冷冻消融在肿瘤免疫治疗中的应用进展

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摘要 冷冻消融(cryoablation)是一种肿瘤微创手术, 对局部肿瘤治疗具有创伤小、治疗精准等优势, 已被广泛运用在肝癌、肾癌、前列腺癌、肺癌、乳腺癌等实体肿瘤的治疗当中。不同于传统的放射治疗(radiation therapy)、微波射频(microwave radiofrequency)等物理治疗, 冷冻消融诱发肿瘤释放的肿瘤抗原更接近天然状态, 能有效激活T细胞对肿瘤抗原的识别, 为产生抗肿瘤免疫的联合治疗提供了基础。因此, 寻找合适的冷冻消融联合治疗药物, 全面增强机体的免疫反应是肿瘤免疫治疗领域的新方向。该文探讨了冷冻消融治疗肿瘤的分子机制以及其所产生的有效抗肿瘤免疫反应, 并综述了近年来冷冻消融结合不同免疫疗法的治疗效果, 以期为肿瘤治疗提供新的治疗策略。

关键词 冷冻消融; 肿瘤免疫治疗; 联合治疗; 免疫机制

The Progress of Cryoablation in Tumor Immunotherapy

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Abstract Cryoablation is a minimally invasive surgery for tumors. It has the advantages of small trauma and precise treatment for local tumor treatment. It has been widely used in the treatment of solid tumors such as liver, kidney, prostate, lung and breast cancer. Different from traditional radiation therapy, microwave radiofrequency and other physical therapies, cryoablation induces tumor antigens released by tumors to be closer to the natural state, which can effectively activate T cells to recognize tumor antigens for the production of anti-tumor immunity. The combination therapy provides the basis. Therefore, it is a new direction in the field of tumor immunotherapy to find suitable cryoablation combined therapy drugs to comprehensively enhance the body's immune response. This review discusses the molecular mechanism of cryoablation in the treatment of tumors and the effective anti-tumor immune response it induces. In addition, it reviews the therapeutic effects of cryoablation combining with different immunotherapies in recent years, hoping to provide new treatment strategies for tumor treatment.

Keywords cryoablation; tumor immunotherapy; combination therapy; immunologic mechanism

自19世纪末研究者们首次使用低温治疗肿瘤以来, 冷冻消融技术已被广泛应用于治疗肝癌、肺癌等各种原发性良恶性肿瘤[1-2]。近年来研究发现, 冷冻消融技术不仅可以对肿瘤组织进行反复冻融循环造成局部损伤, 而且可引起机体的抗肿瘤免疫反应。因此, 其在实体瘤患者的治疗中发挥着越来越

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重要的作用[3-4]。

目前, 临床上常用的消融治疗有射频消融(radiofrequency ablation, RFA)、微波消融(microwave ablation, MWA)、高强度聚焦超声(hiigh intensity focused ultrasound, HIFU)和冷冻消融(cryoablation)。其中, RFA、MWA、HIFU消融治疗都遵循热疗的原则, 只有冷冻消融是一种通过冻融过程引起组织损伤的低温疗法。 YAKKALA等[5]和SHAO等[6]最近研究表明, RFA、MWA、HIFU等方法虽然也会导致肿瘤抗原的原位释放, 但在保存天然肿瘤抗原结构(未变性的抗原)这一方面, 冷冻消融治疗优于其他技术, 且引起的适应性免疫反应更强, 如激活$CD8^+$T细胞活性。除此之外, HE等[7]研究还发现, 冷冻消融治疗不仅可以释放一系列的肿瘤抗原, 激活局部的抗肿瘤免疫, 同时还能引起远端效应。

然而, 虽然冷冻消融可以触发肿瘤的特异性免疫反应, 但这种免疫反应的大小和可持续性不足以完全杀死肿瘤, 以至于肿瘤发生复发和转移。 为了解决这一问题, 越来越多的研究者正将免疫系统与局部治疗结合起来[8-11]。 事实上, 局部治疗联合免疫佐剂[如: 白细胞介素-2(interleukin-2, IL-2)[12]、粒细胞–巨噬细胞集落刺激因子(granulocyte -macrophage colony stimulating factor, GM-CSF)[13]、Toll样受体(toll-like receptor, TLR)激动剂[14]]产生的抗肿瘤免疫反应已经显示出对肿瘤良好的抑制效果。 此外, 进一步研究发现, 树突状细胞(dendritic cells, DCs)、NK细胞(natural killer cell)等细胞类疗法和冷冻治疗也显示出一定的联合治疗前景[15]。最近使用免疫检查点阻断剂(如anti-CTLA-4、anti-PD-1)配合冷冻治疗等局部治疗显示出了持久的肿瘤特异性免疫应答[16-17], 甚至推动了几项针对黑色素瘤[18]、前列腺[19]、乳腺癌[20]和肝癌[21]的临床试验。

因此, 联合免疫治疗是冷冻消融技术在临床应用中的发展趋势。本文将综述近年来冷冻消融治疗肿瘤产生有效免疫反应的作用机制, 以及与免疫疗法联合治疗的效果, 以探讨冷冻消融的治疗前景, 为肿瘤治疗策略提供一个新的选择。

1 冷冻消融治疗肿瘤的基本原理

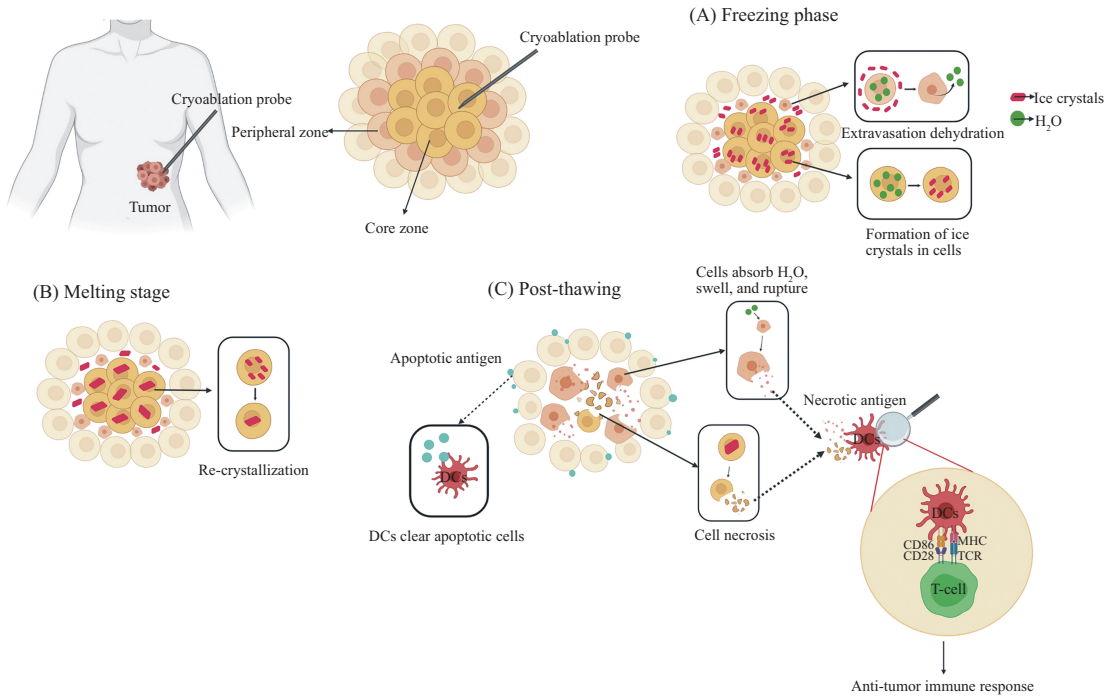
近年来, 冷冻消融技术以其微创、患者痛苦度低、目的性强等潜在优势已成为各种癌症治疗的常用方法。然而, 冷冻消融技术用于肿瘤治疗的具体作用机制目前尚未被完全阐明。早期认为冷冻消融的极端温度会对细胞造成机械损伤, 当细胞因冷冻脱水时, 高浓度的溶质会引起蛋白质受损, 破坏细胞膜和细胞质中的酶, 从而产生治疗效果[22]。 同时, 如果细胞内出现冰晶, 这些冰晶也会对细胞器和细胞膜造成损伤[23-24]。

目前冷冻消融机制主要分为三个阶段: 冷冻阶段、融化阶段、解冻后阶段[5]。冷冻阶段(图1A), 首先在较低的冷冻速率下, 由于细胞膜磷脂双分子层的保护作用, 冰晶主要先在细胞外空间形成, 胞外溶质浓度的逐渐增加, 会产生高渗的细胞外环境, 导致细胞外渗脱水。这可能是细胞的一种决策, 通过浓缩胞内溶质, 以此来抵抗胞内产生冰晶, 但对细胞也会造成严重的损伤。随后在快速的冷冻速率下, 消融核心区随着温度的急剧下降, 细胞来不及外渗脱水, 最终在细胞内形成冰晶[5]。

在融化阶段(图1B), 细胞内的小冰晶由于热力学不稳定, 会融合形成较大的冰晶(再结晶), 这增强了对细胞膜和胞内细胞器的机械损伤[25-26]。当存在大量的冷冻细胞时, 就会堆积成紧密的组织, 从而破坏组织结构。

在解冻后阶段(图1C), 细胞外的冰晶融化, 形成胞内的局部低渗, 造成胞外的溶剂进入胞内, 导致之前脱水的细胞重新膨胀并破裂, 释放细胞内容物。在此过程中, 消融核心处的细胞, 由于产生冰晶而造成细胞坏死。暴露于最外围亚致死温度下消融区的细胞会发生凋亡(apoptosis), 释放凋亡小体, 随后被巨噬细胞(macrophage)或者树突状细胞(DCs)清除。

目前, 普遍认为冷冻消融能够同时导致肿瘤细胞发生细胞凋亡和细胞坏死。其中, 细胞凋亡通常发生在肿瘤外围区, 开始于冷冻后的几小时, 并持续数天。冷冻消融引起的细胞凋亡可以同时由caspase蛋白酶家族参与的内源性和外源性两种细胞凋亡途径介导。研究表明, 冷冻消融能够激活BCL-2蛋白的活性, 引起内源性细胞凋亡, BCL-2促进线粒体外膜通透性(MOMP)的改变、细胞色素c的释放、caspase激活和细胞凋亡。当细胞色素c在ATP依赖性过程中结合APAF1时, 形成多聚体凋亡体。前caspase 9蛋白与凋亡体结合, 被切割并随后被激活, 并激活下游的caspase 3蛋白[27]。这导致DNA片段化, 包括内切核酸酶的激活、核蛋白和细胞骨架的破坏、蛋白质的交联、吞噬细胞配体的表达和凋亡小体的形成, 最



实线箭头代表局部区域放大, 虚线箭头代表DCs细胞识别肿瘤抗原。

The solid arrow represents the local area enlargement, and the dashed arrow represents DCs that recognize tumor antigens.

图1 冷冻消融治疗肿瘤的基本机制(根据参考文献[5]修改)

Fig.1 The basic mechanism of cryoablation in the treatment of tumors (modified from reference [5])

终导致细胞凋亡[28]。外源性细胞凋亡途径由死亡受体配体如TNFα、TRAIL和FAS诱导, 通过caspase 8形成死亡诱导信号复合物(death inducing signal complex, DISC), 最终导致细胞凋亡[29]。

与细胞凋亡不同, 坏死是冷冻消融治疗过程引起的另一种肿瘤细胞死亡形式。当细胞坏死发生时, 细胞中caspase 8表达减少, 受体相互作用丝氨酸/苏氨酸激酶3(receptor interacting serine/threonine kinase 3, RIPK3)和混交激酶域蛋白(mixed lineage kinase domain like pseudokinase protein, MLKL)表达量上调, 从而促进细胞程序性坏死[30]。

此外, 癌细胞已被证明可通过抑制其抗癌作用而抑制多种细胞死亡方式[31]。因此, 冷冻消融治疗是否会引起肿瘤细胞的自噬或使用自噬抑制剂是否会增强治疗效果, 及其他如细胞焦亡是否会引起更强的免疫反应、炎症反应值得我们进一步研究。

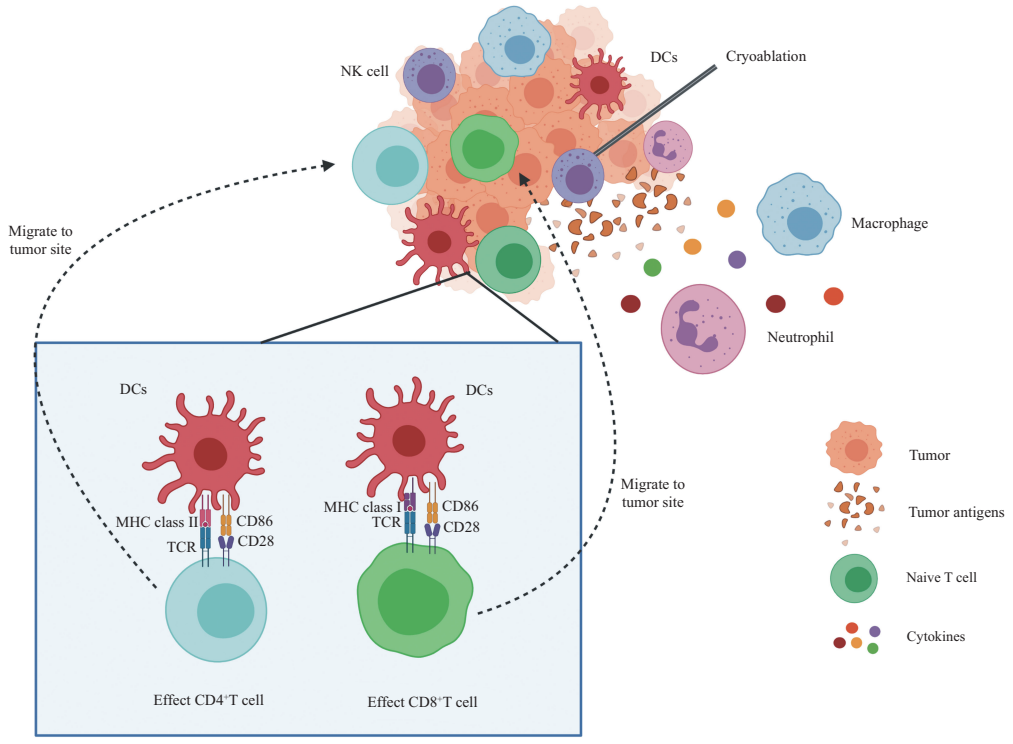
2 冷冻消融引起的抗肿瘤免疫反应

除了上述冷冻消融治疗肿瘤的基本机制外, 冷冻消融治疗肿瘤如何引起机体的免疫反应也是该领域的一个重要研究方向。研究显示, 在各种模型中, 冷冻消融后肿瘤特异性T细胞如$CD4^+$、$CD8^+$[32], NK细胞以及白细胞介素-4(interleukin-4, IL-4)、颗粒酶A(granzyme A, GZMA)、肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)等细胞因子[33-34]水平都有增加(图2)。其中, 激活的$CD8^+$T细胞不仅能通过颗粒酶(granzyme)、穿孔素(perforin)直接杀伤肿瘤, 还可以通过Fas/FasL途径, 激活肿瘤细胞中的caspase 3, 诱导其凋亡。$CD4^+$T细胞包含许多细胞亚群。其中一些细胞亚群如Th1(helper T cell 1, Th1)也具有显著的抗肿瘤活性[35]。

此外, 研究表明肿瘤细胞不同的死亡方式会引起完全不同的抗肿瘤免疫反应。

其中细胞凋亡通常是一种生理现象, 随着细胞染色体DNA降解, 其细胞碎片被巨噬细胞或DCs细胞所吞噬。SCHEFFER等[36]研究表明, 在炎性条件下凋亡的细胞可能相较于坏死的细胞更能刺激抗肿瘤免疫反应, 其可能原因是吞噬凋亡细胞的树突状细胞将凋亡细胞的抗原成分以MHC I类分子限制的方式递呈给细胞毒性T细胞(cytotoxic T cells, CTLs), 尚未清除的凋亡细胞可能会发生二次坏死并释放促炎症信号, 而在此状态下树突状细胞成为表达抗原及诱发效应T细胞的理想状态。

当凋亡细胞不能被及时有效地清除时, 它们会



虚线箭头代表效应CD4⁺T细胞或效应CD8⁺T细胞向肿瘤组织迁移。

The dashed arrow represents effector CD4⁺T cells or effector CD8⁺T cells migrate to tumor tissue.

图2 冷冻消融引起的免疫反应

Fig.2 Immune response caused by cryoablation

因为失去膜的完整性发展为继发性坏死。这会导致损伤相关的分子模式(damage associated molecular patterns, DAMPs)的释放[30]。DAMPs能激活固有免疫细胞, 同时可直接或间接启动适应性免疫反应。坏死中的RIPK3是介导细胞死亡信号通路的关键调控蛋白, 在N-端包含一个激酶结构域, 已被证明具有调节炎症信号通路的作用[37]。除此之外, RIPK3在C-端包含一个RHIM功能结构域, 能与其他相关信号通路同型的RHIM[如RIPK1、Toll/白细胞介素-1 (interleukin-1, IL-1)受体(TIR)结构域]相互作用, 诱导TRIF(TIR domain-containing adaptor inducing IFN-β)通路和DNA依赖的IFN调节因子(DAI)的激活[38], 并触发细胞坏死。肿瘤坏死通常还伴随着组织损伤, 其表现为细胞崩解、细胞核发生改变及细胞内容物[如促炎症细胞因子(pro-inflammatory cytokines)、热休克蛋白(heat shock protein)、DNA、RNA等]的释放[39-40], 这些因子可刺激机体产生强烈的免疫反应(图2)。

冷冻消融可以同时诱导肿瘤细胞发生坏死和凋亡, 更增强了冷冻消融对肿瘤的治疗效果。因此, 阐明冷冻消融诱导何种细胞死亡方式, 以及如何增强肿瘤细胞的死亡是今后冷冻消融研究的一个重要方向。

除此之外, 冷冻消融还能造成血管内皮细胞和周围细胞的损伤, 使得血管内出现停滞, 导致细胞水肿和凝血级联反应, 在血管内形成微血栓, 进而引发组织缺血[41]。组织学研究表明, 消融后肿瘤内会浸润中性粒细胞(neutrophil), 随后募集大量巨噬细胞[42]。

肿瘤冷冻消融可以触发肿瘤特异性免疫反应。然而, 这种免疫反应的大小和可持续性却不足以抵抗肿瘤的再挑战。除此之外, 肿瘤中存在多种免疫逃逸机制来躲避机体的免疫攻击[43-45]。因此, 冷冻消融联合免疫治疗来应对免疫抑制反应, 为治疗癌症治疗患者提供了更多的选择。

3 冷冻消融联合免疫治疗策略

虽然目前冷冻消融技术在多种癌种中取得了不错的临床成效, 然而多年的临床试验表明, 单一的冷冻消融针对晚期肿瘤依然只有有限的治疗效果。其中的一个重要原因在于肿瘤的发生发展涉及多个过程, 期间会形成多种突变, 单一的治疗手段尚不足

以完全治疗肿瘤。因此, 联合治疗成为了冷冻消融的一个重要发展方向。目前临床中已经有冷冻消融与放疗、化疗、激素以及免疫治疗联合的报道, 并且都显示出了积极的治疗效果(表1)[46-53]。

近年来, 随着免疫检查点抑制剂PD-1(programmed cell death-1)/PD-L1(programme-d cell death-ligand 1)、CAR-T(chimeric antigen receptor T cell)、过继性细胞治疗(adoptive cellular immune-therapy, ACI)等新的抗肿瘤免疫疗法的出现, 免疫疗法已成为肿瘤治疗新的方向。此外, 研究表明多种治疗方法与免疫疗法联合使用效果显著[54]。因此, 本文将重点讨论冷冻消融和肿瘤免疫治疗联合使用的效果和机制。

3.1 冷冻消融联合免疫检查点抑制剂

研究显示, 免疫检查点(immune checkpoint)分子在免疫耐受中起着至关重要的作用, 如肿瘤细胞可以利用这些途径逃避免疫攻击。其中阻断免疫检查点分子CTLA-4(cytotoxic T lymphocyte associated protein-4), 可以增强其配体CD80/86与CD28的结合程度, 进而促进T细胞活化[55-56]。肿瘤和肿瘤相关巨噬细胞(tumor-associated macrophages, TAMs)表达PD-L1, 在肿瘤抑制T细胞方面起着关键作用[57]。阻断PD-1或PD-L1的抗体对T细胞引起的抗肿瘤反应起着关键作用。

利用免疫检查点抑制剂联合冷冻消融治疗肿瘤可以增强机体的免疫反应。2018年, BENZON博士团队[58]发现, 在前列腺癌小鼠模型中, 冷冻消融联合CTLA-4治疗时, 死亡率降低4倍。2012年, WAITZ博士团队[17]在另一项冷冻消融联合CTLA-4实验中, 发现有44%的小鼠存活下来。联合应用CTLA-4和PD-1阻断可提高黑色素瘤、肾细胞癌和许多其他癌症[59-60]患者的生存率。

3.2 冷冻消融联合Toll样受体(TLRs)激动剂

TLRs是天然免疫应答中起重要作用的受体, 可识别与微生物和受损宿主细胞相关的模式分子。TLRs信号触发巨噬细胞和树突状细胞(DCs)的代谢转变, 促进其从耐受状态向免疫状态的转变。TLRs与同源配体结合后, 启动细胞内My88或TRIF介导的信号通路。这些信号通路会产生I型干扰素(type I interferon, IFN-I)和炎症反应, 促进T细胞免疫应答[61-62]。

REDONDO博士等[63]研究表明, 咪喹莫特(TLR7 激动剂)联合冷冻消融治疗B16-OVA肿瘤, 小鼠的存活率高达95%, 而单独使用冷冻消融治疗小鼠的存活率仅有30%~35%。因此, TLR激动剂可以作为冷冻消融治疗肿瘤的佐剂, 以产生更强的抗肿瘤免疫反应。但对于不同肿瘤类型的联合治疗效果还需要进一步的研究。

3.3 冷冻消融联合过继性细胞疗法

3.3.1 DCs细胞 树突状细胞在先天免疫反应和适

表1 冷冻消融联合不同治疗方式的临床研究

Table 1 Clinical study of cryoablation combined with different treatment methods

研究对象 Research object	治疗方式 Treatment	治疗效果 Treatment effect	参考文献 References
Non-small cell lung cancer	Cryoablation+allogeneic NK cells	Enhance the patient's immune function, significantly improve the RR (response rate) and DCR (disease control rate)	[46]
Advanced liver cancer	Cryoablation+allogeneic NK cells	Enhance immune function, improve the quality of life of patients, and reduce the expression of AFP	[47]
Unresectable liver cancer	Cryoablation+drugloaded microsphere embolization	The survival rate is significantly improved, the survival rate is 82.7%	[48]
Esophageal cancer and liver metastases	Cryoablation+chemotherapy	Improve OS (overall survival) and QOL (quality of life) of patients	[49]
Advanced renal cell carcinoma	Cryoablation+sorafenib	The patient's immune function indicators are improved, the ORR (objective response rate) and DCR (disease control rate) are significantly higher, and the PFS (progression-free survival) and OS (overall survival) are significantly longer than other groups	[50]
Malignant liver cancer	Cryoablation+radiotherapy	The patient's quality of life is significantly improved and the survival period is prolonged	[51]
Melanoma	Cryoablation+pembrolizumab	No adverse events or major complications of grade 3-4 are observed, NK cells increase and T-reg cells decrease	[52]
Prostate cancer	Cryoablation+androgen	Reduce PSA (prostate specific antigen) and relieve urinary symptoms	[53]

应性免疫反应之间起着桥梁作用。在之前提出的冷冻消融治疗肿瘤的机制中, 冷冻消融后所释放的肿瘤抗原被抗原提呈细胞(antigen presenting cell, APC)所吸收, 其中包括未成熟的树突状细胞。同时, 未成熟的树突状细胞在提取肿瘤的抗原后还需环境刺激从而成熟, 以此来激活机体的免疫应答。在冷冻消融后的肿瘤微环境中, 坏死细胞和炎症细胞因子可刺激DCs细胞的成熟[39]。成熟的DCs细胞能通过MHC I类和II类分子将肿瘤抗原呈递给T细胞, 从而导致肿瘤特异性T细胞的激活和增殖[64]。此外, DCs通过一种被称为交叉递呈的机制有效诱导$CD8^+$T细胞向细胞毒性T细胞(CTLs)分化, 产生肿瘤特异性CTLs是大多数癌症免疫治疗的目标[65]。

2014年, YIN博士团队[66]在胶质瘤中发现, 冷冻消融联合DCs疫苗可促进DCs成熟, 增强DCs抗原呈递功能, 诱导细胞毒性T淋巴细胞(CTLs)杀伤肿瘤细胞。2019年, XU博士团队[67]在另一项临床研究中, 测试了DCs细胞−细胞因子诱导的杀伤细胞(DC cell cytokine induced killer cell, DC-CIK)与冷冻消融联合对结肠癌患者的治疗效果。结果发现, 两者联合治疗缓解率为61.76%, 远高于对照组, T淋巴细胞亚群升高, 术后三个月整体生活质量明显高于对照组, 表明DC-CIK细胞联合冷冻消融可提高结直肠癌肝转移患者的免疫功能和生活质量, 增强对肿瘤的杀伤作用。

3.3.2 *自然杀伤细胞* NK细胞是属于先天免疫系统的非特异性杀伤细胞, 在对抗肿瘤的早期阶段发挥着重要的作用[68]。随着对NK细胞功能的进一步了解, 过继NK细胞转移对各种癌症具有很好的抗肿瘤作用。与细胞毒性T细胞或其他免疫细胞不同, NK细胞通过杀伤细胞免疫球蛋白样受体(killer cell immunoglobulin-like receptor, KIR)在细胞表面识别“非自身”组织相容性抗原。NK细胞产生的免疫活性细胞因子使其成为免疫治疗的吸引工具。

2017年, LIN博士团队[69]在一项临床研究中测试了冷冻消融联合同种异体NK细胞来治疗实体癌患者的效果, 发现同种异体NK细胞联合冷冻消融相较于单独冷冻消融更具有协同效应。这种有效性在非小细胞肺癌(non-small cell lung cancer, NSCLC)、肝细胞癌和肾细胞癌中均有体现, 提示了NK细胞治疗对冷冻消融有增强作用[46,49]。

3.4 冷冻消融联合肿瘤疫苗

肿瘤疫苗通过识别肿瘤特异性抗原(tumor specific antigen, TSA)或肿瘤相关抗原(tumor associated antigen, TAA)激活$CD8^+$T细胞和$CD4^+$T细胞, 诱发全面抗肿瘤免疫应答, 从而达到消除或抑制肿瘤病灶生长、转移或复发的目的[70]。目前常见的肿瘤疫苗有全细胞疫苗(whole cell vaccine, WCV)、多肽疫苗(multi-peptide vaccine, MPV)、DCs肿瘤疫苗(DCs tumor vaccine)等。

2006年, DEN BROK博士团队[14]在研究中发现, 肿瘤原位冷冻消融治疗后获得了肿瘤内抗原模型; 随后与TLR9的联合治疗在小鼠根除局部肿瘤和全身肿瘤方面效果显著, 能诱导机体DCs成熟, 为机体提供一种原位DCs疫苗。2013年, FLOERCKEN博士团队[71]利用患者自身肿瘤细胞裂解物负载DCs治疗肾癌的I/II期临床研究中, 发现DCs疫苗能诱导机体T细胞的增殖, 同时刺激患者的体内出现特异性Th1型免疫应答。此外, 研究显示, 冷冻治疗能在肿瘤局部募集大量的巨噬细胞以及树突状细胞, 这为吞噬肿瘤疫苗奠定了基础[72]。

目前, 随着生物信息学的发展, 以大数据为基础的个性化肿瘤新抗原(neoantigen)疫苗治疗肿瘤已在临床上表现出良好的抗肿瘤效应[73-74]。因此, 冷冻消融联合肿瘤疫苗治疗癌, 也是局部治疗应用的一个新的方向。

4 展望

冷冻消融是一种低温治疗肿瘤的方式, 通过促进肿瘤释放大量抗原, 能有效激活T细胞对肿瘤的识别[5,75]。然而仅使用冷冻消融很难触发足够强烈的抗肿瘤免疫, 该方式一般不足以完全抵抗肿瘤。随着肿瘤免疫研究的不断深入, 研究发现通过冷冻消融不仅可以控制原发肿瘤, 同时利用免疫激活剂还可增强全身抗肿瘤免疫反应, 在机体内产生“原位肿瘤疫苗”, 进而抑制残留肿瘤的转移和复发[76]。这一治疗策略, 已经得到了多个临床试验的验证。然而, 不同的免疫治疗药物对机体的免疫激活效果作用不同, 如PD-1抗体能阻断肿瘤对T细胞的抑制作用[77], 进而激活机体免疫; CTLA-4抗体则能消除Treg对$CD8^+$T细胞的抑制[78], CD47抗体促进巨噬细胞对肿瘤细胞的识别[79]等。因此, 从应用的角度出发, 彻底揭示冷冻消融治疗后肿瘤微环境的变化, 尤其是不同免疫细胞亚型的变化, 对冷冻消融的联合治疗意义重大。

综上, 针对不同患者免疫微环境的不同, 寻找合适的免疫疗法与冷冻消融联合治疗是提高肿瘤治愈率的关键。免疫疗法与冷冻消融的联合使用, 以及改进和优化现有免疫疗法与冷冻消融的联合治疗手段, 将会给肿瘤治疗提供更多的可选工具, 也是一个极具临床价值的研究方向。

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