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免疫原性细胞死亡及其在肝癌治疗应用中的研究进展

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摘　要

免疫原性细胞死亡(immunogenic cell death, ICD)在肝癌的治疗中有重要作用。机体发生ICD时，细胞分泌多种损伤相关分子模式(damage-associated molecular patterns, DAMPs)，包括三磷酸腺苷(adenosine triphosphate, ATP)、钙网蛋白(calreticulin, CRT)、高迁移率族蛋白B1 (high mobility group box protein B1, HMGB1)、热休克蛋白(heat shock protein, HSP)和干扰素(Interferon, IFN)等。DAMPs通过各种模式识别受体吸引自然杀伤细胞、巨噬细胞、树突状细胞等免疫细胞，并促进其成熟、活化从而增强抗肿瘤免疫反应。目前诱导ICD治疗肝癌主要通过药物方式(化疗药物、靶向药物等)、生物方式(溶瘤病毒)及物理方式(纳米脉冲刺激、光动力疗法等)。本文就ICD的相关分子及在肝癌治疗中的研究进展作一综述，以期在临床上为肝癌患者的治疗提供一些有益参考。

关键词

肝细胞癌，免疫原性细胞死亡，三磷酸腺苷，钙网蛋白，高迁移率族蛋白B1

Progress in Research on Immunogenic Cell Death and Its Application in Hepatocellular Carcinoma Therapy

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Abstract

Immunogenic cell death (ICD) has an important role in the treatment of hepatocellular carcinoma. When ICD occurs in the body, cells secrete various damage-associated molecular patterns (DAMPs), and the major DAMPs include adenosine triphosphate (ATP), calreticulin (CRT), high mobility group box protein B1 (HMGB1), heat shock protein (HSP), and interferon (IFN), etc. DAMPs attract natural killer cells, macrophages, dendritic cells and other immune cells through various pattern recognition receptors, and promote their maturation and activation to enhance anti-tumor immune responses. Currently, the induction of ICD in hepatocellular carcinoma treatment is mainly done by pharmacological (chemotherapeutic drugs, targeted drugs, etc.), biological (oncolytic viruses), and physical (nano-pulse stimulation, photodynamic therapy, etc.) modalities. In this article, we present a review of the important molecules related to ICD and the research progress of induced ICD in hepatocellular carcinoma treatment, in order to help design more effective and precise strategies to treat hepatocellular carcinoma in the future.

Keywords

Hepatocellular Carcinoma, Immunogenic Cell Death, Adenosine Triphosphate, Calreticulin, High Mobility Group Box Protein B1

1. 肝癌概述

原发性肝癌是最常见的恶性肿瘤之一，其发病率位居全球第六位，死亡率位居全球第三位[1]。原发性肝癌包括肝细胞癌(hepatocellular carcinoma, HCC)、肝内胆管癌(intrahepatic cholangiocarcinoma, ICC)和混合型肝细胞癌–胆管癌(combined hepatocellular-cholangiocarcinoma, cHCC-CCA)三种不同病理学类型。其中 HCC 最为多见，占原发性肝癌的 75%~85% [2]。诱导肝癌发生的危险因素有很多，包括乙型肝炎病毒或丙型肝炎病毒感染、过量饮酒、非酒精性脂肪性肝病[3]、糖尿病及代谢综合征、吸烟及空气污染[4] [5]等，在我国最主要的危险因素是乙肝病毒感染。目前肝癌的治疗措施包括手术切除、肝移植、射频消融、经动脉化疗栓塞和放射治疗等[6]，其中手术切除为主要治疗措施[7]。一项流行病学研究显示中国肝癌患者 5 年生存率仅在 10%~19%之间，手术后 5 年复发率高达 40%~60% [8] [9]。并且肝癌起病隐匿，大部分患者就诊时已处于中晚期[10]，丧失根治性手术切除机会。近年来随着药物研究与医疗技术的发展，靶向治疗与免疫治疗的出现为中晚期肝癌患者的诊治提供了新的思路[11]。

2. 免疫原性细胞死亡

2.1. 免疫原性细胞死亡概念

2005 年的一项研究[12]发现了一种与自噬、凋亡、焦亡不同的调节性细胞死亡(Regulated cell death, RCD)方式，并将其称为免疫原性细胞死亡(immunogenic cell death, ICD)。ICD 是肿瘤免疫治疗的重要机制之一，在机体发生细胞凋亡时，非免疫原性的细胞能够转化为免疫原性细胞并释放损伤相关分子模式(damage-associated molecular patterns, DAMPs)增强肿瘤免疫效应[13]。在这一过程中，不仅可以杀死由 ICD 诱导剂

诱导产生的细胞，还可以杀死垂死肿瘤细胞，并且这些肿瘤细胞会作为“肿瘤疫苗”引起残留肿瘤细胞的免疫反应。这种方式使得患者从化疗、物理等方式诱导引发的 ICD 过程中获取到长期的临床获益[14]。

2.2. 免疫原性细胞死亡相关分子

2.2.1. 钙网蛋白

内质网(endoplasmic reticulum, ER)中的钙网蛋白(calreticulin, CRT)是一种高度保守的钙结合蛋白，具有分子伴侣、Ca^{2+}稳态维持、基因表达调节等生物学功能[15] [16]，在 ER 内外均发挥作用[17]。在肿瘤细胞 ICD 反应过程中，真核细胞起始因子 2α (eukaryotic translation initiation factor 2α, eIF2α)受外界因素或特定药物的影响磷酸化，导致一系列信号传导反应[18]。包括 caspase-8 的激活、ER 蛋白水解、促凋亡蛋白 BAX 和 BAK 在线粒体外膜上积累、CRT 在 SNAP25 (突触体相关蛋白 25)介导下从内质网转运至高尔基体并通过胞质小泡在细胞表面上表达[19] [20]。细胞膜表面暴露的 CRT (etco-CRT)表达出“吃掉我”的信号作用，并与 CD91 结合后促进树突状细胞(dendritic cells, DCs)的成熟和活化，进而激发肿瘤特异性细胞毒性淋巴 T 细胞(cytotoxic T lymphocyte, CTL)的应答[21]、促炎因子 TNF-α 和 IL-6 的释放。ICD 期间 CRT 的移位和暴露可以触发强大的抗肿瘤免疫应答[22]，并且是 ICD 抗肿瘤免疫的重要标志。

2.2.2. 三磷酸腺苷

ATP，即三磷酸腺苷，作为细胞内部含量极高的代谢产物，在 ICD 过程中，肿瘤细胞通过自噬释放大量 ATP [23]，其中受损的细胞器、细胞质蛋白和其他成分被降解[24]，ATP 经由囊泡释放至胞外。释放至胞外的 ATP 具有“发现我”信号的作用，与 DCs 上的 P2X7 和 P2Y2 受体结合促进 DC 激活和成熟及巨噬细胞的扩增[25]。另外，ATP 同时激活半胱天冬酶 1 (caspase-1)依赖性 NLRP3 炎性小体，引发白介素-1β (interleukin-1β, IL-1β)和白介素-18 (IL-18)的分泌促进抗肿瘤免疫反应[26] [27]。

2.2.3. 高迁移率族蛋白 B1

高迁移率族蛋白 B1 (HMGB1)是一种广泛存在于哺乳动物细胞核中的非组蛋白染色质结合蛋白，具有基因转录调节、蛋白质转录调节和促进 V(D)J 重组等作用[28]-[30]。在细胞内和细胞外的 HMGB1 执行不同的功能。细胞内 HMGB1 与苄氯素 1 (Beclin-1)相互作用以介导自噬。细胞外的 HMGB1 在细胞凋亡的晚期阶段从细胞核中释放和累积，并能通过酶联免疫吸附实验被检测[31]-[33]。细胞外的 HMGB1 作为 DAMP 与模式识别受体(PRR)结合，包括 Toll 样受体 4 (Toll-like receptor 4, TLR4)及晚期糖基化终末产物受体(the receptor of advanced glycation end product, RAGE)从而激活单核细胞或巨噬细胞释放细胞因子增强 DC 的抗原呈递[34]。此外，HMGB2 与 Toll 样受体结合促进核因子-κB (nuclear factor kappa B, NF-κB)的活化[35]，导致促炎细胞因子与血管生成因子增加及肿瘤组织的破坏以进一步促进炎症反应[36]。

2.2.4. 热休克蛋白

热休克蛋白(heat shock protein, HSP)是一种高度保守的保护性蛋白，它的主要功能是协助蛋白质的折叠与成熟并帮助细胞维持正常的生理活性，除此之外它还具有一定的抗凋亡能力[37] [38]。HSP 通常在肿瘤细胞中过表达，其中主要参与肿瘤细胞 ICD 过程的 HSP 是 HSP70 和 HSP90 [39]，HSP70 是参与蛋白质折叠和转运的重要蛋白，它在肿瘤细胞的质膜和细胞外介导肿瘤免疫应答[40]。HSP 90 是一种肿瘤标志物，它参与肿瘤细胞的生长、侵袭、转移、血管生成及蛋白合成和凋亡[41]。在肿瘤细胞 ICD 过程中，HSP70 和 HSP90 与抗原结合形成复合物，不但刺激肿瘤抗原的摄取及 DC 细胞的成熟和活化[42]，还可诱导 CTLs 与 NK 细胞的激活以发挥关键的肿瘤杀伤作用[43]。

2.2.5. 干扰素

干扰素(Interferon, IFN)是具有多种作用的细胞因子，具有抗病毒感染、抗肿瘤活性和免疫调节的作

用。I 型干扰素包括 IFN-α 和 IFN-β 在内的多个亚型，在免疫应答过程中 IFN I 可通过与I型干扰素受体(type I interferon receptor, IFNAR)结合来诱导肿瘤细胞凋亡、抗血管生成和对免疫系统的细胞产生直接影响[44]。IFN-γ 是 II 型干扰素家族中的唯一成员，通过上调组织相容性复合体(major histocompatibility complex, MHC) I 和 MHC II 分子的表达促进效应 T 细胞(effector T cellI Teff)识别病原体来源的抗原、提高 NK 细胞和巨噬细胞的活性。此外，IFN-γ 的信号传导促进 DC 成熟及刺激分子的高表达。

3. 免疫原性细胞死亡在肝癌中的诱导

3.1. 药物诱导 ICD

3.1.1. 化疗药物

一些化疗药物如奥沙利铂、博来霉素、环磷酰胺、硼替佐米和蒽环类药物等已经被证实可以诱发 ICD，并形成持久的保护性免疫。铂类化疗药物(如顺铂或奥沙利铂)单独使用或与其他化疗药物(如吉西他滨)联合使用已被批准用于临床治疗或在临床试验中被评估。在日本和韩国，HAIC (肝动脉灌注化疗)-FOLFOX (氟尿嘧啶、亚叶酸和奥沙利铂)方案在晚期肝癌的转化降期方面表现出了较好的结果[45]。一项研究发现，奥沙利铂(而非顺铂)在人和小鼠的肝癌细胞中通过促进 CRT 暴露、HMGB1 和 ATP 分泌及 DC 成熟来诱导并增强 ICD。除此之外，奥沙利铂与 PD-1 联用有效抑制了肿瘤生长且获得比单药治疗更好的结局[46]。氟尿嘧啶(Fu)尽管已经被证明可以在体内诱导 ICD，但是由于耐药性和低选择性导致不可避免的毒副作用及不理想的免疫应答。近年来出现如石墨烯、共价有机框架、硒纳米颗粒和金纳米颗粒等载体通过负载 Fu 以提高其靶向性及免疫原性[47]。如何更精确的输送化疗药物至肿瘤处以触发肿瘤细胞发生 ICD 亟待更多研究。

3.1.2. 靶向药物

目前用于肝癌治疗的靶向药物有索拉非尼、仑伐替尼、贝伐珠单抗联合阿替利珠单抗和多纳非尼等[48]。其中索拉非尼是美国食品药物管理局批准的第一个血管内皮生长因子(VEGF)通路抑制剂，最初被开发用于治疗肾细胞癌，也在那时被批准用于肝癌[49]。索拉非尼可诱导活性氧(ROS)的过度产生，引发氧化损伤和铁凋亡导致 ICD 的产生[50]。仑伐替尼是一种酪氨酸酶抑制剂，能够抑制多种酪氨酸激酶。一项研究显示，接受仑伐替尼治疗的病人肝癌细胞中 DAMPs 显著增加(包括 CRT 释放、ATP 释放和 HMGB1 释放增加)、DAMPs 下游受体(TLR3 和 TLR4)表达上调和 PD-L1 表达上调[51]。

3.1.3. 纳米药物

纳米颗粒包封可通过精确控制药物剂量以降低药物毒性，并且一些研究发现纳米材料可放大癌细胞死亡的免疫原性[52]，纳米颗粒搭载化疗药物为增强抗癌疗效和减轻副作用提供了有希望的前景。Yin [53]等人构建了靶向肝癌干细胞(LCSCs)的生物响应性 Au-miR-183 抑制剂(Au@miR-183)给药系统。Au@miR-183 首次实现向 LCSCs 的特异性靶向递送，并在微环境中消耗 NADPH 和 H_2O_2 导致氧化还原稳态紊乱，调节 LCSCs 生态诱导 LCSC 的干细胞退化。同时 Au@miR-183 提高了肿瘤部位的免疫原性，增加了 ATP 分泌和 HMGB1 的释放、促进 DC 等抗原呈递细胞成熟、诱导 CD8 + T 细胞的浸润从而激活体内抗肿瘤免疫。Zhou [54]等人设计了一种普鲁士蓝(PB)纳米颗粒(NP)，它将负载索拉非尼的多孔金属有机框架与肝癌特异靶向肽 SP94 和近红外染料花青(Cy)5.5 缀合，实现了索拉非尼的靶向递送和控制释放，减少了索拉非尼的副作用；同时缓解了肝癌缺氧，产生免疫促进的肿瘤微环境。Chen [55]等人发现，与游离多柔比星相比，将多柔比星加载在 H-铁蛋白纳米笼(HFn)中表现出了更优秀的靶向肿瘤递送和肿瘤内渗透能力。HFn 负载多柔比星不但提高了多柔比星的治疗效果，而且显著降低了体内毒性作用，并且使肿瘤细胞对免疫活化敏感，与抗 PD-1 抗体疗法组合进一步增强肝癌的治疗效果。一项研究发现将奥沙利铂(OxP, OX)衍生物和亚叶酸(FnA, FOL)的纳米制剂与含氟尿嘧啶(5-FU, F)的纳米制剂组合使用相比任一单

一纳米制剂增强了 ATP 与 HMGB1 的分泌与释放，使肿瘤微环境由“冷环境”转变为“热环境”。通过联合使用两种纳米制剂，CD8 + T 细胞、CD4 + T 细胞和 DC 细胞在肿瘤中被显著激活，并伴随 IFN-γ、TNF-α 和 IL-12 的上调。此外，这两种纳米制剂与抗 PD-L1 单克隆抗体联用时，与单用两种纳米制剂或单用抗 PD-L1 单克隆抗体相比更显著地抑制肝转移[56]。目前纳米药物的临床数据较少，未来还需更多临床试验验证其对肝癌的治疗效果。

3.2. 溶瘤病毒(Oncolytic Virus, OVs)疗法诱导 ICD

OVs 与传统的抗肿瘤药物相比具有许多优势，因为具有感染和杀死癌细胞而不损害健康组织的生物学特性。OV 介导的溶瘤作用不仅导致肿瘤消退，还向树突状细胞(DC)和其他抗原呈递细胞(APC)提供的关键信号以引发额外的强效抗肿瘤免疫应答[57]。并且 OVs 介导的癌细胞溶瘤过程中还观察到 ICD 的典型特征，如钙网蛋白表面暴露、HMGB1 和 ATP 释放以及内质网应激[58]。据报道，构建有肿瘤诱导和免疫刺激分子的腺病毒在肝癌细胞系中导致肝癌细胞死亡增加及 IFN-γ 表达上调[59]。一项研究表明一种来源于牛痘病毒的溶瘤病毒 Pexa-vec (pexastimogene devacirepvec)改善了不可切除肝癌患者的总生存期，并且患者对这一溶瘤病毒耐受性良好[60]，但是在索拉非尼治疗失败的患者中则没有达到预期的治疗效果[61]。

3.3. 物理疗法诱导 ICD

3.3.1. 纳米脉冲刺激(Nano-Pulse Stimulation, NPS)

NPS，也可以称为纳秒脉冲电场(Nanosecond pulsed electric field, nsPEF)是一种作用于细胞，导致细胞中的膜纳米孔的形成和离子通道的激活、细胞质 Ca^{2+}浓度增加、内质网应激和 ROS 释放增加的 ICD 诱导方式[62]，在特定能量下的 NPS 治疗能够触发三种关键 DAMP 的发射，其水平与阿霉素(DOX)等药物治疗相当。并且多项肝癌小鼠实验表明，NPS 改变了 TME 中的免疫细胞组成导致 TME 中的巨噬细胞增加，从而有效地消除肿瘤延长小鼠的生存时间[63] [64]。此外，Chen [65]等人发现 T 细胞和 NK 细胞的浸润指标颗粒酶-B (granzyme B)在 NPS 后的 TME 中累积，提示 NPS 可能引起 TME 中的 T 细胞和 NK 细胞增加来增强抗肿瘤作用。

3.3.2. 近红外光免疫疗法(Near-Infrared Photo-Immunotherapy, NIR-PIT)

NIR-PIT 是一种用于肿瘤的分子靶向光疗，首先向肿瘤细胞注射一种靶向肿瘤细胞表面表达抗原的单克隆抗体(mAb)与光活化二氧化硅酞菁九光敏剂 IRDye700DX (IR700)共轭物，随后将肿瘤细胞局部暴露于 NIR 光。当肿瘤细胞暴露于 NIR 光时，就会触发一系列选择性细胞毒性作用诱导肿瘤细胞的快速和高度选择性 ICD [66]。Ogawa [67]等人发现 NIR-PIT 会诱导细胞体积快速增大与破裂、CRT 和 HSP 的质膜表达增加、HMGB 1 和 ATP 的快速分泌及 DC 成熟。Hanaoka [68]等人报道了一例在肝癌动物模型中抗磷脂酰肌醇蛋白聚糖-3 的 mAb (YP7)与 IR700 共轭的抗体－光敏剂偶联物进行的 NIR-PIT。在这项研究中，IR700-YP7 在肿瘤中积累，与未处理的对照组相比显著抑制肿瘤生长并在暴露于 NIR 后快速诱导靶向细胞死亡。

3.3.3. 氧增强光动力疗法(Photodynamic Therapy, PDT)

PDT 通过向机体注射光敏剂后用特定波长的光来照射肿瘤部位使 ROS 水平增加来触发肿瘤细胞凋亡[69]。缺氧在实体瘤中会促进肿瘤细胞转移，而缺氧也会极大降低 PDT 的疗效[70]，为了解决缺氧对 PDT 的限制作用，Hou [71]等人开发了锰氧化物交联的牛白蛋白/透明质酸纳米粒子(BHM)，实现阿霉素(DOX)和吲哚菁绿(ICG)的连续负载，获得 DOX/ICG 共载的 BHM 纳米平台(简称 BHMDI)。BHMDI 可通过催化细胞内 H_2O_2 分解为 O_2 来有效缓解肿瘤缺氧，显著提高 PDT 疗效。BHMDI 纳米平台还降低了肿瘤中 M2 巨噬细胞的比例、促进了 DC 的成熟、CD8 + T 细胞的浸润。此外，BHMDI 纳米平台与 PD-1 联合使用不仅根除了原发性肿瘤，而且通过触发强大的全身抗肿瘤免疫抑制了肝癌的肿瘤复发、远端肿瘤

生长和肺转移。Xu [72]等人制备了一种靶向和 ROS 敏感的共负载聚集诱导发射(AIE)光敏剂(TB)和紫杉醇(PTX)的光触发纳米(TB/PTX@RTK)胶束。光照射下，TB/PTX@RTK 胶束可以在局部区域产生高浓度的 ROS 以促进 PTX 的释放，其与 TB 介导的 PDT 有效协同以产生很强的肿瘤杀伤和抗肿瘤免疫激活作用。TB/PTX@ RTK 介导的化学 PDT 不改变肝癌细胞的总 CRT 含量，但比单独的 PDT 显著增加细胞膜表面上 CRT 的表达。与单一治疗模式相比，TB/PTX@RTK 介导的化学 PDT 能更强的诱导 ICD。同时 TB/PTX@RTK 胶束可显著上调肿瘤细胞 PD-L1 的表达，与抗 PD-L1 抗体有效协同诱导远端效应，建立长期免疫记忆，抑制肿瘤的复发和转移。

4. 总结与展望

肝癌因为其起病隐匿，我国早期肝癌发现率不足 30%，70%~80%的肝癌在发现时已到中晚期，治疗选择十分有限，5 年生存率不足 20%。手术、放疗、化疗等的联合使用在一定程度上延长了患者的生存期，但肝癌治疗目前在医学上仍是一大难题。ICD 作为一种增强肿瘤免疫效应的细胞死亡形式，在抗肿瘤治疗方面展现出优异的前景。但目前绝大多数为基础实验与动物实验，ICD 相关的肝癌临床研究仍旧较少，如何在肝癌患者中诱导 ICD 以提高其生存率和治愈率在未来需要更多的探索与研究。

本文就 ICD 的概念、分子机制及在肝癌中诱导方式做一综述以期在临床上为肝癌患者的治疗提供一些有益参考。

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