超声介导的肿瘤免疫治疗

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2017-07-27 收稿, 2017-09-18 修回, 2017-09-22 接受, 2017-11-09 网络版发表

摘要 免疫治疗是一种新兴的肿瘤疗法. 与传统的疗法直接杀伤肿瘤不同, 免疫治疗是通过激活或增强人体免疫系统, 依靠自身免疫功能间接杀灭癌细胞和肿瘤组织的疗法. 考虑到肿瘤的异质性和遗传不稳定性, 单一的疗法不能达到完全治愈肿瘤的效果. 近年来, 有研究表明, 利用超声可以提高机体对肿瘤的免疫反应强度, 加强肿瘤的免疫治疗效果. 聚焦超声在破坏肿瘤的同时可以导致机体在位产生肿瘤碎片和肿瘤相关抗原等物质, 增强肿瘤的免疫原性, 刺激细胞免疫, 诱导机体产生免疫应答. 微泡是临床上使用的超声造影剂, 微泡的加入可以加强聚焦超声对肿瘤的破坏效果, 引发更强的免疫反应. 利用超声和微泡的组合还可以打开血脑屏障, 促进免疫细胞或免疫治疗药物进入血脑屏障发挥作用. 此外, 微泡还是一种常用的基因和药物载体, 利用超声将负载免疫相关基因或抗原的微泡递送到肿瘤细胞或免疫细胞中, 同样可以增强免疫应答反应, 提高肿瘤免疫治疗的疗效.

关键词 超声, 免疫治疗, 肿瘤, 微泡

恶性肿瘤是严重威胁人类生命的主要疾病之一. 目前临床上使用的肿瘤治疗方案主要有手术切除、放射治疗、化学治疗等. 这几种传统治疗手段虽然能对肿瘤原发灶进行很好的根治, 但各自具有一定的缺陷, 并不能完全有效地控制肿瘤的复发和转移. 为了突破肿瘤治疗的瓶颈, 我们迫切需要开发新型的肿瘤治疗方法. 免疫疗法是一种新兴的肿瘤治疗方法, 与以往疗法直接杀伤肿瘤不同, 肿瘤的免疫治疗是通过激活或增强人体免疫系统, 依靠自身免疫功能杀灭癌细胞和肿瘤组织的疗法, 该方法针对的靶标不是肿瘤细胞和组织, 而是人体自身的免疫系统. 2013年, *Science*将免疫抗癌疗法评为年度“十大科技突破”之首^[1].

有效的肿瘤免疫应答有3个步骤^[2]. (1) 树突状细胞(dendritic cells, DC)需要捕获肿瘤抗原, 并在胞内加工处理, 获得“成熟信号”以激活对抗原的免疫反应; (2) 由DC将抗原信息呈递给淋巴组织中未成熟的T细胞使其活化, 由此启动机体的特异性免疫反

应. 如果呈递抗原的DC未被成熟信号激活, 则会诱导T细胞产生耐受性, 从而抵抗免疫反应^[3]; (3) 活化的T细胞在肿瘤组织中浸润, 特异性地识别并杀死癌细胞. 然而, 肿瘤细胞具有免疫逃逸的特性, 能通过多种机制逃避免疫系统地识别和攻击, 因此机体很难通过自身免疫系统有效地杀灭肿瘤^[4].

相较于副作用极大的传统肿瘤治疗方法, 通过激发自身的免疫系统杀灭肿瘤是极具前景的抗肿瘤手段. 当前癌症免疫治疗主要包括以下几种类型: 增强非特异性免疫系统; 体内肿瘤疫苗; 单克隆抗体; 以及过继细胞转移治疗技术(adoptive cell transfer, ACT)^[5].

利用非特异性免疫刺激剂或相关细胞因子可以激活非特异性免疫, 进而增强特异性免疫反应的强度, 如细胞因子 γ 干扰素(IFN- γ)和白细胞介素(IL-2)已经用于癌症的免疫治疗. 2010年, 第一种治疗性肿瘤疫苗Sipuleucel-T(或称Provenge)被美国食品药品监督管理局(FDA)批准用于治疗转移性前列腺癌^[6].

引用格式: 张晓婷, 戴志飞. 超声介导的肿瘤免疫治疗. 科学通报, 2017, 62: 4131–4138

Zhang X T, Dai Z F. Ultrasound induced cancer immunotherapy (in Chinese). Chin Sci Bull, 2017, 62: 4131–4138, doi: 10.1360/N972017-00769

Sipuleucel-T 的耐受性较好, 能产生长期的免疫反应, 有效延长患者的生存期^[7], 相关的联合治疗方法已经处于研究阶段^[8,9]. 2011 年, 首个以免疫检查点为靶点的单抗类药物 Ipilimumab (Yervoy) 经 FDA 批准上市, 用于治疗转移性黑色素瘤^[10]. 到目前为止, FDA 已经批准的免疫检查点抑制剂有 CTLA-4 单抗药物 Ipilimumab, PD-1 单抗药物 Nivolumab (Opdivo) 和 Pembrolizumab (Key-truda), 以及 PD-L1 单抗药物 Atezolizumab (Tecentriq) 等^[11,12]. 过继性免疫细胞转移通过在体外激活自体或异体免疫效应细胞, 再输注给患者, 达到杀伤体内肿瘤细胞的目的, 对血液和免疫系统肿瘤的治疗效果较好^[13~15]. 其中嵌合抗原受体 T 细胞免疫疗法 (CAR-T) 是一种有前景的 ACT 疗法, 在白血病治疗领域取得了较大的成功, 但对于实体瘤的治疗效果并不理想^[16]. 在最新的研究中, 研究人员利用负载 CAR 基因的纳米粒子实现了对 T 细胞的体内编辑, 成功提高了白血病小鼠的生存期. 这一方法省去了提取和体外处理 T 细胞的步骤, 为肿瘤的免疫治疗提供了新的思路^[17].

超声波是频率高于 20 kHz 的声波, 高于人耳可以检测到的频率^[18]. 超声成像是目前世界上应用最广的成像手段之一, 具有安全无创、无放射性、成本低廉、操作简单、实时成像等优点. 除了用于医学成像, 超声在治疗领域也有较好的表现. 单独使用超声波, 或者联合药物, 可以治疗癌症、心血管疾病、感染、血栓、青光眼、神经损伤、皮肤伤口及骨折等^[19,20]. 近年来, 有许多研究表明超声可以用来提高机体对肿瘤的免疫反应. 聚焦超声 (focused ultrasound, FUS) 不仅可以产生热效应, 产生的机械效应和空化效应能提高宿主的抗肿瘤免疫应答. 此外, 利用超声将基因和抗原递送到细胞中同样可以激活抗癌免疫反应. 通过超声将微泡击破时对细胞膜产生的力直接将物质递送到细胞中, 从而规避了各种天然屏障. 用这种方式, 肿瘤抗原和抗原编码基因可被递送到免疫细胞, 免疫应答刺激基因也可以递送到肿瘤细胞.

本文概述了超声如何实现免疫应答, 并讨论未来发展中的潜在的可能性和存在的问题.

1 超声增强免疫治疗效果

1.1 HIFU 消融增强免疫治疗效果

高强度聚焦超声 (high intensity focused ultra-

sound, HIFU) 可以将超声波高度聚焦于体内靶区, 在肿瘤内产生瞬态高温、空化、机械作用等生物学效应, 杀死靶区内的肿瘤细胞, 同时对邻近组织造成较小的伤害, 适用于治疗多种良、恶性实体肿瘤.

Yang 等人^[21]很早就发现 HIFU 可以刺激机体对肿瘤的免疫反应. 他们用 HIFU 治疗荷神经母细胞瘤 C1300 小鼠, 在肿瘤消融后给小鼠再次接种同种肿瘤. 对比之前未接种肿瘤的小鼠或未经 HIFU 治疗的荷瘤小鼠, 经 HIFU 治愈的小鼠再次接种的肿瘤增殖率显著降低. Zhang 等人^[22]发现 HIFU 消融时产生的肿瘤碎片引起肿瘤特异性免疫应答, 可以作为有效的抗肿瘤疫苗.

研究表明 HIFU 激活的免疫细胞过继转移同样具有很好的疗效^[23]. 用 HIFU 处理 H22 荷瘤小鼠, 14 d 后从脾脏中取得 T 细胞, 过继转移到新的 H22 荷瘤小鼠体内. 研究结果表明 HIFU 处理的小鼠外周血中 CD3⁺, CD4⁺, CD4⁺/CD8⁺ 比值, CTL 细胞毒性, IFN- γ 和 TNF- α 分泌均有明显升高. 而将 HIFU 激活的 T 淋巴细胞转移到荷瘤小鼠后, 诱导肿瘤浸润性 T 淋巴细胞和 IFN- γ 分泌细胞的显著增加. 与对照组和假手术组相比, HIFU 激活的淋巴细胞显著抑制了体内肿瘤的生长和进展, 延长了存活时间.

HIFU 杀伤肿瘤组织主要依靠热效应. 产热效果不同的 HIFU 在刺激免疫应答方面具有不同的效果. Liu 等人^[24]的研究表明, 相比于高温消融 (80°C), 较低温度的 HIFU 消融 (55°C) 能更有效地保留抗原, 刺激 DC 的浸润和原位成熟, 引发更强的免疫反应. 另外, 扫描方式同样影响 HIFU 的免疫治疗效果. 在病变组织中留出部分间隙的稀疏扫描方式同样能更好地保留抗原, 因此比密集扫描方式更有效.

Silvestrini 等人^[25]发现, HIFU 与两种免疫疗法 (PD-1 检查点抑制剂和 TLR9 激动剂) 结合治疗上皮癌小鼠模型具有优异的效果. HIFU 只能原位消融肿瘤, 而通过与免疫治疗相结合, 可以更全面地控制肿瘤. 该研究组发现优化免疫治疗和 HIFU 消融的时间顺序对于该疗法至关重要. 在 HIFU 消融之前先进行免疫治疗的效果最好, 90 d 后 80% 的小鼠完全治愈. 但消融后再开始免疫治疗, 疗效则差很多, 这可能是因为 HIFU 消融后肿瘤微环境的机械变化和炎症介导的免疫表型的改变影响了免疫治疗的效果.

HIFU 介导免疫应答的主要机制有: (1) HIFU 消融后残留的肿瘤碎片和释放的肿瘤相关抗原 (tumor-

associated antigen, TAAs)可以共同作为肿瘤疫苗,增强了肿瘤的免疫原性;(2) HIFU治疗会引起Th1型反应,导致细胞免疫的显著变化,增强DC细胞和细胞毒性淋巴细胞的活性;(3) HIFU治疗能够平衡肿瘤微环境中肿瘤诱导的免疫抑制作用^[26~28]。这些效应使得HIFU不仅能直接杀伤肿瘤组织,还能有效促进机体的抗肿瘤免疫反应(图1)。将HIFU与过继细胞疗法、免疫点抑制剂相结合的治疗方式具有更好的疗效,有希望应用到临床上治疗癌症。但HIFU治疗并不是完全没有风险的,皮肤发红、目标区域外的水肿性疼痛和坏死是常见的并发症,在治疗过程中需要加以注意。

1.2 利用声孔效应递送活性物质

声孔效应是由于超声空化伴随发生的冲击波、射流影响细胞膜的完整性,使细胞膜对大分子暂时开放。声孔效应是超声增强药物递送和超声引导基因转染的重要物理基础^[29]。因此,我们可以利用超声的声孔效应进行基因转染或药物递送,辅助肿瘤的免疫细胞治疗。

Casey等人^[30]利用超声的声孔效应将含有粒细胞-巨噬细胞集落刺激因子(GM-CSF)的质粒递送到实体

瘤内。小鼠经过超声介导的基因治疗后存活率为55%,治愈小鼠再次接种肿瘤细胞也未见到肿瘤复发。将从治愈小鼠体内取出的脾细胞过继到健康小鼠体内,健康小鼠同样具有了肿瘤抗性。这些结果均表明超声介导的免疫相关基因的转染具有较好的疗效。

经由超声照射辅助递送单克隆抗体,在体外和体内实验中同样具有较好的效果。体外实验中可以看出超声辐照下单抗的细胞毒性增强^[31],体内实验也已证明超声增强了肿瘤对单抗的摄取^[32~34]。需要注意的是,利用超声造影剂微泡能够增强超声的声孔效应,改善药物递送效率,将药物连接到微泡上还能提高局部药物浓度,这些会在后文2.2中进行介绍。

2 超声微泡辅助免疫治疗

在超声的基础上引入超声造影剂,能够大幅度提高超声成像的图像分辨率和灵敏度。最常用的超声造影剂是含气体的封闭微泡形式,由包膜材料与核心气体构成。近年来,超声造影剂在治疗超声领域的应用也开始被研究。由于微泡可加强空化效应,从而促进超声生物学效应,因此在溶栓、介导基因转移、药物输送等治疗方向上也有应用价值^[35~38]。

图1 高强度聚焦超声(HIFU)的抗肿瘤反应^[28]

Figure 1 Summary of the high-intensity focused ultrasound (HIFU) anti-cancer response^[28]

2.1 微泡加强超声空化效应

由于微泡可以加强空化效应,在超声消融刺激免疫反应的基础上,可以在体系中加入微泡增强治疗效果. Liu等人^[39]验证了没有活性的微泡具有触发免疫应答的潜力. 他们使用声诺维(SonoVue)微泡与聚焦超声(FUS)相结合的方式治疗小鼠实体肿瘤CT-26. 静脉注射微泡后立即用超声照射肿瘤. 与仅用超声治疗相比,微泡加超声的治疗方式导致肿瘤生长减慢,免疫细胞对肿瘤组织的浸润增加.

Wei等人^[40]利用聚焦超声和微泡的组合诱导打开血脑屏障,以增强白细胞介素12(IL-12)在C-6胶质瘤大鼠中的递送. 血脑屏障(blood-brain barrier, BBB)是大脑血管壁上的一层紧密细胞,它能保护大脑抵御感染、毒素和其他威胁. 不过,血脑屏障的存在同样也阻碍了药物进入大脑,超声与微泡结合能够短暂打开血脑屏障帮助药物进入大脑^[41-43]. 聚焦超声诱导的BBB开放对正常动物的T细胞没有明显影响,但是它引起肿瘤浸润淋巴细胞(TIL)的轻度变化. IL-12给药会引起TIL细胞的显著增加,而FUS引导微泡打开BBB后与IL-12联合给药产生的TIL细胞的增加最为显著,从而有助于抑制肿瘤生长,增加动物的存活率.

聚焦超声诱导BBB开放为中枢神经系统免疫治

疗提供了新的可能性,主要通过以下3种机制^[44]: (1) 通过增加局部血脑屏障通透性以促进免疫调节相关的单抗或细胞因子的渗透; (2) 通过招募或调整所需的免疫细胞以渗透和靶向到病变部位; (3) 通过激活神经胶质细胞和其他先天细胞使微环境具有一定抗肿瘤性. 这3个途径表明应用聚焦超声引导微泡打开BBB可以增强药物对大脑的免疫治疗作用,在神经免疫调节和神经免疫治疗上的应用前景广阔(图2).

2.2 微泡递送活性物质

微泡是研究中常用的基因和药物载体. 用微泡递送活性物质最简单方法是共注射,将微泡和生物活性物质在离体溶液中混合,然后注射混合物,并在体内使用US辐照促进递送. Sakakima等人^[45]选择了脂质包裹的全氟丙烷微泡,与编码干扰素IFN的质粒一起使用. 在体外实验中,IFN-pDNA和超声的组合治疗使得IFN的表达增强,而肿瘤细胞的存活率显著下降. 体内实验也证明该方法可以减缓小鼠的肿瘤生长速度.

这种共注射方式的潜在问题是两者在体内的分布并不完全相同,会导致药物的利用率变低. 因此,更好的方法是将微泡与活性物质偶联,不仅保证了两者的生物分布相同,还提高了活性物质的局部浓

图2 (网络版彩色)聚焦超声诱导血脑屏障的开放以及对中枢神经系统免疫调节和免疫治疗的潜在影响^[44]

Figure 2 (Color online) Schematic showing FUS-induced BBB opening with its potential effect in CNS immune modulation and immunotherapy^[44]

度,降低了用量. 超声微泡在药物递送方面的应用很广,利用这种方式可以递送各种类型的大分子,比如将 mRNA 递送到 DC^[46],将 pDNA 递送到肿瘤细胞^[45,47,48],以及将各种肿瘤抗原递送给 DC^[49~51]. 靶向微泡与超声的组合则提供了更复杂有效的递送系统,这种治疗系统不仅具有位点特异性,同时也具有细胞特异性,可以更高效地激活免疫系统. 例如,修饰了甘露糖的靶向脂质微泡,在超声的作用下可以更加有效地将基因递送到含甘露糖受体的 DC^[52]. 经 pDNA 和甘露糖修饰的靶向脂质微泡在体内实验中也显示出较好的免疫治疗效果,导致肿瘤生长减少和存活率增加^[53,54].

Dewitte 等人^[55]利用微泡在体外将抗原 mRNA 以及免疫调节 TriMix mRNA 转染进 DC (图3). 将处理后的 DC 作为特异性疫苗接种给荷瘤小鼠,能有效诱导抗原特异性 T 细胞的产生,促进抗原表达细胞的特异性裂解,导致肿瘤生长减慢,总生存期显著增加. 此外,有 30% 的接种小鼠中观察到肿瘤的完全消融,证明这种方法可以引起长期的抗原特异性免疫记忆,具有用于开发 DC 疫苗的潜在价值.

微泡作为免疫治疗中的基因或抗原递送载体也有一定的缺点. 首先,微泡直径一般在 2~5 μm ,不能有效地穿透肿瘤组织;其次,因为包裹气体的原因,微泡在体内半衰期较短. 另一个潜在的问题是制备方法复杂,使用前需要预处理. 这些缺点一定程度上限制了微泡在免疫治疗中的应用.

3 总结

近年来肿瘤免疫治疗取得了令人振奋的成绩,尤其是为进展性晚期癌症患者带来了希望. 但由于人体的免疫系统具有高度的异质性及特异性,目前肿瘤的免疫疗法还不十分完善,不能普遍应用到临床治疗中.

超声具有无创、穿透性好、可以局部辐照、产生空化效应等特点,在肿瘤消融和药物递送方面具有很大的优势. 单独使用超声、或利用超声递送免疫刺激物均可以诱发机体的抗肿瘤免疫应答. 与单独的免疫治疗相比,超声与免疫治疗的联合治疗具有更好的效果.

超声介导免疫治疗这一领域相当年轻,许多机制尚未完全了解,需要开展进一步的研究. 首先,肿瘤免疫治疗需要考虑很多因素,如患者的癌症类型、遗传背景、性别、年龄及种族,不同治疗药物的作用位点、适应证及作用机制等,并以此为依据搭配合适的药物组合. 其次,较低温度的 HIFU 消融能引发更强的免疫应答,但对于肿瘤消融和治愈原发性疾病来说,高温消融更有效,因此在使用 HIFU 时需要认真权衡肿瘤消融与免疫治疗的利弊. 此外,微泡作为免疫治疗中的基因或抗原的递送载体也有制备复杂、半衰期短、不能有效穿透肿瘤组织等问题亟待解决. 但不可否认的是,随着对免疫治疗、HIFU 消融、微泡介导药物递送等机制的研究逐渐透彻,超声与免疫治疗的联合治疗前景十分广阔.

图3 (网络版彩色)使用负载mRNA的微泡和超声将基因转染进树突状细胞,用于治疗小鼠肿瘤^[55]

Figure 3 (Color online) The use of mRNA-loaded microbubbles and ultrasound for gene transfection of DCs to treat tumor *in vivo*^[55]

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Summary for “超声介导的肿瘤免疫治疗”

Ultrasound induced cancer immunotherapy

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Cancer immunotherapy is rapidly becoming one of the pillars of anti-cancer therapy. Unlike previous cancer therapies, immunotherapy aims at activating or enhancing the natural immune response of the patient to kill cancer cells and tissues. It means that the immune system can be activated to attack the cancer cells but leaving the normal, healthy cells intact. Immunotherapy can be realised in several treatment approaches, such as the increase of the non-specific immune system, by cancer vaccines, adoptive cell transfer and monoclonal antibodies. Considering the heterogenicity and genetic instability of tumors, a certain kind of treatment may not achieve ideal therapeutic effect all the time. In recent years, using ultrasound to achieve immune response towards cancer shows great promise. Ultrasound is one of the most commonly used imaging diagnostic techniques for cancer diseases. Besides, ultrasound has attraction for both direct US treatment and activating drug delivery due to its biological effects. Several studies suggest that ultrasound can be used to boost the host anti-tumor immune responses. The recent advances, the potential and existing problems of US induced cancer immunotherapy are reviewed.

Focused ultrasound (FUS) can achieve a high US energy with in a small focal volume, which causes local destruction of tumor tissues with minimal damage to surrounding normal tissues. Destruction of tumors caused by FUS may lead to generate tumor debris and tumor-associated antigens (TAAs) *in situ*, which can facilitate and amplify the anti-tumor immune responses, and protect the body from tumorigenesis when re-challenged. HIFU also has effects on other than the destruction of the tissue. HIFU treatment, which triggers a Th1 type response, leads to significant changes in cell-mediated immunity. HIFU treatment also acts by balancing the cancer-induced immuno-suppression in tumor micro-environment.

Microbubbles (MBs), a kind of US contrast agents, can improve the therapeutic efficacy of FUS because of their intensity reflection and scattering of ultrasonic waves, which shows great value in clinical use. The addition of MBs to FUS can help generate more local destruction in the area of focus. Combination FUS with microbubbles has been found to increase the permeability of the blood-brain barrier (BBB) and provide a temporary and targeted opening of the BBB without inflicting brain damage or inflammation, thus is an attractive means to deliver immune cells and drugs.

Sonoporation is the use of sound (typically ultrasonic frequencies) for modifying the permeability of the cell plasma membrane. Sonoporation employs the acoustic cavitation of microbubbles to enhance delivery of large molecules. This technique is usually used in gene therapy and drug delivery. Another way to achieve anti-tumor immune responses is using US and MBs to deliver immune-stimulating substances to immune cells or tumor cells. Drug delivery that using a combination of MBs and US has been explored. Non-destructive US with or without MBs can increase the delivery of active substances including antigens and immune-stimulating genes. Combination of microbubbles with cell-targeting ligands and US provides an even more smart delivery system, therefore, the therapy is not only site specific but also cell specific. Overall, the use of US to achieve immune response towards cancer is showing promise. The field is fairly young and many mechanisms are still not fully understood, thus further researches are needed.

ultrasound, immunotherapy, tumor, microbubbles

doi: 10.1360/N972017-00769