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靶向复制应激联合免疫检查点抑制剂在肿瘤治疗中的作用机制研究

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摘要 复制应激(replication stress, RS)是指由于各种因素导致DNA复制叉的推进受阻、停滞或崩溃, 这会激活细胞复制应激反应来应对复制应激, 但是如果复制应激超过细胞的修复能力, 就会引发基因组不稳定甚至细胞死亡。靶向RS的药物即以此为机制杀伤肿瘤细胞。最近有研究发现, 靶向RS能够协同免疫检查点抑制剂(immune checkpoint inhibitors, ICIs)增强对肿瘤的杀伤作用, 但其机制尚未完全阐明。该篇综述重点回顾了靶向复制应激与免疫检查点抑制剂联合治疗肿瘤的研究进展, 为开发更高效的肿瘤治疗策略提供了理论依据。

关键词 复制应激; 免疫检查点抑制剂; 联合治疗; 机制研究

Studies on the Mechanism of Targeting Replication Stress Combined with Immune Checkpoint Inhibitors in Tumor Therapy

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Abstract RS (replication stress) refers to the slowing down, stopping or collapsing of DNA replication forks due to various factors, which will activate the cellular replication stress response to cope with the replication stress. However, if the replication stress exceeds the cell's ability to repair itself, genomic instability or even cell death will be triggered. Drugs targeting RS kill tumor cells by this mechanism. Recently, it has been found that targeting RS can synergize with ICIs (immune checkpoint inhibitors) to enhance tumor killing, but the mechanism has not been fully elucidated. This review focuses on reviewing the research progress of combining targeted replicative stress with immune checkpoint inhibitors for the treatment of tumors to provide a theoretical basis for the development of more efficient tumor treatment strategies.

Keywords replication stress; immune checkpoint inhibitor; combination therapy; mechanism research

在真核细胞中, DNA的复制过程会由于各种原因出现复制应激(replication stress, RS)[1], 进而引发复制应激反应(replication stress response, RSR), 使DNA复制叉推进减慢或停滞以便于进行损伤修复[2]。RSR主要由共济失调毛细血管扩张和Rad3相关–检查点激酶1(ataxia-telangiectasia and rad3-related-checkpoint

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kinase 1, ATR-CHK1)通路和共济失调毛细血管扩张症突变–检查点激酶2(ataxia-telangiectasia mutated-checkpoint kinase 2, ATM-CHK2)通路协调控制[3], 此外还涉及DNA损伤修复和耐受机制。癌细胞内存在持续的复制应激, 如果抑制RSR中的关键蛋白活性, 增强复制应激反应, 就会诱发癌细胞有丝分裂灾难甚至细胞死亡。

免疫检查点阻断(immune checkpoint blockade, ICB)在肿瘤治疗中已取得不错疗效, ICIs主要包括抗细胞毒性T淋巴细胞相关抗原4(cytotoxic T-lymphocyte associated antigen-4, CTLA-4)、程序性死亡配体-1(programmed death-ligand 1, PD-L1)和程序性死亡受体-1(programmed death-1, PD-1), 它们能够解除肿瘤细胞对T细胞的抑制, 从而使T细胞更加有效地杀伤肿瘤细胞[4], 然而ICIs会使机体出现以炎性综合征为主要表现的免疫相关不良反应(immune-related adverse events, irAE)[5]并产生耐药性[6], 亟需相关研究来减轻不良影响。

现有研究表明, 靶向复制应激的部分抑制剂会将核内DNA片段释放到细胞质, 引起先天免疫反应[7], 且抑制剂对PD-L1的表达具有调节作用, 若将其与免疫检查点抑制剂联合应用可能会导致对肿瘤杀伤的协同效应, 并克服单独使用时的耐药性。然而二者联合应用的具体机制有待进一步明确, 效果也仍存争议, 针对相关靶点的生物标志物还需要深入挖掘。本文就靶向RS联合ICIs治疗肿瘤的相关研究进展进行综述, 为临床研发更高效的肿瘤治疗策略提供参考。

1 复制应激的来源及细胞反应

1.1 复制应激的来源

各种限制复制叉进展和阻碍DNA复制的内源性或外源性事件都可以导致复制应激。在肿瘤细胞中常见的内源性事件是未能及时修复的DNA损伤, 它会阻碍复制叉的前进。此外, 致癌基因激活会导致核苷酸库耗尽、复制叉与转录复合物冲突和起源放电增加, G_1/S检查点的丢失和过早进入S期也会影响DNA复制过程而导致复制应激[7]。还有一些在正常细胞中同时存在的内源性因素, 如活性氧对DNA结构的损伤、重复DNA序列区域出现复杂的二级结构如DNA发夹(DNA hairpin)或四链体(G-quadruplex)、DNA修复过程形成的中间产物、新生RNA与DNA杂交形成R环(R-loop)和核糖核苷酸错误掺入导致DNA链结构异常等都会阻碍复制过程的顺利进行[1]。外源性事件如化疗药物、细胞毒性物质、辐射等引起的DNA损伤, 这些外源性因素可能直接破坏DNA分子的结构, 使复制叉在遇到损伤部位时无法正常推进, 从而引发复制应激[8]。这些内源性或外源性因素可以导致DNA单链或双链断裂的积累, 进而形成未能及时修复的DNA损伤(图1)。

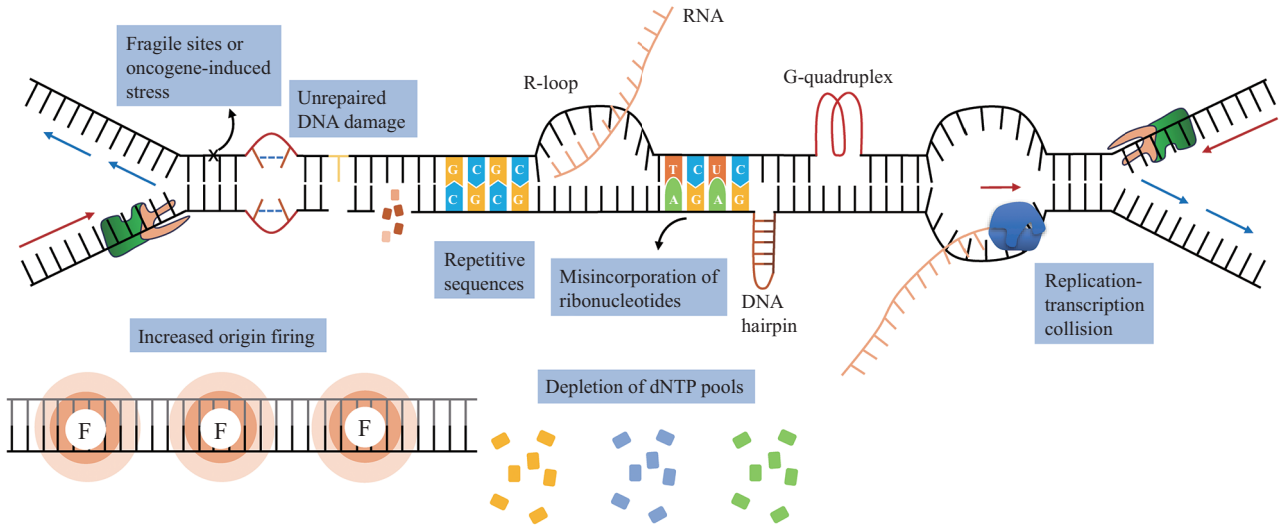
1.2 复制应激的细胞反应

复制应激发生时, 细胞会启动一系列复杂的反应。首先是细胞周期检查点的激活, 如ATR-CHK1和ATM-CHK2通路的激活。ATR主要应对复制叉停滞时产生的单链DNA(single-stranded DNA, ssDNA), ATR通过激活CHK1, 使细胞周期进程暂停在S期或G_2/M期, 为修复争取时间[9-10]。ATR-CHK1通路能够抑制复制起始放电、维持复制叉稳定、促进复制叉修复和重启[11]。ATR-CHK1通路激活后还可以磷酸化蛋白激酶WEE1, WEE1通过抑制细胞周期蛋白依赖性激酶(cyclin-dependent kinase, CDK)活性来进一步减缓细胞周期进程[12-14]。ATM则更多地针对DNA双链断裂(double-strand breaks, DSBs), 通过磷酸化CHK2调控细胞周期停滞在G_1期, 直到损伤被修复或者不利因素被消除[15-16]。ATM可以通过调控DNA Werner综合征解旋酶(Werner syndrome helicase, WRN)和Bloom综合征解旋酶(Bloom syndrome helicase, BLM)来促进停滞的复制叉重启[17-18]。ATM和CHK2还通过稳定肿瘤抑制基因*P53*以增强其对下游基因的转录激活驱动[19]而保护复制应激区域。同时, 细胞会通过同源重组修复和非同源末端连接等途径参与修复复制应激导致的DNA损伤, 以维持基因组稳定性。细胞还存在DNA损伤耐受(DNA damage tolerance, DDT)途径, 在一定程度上允许细胞在存在DNA损伤的情况下继续存活和复制, 但这可能增加基因组不稳定性和突变的风险[7,20]。

在长期或严重复制应激下, 如果修复失败, 细胞可能会走向凋亡或衰老。这些反应是细胞为了应对复制应激维持自身生存和基因组完整性的重要策略, 但在肿瘤细胞中, 这些机制可能被异常调控, 影响肿瘤的发展和治疗效果。

2 复制应激与肿瘤进展的关系

原癌基因如*MYC*等不仅能诱导细胞持续增殖,



复制叉主要由解旋酶(橙色)、聚合酶(绿色)、前导链(红色箭头)、后随链(蓝色箭头)组成。RNA聚合酶为蓝色, 新合成的RNA分子为淡黄色。起源放电增加由标有字母F的淡橙色圆圈表示。

The replication fork mainly consists of helicase (orange), polymerase (green), the leading strand (red arrow), and the lagging strand (blue arrow). RNA polymerase is blue, and the newly synthesized RNA molecules are pale yellow. The pale orange circles labelled with the letter F show the increased origin firing.

图1 DNA复制应激的原因(根据参考文献[7]修改)

Fig.1 Causes of replication stress (modified from reference [7])

其过表达还会使转录速率增加, 提升复制叉与转录复合物相遇并发生冲突的概率, 导致细胞出现复制应激[21]。复制起点结构在一定程度上能够稳定DNA中易发生断裂的脆性位点, 癌基因的激活等使染色质中存在更多缺乏休眠复制起点的脆性位点, 在受到内外因素干扰后更易发生DNA断裂, 这进一步增加了复制叉停滞和DNA损伤事件的发生概率[22]。由于复制应激使基因组不稳定, 这成为了细胞转化为癌细胞过程中的早期事件[21]。癌细胞在长期的复制应激环境下, 会通过如RSR通路(如ATR-CHK1通路)等机制间接抑制凋亡, 使肿瘤细胞能够在持续的复制应激和DNA损伤情况下存活下来。复制应激还会影响肿瘤细胞的坏死和自噬过程, 当出现严重的复制应激, 例如过度的DNA损伤或长时间的复制叉停滞且无法修复时, 肿瘤细胞会发生坏死, 临床化疗药物顺铂(Cisplatin)即通过此机制杀伤肿瘤细胞[23]。

3 靶向复制应激的药物及机制

靶向复制应激反应的药物为肿瘤治疗提供了新策略。ATR抑制剂(如AZD6738)、CHK1抑制剂(如UCN-01)和WEE1抑制剂(如AZD1775)都会激活CDK并允许细胞进入有丝分裂, 癌细胞无法留出足够的时间修复受损DNA, 从而过早进入有丝分裂而死亡[24-26]。聚腺苷二磷酸–核糖聚合酶(poly-ADP-ribose polymerase, PARP)可检测和修复DNA单链损伤, 乳腺癌易感基因1和2(breast cancer susceptibility gene 1 and 2, BRCA1 and BRCA2)是用于修复DNA双链损伤的同源重组修复(homologous recombination repair, HRR)中的关键酶, PARP抑制剂在BRCA1/2缺陷肿瘤中因合成致死效应导致肿瘤细胞死亡。ATR/CHK1/WEE1抑制剂会导致DNA在S期和G_2期的HRR受损, 并诱导双链断裂[27-31], 可以用于因为恢复HRR功能而对PARP抑制剂(如奥拉帕利)产生获得性耐药的肿瘤[32-35]。肿瘤细胞因长期使用靶向复制应激药物而产生耐药性, 其原因可能是肿瘤细胞内其他补偿性信号通路被激活, 或者是药物靶点本身发生突变导致药物失效。这表明在治疗期间, 需要同时对潜在的耐药机制进行监测和干预, 比如联合使用针对多种耐药相关途径的药物。跨损伤合成(translesion synthesis, TLS)是一个主要的DDT途径, 而TLS聚合酶的小分子抑制剂(如吲哚硫代巴比妥酸及其衍生物)可通过干扰肿瘤细胞的DNA损伤耐受机制, 增加DNA损伤积累, 使肿瘤细胞对化疗药物诱导的DNA损伤更加敏感, 可以协同常规化疗药物促进其对肿瘤的杀伤作用[36-37]。

部分化疗药物能够诱导复制应激杀死肿瘤细

胞。例如，铂类药物(如顺铂)通过与DNA结合形成加合物，引发复制叉停滞，诱导复制应激[38]。抗代谢药(如吉西他滨)干扰核苷酸合成，使复制过程因原料不足而应激[39-40]。拓扑异构酶抑制剂(如喜树碱衍生物)则是通过控制DNA解螺旋过程阻碍前进的复制叉，诱导DNA双链断裂，并触发复制应激相关的细胞杀伤机制[41]。烷化剂(如环磷酰胺)能够与DNA链上的碱基发生共价结合，形成DNA链间或链内交联，导致DNA链断裂[42]，使细胞无法复制DNA而死亡。这些药物能够诱导肿瘤细胞出现复制应激，因此一个重要的研究方向是将其与ATR/CHK1/WEE1抑制剂协同组合，以增强其对肿瘤的杀伤作用。

由于肿瘤细胞的异质性以及复制应激反应网络的复杂性，单一药物往往难以完全抑制肿瘤生长，因此联合不同的靶向复制应激药物或将这些靶向药物与传统化疗、放疗或免疫治疗联合也是极具前景的方向。例如，在免疫治疗后使用ATR抑制剂，利用ATR抑制剂干扰肿瘤细胞对免疫检查点抑制剂诱导的复制应激进行修复，进而提高抗肿瘤的整体效果。

4 靶向复制应激和ICIs的联合应用

4.1 复制应激与免疫反应的关联及在DDR缺陷肿瘤中的意义

复制应激可使胞质中DNA片段或微核数量增加，激活环鸟苷酸–腺苷酸合成酶(cyclic GMP-AMP synthase, cGAS)/干扰素基因刺激蛋白(stimulator of interferon genes, STING)通路，诱导I型干扰素(interferon I, IFN I)和促炎细胞因子的产生，并上调PD-L1表达，IFN I还促使趋化因子CCL5和CXCL10表达水平增加，促进肿瘤中效应性T细胞的浸润[43-45]。靶向复制应激还可导致肿瘤细胞内源性免疫原性增强，如增加肿瘤抗原的表达和释放。免疫检查点抑制剂可解除肿瘤微环境中的免疫抑制状态，使免疫系统能够有效识别并杀伤这些经靶向治疗后“暴露”的肿瘤细胞。二者联合可产生协同作用，增强T细胞对肿瘤细胞的杀伤活性，提高抗肿瘤免疫效应。

在DNA损伤修复(DNA damage repair, DDR)缺陷肿瘤中，这种关联有着更高的研究价值。DDR缺陷会使肿瘤细胞基因组变得不稳定，产生更多新抗原并上调PD-L1的表达，这不仅增强了适应性免疫系统对肿瘤的识别能力，还会与cGAS-STING信号转导相互作用[46-47]。例如在DNA损伤反应缺陷型乳腺癌中，S期DNA损伤以STING依赖方式激活了PD-L1表达[48]，使用特异性抑制剂靶向RSR有可能放大这种反应并促进对乳腺癌细胞的清除。在患有BRCA1缺陷型卵巢肿瘤的小鼠中，奥拉帕利通过STING依赖性抗肿瘤免疫反应发挥作用，将PARP抑制剂和PD-1阻断剂联合使用时，显示出了更好的抗肿瘤效果[49]。由于DDR通路的改变与肿瘤对ICI的易感性高度相关，将靶向DDR通路的药物与ICI相结合成为研究较多且有吸引力的治疗策略[47,50]。

4.2 ICB与靶向RSR通路蛋白联合治疗肿瘤的机制研究进展

免疫和DNA复制应激反应途径存在交叉[51]，如RSR关键成员DNA-PK、MRE11和RAD50同时也是启动STING依赖性信号和T细胞活化的传感器[52-54]，这种交叉作用为联合治疗提供了理论研究基础。在这方面，以CHK1为代表的RSR通路蛋白抑制剂的研究得到了快速发展。MCGRAIL等[55]发现抑制CHK1/2诱导RSR缺陷能够增加异常复制起始点，促进免疫刺激性细胞溶质DNA的积累，改善肿瘤模型中的ICB反应，CHK1和CHK2选择性抑制剂Prexasertib有希望增加免疫疗法的有效性[56]。一项临床前数据显示，CHK1抑制剂SRA737促进了肺癌中细胞毒性T淋巴细胞激活，SRA737+抗PD-L1+吉西他滨三联疗法可以显著提升抗肿瘤效果[57]。除了CHK1外，ZHANG等[58]发现在使用YKL-5-124抑制细胞周期蛋白依赖性激酶7(cyclin-dependent kinase 7, CDK7)后，小细胞肺癌(small cell lung cancer, SCLC)的基因组会不稳定，因为这增强了复制应激和$CD4^+$T细胞的浸润，将YKL-5-124与抗PD-1联合使用能够明显抑制肿瘤的生长。LIU等[59]发现细胞分裂周期7激酶(cell division cycle 7 kinase, CDC7)的抑制能够使肿瘤细胞对复制应激的反应减弱，并能抑制肿瘤细胞的DNA复制，其与肿瘤免疫微环境的关系值得进一步研究，包括CDC7抑制剂与免疫检查点抑制剂联合应用的具体机制和效果。DNA损伤反应机制与免疫调节之间存在紧密的联系，在药物联合治疗中可以通过调节这些关键蛋白和信号通路来增强抗肿瘤效果，但其中涉及的复杂分子机制和细胞内调控网络仍需要更多的研究来解析。

4.3 非复制应激反应靶点沉默对肿瘤复制应激和免疫反应的影响

其他一些非复制应激反应靶点的沉默也促进

了复制应激反应及免疫治疗。抑制组蛋白去甲基化酶KDM4A可以阻止鳞状细胞癌中的DNA复制，增强复制应激并诱导胞质DNA积累，激活肿瘤细胞内源性cGAS-STING信号转导，还可与PD-1阻断剂协同募集和激活$CD8^+$T细胞，以此抑制鳞状细胞癌的生长和转移[60]。沉默癌细胞重复元件的阻遏物FBXO44及其辅因子SUV39H1可刺激癌细胞复制应激并改善抗PD-1治疗反应，促使癌细胞DNA双链断裂和IFN信号转导，最终促进肿瘤死亡[61-62]。SMARCB1是染色质重塑复合物的核心成员，能够调节基因的可及性，MSAOUEL等[63]发现在肾髓质癌中，SMARCB1缺失不仅导致胞质dsDNA增加促进cGAS/STING通路激活，提升了肿瘤的免疫原性，还激活c-MYC通路增强了复制应激，最终导致了肿瘤细胞DNA损伤，这提示我们将*SMARCB1*敲除与ICIs疗法相结合或许会产生有益效果。未来的研究可进一步探究*SMARCB1*等基因在不同肿瘤类型中的作用、与ICI疗法联合应用的可行性及潜在机制，同时关注这种联合治疗可能引发的不良反应和潜在风险。

4.4 联合治疗的复杂性

在一些肿瘤细胞中，使用DNA损伤剂处理后，PD-L1的含量会以依赖ATM/ATR/CHK1途径的方式上调，当使用ATM、ATR或CHK1的特异性抑制剂后，这种上调趋势也会受到抑制[64]，而且ATR抑制甚至会使PD-L1水平下降[65]，这可能会让DNA损伤检查点抑制剂和PD-1/PD-L1阻断之间失去协同作用。这表明在肿瘤细胞的免疫调控网络中，ATM/ATR/CHK1途径与PD-L1的表达之间存在着紧密且微妙的联系，这种联系可能不仅局限于简单的线性调控，或许涉及到更为复杂的信号交叉和反馈机制。WAYNE等[66]指出ATR、CHK1或WEE1的抑制不能激活IFN I应答，其中ATR抑制虽然激活了cGAS-STING途径，但是通过激活CDK1-SPOP途径又会导致PD-L1蛋白降解[67]，这可能涉及SPOP蛋白对PD-L1的泛素化修饰和降解过程的调控。尽管目前具体机制仍不清楚，但可以推测这是细胞在应对复制应激和免疫压力时一种精细的平衡调节机制。这种平衡可能因联合治疗中的药物干预而被打破，从而导致治疗效果的不确定性。这也意味着在开发更有效的联合治疗方案时，需要深入研究这些复杂的相互作用，以避免因单一靶点抑制而引发的负面效应。此外，BURLEIGH等[68]确定DNA-PK为不依赖STING的DNA传感器，对其抑制可能影响先天免疫激活。这意味着当使用靶向复制应激药物抑制DNA-PK活性时，可能在抑制肿瘤细胞增殖的同时，也削弱了机体对肿瘤细胞的先天性免疫识别和攻击能力，从而影响了患者的整体治疗效果。

5 潜在生物标志物

5.1 RSR途径主要生物标志物

细胞周期蛋白E1(cyclin E1, CCNE1)扩增的肿瘤模型对ATR、CHK1和WEE1这三种抑制剂表现出了更高的敏感性，作用机制可能涉及CCNE1扩增导致细胞周期调控紊乱，使肿瘤细胞对复制应激更加敏感，从而对特定的抑制剂产生更强的反应，这提示CCNE1能够成为RSR途径靶向治疗的生物标志物[33,69]。在临床应用方面，CCNE1扩增的肿瘤患者在早期临床试验中也对WEE1抑制剂和CHK1抑制剂呈现出更高的反应率[70-71]。

WEE1抑制剂可以使范可尼贫血(Fanconi anemia, *FA*)/同源重组(homologous recombination, *HR*)基因沉默的肿瘤中核苷酸库耗竭并促进RS，导致肿瘤细胞进入异常有丝分裂而死亡[29]；*TP53*突变会造成G_1/S检查点出现缺陷，从而增加肿瘤细胞对WEE1调控的G_2/M检查点的依赖[72]；肿瘤细胞中功能丧失的SET结构域2(SET domain containing 2, SETD2)与WEE1抑制剂共同作用能够对肿瘤细胞产生合成致死效应，这可能与SETD2在染色质修饰和基因表达调控中的作用有关，当SETD2功能丧失时，肿瘤细胞对WEE1抑制剂会更加敏感[14]。以上三者都可作为WEE1靶向治疗的生物标志物。

肿瘤细胞中ATM功能丧失会增强肿瘤细胞对ATR通路的依赖，二者功能的同时丧失会使肿瘤细胞出现合成致死的现象[73]，ATM缺陷型肿瘤对ATR抑制剂ceralasertib和吉西他滨联合用药敏感性更强[74]，因此ATM缺失可被用作一个选择性生物标志物。类似地，RAD51缺失后，对ATR或CHK1的抑制会造成同源重组缺陷细胞的死亡[75]。

5.2 HRR通路相关基因标志物

BRCA2缺陷会使PD-L1/PD-1和肿瘤浸润淋巴细胞(tumor-infiltrating lymphocytes, TILs)表达上调[76]，并且BRCA1/2突变患者对ICIs有更好的治疗反应[77]，提示*BRCA*基因可作为ICIs治疗的生物标志物或者

靶向位点。在BRCA1相关蛋白1(BRCA1 associated protein 1, BAP1)突变的间皮瘤中，免疫检查点激活和TILs水平明显更高[78-79]，说明BRCA1可作为间皮瘤患者ICIs治疗的潜在生物标志物。

5.3 ICI免疫疗法相关复制应激生物标志物

在临床前研究中，发现DNA聚合酶ε(DNA polymerase epsilon, *POLE*)突变不仅导致复制叉推进减慢或停滞引起复制应激，还使肿瘤中免疫检查点相关蛋白(如PD-L1和PD-L2)以及T细胞标志物(如CD8A、PD-1和CTLA-4)的表达水平升高，提示*POLE*突变可能成为ICI免疫治疗的一个生物标志物[80]。

临床前研究中选中的预测性生物标志物需要临床活检样本进行验证以确定其有效性，并且由于肿瘤微环境的复杂性，对靶向RS或者ICI治疗结果的预测可能需要与其他遗传缺陷进行组合应用。例如，肿瘤细胞的异质性可能导致不同区域的肿瘤细胞对治疗的反应不同，免疫细胞的类型和功能状态也会影响治疗效果。对治疗前后的肿瘤样本进行遗传分析能够显示靶向药物治疗反应的动态变化，这样不仅有助于发现新的生物标志物还有助于治疗前对患者的选择。

6 展望

复制应激可通过多种途径影响肿瘤免疫微环境，ICB联合靶向RSR通路蛋白(如CHK1抑制剂等)或其他复制应激相关靶点(如CDK7、CDC7、KDM4A、SMARCB1等)的研究，都显示出了这种联合治疗策略在增强抗肿瘤效果上的潜力。然而，研究中出现的矛盾结果表明联合治疗的分子机制比预期更为复杂，且整体上仍缺乏可靠且全面的标志物来准确筛选能从联合治疗中获益的患者。在未来的研究中，需要进一步深入解析联合治疗的复杂分子机制，加大力度寻找更具特异性和敏感性的标志物。从临床应用角度，应积极开展更多高质量的临床试验。总之，靶向复制应激联合免疫检查点抑制剂在肿瘤治疗领域前景广阔，但仍需要大量深入的研究工作来克服当前面临的挑战。

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