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肿瘤巨胞饮的分子调控机制研究进展

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摘要 巨胞饮是一个在真核生物中高度保守的、依赖肌动蛋白和溶酶体的内吞途径, 通过形成巨胞饮小体非选择性地摄取细胞外可溶性物质. 这一过程在多种生物学功能中发挥重要作用, 包括抗原递呈、细胞代谢调控以及药物递送等. 近年来, 巨胞饮因其在胰腺导管腺癌等恶性肿瘤发展和转移中的关键作用而受到广泛关注, 阻断巨胞饮能显著抑制肿瘤发展, 靶向巨胞饮治疗肿瘤具有重要的临床转化价值, 有关肿瘤巨胞饮发生的分子调控机制研究取得了许多重要研究进展. 本文在简要概述巨胞饮发生过程的基础上, 综述并讨论了最近关于巨胞饮在肿瘤细胞或肿瘤相关成纤维细胞响应营养压力时的信号转导途径, 其中包括EGFR-PAK途径、mTOR、Ca^{2+}-CaMKK2-AMPK-RAC1 途径、Ca^{2+}-ARHGEF2-RAC1 和DDR1-NF-κB-p62/SQSTM1-NRF2途径, 抗氧化因子NRF2介导的巨胞饮转录调控机制, 巨胞饮与另一营养获取途径细胞自噬的相互调控关系, 以及新发现的巨胞饮相关蛋白, 包括V-ATPase、SDC1、LYSET、TPC、TMEM206和Slc15A调控巨胞饮发生的作用和机制. 此外, 本文还讨论了未来巨胞饮在发生机制研究和抑制剂开发方面所面临的问题及可能的解决策略. 理解肿瘤巨胞饮发生的分子调控机制对于建立靶向巨胞饮途径的抗肿瘤策略具有十分重要的意义.

关键词 膜褶皱, 巨胞饮, RAC1, DDR1, NRF2, 肿瘤

生物进化早期, 生物体结构极其简单, 单细胞生物如阿米巴原虫(amoeba)能通过内吞作用(endocytosis)摄取细胞外物质, 维持体内的能量供应[1,2]. 尽管高等动物有完善的消化系统, 但是依然保留利用内吞作用摄取胞外物质的方式, 表明内吞作用在进化上高度保守, 在细胞内外物质交换和信号传递中具有独特作用[3,4]. 巨胞饮(macropinocytosis)作为一种特殊的内吞方式, 其发现距今已有近百年历史, 因其在免疫应答、能量代谢、药物递送, 尤其是调控胰腺导管腺癌(pancreatic ductal adenocarcinoma, PDAC)等恶性肿瘤生长方面的重要作用, 近年来受到高度关注[5~8]. 1883年, “固有免疫之父”俄罗斯科学家Élie Metchnikoff发现, 巨噬细胞能利用细胞质膜包围细胞外颗粒物质形成“吞噬体”(phagosome), 将细胞外物质摄入细胞内, Metchnikoff将这一过程称之为细胞吞噬(phagocytosis)[9]. Phagocytosis一词的词根来自古希腊语短语“φαγεῖν”(英: phagein译指“吃”)和“κύτος”(英: kytos译指“细胞”), 表示一种与摄食相关的过程, 即细胞摄(吃)入细胞外颗粒(大分子)的过程. 受Phagocytosis的启发, 1904年美国生理学家Samuel James Meltzer认为, 既然细胞能“吃”进细胞外物

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质, 那么细胞是否能喝(drinking)入细胞外物质? Meltzer提出“Cell drinking”的概念是巨胞饮过程的最早假说, 并无实验支持. 1931年胚胎学家Warren Lewis利用显微镜延时摄影技术首次在巨噬细胞和癌症细胞中观察到细胞通过细胞膜的褶皱和杯状结构摄取介质中的小滴, 并将其命名为胞饮作用(pinocytosis)[10]. “pino”源自希腊语“pinein”表示喝, “cyto”表示细胞, “osis”代表过程和状态. 随着电子显微镜和荧光显微镜技术的应用和进步, 巨胞饮过程已被清晰地观测到. 目前认为, 在细胞表面肌动蛋白的驱动下, 细胞膜形成褶皱, 进而包裹成一个直径约0.2~5 μm的巨胞饮小体(macropinosome), 非选择性地将胞外可溶性物质吞饮进入胞内, 一部分巨胞饮小体能重新循环至细胞膜并与其融合, 一部分巨胞饮小体与溶酶体融合后被降解. 巨胞饮与小胞饮(micropinocytosis)和吞噬作用的发生过程相似, 但又有显著区别. 三者都是细胞通过胞膜形成囊泡, 将外界的物质摄取进入细胞内部的过程, 都依赖细胞骨架的活动, 尤其是微管和微丝作用, 来驱动膜的改变和囊泡的形成. 与巨胞饮和吞噬作用相比, 小胞饮通常依赖网格蛋白小窝(clathrin-coated pits)形成膜泡, 且膜泡直径较小, 一般为100 nm[11], 而巨胞饮和吞噬作用均为依赖于肌动蛋白的内吞过程, 且膜泡直径较大, 0.2~5 μm. 小胞饮和吞噬作用对内吞底物有选择性, 底物需要与细胞膜受体结合, 即通过配体-受体结合实现内吞[12], 而巨胞饮非选择性地摄取可溶性物质[13]. 此外, 吞噬作用通常发生在特定的细胞中, 如中性粒细胞、巨噬细胞、单核细胞和树突状细胞等, 一般在免疫反应, 清除病原和细胞碎片中发挥作用[14], 而巨胞饮和小胞饮作用广泛存在于真核细胞中.

巨胞饮在多个生理和病理过程中发挥重要作用. 免疫细胞(如树突状细胞和巨噬细胞)能通过巨胞饮对病毒和细菌等外来抗原进行摄取和加工, 利于抗原呈递和免疫监视[5]; T细胞活化后, 其巨胞饮活性的增强能促进T细胞生长和增殖; 巨胞饮通过内吞细胞膜上的受体及其配体, 参与调节信号转导途径, 如表皮生长因子受体和胰岛素受体信号途径[15]; 巨胞饮小体循环回细胞膜能动态更新细胞膜成分, 参与细胞内膜-蛋白平衡的调节, 维持细胞形态[16]; 在炎症或损伤后, 巨胞饮有助于清除坏死细胞碎片和病原体, 维持组织稳态[17]; 通过摄取环境中的蛋白质(如白蛋白、血清蛋白等)、葡萄糖和脂类(如胆固醇)等营养物质, 巨胞饮在维持细胞代谢需求, 尤其是在维持肿瘤细胞的代谢稳态中发挥关键作用[6,18]. 恶性肿瘤细胞如PDAC肿瘤细胞具有高水平的巨胞饮活性, 能摄取肿瘤微环境中丰富的胶原蛋白并将它们转化为三羧酸循环的中间代谢产物、氨基酸和ATP(adenosine triphosphate)为细胞供能, 以适应自身的快速增殖和异常的血管微环境[19]. 基于巨胞饮的药物递送系统和靶向巨胞饮途径的抗肿瘤药物研发显示出巨大前景[20,21]. 基于白蛋白或脂蛋白的仿生纳米颗粒包裹的抗肿瘤药物能通过巨胞饮被肿瘤细胞吞饮进入胞内, 从而起到靶向杀伤肿瘤的作用. 近年来, 关于巨胞饮在肿瘤发生发展中的分子调控机制取得了巨大进步, 在巨胞饮启动的营养信号传导、转录调控机制、与另一营养获取途径细胞自噬的相互作用, 以及巨胞饮相关蛋白鉴定方面涌现出了大量优秀研究成果[6,20,22~28]. 本综述将在简述巨胞饮发生过程的基础上, 重点总结和讨论关于肿瘤巨胞饮分子调控机制的最新研究成果. 此外, 本综述还将讨论巨胞饮在发生机制和特异性抑制剂开发方面所面临的问题和可能的解决策略. 深入理解肿瘤巨胞饮发生的分子调控机制将有助于新的抗肿瘤靶点的发现和靶向巨胞饮途径抗肿瘤策略的研发.

1 巨胞饮的发生过程

中外学者的许多优秀综述中已经详尽阐述了巨胞饮的发生过程[29~31], 为了保证本文的可阅读性, 我们将简要总结巨胞饮的发生过程. 总体而言, 由于缺乏特异的巨胞饮小体膜标志蛋白, 人们对巨胞饮发生过程的机制还知之甚少, 研究进展缓慢. 巨胞饮的发生过程大致分为以下几个阶段(图1).

(1) 细胞膜的局部扩展和内陷. 肌动蛋白细胞骨架重排启动了巨胞饮的发生. 细胞膜的局部扩展, 也被称为膜褶皱(membrane ruffles), 由肌动蛋白的聚合形成. 细胞外信号, 如表皮生长因子(epidermal growth factor, EGF)与EGF受体(epidermal growth factor receptor, EGFR)结合[15,32]或GAS6(growth arrest-specific 6)与其受体UFO (tyrosine-protein kinase receptor UFO)结合[33], 激活小GTP酶RAS(rat sarcoma)和磷脂酰肌醇-3-激酶(phosphoinositide 3-kinase, PI3K). 激活的PI3K磷酸化4,5-二磷酸肌醇(phosphatidylinositol (4,5) bisphosphate, PI(4,5)P2), 生成的3,4,5-三磷酸肌醇PI(3,4,5)P3(phosphatidylinositol-3,4,5-trisphosphate, PIP3)在膜皱褶中不断增加[34]. PIP3在巨胞饮发生中的确切作用机制目前还不清楚, 其在局部区域的聚集可能招募带有PH结构

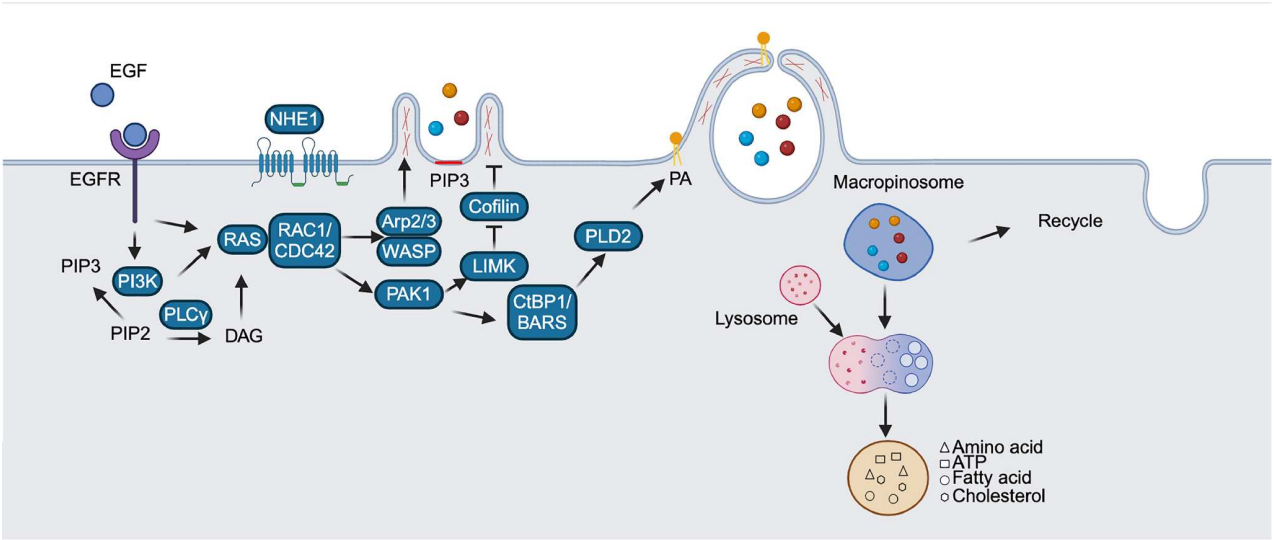

图 1 巨胞饮的发生过程(由BioRender.com制作). 在胞外信号如EGF作用下, 激活的EGFR能促进PI3K和RAS活性, 后者通过激活CDC42/RAC1活化Arp2/3复合物和PAK1, 从而促进肌动蛋白多聚化和杯状结构形成; 局部细胞膜内陷继续扩展, 形成一个较大的囊泡, PAK1通过活化CtBP1/BARS激活PLD2产生磷脂酸PA, 促进杯状结构封闭, 形成巨胞饮小体; 形成的巨胞饮小体一部分能与溶酶体融合使底物降解, 一部分能循环回细胞膜表面, 维持细胞膜的完整性

Figure 1 Macropinocytosis (created with BioRender.com). When stimulated by extracellular signals such as EGF, activated EGFR promotes the activation of PI3K and RAS, which in turn activate CDC42/RAC1, the Arp2/3 complex, and PAK1. This leads to actin polymerization and the formation of cup-shaped structures. The local invagination of the cell membrane expands, forming a larger vesicle. PAK1 activates CtBP1/BARS, which in turn activates PLD2 to produce phosphatidic acid (PA), promoting the closure of the cup-shaped structure and the formation of a macropinosome. The macropinosome fuses with lysosomes for substrate degradation or recycles back to the cell membrane to maintain membrane integrity

域的效应蛋白, 如磷酸肌醇磷脂酶C(phospholipase Cγ, PLCγ)和蛋白激酶B(protein kinase B, PKB/AKT)等[35,36], PLCγ催化PIP2产生1,2-二酰甘油(diacylglycerol, DAG), 后者能激活RAS[37]. Leep2(leading edge-enriched protein2)被发现是盘基网柄菌(*Dictyostelium discoideum*, 一种真核微生物, 常用于研究巨胞饮)中RAS蛋白的GTP酶激活蛋白(GTPase-activating protein, GAP), 通过水解GTP产生GDP调控RAS活性和巨胞饮[38]. 激活的RAS蛋白能活化小GTP酶RAC1/CDC42 (ras-related C3 botulinum toxin substrate 1/cell division cycle 42)[39], 后者与PI(4,5)P2结合并激活Wiskott-Aldrich综合征蛋白(Wiskott-Aldrich syndrome protein, WASP)[40]. RAC1/CDC42对局部pH高度敏感, 在弱碱环境(pH 7.8)时活性最高. EGF能激活细胞膜定位的钠离子(Na^+)和氢离子(H^+)交换泵NHE1(sodium-hydrogen exchanger 1), 促进Na^+泵入细胞内, H^+泵出细胞外, 从而升高膜局部pH(pH 7.8), 维持RAC1/CDC42活性[41]. 活化的WASP蛋白暴露其C-端结构域结合并激活肌动蛋白相关蛋白2/3复合物(actin related protein 2/3 complex, Arp2/3 complex). 活化的Arp2/3复合物作为成核位点, 与单体肌动蛋白结合促进肌动蛋白的多聚化[42]. 此外, CDC42能促进p21蛋白激活激酶1(p21 activated kinase 1, PAK1)活性, 从而活化肌动蛋白结合激酶LIM激酶(LIM domain kinase, LIMK), 后者通过直接磷酸化肌动蛋白解聚因子Cofilin使其失去活性, 从而促进肌动蛋白多聚化和膜褶皱形成[43]. 随着膜褶皱的增大, 它们会进一步向细胞质内陷, 形成巨胞饮杯状结构(macropinocytic cups)来捕捉外部可溶性物质[13]. 在一些情况下, 膜褶皱可能在局部区域聚集, 形成密集的巨胞饮斑块(macropinocytic plaques). 这些斑块区域主要富含三大类物质, 分别是细胞骨架蛋白(如F-actin、Arp2/3复合物、WASP)、小GTP酶(如RAS、RAC1/CDC42等)和磷脂酰肌醇[44,45]. 这些斑块的形成可能与膜的局部物理拉伸以及和不同蛋白质的相互作用有关, 它们可能为膜提供更大的弹性或支撑, 从而促进更大范围的内陷以捕获外界物质.

(2) 形成巨胞饮小体. 局部细胞膜内陷继续扩展, 最终形成一个较大的囊泡, 被称为巨胞饮小体(macropinosome), 包裹了大量细胞外可溶性物质. 激活的PAK1通过磷酸化活化CtBP1/BARS(C-terminal binding

protein-1/brefeldin A-ADP ribosylated substrate), 后者通过结合并激活磷脂酶PLD2(phospholipase D2)在巨胞饮小体上产生磷脂酸(phosphatidic acid, PA), 促进巨胞饮小体的封闭[46,47]. 杯状结构的封闭还与磷酸肌醇(phosphoinositide, PIP)信号途径密切相关. 在生长因子等的作用下, PI(4,5)P2在形成膜褶皱的位置增加约2倍, 随后在激活的PI3K作用下, 形成PI(3,4,5)P3, PIP3在杯状结构闭合前达到顶峰[32]. 在盘基网柄菌中发现, PI3K既与膜褶皱的形成有关, 也与杯状结构的闭合有关. PIP3在磷脂酰肌醇磷酸酶(SH2-containing inositol 5'-phosphatase 2/inositol polyphosphate phosphatase-like 1, SHIP2/INPPL1)、II型肌醇多磷酸四磷酸酶(inositol polyphosphate-4-phosphatase type II B, INPP4B)、肌小管蛋白相关蛋白6(myotubularin-related protein 6, MTMR6)和肌小管蛋白相关蛋白9(myotubularin-related protein 6, MTMR9)的催化作用下脱磷酸化, 生成PI(3,4)P2、PI3P, 并最终生成PI. 在人表皮癌细胞(A431)中, 抑制上述磷酸酶活性, 能抑制杯状结构闭合, 但不影响膜褶皱的形成. PI3P能直接激活钙离子激活的钾离子通道KCa3.1, 以目前未知的机制促进杯状结构的闭合[34].

Rab(ras-related protein)家族成员小GTP酶Rab5也被发现与杯状结构的闭合有关. Rab5招募的III型磷脂酰肌醇3-激酶(class III phosphatidylinositol 3-kinase, PI3K-III)VPS34能磷酸化PI产生PI3P, 后者招募含有FYVE(Fab-1, YGL023, Vps27, and EEA1)或PX/PH(phox homology domain/pleckstrin homology domain)等能结合PI3P结构域的效应蛋白质, 从而促进杯状结构封闭形成巨胞饮小体[48]. 杯状结构封闭的同时还会发生巨胞饮小体的收缩过程. 最近的研究发现, 电解质和渗透压平衡是调控巨胞饮小体收缩的关键. 在吞饮过程中, 大量吞饮进入巨胞饮小体的Na^+会被两孔通道蛋白(two-pore channel, TPC)泵出巨胞饮小体[49], 为了维持电解质平衡, 氯离子(Cl^-)也在阴离子泵作用下由巨胞饮小体泵入细胞质, 巨胞饮小体中的水也会随之排入细胞质以维持渗透压平衡, 从而促使巨胞饮小体收缩[50,51].

(3) 巨胞饮小体与溶酶体融合. 在盘基网柄菌中, Rab5到Rab7的转换在巨胞饮小体与溶酶体的融合过程中发挥重要作用. Rab5定位的巨胞饮小体能与Rab7标记的晚期内体/溶酶体融合, 这一过程受PI(3,4)P2、PripA(phox homology domain-containing protein)和TbcrA (T cell receptor alpha chain)蛋白复合物调控[27]. PripA是Rab7结合蛋白, TbcrA是Rab5的GAP蛋白. PripA通过与PI(3,4)P2和Rab7相互作用, 连接巨胞饮小体和晚期内体/溶酶体. PripA招募TbcrA, 水解GTP为GDP, 使得Rab5失活, 从而实现Rab5到Rab7的转换, 促进巨胞饮小体内容物在溶酶体的降解. 但是在A431细胞中, 巨胞饮小体可能与质膜或溶酶体融合, 但不与早期或晚期内体融合[52], 提示高等真核生物存在不同于低等生物的巨胞饮小体成熟机制和独立的巨胞饮小体酸化机制. SNARE(small NF90-associated RNA E)蛋白复合物介导细胞内囊膜之间的融合[53]. 尽管何种SNARE复合物介导巨胞饮小体和溶酶体融合目前还不清楚, 但在肠道上皮细胞中, SNAP25(synaptosomal-associated protein 25)和Syntaxin 4被发现能介导巨胞饮小体和含有沙门氏菌的囊泡融合[54], 在犬肾上皮细胞中, SEPT2能结合PI(3,5)P2阳性的巨胞饮小体, 敲降SEPT2(septin 2)能抑制巨胞饮小体和溶酶体的共定位[55], 提示SEPT2能调控巨胞饮小体和溶酶体融合. 此外, 溶酶体定位的、由PI(3,5)P2控制的钙离子(Ca^{2+})通道TRPML1缺失也能显著抑制巨胞饮小体与溶酶体融合[56]. 然而, SEPT2和TRPML1(transient receptor potential mucolipin 1)介导的巨胞饮小体和溶酶体融合是否具有普适性以及它们的确切机制还有待深入研究.

(4) 巨胞饮小体循环. 巨胞饮小体能循环回细胞膜表面以维持细胞膜的完整性并补充细胞表面受体. 逆转运分选复合物和WASH复合物(WASP和SCAR homologue)在这一过程中发挥重要作用[57]. 逆转运复合物由3个Vps亚基(Vps35、Vps26和Vsp29)和一个分选连接蛋白异源二聚体(SNX1/SNX2和SNX5/SNX6)(sorting nexin, SNX)组成. 巨胞饮小体脱水收缩后能形成小管(tubule), 这些小管具有很高的弯曲度, 从而能招募含有曲度感受结构域(bin amphiphysin rvs, BAR)的蛋白SNX1、SNX2、SNX5和SNX6[58]. WASH和逆转运复合物直接相互作用, 将逆转运蛋白及其底物隔离到囊泡表面的肌动蛋白亚结构域中, 并驱动它们逆转运进入回收囊泡中[59]. 在乳腺癌细胞中, SNX1被发现是由EGF激活的巨胞饮将E-cadherin蛋白循环回细胞膜所必需的[60].

2 肿瘤巨胞饮的分子调控机制

肿瘤细胞的快速增殖使其自身和基质细胞处于营养相对匮乏的微环境中. 近年来研究发现, 营养压力

(葡萄糖或氨基酸饥饿)诱导的巨胞饮对维持肿瘤细胞和肿瘤微环境的代谢稳态至关重要[61]. PDAC的肿瘤血管形成较少, 肿瘤细胞具有高水平的巨胞饮活性, 并且高度依赖谷氨酰胺. 阻断谷氨酰胺缺乏诱导的巨胞饮能显著抑制PDAC发展[6,62]. 因此, 理解营养压力下, 肿瘤巨胞饮的分子调控机制对研发靶向巨胞饮的抗肿瘤策略至关重要. 我们将总结和讨论最近关于肿瘤应对营养压力时巨胞饮发生的分子调控途径(图2)和新发现的巨胞饮相关蛋白在巨胞饮发生中的作用(表1).

2.1 EGFR-PAK途径和mTOR

诸多研究显示, PDAC肿瘤细胞的生长对环境中的谷氨酰胺水平高度敏感[7], 在营养匮乏时, 肿瘤细胞通过巨胞饮摄取并代谢胞外物质以产生氨基酸维持自身生长[6]. 局部营养压力如何调控巨胞饮是一个长期受关注的重要科学问题. Lee等人[24]的研究发现, 肿瘤核心区域的氨基酸水平低于周边区域, 而巨胞饮活性则呈相反趋势, 谷氨酰胺而非其他非必需氨基酸的缺乏能够特异性激活EGFR-PAK途径, 促使巨胞饮发生. 然而, EGFR主要对生长因子信号, 如EGF做出响应, 促进巨

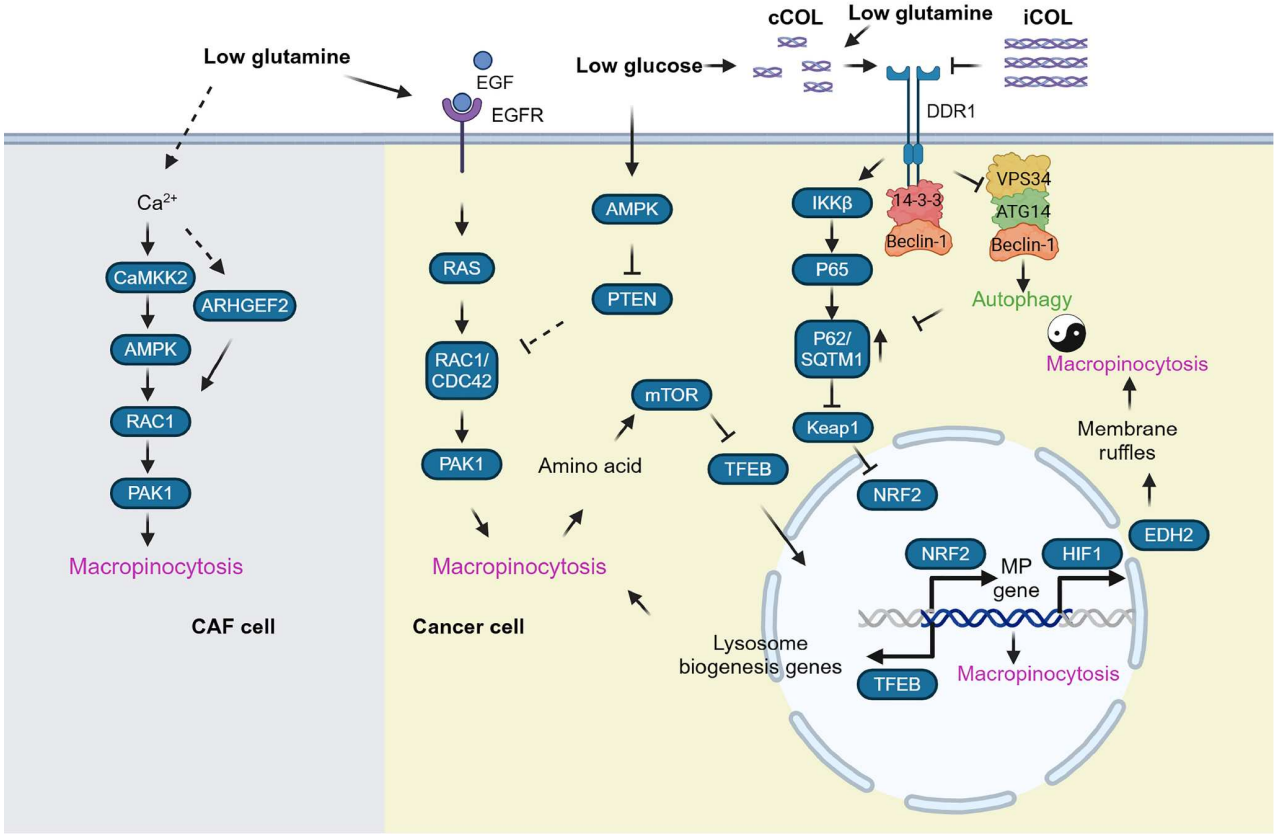

图 2 肿瘤巨胞饮的分子调控机制(由BioRender.com制作). 低谷氨酰胺(low glutamine)状态下, 肿瘤相关成纤维细胞(CAF)巨胞饮的激活依赖于Ca^{2+}-CaMKK2-AMPK-RAC1途径和Ca^{2+}-ARHGEF2-RAC1途径, 肿瘤细胞(cancer cell)通过EGFR-RAC1-PAK1途径激活巨胞饮; 低葡萄糖(low glucose)或低谷氨酰胺状态下, 裂解型胶原蛋白(cCOL)通过激活DDR1-IKKβ-p62/SQSTM1-NRF2途径上调巨胞饮相关基因转录, 促进巨胞饮发生, 全长型胶原(iCOL)则抑制该途径; DDR1通过与14-3-3和Beclin-1形成复合物抑制VPS34-ATG14-Beclin-1复合物的形成, 抑制细胞自噬发生; 自噬受抑制也能通过p62/SQSTM1-NRF2途径促进巨胞饮; 巨胞饮途径产生的氨基酸能促进mTOR活性, mTOR能抑制控制溶酶体发生的转录因子TFEB活性, 从而抑制溶酶体发生和巨胞饮

Figure 2 Molecular regulatory mechanisms of tumor macropinocytosis (created with BioRender.com). Under low-glutamine conditions, initiation of macropinocytosis in tumor-associated fibroblasts (CAFs) depends on the Ca^{2+}-CaMKK2-AMPK-RAC1 and Ca^{2+}-ARHGEF2-RAC1 pathways, whereas tumor cells activate macropinocytosis via the EGFR-RAC1-PAK1 pathway. Under low-glucose or low-glutamine conditions, cleaved collagen upregulates the transcription of macropinocytosis-related genes via the DDR1-IKKβ-p62/SQSTM1-NRF2 pathway, thereby promoting macropinocytosis in tumor cells. By contrast, full-length collagen inhibits this pathway. DDR1 suppresses autophagy by forming a complex with 14-3-3 and Beclin-1, thereby inhibiting the formation of the VPS34-ATG14-Beclin-1 complex. Inhibition of autophagy promotes macropinocytosis via the p62/SQSTM1-NRF2 pathway. Amino acids generated by the macropinocytosis pathway activate mTOR, which in turn inhibits the activity of the transcription factor TFEB, a key regulator of lysosome biogenesis, thus suppressing both lysosome formation and macropinocytosis

表 1 新发现的巨胞饮相关蛋白在巨胞饮发生中的作用

Table 1 Role of newly discovered macropinocytosis-associated proteins in the occurrence of macropinocytosis

新发现的巨胞饮相关蛋白	功能
V-ATPase	促进胆固醇向细胞膜运输, 激活RAC1[23]
SDC1	受ARF6和ACSS2调控, 能促进RAC1活性[22,63]
LYSET	促进溶酶体分解代谢酶由高尔基体向溶酶体转运[64]
TPC	介导Na^+由巨胞饮小体泵到细胞质[49]
TMEM206	介导Cl^-由巨胞饮小体泵到细胞质[50]
Slc15A	定位于细胞膜和早期巨胞饮小体, 促进寡肽摄取[65]

胞饮发生, 谷氨酰胺饥饿如何促进生长因子激活EGFR仍需进一步探究.

哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)是细胞中氨基酸的感受器, 是控制细胞生长和蛋白质翻译的关键蛋白质激酶[66]. 诸多研究表明, 抑制mTOR活性能显著促进依赖胞外蛋白作为营养来源细胞的巨胞饮活性[67,68]. mTOR能抑制控制溶酶体发生的转录因子TFEB(transcription factor EB)的细胞核定位, 这可能是mTOR抑制巨胞饮活性的机制之一[56,68~71]. 巨胞饮的持续激活可能引起巨胞饮小体的融合形成大囊泡导致细胞死亡, 被称为巨泡式死亡(methuosis)[31,72,73]. 随着巨胞饮摄取的胞外蛋白质在溶酶体分解代谢, 产生的氨基酸如谷氨酰胺和天冬氨酸等能激活mTOR[74,75], 这可能是肿瘤细胞防止巨胞饮过度激活和维持自身快速增殖的机制.

2.2 Ca^{2+}-CaMKK2-AMPK-RAC1途径和Ca^{2+}-ARHGEF2-RAC1途径

谷氨酰胺饥饿不仅能促进肿瘤细胞的巨胞饮, 还能提高基质中肿瘤相关成纤维细胞(cancer-associated fibroblasts, CAF)的巨胞饮活性. 然而, CAF中谷氨酰胺饥饿诱导的巨胞饮活性不受EGFR-PAK途径调控. 谷氨酰胺缺乏能提高CAF中的Ca^{2+}水平, 进而促使钙调蛋白依赖性蛋白激酶激酶2(calcium-dependent protein kinase kinase 2, CaMKK2)激活能量感受器磷酸腺苷活化蛋白质激酶(AMP-activated protein kinase, AMPK)-RAC1途径, 促进巨胞饮发生[76]. 此外, 低谷氨酰胺还能促进Rho/RAC1鸟嘌呤核苷酸交换因子2(Rho/Rac guanine nucleotide exchange factor 2, ARHGEF2)与RAC1结合, 从而激活RAC1[76]. 然而, ARHGEF2与RAC1的结合不受AMPK调控[76]. 目前, 低谷氨酰胺提高细胞内Ca^{2+}水平的具体机制尚不明确, 一种可能的原因是营养缺乏引起内质网应激, 促进了内质网Ca^{2+}释放, 从而提高了细胞质Ca^{2+}水平[77,78]. 此外, Ca^{2+}如何促进ARHGEF2与RAC1的结合也有待深入研究. 葡萄糖饥饿引起的AMPK激活已得到广泛证实[79,80]. 但值得注意的是, 葡萄糖饥饿无法激活CAF中的巨胞饮活性, 解析AMPK如何区分响应葡萄糖或谷氨酰胺饥饿信号产生不同的下游效应对理解不同营养物质缺乏诱导的巨胞饮调控机制至关重要. 谷氨酰胺饥饿诱导的肿瘤细胞和CAF中的巨胞饮途径受不同信号调控, 这表明深入研究不同细胞类型中的巨胞饮途径调控机制十分必要.

2.3 DDR1-NF-κB-p62/SQSTM1-NRF2途径

除了以上氨基酸匮乏诱导的巨胞饮途径外, 大量研究发现, 葡萄糖剥夺能显著促进肿瘤细胞的巨胞饮活性[25,26,81~83]. 葡萄糖缺乏能显著降低细胞中ATP水平, 激活AMPK[84,85]. 在低葡萄糖状态下, 磷酸酶PTEN(phosphatase and tensin homolog)缺失诱导的前列腺癌肿瘤细胞巨胞饮依赖于AMPK活性, 抑制RAC1或PI3K活性能阻断PTEN缺失诱导的巨胞饮. 然而, 有研究显示, AMPK能抑制AKT和PI3K活性[86]. 因此, 低营养状态下, AMPK如何调控巨胞饮活性有待进一步研究. 最近的研究表明, 低葡萄糖和低氨基酸都能显著诱导PDAC肿瘤细胞通过巨胞饮途径从细胞外摄取胶原蛋白为自身提供营养, 胶原蛋白不仅能作为巨胞饮底物被内吞, 还能作为信号分子调控巨胞饮活性. 全长型胶原蛋白(intact collagen, iCOL)分泌到细胞外后被金属蛋白酶(matrix metalloproteinases, MMPs)裂解产生的3/4型胶原和1/4型胶原, 被称为裂解型胶原(cleaved collagen, cCOL)[87,88]. 研究显示[25,26,87,88], cCOL能激活其受体DDR1(discoidin domain receptor 1), 后者通过提高核因子κB激酶抑制因子β亚基(ihibitor of NF-κB kinase subunit β, IKKβ)活性促进NF-κB p65的细胞核定位, 进而促进了泛素结合蛋白/自噬底物受体p62/SQSTM1表达, p62/SQSTM1累积能抑制抗氧化转录因子(nuclear

factor erythroid 2-related factor 2, NRF2)的E3泛素连接酶Keap1(Kelch-like ECH-associated protein 1)活性, 从而显著提高NRF2的转录活性[89]. 激活的NRF2能直接转录巨胞饮相关基因CDC42、SDC1、NHE1和PIK3CG(phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma)的表达, 促进巨胞饮发生; 而iCOL能抑制DDR1活性阻断巨胞饮发生. cCOL-DDR1-NF-κB-p62/SQSTM1-NRF2途径激活的巨胞饮在PDAC临床样本中也获得了证实, 并且与PDAC患者的预后不良呈正相关, 而抑制该途径蛋白的活性或表达能显著抑制PDAC的发展和肝转移. 这些研究结果表明, 靶向DDR1-NRF2轴介导的巨胞饮途径治疗肿瘤具有良好的发展前景.

细胞自噬是真核生物中高度保守的依赖于溶酶体的细胞代谢途径, 通过形成双层膜包裹的自噬小体将受损的细胞器或蛋白质送至溶酶体降解, 实现物质的循环再利用, 对维持细胞稳态至关重要[84,85,90~92]. 肿瘤细胞通常具有高水平的自噬活性, 比如, 相较于正常细胞, PDAC肿瘤细胞的自噬相关蛋白表达显著升高. 自噬抑制剂被认为是治疗肿瘤的重要策略. 然而, 自噬抑制剂并未显著提高PDAC患者的生存期[93,94]. 近期的研究发现, 抑制细胞自噬导致p62/SQSTM1的大量累积通过激活NRF2显著提高了巨胞饮相关基因的转录, 从而促进了巨胞饮活性, 联合抑制自噬和巨胞饮能显著抑制PDAC发展. 此外, 激活NRF2的其他因素, 如致癌突变、低氧和氧化应激等[89,95], 也能显著促进巨胞饮活性. 有趣的是, DDR1通过与14-3-3、AKT和Beclin-1形成复合物, 抑制了自噬启动的重要激酶复合物Beclin-1:VPS34:ATG14的形成[96], 从而抑制了自噬, 提示DDR1还能通过抑制自噬促进巨胞饮活性. 这些结果表明, 自噬和巨胞饮这两个营养获取途径能相互调控, DDR1-NRF2是两途径相互作用的重要纽带. 自噬和巨胞饮是否共享某些膜蛋白分子在两途径中各自发挥作用, 以及营养压力如何启动DDR1-NRF2轴促进巨胞饮活性是有待深入研究的重要方向.

EHD2(EH-domain containing 2)蛋白是参与膜褶皱形成的重要蛋白, 它的N端与细胞膜结合, C端与肌动蛋白结合蛋白(EH domain-binding protein 1, EHBP1)结合, 促进细胞骨架重排、膜弯曲、膜褶皱形成和内吞作用. 研究显示, 低氧条件下, 低氧诱导因子(hypoxia-inducible factor 1, HIF-1)能通过转录上调EHD2表达促进肝细胞肝癌(hepatocellular carcinoma, HCC)的巨胞饮活性, 敲除HIF-1或EHD2能抑制低氧诱导的巨胞饮[97]. 在多个肿瘤中, NRF2被发现能转录上调HIF-1表达[98~100], 提示DDR1-NRF2轴也能通过上调EHD2表达, 促进巨胞饮.

2.4 新发现的巨胞饮相关蛋白

2.4.1 V-ATPase和SDC1

RAS基因突变在所有肿瘤中占比最高, 其激活RAC1增强肿瘤细胞巨胞饮活性是促进癌症发展的重要机制[6,101]. 虽然巨胞饮小体的脂质组成尚未明确, 但膜皱褶富含特定的脂筏和磷酸肌醇, 缺乏胆固醇能阻断RAC1的细胞膜定位抑制巨胞饮小体形成[102]. Ramirez等人[23]的研究显示, RAS激活突变通过增强囊泡ATP酶(V-ATPase)的细胞膜定位促进胆固醇向细胞膜运输, 从而激活RAC1, 促进巨胞饮. 虽然V-ATPase运输胆固醇的机制目前还不清楚, 但是已知不依赖于其调控pH的活性. Yao等人[22]的研究发现, RAS激活突变通过促进ARF6特异性鸟嘌呤核苷交换因子(guanine nucleotide exchange factor, GEF)PSD4的表达激活小GTP酶ARF6, 促进Syndecan 1(SDC1)的细胞膜定位. 细胞膜定位的SDC1能激活RAC1, 从而能促进巨胞饮活性. RAS-RAC1和RAS-ARF6-SDC1-RAC1介导的巨胞饮过程可能在持续时间上存在差异. 有研究认为, ARF6参与的巨胞饮存续时间短, 它能调控RAS、RAC1、CDC42和SDC1返回细胞表面[22,103]. 此外, 脂质代谢途径的重要激酶乙酰辅酶A合成酶短链家族成员2(acetyl-CoA synthetase short chain family member 2, ACSS2)也被发现通过转录因子ETV4(ETS variant transcription factor 4)促进锌离子转运蛋白ZIP4(zinc transporter protein 4)的表达, 进而增强SDC1的细胞膜定位, 从而促进巨胞饮发生[63]. 尽管ZIP4调控SDC1的膜定位机制还不清楚, 但是ACSS2参与调控巨胞饮发生提示, 代谢重编程和巨胞饮能相互调控.

2.4.2 LYSET

巨胞饮小体与溶酶体融合将摄取的外来物质降解才能为肿瘤细胞提供营养, 因此, 溶酶体中的分解代谢酶对巨胞饮流(macropinocytic flux)至关重要. 通过遗传筛选, Pechinch等人[64]发现, 溶酶体酶运输因子(lysosomal enzyme trafficking factor, LYSET/TMEM251)在溶酶体分解酶由高尔基体转运至溶酶体过程中发挥关键作用. LYSET能与N-乙酰葡糖胺-1-磷酸转移酶(GlcNAc-1-phosphotransferase)结合并帮助其定位于高

尔基体, 后者能将溶酶体定位信号6-磷酸-甘露糖添加至溶酶体分解代谢酶, 从而促进分解代谢酶向溶酶体的运输; 而LYSET缺失能使溶酶体分解代谢酶定位错误, 定位至细胞膜, 从而显著抑制溶酶体降解和肿瘤细胞的成瘤能力. 因此, LYSET是肿瘤细胞适应营养压力的关键蛋白, 是具有重要潜力的抗肿瘤靶点.

2.4.3 TPC和TMEM206

尽管持续性吞饮外界流动性物质, 但是巨胞饮小体形成过程中的体积始终维持恒定. 最近的两项研究揭示了这一现象背后的机制. 巨胞饮途径从细胞外摄取的主要阳离子是钠离子, 在巨噬细胞中它们会通过TPC由巨胞饮小体前体泵入细胞质, 同时伴随着氯离子和水由巨胞饮小体泵入细胞质以维持电解质和渗透压平衡[49]. 这一脱水消退过程既维持了巨胞饮小体形成过程中体积的恒定, 也促进了膜褶皱的形成和曲率感受蛋白的招募. 随后的研究发现, 巨噬细胞中负责将氯离子泵出巨胞饮小体的蛋白是质子门控阴离子通道ASOR/TMEM206(acid-sensitive outwardly rectifying anion channel/transmembrane protein 206), 由质子氯离子转运通道CLC-5启动激活ASOR/TMEM206[50]. 然而, 巨噬细胞中存在持续型和诱导型巨胞饮, 肿瘤细胞和其他细胞中的巨胞饮是否依赖于TPC和TMEM206执行消退功能目前还不清楚. CLC-5和TMEM206缺失不妨碍肿瘤细胞巨胞饮小体的形成、酸化和降解, 并且TMEM206缺失能增强血清白蛋白依赖的肿瘤细胞存活. 这提示肿瘤细胞可能存在不同于巨噬细胞的机制调控巨胞饮小体消退和囊泡酸化.

2.4.4 Slc15A

盘基网柄菌是一种社会性阿米巴原虫, 在营养丰富的条件下以单细胞形式存在, 当营养匮乏时, 会聚集形成多细胞体. 它是一种常用的模式生物, 在细胞生物学、发育生物学和免疫学等领域的研究中具有重要作用. 通过在盘基网柄菌中建立高通量成像遗传筛选系统, 中国学者鉴定到一个重要的巨胞饮途径调节因子—寡肽转运蛋白Slc15A(solute carrier family 15 member 1), 它能定位于细胞膜和早期巨胞饮小体[65]. Slc15A介导的巨胞饮作用能帮助细胞获取寡肽为自身提供关键氨基酸, 而Slc15A缺失能显著抑制巨胞饮作用和细胞生长, 并且促进细胞自噬的发生, 这一结果为自噬和巨胞饮途径的相互调控提供了新证据[65]. 然而, Slc15A受何种机制调控参与巨胞饮以及如何调控巨胞饮途径发生还有待于进一步研究. 研究显示, Slc15A1在肺腺癌和非小细胞肺癌中高表达, Slc15A4能作为肺腺癌的预后标志物[104], 提示Slc15A介导的巨胞饮可能在人的肿瘤发展中发挥重要作用. 此外, 在盘基网柄菌的生长阶段, 转录因子Hbx5(hepatitis B virus X protein 5)和MybG(myb domain-containing protein)在细胞核中形成功能性复合物促进巨胞饮相关基因转录和巨胞饮发生. 而在饥饿诱导的多细胞盘基网柄菌中, 转录因子复合物在动态环腺苷3′,5′-单磷酸(cAMP)信号作用下进行核质穿梭, 使得复合物在细胞质中的停留时间增加, 从而下调巨胞饮作用. 巨胞饮相关基因表达与cAMP动态信号相结合有利于细胞间的长距离通讯, Hbx5-MybG 复合物的核质穿梭能从群体水平调控巨胞饮活性适应不断变化的周围环境[105].

3 总结和展望

从1931年胚胎学家Warren Lewis观察到巨胞饮途径并提出Pinocytosis, 巨胞饮的研究距今已有90多年历史. 经过近一个世纪沉淀, 科学家对巨胞饮的功能有了较为全面的认识, 尤其是巨胞饮在肿瘤发生中的作用和分子调控机制近年来得到了高度关注和迅猛发展. 不同刺激信号(营养匮乏、胶原蛋白不同裂解状态等)作用下调控巨胞饮发生的信号传递途径、转录机制和自噬与巨胞饮的相互调控机理得到揭示, 许多新的巨胞饮相关蛋白被发现, 这些研究成果极大地加深了我们对巨胞饮发生过程的理解, 并推动了靶向巨胞饮途径抗肿瘤药物的研发. 然而, 科学家对巨胞饮发生分子机制的认识还只是冰山一角. 目前最常用的巨胞饮检测手段依然是荧光染料标记的分子量为70000 Da的葡聚糖, 缺乏能特异性标记巨胞饮小体的膜定位蛋白. 巨胞饮的核心特征是膜的动态重构, 涉及一系列膜定位蛋白的协作. 未来研究可进一步挖掘定位在巨胞饮小体上的特异性膜蛋白或脂质分子(如磷脂酰肌醇修饰蛋白), 通过超高分辨率成像探索它们在膜皱褶形成、闭合、巨胞饮小体运输以及在巨胞饮小体和溶酶体融合中的功能; 探索它们的时空调控, 揭示膜定位蛋白如何在巨胞饮途径的各阶段(例如膜突起形成和封闭)中实现精准的时间与空间控制; 研究膜定位蛋白的翻译后修饰(如磷酸化、糖基化)在巨胞饮调控中的作用.

溶酶体内的酶在酸性环境下才能正常发挥作用, 其中一类叫作酸性水解酶(如蛋白酶、脂肪酶和糖苷酶), 这些酶的活性依赖于低pH环境(pH在4.5~5.0范围内)[106,107]. 因此, 囊泡在与溶酶体融合前需要酸化. 例

如, 在内体成熟过程中, 内体的pH逐渐下降, 由接近中性的pH 6.0~6.5降至更酸性的pH 5.0~5.5, 这一酸性环境有助于其与溶酶体融合并激活溶酶体酶, 使其能够有效地降解内体中的物质[108,109]. 尽管NHE1被发现能调控局部细胞膜的pH以维持RAC1活性, 但是它不能定位于巨胞饮小体. 调控溶酶体酸化的质子泵V-ATPase也被发现能定位于细胞膜, 但是与其质子泵功能无关, 主要负责运输胆固醇. 巨胞饮小体的酸化关系到其能否与溶酶体融合, 需进一步研究V-ATPase是否能定位于巨胞饮小体并介导其酸化, 同时需要对细胞内膜定位蛋白进行遗传筛选以期获得能定位于巨胞饮小体且能调控酸化的质子泵.

自噬与巨胞饮是肿瘤细胞维持代谢稳态的重要途径, 抑制自噬通过激活NRF2上调巨胞饮相关基因转录, 促进巨胞饮; 抑制巨胞饮降低细胞的能量水平也能激活自噬发生. 此外, DDR1既能通过激活NRF2促进巨胞饮, 也能通过与Beclin-1形成复合物抑制自噬. 尽管巨胞饮和自噬之间的相互调控已经得到证实, 但是二者之间是否共享某些膜蛋白质或脂质分子直接参与巨胞饮小体和自噬小体的形成还有待于深入研究. 除细胞膜和内体-溶酶体外, 细胞内膜系统如内质网等细胞器是否参与了巨胞饮小体的形成也是未来巨胞饮研究的重要方向.

巨胞饮已被发现在PDAC、HCC、前列腺癌、乳腺癌和结直肠癌等诸多恶性肿瘤的发生发展中发挥重要作用[6,18,110~112]. 随着肿瘤巨胞饮调控机制的深入研究, 利用巨胞饮递送抗肿瘤药物和靶向巨胞饮途径的抗肿瘤抑制剂开发也在如火如荼地进行. 比如, 白蛋白与抗肿瘤药物的偶联体, 能通过巨胞饮途径被肿瘤细胞摄取, 但是该策略的靶向性较差. T细胞等免疫细胞活化后也具有较强的巨胞饮活性, 如何使抗肿瘤药物特异性被肿瘤细胞摄取是亟需解决的科学问题. 尽管巨胞饮本身是非选择性的, 但可以通过在药物载体表面修饰靶向分子(如抗体、配体等)来增加对特定细胞的靶向性. 通过这种方式, 药物载体能够增强对目标细胞的亲和力, 从而提高药物递送的准确性和效率. 虽然诸多在临床上已经用于治疗肿瘤或其他疾病的药物, 如匹伐他汀、索拉非尼、吉非替尼和利坦色林等被发现具有抑制巨胞饮活性的作用[111,113~115], 但是这些药物缺乏特异性且抑制巨胞饮的机制不明确. 研究这些药物的巨胞饮抑制机制将有利于发现新的巨胞饮相关蛋白, 从而为巨胞饮特异性抑制剂的开发提供新的靶点. 目前最为常用的巨胞饮抑制剂是治疗高血压药物阿米洛利(amiloride)的衍生物EIPA(ethylisopropyl amiloride), 它能选择性抑制NHE1, 但是对NHE家族的其他成员仍具有一定的抑制作用, 并且半数抑制浓度(IC_{50})较高, 限制了其在临床上的应用. 另外, V-ATPase的抑制剂249C(一种二氢吡唑-5-羧酰胺化合物)虽然具有很强的溶酶体抑制活性, 但也缺乏抑制巨胞饮的特异性, 易引起较强的副作用. 缺乏特异的巨胞饮小体膜蛋白是妨碍巨胞饮高效特异抑制剂开发的重要原因. 通过评估对大分子葡聚糖或白蛋白摄取的抑制作用, 近期多个研究组鉴定到能高效抑制巨胞饮的抑制剂[116~118]. 未来通过整合高分辨率质谱、超高分辨率激光共聚焦显微镜和结构生物学技术深入研究巨胞饮小体的膜组成成分并鉴定高效巨胞饮抑制剂的作用机制, 将推动巨胞饮高效特异性抑制剂的开发. 总之, 这些问题的研究不仅将更全面地揭示巨胞饮的形成和分子调控机制, 还将加速巨胞饮途径从基础生物学到临床应用的转化.

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Summary for “肿瘤巨胞饮的分子调控机制研究进展”

Insights into the molecular regulatory mechanisms of tumor macropinocytosis

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Macropinocytosis is a highly conserved, actin-dependent endocytic pathway in eukaryotic cells that facilitates the non-selective engulfment of extracellular soluble substances by macropinosomes. These macropinosomes can either fuse with lysosomes for degradation or recycle back to the plasma membrane, to replenish membrane receptors. As a vital cellular process, macropinocytosis is important in various biological functions, including immune responses, cellular metabolism, and the progression of several types of advanced cancers.

The function of macropinocytosis in the progression of malignant tumors, such as pancreatic ductal adenocarcinoma (PDAC), has been a focus of research. An intriguing aspect of PDAC is how tumor cells exploit macropinocytosis to scavenge nutrients from the surrounding tumor microenvironment. Specifically, collagen, a major component of the extracellular matrix, is an important source of nutrients. Tumor cells internalize collagen via macropinocytosis, followed by its degradation into amino acids and intermediates of the tricarboxylic acid (TCA) cycle. These metabolites facilitate tumor growth and metastasis, particularly to the liver. Inhibitors of macropinocytosis, alone or in combination with other antitumor therapies such as autophagy inhibitors, chemotherapy, or immune checkpoint inhibitors, significantly reduce tumor growth and metastasis. These findings suggest that targeting the macropinocytosis pathway is a promising therapeutic approach. Understanding the molecular regulatory mechanisms that underlie macropinocytosis in tumors is thus essential for the development of novel treatments for cancer.

There has been considerable progress in understanding the molecular regulatory mechanisms that control macropinocytosis, particularly in the context of tumor biology. This review first focuses on macropinocytosis to provide readers with insight into this physiological pathway, followed by a discussion of recent findings related to its molecular regulation. Tumor cells often experience nutrient stress due to their rapid growth and the abnormal vascularization of solid tumors. The activation of macropinocytosis in response to this stress promotes the acquisition of extracellular nutrients, such as amino acids and glucose, to meet the metabolic demands of the tumor. Several key signaling pathways regulate macropinocytosis in tumors under nutrient stress. One important pathway is the EGFR-PAK axis, which activates macropinocytosis in tumor cells, in response to glutamine deprivation. Unlike cancer cells, in cancer-associated fibroblasts (CAFs), macropinocytosis is initiated by calcium ion (Ca^{2+})-dependent pathways, such as Ca^{2+}-CaMKK2-AMPK-RAC1 and Ca^{2+}-ARHGEF2-RAC1 under glutamine starvation. Furthermore, the DDR1-NF-κB-p62/SQSTM1-NRF2 pathway mediates macropinocytosis under both glutamine-deprivation and glucose-depletion conditions. Notably, NRF2 is a critical regulator of the transcription of macropinocytosis-related genes, which promote the survival of cancer cells when autophagy, another nutrient-scavenging pathway, is inhibited. By contrast, when macropinocytosis is downregulated, the resulting low ATP level activates autophagy, suggesting a compensatory relationship between these two pathways. In addition to these pathways, several novel macropinocytosis-related proteins have been identified, including V-ATPase, SDC1, LYSET, TPC, TMEM206, and Slc15A. These proteins are involved in several aspects of macropinocytosis, such as cholesterol trafficking, RAC1 activation, lysosomal enzyme trafficking, macropinosome resolution, and macropinocytosis activation in *Dictyostelium discoideum*.

Despite the above-mentioned advancements, our understanding of macropinocytosis is incomplete. More research is needed to identify the proteins localized to macropinosomes, the mechanisms of macropinosome acidification, and the molecular machinery that connects macropinocytosis and autophagy. Additionally, the identification of highly specific and effective inhibitors of macropinocytosis would enable the development of novel therapeutics. Future research will benefit from the integration of high-resolution mass spectrometry, ultra-high-resolution confocal microscopy, and structural biology techniques. These advanced tools will enable a detailed examination of the membrane composition of macropinosomes and identification of the mechanisms by which macropinocytosis inhibitors exert their effects. Further exploration of these issues will not only provide a more comprehensive understanding of the formation and molecular regulatory mechanisms of macropinocytosis but also accelerate the translation of these pathways from basic biology to clinical applications.

membrane ruffles, macropinocytosis, RAC1, DDR1, NRF2, tumor

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