

综述

乙酰胆碱信号通路在肺癌中的作用

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摘要: 肺癌作为当今世界最为致命的疾病之一, 严重地威胁着人类的生命健康。神经递质乙酰胆碱(acetylcholine, ACh)作为肺癌的自分泌和旁分泌生长因子, 可与肺癌细胞上的烟碱型乙酰胆碱受体(nicotinic acetylcholine receptor, nAChRs)和毒蕈碱型乙酰胆碱受体(muscarinic acetylcholine receptors, mAChRs)结合, 促进肺癌细胞的增殖、迁移和侵袭。近年来, 全基因组数据研究表明, nAChRs与吸烟相关肺癌风险密切相关, 因此其在肺癌发生发展中的作用机制的研究对肺癌药物的研发具有重要意义。本文对乙酰胆碱信号通路在肺癌进展中的作用机制进行综述, 为其成为肺癌治疗新分子靶点和药物的开发提供依据。

关键词: 乙酰胆碱信号通路; 烟碱型乙酰胆碱受体; 毒蕈碱型乙酰胆碱受体; 肺癌

Roles of acetylcholine signaling pathway in lung cancer

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Abstract: Lung cancer is one of the most fatal diseases in the world today, which seriously threatens human life and health. The neurotransmitter acetylcholine (ACh) acts as an autocrine and paracrine growth factor for human lung cancer, which binds to nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs) to accelerate lung cancer cell proliferation, migration and invasion. In recent years, genome-wide data studies have shown that nAChRs are closely related to the smoking-related risk of lung cancer. Therefore, the study of nAChRs is significant in the development of drugs for the treatment of lung cancer. This work reviews the mechanism of acetylcholine signaling pathway in lung cancer progression, which provides a basis for its development as a new molecular drug-target for lung cancer treatment.

Key Words: acetylcholine signaling pathway; nicotinic acetylcholine receptor; muscarinic acetylcholine receptor; lung cancer

肺癌是全球最常见的恶性肿瘤之一, 主要由非小细胞肺癌(non-small-cell lung cancers, NSCLC)和小细胞肺癌(small-cell lung cancer, SCLC)两种

病理类型组成, 两者分别占有所有病例的70%~80%和10%~15%。大多数肺癌患者就诊时已处于中晚期, 5年总体生存率约为17.8%。靶向治疗在晚期

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NSCLC的治疗中发挥了极其重要的作用,但由于个体差异性和药物机制差异性的存在,实现肺的精准治疗还需要不断探索新的靶向药物。乙酰胆碱(acetylcholine, ACh)作为中枢神经系统和周围神经系统的关键神经递质,通过乙酰胆碱受体(acetylcholine receptors, AChRs)介导神经元之间以及神经元和肌肉细胞之间的通信,调节运动、心率、消化、呼吸和其他自主功能^[1]。AChRs除了在中枢神经系统和外周神经系统、自主神经系统和神经肌肉接头中发挥重要的生理功能外,还在人体不同器官中广泛表达。ACh作用广泛,其与AChRs结合介导不同类型肿瘤细胞的增殖、凋亡、血管生成及上皮-间质转化(epithelial-mesenchymal transition, EMT)等过程^[2]。在肺癌中,ACh通过与烟碱型乙酰胆碱受体(nicotinic acetylcholine receptor, nAChRs)和毒蕈碱型乙酰胆碱受体(muscarinic acetylcholine receptors, mAChRs)结合,促进肺癌细胞的增殖和转移,表明ACh信号通路参与肺癌的发生。本文综述了ACh信号系统在肺癌进展中的作用及展望,对肺癌分子靶点和药物的研究具有重要意义。

1 ACh通路概述及其在肺癌中的代谢和作用

ACh和胆碱能蛋白在非神经组织中表达,如肺、结肠、胰腺、皮肤、胆囊、小肠和大肠组织^[3]。在肺部,ACh作为自分泌和旁分泌生长因子发挥作用,调节气道重塑、气道肌肉收缩、黏液分泌和肺免疫功能^[4]。支气管上皮合成、运输和降解ACh。ACh在胆碱乙酰转移酶(choline

acetyltransferase, ChAT)的作用下由胆碱和乙酰辅酶A在细胞质中合成。肉碱乙酰基转移酶(carnitine acetyltransferase, CrAT)催化合成ACh^[5]。合成的ACh被囊泡ACh转运蛋白(recombinant vesicular acetylcholine transporter, VAChT)通过内吞作用储存在囊泡中,并被运输到质膜。在质膜中,通过胞吐作用被释放到细胞外^[6]。细胞外的ACh与靶细胞上的nAChRs和mAChRs结合。过量的ACh被乙酰胆碱酯酶(acetylcholine esterase, AChE)和丁酰胆碱酯酶(butyrylcholine esterase, BChE)迅速降解生成胆碱。胆碱通过胆碱转运蛋白1(choline transporter 1, ChT1)转运回细胞质,进行新一轮ACh的合成。胆碱转运蛋白样蛋白1-5(choline transporter like protein 1-5, CTL1-5)在非神经元细胞的胆碱摄取及其向细胞质的转运中发挥作用,多特异性有机阳离子转运体1-2(organic cation transporters 1-2, OCT1-2)促进胆碱和ACh在肺细胞中的双向转运(图1)^[7]。

1.1 ACh通路在肺癌细胞中的代谢

ACh主要由ChAT催化合成,ChAT在人类肺上皮的多种细胞中表达,如永生化的正常肺上皮细胞、原代正常人肺泡上皮细胞、正常的支气管上皮细胞(bronchial epithelial cells, BECs)。ChAT在BECs上的表达受烟草成分、炎症刺激的调节^[8]。尼古丁和转化生长因子- β 1(transforming growth factor- β 1, TGF- β 1)刺激肺腺癌(lung adenocarcinoma, LUAD)细胞中ChAT水平,促进ACh生成和肺癌细胞增殖。ChAT拮抗剂BW813U在人LUAD裸鼠模型具有良好的抗癌作用^[9],提示

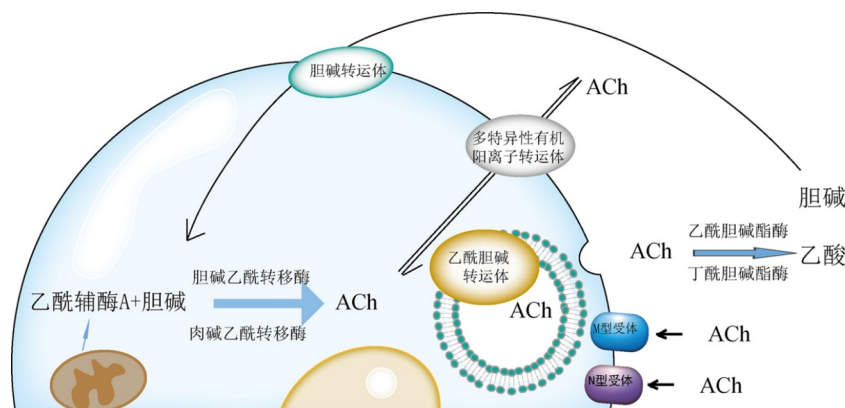


图1 乙酰胆碱(ACh)的合成、运输、转运及降解过程

抑制ChAT的表达可能是人类肺癌的治疗策略之一。VACHT转运胞内ACh, 调节肺部炎症和气道重塑, 降低组织损伤和死亡^[10]。VACHT低表达的小鼠在香烟烟雾的暴露下更容易发生肺气肿, 肺部炎症细胞浸润明显增加^[11]。AChE不仅参与水解ACh, 在调节细胞凋亡、参与炎症及肿瘤进展中也发挥作用^[12]。AChE在正常人肺成纤维细胞(human lung fibroblasts, HLF)中处于低水平表达, 而在细胞凋亡或应激状态下, AChE的表达水平升高。AChE通过促进凋亡蛋白酶激活因子-1和细胞色素c之间的相互作用, 促进凋亡小体的形成。在SCC-L中, AChE的表达降低, 凋亡小体减少, 从而促进肿瘤细胞增殖^[13]。AChE激动剂促进AChE在癌细胞中的表达, 减弱了ACh促肿瘤进展作用, 同时增强AChE的促凋亡作用。AChE激活剂可作为单一药物或与其他分子靶向药物联合应用, 成为肺癌靶向治疗重要策略之一。

1.2 胆碱转运在肺癌进展中发挥重要作用

胆碱是ACh的前体, 肺癌细胞中胆碱水平升高^[14]。胆碱转运蛋白CTL1在各种类型的器官和肿瘤中广泛表达, 包括大脑、微血管内皮和胎盘, 负责细胞膜中细胞外胆碱的摄取。CTL1转运胆碱具有亲和性、Na⁺不依赖性和pH依赖性的特点。CTL2也在各种类型的器官和肿瘤中表达, 调节胆碱向线粒体的运输^[15]。CTL1和CTL2在人类SCLC和LUAD中介导胆碱转运, 肿瘤细胞胆碱代谢增强, 胆碱放射性示踪剂(11c-胆碱)广泛用于肿瘤的临床检测和评估预后^[16]。胆碱也是磷脂合成的重要成分, 靶向胆碱摄取的药物会通过两种机制抑制肺癌细胞的生长, 一是阻断ACh的产生, 二是抑制肺癌细胞的细胞膜合成。这提示胆碱转运蛋白在肺癌进展中发挥重要作用, 为肺癌药物靶点的选择拓展了思路。

1.3 ACh通路在肺癌进展中的作用

在肺癌中, ACh自分泌增加, 促进肺癌细胞迁移和侵袭。ACh激活小细胞肺癌H82细胞中的有丝分裂发生通路, 即有丝分裂原激活蛋白激酶(MAPK)通路、细胞内钙通路和Akt通路^[17]。ACh可诱导A549细胞产生细胞因子IL-1、IL-6、IL-8, 这些细胞因子可促进人NSCLC的增殖和转移。ACh激活A549和L78细胞中的磷酸肌醇-3激酶(PI3K)/

Akt信号通路, 增强细胞中基质金属蛋白酶-9(matrix metalloproteinase-9, MMP-9)的表达和功能活性, 降低E-钙黏素(E-cadherin)的表达^[18]。MMP在人类肺癌的迁移和侵袭发挥重要作用, E-cadherin的下调是EMT的标志, 表明ACh在人类NSCLC的进展中起重要作用。

2 nAChR信号通路介导肺癌进展

nAChR是由5个亚基组成的同源或异源蛋白聚体。在原发性肺肿瘤细胞中, 由相同亚基(同源 $\alpha 7$ -nAChR和 $\alpha 9$ -nAChR)或不同 α 和 β 亚基(异构体 $\alpha 3\beta 4\alpha 5$ -nAChR、 $\alpha 3\beta 2\alpha 5$ -nAChR和 $\alpha 4\beta 2$ -nAChR)组成了多种nAChR亚型^[19]。nAChR参与肿瘤细胞增殖, 并减少应激信号如化疗药物、电离辐射、EGFR酪氨酸激酶抑制剂(epidermal growth factor receptor tyrosine kinase inhibitor, EGFR-TKI)诱导的细胞死亡^[20]。

2.1 $\alpha 5$ -nAChR介导肺癌细胞免疫逃逸和细胞凋亡

全基因组研究表明, 编码 $\alpha 5$ -nAChR的CHRNA5与肺癌风险和尼古丁依赖高度相关^[21]。我们课题组前期研究表明, $\alpha 5$ -nAChR与NSCLC的发生发展密切相关^[22]。 $\alpha 5$ -nAChR介导尼古丁相关性肺癌的发生和发展, $\alpha 5$ -nAChR通过激活JAK2/STAT3/Jab1信号通路, 促进肺癌细胞的增殖、侵袭和免疫逃逸^[23,24]。免疫治疗已被证明是治疗NSCLC的一种有前途的治疗方法。在LUAD中, $\alpha 5$ -nAChR的表达与PD-L1呈正相关。生理状态下, T细胞选择性地清除病原体 and 异常细胞, 避免攻击正常细胞, 维持免疫稳态。细胞程序性死亡-1(PD-1, 由PDCDI编码)和程序性死亡配体1(PD-L1, 由CD274编码)是维持免疫稳态的重要蛋白质。在肿瘤微环境中, PD-1/PD-L1轴被癌细胞阻断以逃避免疫监视, 促进肿瘤增殖^[25]。PD-1单抗或PD-L1单抗已经用于晚期肺癌的一线治疗, PD-1/PD-L1通路是治疗肿瘤的研究热点。在LUAD中, $\alpha 5$ -nAChR的表达与PD-L1呈正相关, $\alpha 5$ -nAChR-STAT3-Jab1轴通过调节PD-L1在肺癌中的表达介导免疫逃逸, 促进肺癌进展^[26], 表明 $\alpha 5$ -nAChR为NSCLC诊断和免疫治疗的潜在靶点提供依据。

我们的基因表达谱显示, $\alpha 5$ -nAChR通过调控

细胞周期相关基因(细胞周期蛋白D1、E2和D3)的表达,在细胞周期进展和细胞凋亡中发挥作用^[27]。 $\alpha 5$ -nAChR下调抑制细胞周期蛋白D1、E2和D3的表达,促进肺癌细胞从G₀/G₁期到S期的转变,因此, $\alpha 5$ -nAChR在肿瘤进展中发挥重要作用。Survivin是凋亡抑制基因家族中的一员,在人类所有癌症中差异表达。Survivin促进肿瘤细胞增殖和侵袭,抑制细胞凋亡,与多种癌症的预后不良有关。 $\alpha 5$ -nAChR与Survivin在肺癌中的表达呈正相关,同时检测 $\alpha 5$ -nAChR和Survivin的表达可更好地评估患者的预后^[28],提示靶向 $\alpha 5$ -nAChRs治疗对肺癌防治具有重要意义。

2.2 $\alpha 7$ -nAChR促进尼古丁相关肺癌发生发展

有研究发现, $\alpha 7$ -nAChR参与尼古丁相关肺癌细胞增殖和转移^[29]。尼古丁与 $\alpha 7$ -nAChR结合,导致适配蛋白 β -arrestin的募集,诱导Src激酶的激活。Src激酶的激活触发了由Raf通路、PI3K/Akt和MAPK激酶通路组成的有丝分裂信号级联反应,促进细胞增殖^[29]。尼古丁及衍生物4-甲基亚硝胺基-1-3-吡啶基-1-丁酮[4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone, NNK]长期暴露可提高 $\alpha 7$ -nAChR的水平,参与肺癌的发生^[30]。尼古丁在转录水平诱导 $\alpha 7$ -nAChR上调, $\alpha 7$ -nAChR启动子受多种调控元件和转录因子的调控。转录因子E2F1激活 $\alpha 7$ -nAChR转录,激活下游信号分子,如Src、MEK、PI3K/Akt和CDK4/6,形成一个自我调节的前馈环路,增加 $\alpha 7$ -nAChRs的表达^[31]。在NSCLC细胞系H1299中, $\alpha 7$ -nAChR拮抗剂D-tubocurarine通过抑制ERK-MAPK通路,抑制癌细胞生长^[32]。因此,靶向 $\alpha 7$ -nAChR药物的研发,可能为肺癌患者的治疗提供策略。

2.3 nAChRs变构调节剂在肺癌中的作用

淋巴细胞抗原6(lymphocyte antigen 6, Ly6)蛋白家族是nAChR的变构调节剂,其家族成员Ly6/神经毒素1(lymphocyte antigen 6/neurotoxin 1, Lynx1)和Ly6/神经毒素2(lymphocyte antigen 6/neurotoxin 2, Lynx2)是多个nAChR亚基的负变构调节因子,在气道中表达,并与 $\alpha 7$ -nAChR共定位^[33]。重组水溶剂Lynx1变体与细胞膜上的 $\alpha 7$ -nAChR相互作用,激活PLC(PKC/IP3)和ERK信号通路、促进转录因子NF- κ B表达,进而激活JAK、JNK、p38 α 、

Src、PI3K和Akt激酶,促进细胞凋亡,抑制肺癌进展,提示靶向 $\alpha 7$ -nAChR是肺癌治疗的策略之一^[34]。

3 mAChRs信号通路介导肺癌进展

mAChRs是一种G蛋白偶联受体(G-protein coupled receptor, GPCR),存在M1R、M2R、M3R、M4R、M5R等5种亚型。根据G蛋白偶联特性,M型受体可以大致分为2类:M1R、M3R和M5R与Gq型蛋白偶联,激活磷脂酶C募集三磷酸肌醇信号通路;M2R和M4R受体与百日咳毒素敏感的Gi/o蛋白偶联,抑制腺苷环化酶活性^[35]。M受体系统调节多种肺功能,包括气道重塑、气道平滑肌收缩、炎症和创面愈合等^[36]。

3.1 M3R介导肺癌细胞黏附和侵袭

M3R在肺癌细胞高表达。M3R的表达与肿瘤分期、Ki67表达、肿瘤大小、淋巴结转移密切相关。相对于肺癌I期,M3R的表达在II期和III期NSCLC中升高。与低M3R表达的NSCLC患者相比,高表达M3R的NSCLC患者的无病生存期和总生存期更低^[37]。在H82细胞中,ACh与M3R结合诱导MAPK激活和细胞内钙浓度的升高,促进肺癌细胞的增殖,参与肺癌细胞的黏附和侵袭^[38]。卡巴胆碱是一种胆碱能激动剂,在A549细胞中,卡巴胆碱激活下游Smad和ERK通路,刺激分泌TGF- β 1和MMP-9^[39],诱导EMT促进肺癌迁移和侵袭。卡巴胆碱生物学活性由M1R和M3R介导,M1R拮抗剂哌仑西平和M3R拮抗剂4-二苯基乙酰氧基-N-甲基-哌啶(4-diphenylacetoxy-N-methylpiperidine-methiodide, 4-DAMP)抑制了卡巴胆碱诱导的MMP-9的表达,抑制肿瘤的转移^[40]。M3R与表皮生长因子受体(epidermal growth factor receptor, EGFR)可协同促进肺癌的增殖和转移,与肺癌预后密切相关^[41]。因此,M3R可能成为肺癌治疗的靶点之一。

3.2 靶向M3R抑制肺癌进展

M型乙酰胆碱信号系统已被广泛用于抗癌药物的研发,非特异性M受体拮抗剂阿托品降低人小细胞肺癌细胞的活力,阻断TGF- β 1诱导的A549肺癌细胞的EMT^[42]。新型M3R拮抗剂溴氰化铵(acridinium bromide)在人类NSCLC中发挥抗肿瘤作

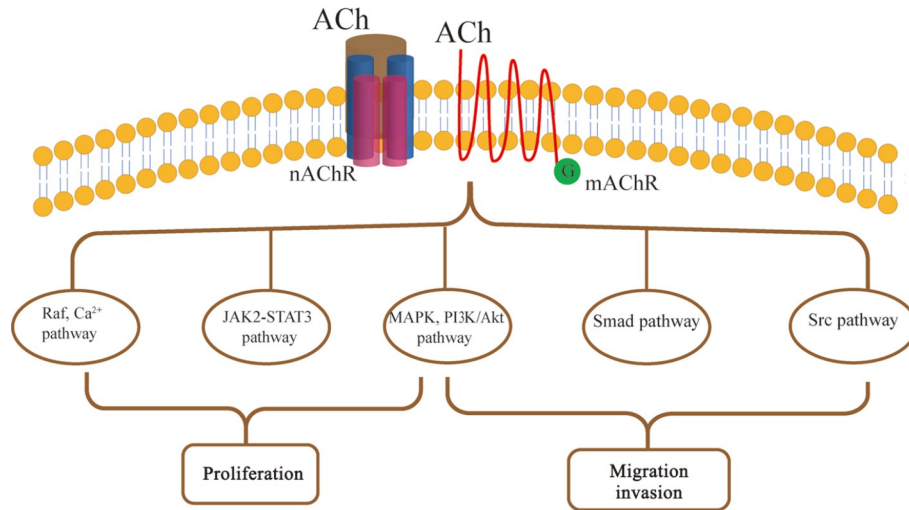


图2 ACh/AChR通路介导肺癌进展中的作用机制

用。溴氰化铵通过调节PI3K/Akt信号通路来抑制肺癌细胞的增殖,抑制Bcl-2表达,促进细胞凋亡,减少肿瘤细胞迁移和侵袭^[43]。M3R调节EGFR突变肺癌药物耐受性,在耐药癌细胞中,ACh-M3R-WNT轴介导药物耐受。M3R拮抗剂darifenin可显著抑制小鼠的肿瘤复发^[44]。因此,M3R是一个有前景的肺癌治疗靶点,靶向M3R的研究可以为肺癌靶向药物的研发提供证据。

4 总结

流行病学数据表明,吸烟与所有组织学类型肺癌的发生有很强的病因学联系。胆碱能通路在肺癌发生发展中发挥重要作用,在肺癌细胞中,ChAT、VACHT、胆碱转运体(choline transporters, ChTs)等胆碱能蛋白表达增加,AChE的表达减少。ACh作为肺癌细胞的自分泌和旁分泌生长因子,促进肿瘤细胞的增殖、迁移和侵袭。ACh通过与nAChRs和mAChRs结合在肺癌进展中发挥作用,其中nAChRs是研究热点。ACh通过激活多种通路(如MAPK通路、细胞内钙通路和Akt通路)促进肿瘤细胞增殖,同时通过激活Smad信号通路、Src通路介导EMT,促进肺癌进展(图2)。因此,胆碱能通路中的胆碱能蛋白可能成为肺癌治疗的潜在靶点,其研究为肺癌治疗新型药物和靶向治疗提供新思路。

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