长链非编码RNA UCA1与肿瘤耐药

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摘要 作为针对恶性肿瘤的有效治疗方式, 化疗已被广泛用于治疗各种恶性肿瘤。虽然化学治疗提高了患者的存活率及预后水平, 但肿瘤迅速形成的多药耐药会导致治疗失败。近年发现, 作为促多药耐药基因的lncRNA UCA1介导多种肿瘤形成耐药。该文回顾了UCA1在肿瘤耐药中的研究进展, 并展望了该领域未来的发展及面临的挑战。

关键词 长链非编码RNA; UCA1; 肿瘤; 耐药

Long Non-Coding RNA UCA1 and Tumor Drug Resistance

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Abstract As an efficient treatment for malignant tumor, chemotherapy has been used widely in treating various tumors. Although the use of chemotherapy drugs has significantly improved patients' survival rate and prognosis, the rapid development of multidrug resistance will lead to therapeutic failure. In the past few years, lncRNA UCA1, as a multi-drug resistance gene, has been found to mediate drug resistance in a great number of tumors. This manuscript reviews the progress of UCA1 in drug resistance and prospects the development and challenge in the future.

Keywords long non-coding RNA; UCA1; tumor; drug resistance

目前, 恶性肿瘤已是全球范围内引起死亡的最主要原因。尽管化疗是癌症手术后或非手术保守治疗的主要治疗手段, 但药物的弱靶向性及无靶向性以及肿瘤快速建立的多药抗药(multidrug resistance, MDR)常导致药物毒性加大、疗效下降及治疗失败, 并使预后不良。长链非编码RNA尿路上皮癌胚抗原1(urothelial carcinoma antigen 1, UCA1)已被发现参与调控多种肿瘤耐药, 因此深入了解UCA1介导的耐药机制将为今后改善恶性肿瘤药物敏感性打下理论基础。

1 UCA1

2006年, YANG等[1]首先报道了于膀胱癌细胞系BLS-211及BLZ-211中新发现的表达序列标签(expressed sequence tag, EST)片段(Genbank: DR159656)。对该片段进行cDNA末端快速扩增技术(rapid amplification of cDNA ends, RACE)后, 他们获得了该EST片段的全长cDNA, 命名为UCA1。UCA1定位于19p13.12, 有3个外显子, 其中前2个外显子组成ERV1家族长末端重复序列(long terminal repeat, LTR)部分。

收稿日期: 2019-05-07 接受日期: 2019-08-06
国家自然科学基金(批准号: 81402344)、陕西省青年科技新星项目(批准号: 2018KJXX-096)、陕西省自然科学基础研究计划(批准号: 2015JQ8308)和陕西省教育厅科学研究计划(自然科学专项)(批准号: 16JK1221)资助的课题
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Received: May 7, 2019 Accepted: August 6, 2019
This work was supported by the National Natural Science Foundation of China (Grant No.81402344), the Program of Young Technology Star of Shaanxi Province (Grant No.2018KJXX-096), the Program of Science and Technology of Shaanxi Province (Grant No.2015JQ8308) and the Program of Education of Shaanxi Province (Grant No.16JK1221)
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作为非编码RNA, UCA1无开放阅读框(open reading frame, ORF)编码蛋白质。RNA印迹(Northern blot)分析UCA1序列时发现, UCA1存在3个不同的剪接变异体, 长度分别约1 400 bp、2 200 bp及2 700 bp。这其中长度约1 400 bp的转录本最为丰富。另外, RT-PCR分析UCA1的时间及空间表达模式时, 发现UCA1在多种胚胎及肿瘤组织, 如膀胱癌、结直肠癌等组织中高表达[2], 这表明, UCA1与肿瘤发生发展过程有关。

2 肿瘤耐药相关机制

无论靶向还是非靶向药物治疗, 内源性及获得性肿瘤耐药都已严重影响了治疗效果。以往的研究表明, 导致肿瘤耐药的机制包括抗凋亡蛋白与药物外排泵的过度表达、肿瘤细胞促生存通路协同激活等[3]。除上述传统机制, 线粒体代谢和氧化磷酸功能状态[4]、ERα突变[5]、增强子重塑[6]、外泌体(exosome)诱导耐药[7]以及肿瘤MDR相关基因lncRNA, 如HOTAIR(HOX transcript antisense RNA)[8]、ANRIL(antisense non-coding RNA in the INK4 locus)[9]、UCA1等诱导的肿瘤耐药机制也在不断阐明。在众多lncRNA中, 由于UCA1在多种肿瘤中高表达, 且已发现介导多种肿瘤形成耐药[10], 因此对其进行介绍。

3 UCA1与肿瘤耐药

3.1 UCA1与呼吸系统肿瘤耐药

目前, 化疗已成为错失手术时机的非小细胞肺癌(non-small cell lung carcinomas, NSCLC)患者治疗的标准方案, 随着顺铂、吉非替尼等化疗药物的应用, 肺癌耐药也已越发普遍。以往的数据显示, 长期应用顺铂后, 肿瘤反应性明显降低且超过60%患者会复发[11]。生物信息学筛选及qRT-PCR分析后发现, 顺铂及酪氨酸激酶抑制剂(tyrosine kinase inhibitors, TKI)耐药的肺腺癌及NSCLC组织UCA1明显表达明显增高; CCK-8及MTT法分析发现, 包括肺腺癌在内的NSCLC耐药细胞IC_{50}明显高于非耐药细胞, 且与UCA1表达程度呈正相关, 在敲低UCA1表达后, IC_{50}明显下降[12-14]。这表明, 下调UCA1表达可逆转肺癌细胞对顺铂的低反应性。已有研究报道, 上皮间质转化(epithelial-mesenchymal transition, EMT)程度与肿瘤化疗敏感性有关[15-16]。蛋白质印迹(Western blot)发现, 耐药NSCLC细胞的EMT相关蛋白N-cadherin、Vimentin及Snail表达明显增高, 而敲低UCA1后, 上述蛋白表达下调[12,14]。这表明, UCA1可能通过调节EMT参与肿瘤耐药。

数据库分析发现, mTOR信号通路对NSCLC形成TKI耐药发挥重要作用[14]。已知PI3K/AKT/mTOR与ERK通路是EGFR的2条关键下游信号通路[17]。CHENG等[14]检测耐药细胞中该通路蛋白表达水平发现, 敲低UCA1后, p-EGFR(p-epidermal growth factor receptor)、p-AKT、p-ERK(p-extracellular signal regulated kinase)及p-mTOR(p-mammalian target of rapamycin)表达水平都明显下降。另外, 应用mTOR抑制剂后, TKI耐药细胞的IC_{50}也明显降低。据此推测, UCA1可能通过PI3K/AKT/mTOR通路调节NSCLC耐药。

敲低在体TKI耐药NSCLC细胞UCA1表达后, 耐药细胞凋亡率及半胱氨酸蛋白酶-3(caspase-3)、半胱氨酸蛋白酶-8(caspase-8)明显增高。这表明, 敲低UCA1对在体肿瘤耐药也有逆转作用[14]。上述研究表明, UCA1可通过多种途径介导肺腺癌及NSCLC耐药, 但目前这些研究在UCA1激活EMT及mTOR通路的具体方式上仍不明确。

3.2 UCA1与消化系统肿瘤耐药

3.2.1 UCA1与胃癌耐药　作为消化系统最常见的恶性肿瘤, 胃癌的治疗方法主要为手术治疗、化疗及生物治疗。而使用化疗药物所产生的MDR现象常导致化疗失败[18-19]。近年来研究发现, UCA1可通过调节miRNA介导胃癌细胞耐药。多项研究表明, UCA1在阿霉素(ADR)及5-氟尿嘧啶(5-FU)耐药的胃癌细胞中表达上调, 且敲低耐药细胞UCA1后, 耐药胃癌细胞的IC_{50}明显降低而凋亡率升高。Western blot证实, 凋亡相关蛋白caspase-3及PARP表达升高而Bcl-2明显降低[19-21]。这表明, 敲低UCA1也可逆转胃癌耐药。FANG等[19]发现, 在胃癌耐药细胞中miR-27b表达明显下调, 且与UCA1表达水平呈负相关。已有研究报道, 作为胃癌抑制因子的miR-27b在胃癌中的表达常降低, 并可参与调节胃癌MDR[22]。应用生物信息学分析发现, UCA1有2个miR-27b潜在结合位点。在过表达miR-27b后, 耐药胃癌细胞IC_{50}同样降低[19]。上述结果表明, UCA1/miR-27b轴可介导胃癌耐药形成。然而, miR-27b是否存在下游靶基因使UCA1通过内源竞争RNA(competing endogenous RNA, ceRNA)方式调节耐药有待探明。

3.2.2 UCA1与结直肠癌耐药 目前, 西妥昔单抗及5-FU常作为结直肠癌患者基础治疗及转移后的辅助治疗。但由于日益普遍的耐药现象, 化疗疗效越发减弱[23-24]。UCA1可通过外泌体转导及ceRNA方式介导结直肠癌耐药。BIAN等[23]分析结直肠癌细胞UCA1基因时, 发现了与以往报道的UCA1转录片段(NR_015379.3、GU799565)不同的UCA1转录本, 此转录本全长1 456 bp, 并于第二个外显子处多出47 bp。这说明, 结直肠癌中存在发挥相应功能的新UCA1转录本。qRT-PCR发现, UCA1表达水平在西妥昔单抗及5-FU耐药的结直肠癌细胞中均明显上调。用5-FU处理UCA1高表达结直肠癌细胞后, 凋亡率显著降低[23-24]。这表明, UCA1可通过降低肿瘤细胞凋亡而耐受5-FU。众所周知, UCA1可以ceRNA方式通过海绵样吸附于miRNA而影响下游通路。经生物学预测分析后, 他们发现, miR-204-5p及CREB1(cAMP-response element binding protein 1)是UCA1作为ceRNA调节通路中的潜在靶分子。荧光素酶检测证实, miR-204-5p可分别与UCA1及CREB1结合。另外, 在结合方式上, miR-204-5P可通过含Ago-2的microRNA核酸蛋白复合体(miRNA ribonucleoprotein complexes, miRNPs)与UCA1结合[23], 表明UCA1/miR-204-5P/*CREB1*轴可介导结直肠癌对5-FU耐药。除*CREB1*, miR-204-5p其他的靶基因*BCL-2*、*RAB22A*表达水平也随UCA1表达增高而增高。但经CCK-8分析发现, 沉默*BCL-2*及*RAB22A*只部分抑制UCA1的促肿瘤生长及凋亡抑制作用。因此是否存在UCA1/miR-204-5P/*CREB1*、*BCL-2*、*RAB22A*调控网络仍需研究[20]。

近年来研究发现, 含有UCA1的外泌体可在肿瘤细胞间转导耐药表型并用于预测肿瘤的化疗敏感性。外泌体是直径30~100 nm的脂质体, 它可直接从质膜表面出芽或由多泡体与质膜融合而分泌[25]。YANG等[24]发现, UCA1表达水平在西妥昔单抗耐药细胞分离的外泌体中明显升高。有研究报道, 细胞分泌的外泌体可为邻近细胞所整合[26]。荧光成像分析发现, 标记的外泌体呈时间依赖性整合于受体细胞, 同时标记的UCA1与外泌体也共定位于受体细胞。经证实, 受体非耐药细胞的UCA1水平明显增加。这说明, 耐药细胞中含UCA1的外泌体转至非耐药细胞后, 可改变受体细胞UCA1表达。值得注意, 虽然检测发现共同培养的非耐药细胞凋亡率明显降低, 但凋亡率降低水平与加入的外泌体数量呈负相关。这些结果说明, 非耐药结直肠癌细胞可通过转入的含UCA1外泌体产生耐药, 但外泌体介导的耐药可能并不占主导地位。

有关外泌体的临床研究发现, 耐药结直肠癌患者血清中的外泌体数量及其UCA1表达水平明显高于药物敏感患者。qRT-PCR显示, 即使受强酸、强碱及低温处理, 外泌体UCA1表达依旧稳定[24]。这表明, 外泌体UCA1表达水平可作为预测结直肠癌患者耐药的一项指标。

3.3 UCA1与泌尿系统肿瘤耐药

3.3.1 膀胱癌耐药 化疗是晚期膀胱癌患者的标准治疗方式, 使用中形成的耐药会使超过50%的患者治疗失败[27]。近期研究表明, UCA1可通过ceRNA方式激活膀胱癌细胞自噬从而形成耐药。WU等[27]发现, 膀胱癌细胞的UCA1表达水平明显上调, 他们用生物信息学预测发现, UCA1包含miR-582-5p结合位点, 且过表达UCA1后miR-582-5p明显下调。Western blot显示, 敲低UCA1或过表达miR-582-5p后多药耐药相关蛋白TOPO-II(DNA topoisomerase-II)表达增高, 而MRP1(multidrug resistance protein 1)、LRP(lipoprotein receptor-related protein)、GST(glutathione *S*-transferase)表达下调。以往研究表明, MRP1、LRP、GST及TOPO-II蛋白对肿瘤耐药十分重要, 除TOPO-II外, 其余蛋白表达水平都与耐药程度呈正相关[28], 这表明, UCA1可能通过miR-582-5p介导膀胱癌耐药。他们进一步发现, 敲低UCA1后自噬标记物LC3-II(light chain 3-II)表达明显下调且LC3-ILC3-I也明显降低, 同时自噬底物p62表达上调, 表明敲低UCA1可抑制膀胱癌自噬。通过生物信息学预测, *ATG7*包含miR-582-5p结合位点[27]。ATG7(autophagy related gene 7)是参与泛素系统的E1样激活酶, 其可诱导膀胱癌自噬而耐受抗肿瘤治疗[29]。荧光素酶检测发现, 共转染ATG7-WT及miR-582-5p后, 荧光素酶活性明显减弱。且RT-PCR显示, 上调miR-582-5p表达后*ATG7*表达明显下降[27], 提示UCA1/miR-582-5p/*ATG7*轴可引起自噬而使膀胱癌耐药。

除此之外, PAN等[30]发现, 过表达UCA1后miR-196-5p表达水平也随之升高。他们发现, miR-196-5p包含多个*CREB*结合位点, 敲低*CREB*或突变miR-196-5p结合位点都可使依赖UCA1的启动子活性明显降低, 而敲低miR-196-5p后$P27^{kip1}$表达也随之下

调。同时, 共转染miR-196-5p与*P27*^{kip1} 3′UTR-WT可明显降低荧光素酶活性。这表明, miR-196-5p可与*P27*^{kip1}结合。另外, 流式细胞仪分析显示, 敲低miR-196-5p会使吉西他滨与顺铂诱导的肿瘤细胞凋亡明显增高。上述结果表明, 膀胱癌可通过UCA1/*CREB*/miR-196-5p/*P27*^{kip1}轴介导耐药, 这也为研究UCA1耐药的分子机制提供了新思路。

另有研究发现, UCA1可通过作用于Wnt通路促使肿瘤细胞产生耐药。FAN等[31]应用Western blot发现, 耐药细胞中Wnt6蛋白表达水平与UCA1表达呈正相关。他们探寻UCA1与Wnt通路关系时发现, 过表达UCA1可增加TOP Flash系统中Wnt诱导的荧光素酶活性。因Wnt6可激活Wnt通路[32], 经MTT法验证, 敲低*Wnt6*表达可使过表达UCA1所激活的Wnt通路活性减弱, 同时也使受顺铂处理后的肿瘤细胞活性明显降低[31]。这表明, UCA1可通过上调Wnt6表达并激活Wnt信号通路介导膀胱癌耐药。

3.3.2 前列腺癌耐药 前列腺癌也可以ceRNA方式对多西他赛耐药。WANG等[33]发现, 耐药前列腺癌细胞UCA1表达明显升高, 而miR-204表达却明显降低。有研究报道, *Sirt1*(silent mating type information regulation 2 homolog-1)作为miR-204的直接靶点, 常在前列腺癌细胞中过表达, 且过表达的*Sirt1*与前列腺癌化疗耐药有关[34]。qRT-PCR显示, *Sirt1*在耐药前列腺癌细胞中明显上调, 并且其表达水平随miR-204升高而减弱。在耐药细胞中分别敲低UCA1、*Sirt1*或过表达miR-204后, 耐药细胞IC_{50}明显降低; 同时, 导致肿瘤耐药的重要外排转运体P-gp蛋白表达水平也随之下调[33]。上述实验并未采用荧光素酶报告基因及RNA免疫沉淀(RNA binding protein immunoprecipitation, RIP)等实验验证UCA1、miR-204及*Sirt1*之间能否结合及结合形式, 因此只能部分说明UCA1/miR-204/*Sirt1*轴在前列腺癌耐药中的作用。

3.4 UCA1与女性生殖系统肿瘤耐药

3.4.1 乳腺癌耐药 乳腺癌是女性第二位致死性肿瘤, 大部分雌激素受体(estrogen receptor, ER)和孕激素受体(progesterone receptor, PR)阳性的乳腺癌患者在应用曲妥珠单抗及他莫昔芬后会发生耐药, 最终导致治疗失败[35]。多项研究发现, 耐药乳腺癌细胞中UCA1明显上调。在敲低UCA1表达后, 耐药细胞的IC_{50}明显降低而凋亡率升高[36-39]。ZHU等[35]发现, 敲低*HER-2*(human epidermal growth factor receptor 2)阳性的曲妥珠单抗耐药细胞中UCA1表达后, miR-18a表达明显增强而前体miR-18a并无改变, 这说明UCA1在转录后水平调节miR-18a。通过预测发现, *YAP1*(yes-associated protein 1)是miR-18a的潜在靶基因。以往的研究报道, YAP1是调节药物敏感性的重要蛋白[40]。他们将miR-18a与YAP1-UTR-WT共转染细胞后, 荧光素酶活性明显减弱。同时qRT-PCR及Western blot发现, 上调耐药细胞miR-18a后*YAP1*及其靶分子CDK6表达下调, 而抑癌基因*PTEN*(phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase)却明显上调[35,40-41]。这表明, UCA1可通过UCA1/miR-18a/*YAP1*轴调控HER-2阳性乳腺癌耐药, 但*YAP1*能否通过CDK6及*PTEN*继续调控耐药仍需研究。不仅如此, miR-18a也可反向调节UCA1。ZHU等[35]对曲妥珠单抗耐药细胞应用miR-18a抑制剂后, 发现UCA1表达明显上调。通过预测及荧光素酶检测验证UCA1可与miR-18a结合, 且RNA沉淀法(RNA precipitation)证实, miR-18a可与整合UCA1 RNA探针的Ago-2蛋白共沉淀。有研究报道, miRNA可与AGO2装配形成AGO2-RISC使基因沉默[42]。这表明, miR-18a可能通过RISC抑制UCA1表达, 同时*CA1*/miR-18a/*YAP1*通路存在的反馈可加强UCA1表达而加重耐药。另一项研究还发现, 在ER阳性的他莫昔芬耐药乳腺癌细胞中, UCA1同样可抑制miR-18a表达, 但LI等[39]发现, 耐药细胞过表达miR-18a可明显抑制他莫昔芬诱导上调的*HIF1α*(hypoxia inducible factor-1)的表达。以往曾报道, miR-18a可直接结合*HIF1α* 3′UTR区并抑制其表达[43]。这表明, UCA1也可通过UCA1/miR-18a/*HIF1α*轴调节ER阳性乳腺癌细胞对他莫昔芬耐药。同时, 该通路也存在反馈调节。qRT-PCR分析发现, 敲低耐药细胞*HIF1α*表达后, 他莫昔芬诱导上调的UCA1表达水平明显被抑制[39]。其机制可能与*HIF1α*结合于UCA1启动子缺氧反应元件(hypoxia response elements, HREs)而增强其表达有关。通过该反馈UCA1的表达水平将进一步增高, 从而不断抬高乳腺癌对他莫昔芬的耐药性。

除通过miRNA, UCA1也可通过如下通路参与形成耐药: LI等[36]发现, 敲低UCA1表达后ER阳性的他莫昔芬耐药细胞终止于G_2/M期, 且细胞周期相关因子p21表达上调。他们通过RIP及染色质免疫共沉淀技术(CHIP)证实, UCA1可招募*EZH2*至p21启动子, 并抑制p21表达。这表明, UCA1/*EZH2*/p21轴可

能参与调节耐药。不仅如此, qRT-PCR还发现敲低耐药细胞UCA1后, CREB与p-CREB、AKT与p-AKT表达都明显降低, 且应用PI3K抑制剂处理耐药细胞后, p-CREB、p-AKT表达也明显下调。PI3K/AKT信号通路对正常细胞周期过程十分重要, 且可调节CREB表达, 而作为原癌转录因子, CREB是调节乳腺癌细胞周期的关键分子[44-45]。这说明, UCA1可通过PI3K/AKT信号通路调节CREB, 并影响耐药细胞凋亡而形成耐药。同时, 敲低UCA1也可降低ER阳性的他莫昔芬耐药细胞p-AKT及p-mTOR表达水平, 在应用mTOR抑制剂雷帕霉素后, UCA1高表达产生的肿瘤细胞化疗耐受明显减弱[46]。研究发现, 活化AKT/mTOR通路可使乳腺癌形成他莫昔芬耐药[47]。这表明, UCA1可能通过AKT/mTOR通路影响耐药。另外LIU等[38]用免疫细胞化学法发现, 主要于核内翻译的β-catenin在敲低UCA1后大量出现于细胞质, 且他们敲低UCA1后发现, Wnt/β-catenin通路活性也明显受抑制。该实验说明, UCA1可通过促进核内β-catenin翻译增加Wnt/β-catenin通路活性, 重分布核外ER从而介导他莫昔芬耐药。上述实验对UCA1调节机制的研究并不深入, 但可为今后实验提供方向。

外泌体也参与了ER阳性乳腺癌耐药。XU等[48]发现, 他莫昔芬耐药细胞的外泌体UCA1明显增高; 与外泌体共同培养的化疗敏感细胞受他莫昔芬处理后, caspase-3及凋亡率都明显下降。虽然外泌体参与乳腺癌耐药, 但将其作为耐药预测指标需从耐药及敏感患者中采样, 并分析预测效能。

3.4.2 *卵巢癌耐药* 卵巢癌对顺铂及紫杉醇反应较好, 但长期使用形成耐药后, 大部分患者会复发甚至死亡[49]。UCA1同样以ceRNA方式调节卵巢上皮癌耐药并可作为预测化疗敏感性的生物标记物。qRT-PCR发现, 耐药卵巢上皮癌细胞UCA1表达上调[50-51]。WANG等[50]发现, 敲低UCA1可显著上调miR-129, 同时耐药细胞IC_{50}明显降低而凋亡率显著提高。他们通过预测发现, UCA1存在miR-129结合位点, 且共转染miR-129与UCA1-WT后的荧光素酶活性明显减弱。这表明, UCA1可与miR-129结合。以相同方法发现, miR-129也可与*ABCB1*(ATP-binding cassette subfamily B member 1)结合。Western blot显示, 敲低UCA1或过表达miR-129都可抑制*ABCB1*表达, 同样过表达miR-129或敲低*ABCB1*也都可降低耐药细胞IC_{50}。这表明, UCA1/miR-129/*ABCB1*轴可介导卵巢上皮癌耐药。另外, 多变量分析发现, UCA1表达水平与化疗反应性有关, 并可作为评价低反应性患者预后的独立指标[51]。因此, UCA1可用于临床预测卵巢上皮癌化疗敏感性。

3.5 UCA1与口腔鳞癌耐药

口腔鳞癌是最常见的头颈部肿瘤, 5年存活率不足50%, 而且口腔鳞癌中越发普遍的化疗耐药也常导致治疗失败。同样好发的舌鳞癌也面临此问题[52-53]。口腔鳞癌也存在多条UCA1介导的调节通路。qRT-PCR显示, 顺铂耐药的口腔鳞癌及舌鳞癌中UCA1表达明显增高; 同时Western blot及流式细胞仪分析发现, 敲低UCA1使耐药细胞凋亡率明显升高且caspase-3、Bax蛋白表达上调, 而Bcl-2表达明显下调[54-55]。通过预测, FANG等[54]发现, UCA1存在miR-184结合位点且在过表达UCA1后miR-184表达下调。经过荧光素酶检测确认, miR-184可与UCA1-WT结合。另外, 敲低miR-184可逆转低表达UCA1产生的高药物敏感性。经相同方法确认, *SF1*(steroidogenic factor 1)为miR-184下游靶分子且其表达水平与miR-184呈负相关, 表明UCA1/miR-184/*SF1*轴可调节口腔鳞癌对顺铂耐药。在针对舌鳞癌研究中, Wang等[55]发现, 敲低UCA1可抑制顺铂诱导激活PI3K及Akt473位丝氨酸磷酸化。这表明, UCA1可能通过PI3K/Akt通路影响凋亡而使舌鳞癌耐药。但很显然, 目前关于UCA1如何激活PI3K/Akt通路的具体分子机制并不明确。

3.6 UCA1与白血病耐药

*BCR-ABL*融合基因抑制剂伊马替尼(imatinib, IM)已广泛用于治疗慢性粒细胞白血病(chronic myeloid leukemia, CML)慢性期患者, 对改善患者预后效果显著。然而晚期患者常因IM耐药而影响疗效[56]。UCA1同样以ceRNA方式介导CML对IM耐药。XIAO等[57]发现, 耐药细胞UCA1及*MDR1*表达增高且其表达程度与IM浓度呈正相关。另外, 过表达UCA1后*MDR1*表达也随之升高。以往研究报道, MDR1表达异常上调被认为是IM耐药的主要原因[58]。他们经预测发现, miR-16存在结合UCA1及*MDR1*的潜在位点。同时RIP分析发现, miR-16过表达细胞中AGO2沉淀的内源性UCA1明显增多, 但miR-16并不影响UCA1水平。这说明, UCA1以AGO2依赖性方式调节miR-16, 且其表达并不受miR-16影响。另外荧光

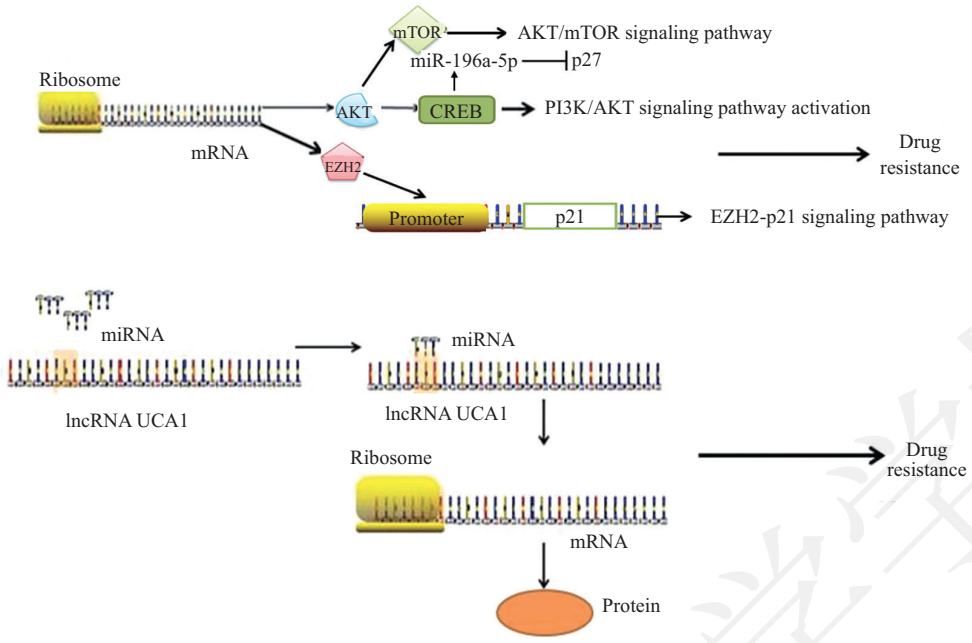

图1 UCA1参与的肿瘤耐药信号通路

Fig.1 Signaling pathways of tumor drug resistance mediated by UCA1

素检测发现, miR-16可与MDR1 3′UTR-WT结合, 且RT-PCR与Western blot证实, 上调miR-16表达可明显抑制MDR1表达[57]。这表明, UCA1/miR-16/*MDR1*轴对IM 耐药CML起了重要作用。

如图1所示, UCA1参与的信号通路可在一定程度上反映其参与的肿瘤耐药机制。 UCA1相关的肿瘤耐药研究见表1。

4 肿瘤生物标记物UCA1

UCA1只在胚胎及肿瘤组织中表达, 而在大部分正常组织并无表达。这表明, UCA1可作为潜在的肿瘤诊断及预后标记物。同时, 多项meta分析发现, UCA1表达水平与多种肿瘤的总生存期(overall survival, OS)及无进展生存期(progression-free survival, PFS)呈负相关[59-62], 表明UCA1与肿瘤预后密切相关。另外, UCA1对多种肿瘤的诊断也有一定意义。多项研究发现, UCA1表达水平在肺癌、胃癌、直肠癌等肿瘤患者血浆及膀胱癌患者尿液中明显增高, 且ROC(receiver operating characteristic)分析表明, UCA1对上述肿瘤的诊断有较高的准确性; 然而, 在其他如卵巢癌、前列腺癌等肿瘤中, UCA1的诊断与预后检测效能仍有待确认[63-67]。此外, LI等[68]已建立利用体液(如血液、尿液等)进行体外检测UCA1基因的荧光定量PCR技术, 且敏感性、特异性、稳定性和可重复性都较好, 这为今后肿瘤诊断提供了新方法。

5 CRISPR与UCA1检测及治疗

目前, 利用CRISPR技术对耐药lncRNA进行检测及干预的相关研究也已开展。HO等[69]在CRISPR/Cas系统基础上通过同源重组及双导RNA载体在人结肠癌细胞中成功敲除了UCA1, 敲除UCA1、嘌呤霉素处理后的结肠癌细胞集落形成能力明显提高。之后, ZHEN等[70]也在CRISPR/Cas9系统基础上利用UCA1特异引导RNA(guide RNA, gRNA)敲低了人膀胱癌UCA1表达, 在体及离体条件下, 膀胱癌细胞增殖、迁移、侵袭能力明显受到抑制。另外, BESTER等[71]通过构建双蛋白编码和非编码整合的CRISPRa筛选(DICaS)平台成功辨识了肿瘤耐药相关的编码和非编码整合途径。上述研究为探明未知耐药通路及今后针对UCA1的靶向治疗提供了帮助。

作为分子生物学领域的革命性技术, CRISPR技术虽然可以编辑基因及调节转录, 但该技术在编辑lncRNA时仍存在问题: (1)Cas9-sgRNA复合体在靶向结合DNA时的特异性及作用效能是否良好? (2)编辑过程是否会影响基因座位上的相邻及重叠基

表1 LncRNA UCA1在肿瘤耐药中的机制

Table 1 The mechanism of lncRNA UCA1 in cancer drug resistance

肿瘤类型 Cancer types	耐药种类 Drug resistance types	分子机制 Mechanisms	参考文献 References
NSCLC	Cisplatin/EGFR-TKIs	EMT	[12, 14]
	EGFR-TKIs	PI3K/AKT/mTOR	[14]
Gastric cancer	DDP/5-FU/ADR	miR27b	[19]
	Adriamycin	Bcl-2, caspase-3	[20-21]
Colorectal cancer	5-FU	miR-204-5P/*CREB1*, *BCL2*, *RAB22A*	[23]
	Cetuximab	Exosome	[24]
Bladder cancer	Cisplatin/gemcitabine	*CREB*/miR-196-5p/$P27^{kip1}$	[30]
	Cisplatin	Wnt6/Wnt pathway	[31]
	Rapamycin	miR-582-5p/*ATG7*	[27]
Prostate cancer	Docetaxel	miR-204/*Sirt1*, P-gp	[33]
Breast cancer	Trastuzumab	miR-18a/*YAP1*	[35]
	Tamoxifen	miR-18a/*HIF*α	[39]
	Tamoxifen	EZH2/p21, PI3K/AKT, CREB	[36]
	Tamoxifen	Wnt/β-catenin	[38]
	Tamoxifen	AKT/mTOR pathway	[46]
	Tamoxifen	Exosome	[48]
Ovarian cancer	Paclitaxel	miR-129/*ABCB1*	[50]
OSCC	Cisplatin	miR-184/*SF1*	[54]
TSCC	Cisplatin	PI3K/AKT pathway	[55]
CML	IM	miR-16/*MDR1*	[57]

NSCLC: 非小细胞肺癌; OSCC: 口腔鳞状细胞癌; TSCC: 舌鳞状细胞癌; CML: 慢性粒细胞白血病; EMT: 上皮间质转化。

NSCLC: non-small cell lung cancer; OSCC: oral squamous cell carcinomas; TSCC: tongue squamous cell carcinoma; CML: chronic myeloid leukemia; EMT: epithelial-mesenchymal transition.

因? 因此, 在设计sgRNA时, 应认识到靶基因定位的复杂性, 以降低风险。

6 问题与展望

结合上文, 我们发现UCA1可通过多条通路调控肿瘤耐药, 这说明, UCA1有很大潜力作为今后的治疗靶点。但不可否认, 目前研究仍存在问题: (1)把在多种正常及肿瘤组织中都有表达的UCA1作为特定肿瘤的耐药标记物, 其特异性是否理想? (2)除通过miRNA介导耐药, UCA1是否还存在其他耐药调控通路? (3)活体组织是否也适用离体细胞涉及的分子机制? 为解决这些问题, 今后研究可从以下几个方面进行: (1)由于包括UCA1在内的MDR基因在肿瘤转录或表达中并不十分特异, 因此解决此问题主要在于实现药物的靶向性递送; (2)作为MDR相关基因, 将UCA1作为抗MDR靶点关键在于利用以靶向分子片段导向的脂质体或其他可用作靶向运载工具进行多药及多靶点用药的联合研究; (3)探寻能与UCA1联合作为耐药分子标记物的其他ncRNA, 并进一步阐明相关分子机制; (4)构建相关多种肿瘤耐药的动物模型以及逐步开展临床试验; (5)丰富实验手段、建立全面的数据库并以此预测和研究UCA1参与的耐药调控网络。

虽然现有的研究仍有不足之处, 但随着研究的深入及上述问题的解决, UCA1在抗肿瘤耐药中的突出价值将为今后的临床预测与治疗作出贡献。

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