不可逆电穿孔联合免疫疗法治疗实体瘤的研究进展

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摘要 不可逆电穿孔(irreversible electroporation, IRE)是近年来新兴的非热消融技术, 因其不引起热损伤, 避免了“热沉效应”, 在实体瘤的治疗上具有很好的应用前景. 但IRE因消融效果不彻底、易出现复发等因素限制了其临床应用. 然而IRE通过高频短脉冲在细胞膜上形成不可逆的纳米级穿孔, 不仅可直接杀伤肿瘤细胞, 且细胞内隐藏肿瘤抗原的大量释放可促使肿瘤细胞变成原位“肿瘤疫苗”, 诱发潜在的抗肿瘤免疫反应, 有助于杀伤消融后残余的肿瘤细胞并抑制肿瘤的局部复发与转移, 为IRE联合免疫疗法治疗恶性肿瘤奠定了基础. 本文主要围绕IRE诱导肿瘤机体的肿瘤局部和全身性免疫反应的特点及其相关机制, 以及IRE联合免疫疗法治疗实体瘤效果的研究进展进行综述, 以期能为IRE联合免疫疗法治疗实体瘤的临床应用提供理论与实践指导.

关键词 不可逆电穿孔, 免疫疗法, 实体瘤, 联合治疗

不可逆电穿孔(irreversible electroporation, IRE)是一种新型局部非热物理消融技术, 主要用于恶性实体瘤的治疗^[1]. 该技术以Nanoknife®(Angiodynamics)的商品名上市, 并于2012年获美国食品药品监督管理局(Food and Drug Administration, FDA)批准进行临床试用, 同年12月获得欧洲联盟临床许可, 2015年获得国家食品药品监督管理总局(China Food and Drug Administration, CFDA)批准进入中国市场开展相关临床试验研究^[2-4]. IRE主要通过图像引导、腹腔镜下或剖腹术, 在肿瘤组织内部和周围插入两根或多根电极, 通过电极对肿瘤组织施加高压脉冲电场(pulsed electric field, PEF). 当PEF强度超过一定阈值时, 可在细胞膜磷脂双分子层中形成不可逆的纳米级穿孔道, 导致细胞膜永久性裂解或细胞稳态丧失, 诱导细胞坏死^[5]. IRE主要

损伤靶组织内细胞膜性结构, 具有非热消融的特性, 对细胞外大分子和结缔组织成分无损伤作用, 可保存组织的结构完整性. 因此, IRE对在大血管、肠道、胆道或泌尿道等重要管道结构附近肿瘤的治疗具有很好的应用前景. 临床研究表明, IRE在胰腺癌、肝癌、前列腺癌等实体瘤的治疗中具有很好的疗效, 可显著延长患者的生存期^[1,6,7]. 然而, 由于不同肿瘤组织介电异质性引起的治疗电场分布不均, 导致肿瘤组织消融不彻底, 增加肿瘤复发的风险. 但IRE在细胞膜上形成的不可逆的纳米级孔道, 不仅可诱导肿瘤细胞死亡, 同时细胞膜穿孔后的肿瘤细胞内隐藏肿瘤抗原的大量释放促使肿瘤细胞变成原位肿瘤疫苗, 进一步诱发机体产生免疫反应, 有效地提高机体抗肿瘤免疫应答杀伤消融后残余的肿瘤细胞并抑制肿瘤的复发, 为IRE联合免疫

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疗法治疗肿瘤提供了基础。Neal等人^[8]在IRE治疗小鼠皮下肾癌的研究中发现, IRE可显著延长免疫功能正常的荷瘤小鼠总生存期(overall survival, OS); 在IRE治疗后的肿瘤组织中, 存活和死亡肿瘤组织的交界处有大量CD3⁺ T淋巴细胞浸润, 而在免疫缺陷的裸鼠体内无此作用, 提示IRE诱导荷瘤宿主的免疫系统的反应发挥了重要作用。近年来, 越来越多的研究表明, IRE可诱导荷瘤机体产生抗肿瘤免疫反应, 而联合免疫治疗可能增强IRE诱导的这种免疫反应, 促进免疫细胞对肿瘤细胞的杀伤作用。鉴于此, 本文主要围绕IRE所致的免疫反应的机制与特点, 以及IRE联合免疫疗法治疗肿瘤效果的研究进展进行综述。

1 IRE消融肿瘤诱导免疫反应的特点

1.1 IRE诱导的免疫反应强于其他肿瘤消融疗法

由于IRE仅作用于细胞膜的脂质双层, 对膜蛋白等其他分子的影响甚微^[9], 而其他消融疗法如冷冻或热消融可导致蛋白变性, 因此IRE消融后诱导形成的肿瘤抗原性较其他消融术后肿瘤抗原性更强, 可诱导更强烈的免疫反应^[10,11]。Shao等人^[11]将B16黑色素瘤细胞经热(50°C, 30 min)、冷冻(80°C, 30 min)和IRE(12 V/cm, 99个脉冲, 50 ms脉冲, 间隔1 Hz)处理后, 收集分析细胞裂解物发现, IRE处理组细胞释放的B16蛋白和酪氨酸酶相关蛋白-2抗原最多, 其次是低温和高温处理组。体外混合培养结果表明, 相较于低温和热处理, IRE处理的肿瘤细胞对CD8⁺ T促增殖和促活化作用最强。Sugimoto等人^[12]探究了IRE($n = 10$)和射频消融(radiofrequency ablation, RFA)($n = 11$)疗法在肝癌患者体内诱导的全身性免疫反应。与RFA组相比, IRE组的巨噬细胞移动抑制因子(macrophage migration inhibitory factor, MIF)血清水平在IRE治疗后显著升高, 且在消融后1年, IRE的消融区轴向直径和面积明显小于射频消融区, 提示IRE介导的MIF早期的显著增加有助于早期修复过程, 并导致消融区的显著收缩。Bulvik等人^[13]通过RFA或IRE消融正常肝脏组织发现, RFA治疗后的肝脏组织仅在消融区域周围形成清晰的炎性边缘, 而在IRE消融的组织中, 炎性细胞可浸润穿透整个消融区, 大部分细胞沿着残存的血管分布。且IRE介导的IL-6的分泌水平是RFA组的3.3倍。他们进一步探究了二者在肝癌荷瘤小鼠体内的消融效果, 结果表明, IRE对肝脏局部肿瘤的消融效果优于RFA, 对远隔部位的皮下移植瘤也具有抑制

作用; IRE消融区域内血管的持续通畅增加了免疫细胞浸润的面积, 且浸润细胞密度与血清IL-6水平呈正相关, 提示IRE消融肝脏肿瘤可诱导更强烈的局部和全身性的免疫效应。White等人^[14]分析了IRE和冷冻消融小鼠原位胰腺癌诱导的早期免疫应答的差异, 结果表明, IRE消融组CD3⁺ T细胞分别在6、12和24 h消融肿瘤组织中的浸润显著多于冷冻消融组, 巨噬细胞在12和24 h的浸润多于冷冻消融组, 自然杀伤细胞(natural killer cell, NK)在两组间的浸润无显著差异。由以上研究结果可知, 相较于其他消融疗法, IRE消融可诱导更强烈的免疫反应。

1.2 IRE在实体瘤中诱导的免疫反应的特点

表1总结了IRE在不同肿瘤动物模型及不同肿瘤患者体内诱导免疫反应的改变情况。一项小鼠皮下肉瘤模型^[15]研究发现, 在IRE消融前、消融2及6 h后的肿瘤组织中未出现免疫细胞的浸润, 即IRE不能诱导免疫反应。而Li等人^[16]采用流式细胞术(flow cytometry, FCM)检测了IRE治疗大鼠皮下骨肉瘤7 d后外周血中T淋巴细胞亚群的变化。结果表明, 相比假手术组与对照组, IRE治疗组CD3⁺ T、CD4⁺ T细胞的比例及CD4⁺ T/CD8⁺ T比值均显著增加; 脾细胞中IFN- γ ⁺的细胞比例显著增加; 血清sIL-2R的分泌水平随时间延长而降低。以上结果表明, 在IRE消融肉瘤早期, IRE诱导的免疫反应可能还未发生显著改变, 而随着消融时间延长, IRE在荷瘤机体内可同时介导细胞及体液免疫反应。Ringel-Scaia等人^[17]在小鼠原位乳腺癌模型中研究发现, IRE消融后2 d即可在消融的肿瘤组织中观察到中性粒细胞下降了8.7%, T细胞群减少了12.5%, 调节性T细胞(regulatory T cell, Treg)亚群增加了0.15%, 骨髓来源的抑制性细胞(myeloid-derived suppressor cells, MDSCs)及肿瘤相关巨噬细胞(tumor-associated macrophages, TAMs)亚群在IRE治疗后未出现差异性改变。

多项小鼠肝癌模型的研究表明, IRE消融后可诱导CD8⁺ T和树突状细胞(dendritic cell, DC)向消融周围区域浸润增加, 并伴有Treg亚群浸润减少^[18-20], 脾脏中Treg和PD-1⁺ T细胞减少, CD8⁺ IFN- γ ⁺ T细胞比例增加^[18]; 外周血CD8⁺ T细胞比例均增多, 而Treg比例明显下降^[19]。Chen等人^[21]研究发现, IRE消融小鼠肝癌组织可诱导多种细胞因子和趋化因子激活, IRE可将荷瘤小鼠体内Th2型免疫反应占优势的状态逆转为Th1型免疫反应占优势, 增强机体的抗肿瘤活性; IRE消融区出现

表 1 IRE消融不同肿瘤诱导的免疫反应的特点^{a)}

Table 1 Characteristics of immune response induced by IRE ablation in different kinds of tumors

文献	种属	肿瘤模型	检测时间	检测方法	免疫反应的特点		
					局部(肿瘤组织)	全身(外周血)	全身(脾脏)
[15]	小鼠	皮下肉瘤	Pre-IRE, IRE 2 h, 6 h	IHC	无免疫细胞浸润	NA	NA
[16]	大鼠	皮下骨肉瘤	IRE 1 d, 3 d, 7 d, 14 d, 21 d	FCM, ELISA	NA	CD3 ⁺ T, CD4 ⁺ T和CD4 ⁺ / CD8 ⁺ T ⁺ , IL-2R ⁺	IFN- γ ⁺
[17]	小鼠	原位乳腺癌	IRE 2 d	FCM	中性粒细胞和CD4 ⁺ CD45 ⁺ T ⁺ , Treg ⁺	NA	NA
[18]	小鼠	皮下肝癌	IRE 7 d	IHC, FCM	CD8 ⁺ T和DC ⁺ , Treg 和PD-1 ⁺ T ⁺	NA	CD8 ⁺ IFN- γ ⁺ T ⁺ , Treg和PD-1 ⁺ T ⁺
[19]	小鼠	皮下肝癌	IRE 1 d, 3 d, 7 d, 14 d	FCM, IHC	CD8 ⁺ T ⁺ , Treg ⁺	CD8 ⁺ T ⁺ , Treg ⁺	NA
[20]	小鼠	原位肝癌	IRE-3 d, 7 d, 14 d	FCM, CBA	CD8 ⁺ T ⁺ , Treg ⁺	IFN- γ , IL-2, TNF- α 和IL- 1 β , IL-10 ⁺	NA
	人	原位肝癌	IRE 1 d, 3 d, 7 d	FCM	NA	活化的T细胞, 中性粒细 胞, 单核细胞和NK ⁺ , 淋 巴细胞, CD4 ⁺ T和Treg ⁺	NA
[21]	小鼠	原位肝癌	IRE 2 h, 2 d, 7 d, 14 d, 90 d	IHC, ELISA	巨噬细胞 ⁺	IL-3, IL-13, IL-15, IL- 17A, IL-22, IL-28, IL-31 和IFN- γ ⁺	NA
[22]	大鼠与小鼠	原位肝癌	IRE 2 h	IHC	CD8 ⁺ T ⁺	CD8 ⁺ T ⁺	NA
[23]	狗	原位肝癌	IRE 4 d	IHC	CD3 ⁺ T ⁺	NA	NA
[24]	小鼠	原位胰腺癌	IRE 7 d	IHC, FCM	CD45 ⁺ T, CD8 ⁺ T ⁺ , MDSCs ⁺ IFN- γ , CCL1和IL-2 ⁺ IL-4和IL-6 ⁺	NA	NA
[25,26]	小鼠	原位与皮下 胰腺癌	IRE 7 d	IF, IHC, FCM	CD8 ⁺ T和M1型巨噬细胞 ⁺	CD8 ⁺ T和CD8记忆T ⁺	CD8 ⁺ T, CD8记忆T 和M1型巨噬细胞 ⁺
[27]	人	胰腺癌	Pre-IRE, IRE-2 周, IRE-3个月	FCM	NA	活化PD-1 ⁺ T ⁺ , Treg ⁺	NA
[28]	人	胰腺癌	Pre-IRE, IRE 1 d, 3 d, 5 d	FCM	NA	Treg ⁺	NA

a) IHC: Immunohistochemistry, 免疫组化; ELISA: Enzyme linked immunosorbent assay, 酶联免疫吸附法; CBA: Cytometric bead array, 流式微球分析; NA: Not available, 未研究

大量巨噬细胞浸润, 有助于肝脏组织修复及宿主存活。具有促炎活性的细胞因子IFN- γ 、IL-2、TNF- α 和IL-1 β 在IRE消融组显著高于对照组, 而具有免疫抑制活性的细胞因子IL-10水平在IRE组降低^[22]。此外, IRE消融诱导的免疫反应可抑制继发肿瘤的形成。研究人员使用IRE-处理的H22细胞裂解物作为“疫苗”接种小鼠, 可预防小鼠肿瘤发生, 而通过耗竭IRE治疗后CD8⁺ T细胞可诱导局部肿瘤再次生长和远处转移^[18]。类似的研究表

明, nsPEF消融肝癌可诱导CD8⁺ T细胞的产生, 并且以CD8⁺依赖性方式抑制继发性肿瘤生长^[22]。Partridge等人^[23]对3例肝癌犬经皮IRE治疗, 在IRE消融区仅有CD3⁺ T淋巴细胞浸润, 而无CD4⁺、CD8⁺ T及B淋巴细胞的浸润。Guo等人^[20]探究了IRE消融11例患者肝癌组织诱导的免疫应答的改变, 在IRE 1 d后, 患者中性粒细胞和单核细胞增加而淋巴细胞计数减少, 并可在7 d内恢复至基线值。同时, IRE治疗1 d后外周血中循环

CD4⁺ T细胞亚群减少, Treg减少, 活化的T细胞和NK细胞比例增加。

IRE消融小鼠原位胰腺癌的相关研究表明, IRE可明显抑制小鼠原位胰腺癌的生长, 增加肿瘤局部CD8⁺ T细胞的浸润^[24,25], 诱导的巨噬细胞向M1型极化^[26], 脾细胞中CD8⁺ T细胞和记忆T细胞比例显著增加^[25]。此外, 经IRE处理的肿瘤细胞上清液可抑制肿瘤的生长, 且IRE诱导的免疫反应可抑制远隔部位肿瘤生长, 表明IRE激活的系统性免疫应答具有全身性的抗肿瘤作用^[25]。Narayanan等人^[24]分析了IRE消融小鼠原位胰腺癌1周时肿瘤组织内细胞因子的分泌情况, 具有抑癌作用的Th1型免疫反应细胞因子IFN- γ 、CCL1和IL-2均显著增加; 促癌作用的Th2免疫反应细胞因子IL-4和IL-6均降低。发挥募集MDSCs和阻碍CD8⁺ T细胞浸润的趋化因子CXCL1显著降低, 诱导的单核细胞的趋化因子CXCL3增加。但免疫组织化学(immunohistochemistry, IHC)检测结果表明, IRE可促使肿瘤组织消融区CD45⁺ T和CD8⁺ T细胞的浸润明显增加。FCM检测肿瘤组织免疫细胞结果显示, CD45⁺ T、CD8⁺ T、CD8⁺ IFN γ ⁺ T细胞以及促炎M1型巨噬细胞与M2型巨噬细胞的比值在IRE消融组及对照组间未出现显著差异, 仅MDSCs细胞亚群在IRE消融组显著降低^[24]。这表明检测方法对判断IRE介导的肿瘤免疫反应的特点有显著影响。两项临床研究发现^[27,28], IRE消融局部晚期胰腺癌(locally advanced pancreatic cancer, LAPC)可促使患者外周血中Treg一过性减少和活化PD-1⁺ T细胞一过性的增加, 其诱导的特异性T细胞应答与患者更长的OS有关, 且IRE消融LAPC介导Treg减少集中在IRE消融后3~5 d。这表明IRE可一过性缓解LAPC的免疫抑制, 为与免疫治疗联合增强临床疗效创造临床窗口期。

1.3 IRE在实体瘤中诱导的免疫反应差异分析

由以上研究结果可知, IRE的确可诱导显著的免疫反应, 但在不同研究中, 其诱导的免疫反应存在显著差异, 甚至相互矛盾。导致研究结果存在诸多差异的主要原因包括: (1) 采用的肿瘤动物模型不同。以往的研究多采用皮下移植瘤模型或原位移植瘤模型, 皮肤与消化道组织分别起源于外胚层和内胚层, 其形成的组织差异明显。因此不同的肿瘤模型可导致研究结果存在差异。而动物原位移植瘤模型更符合探究免疫反应的肿瘤模型, 因此在后续类似研究中应采用原位肿瘤模型进行探究。(2) 所选用的肿瘤细胞系与小鼠品系不同

源。由不同品系所致的免疫排斥反应并未被考虑, 如小鼠肝癌H22细胞系可在多种品系(如C57BL/6与BALB/c)小鼠体内快速成瘤, 但其本身源于C3HA小鼠, 而该品系的小鼠目前并不常用于肿瘤研究中, 以往应用H22细胞系建立的小鼠肝癌模型多采用C57BL/6与BALB/c小鼠。Hep1-6小鼠肝癌细胞源于C57L小鼠, 其常被用于接种不同品系的C57BL/6与BALB/c小鼠, 建立肿瘤模型进行研究。在此类动物模型中, IRE均可诱导显著的免疫反应^[18,19,29]。(3) 多项研究均在IRE与其他疗法联合治疗后, 对荷瘤机体的免疫细胞亚群进行了检测^[24,30], 而其他疗法对机体免疫反应的影响程度还有待进一步研究, 因此需设计严格的由IRE单一疗法诱导的免疫反应相关实验进行探究。此外, 肿瘤组织的取材部位、IRE治疗后的检测免疫反应时间、检测时所采用的方法及所侧重的免疫细胞亚群, 以及所选择的表征免疫的标记物等均会导致研究结果存在差异。综上, 在后续研究中, 应全面考虑相关影响因素以保证研究结果的可靠性和准确性。

2 IRE消融肿瘤组织诱导免疫反应的机制

2.1 IRE介导损伤相关分子模式的释放诱导免疫反应

与手术切除不同, IRE消融后凋亡或坏死细胞残留物仍可被DC摄取, 诱导有效的全身性抗肿瘤免疫反应^[31]。IRE消融局部肿瘤组织后, 濒死细胞可释放大量的内源性危险信号分子, 即损伤相关分子模式(damage associated molecular patterns, DAMP), 驻留在肿瘤组织中的DC可摄取这些抗原分子迁移至引流淋巴结, 并重新激活肿瘤抗原特异性T细胞, 影响免疫抑制性T细胞亚群的扩增, 活化的肿瘤抗原特异性T细胞可能归巢到体内更远的部位, 消除转移灶, 抑制肿瘤的发展^[32,33]。大量研究表明, IRE介导的细胞膜穿孔可增加DAMP的合成和分泌, 如ATP、HMGB1(high mobility group box 1)、钙网蛋白及HSP70(heat shock protein)等^[25,26,30,34], 诱导肿瘤细胞的免疫原性细胞死亡, 增强抗肿瘤免疫反应^[25]。而且, DAMP的释放随电场强度的增加而增加, 其分泌水平与细胞死亡呈正相关^[34]。He等人^[26]研究发现, IRE可增加HMGB1的释放, 通过与晚期糖基化终末产物(receptor for advanced glycation end products, RAGE)受体结合, 激活MAPK-p38通路, 导致巨噬细胞向M1型极化。此外, 巨噬细胞中的HMGB1和RAGE释

放通过MAPK-ERK途径的正反馈,促进巨噬细胞M1型极化发挥抗肿瘤的效果。

2.2 IRE消融可改善肿瘤微环境

IRE消融可保持组织中较大血管完整的结构和功能,可促使随后致敏的效应T细胞向病变消融肿瘤残存部位浸润^[13,35]。Zhao等人^[30]研究发现,IRE消融后的肿瘤组织中心发生大片坏死,而边缘存活的肿瘤组织中微血管密度增加,且血管的通透性也增强,在IRE治疗6 d后微血管密度逐渐减少,于第9天恢复至基线水平。此外,IRE治疗4 d后肿瘤存活区域的缺氧标志物——缺氧诱导因子1- α (hypoxia inducible factor1- α , HIF1- α)及碳酸酐酶-9(carbonic Anhydrase IX, CA-IX)表达分别为对照组的53%与24%。基质透明质酸的标志物——透明质酸结合蛋白-1(hyaluronan binding protein 1, HABP1)为对照组的70%,细胞外基质硬度的标志物——赖氨酰氧化酶(lysyl oxidase, LOX)为对照组的41%,PD-L1为对照组的18%。然而,这些蛋白的表达在IRE治疗6 d后又逐渐增加,表明IRE可以通过增加肿瘤血管的密度与通透性、软化细胞外基质和减轻缺氧来短暂调节肿瘤微环境(tumor microenvironment, TME),有利于免疫细胞向残存肿瘤组织中浸润^[36,37]。胸腺基质淋巴细胞生成素(thymic stromal lymphopoietin, TSLP)已被证实在多种肿瘤中可驱动免疫细胞向促癌免疫表型Th2型细胞极化^[38-40]。Goswami等人^[41]研究发现,IRE可在小鼠与人的乳腺癌细胞微环境中抑制促癌炎症分子TSLP的表达。以上研究结果表明,IRE可改善TME,有助于抗肿瘤免疫反应的发生,从而抑制消融残存的肿瘤细胞。

3 IRE联合免疫疗法治疗实体瘤的疗效

IRE因其不引起热损伤,避免了“热沉效应”,可用于解剖关系复杂、侵犯邻近血管和神经结构的肿瘤治疗。国内外多个研究中心开展了针对IRE治疗恶性肿瘤有效性的研究。临床研究结果表明,IRE可显著延长胰腺癌患者的OS^[42],对肾癌的消融治疗是安全有效的,且具备对小肾癌进行根治性治疗的潜力^[43]。Distelmaier等人^[44]证实了IRE可用于邻近门静脉或肝静脉的肝脏肿瘤的消融治疗,不受热沉效应影响。Blazevski等人^[7]综述分析了IRE对283例前列腺癌患者的治疗效果,91%~100%的患者保留了控尿功能,79%~100%的男性患者保留了勃起功能,复发率为0~33%。结果表明,IRE

在有效清除肿瘤的同时还可保障患者的生活质量,是一种极具前景的前列腺癌的治疗方案。

胰腺癌对免疫治疗不敏感与其高度免疫抑制的TME密切相关。Yang等人^[45]研究发现,IRE消融小鼠原位胰腺癌可以克服TME免疫抑制,提高DC疫苗接种的有效性,IRE联合DC疫苗可促使CD8⁺ T细胞和颗粒酶B⁺ T细胞向肿瘤组织肿瘤浸润增加,克服胰腺癌免疫抑制的TME,有效延长荷瘤小鼠的中位生存期,且1/3小鼠的无瘤生存率可达90 d。国内外研究均发现,IRE联合PD-1抑制剂可促进具有抗肿瘤作用的免疫细胞(如CD8⁺ T、Th1、M1型细胞)比例增加,并向消融残存的肿瘤组织中浸润,而具有免疫抑制活性的细胞(如Treg、MDSCs及M2型细胞)比例减少,并可诱导长期记忆性的抗肿瘤免疫应答反应,从而显著延长胰腺癌荷瘤小鼠的OS^[24,30]。Neill等人^[46]研究发现,IRE消融小鼠原位胰腺癌残存肿瘤细胞PD-L1的表达增加,并进一步采用IRE联合纳武单抗(FDA批准的PD-1抑制剂临床应用)对10例Ⅲ期不可手术切除的胰腺癌患者进行治疗,患者疾病进展的平均时间为6.3个月,中位OS为18.0个月。Qian等人^[47]研究发现,nsPEF治疗消融肝癌可诱导肝癌细胞膜PD-L1的易位和释放,抑制了CD8⁺ T细胞功能障碍,因此通过PD-L1抑制剂阻断PD-L1可有效抑制肿瘤生长,并改善荷瘤小鼠的生存期。侯思楠等人^[19]研究发现,IRE联合PD-1抑制剂能有效减少肿瘤负荷,并引起TME改变。最新的一项小鼠前列腺癌的研究发现,IRE联合抗CTLA-4抑制剂可促进血液、肿瘤和非淋巴组织中肿瘤特异性CD8⁺ T细胞的扩增,有助于抑制肿瘤生长并避免新发肿瘤的形成,且IRE与CTLA-4抑制剂双重治疗后,再加入PD-1抑制剂可有效抑制残存肿瘤细胞的生长^[48]。

多项临床研究已证实,IRE联合免疫疗法可有效改善肿瘤患者的生存预后。我国学者探究了IRE联合NK细胞免疫疗法用于不可切除肝癌的治疗,证实了联合疗法下肝癌患者的无病生存期(disease-free survival, DFS)和OS明显优于单一疗法^[49]。有研究采用IRE联合同种异体NK细胞治疗Ⅳ期肝细胞癌^[50]及Ⅲ/Ⅳ期胰腺癌^[51]患者。结果表明,IRE联合NK细胞对肝癌有协同的治疗效果,不仅增强了患者的免疫功能,还降低了甲胎蛋白的表达并可显著延长患者的中位OS。在Ⅲ或Ⅳ期胰腺癌患者治疗中,联合治疗组患者的无进展生存期(progression-free survival, PFS)和OS均显著高于单一治疗组,而不良反应在3组间无明显差异。学者进一步探

究了IRE联合同种异体 $\gamma\delta$ T细胞输注治疗LAPC, 结果表明, 联合治疗组患者OS较IRE单一疗法延长了13.5个月^[52]. Pan等人^[53]的研究发现, IRE联合NK细胞疗法较IRE单一疗法治疗晚期胰腺癌并不能显著延长患者DFS和OS, 但可显著提升短期有效率, 并改善患者的免疫功能, 降低肿瘤相关标记物的表达水平. 最近的一项研究表明, 应用IRE联合Alphavirus M1溶瘤病毒治疗胰腺癌时, 治疗效果具有协同增强的作用. IRE将具有免疫沉默的“冷肿瘤”变为以T细胞活化为特征的“热肿瘤”, 促进M1的感染来增强其溶瘤作用^[54]. 以上研究结果表明, IRE联合免疫疗法治疗临床恶性肿瘤具有一定的有效性和安全性. 图1展示了IRE消融联合瘤内和/或全身免疫疗法治疗胰腺癌的策略及机制^[55]. 由于胰腺癌TME中富集具有免疫抑制作用的Treg、M2巨噬细胞、Th2和抑制性细胞因子(红色图标)易形成免疫抑制的微环境. 将具有免疫刺激作用的CpG直接注射到肿瘤组织中, 其可扩散至周围引流淋巴结, 结合未成熟DC上的Toll样受体9(橙色受体), 促使DC成熟(紫色细胞), 激活细胞毒性和Th1细胞(绿色图标)促进1型干扰素(IFN, 蓝色图标)的释放. 同时, IRE消融诱导大量肿瘤细胞死亡, 介导肿瘤抗原释放, 可被DC摄取并转运至淋巴结, 在淋巴结中形成适应性抗肿瘤特异性T细胞免疫应答(绿色图标). IRE消融肿瘤组织降低肿瘤负荷, 促使免疫抑制性作用的细胞减少以及相关细胞因子分

泌减少, 因此IRE可将免疫抑制TME逆转(红色图标)到免疫活化(绿色图标)的TME. 此外, 由于肿瘤细胞表面的PD-L1与免疫细胞表面的PD-1结合, 从而抑制免疫细胞活性. 检查点抑制剂(checkpoint inhibitor, CPI)结合肿瘤细胞或T细胞上的免疫检查点受体, 通过将CPI静脉注射可阻断这种调节相互作用, 使T细胞攻击肿瘤细胞. 因此, 将此3种治疗方式协同作用可共同改变TME, 诱导全身性的免疫应答, 并在远处转移病灶中产生远位免疫抑制效应(图1中肝脏所示). 以上研究结果表明, IRE联合免疫疗法是一种极具前景的恶性实体瘤的治疗策略.

4 结论与展望

近几年来, 由IRE诱发的免疫反应受到越来越多的关注. 大量基础及临床研究表明, IRE可在荷瘤机体内诱导强烈的免疫反应. IRE消融联合免疫疗法治疗实体瘤已显示出巨大的临床应用价值和发展空间, 但目前基础研究结果差异显著, IRE联合各类免疫疗法的大规模临床数据仍缺乏, 尚缺少充足有力的研究结果支撑其临床应用. 因此未来基础研究仍需深入探索IRE联合免疫治疗的作用机制, 探究IRE联合免疫治疗的最佳时机. 而临床研究需集中于患者的选择, 及两种疗法的结合时机、用量等以期寻求最佳的联合治疗模式, 从而使更多恶性肿瘤患者受益.

图1 IRE联合免疫疗法治疗胰腺癌的策略与机制^[55]. 1, 瘤内CpG注射; 2, IRE消融; 3, CPI静脉内注射

Figure 1 Strategies and mechanisms of IRE combined with immunotherapy in the treatment of pancreatic cancer^[55]. 1, Intratumoral CpG injection; 2, IRE ablation; 3, CPI intravenous injection

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Summary for “不可逆电穿孔联合免疫疗法治疗实体瘤的研究进展”

Progress on irreversible electroporation combined with immunotherapy in the treatment of solid tumors

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The advent of medical engineering technologies such as electromagnetism, acoustics or optics tumor ablation technology coupled with their clinical applications has gained a lot of interest among scientists. Irreversible electroporation (IRE), a novel physical ablation technique, is widely utilized to ablate the unresectable solid tumors and demonstrates a favorable safety and high efficacy in clinical applications, which is a promising therapy in clinical cancer treatment. As opposed to thermal ablative techniques, IRE induces cell death by the delivery of high-voltage electrical pulses, which possesses several advantages as a non-thermal ablation method: (1) Less collateral thermal damage, especially for the vital nerves/vessels and luminal structure; (2) no heat sink effect, it avoids the energy reduction caused by blood flow that would lead to the incomplete ablation; (3) preservation of extracellular matrix (ECM) scaffold, it is important for promoting rapid postoperative recovery. While a major drawback, the challenge for IRE, is the susceptibility to recurrence resulting from the incomplete ablation, which limits the clinical application of IRE.

However, IRE utilizes electric pulses to alter the transmembrane voltage on the cell membrane, which causes nanometer-sized membrane defects or pores in the cells and leads to loss of cell homeostasis. IRE has been demonstrated to not only kill tumor cells directly, but also promote the massive release of intracellular concealed tumor antigens that become an *in situ* “tumor vaccine”, inducing a potential anti-tumor immune response to kill residual tumor cells after ablation and inhibiting the local recurrence of tumors. Therefore, IRE can be regarded as a potential immunomodulatory therapy. IRE combined with immunotherapy will be a promising therapeutic strategy for malignant tumors. Recently, researchers paid much more attention to studying the immune response induced by IRE. And several studies have confirmed that IRE can induce a significant immune response in tumor-bearing organisms and significantly improve the anti-tumor efficacy of IRE. Additionally, cancer treatment has entered the era of clinical multidisciplinary comprehensive treatment, and the prognosis of patients with different tumor has been significantly improved. Thus, IRE combined with immunotherapy may have a synergistic effect on malignant tumor treatment. This article reviewed the characteristics of local and systemic immune response induced by IRE in tumor-bearing organisms, and its related mechanisms, and the research progress on the efficiency of IRE combined with immunotherapy in the treatment of solid tumors. The review aimed at elucidating the characteristics of IRE-induced immune response, and the mechanism behind it, and its potential in combination with immunotherapy for the treatment of solid tumors. It will provide a theoretical and practical guidance for clinical application of IRE combined with immunotherapy in the treatment of solid tumors.

irreversible electroporation, immunotherapy, solid tumors, combination therapy

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