恶性肿瘤免疫检查点抑制剂耐药机制及临床应用

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摘　要:与以往肿瘤经典治疗手段不同,肿瘤免疫治疗的焦点从肿瘤本身转为动员宿主免疫系统去识别并消灭肿瘤细胞。近年来,针对免疫检查点的单克隆抗体在临床应用中取得了飞速进展,随之而来的是各种耐药问题的出现。文章针对各种耐药分子机制以及相关临床应用作一具体阐述。

关键词:免疫治疗;免疫检查点抑制剂;耐药机制;肿瘤;临床应用
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Clinical Application of Cancer Immune Checkpoint Inhibitors and Related Mechanism of Drug Resistance

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Abstract:The focus of tumor immunotherapy has been shifted from tumor itself to host immune system,which is different from conventional tumor therapy. In recent years,the monoclonal antibodies against immune checkpoints have made rapid progress in clinical applications,nevertheless the related drug resistance also appears. This article reviews the resistance mechanism of immune checkpoints drugs and related clinical implications.

Key words:immunotherapy;immune checkpoint inhibitors;resistance mechanism;tumor;clinical application

对大多数患者来说，转移性恶性肿瘤仍然是一种不可治愈的疾病，癌细胞内在基因组的不稳定性有助于癌细胞逃脱机体免疫系统的监测及外界干预治疗手段。随着肿瘤免疫检查点和抑制剂的发现[1]，肿瘤免疫生物学及其治疗都取得了重大突破。

Brunet 等[2]在活化的效应 T 细胞和调节性 T 细胞(Tregs)上发现了 CTLA-4 受体。随后 Krummel 等[3]发现 CTLA-4 与 CD28 竞争 B7 配体可抑制 T 细胞 IL-2 的分泌。之后 2011 年 ipilimumab 被批准用于治疗晚期黑色素瘤，成为第一类 FDA 批准的 CTLA-4 检查点抑制剂[4]。

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1992 年,Ishida 等[5]在活化 T 细胞内克隆出另一个重要的检查点受体 PD-1,随后其配体 PD-L1 也被发现[6]。PD-L1 高表达可诱导肿瘤细胞免疫逃逸。PD-1 和 PD-L1 抑制剂能激活肿瘤反应性 T 细胞并在多种肿瘤组织中产生长期的抗肿瘤反应[7],包括部分免疫治疗不敏感的肿瘤类型。目前有十余种 PD-1 和 PD-L1 抗体已获批或正处于临床试验阶段。

随着免疫检查点抑制剂应用增加，越来越多的恶性肿瘤患者出现临床耐药问题。免疫检查点抑制剂耐药包括原发性、适应性以及获得性耐药。在患者疾病发展过程中多种因素不断影响抗肿瘤免疫应答的进展，免疫相关的耐药问题也将会成为未来免疫治疗的挑战。

1 原发性、适应性耐药

原发性耐药指患者对检查点抑制剂初始治疗无应答。适应性耐药指患者在有效治疗一段时间后产生耐药性。有研究表明肿瘤细胞的内、外在因素都参与该耐药机制。缺乏肿瘤抗原而不能被 T 细胞识别是肿瘤患者对免疫检查点阻滞治疗无应答的最直接原因[8]。另外,表达肿瘤抗原的癌细胞也可通过改变 β_2 微球蛋白或主要组织相容性复合体(MHC)本身破坏抗原提呈过程[9]。

1.1 肿瘤细胞内在因素

肿瘤细胞内在因素指细胞本身通过表达或沉默某些基因和通路、抑制免疫细胞浸润到肿瘤微环境,来促进免疫治疗原发性或适应性耐药的形成。肿瘤细胞可通过激活丝裂原活化蛋白激酶(MAPK)信号转导通路及抑制 PTEN 的表达,进一步增强 PI3K 信号、Wnt/β 连环蛋白信号通路,抑制干扰素 γ(IFN-γ)信号通路以及肿瘤抗原的表达。MAPK 致癌信号通路可通过表达 VEGF 和 IL-8 抑制 T 细胞的募集和功能[10]。多种肿瘤 PTEN 的缺失可增强 PI3K 信号,进而导致免疫治疗耐药[11]。TCGA 黑色素瘤相关数据显示 PTEN 的丢失可降低 IFN-γ、颗粒酶 B 的基因表达以及 CD8 T 细胞的浸润。致癌信号通路介导的 T 细胞免疫逃逸可能与 Wnt 信号通路的 β 连环蛋白的稳定性相关[12]。

癌细胞高表达 PD-L1 配体能有效抑制 T 细胞的抗肿瘤反应。PDJ 扩增是指 9 号染色体的 *PD-L1*、*PD-L2* 基因和 IFN-γ 受体信号转导分子激酶 2(*JAK2*)基因的联合扩增[13]。在霍奇金淋巴瘤的恶性 Reed Sternberg 细胞中发现了 PDJ 扩增,且抗 PD-1 治疗对超过 80%的化疗难治性霍奇金病患者有效[14]。*PTEN* 缺失、*PI3K* 或 *Akt* 基因突变[15]、*EGFR* 突变[16]、*MYC* 过度表达[17]、*CDK5* 破坏[18]、*PD-L1* 基因 3'端非编码区的缺失都可导致癌细胞 PD-L1 表达水平的改变[19]。目前尚不清楚这些致癌信号表达的 PD-L1 是否与临床抗 PD-1、抗 PD-L1 治疗耐药性的产生相关。

IFN-γ 信号通路是免疫治疗耐药性形成的一个关键通路[20-23],其在抗肿瘤免疫应答中具有双重作用。以癌细胞或 APCs 同源抗原为靶向的肿瘤特异性 T 细胞分泌的 IFN-γ,可促进 MHC 分子的表达、参与并增强肿瘤抗原提呈、招募其他免疫细胞、直接抑制癌细胞增殖及促癌细胞凋亡[24]。但持续 IFN-γ 暴露也会诱导癌细胞重新编辑并诱导免疫逃逸[25,26]。癌细胞可通过 IFN-γ 受体链 *JAK1* 和 *JAK2*、信号转导分子及转录激活分子(*STATs*)的突变或减少表达以逃脱 IFN-γ 效应[27]。在细胞系和动物模型中,IFN 受体信号通路分子的突变或表观遗传沉默都会导致 IFN-γ 失去抗肿瘤作用[28,29]。分析显示对 CTLA-4 抗体 ipilimumab 治疗无应答的肿瘤患者体内 IFN-γ 受体 1、2 基因 (*IFNGR1* 和 *IFNGR2*)、*JAK2* 和干扰素调节因子 1(*IRF1*)有高频突变。这些突变能影响IFN-γ 的信号转导并使肿瘤细胞逃脱 T 细胞攻击,诱导抗 CTLA-4 治疗原发性耐药;同时也会诱导癌细胞 *PD-L1* 基因沉默,导致 IFN-γ 暴露下 PD-L1 表达减少及原发性耐药[30]。癌细胞 DNA 的表观遗传修饰可能会改变免疫相关基因的表达并影响抗原加工、提呈和免疫逃逸[31,32]。去甲基化剂能使免疫相关基因重新表达,与免疫疗法联用时有潜在的治疗作用[33]。一些临床前期数据表明逆转癌细胞表观遗传变异可增强免疫治疗的识别和应答。

1.2 肿瘤细胞外在因素

肿瘤细胞原发性、适应性耐药的外在因素包括肿瘤微环境中除肿瘤细胞的其他成分,如 Tregs、髓源抑制性细胞(MDSCs)、M2 型巨噬细胞和其他抑制性免疫检查点。Tregs 通过分泌 IL-10、TGF-β 和 IL-35 等抑制性细胞因子或直接细胞接触途径抑制效应 T 细胞(Teffs),在维持自身免疫耐受中起重要作用[34]。研究表明,肿瘤微环境的 Tregs 耗竭可以恢复或提高抗肿瘤免疫[35,36],不能增加原发或复发肿瘤中 Teffs/Tregs 比值的免疫治疗更易产生耐药性。MDSCs 已成为包括肿瘤在内的各种病理条件下免疫应答的主要调节者。研究表明,肿瘤微环境中的 MDSCs 可导致免疫检查点抑制剂[37]、过继性 T 细胞治疗和 DC 疫苗等免疫治疗效果下降[38,39]。因此,消除或重新编辑 MDSCs 有望提高免疫治疗的疗效。

在荷瘤小鼠模型中,巨噬细胞 PI3Kγ 的选择性失活可协同免疫检查点抑制剂促进肿瘤的消退并提高生存率[40]。缺乏 PI3Kγ 的小鼠或 PI3Kγ 抑制剂(TG100-115 或 IPI-549)治疗的荷瘤小鼠肿瘤生长受抑,且该荷瘤小鼠巨噬细胞中的免疫活化相关基因和蛋白明显上调。这些数据说明 PI3Kγ 作为一个分

子转换器可以调节巨噬细胞功能。研究人员还发现PI3Kγ抑制剂(TG100-115)与抗PD-1联用可改善肿瘤免疫排斥现象并提高荷瘤小鼠的生存期。在另一项研究中,与抗CTLA-4和抗PD-1联合治疗的荷瘤小鼠相比,PI3Kγ抑制剂、抗CTLA-4和抗PD-1三联疗法加快了肿瘤的消退并提高了荷瘤小鼠的长期生存率[41]。这些研究说明PI3Kγ抑制剂联合免疫检查点抑制剂治疗恶性肿瘤具有进一步研究的价值。

肿瘤相关巨噬细胞(TAM)是另一类影响免疫应答的细胞。TAM有促进抗肿瘤免疫的M1型巨噬细胞和具有亲致瘤特性的M2巨噬细胞两种[42]。在化学因素诱导肺腺癌的小鼠模型中,由于CCL2和/或CCR2信号的失活抑制了M2型巨噬细胞的募集,从而限制肿瘤的生长[43]。同样的,在皮肤T细胞淋巴瘤[44]、结肠癌、乳腺癌[45]、黑色素瘤等荷瘤小鼠模型中M2型巨噬细胞缺失也表现出相似的效应[46,47]。研究表明,巨噬细胞可通过调节肝细胞癌PD-L1和卵巢癌B7-H4的表达直接抑制T细胞应答[48,49]。

2 获得性耐药

诱导长期、持续性的抗肿瘤应答是肿瘤免疫治疗的标志。然而,随着免疫治疗的广泛使用,获得性耐药的患者逐渐增加。对抗CTLA-4、抗PD-1治疗有客观应答的部分转移性黑色素瘤患者在持续性治疗过程中仍会复发[50]。复发的潜在机制有T细胞功能的丧失、肿瘤抗原提呈下降致T细胞识别受限、肿瘤免疫逃逸细胞株的形成。以上机制均能导致检查点抑制剂和过继细胞治疗(adoptive cell therapy,ACT)的获得性耐药。

有证据表明,由于HLA Ⅰ类分子共享组件B2M的丢失,对IL-2或TIL-ACT免疫疗法有初始应答的患者会出现获得性耐药。B2M参与HLA Ⅰ类折叠及运输[51,52],其遗传缺陷会导致$CD8^+$T细胞识别受限。在1例抗PD-1晚期获得性耐药患者的耐药细胞中发现B2M的纯合子突变及细胞表面HLA Ⅰ类分子表达的缺失[23]。同时在另外2例复发肿瘤研究中发现JAK1、JAK2野生型等位基因杂合性缺失并丧失相关功能。以上突变可诱导癌细胞逃脱IFN-γ的抗增殖作用[23]。在1例TIL-ACT治疗有效的转移性结直肠癌研究中发现抗原提呈物质的丢失参与获得性耐药的形成。因此,抗原提呈物质以及IFN-γ信号转导通路相关遗传物质的改变,可能促进抗PD-1及ACT治疗获得性耐药的发生。

抗肿瘤T细胞以肿瘤细胞表达的同源抗原为靶点,肿瘤细胞减少抗原表达或使其突变则可形成获得性耐药。数据显示检查点抑制剂启动的抗肿瘤T细胞主要识别突变新抗原[53,54]。基因缺失、突变或表观遗传的改变都会导致MHC呈递的突变新抗原下降,进而形成获得性耐药。临床证据表明抗肿瘤T细胞靶点的丢失会增加肿瘤免疫治疗的耐药。

3 监测耐药机制

总肿瘤突变负荷是目前最好的预测检查点抑制剂疗效的生物标志物[55,56]。高突变负荷的恶性肿瘤分泌高水平的新抗原并诱导有效的抗肿瘤免疫反应,并与多种恶性肿瘤免疫检查点抑制剂应答增强相关[57,58]。除了基因组标志物和免疫调节基因表达谱外[59],包括$CD8^+$T淋巴细胞的密度和分布、T细胞克隆能力、PD-L1表达在内的免疫标志物都与免疫检查点抑制剂的疗效区别相关。目前,肿瘤免疫学能同时分析肿瘤微环境的以下7大特征:肿瘤对免疫效应细胞的敏感性、肿瘤的异质性、全身免疫状态、免疫细胞浸润、检查点分子表达缺失、可溶解抑制剂如IL-1和IL-6的缺失以及肿瘤代谢抑制剂的缺失[60]。这些研究将有助于肿瘤免疫治疗的个体化。

4 克服免疫治疗耐药

随着耐药机制的阐明,更多的研究致力于如何克服免疫治疗耐药,如通过一些手段将免疫冷肿瘤转化为热肿瘤[61~63],或通过TILs体外扩增回输[64]、抗原特异性T细胞过继疗法以增强内源性T细胞功能等[65~69]。已有研究利用联合用药模式尝试减少免疫耐药的发生[70~72],如联用抗CTLA-4和抗PD-1能大大提高免疫治疗反应率并延长转移黑色素瘤患者的生存期[73~75]。联合用药的基本原理是多效应叠加,肿瘤特异性T细胞检查点抑制剂都具有重叠性和特异性,联合使用具有协同作用。

免疫反应具有双重性,可导致免疫编辑和免疫逃逸,这将成为管理免疫治疗药物、推动抗肿瘤免疫

应答的重要考量因素。一些临床实验正在检测肿瘤微环境中 LAG-3、TIGIT、ISTA 等抑制性免疫检查点以及相关抗体单一或联合使用的疗效[76~78]。我们需要通过更多的临床试验去发现针对其他抑制性通路、有效克服耐药并能调节免疫反应的二联或三联疗法,以获取额外临床效益。

传统理论认为化疗会减少淋巴细胞数量或影响其功能导致免疫抑制,但是随着研究的不断深入,有证据提示化疗药物可增加肿瘤细胞抗原性[79]、免疫原性及敏感性[80],也可抑制 Tregs 及 MDSCs[81,82]并促进 DCs 功能[83]。放疗可引起免疫源性细胞死亡,并可促进肿瘤微环境中 T 细胞的募集及其功能,有协同免疫治疗的作用[84]。有研究显示对肿瘤进行局部放疗可将对抗 CTLA-4 抗体耐药的肿瘤转变为对抗 CTLA-4 抗体敏感的肿瘤[85]。局部大剂量放疗后会诱导共抑制分子(如 PD-L1)的表达[86]。小鼠胶质瘤模型中, 联合放疗和抗 PD-1 治疗显著增加了中位生存期,部分存在抗肿瘤记忆应答[87]。目前,已有部分放疗、化疗联合免疫治疗的研究结果陆续公布,但是该模式的治疗纵然有较为广阔的前景, 其全面推广仍尚需时日。

靶向治疗可能通过破坏癌基因依赖和加强抗肿瘤免疫作用致肿瘤细胞衰老并促进 T 细胞清除癌细胞[88]。联合分子靶向治疗与免疫治疗具有完善的临床和预临床基本理论[89~91]。有研究表明尽管针对黑色素瘤 *BRAF* 致癌基因的靶向治疗单用无法长期控制肿瘤[92~94],但却能通过作用于肿瘤微环境,如增加抗原分泌[95]、增加 HLA 表达[96]、增强 T 细胞浸润、减少免疫抑制细胞因子的释放和改善 T 细胞功能[97~99],真正将肿瘤“冷”微环境转为“热”微环境,以便于支持包括免疫治疗在内的多模式治疗手段。

5 总 结

免疫治疗是近年来抗肿瘤研究新的热点和可能,随着免疫治疗分子机制的研究进展,免疫检查点抑制剂得到了广泛应用。同时,如何克服免疫治疗耐药需要更充足的理论研究及应对策略。免疫治疗与多模式治疗手段的联合以及其他免疫检查点的发现,将成为抗肿瘤治疗中极其重要的研究方向。

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