
<th>Macropinocytic cargo</th>
<th>Signaling Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pancreatic cancer</td>
<td>KRAS</td>
<td>proteins</td>
<td>RAS, PI3K</td>
<td>[1, 9]</td>
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<td></td>
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<td>proteins, ATP</td>
<td>RAS, PI3K, Rac</td>
<td>[26, 28-31]</td>
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<td>KRAS</td>
<td>proteins</td>
<td>RAS, PI3K, Wnt</td>
<td>[39, 42, 43]</td>
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<td>HRAS</td>
<td>proteins</td>
<td>RAS, PI3K, Wnt</td>
<td>[46, 48]</td>
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<tr>
<td>brain and nervous system cancers</td>
<td>HRAS</td>
<td>proteins</td>
<td>RAS, PI3K</td>
<td>[49]</td>
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<td>GBM</td>
<td>HRAS</td>
<td>proteins</td>
<td>RAS, PI3K</td>
<td>[49]</td>
</tr>
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<td>neuroblastoma</td>
<td>IGF-1</td>
<td>proteins</td>
<td>PI3K</td>
<td>[57]</td>
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<td>TrkA</td>
<td>proteins</td>
<td>?</td>
<td>[58]</td>
</tr>
<tr>
<td>prostate cancer</td>
<td>PTEN</td>
<td>necrotic cell debris</td>
<td>PI3K</td>
<td>[7, 27, 61-63]</td>
</tr>
</tbody>
</table>

\(^{G12D/-}\cdot p53^{loxP/loxP}\) (KP) mice models could progress to PDAC via restricting the expression of the pancreatic cre-recombinase. In vitro, the levels of macropinocytic induction in KP mice were significantly higher than those in the wild-type group [12]. Similarly, the stimulation of macropinocytosis was also clearly increased in all human PDAC specimens [14]. Therefore, macropinocytosis is a PDAC metabolic adaptation that allows PDAC cells to uptake extracellular proteins and other small molecules [15].

As one of the extracellular proteins, serum albumin is the most abundant. It is a legitimate inference that PDAC cells afford to take up extracellular serum albumin by macropinocytosis [16]. Using immunofluorescence microscopy that tracked labeled amino acids and albumin, an in vitro study demonstrated that the presence of macropinosomes is involved in the degradation of proteins in lysosomes [14]. The free amino acids, such as glutamine, are produced by breakdown of proteins. As a consequence, PDAC cells have the capacity to conduct tricarboxylic acid (TCA) cycle due to the presence of these free amino acids [17]. Taken together, PDAC cells adapt to promote the catabolism of serum albumin when essential amino acids are lacking.

Table 1. Example of micropinocytosis in different cancer types
is activated by amino acids that are produced by lysosomal degradation of internalized extracellular proteins and in response to growth factors, signaling. Surprisingly, the activity mTORC1 can not only stimulate biosynthetic pathways but also inhibit lysosomal proteins’ degradation [21, 22]. Importantly, one particular study has shown that mTORC1 inhibition could significantly increase lysosomal degradation of internalized proteins from the extracellular environment [2]. Moreover, mTORC2 plays a significant role in macropinocytosis and lysosomal degradation of extracellular proteins. One particular study demonstrated that mTORC2 disruption can deprive the ability of lysosomes to scavenge proteins, leading to the inhibition of proliferation and induction of apoptosis [23].

Lung cancer

Similarly, KRAS oncogene mutations are also the most common in non-small cell lung cancer (NSCLC) [24, 25]. It is known that NSCLC cells, driven by KRAS mutations, stimulate macropinocytosis to survive and proliferate in nutrient-deprived environments (Table 1) [26]. Interestingly, KRAS addiction refers to NSCLC cell lines that carry KRAS mutations and that are dependent on the expression of the KRAS oncogene for their viability [27]. A recent report showed that KRAS-addicted NSCLC cells trigger macropinocytosis following direct binding between galectin-3 and integrin αvβ3 receptor on their cell surfaces [28]. Integrin αvβ3-positive cells induce the formation of macropinosomes that promote the internalization of extracellular proteins. This process depends on galectin-3 and integrin αvβ3, as the consumption of either molecule reduces the activity of macropinocytosis (Figure 1). In addition, AMP-activated protein kinase (AMPK) also plays a significant role in lysosomal induction in KRAS-mutant NSCLC. For example, a study has elaborated that energy depletion could activate AMPK that prompts the dephosphorylation and nuclear transport of transcription factors, such as Tfe2 and Tfe3. Importantly, activated Tfe3 can promote the expression of lysosomal genes in the nucleus (Table 1; Figure 1) [29]. Hence, AMPK is required for the growth of KRAS-driven NSCLC by indirectly regulating the expression of lysosomal genes. Remarkably, a recent report has also demonstrated that NSCLC cell lines could survive in glucose starvation conditions through Rac-driven macropinocytosis which facilitated the internalization of extracellular proteins. Noteworthy, PI3K was shown to be the crucial upstream activator of macropinocytosis that was mediated by the Rac-Pak signaling (Table 1; Figure 1) [30].

In addition to the internalization of extracellular proteins, lung cancer cells could also assimilate extracellular ATP through macropinocytosis to maintain viability under low energy environments (Table 1) [31]. Therefore, the levels of extracellular ATP in lung cancer are much higher than those in normal tissues. Furthermore, several studies indicated that NSCLC cells could internalize extracellular ATP by macropinocytosis to enhance their intracellular ATP levels, which lead to cancer cell growth and drug resistance [32-34]. In summary, ATP internalization through macropinocytosis is a major example of macropinocytic cargo changes in cancer cells. According to different circumstances, the identification of new macropinocytic cargoes that play important roles in cancer cells’ proliferation or drug resistance can result in the development of novel cancer targeted therapies.

Colorectal cancer

Similar to lung and pancreatic cancers, mutations in KRAS oncogene are also the predominant oncogenic alterations in colorectal cancer [35]. It is well known that macropinocytosis plays a critical role in the proliferation and growth of cancer cells driven by oncogenic KRAS. Several studies demonstrated that macropinocytosis was conducive to cancer cells growth by improving the activity of mTORC1 [36-38]. Interestingly, the activation of the PI3K/Akt signaling pathway is closely linked to mTORC1 activity [20]. Furthermore, study showed that the activation of the protein kinase CK2 in colorectal cancer plays an important role in the PI3K/Akt signaling pathway that could activate mTORC1. By activating Akt through phosphorylation, CK2 could enhance the PI3K/Akt signaling pathway (Table 1) [39]. Subsequently, Akt inactivates tuberous sclerosis complex 2 (TSC2) through phosphorylation, leading to TSC1/2 lysosomal separation and the activation of Rheb that activates mTORC1 on the lysosomal membrane [40]. In addition, another study demonstrated that the Rag activation can recruit mTORC1 to the surface of the lysosome (Figure 2) [41]. Therefore, small
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GTPases Rag or Rheb plays a vital role in regulating mTORC1 activity, which stimulates protein synthesis and cell growth [20].

Moreover, the Wnt signaling pathway is also an important player in colorectal cancer through its role in switching metabolic pathways according to nutrient stress requirements. For instance, a recent report indicated that the activation of the Wnt signaling pathway and the tumor suppressors APC, or Axin deletions, markedly increase macropinocytosis in colorectal cancer cells (Table 1) [42]. Interestingly, Wnt growth factors induce the initiation of macropinocytosis by binding to co-receptors Frizzled (Fz) and LDL receptor-related protein 6 (Lrp6) on the cell surface. The canonical Wnt pathway signals target the activation of WNT genes by stabilizing the transcriptional activator β-catenin, which can translocate into the nucleus and bind to the TCF transcriptional factors (Table 1) [43]. In colorectal cancer cells, the study demonstrated that β-catenin stabilization benefits from the functional incapacitation of the complex components, APC, Axin1, GSK3β, and CK1, that could destroy β-catenin. Importantly, it was also found that the ATPase vacuolar protein sorting 4 (Vps4), contributes to the accumulation of β-catenin in colorectal cancer cells [42]. The increased expression of Wnt3a, a WNT gene expression product, resulted in an increased uptake of extracellular proteins by stimulating actin rearrangements, which could be blocked by the inhibitor of NHE [42]. Fortunately, previous studies have shown that the protein arginine methyltransferase 1 (PRMT1) is important for Wnt signaling [44, 45]. A study provided an intriguing evidence showing that Wnt-driven macropinocytosis requires PRMT1 in colorectal cancer cells (Table 1) [42]. Therefore, under nutrient-limited conditions, the Wnt signaling pathway plays an important role in macropinocytosis in colorectal cancer cells (Figure 3).

Bladder cancer

Interestingly, bladder cancer cells that are most commonly driven by HRAS mutations, also exhibit enhanced macropinocytosis for extracellular proteins uptake [13]. Bacille Calmette-Guérin (BCG), an attenuated strain of Mycobacterium bovis, is absorbed into bladder cancer cells by macropinocytosis. It is also well known that BCG can be effectively used in the treatment of superficial bladder cancer [46]. However, BCG mechanism of action in the treatment of bladder cancer is still unclear [47]. Interestingly, these HRAS-driven or KRAS-driven bladder cancer cells allowed the assimili-
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Figure 3. The Wnt growth factor triggers macroinocytosis by binding to co-receptors Frizzled (Fz) and LDL receptor-related protein 6 (Lrp6) on the cell surface. The stabilized upstream effector, β-catenin, can translocate into the nucleus and bind with TCF transcriptional factors to promote the expression of the downstream effector, Wnt3a, which can increase the uptake of extracellular proteins. The stabilization of β-catenin benefits from the functional incapacitation of the complex components, APC, Axin1, GSK3β, and CK1, that could destruct β-catenin. The vacuolar protein sorting 4 (Vps4) contributes to β-catenin accumulation in cancer cells. In addition, Wnt-driven macroinocytosis may require the protein arginine methyltransferase 1 (PRMT1) and Pak1 in cancer cells. However, the upstream effectors of PRMT1 and Pak1 in Wnt signaling pathway, are still unclear.

In addition to Ras signaling pathway, the activation of the Wnt signaling pathway has also been demonstrated to induce macroinocytic uptake in bladder cancer cells. Interestingly, several studies demonstrated that five negative regulators (DKK2, KREMEN1, Nkd1, SMAD4, and MAPK91) of the Wnt signaling pathway were identified by performing a whole-genome shRNA screen in RAS-wild-type bladder cancer cells [48]. The knockdown of these genes caused activation of the canonical Wnt signaling pathway, which stimulated macroinocytic uptake through β-catenin accumulation and translocation. The use of a recombinant Wnt3a protein or the expression of a constitutively active form of β-catenin resulted in robust macroinocytosis in bladder cancer cells. Similar to the Ras pathway, it was also found that the Wnt pathway stimulates macroinocytosis in a Pak1-dependent mode that was demonstrated using the Pak1 inhibitor IPA-3 (Table 2; Figure 3). Therefore, the activation of the Wnt pathway is necessary to oncogenic Ras-driven macroinocytosis in bladder cancer cells (Table 1) [48]. In short, the Wnt signaling pathway may be closely related to Ras and PI3K signaling pathways through pak1, suggesting that these sig-
## Table 2. Example of therapeutic modalities by exploiting micropinocytosis in cancers

<table>
<thead>
<tr>
<th>Therapeutic modalities</th>
<th>drugs</th>
<th>Mechanism</th>
<th>Type of cancer</th>
<th>Reference</th>
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<td></td>
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<td>NHE inhibitor</td>
<td>EIPA</td>
<td>impact on submembranous alkaline pH</td>
<td><em>RAS</em>-driven cancer</td>
<td>[1, 42, 73]</td>
</tr>
<tr>
<td>v-ATPase inhibitor</td>
<td>bafilomycin A1</td>
<td>impact on lysosomal acidic pH</td>
<td><em>RAS</em>-driven cancer</td>
<td>[74]</td>
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<td>EGFR inhibitor</td>
<td>gefitinib</td>
<td>inhibit the macropinocytosis pathway</td>
<td>NSCLC</td>
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<td>Galectin-3 inhibitor</td>
<td>GCS-100</td>
<td>inhibit the macropinocytosis pathway</td>
<td>Lung cancer or pancreatic cancer</td>
<td>[28]</td>
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<td>DOCK1 inhibitor</td>
<td>TB00P</td>
<td>repress DOCK1-mediated macropinocytosis</td>
<td><em>RAS</em>-driven cancer</td>
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<td>actin inhibitors</td>
<td>blebbstatin</td>
<td>inhibit actin polymerization</td>
<td><em>RAS</em>-driven cancer</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>cytochalasin D</td>
<td>inhibit actin polymerization</td>
<td><em>RAS</em>-driven cancer</td>
<td>[78]</td>
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<td>PI3K inhibitors</td>
<td>Wortmannin, LY294002</td>
<td>inhibit PI3K signaling pathway</td>
<td><em>RAS</em>-driven cancer</td>
<td>[79]</td>
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<td>Pak1 inhibitor</td>
<td>IPA-3</td>
<td>impact on actin polymerization</td>
<td><em>RAS</em>-driven and WNT-driven cancer</td>
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<td>suppress proteins scavenging</td>
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<td>AMPK activator</td>
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<td>block mTORC1</td>
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<td>anti-cancer agents conjugation</td>
<td>albumin-conjugated DOX</td>
<td>increase anti-cancer drugs to cancer cells through micropinocytosis</td>
<td>PDAC</td>
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<td></td>
<td>nab-paclitaxel with gemcitabine</td>
<td>increase anti-cancer drugs to cancer cells through micropinocytosis</td>
<td>PDAC</td>
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<td>T-UPSM</td>
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<td>using macropinocytosis</td>
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<td>pancreatic cancer</td>
<td>[106]</td>
</tr>
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</table>
naling pathways work together in cancer macropinocytosis.

**Brain and nervous system cancers**

The activation of the Ras and PI3K signaling pathways are also significantly involved in the induction of macropinocytosis in glioblastoma (GBM) cells [49]. EGFR and PDGFR are activators of macropinocytosis in GBM cells [49]. Interestingly, previous studies have demonstrated that EGFR and PDGFR were upregulated in gliomas [50-52]. In gliomas, K-Ras and H-Ras are activated after stimulation by EGFR and PDGFR. Subsequently, Rac1 and Cdc42 activate both Arf6 and Pak1 that are initiators of actin polymerization. In addition, the transformation of PI (3, 4, 5) P3 by PI (3, 4) P2 under the control of PI3K, plays an important role in the formation of macropinosomes (Table 1) [49]. Therefore, the activated Ras in GBM cells can enhance the levels of intracellular macropinosomes and extracellular small molecules' internalizations in nutrient-poor environments (Figure 1). Fortunately, this property has been used to study the absorption of potential therapeutic nano-drugs that could cross the blood-brain barrier [53]. However, the hyperstimulation of macropinocytosis in GBM cells that was induced by over-expressed Ras or by small molecules, could lead to methuosis, a form of cell death [54]. For example, one particular study has demonstrated that the 3-(5-methoxy-2-methyl-1Hindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (MOMIPP) can induce massive macropinocytosis, leading to methuosis in GBM cells (Table 2) [55]. Similarly, uncontrolled macropinocytotic effects were observed in GBM cells that were treated with Bacoside A, and that resulted in tumor cell death (Table 2) [56]. This event may be dependent on high intracellular calcium mediated by the phosphorylated calcium/calmodulin-dependent protein kinase IIA (CAMK2A).

Furthermore, macropinocytosis is also easy to find in medulloblastoma and neuroblastoma. For example, two different types of macropinocytosis were found in neuroblastoma cells, which were induced by insulin-like growth factor 1 (IGF-1) and phorbol 12-myristate 13-acetate (PMA) (Table 1) [57]. Interestingly, IGF-1 induced macropinocytosis predominantly occurred in the cell bodies and required the PI3K signaling for macropinosomes formation, while PMA induced macropinocytosis in the neurites did not require PI3K signaling. Other than GBM, the hyperstimulation of macropinocytosis, in medulloblastoma and neuroblastoma, have also been observed to result in cell death. In fact, uncontrolled macropinocytosis has been detected when medulloblastoma cells, with TrkA overexpression, were treated with nerve growth factor (NGF), leading to tumor cell death (Tables 1, 2) [58]. Moreover, in neuroblastoma cells, methamphetamine (MET) induced hyperstimulation of macropinocytosis that was mediated by activated Ras/Rac1, in response to PI3K signaling pathways, eventually resulting in tumor cell death (Table 2) [59].

**Prostate cancer**

Loss of the tumor suppressor gene PTEN is most common in prostate cancer [60]. PTEN is as a lipid phosphatase that plays an important role in opposing the activity of the PI3K pathway that signals through transforming PI (3, 4, 5) P3 into PI (4, 5) P2 (Table 1) [27, 61]. Therefore, PTEN loss in prostate cancer results in increased PI (3, 4, 5) P3 [62]. Because PI (3, 4, 5) P3 is necessary for the plasma membrane ruffling and macropinosomes closure, PTEN loss in prostate cancer cells may be conducive in exploiting macropinocytosis to maintain survival and proliferation of cancer cells in nutrient-depleted conditions. However, the loss of PTEN alone is not enough to trigger macropinocytosis. Cells can effectively respond to energy shortages by activating AMPK (Table 1) [63]. The induction of macropinocytosis is also mediated by AMPK, which functions to activate Rac1 that promotes macropinosomes’ formation (Table 1: Figure 1) [7]. Strikingly, AMPK can also improve the effective lysosomal degradation of internalized proteins by antagonizing the mTORC1 pathway (Figure 1) [9]. One particular study has provided that sepantronium bromide could block mTORC1 through AMPK activation (Table 2) [64].

To proliferate and survive in nutrient poor environments, PTEN-deficient prostate cancer cells internalize and catabolize necrotic debris and extracellular proteins by macropinocytosis [65]. It is surprising that serum albumin uptake in PTEN-deficient prostate cancer cells is independent of macropinocytosis, and potentially depends on macropinocytosis-independent
pathways for its internalization [7]. Interestingly, several studies indicated that prostate cancer associated fibroblasts could afford to ingest serum albumin to support cancer cells growth via macropinocytosis [66, 67]. In fact, PTEN-deficient prostate cancer cells assimilate necrotic cell debris in nutrient-limiting conditions, solely by macropinocytosis. In prostate cancer cells, necrotic cell debris, as macropinocytic cargoes, are degraded to produce amino acids and lipids (Table 1; Figure 1) [7]. Both amino acids and lipids that are generated by catabolism of necrotic cell debris are beneficial to biosynthetic metabolism and material storage. Therefore, further studies are needed to explore the extent AMPK effects on cellular macropinocytosis in other pathological contexts and whether necrotic cell debris could be exploited as a nutrient source to support growth in other cancer types [68].

**Therapeutic modalities for exploiting cancers macropinocytosis**

**Chemotherapy**

Since cancer cells can absorb extracellular small molecules, such as serum albumin, necrotic debris and ATP, through macropinocytosis, small molecule drugs can therefore be used to block the macropinocytosis pathway and treat cancers by disrupting the metabolic activity of cancer cells [69-72]. For example, numerous studies have shown that 5-(N-ethyl-N-propyl) amiloride (EIPA) can inhibit both macropinocytosis and aevin polymerization by blocking NHE (Table 2) [1, 42, 73]. Similarly, v-ATPase inhibitors, such as bafilomycin A1, that have an impact on lysosomal acidic pH, can also result in the inhibition of macropinocytosis and reduction of intracellular amino acid levels (Table 2) [74]. In addition, inhibitors of the signaling pathway networks that mediate the activity of macropinocytosis are also effectively used in anti-cancer treatment. For instance, a recent study indicated that the EGFR inhibitor, gefitinib, can suppress the macropinocytosis pathway in NSCLC cells (Table 2) [75]. Analogously, one particular study has demonstrated that the Galectin-3 inhibitor, GCS-100, could disrupt the interaction between Galectin-3 and integrin αvβ3, thereby inhibiting the macropinocytosis pathway in KRAS-addicted lung and pancreatic cancer cells (Table 2) [28]. In fact, DOCK1 is a Rac-specific guanine nucleotide exchange factor (GEF) and the selective inhibitor of DOCK1, 1-(2-(3'-trifluoromethyl)-1,1'-biphenyl)-4-yl)-2-oxoethyl)-5-pyrrolidinyl-sulfonyl-2(1H)-pyridone (TBOPP), can repress DOCK1-mediated macropinocytosis in RAS-transformed cancer cells (Table 2) [76]. There are other frequently used small molecule inhibitors of macropinocytosis, such as actin inhibitors (blebbstatin [77], cytochalasin D [78]) and PI3K inhibitors (Wortmannin, LY294002 [79]) (Table 2). Moreover, recent studies reported that a novel lysosomal inhibitor, palmitoyl-protein thioesterase 1 (PPT1), can suppress lysosomal activity that play a critical role in degrading proteins during macropinocytosis (Table 2) [80, 81]. Interestingly, it was shown that better effects could be obtained when mTOR inhibitors are delivered in combination with inhibitors that could suppress the uptake or the process of lysosomal extracellular proteins scavenging [2, 23].

In addition to blocking cancer cell macropinocytosis, another anti-cancer method is to cause a significant increase in cancer cell catastrophic macropinocytosis by using related inhibitors or inducers. For example, one particular study has demonstrated that treatment with the CK2 inhibitor, silmitasertib, and gives rise to large number of vacuoles that derived from massive macropinocytosis, resulting in colorectal cancer cells methuosis-like death (Table 2; Figure 2) [39]. Similarly, several studies have shown that inducers can also cause massive macropinocytosis that lead to methuosis in brain and nervous system cancers cells (Table 2) [55, 56, 58, 59].

Lastly, researchers also made great efforts to exploit macropinocytosis for delivering anti-cancer agents into cancer cells [71]. For example, a study demonstrated that albumin-conjugated doxorubicin (DOX) could be internalized into KRAS-driven PDAC cells by macropinocytosis, thereby releasing DOX in the PDAC cells to exert its toxic effect (Table 2) [82]. Similarly, it has been shown that macropinocytosis can be exploited for internalization of drug that combined albumin nanoparticles (nab-paclitaxel) with gemcitabine for PDAC therapy (Table 2) [83]. Interestingly, a recent research indicated that triptolide prodrug-loaded UPSM (T-UPSM) could be absorbed into KRAS-mutant PDAC cells through macropinocytosis, which pH triggered the rapid release of drug in lysosomes.
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(Table 2) [84]. Furthermore, Tubeimoside-1 (TBM1) can induce macropinocytosis and increase 5-FU transport into colorectal cancer cells where it promotes synergistic anti-cancer effects (Table 2) [85]. Analogously, a recent study has shown that MOMIPP could also induce massive macropinocytosis, leading to increased uptake of temozolomide by GBM cells and resulting in additional anti-cancer effects (Table 2) [49]. Therefore, understanding the mechanism of cancer cells’ macropinocytosis is very helpful for cancer treatment.

**Immunotherapy**

Currently, therapeutic methods, such as monoclonal antibodies (mAbs) and vaccines, are widely used in cancer immunotherapy [86]. Interestingly, recent studies have elaborated that mAbs could be internalized into cancer cells through macropinocytosis [87, 88]. In addition, macropinocytosis can be selectively upregulated in different types of cancer. Therefore, mAbs that can be effectively internalized via macropinocytosis are meaningful for the development of cancer therapies. For instance, bevacizumab nanoparticles, which can be internalized by macropinocytosis, were applied to target intracellular VEGF in NSCLC (Table 2) [89]. In recent years, more studies have reported on the application of antibody-drug conjugates (ADCs) in cancer therapy [90-92]. Intriguingly, one particular study has indicated that the ScFv antibody that was based on albumin domain and its cytotoxic conjugate, exhibits characteristics of targeted-EGFR, intensive-macropinocytosis and cytotoxicity, resulting in apparent growth inhibition of KRAS-mutant pancreatic cancer (Table 2) [93].

In addition to mAbs, vaccines that are associated with macropinocytosis also play an important role in cancer immunotherapy. For example, one particular study has shown that onco- geneic activation of macropinocytosis resulted in BCG internalization into bladder cancer cells (Table 2) [46]. Additionally, mycobacterium tuberculosis vaccine (MTBVAC) could also be internalized into bladder cancer cells through macropinocytosis, resulting in the inhibition of cancer cells’ growth (Table 2) [94]. Thus, MTBVAC can be used as a new immunotherapy drug for bladder cancer. Similarly, ApoE3-incorporated biomimetic nanoparticle is also very likely to be used as a safe and effective nanovaccine for cancer immunotherapy by exploiting the macropinocytosis pathway (Table 2) [95]. Briefly, both mAbs and vaccines can be effectively applied to cancer immunotherapy through exploiting macropinocytosis.

**Nucleic acid therapy**

Nucleic acid therapy may become another emerging therapeutic modality that could use the role of macropinocytosis in cancer cells [10]. Currently, the fastest clinically progressing nucleic acid drugs for cancer therapy are antisense oligonucleotides (ASOs), DNA, short interfering RNA (siRNA), microRNA (miRNA), and messenger RNA (mRNA) (Figure 4) [96]. However, due to the cell membrane barrier, these nucleic acids require carriers to enter the cell. Fortunately, there are several delivery systems, such as lipids [97, 98], polymers [99], and peptides [100], that can be used to carry nucleic acids into cancer cells and via macropinocytosis. For example, translationally controlled tumor protein (TCTP) ASOs that carried by lipids can be internalized into castration resistant prostate cancer cells through macropinocytosis, resulting in decreased expression of TCTP, which is tightly linked to cell growth (Table 2) [97]. Interestingly, siRNA, which is carried by lipofectamine, can drive the suppression of the transcription factor EB (TFEB) in KRAS-mutant cancer cells, leading to a substantial reduction in the lysosomal ability to degrade extracellular proteins (Table 2) [101]. Similarly, AGMA1 polyamidoamine that effectively carry siRNAs can be absorbed into prostate cancer cells by macropinocytosis and cause gene silencing without inducing cytotoxicity [102]. Furthermore, one particular study demonstrated that gold nanoparticles modified by cell-penetrating peptides (CPPs) can significantly increase cellular uptake and the absorption rate of nucleic acid drugs [103].

Interestingly, in addition to these carriers (e.g., lipids, polymers, and peptides), inorganic nanoparticles [53] and extracellular vesicles (EVs) [104-106] can also carry nucleic acids into cancer cells through macropinocytosis. For example, the nanostructure, lipoprotein, which carries the activating transcription factor-5 (ATF5) siRNA, can cross the blood-brain barrier, and be internalized by Ras-driven GBM cells via macropinocytosis. Subsequently, the release of ATF5 siRNA in the GBM cells causes the inhibi-
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In addition, exosomes can carry siRNA that targets oncogenic \( KRAS^{G12D} \), into pancreatic cancer cells through macropinocytosis, which inhibits cancer cell growth and increases overall survival (Table 2) [106]. Therefore, research on the use of macropinocytosis for intracellular delivery of nucleic acids drugs to cancer cells, is needed in the future.

Conclusions and perspectives

Under nutritional stress, cancer cells can initiate macropinocytosis through the activation of oncogenes and related complex signaling pathways, or the deactivation of tumor suppressor genes. The macropinocytic cargos may be extracellular proteins, ATP, necrotic cell debris or other macromolecules. Fortunately, an enhanced macropinocytic activity has been observed in various types of cancer. Not only does macropinocytosis provide a survival possibility under nutritional deficiencies, but it also provides the potential for tumors to limitlessly grow in harsh tumor microenvironments. In fact, the molecular mechanism that drive macropinocytosis in different cancers is quite complex. For example, the Ras, PI3K, and Wnt signaling pathways play significant roles in triggering macropinocytosis in different cancers. Therefore, it is extremely useful to better understand the molecular mechanism of macropinocytosis to enable the development of cancer targeted therapies.

At present, there are three main therapeutic modalities that exploit cancer macropinocytosis, including chemotherapy, immunotherapy,
and nucleic acid therapy. For this reason, developing novel, specific and effective drugs (e.g., small molecules, mAbs, vaccines, and nucleic acids) for targeting cancer macropinocytosis will be the focus of future research. For instance, in addition to the molecular targets in Table 2, these molecules (e.g., Rac1, Cdc42, SDC1, Rag, Rheb, Fz, Lrp6, Vps4, β-catenin, Wnt3a, and PRMT1) could be used as cancer therapeutic targets by exploiting macropinocytosis.

However, macropinocytosis is not the only cancerous metabolic pathway in nutrient-poor conditions. For example, there are other metabolic pathways, such as clathrin-mediated endocytosis (CME) [107, 108] and caveolae-mediated endocytosis (CVE) [108, 109], that can also effectively internalize small particles into cancer cells. Therefore, studies that focused on combining macropinocytosis inhibitors with other metabolic pathway inhibitors may improve cancers therapeutic outcomes.

Acknowledgements

The work was supported by The National Natural Sciences Foundations of China (No. 819-60456 and 81960457) and Jiangxi Provincial Natural Science Foundation (No. 20202ACB-216004). Thank you very much for the language editing service provided by editsprings.

Disclosure of conflict of interest

None.

Abbreviations

Pak1, p21-activated kinase 1; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinases; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; PDAC, pancreatic ductal adenocarcinomas; KP, KrasG12DΔN; p53loxP/loxP; TCA, tricarboxylic acid; NHE, Na⁺/H⁺ exchanger; v-ATPase, vacuolar H⁺-ATPase; SDC1, syndecan 1; mTOR, mechanistic targeting of rapamycin; NSCLC, non-small cell lung cancer; AMPK, AMP-activated protein kinase; TSC, tuberous sclerosis complex; Fz, Frizzled; Lrp6, LDL receptor-related protein 6; Vps4, vacuolar protein sorting 4; PRMT1, protein arginine methyltransferase 1; BCG, Bacille Calmette-Guerin; GBM, glioblastoma; MOMIPP, 3-(5-methoxy-2-methyl-1Hindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one; CAMK2A, calcium/calmodulin-dependent protein kinase IIa; IGF-1, insulin-like growth factor 1; PMA, phorbol 12-myristate 13-acetate; NGF, nerve growth factor; T-UPSM, triptolide prodrug-loaded UPSM; TBM1, Tubeimoside-1; mAbs, monoclonal antibodies; ADCs, antibody-drug conjugates; MTB-VAC, mycobacterium tuberculosis vaccine; siRNA, short interfering RNA; miRNA, microRNA; mRNA, messenger RNA; TCTP, translationally controlled tumor protein; TFEB, transcription factor EB; CPPs, cell-penetrating peptides; EVs, extracellular vesicles; ATF5, activating transcription factor-5; CME, clathrin-mediated endocytosis; CVE, caveolae-mediated endocytosis; RISC, RNA-induced silencing complex.

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