POLE/POLD1突变与肿瘤免疫治疗研究进展

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摘要 阐述了DNA聚合酶ε(polymerase epsilon,POLE)和DNA聚合酶delta 1(polymerase delta 1,POLD1)基因编码的亚基参与DNA复制和校对,总结了POLE/POLD1突变对肿瘤突变负荷以及肿瘤内免疫细胞浸润的影响,综述了POLE/POLD1突变在子宫内膜癌、结直肠癌、肺癌中的研究进展,并讨论了POLE/POLD1突变作为潜在的免疫检查点抑制剂疗效预测分子标志物面临的诸多挑战。

关键词 POLE;POLD1;基因突变;免疫治疗;分子标志物

目前癌症发病率和死亡率在全世界不断上升,癌症是全世界最常见的死亡原因之一,2020年全球有近1000万的癌症死亡病例[1],2015年中国也有高约281.4万例的癌症患者死亡[2]。因此癌症防治已成为重大的公共卫生问题。以往癌症治疗方法包括手术、放疗、化疗,但都存在一定的局限性。

近年来,以免疫检查点抑制剂(immune checkpoint inhibitors,ICI)为代表的免疫疗法在恶性肿瘤治疗中取得重大进展,其中程序性死亡受体1(programmed death 1,PD-1)抗体应用最为广泛,包括帕博利珠单抗、纳武单抗等。与传统的化疗及靶向治疗相比,有研究发现即使停止ICI治疗后,部分患者仍可出现持续的病情缓解,如非小细胞肺癌患者接受ICI治疗后5年的总生存率上升至16%[3],有研究显示ICI治疗可以产生持久的肿瘤特异性免疫记忆[4],这为提高肿瘤治疗疗效以及患者长期生存带来了曙光。然而ICI的疗效仍未令人满意,2012年一项纳入多个癌种免疫治疗的临床研究显示,仅有28%晚期黑色素瘤、27%肾细胞癌以及18%晚期非小细胞肺癌患者在接受纳武单抗治疗后获得客观缓解[5]。因此找到更多可靠的ICI疗效预测分子标志物,准确区分ICI治疗有效人群,对提高免疫疗效以及减少过度治疗非常关键。

错配修复缺陷(deficient mismatch repair,

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dMMR)/微卫星高度不稳定(microsatellite instable high,MSI-H)是首个被发现的泛癌种ICI治疗的疗效预测分子标志物[6]。2017年,Le等进行的一项纳入12种肿瘤类型的dMMR晚期癌症患者临床研究显示,dMMR晚期肿瘤PD-1单抗治疗客观缓解率(objective response rate,ORR)可达53%,而且有21%患者获得完全缓解[7]。不过有研究显示肿瘤中dMMR/MSI-H分型的比例较低[8]。肿瘤突变负荷高(tumor mutation burden high,TMB-H)是第二个泛癌种ICI疗效预测分子标志物,其在Ⅱ期临床试验KEYNOTE-158表现优异[9-11],并有多项研究显示其与免疫治疗获益显著相关[12-14]。除此之外,还有多个与ICI疗效相关的生物标志物,例如PD-L1的表达、肿瘤内T细胞浸润程度、体细胞拷贝数改变、表观遗传改变、肠道菌群等[15-17]。然而这些生物标志物都有各自的局限性,因此探索更有效的ICI疗效预测标志物仍然十分重要。现有研究表明POLE/POLD1的突变不仅与肿瘤形成密切相关,而且是潜在的泛癌种免疫治疗疗效预测分子标志物。以下将对其功能、对肿瘤发生以及肿瘤免疫治疗的影响进行综述。

1　POLE和POLD1的功能

POLE和POLD1的基因分别编码DNA聚合酶ε(polymerase ε,Pol ε)和聚合酶δ(polymerase δ,Pol δ)的催化和校对亚基,其中Pol ε和Pol δ是真核细胞中的B类DNA聚合酶,两者都具有3′-5′的核酸外切酶活性和聚合酶活性,分别指导DNA前导链和后随链的合成[18-21]。Pol ε和Pol δ具有碱基切除修复[22]、核苷酸切除修复[23]、错配修复[24-25]以及双链断裂修复[26]功能,而真核生物DNA每复制10^9到10^{10}个核苷酸就会出现1个错误,因此POLE和POLD1不仅参与DNA的复制,而且对维持DNA复制的保真度有着至关重要的作用[27]。

2　POLE/POLD1突变与肿瘤发生

负责校对功能的Pol ε和Pol δ如出现致病性突变将会影响基因组的稳定性,可导致突变增加和肿瘤形成[20, 28]。既往有相关研究表明,携带POLE基因单碱基突变(P286R)的小鼠具有更高的胚胎成纤维细胞永生化效率,而该位点突变所带来的整个基因组的超突变在小鼠体内诱导出不同的恶性肿瘤,最常见的是肺腺癌和侵袭性T细胞淋巴瘤,其他恶性肿瘤包括多种不同类型的肉瘤和癌(如神经内分泌癌、乳腺癌、子宫肿瘤、结肠癌和鳞状细胞癌等)[29]。在临床中也有发生结直肠癌、卵巢癌和乳腺癌等三原发癌患者合并有POLE胚系突变的报道[30]。此外,POLE和POLD1突变是结直肠癌、子宫内膜癌、卵巢癌、脑瘤的易感因素[31-36],也是肿瘤潜在的预后指标[37]。

3　POLE/POLD1突变与肿瘤免疫治疗

POLE和POLD1的致病性突变不仅参与肿瘤形成,并且与TMB-H密切相关。目前TMB-H已被普遍认为是免疫治疗获益的分子标志物,有研究表明其机制与TMB-H可以导致肿瘤免疫原性增高、T细胞活性和抗肿瘤反应增加有关[38-40]。为了明确POLE和POLD1基因突变发生率及其对免疫治疗疗效的影响,本研究团队进行了一项大样本分析,收集并分析了47721名不同类型癌症患者的基因检测数据,发现POLE和POLD1的突变频率分别为2.79%和1.37%,其中非黑色素瘤皮肤癌患者的POLE/POLD1突变率最高(16.59%),其他常见癌种包括子宫内膜癌(14.8%)、黑色素瘤(14.7%)、结直肠癌(7.4%)、膀胱癌(7.2%)、食管癌(7.2%)、肺癌(5.9%)等(表1[41])。并且在大多数类型的癌症中,存在POLE/POLD1突变的患者TMB显著高于没有突变的患者。进一步分析接受ICI治疗的队列,发现POLE/POLD1突变患者的总生存期(overall survival,OS)较POLE/POLD1野生型的患者显著延长(分别为34个月和18个月)。且在POLE/POD1突变的非MSI-H肿瘤患者总生存期中,亦有类似结果(突变型患者和野生型患者的OS分别是28个月和16个月)。通过校正微卫星不稳定(microsatellite instable,MSI)状态和瘤种后的多变量Cox回归分析发现,POLE/POLD1是免疫治疗获益的独立危

表1 POLE/POLD1在不同癌种中的突变频率

癌种名称	POLE/POLD1突变频率/% *n*=47721
非黑色素瘤皮肤癌	16.59
子宫内膜癌	14.85
黑色素瘤	14.73
结直肠癌	7.37
膀胱癌	7.21
食管癌	7.15
肺癌	5.89
宫颈癌	5.34
胆管癌	5.16
肾上腺皮质癌	4.37
头颈癌	4.24
肝癌	2.84
肾癌	2.44
肉瘤	2.39
生殖细胞瘤	2.00
卵巢癌	1.90
胰腺癌	1.79
前列腺癌	1.79
乳腺癌	1.43
中枢神经系统肿瘤	1.30
白血病/淋巴瘤	1.15
原发灶不明癌	1.02
甲状腺癌	0.92

险因素(P=0.047)[41]。基于该研究,提出POLE/POLD1突变可能是免疫治疗疗效预测的分子标志物。

多项研究提示POLE/POLD1突变与TMB-H相关[42-44],然而POLE/POLD1突变、错配修复(mismatch repair,MMR)、MSI、TMB之间关系的相关报道仍较少。2017年Brittany B. Campbell等一项纳入儿童及成人肿瘤样本总数超过8.1万例的分析研究显示,POLE/POLD1突变、dMMR、MSI-H均与TMB-H相关。并观察到MSI-H肿瘤的TMB范围约为10~100 mut/Mb(突变/兆碱基),而TMB>100 mut/Mb的情况却更多发生于合并POLE/POLD1突变的微卫星稳定(microsatellite stable, MSS)肿瘤中。而且通过定义仅在超突变肿瘤出现的突变位点是驱动突变,进一步分析发现POLE/POLD1的驱动性突变只分布于核酸外切酶区域。并在聚类时发现可将复制修复缺陷相关的肿瘤分为3个亚簇,进一步分析发现各亚簇与dMMR以及POEL/POLD1突变发生的先后顺序有关,并各有其相应的突变累积模型[45]。2018年Haradhvala等进行的子宫内膜癌研究结果显示,POLE常见突变位点P286和V411L较少出现MSI-H(2/30),主要是核酸外切酶区域其他位点突变出现MSI-H(13/20)。并且通过对POLE突变-MSI和POLD1突变-MSI肿瘤复制链突变的不对称分析发现,POLE突变-MSI肿瘤与POLE突变-MSS相似,其突变主要发生前导链模板;而POLD1突变-MSI肿瘤与POLD1野生型的MSI肿瘤相似,其突变主要发生在后导链模板。经进一步分析,也发现POLE/POLD1突变和dMMR的发生存在先后顺序[46]。Andrianova等发表的一项肿瘤不对称突变研究中,也有类似发现[47]。此外,2019年Yao等的一项纳入多个癌种共1392名患者研究显示,与POLE/POLD1野生型肿瘤相比,POLE核酸外切酶区域突变、POLD1核酸外切酶和非核酸外切酶区域突变患者的TMB显著升高,POLE非核酸外切酶区域突变患者的TMB则无统计学差异[48]。2020年Chang等的研究也提示POLE/POLD1突变与MSI-H相关[49]。因此POLE/POLD1突变作为ICI疗效预测分子标志物,具有良好应用前景。不过目前相关研究较少,主要集中在子宫内膜癌、结直肠癌、肺癌3个癌种。

3.1 POLE/POLD1突变与子宫内膜癌免疫治疗

相比于POLE突变,在子宫内膜癌中POLD1发生率更低,因此在子宫内膜癌中对POLE突变的相关研究较为多见。2013年,Kandoth等根据癌症基因组图谱(cancer genome atlas,TCGA)数据发现可将子宫内膜癌分成POLE突变型、MSI型、低拷贝数型、高拷贝数型4个不同具有预后意义的分子亚组[50],并因此引发了更多对POLE突变子宫内膜癌的研究。同年,Briggs等报道子宫内膜癌中POLE/POLD1存在体细胞突变以及胚系突变,并导致子宫内膜癌发生超突变[51]。POLE突变在子宫内膜癌中有以下特点:POLE突变患者发病年龄较小(<60岁)[52],而且主要见于I期子宫内膜癌[53];POLE突变与良好的临床预后相关,其突变常发生在核酸外切酶结构域的P286位点[54];POLE突变与dMMR无

关[55],其突变特征也与MSI不同[56]。2019年Timmerman等报道了一项纳入108例子宫内膜癌的研究,其结果显示dMMR与MSI具有高度一致性,而POLE核酸外切酶区域突变则并不导致MSI[57]。此外,POLE突变的子宫内膜癌中PD-1及细胞毒性T淋巴细胞相关抗原4(cytotoxic T lymphocyte associated antigen-4,CTLA-4)等免疫检查点相关蛋白表达升高,而且肿瘤浸润T细胞增加,其机制可能是POLE突变导致肿瘤超突变,使肿瘤新抗原的表达增加[58-61]。2015年,Howitt等的研究显示POLE突变的子宫内膜癌产生的肿瘤新抗原是MSI的15倍,MSS的100余倍[61]。Ib期临床试验KEYNOTE-028研究曾报道一名POLE突变型晚期子宫内膜癌患者在接受帕博利珠单抗治疗8周后,取得部分缓解的疗效,并获得超过14个月的持续响应[62]。

上述研究提示POLE突变不仅与子宫内膜癌预后相关,而且与免疫治疗获益相关,因此POLE突变是子宫内膜癌免疫治疗获益的潜在预测指标。

3.2　POLE/POLD1突变与结直肠癌免疫治疗

结直肠癌是中国最常见的恶性肿瘤之一,仅2015年就有37.6万例新增病例和19.1万人死亡[2]。目前晚期结直肠癌的5年生存率仍较低[63],ICI治疗的出现为延长晚期结直肠癌患者的生存期带来了曙光。2015年KEYNOTE-016试验报道了dMMR/MSI-H转移性结直肠癌患者接受帕博利珠单抗单药治疗获益[64]。2019年KEYNOTE-164研究结果显示,标准治疗失败的MSI-H转移性结直肠癌患者接受帕博利珠单抗单药治疗也可取得32%的ORR,而且安全性可控[65]。此外,CheckMate-142[66]、KEYNOTE-177[67]等试验结果也支持dMMR/MSI-H结直肠癌患者接受ICI治疗可获得良好疗效。然而晚期结直肠癌患者的MSI-H发生率仅约5%[68],而且相较于MSI-H的良好疗效,MSS型晚期结直肠癌患者接受ICI治疗的ORR为0[66]。因此POLE/POLD1突变的发现不仅为结直肠癌的发生发展揭示了更多的分子机制,也为鉴别出更多ICI治疗潜在获益患者带来希望。

研究显示,POLE和POLD1与结直肠癌超突变相关[69-70],并有研究报道其参与家族性结直肠癌[71]、息肉性肠病[72]以及早发肠癌[73]。2016年,Glaire等一项入组6517例结直肠癌的研究发现,POLE体细胞突变发生率为1.0%,而且更多见于男性、右半结肠、分期早的患者,并与预后良好相关[74]。2016年,Domingo等的研究显示,POLE突变的结直肠癌也较野生型患者发病年龄更小(中位年龄54.5岁vs 67.2岁),肿瘤内浸润的CD8+T细胞及表达的新抗原也较错配修复(proficient mismatch repair,pMMR)患者显著增加,而且是相对BRAF和KRAS突变独立的预后因素[75]。此外,2020年Mo等发现POLE核酸外切酶区域突变的结直肠癌更容易出现MSI-H,并且所有患者存在高TMB,TMB平均为200.8 mut/Mb[76]。2019年的蛋白质组学研究结果也显示MSI-H的结肠癌主要富集在错配修复通路、POLE以及BRAF突变中[77]。

综上,POLE/POLD1突变影响结直肠癌的发生发展,并且预示免疫治疗可能获益。不过目前相关的大肠癌免疫治疗资料主要来自病例报道,如Gong等在2017年曾报道一名81岁MSS型转移性结直肠癌患者帕博利珠单抗治疗有效[78];2020年,Forgó等也曾报道一名POLE突变型mCRC患者接受帕博利珠单抗后,在随访的32个月里持续响应[79]。2020年,Kim等报道了一项阿维鲁单抗单药治疗dMMR/MSI-H或POLE突变的晚期结直肠癌II期研究结果,该项研究中入组了30例dMMR/MSI-H以及3例POLE突变晚期肠癌患者,取得了24.2%的ORR,然而试验中治疗有反应的患者都是dMMR/MSI-H[80]。因此,虽然POLE/POLD1突变的发现为MSS结直肠癌患者带来了免疫治疗获益的希望,但仍需要更多的研究来了解其有效性。

3.3　POLE/POLD1突变与肺癌免疫治疗

目前肺癌免疫治疗显示出良好的疗效,但ICI疗效预测因子的相关研究仍然关键。因此POLE/POLD1也在肺癌研究中受到广泛关注,然而目前多为POLE突变的相关报道。2018年,Liu等一项纳入了513例肺腺癌和497例肺鳞状细胞癌标本的癌症基因图谱研究发现,POLE突变是肺鳞癌患者良好预后的生物标志物,但与肺腺癌的预后不相关,而合并高PD-L1表达的POLE突变肺腺癌则有

生存改善,进一步分析发现患者的生存改善与抗肿瘤免疫反应途径的激活有关[81]。2020年,Min等研究也显示,POLE突变的非小细胞肺癌良好预后与肿瘤内浸润T细胞及肿瘤突变负荷增加相关,并报道了POLE p.V1446fs位点的移码突变是发生率最高的突变位点(56.8%)[82]。2018年,Song等一项纳入319例非小细胞肺癌研究也有类似发现,该项研究中发生POLE突变的都为pMMR/MSS型肺腺癌患者(9/319,2.8%)。此外,POLE突变患者的TMB、CD8+T细胞、PD-L1表达均高于POLE野生型患者。其中POLE突变和POLE野生型中位TMB分别为12.2和7.8 mut/Mb。文中并报道了一名化疗失败的晚期肺腺癌患者使用帕博利珠单抗治疗取得部分缓解的疗效且无进展生存时间超过了8个月[83]。Rizvi等一项分析帕博利珠单抗治疗持续临床获益及无临床获益患者的肿瘤基因测序结果队列研究,也报道了4例POLE/POLD1突变患者免疫治疗后取得了8~14个月无进展生存的良好疗效[84]。这提示合并POLE突变的非小细胞肺癌对PD-1单抗预期疗效较好。因此,POLE突变是非小细胞肺癌患者免疫治疗反应的候选生物标志物。

4 结论

免疫治疗显著改善了抗肿瘤疗效,其中以PD-1抗体为代表的ICI应用最为广泛。然而目前获益人群有限,现有的预测免疫治疗疗效的标志物也都有各自的局限性,因此寻找更多有效的分子标志物对提高免疫治疗疗效尤为重要。编码DNA聚合酶的POLE/POLD1突变参与多种肿瘤的发生发展,并在多个瘤种中发现其与肿瘤超突变、TMB升高、新生抗原增多、肿瘤内免疫细胞浸润增加等密切相关,已有研究表明这些与良好的免疫疗效密切相关[85],这也预示POLE/POLD1突变有望成为新的泛癌种ICI疗效预测的分子标志物。虽然POLE/POLD1突变应用前景较好,但目前存在以下6方面问题。(1)目前POLE/POLD1突变的ICI治疗数据仍较少,多为个别瘤种的病例报道,仍需要更多的临床研究来探讨其可行性及安全性。(2)现有的免疫治疗相关报道多为ICI单药治疗的病例,而其他免疫治疗手段及联合治疗的有效性及安全性仍有待探讨。(3)POLE/POLD1的突变位点与TMB密切相关,如Hühns等研究发现POLE p.P286R可导致TMB-H,POLD1 p.E279K则为肿瘤突变负荷低(tumor mutation burden low, TMB-L),POLE p.V411L则存在TMB-L及TMB-H[86],而且有报道即便都为POLE p.P286R,其疗效也有差异,而POLE p.V411位点突变却达到完全缓解的满意疗效[87]。因此POLE/POLD1不同的突变位点对免疫疗效的影响仍需更多的研究。(4)个别POLE突变子宫内膜癌病例瘤内CD8+T细胞较野生型无明显差异[88],因此仍需更大样本量来研究其免疫浸润特征。(5)POLE突变频率存在人种和地区[89-90]差异,因此需要进行不同人种及地区的研究。(6)部分罕见瘤种治疗手段较少且疗效较差,而ICI为提高其疗效带来了希望。有报道显示部分罕见肿瘤中也观察到POLE突变,如肉瘤[91]、炎性乳癌[92]等,但相关研究较少。

综上,现有研究表明POLE或POLD1基因突变是预测免疫治疗疗效的潜在生物标志物,但需要更多的临床数据支持。因此本研究团队开展了一项泛瘤种的POLE或POLD1基因突变患者的二期临床研究,期待可以为这些患者的免疫治疗提供更多的证据。

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Advances in research of POLE/POLD1 mutations in cancer immunotherapy

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Abstract This article expounds the involvement of subunits encoded by DNA polymerase (POLE) and DNA polymerase delta 1 (POLD1) in DNA replication and proofreading, summarizes the effects of POLE/POLD1 mutations on tumor mutation load and intratumoral immune cell infiltration, and reviews the research progress of POLE/POLD1 mutations in immunotherapy of endometrial cancer, colorectal cancer and lung cancer. This article also discusses the challenges faced by POLE/POLD1 mutations as potential molecular markers for the predicting efficacy of immune checkpoint inhibitors.

Keywords POLE; POLD1; mutation; immunotherapy; biomarker

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