中国科学: 生命科学 2021 年 第 51 卷 第 12 期: 1646 ~ 1656
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS

评 述 大连医科大学肿瘤干细胞研究院十周年庆专题

RNA可变剪接与肺癌的发生发展

陈磊, 陈超群, 张文静*, 汪洋*

大连医科大学肿瘤干细胞研究院, 大连 116044
* 联系人, E-mail: zhangwj@dmu.edu.cn; yangwang@dmu.edu.cn

收稿日期: 2021-10-08; 接受日期: 2021-10-29; 网络版发表日期: 2021-12-14

摘要 在人类的基因组中, 超过90%的基因都会经历RNA可变剪接, 可变剪接贯穿生命活动的始终. RNA剪接异常与人类的疾病密切相关, 其中包括癌症. 作为第二大致死因素, 癌症对公共健康造成了严重的危害. 在每年癌症新增及死亡病例中, 肺癌均位于首位. 研究发现, 肺癌中存在大量剪接事件异常, 可变剪接参与调控肺癌的发生和发展. 在肺癌中, 剪接因子存在突变、异常表达等情况, 这些因素驱动了肺癌的进程. 此外, 可变剪接在肿瘤的耐药、放疗抵抗、血管生成及代谢等方面都发挥了不可替代的作用.

关键词 肿瘤, 肺癌, 可变剪接, 剪接异常

根据遗传学中心法则, 遗传信息的传递是由DNA到RNA, 最后到蛋白质的过程. 在人类基因组中, 大约有24000个编码蛋白的基因, 但是却可以合成10万多种功能各异的蛋白质[1], RNA可变剪接在其中扮演着重要的角色. 研究表明, 超过90%的人类基因会发生可变剪接, 基因转录形成的前体RNA(pre-mRNA)经剪接可形成不同的mRNA亚型, 这是形成蛋白质多样性的重要来源之一[1~3].

RNA可变剪接发生在被称作剪接体的大型核糖核蛋白(ribonucleoprotein, RNP)复合体中[4,5]. 剪接体由5个小核糖核蛋白颗粒组成, 即U1, U2, U4/U6和U5. 在剪接发生时, U1 snRNA(small nuclear RNA)以碱基互补的方式识别mRNA前体5′剪接位点, 而结合在3′剪接点上游富含嘧啶区的U2AF识别3′剪接位点并引导U2 snRNP与分支点相结合, 形成剪接前体. 随后, 剪接前体与U4, U5, U6 snRNP三聚体相结合, 形成60S的剪接体, 此时内含子弯曲成套索状, 上下游的外显子相靠近, 释放U1, U4和U5, U2和U6形成催化中心, 发生转酯反应, 引起前体RNA分子发生剪接[6].

RNA可变剪接参与多种生物学过程, 剪接事件的异常通常会导致多种疾病的发生发展, 其中包括癌症[7,8]. 越来越多的研究表明, RNA可变剪接在癌症的发生与进展过程中发挥重要功能[9]. 根据世界卫生组织国际癌症研究机构(International Agency for Research on Cancer, IARC)最新发表的数据, 2020年, 约有1930万癌症新发病例, 近1000万癌症死亡病例. 值得注意的是, 约50%的新发病例和58%的死亡病例出现在亚洲. 就特定国家而言, 中国占全球新诊断病例的24%, 死亡人数的30%. 在中国, 肺癌依然是最常见的癌症之一. 2020年, 中国新增肺癌病例约81.6万, 近71.5万人死于肺癌, 肺癌死亡率居于首位[10]. 肺癌患者的5年生存率在21%左右, 且在低生存率的患者中, 57%被诊断为转

引用格式: 陈磊, 陈超群, 张文静, 等. RNA可变剪接与肺癌的发生发展. 中国科学: 生命科学, 2021, 51: 1646–1656
Chen L, Chen C Q, Zhang W J, et al. Roles of RNA alternative splicing during the occurrence and development of lung cancer (in Chinese). Sci Sin Vitae, 2021, 51: 1646–1656, doi: 10.1360/SSV-2021-0407

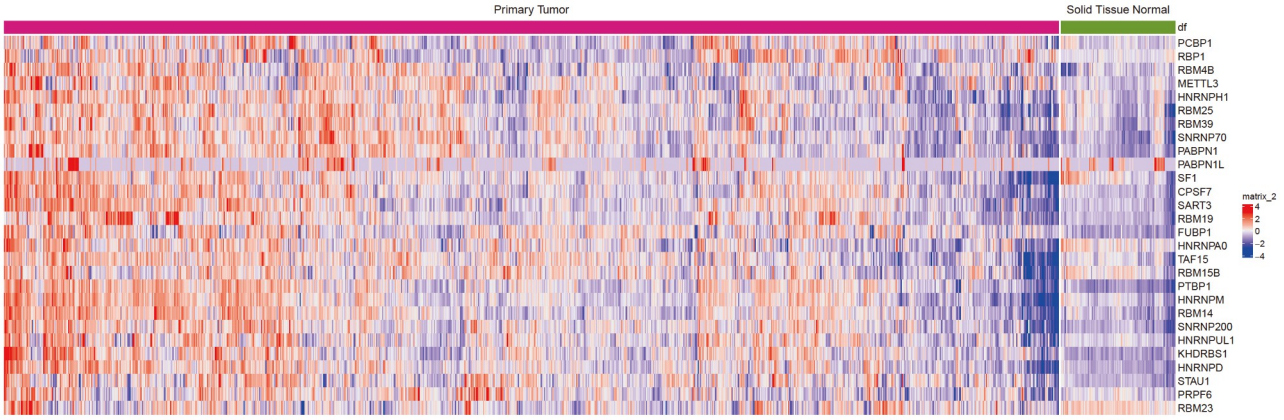

图 1　剪接因子在肺癌中表达情况. 肺癌中存在大量剪接因子的表达异常

Figure 1　Expression of splicing factors in lung cancer. A large number of splicing factors are abnormally expressed in lung cancer

移性疾病[11]. 在肺癌中, 大量的剪接因子存在异常表达的情况(图1), 近年来可变剪接在肺癌中的功能也得到了越来越多的关注和重视. 如在肺腺癌中, SAM68的表达高于正常的肺组织, 通过介导丙酮酸激酶(pyruvate kinase, PKM)前体mRNA的可变剪接, SAM68可促进PKM2的形成, 驱动癌症代谢[12]. 剪接因子的突变、异常表达可能是肺癌的驱动因素之一. 而RNA可变剪接也可参与调控肺癌细胞的增殖、转移、凋亡、放化疗、血管生成等诸多生命活动过程.

1　剪接因子突变、异常表达驱动肺癌的进展

作为调控前体RNA剪接的因子, RBM5, RBM6和RBM10的氨基酸序列具有30%~50%的相似性. 在重度吸烟者、肺癌及其他癌症患者中, 往往伴随着RBM5和RBM6的缺失[13], 并且在75%的原发性肺癌患者中, RBM5的表达会有显著的下调[14]. 此外, 在肺腺癌患者中, RBM10被鉴定为最常见的突变基因之一[15]. 在肺癌中, *NUMB*基因受到RBM5, RBM6和RBM10的共同调控[16]. 以RBM10为例, 作为剪接因子, RBM10通过结合相邻的内含子区域来促进外显子跳读[17~19]. 肺腺癌患者中, RBM10的突变率在8%左右, 该突变在肺腺癌发生的早期出现富集, 且为功能性缺失突变[20~22]. 在中国癌症数据库及TCGA数据库中, 携带RBM10突变的肺腺癌患者, EGFR和KRAS的突变率在两个数据库中分别为93%和78%. 而剩余携带RBM10突变的患者, 则无其他已知的致癌基因突变, 这提示RBM10突变可能是肺腺癌的主要驱动因素之一. RBM10可调控癌症相关基因的剪接, *EIF4H*为其中一个重要的靶基因. 简言之, RBM10可以通过调控*EIF4H*的第5号外显子跳读, 从而使其形成EIF4H-L和EIF4H-S两种不同的剪接本, 其中EIF4H-L可以促进肺腺癌细胞的增殖, 而EIF4H-S则能抑制肺腺癌细胞的生长. 在癌旁组织及正常肺上皮细胞中, EIF4H-L的表达量较低, 而在RBM10突变的肺腺癌患者中, EIF4H-L的形成则会显著增加(图2)[23]. 突变的RBM10不仅驱动肺腺癌的进展, 导致患者的不良预后, 同时也表明其可作为肺腺癌治疗的一个潜在靶点.

2　可变剪接与肺癌细胞凋亡

细胞凋亡是生物体内重要的生理过程之一, 机体依赖细胞凋亡来清除衰老及异常的细胞[24]. 凋亡相关因子BCL-x是BCL-2蛋白家族的成员, 为一种线粒体跨膜蛋白. 其编码基因*BCL-x*的第2号外显子的可变剪接, 导致生成功能相反的两种剪接异构体, 即抑制细胞凋亡的BCL-xL(长亚型)和促进细胞凋亡的BCL-xS(短亚型), 其中BCL-xL是最丰富的BCL-x蛋白, 可以通过多种不同的机制抑制细胞凋亡, 进而促进肿瘤细胞的存活[25].

在多数肿瘤细胞中, 细胞凋亡通常呈现被抑制的特性, 在肺癌中也不例外. 特定RNA剪接因子的异常表达被认为是导致肿瘤细胞凋亡受到抑制的重要原因

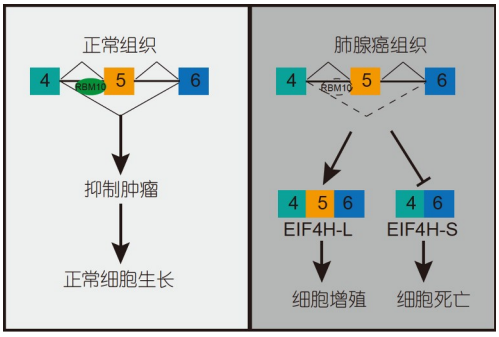

图 2 RBM10发挥抑癌作用的机制. 在正常的组织中, RBM10发挥抑制癌症的功能, 预防细胞癌变. 而在RBM10突变的肺腺癌患者中, EIF4H-L的形成增多, EIF4H-S的表达被抑制, 促进了肺癌细胞的存活

Figure 2 Role and mechanism of RBM10 in inhibiting cancer cell growth. RBM10 functions as a tumor suppressor in normal tissues, preventing cells from becoming cancerous. However, in patients with lung adenocarcinoma and RBM10 mutations, EIF4H-L production increased while EIF4H-S yield reduced, promoting the survival of lung cancer cells

之一[26,27]. 同正常的人支气管上皮细胞相比, 剪接因子RBM4在非小细胞肺癌(non-small cell lung carcinoma, NSCLC)细胞系中呈低表达的状态. 在BCL-x的前体RNA中, 存在RBM4的结合基序CGGCGG. RBM4可识别并结合位于两个5′可变剪接位点之间的该基序, 通过调控剪接, 使具有促凋亡功能的短剪接本BCL-xS显著增加, 从而促进肿瘤细胞凋亡[28]. 在非小细胞肺癌患者中, RBM4的低表达导致促凋亡的BCL-xS剪接本的形成减少, 而抑制凋亡的剪接本BCL-xL的含量则增加. 这一现象促进了肺癌细胞的存活, 加重了肺癌的恶性表型.

此外, 凋亡相关基因*MCL-1*(髓细胞白血病-1)同样受到可变剪接的调控. *MCL-1*是*BCL-2*家族中重要的抗凋亡成员之一. *MCL-1*最初被鉴定为ML-1人髓系白血病细胞系分化过程中一种早期、即时表达基因[29]. 通过发生可变剪接, *MCL-1*基因可以分别编码抑制凋亡的MCL-1_L和促进凋亡的MCL-1_S蛋白. 在肿瘤细胞中, MCL-1_L/MCL-1_S的比值很高, 这也提示肿瘤细胞具有很强的抗凋亡能力[30,31]. 在非小细胞肺癌细胞中, MCL-1_L的蛋白表达普遍增高[32]. 研究指出, SF3B1(剪接因子3B1)在多种肿瘤中都发挥重要作用[33~35], 且可以参与调控MCL-1的可变剪接[36,37]. SF3B1是U2型剪接体U2 snRNP的基本亚基, 且作为一种反式作用剪接因子, SF3B1还可抑制肺癌细胞A549中BCL-xL的生成[38,39]. 肺癌患者的肿瘤细胞中低表达SF3B1, 导致肺癌患者异常RNA可变剪接的发生, BCL-xS和MCL-1_S的表达降低, 其促凋亡能力被抑制(图3), 导致肺癌患者的不良预后.

除剪接因子RBM4和SF3B1外, 其他诸多的剪接因子, 如SRSF1[40], RBM25[41], PTBP1, TRA2β[42]等皆可参与调控肿瘤细胞凋亡的过程. 鉴于可变剪接在肿瘤细胞凋亡中的重要功能, 针对异常可变剪接的肿瘤治疗方案也越来越受重视.

3 可变剪接与肺癌细胞侵袭和转移

肿瘤细胞的侵袭和转移是造成患者治疗效果差, 产生不良预后的重要因素之一, 同样也是导致实体瘤患者死亡的主要原因之一[43]. 一旦肿瘤细胞发生转移, 现有的治疗手段将很难发挥作用[44]. 在肺癌的临床治疗中, 部分肺癌患者甚至会发生脑转移、骨转移及淋巴结转移等.

人类同源哺乳动物激活蛋白(Mena), 与VASP和EVL一起组成肌动蛋白调控蛋白家族中的Ena/VASP. Mena可参与调控细胞生命活动的多个过程, 包括细胞黏附及迁移[45]. 在人类肿瘤细胞中, Mena存在多种剪接异构体, 其中包括Mena11a这一亚型[46]. Mena11a能抑制旁分泌介导的肿瘤侵袭过程[47]. 此外, 在乳腺癌中, 上皮剪接调控蛋白(ESRPs)的低表达可以显著抑制Mena11a蛋白表达[48], 但有关Mena11可变剪接的研究却鲜有报道. 近来研究表明, PTBP1可增强Mena的pre-mRNA中外显子11a的跳读, 进而促进肺癌的侵袭和迁

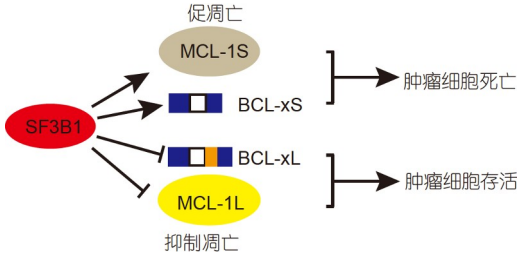

图 3 SF3B1调控肺癌细胞凋亡的机制. 通过可变剪接, SF3B1可以增加促凋亡因子MCL-1_S和BCL-xS的生成, 同时抑制凋亡抑制因子MCL-1_L和BCL-xL的表达

Figure 3 Mechanism of SF3B1 regulating apoptosis of lung cancer cells. SF3B1 can regulate MCL-1 and BCL-x alternative splicing, promoting the production of proapoptotic factors, MCL-1_S and BCL-xS, while decreasing the expression of antiapoptotic factors, MCL-1_L and BCL-xL

移[49]. 在肺腺癌组织中, PTBP1的表达显著增高, 且高表达PTBP1的患者常常具有较差的预后. 在TGF-β诱导的EMT模型中, PTBP1的表达显著上调, 且间质细胞指征蛋白N-cadherin和Vimentin的表达增加, 而上皮细胞指征蛋白E-cadherin表达减少, 说明PTBP1促进肺癌细胞的迁移和侵袭. 进一步研究显示, 在Mena第11号内含子的3′剪接位点上游5 nt的序列TTTTCCCCTT, 以及其5′剪接位点下游101 nt的序列TTTTTTT-TTCTTT皆为PTBP1的结合序列. PTBP1对于这两个位点的识别与结合, 促进Mena的第11号外显子跳读, Mena的短剪接本增加, 提高肺癌细胞侵袭和迁移的能力, 在临床上则表现为肿瘤细胞的远端转移[49].

此外, 研究人员还发现, 在高转移的肺癌细胞A549及NCI-H1299中, KHSRP的mRNA及蛋白的表达水平远高于转移能力低的细胞NCI-H358和NCI-H292. 一系列的细胞表型实验也证明, 敲低KHSRP可以抑制肺癌细胞的侵袭和迁移能力, 而过表达KHSRP则能促进肺癌细胞的侵袭和迁移[50]. 另外, 在非小细胞肺癌患者中, KHSRP同HNRNPC的表达具有相似性, 都在肿瘤组织中异常高表达, 且HNRNPC同样可以促进肿瘤细胞的侵袭和迁移, 二者皆高表达的患者预后往往是最差的. HNRNPC是位于细胞核的RNA结合蛋白, 属于不均一核糖核蛋白(heterogeneous nuclear ribonucleoproteins, hnRNPs). HNRNPC与细胞核内的pre-mRNA相关, 在转录后调控中发挥重要作用, 尤其是RNA可变剪接[51,52]. KHSRP与HNRNPC可相互作用, 可能共同参与细胞侵袭、迁移相关剪接事件的调控.

4　可变剪接与肺癌细胞耐药

在经过化疗药物或靶向药物治疗后, 部分肿瘤细胞会产生耐药机制. 在肺癌患者临床治疗过程中, 耐药也时常出现, 肿瘤细胞耐药已成为肺癌治疗的一大难题. 在肿瘤细胞耐药形成过程中, 可变剪接同样发挥重要的作用.

酪氨酸酶抑制剂(EGFR-TK1)已被广泛应用于肺癌治疗, 第一代可逆的EGFR抑制剂, 如吉非替尼和埃罗替尼对EGFR激活突变(如L858R点突变及第19号外显子缺失)的非小细胞肺癌患者具有良好疗效[53,54]. 但是EGFR-T790M gatekeeper突变则会对第一代药物产生耐药性, 且50%~60%的耐药患者中存在EGFR-T790M gatekeeper型突变[55,56]. 虽然第三代EGFR抑制剂奥斯替尼对存在上述三种突变的患者皆具有不错的疗效, 但是对于EGFR野生型的患者疗效较差[57]. 此外, 第二代EGFR抑制剂对于EGFR-T790M突变型的患者并未显示出良好的治疗效果[58]. 研究证实, EGFR抑制剂功能的实现依赖细胞凋亡调控蛋白BIM, 且BIM和耐药密切相关[59]. 在EGFR突变的非小细胞肺癌患者中, BIM存在2903 bp大小的缺失, 这使BIM的pre-mRNA中的第3号外显子优先于BH3编码的第4号外显子被剪接, 导致缺乏BH3结构域的非活性BIM亚型产生. EGFR突变的肺癌细胞株中促凋亡BIM蛋白亚型表达被抑制, 最终导致对EGFR抑制剂的治疗抵抗[60].

此外, 剪接因子SRSF1同样参与肿瘤耐药相关剪接事件的调控. 在Casepase9的pre-mRNA中, 可能被SRSF1识别的13种RNA顺式作用元件均发生突变, 其中第6号内含子的RNA顺式作用元件(C9-I6/ISE)是一种强剪接增强子, SRSF1可以特异性识别这一序列[61]. 由于缺乏caspase的激活机制, 非小细胞肺癌细胞易表现出对化疗药物的耐药增强[62]. 在A549细胞中, 由于可变剪接的偏好性, SRSF1会促进caspase-9a的增加, 抑制caspase-9b的生成, 若敲低SRSF1, caspase-9a/9b的比值则会降低(图4). 非小细胞肺癌细胞中caspase-9a的高表达, 导致肿瘤细胞对化疗药物柔红霉素、顺铂和紫杉醇的IC_{50}值增加, 从而表现为对化疗药物耐受[61]. 针对SRSF1-caspase9信号轴的药物研发, 为缓解、克服肿瘤细胞耐药的研究提供了新思路.

5　可变剪接与放疗抵抗

虽然当前肿瘤治疗手段日新月异, 但是传统的手术切除、化学治疗以及放射治疗仍是肿瘤治疗的主要手段. 到目前为止, 放疗在肺癌不同阶段的治疗中起到至关重要的作用, 可以显著延长患者的生存时间, 是最有效的方法之一[63]. 然而, 肺癌细胞的放疗抵抗被认为是引起肺癌远端转移和局部复发的重要因素. 放疗抵抗依然是肿瘤临床治疗过程中面临的棘手问题. 肺癌的放疗抵抗涉及诸多生物学过程, 如氧化应激、泛素化、RNA甲基化等[64~66]. 但是对于可变剪接参与的肺癌放疗抵抗, 人们对其仍知之甚少.

肺癌发生放疗抵抗的过程中, 也伴随着剪接因子的异常表达. 有研究表明, 非小细胞肺癌细胞A549及

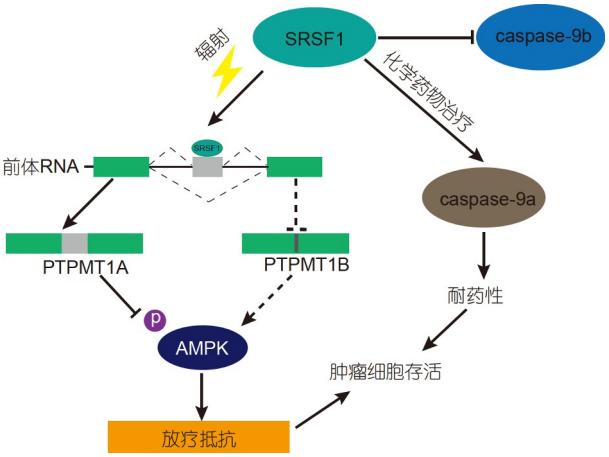

图 4 SRSF1在放疗抵抗和化疗抵抗中的作用. 放疗过程中, SRSF1调控PTPMT1的可变剪接, 使PTPMT1A剪接形式增多, 而抑制PTPMT1B的表达. PTPMT1A抑制AMPK的磷酸化, 最终导致放疗抵抗. 此外, SRSF1还可以促使caspase-9a增加, 抑制caspase-9b表达, 导致细胞耐药性. 二者共同促进了肿瘤细胞的生长

Figure 4 Role of SRSF1 in radioresistance and drug resistance. During radiotherapy, SRSF1 regulates PTPMT1 alternative splicing, increasing the splicing forms of PTPMT1A while inhibiting PTPMT1B expression. PTPMT1A inhibits AMPK phosphorylation, leading to radioresistance. Furthermore, SRSF1 can enhance the expression of caspase-9a while inhibiting the expression of caspase-9b, leading to drug resistance of cancer cells. Together, they coordinate to promote tumor cell growth

H1299经过辐射后, 剪接因子SRSF1的表达会有显著增加, 若敲低SRSF1可以增强肿瘤细胞对辐射的敏感性. 通过筛选, 研究人员发现, SRSF1的靶基因*PTPMT1*在放疗抵抗中发挥重要的功能. PTPMT1是一种类似PTEN的线粒体激酶, 经过SRSF1的剪接调控, 其可以形成两种剪接异构体PTPMT1A和PTPMT1B. 由于放疗后肺癌中的SRSF1表达水平明显增加, 导致长亚型PTPMT1A增加, 若抑制SRSF1的表达, 则会促进放疗状态下短亚型PTPMP1B的生成. PTPMT1B会使AMPK磷酸化增加, 诱导DNA双链断裂, 使癌细胞对辐射增敏(图4). 在癌症样本中, PTPMT1B的表达抑制, 也是造成患者不良预后的重要因素之一[67].

6 可变剪接与肺癌血管生成

血管生成是一个复杂的过程, 可以导致包括肿瘤在内多种疾病的发生与进展[68]. 实体瘤的生长处于低氧环境, 低氧诱导因子(hypoxia-inducible factor, HIF)通路激活并调控血管内皮生长因子(vascular endothelial growth factor, VEGF)及血管生成素-1(angiopoietin-1, ANGPT1)等数百个基因的表达. 血管内皮生长因子-A(VEGF-A)可以同酪氨酸激酶受体VEGFR-1(FIt-1)或VEGFR-2(FIk-1/KDR)结合, 控制新血管生成过程中内皮细胞的增殖、生长和迁移[69]. VEGF-A可被可变剪接广泛地调控, 产生多个剪接异构体. 其中$VEGF_{xxx}$和$VEGF_{xxx}$b家族可编码不同的VEGF-A剪接异构体. $VEGF_{xxx}$可编码包含外显子8a的VEGFA165, 而$VEGF_{xxx}$b则编码包含外显子8b的VEGF165b, 二者只是其C末端的6个氨基酸有所差异. 这两种剪接异构体都能结合VEGFR2, 但是功能却是完全相反的. VEGFA165可以促进肿瘤细胞血管生成, 而VEGF165b则能抑制肿瘤血管的生成[70]. 研究表明, 在36%的非小细胞肺癌中, VEGF165b表达增高, 且VEGF165b/VEGF165的高比值与NSCLC、肺腺癌(46%)淋巴结转移密切相关. VEGF165b可激活侵袭性β1整合素/VEGFR2信号通路, 导致肿瘤细胞的转移, 所以在VEGFA165b较高表达的肺癌细胞中, 会降低传统抗血管生成药物的治疗效果[71].

7 可变剪接与肺癌细胞代谢

1924年, 奥地利科学家Otto Warburg指出, 与正常的细胞相比, 肿瘤细胞使用有氧糖酵解, 减少线粒体氧化磷酸化来进行葡萄糖代谢, 肿瘤细胞这种代谢偏好即为著名的Warburg效应[72], Warburg效应广泛存在于各种肿瘤细胞中. 在营养匮乏、低氧和酸性的环境下, 通过转录重编程, 癌前病变细胞表现Warburg表型, 帮助肿瘤细胞在不利的环境下生长[73]. Warburg效应在肿瘤细胞转移、增殖、免疫逃逸、耐药等方面发挥重要作用[74~77], 同时该效应也被诸多因素所影响, 其中包括可变剪接.

微阵列研究表明, 糖酵解基因是癌症中上调率最高的基因之一, 其中包括糖酵解最后一步的限速酶——丙酮酸激酶[78,79]. 哺乳动物中存在四种丙酮酸激酶亚型, L型和R型在肝脏和红细胞中表达, M1亚型在成人大多数组织中表达, 而M2亚型是M1亚型的剪接异构体, 在胚胎发育过程中表达[80]. PKM1主要在癌前病变的组织中表达, 而PKM2主要在肿瘤组织中表达, 且在肺癌细胞系A549中, PKM2高表达, PKM1则几乎不表达[81]. 这提示PKM1和PKM2两种剪接亚型, 在肿

瘤进展中可能发挥重要的功能. 若在肺癌细胞H1299中敲低PKM2, 会降低细胞葡萄糖代谢, 并抑制肿瘤细胞增殖. 过表达PKM2后, 肺癌细胞有氧糖酵解的能力会增强, 乳酸和丙酮酸生成增多, 果糖2,6-二磷酸水平降低. 在肺癌中, PKM1的功能和PKM2则相反, PKM1促进肿瘤细胞的氧化磷酸化, 抑制肿瘤细胞的生长. PKM2亚型对于肿瘤细胞的Warburg效应是必需的, 肿瘤细胞的代谢偏好为肿瘤细胞在体内提供可变生长优势. 鉴于肿瘤细胞的代谢偏好, 针对肿瘤代谢重编程的研究, 为抗肿瘤药物研发提供了更多的可能.

8　肺癌中lncRNA可变剪接

除了mRNA可以发生可变剪接, 长链非编码RNA (long non-coding RNA, lncRNA)同样受到可变剪接的调控[82]. lncRNA的可变剪接在肿瘤的发生和发展过程中同样发挥重要功能[83,84]. 研究发现, 通过可变剪接, 人肺腺癌中均可以产生PD-L1的一种长链非编码RNA亚型, 即PD-L1-lnc. 同PD-L1的mRNA相似, 在IFN-γ诱导下, PD-L1-lnc在多种肺腺癌组织中表达增高. 研究结果显示, PD-L1-lnc可直接同c-Myc相结合, 增强c-Myc的转录活性, 促进肺腺癌细胞的增殖和侵袭, 导致肺癌的恶性表型[85]. 因此, PD-L1-lnc可作为肺腺癌的检测指标, 预测患者的预后情况.

9　可变剪接与肺癌中外泌体形成

外泌体是细胞外囊泡的一部分, 其可被脂质双分子层包裹, 直径在40~160 nm, 大多数真核细胞可形成外泌体[86]. 外泌体在肿瘤中的功能也被越来越多地报道, 其在肿瘤的发展、转移和免疫等方面皆发挥重要的作用[87]. 鉴于RNA可变剪接的普遍性, 且外泌体中包含多种RNA, 可变剪接对于外泌体的调控同样引起了人们的重视. 如HBS1L的短剪接形式, 其和外泌体及SKI复合物的形成密切相关[88]. 但是外泌体中是否包含经过可变剪接而产生的RNA分子, 目前尚不清楚.

10　可变剪接与肺肿瘤微环境

肿瘤微环境(tumor microenvironment, TME)是一个复杂且不断发展的系统. 除了基质细胞、成纤维细胞和内皮细胞, TME还包含诸多先天性和适应性免疫细胞. 由于TME中细胞组成的复杂性, 血管生成有限且分化较差, 导致营养、氧气输送和废物清理效率低下[89]. TME与肿瘤诸多生命活动关系密切, 如肿瘤转移[90]、肿瘤细胞耐药[90]、放化疗[91,92]、免疫治疗等[90,93]. 此外, 越来越多的证据表明, 可变剪接同样可以参与肿瘤微环境的调控. 可变剪接在乳腺癌、胃癌、恶性胶质瘤、肺癌等恶性肿瘤微环境中的功能越来越多地被报道[94~98].

以肺癌为例, 在肺癌中, Slit2的表达下调, 且Slit2存在两种剪接异构体Slit2-WT和Slit2-Δ15. 研究表明, 在$Kras^{G12D}$人为诱导的小鼠肺癌模型中, Slit2-WT/Slit2-Δ15的比值增加, 总Slit2的表达增加, 抗肿瘤免疫被抑制. 而在尾静脉注射形成的肿瘤模型中, Slit2-WT/Slit2-Δ15的比值会减小, 总Slit2表达降低. 此外, 在脂多糖(lipopolysaccharide, LPS)诱导的肺炎模型中, Slit2-WT/Slit2-Δ15的比值同样会减小, Slit2的表达被抑制, 这提示Slit2-Δ15可能会促进炎症的发生. 在TME中, 具有抗炎症功能的Slit2-WT亚型表达增加, 导致抗肿瘤免疫抑制, 肿瘤细胞的生长逃离监控, 促进肿瘤细胞生长[99]. 但值得注意的是, 肺癌微环境中的其他细胞, 如肺成纤维细胞、免疫浸润细胞中, 可变剪接相关分子的表达情况、RNA剪接事件的变化人们仍然无从知晓, 针对肺癌肿瘤微环境中各类型细胞可变剪接的研究亟待开展.

作为细胞生命活动中的重要一环, 可变剪接对生命活动的精准调控是细胞发挥正常功能的基础之一. 可变剪接是蛋白多样性的重要来源, 同一基因通过可变剪接可产生功能相似或相反的剪接异构体. 如今肿瘤仍然是公共卫生面临的巨大问题, 虽然治疗手段、技术在不断进步, 但是肿瘤的发病率和死亡率依然居高不下. 作为发病率和死亡率皆居前列的癌症, 肺癌是人类健康面临的巨大威胁之一. 随着研究的深入, 可变剪接在肺癌中的作用受到了更多的重视. 虽然技术手段的进步使人们对RNA可变剪接的了解更加全面, 但相对于庞大的基因组, 目前检测到的剪接产物是不全面的. 覆盖范围更广的测序技术, 更深度的测序方法, 迫切地需要相关从业人员去开发. 此外, 人们需要清楚剪接异构体的生物学功能, 了解其生物学意义. 针对可变剪接的研究, 使人们对肿瘤的认识更加全面, 也为肿瘤药物的开发提供新的研究方向.

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Roles of RNA alternative splicing during the occurrence and development of lung cancer

CHEN Lei, CHEN ChaoQun, ZHANG WenJing & WANG Yang

Institute of Cancer Stem Cell, Dalian Medical University, Dalian 116044, China

In the human genome, more than 90% of the human genes undergo alternative splicing, which participates in the entire life activity. Abnormal RNA splicing is closely associated with human diseases, including cancer. Cancer is still a serious public health concern as the second leading cause of death. Every year, lung cancer ranks first in the number of new cancer cases and deaths. Abnormal alternative splicing occurs extensively in lung cancer, regulating tumorigenesis and lung cancer progression. Splicing factor mutations and aberrant expression are well-known drivers of lung cancer progression. Furthermore, alternative splicing plays a critical role in drug resistance, radiotherapy resistance, angiogenesis, and tumor metabolism.

tumor, lung cancer, alternative splicing, abnormal splicing

doi: 10.1360/SSV-2021-0407