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肝癌微环境重塑与免疫治疗策略*

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摘要　肝细胞癌是消化系统常见的恶性肿瘤之一，局部免疫微环境与肿瘤疾病的发生发展及治疗效果密切相关。肿瘤细胞可诱导免疫细胞表型和功能改变塑造免疫抑制微环境，减弱免疫系统杀伤功能。同时，免疫细胞代谢调节对机体抗肿瘤免疫应答至关重要。肝癌微环境中的代谢产物及肠道菌群代谢物失衡可通过调控免疫细胞表型和功能重塑影响肝癌进展。目前，肝癌细胞与微环境免疫细胞之间相互作用导致肿瘤免疫逃逸已成为当前研究的热点。本文总结了肝癌免疫抑制微环境的构成及其代谢调控，并探讨了靶向微环境重塑治疗肝癌的潜在策略，为肿瘤免疫治疗提供新思路。

关键词　肝细胞癌，免疫微环境，免疫逃逸，免疫代谢，免疫治疗
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肝癌是一种多因素诱导、多基因参与、机制复杂的消化系统恶性肿瘤，其发病率位于中国癌症的第4位，死亡率高居第2位，其中肝细胞癌（hepatocellular carcinoma，HCC）占原发性肝癌的85%以上[1]。近年来免疫治疗获得令人瞩目的进展，并在晚期肝癌患者中取得了较好的治疗效果，然而仅有不到30%的肝癌患者生存获益[2]。免疫炎症微环境驱动的肿瘤发生发展及治疗抵抗是当前肝癌防治领域所面临的重大挑战之一，因此，深入理解肿瘤微环境免疫调控机制，发现和阐述微环境中免疫细胞特性、功能重塑及其对肿瘤进展的机制具有重要的科学意义。

肿瘤免疫微环境（tumor immune microenvironment，TIME）由多种细胞与非细胞成分组成包括髓源性抑制细胞（myeloid-derived suppressor cell，MDSC）、肿瘤相关巨噬细胞（tumor-associated macrophage，TAM）、肿瘤相关中性粒细胞（tumor-associated neutrophils，TAN）、调节性T细胞（regulatory T cell，Treg）、固有淋巴样细胞（innate lymphoid cell，ILC）以及生长因子、蛋白水解酶和细胞外基质蛋白等，与患者预后直接相关。肝脏作为一个天然免疫优势器官，长期受到肝门静脉所携带的细菌成分、食源性抗原及大量外来的抗原性物质影响，肝脏微环境表现出一定的免疫抑制性，以抵制非致病性肠道环境引起的过度炎症[3]。肿瘤细胞可通过代谢重编程和分泌抑制性细胞因子等方式诱导肿瘤浸润免疫细胞表型和功能转变，最终塑造抑制性TIME，导致肿瘤免疫逃逸和治疗疗效不佳。本文将从肝癌微环境免疫重塑和微环境中代谢产物变化入手，探讨靶向肝癌免疫抑制微环境增强免疫治疗的策略。

1　癌症免疫周期（cancer-immunity cycle，CIC）

抗肿瘤免疫分为七个连续的过程，统称为“癌症免疫周期”，主要包括：癌细胞释放抗原、树突状细胞呈递抗原、激活并扩增特异性T细胞、活化的T细胞定位并进入肿瘤组织、活化的T细胞浸润肿瘤、T细胞识别和释放细胞毒素，最终发挥杀伤

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癌细胞的作用[4]。癌症免疫周期中任何一个环节缺陷均会导致肿瘤逃避免疫监视，因此，充分理解癌症免疫周期各个过程的发生机制及相互联系可更为有效地指导癌症免疫治疗[5]。

肿瘤微环境中特异性T细胞的浸润和激活对于抗肿瘤免疫至关重要，其中，树突状细胞（dendritic cells，DCs）在抗肿瘤T细胞免疫的启动和维持中起着核心作用。肿瘤浸润DC可提呈大量抗原肽传递至T细胞，并通过分泌细胞因子或表达共刺激分子激活和增强T细胞抗肿瘤免疫应答，进一步促使引流淋巴结（draining lymph node，dLNs）衍生的T细胞活化、增殖并进入肿瘤组织，进而识别和杀伤肿瘤细胞[6]。然而，肿瘤微环境代谢紊乱、长期抗原暴露等因素可导致T细胞功能障碍，呈现免疫耗竭状态，称为耗竭型T细胞（exhaustion T cell，Tex）。Tex细胞的主要特征包括：效应功能丧失，细胞因子（如IFN-γ、TNF-α、IL-2）分泌减少；细胞增殖能力降低，难以有效扩增；细胞程序性死亡受体1（programmed death-1，PD-1）、细胞毒性T淋巴细胞相关抗原4（cytotoxic T lymphocyte-associated antigen-4，CTLA-4）、淋巴细胞活化基因3（lymphocyte-activation gene 3，LAG-3）、T细胞免疫球蛋白黏液素3（T cell immunoglobulin and mucin domain-containing protein 3，TIM-3）等免疫检查点分子表达增加，无法有效杀伤肿瘤细胞，导致癌症免疫周期缺陷，造成肿瘤免疫逃逸[7-9]。

除T细胞外，在癌症免疫周期中，巨噬细胞、中性粒细胞、固有淋巴样细胞等免疫细胞也发挥了关键作用，根据这些细胞的空间分布和功能状态，可将肿瘤微环境分为三种基本的免疫表型：免疫炎症型、免疫排斥型和免疫荒漠型。免疫炎症型肿瘤微环境有较多的T细胞、M1样TAM等具有抗肿瘤功能的细胞浸润，对于免疫检查点抑制剂敏感；免疫排斥型肿瘤微环境中T细胞仅浸润于肿瘤基质而非肿瘤实质；免疫荒漠型肿瘤微环境中含有大量Treg、TAN等免疫抑制细胞，对免疫检查点抑制剂响应不佳（图1）[10-12]。免疫表型对于个体化抗肿瘤免疫治疗决策，监测治疗疗效和疾病发展，以及探究免疫检查点抑制剂耐药机制具有重要的指导意义。

近年来，免疫治疗已成为肿瘤治疗领域的焦点，多种类型的免疫治疗药物和联合治疗方案相继涌现，包括肿瘤疫苗、细胞免疫治疗、免疫检查点抑制剂等，均涉及癌症免疫周期的重新激活，尤其是促进肿瘤微环境中$CD8^+$ T细胞的浸润和效应功能。然而，免疫治疗获益群体仍十分有限且易继发耐药。因此，深度探究肿瘤微环境中各成分表型和功能变化，阐明肿瘤细胞与免疫系统之间的相互作用，可更有效地指导癌症免疫治疗改善现有的肿瘤治疗方案。

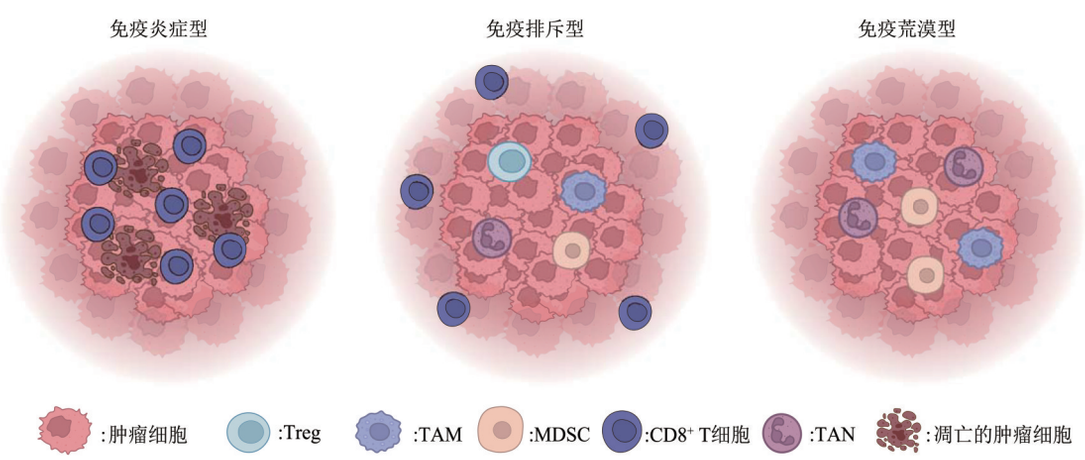

Fig. 1　Immunotypes of the tumor

图1　肿瘤免疫表型

免疫炎症型肿瘤：淋巴细胞浸润丰富，对免疫检查点抑制剂响应良好；免疫排斥型肿瘤：淋巴细胞被细胞外基质阻隔，肿瘤实质中几乎没有淋巴细胞；免疫荒漠型肿瘤：缺乏免疫细胞浸润，对免疫检查点抑制剂响应较差。Treg：调节性T细胞；TAM：肿瘤相关巨噬细胞；MDSC：髓源性抑制细胞；TAN：肿瘤相关中性粒细胞。

2　微环境的组成成分对肝癌TIME的影响

理想状态下，癌症免疫周期能够有效发挥作用，从而消除肿瘤细胞。然而，TIME内存在抑制性免疫细胞和间质、耗竭型T细胞和代谢紊乱，导致癌症免疫周期缺陷和肿瘤进展。肝癌微环境受遗传、病毒、炎症及环境等多重因素的调控呈现高度异质性，其免疫细胞组成及功能的改变，可严重影响肝癌免疫周期及患者对治疗的应答[13]。基于此，阐明肝癌微环境中的组成成分及涉及的复杂信号通路网络系统，将有望为肝癌的治疗提供潜在治疗靶点和有效的治疗手段（图2）。

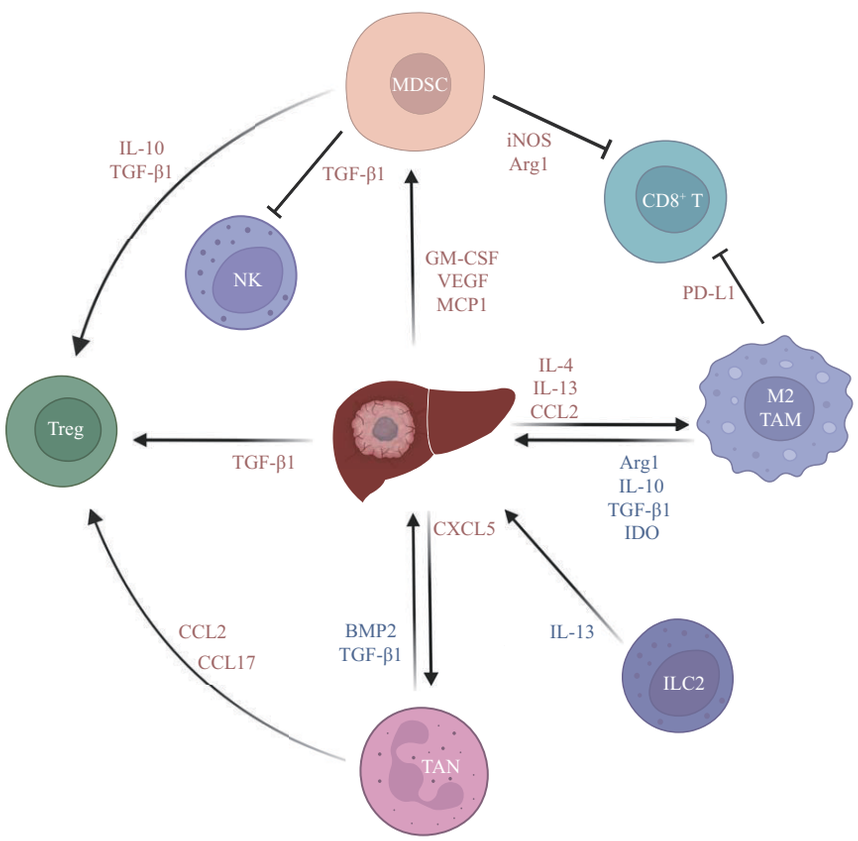

Fig. 2　Hepatocellular carcinoma cells interact with immune cells to promote remodeling of the immune microenvironment

图2　肝癌细胞与免疫细胞相互作用促进免疫微环境重塑

肿瘤细胞分泌CCL2、GM-CSF和TGF-β1细胞因子招募免疫抑制性细胞，诱导免疫细胞向促癌表型转化。免疫抑制性细胞分泌TGF-β1和IL-10细胞因子抑制效应细胞功能，促进肿瘤生长。NK：自然杀伤细胞；GM-CSF：粒细胞-巨噬细胞集落刺激因子；VEGF：血管内皮生长因子；MCP-1：单核细胞趋化蛋白-1；TGF-β1：转化生长因子β1；iNOS：诱导型一氧化氮合酶；Arg1：精氨酸酶1；IDO：吲哚胺2,3-双加氧酶；IL-4：白介素-4；IL-10：白介素-10；IL-13：白介素-13；CCL2：趋化因子配体2；CCL17：趋化因子配体17；BMP2：骨形态发生蛋白2；CXCL5：趋化因子配体5。

2.1　髓源性抑制细胞（MDSC）

MDSC是一群未成熟、具有免疫抑制功能的髓系细胞，在肝癌微环境重塑中起重要作用[14]。肝癌局部微环境缺氧导致缺氧诱导因子1α（hypoxia inducible factor-1α，HIF-1α）增加，进而上调肝癌细胞中ENTPD2蛋白表达，导致胞外5'-AMP的浓度增加，促进TIME中MDSC募集；同时，肝细胞铁死亡可释放大量高迁移率族蛋白B1（high mobility group box-1 protein，HMGB1），触发NF-κB信号通路趋化MDSC浸润诱导免疫抑制[15-16]。而在小鼠原位肝癌模型中，肿瘤相关细胞因子（IL-6、IL-1β、GM-CSF、G-CSF、VEGF、MCP-1和MIF）可直接促进MDSC的募集与扩增[17-19]。肝癌微环境内积累的MDSC通过营养剥夺、细胞因子分泌、细胞间直接接触等方式发挥免疫抑制作用[20]。MDSC中诱导型一氧化氮合酶（inducible nitric oxide synthase，iNOS）与精氨酸酶1（arginase 1，Arg1）的表达显著上调，可直接抑制

T细胞的抗肿瘤功能[21]。另外，MDSC表面的程序性死亡受体配体1（programmed death-ligand 1，PD-L1）与T细胞的PD-1结合，也能抑制T细胞抗肿瘤应答[22]。在小鼠肝癌模型中，MDSC分泌的TGF-β1显著降低NK细胞NKG2D的表达和IFN-γ的分泌，而中和TGF-β1则能恢复NK细胞的效应功能[23]。此外，MDSC分泌的免疫抑制性细胞因子IL-10和TGF-β1还可促进Treg扩增，进一步抑制NK细胞和$CD8^+$ T细胞效应功能[24]。综上所述，MDSC在肝癌微环境中起着重要的免疫抑制作用，针对MDSC的靶向治疗将有助于提高肝癌免疫治疗疗效。

2.2 肿瘤相关巨噬细胞（TAM）

作为肝癌微环境中的重要细胞成分，TAM具有高度异质性，兼具促肿瘤和抗肿瘤的双重功能[8]。一方面，M1样TAM主要执行免疫监视、抗原提呈等抗肿瘤免疫功能；另一方面，M2样TAM可塑造免疫抑制性微环境，这是导致肝癌复发转移、治疗抵抗的重要原因之一[25]。其中，M2样TAM通过分泌Arg1、IL-10、TGF-β1、IDO、VEGF和IL-6等细胞因子抑制肿瘤免疫应答、促进肿瘤微血管生成、增强肿瘤细胞的增殖及侵袭能力[26-27]。值得一提的是，TAM表面抑制分子部分来源于肝癌细胞分泌的外泌体且受高尔基体膜蛋白1调控[28]。而TAM分泌的CCL5、CCL20和CCL22等趋化因子在Treg的募集中也起到关键作用[29]。此外，细胞间直接接触是TAM抑制T细胞的重要途径之一，TAM不仅通过细胞表面HLA-A、HLA-E与T细胞表面ILT2、CD94结合抑制T细胞功能，还通过PD-L1、B7与T细胞表面PD-1、CTLA-4结合进一步发挥免疫抑制作用[30]。

TAM的表型转换受到肿瘤微环境调控，可通过多种方式诱导TAM的M2样极化。首先，肝癌细胞来源的TGF-β1、IL-4、IL-13、CSF-1、CCL2、CXCL12和CTGF等细胞因子可直接促进M2样TAM极化[30-32]。在肝癌中，肿瘤细胞来源的Wnt配体可激活TAM的Wnt/β-catenin信号诱导其向M2样极化，进一步促进肿瘤的生长、侵袭、转移和免疫逃逸[33]。此外，肝癌源性外泌体可包裹lncRNA miR4458HG并作用于TAM促进M2样极化[34]。近期研究表明，锌、铁等微量元素在TAM的表型转变中也发挥着重要作用[35-36]，肝癌细胞可与巨噬细胞竞争性摄取亚铁离子，导致肝癌组织中TAM内转铁蛋白表达下调，HIF-1α上调促进M2样极化，同时，动物实验表明铁缺乏会诱导小鼠巨噬细胞M2极化促进肿瘤进展[36]。

2.3 肿瘤相关中性粒细胞（TAN）

中性粒细胞是慢性炎症的重要介质，在慢性肝病和肝癌的免疫浸润中占比相对较高，目前中性粒细胞在癌症中的病理作用逐渐被深入研究。干细胞样癌细胞是肝癌进展和治疗抵抗的关键驱动因素，相关文献报道TAN通过分泌骨形态发生蛋白2（bone morphogenetic protein 2，BMP2）和炎症因子TGF-β1诱导肝癌细胞表达miR-301-3p，促进肝癌干细胞样细胞特征从而增强肝癌细胞的增殖、侵袭等能力[37]。与此同时，上述肝癌细胞可上调趋化因子CXCL5的表达，增加肿瘤组织中TAN的浸润，导致炎症微环境形成，促进肝癌转移复发[37-38]。TAN可通过分泌趋化因子CCL2、CCL17募集TAM与Treg浸润促进肝癌进程[39]。

最新研究发现，肝癌患者中存在与抗PD-1治疗耐药相关的$CD10^+ALPL^+$中性粒亚群，肿瘤细胞通过NAMPT-NTRK信号通路对该中性粒细胞进行重编程，维持其未成熟状态，进而导致T细胞终末耗竭，造成患者治疗耐药，这一研究揭示了中性粒细胞诱发的抗PD-1治疗耐药机制，为新的免疫治疗靶点和联合治疗方案提供了新的参考[40]。此外，CT10激酶样调节基因（regulator of kinase like protein，CRKL）在PD-1单抗耐药的肝癌患者组织中表达上调，CRKL通过招募TAN重塑TIME导致PD-1单抗耐药[41]，以上发现表明使用小分子药物和免疫疗法靶向干预中性粒细胞可作为肝癌治疗的未来研究方向。

2.4 调节性T细胞（Treg）

Tregs是一类免疫抑制性$CD4^+$ T细胞，在维持体内免疫耐受中起到重要作用。研究报道肝癌患者中Tregs可通过多种机制触发免疫反应，早期研究揭示，在肝癌组织和外周血中$CD4^+CD25^+FoxP3^+$Tregs数量显著增多，该典型的细胞亚群可通过抑制颗粒酶、穿孔素的产生和释放，从而削弱$CD8^+$ T细胞的杀伤功能，且循环Tregs通过CCR6-CCL20轴向肿瘤微环境迁移，促进肿瘤免疫逃逸，这与不良临床结局正相关[42-43]。双调蛋白（amphiregulin，AREG）可激活$CD4^+FoxP3^+$Tregs，经慢病毒或AREG中和抗体阻断其蛋白质表达及功能进而抑制Tregs激活，机制研究进一步揭示AREG可激活Tregs中雷帕霉素靶蛋白复合物1（mechanistic target of rapamycin complex 1，mTORC1）信号通

路，经雷帕霉素（mTORC1抑制剂）治疗可导致Tregs功能受损以增强$CD8^+$ T细胞的抗肿瘤功能；此外，吉非替尼（gefitinib）通过阻断Tregs中AREG/EGFR信号通路增强抗肿瘤免疫可抑制小鼠异种移植肿瘤模型中的肿瘤大小[44]。最近研究发现，糖酵解过程中的重要代谢酶果糖-1,6-二磷酸醛缩酶B（fructose-1，6-bisphosphate aldolase B，ALDOB）在肝癌组织中低表达，而低水平的ALDOB可促进*TGF-β1*基因启动子区域的H3K9乙酰化，继而导致促肿瘤因子TGF-β1的表达上调，增加Tregs的数量，减少$CD8^+$ T细胞浸润并呈现免疫耗竭状态[45]。最新研究表明，缺氧条件下肿瘤血管内皮细胞特异性高表达二酰基甘油激酶γ（diacylglycerol kinase gamma，DGKG）以募集泛素特异性肽酶，促进锌指E-box结合同源盒使其去泛素化并正反馈激活TGF-β1，最终导致肿瘤血管生成、Treg分化进而加速肝癌进展[46]。

2.5　固有淋巴样细胞（ILC）

ILC是固有免疫中重要的淋巴细胞群，包括NK、ILC1、ILC2、ILC3和ILCreg 5个亚群[47]。在肝脏中，固有淋巴细胞占淋巴细胞数量的2/3以上，在维持免疫稳态方面发挥关键作用[48]。ILC具有高度可塑性，兼具抑癌与促癌功能。NK细胞通过分泌IFN-γ、TNF-α、颗粒酶和穿孔素发挥抗肿瘤功能。ILC1功能与NK细胞类似，但细胞毒性较弱[49-50]。研究发现，TGF-β是驱动NK向ILC1转化的关键细胞因子，肝癌微环境中高浓度的TGF-β诱导NK细胞向ILC1转变以降低其细胞毒性，从而削弱机体的免疫监视，促进肝癌的发展与免疫逃逸[50-51]。ILC2可表达多种细胞因子与趋化因子，对肿瘤产生不同程度的影响[52]。其中，$ICOS^+$ILC2与肝癌患者预后负相关[53-54]，其通过分泌IL-13作用于MDSC形成抑制性TIME进而促进肝癌进展[53]。此外，在肿瘤组织中ILC2可通过促进中性粒细胞增殖，抑制$CD8^+$ T细胞的增殖和杀伤作用来诱导抑制性TIME的形成[54]。

3　代谢紊乱对肝癌TIME的影响

细胞代谢与细胞增殖、存活及分化密切相关，微环境代谢紊乱是阻止癌症免疫周期的最重要因素之一。为实现快速增殖和分化，肿瘤细胞通过改变其代谢模式，即“代谢重编程”，与抗肿瘤免疫细胞竞争葡萄糖、脂质和核苷酸等关键营养物质。同时，代谢物异常消耗导致局部缺氧、pH值降低和代谢废物的积累，从而抑制免疫细胞的增殖和效应功能，最终促进免疫逃逸和肿瘤的进展（图3）。

3.1　肝癌细胞代谢产物对肝癌TIME的影响

3.1.1　糖代谢重编程重塑肝癌TIME

即使在氧气充足的环境中，肿瘤细胞仍通过大量消耗葡萄糖进行糖酵解供能，这种现象称为“Warburg效应”[55-57]。由于糖酵解供能效率远低于氧化磷酸化，肝癌细胞为获取足量的能量和物质以实现快速增殖，其葡萄糖摄取和利用能力显著增强[56]。研究表明，肝癌细胞中葡萄糖转运蛋白（glucose transporters，GLUTs）表达显著上调，并与患者预后负相关[58-59]；糖酵解关键限速酶己糖激酶2（hexokinase 2，HK2）表达上调，干扰HK2蛋白水平可有效抑制肝癌细胞增殖[60-63]。乳酸作为糖酵解的主要产物，是诱导肿瘤免疫逃逸的重要因子，在肝癌TIME重塑中扮演重要角色。乳酸可抑制T细胞和NK细胞中活化T细胞核因子（nuclear factor of activated T cells，NFAT）表达，导致其效应功能降低及IFN-γ分泌减少[64-65]。此外，乳酸可通过调控组蛋白乳酰化修饰促进巨噬细胞M2样极化、诱导$CD8^+$ T细胞免疫耗竭，促进肿瘤免疫逃逸[66-67]。研究报道，肝癌细胞MCT4表达上调可促进乳酸输出和TIME酸化，诱导免疫抑制微环境[68]，而NAD^+依赖性脱乙酰酶（sirtuin-3，SIRT3）可抑制细胞周期蛋白乳酸化修饰，阻止肝癌进展[69]。因此，靶向干预糖酵解和乳酸代谢关键信号分子，可为肝癌治疗提供潜在的策略。

3.1.2　异常脂质代谢重编程重塑肝癌TIME

除了葡萄糖代谢和乳酸代谢外，脂代谢重编程也在肝癌进程中发挥重要作用[68，70]。非酒精性脂肪性肝中亚油酸堆积，促进线粒体活性氧类（ROS）产生，导致$CD4^+$ T细胞功能丧失[71]。花生四烯酸生物活性代谢产物前列腺素E2（prostaglandin E2，PGE2）与肿瘤免疫逃逸密切相关，肝癌细胞中PGE2合成的限速酶COX2表达上调可抑制cDC1功能、诱导巨噬细胞M2样极化并抑制$CD8^+$ T细胞功能，塑造抑制性肝癌免疫微环境[72-75]。最新研究报道，人肝癌组织存在大量富含脂滴的巨噬细胞浸润并与肝癌疾病进展正相关。进一步研究发现，肝癌细胞条件培养基可促进巨噬细胞摄取甘油三酯和脂滴形成进而诱导巨噬细胞CCL20分泌增加，招募$CCR6^+$ Treg形成免疫抑制微环境；靶向阻断甘油三酯合成关键酶可显著抑制肝癌进展[76]。

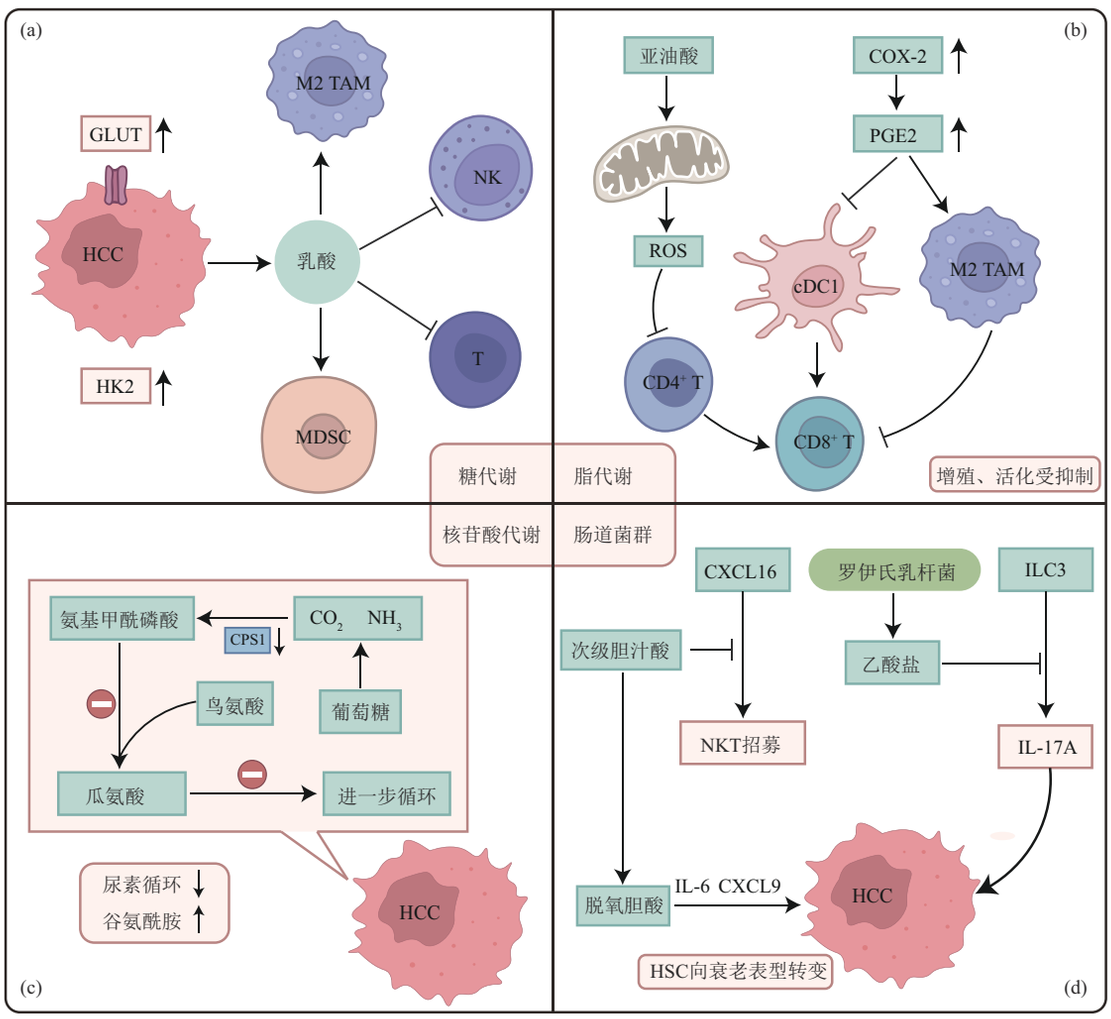

Fig. 3　Metabolic reprogramming in the microenvironment of hepatocellular carcinoma

图3　肝癌微环境中的代谢重编程

（a）肝癌细胞糖酵解水平增高，代谢产物乳酸增多促进免疫抑制性细胞富集、抑制效应T细胞和NK细胞功能；（b）异常脂代谢产物可促进ROS和PGE2生成，抑制T细胞增殖活化；（c）肝癌细胞核苷酸代谢上调，促进肿瘤细胞增殖；（d）肠道菌群衍生的代谢产物调控HSC细胞表型和ILC细胞功能促进肿瘤生长。HCC：肝细胞癌；GLUT：葡萄糖转运蛋白；HK2：己糖激酶2；ROS：活性氧类；COX-2：环氧合酶2；PGE2：前列腺素E2；CPS1：氨基甲酰磷酸合成酶1；CXCL16：趋化因子配体16；CXCL9：趋化因子配体9；IL-17A：白介素-17A；IL-6：白介素-6；HSC：肝星状细胞。

3.1.3　核苷酸代谢重编程重塑肝癌TIME

为适应快速的细胞分裂肝癌细胞中核苷酸代谢显著上调，这与肝癌患者总生存期负相关[77-79]。研究报道，核苷酸代谢异常不仅会加速肿瘤的进展，还可改变肿瘤微环境的免疫反应。肝癌组织中垂体肿瘤转化基因（pituitary tumor transforming gene，PTTG1）表达异常升高，其通过与*ASNS*启动子区域结合上调*ASNS*的表达水平，进一步促进核苷酸合成所需原料天冬氨酸代谢进而激活mTOR信号通路，导致肝癌细胞增殖及肝癌进展[80]，而剥夺天冬氨酸可促进$CD8^+$ T细胞增殖和IFN-γ分泌，重塑$CD8^+$效应T细胞的识别和杀伤功能，发挥抗肿瘤免疫功能[81]。谷氨酰胺代谢也是癌细胞代谢的关键因素之一，其中尿素循环是其代谢的关键途径。在肝癌细胞中，尿素循环的限速酶氨基甲酰磷酸合成酶1（carbamoyl phosphate synthetase 1，CPS1）基因启动子区域高度甲基化，导致CPS1表达显著减少致使谷氨酰胺堆积，而恢复CPS1表达水平可重启尿素循环来抑制核苷酸代谢进而阻止肝癌进程[82]。此外，谷氨酰胺代谢可激活免疫细胞并调节T细胞向炎症亚型转变以促进癌细胞生长，而阻断谷氨酰胺代谢可显著增加$CD8^+$ T细胞的浸润从

而抑制肝癌进程[83]。

3.2　肠道菌群衍生代谢物对肝癌TIME的影响

肠道与肝脏在解剖位置和生理功能上紧密相连，通过胆道、门静脉和体循环进行双向交流，这种相互作用被称为“肠-肝轴”。生理状态下，门静脉将肝脏合成的胆汁及抗体分泌至肠腔，同时肠道菌群及其代谢产物、营养物质等由门静脉血液转移至肝脏。然而，在肿瘤或慢性炎症等病理状态下肠黏膜屏障破坏，肠道菌群失调及其异常代谢产物可通过门静脉进入肝脏，促进肝癌进展。

胆汁酸循环在肠道菌群代谢异常介导的肝癌免疫逃逸中发挥关键作用。肠道菌群可将初级胆汁酸代谢为次级胆汁酸，由“肠-肝轴”再循环到肝脏。食源性和遗传性肥胖可引起肠道菌群失衡，从而影响胆汁酸循环，促进脱氧胆酸产生和再吸收，导致DNA突变和细胞癌变[84]。同时，脱氧胆酸还能促进IL-6、Gro-α和CXCL9分泌，诱导肝星状细胞（hepatic stellate cells，HSC）向衰老表型转换[85]。次级胆汁酸可下调肝窦内皮细胞中趋化因子CXCL16表达，导致肝脏难以招募NK细胞等效应细胞，从而抑制抗肿瘤免疫反应[86]。此外，部分胆汁酸循环产物可逆转肝脏癌前病变进而发挥抗癌作用。研究表明，单形拟杆菌合成3-琥珀酰化胆酸诱导菌群重塑，促进益生菌嗜黏蛋白阿克曼氏菌的生长，从而改善肠屏障功能，缓解慢性炎症并逆转小鼠代谢相关脂肪性肝炎表型[84]。

肝癌TIME重塑过程中短链脂肪酸也发挥了重要作用。研究发现，小鼠肝癌模型中肠道菌群罗伊氏乳杆菌丰度降低，短链脂肪酸尤其是乙酸水平合成减少，导致肝脏微环境中乙酸缺乏而IL-17A分泌增加，最终抑制ILC3抗肿瘤功能，移植健康小鼠粪便菌群或罗伊氏乳杆菌均可提升乙酸水平促进肝癌TIME重塑，进而恢复ILC3细胞的抗肿瘤功能，抑制肿瘤进展[87]。

4　靶向TIME治疗肝癌

肝脏局部免疫失衡和代谢紊乱共同塑造了肝癌特殊的微环境，导致免疫抑制性细胞因子、细胞外基质及异常代谢产物累积，诱导肿瘤异常血管生成，免疫抑制细胞募集，T细胞浸润减少和抗肿瘤功能降低，造成癌症免疫周期缺陷，肝癌免疫逃逸和肿瘤进展。肝癌的免疫治疗正是基于以上TIME的特征，对肝癌免疫微环境进行重编程，以重新激活癌症免疫周期，恢复抗肿瘤免疫应答。而随着对癌症免疫周期认知的不断深入，将进一步为肝癌的临床治疗提供新的辅助疗法以提高治疗效果（图4）。

4.1　免疫检查点抑制剂

免疫检查点是调节T细胞受体抗原识别的重要信号分子，在维持机体的免疫稳态中起到关键作用。常见的免疫检查点包括CTLA-4、PD-1、TIM3和LAG3等，其中CTLA-4和PD-1/PD-L1是肝癌免疫治疗中最常用的靶点，针对以上靶点研发的免疫检查点抑制剂（immune checkpoint inhibitor，ICI）可选择性调控T细胞亚群功能，通过阻断共抑制信号重塑肝癌TIME，恢复T细胞的“杀敌”功能，改善中晚期肝癌患者临床预后，目前已成为免疫治疗领域的“明星药物”。

CTLA-4是一种主要表达在活化的T细胞表面的免疫抑制性受体，其与配体B7-1（CD80）/B7-2（CD86）结合产生抑制性信号，阻止T细胞的活化并降低免疫应答能力，阻断CTLA-4可恢复T细胞抗肿瘤免疫应答。目前，曲美木单抗（Tremelimumab）和伊匹木单抗（Ipilimumab）对肝癌患者有显著的治疗效果，其中Tremelimumab作为首个上市的CTLA-4抑制剂，既可通过阻断CTLA-4解除抑制T细胞活化的信号，重新激活T细胞分化、增殖为效应细胞，并增强了IFN-γ、IL-2细胞因子的分泌，还可通过抑制Tregs改善免疫抑制肿瘤微环境，增强抗肿瘤免疫[88]。一项包括20例肝癌患者的临床研究显示，在接受Tremelimumab治疗后，患者的客观缓解率（objective response rate，*ORR*）为17.6%，此外，患者在治疗后6个月和1年的总生存率分别为64%和43%[89]。另一项研究揭示了Tremelimumab联合消融对进展期的肝癌患者的治疗情况，临床结果显示19例患者中5例患者获得了缓解[90]，以上临床试验表明靶向CTLA-4研发特异性的抑制剂可能为肝癌的治疗提供新的策略。

近年来，以PD-1/PD-L1作为靶点的免疫检查点抑制剂在肝癌临床免疫治疗中得到广泛应用，主要包括纳武利尤单抗（Nivolumab）、帕博利珠单抗（Embrolizumab）及卡瑞利珠单抗（Camrelizumab）等。PD-1/PD-L1单抗可通过阻断PD-1与PD-L1的相互作用，逆转TIME中T细胞免疫耗竭，而活化的T细胞通过释放炎症因子和细胞毒性颗粒降低肿瘤免疫微环境的免疫抑制性，重新激活免疫系统的功能起到清除肿瘤细胞的作用。Nivolumab和

Pembrolizumab已被美国食品药品监督管理局（food and drug administration，FDA）批准用于索拉非尼治疗失败的晚期肝癌患者，两者在治疗晚期肝癌患者中均显示出了较强的安全性[91]。然而基于KEYNOTE-240临床试验对治疗效果和安全性进行评估显示，经Nivolumab和Pembrolizumab治疗的患者总生存期和无进展生存期并未达到预先规定的统计学显著性差异和标准[92]。因此，针对两者在肝癌晚期患者治疗中的局限性，一方面正在研发更具潜力的PD-1/PD-L1抑制剂如Camrelizumab，该药目前已经取得了良好的临床试验数据，但其长期疗效仍需进一步跟踪验证[93]，另一方面，鉴于单一ICI在晚期肝癌治疗中效果并不理想，联合免疫治疗可能为ICI在晚期肝癌的治疗提供了更有前景的方向。

4.2 联合治疗

大多数肝癌晚期患者对ICI单药治疗的*ORR*不高导致了病情的进一步发展。因此，研究人员试图通过联合用药增强ICI疗效。联合免疫治疗是通过不同作用机制的药物协同发挥作用以提高患者生存期、反应率及抗肿瘤效果。目前，已开发出几种新的ICI联合给药方案，主要包括PD-1/PD-L1抗体与CTLA-4、抗血管生成抑制剂、代谢检查点抑制剂等联合治疗[94-96]。

双免疫检查点抑制剂如PD-1/PD-L1抗体与CTLA-4抗体的联用在肿瘤免疫治疗中展现出显著疗效。在晚期黑色素瘤中，两者联用显示出良好的治疗效果[97]，这极大地推进了联合用药在肝癌中的临床研究。在PD-1抑制剂（Nivolumab）联合CTLA-4抑制剂（Ipilimumab）治疗晚期肝癌患者的临床试验中，联合用药治疗相比于Nivolumab单药组延长了10个月生存期，初步显示出双药联用可提高晚期肝癌患者的治疗效果[98]。此外，近期研究评估了一项关于头颈癌患者接受免疫联合治疗的响应情况，结果显示，PD-L1抗体主要引起$CD8^+$ T细胞增殖，而PD-L1抗体联合CTLA-4抗体治疗会导致$CD4^+$ T和$CD8^+$ T细胞同时增殖，表明联合疗法将更大程度地调节肿瘤TIME，更有效地通过对免疫微环境重塑实现对癌细胞的杀伤作用[99]。

血管生成因子如VEGF已被证实通过上调免疫检查点诱导T细胞耗竭导致癌症免疫逃逸。VEGF抑制剂可通过促进T细胞浸润、下调免疫检查点、减少免疫抑制性细胞的聚集，诱导肝癌中肿瘤血管的正常化重塑免疫抑制性TIME[100]，其与PD-1/PD-L1联用改善晚期肝癌疗效提供了可能。IMbrave-150 III期临床试验显示，晚期肝癌患者接受阿替利珠单抗（Atezolizumab，PD-L1抑制剂）和贝伐珠单抗（Bevacizumab，VEGF抑制剂）联合治疗的中位数生存期为19.2个月，而索拉非尼治疗组的中位数生存期为13.4个月。在18个月时，联合治疗组的存活率为52%，显著高于索拉非尼治疗组40%的存活率。因此，PD-1/PD-L1抗体与VEGF抑制剂的联用为靶向TIME治疗肝癌提供了新的临床治疗策略。

CD73是催化AMP生成胞外核苷的主要酶，通过产生腺苷影响抗肿瘤免疫反应，导致肝癌TIME中的免疫抑制[101]，CD73抑制剂为代谢免疫检查点抑制剂，其可通过阻断细胞外腺苷产生来维持细胞核苷酸代谢稳态进而逆转肝癌微环境的免疫抑制。大量研究表明，肿瘤细胞可表达CD73并释放腺苷以介导免疫逃逸[102-103]。此外，临床研究证实CD73可作为肝癌免疫靶点，但单独靶向CD73治疗会损伤正常组织，而其与PD-L1联用具有更强的免疫激活药效，在晚期肝癌中展现出了良好的应用前景[104]。

4.3 溶瘤病毒疗法

溶瘤病毒（oncolytic virus，OV）是一类能特异性复制并引起肿瘤细胞凋亡而不损伤正常细胞的病毒，OV可通过直接裂解肿瘤细胞、诱导抗肿瘤免疫应答、破坏肿瘤血管发挥抗血管生成进而起到抗肿瘤作用[105-106]。目前，经过基因重组的牛痘病毒、呼肠孤病毒、单纯疱疹病毒与腺病毒具有较好的临床疗效。

JX-594是一种携带GM-CSF的转基因牛痘病毒，已被批准用于临床治疗[107]。JX-594可在缺失胸苷激酶（thymidine kinase，*TK*）基因的肿瘤细胞中选择性复制，同时表达GM-CSF和β半乳糖苷酶激活免疫反应，并通过将子代病毒释放到肿瘤微环境中介导肿瘤细胞裂解导致炎性免疫反应，使免疫抑制的肿瘤微环境转变为炎性反应肿瘤微环境，激活肿瘤免疫应答功能，引起较强的抗肿瘤免疫反应[108-109]。在一项I期临床试验（NCT00629759）和随后的II期临床试验（NCT00554372）中，肝内注射JX-594后肝癌患者均表现出积极的治疗效果[110]。尽管如此，溶瘤病毒作为单一抗肿瘤药物的疗效并不理想，因此常与各类药物联合治疗肝癌[111]。此外，联合用药的安全性也是目前亟需解

决的问题。例如，JX-594联合Nivolumab作为一线治疗获得了33.3%的*ORR*，但由于治疗过程中频繁出现严重副作用，该研究最后被终止。但随着基因工程技术的不断发展，OV疗法也将不断完善，将为更多肝癌患者带来新的希望。

4.4　过继细胞疗法

过继细胞疗法（adoptive cell transfer therapy，ACT）是将自体或异体的免疫细胞分离，经体外基因工程修饰、激活并扩增出足量的具有抑制肿瘤活性的免疫细胞后，再将其回输到肿瘤患者体内达到抗肿瘤效果的免疫疗法。目前ACT的研究多聚焦在嵌合抗原受体T细胞（chimeric antigen receptor T-cells，CAR-T）治疗、T细胞受体工程化T细胞（T-cell receptor engineered T cells，TCR-T）治疗、嵌合抗原受体自然杀伤细胞（chimeric antigen receptor-natural killer cell，CAR-NK）治疗等。

近年来CAR-T疗法是ACT研究中具有较好临床前景的新型细胞免疫疗法[112]。CAR-T是指将靶向特异性肿瘤抗原的scFv段与*CD3*基因序列重组并导入T淋巴细胞中形成的具有肿瘤特异性杀伤作用的免疫细胞。此外，CAR-T细胞还导入共刺激分子可进一步增强T细胞的功能与增殖能力[113-114]。目前，CAR-T疗法在白血病的治疗中已取得显著的疗效，被认为是最有前景的肿瘤治疗方式之一，且在肝癌中的治疗作用也初步被证实[115]。有研究表明，通过在CAR-T中增加细胞因子IL-7和CCL19的表达，可增强DC和T淋巴细胞在肿瘤组织中的浸润，改善肿瘤免疫抑制微环境启动抗肿瘤免疫反应，间接增强CAR-T的抗肿瘤活性，随后该研究的有效性也在肝癌细胞中得到了证实[116]。目前，靶向磷脂酰肌醇蛋白聚糖3（glypican-3，GPC-3）的CAR-T细胞疗法治疗肝癌的I期临床研

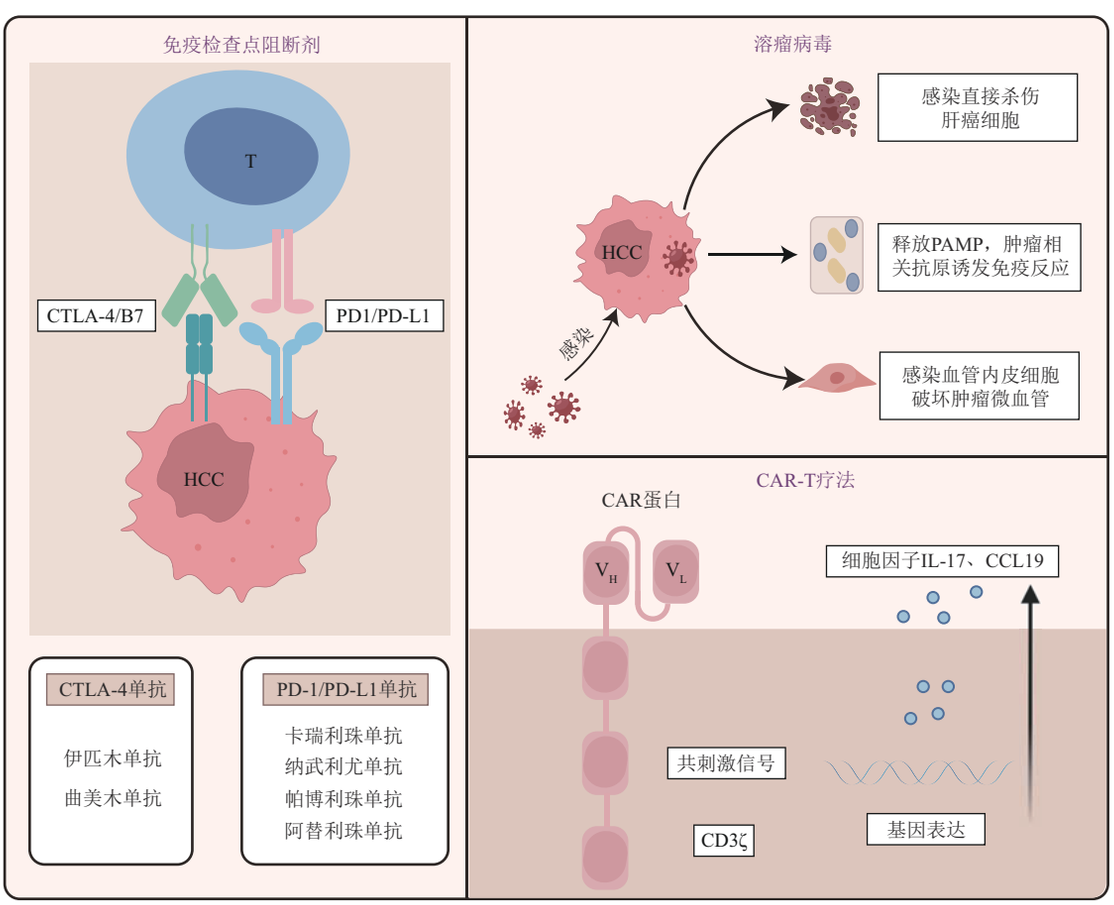

Fig. 4　Immune checkpoint inhibitors, oncolytic viruses, and CAR-T therapy in treating hepatocellular carcinoma

图4　免疫检查点抑制剂、溶瘤病毒、CAR-T疗法治疗肝细胞癌

免疫检查点抑制剂通过阻断PD-1/PD-L1、CTLA-4/B7等信号维持T细胞活性；溶瘤病毒特异性破坏肿瘤细胞、微血管内皮细胞并激活免疫反应发挥抗肿瘤作用；CAR-T细胞特异性识别肿瘤，并通过转基因表达共刺激分子、细胞因子增强其杀伤能力。CTLA-4：细胞毒性T淋巴细胞相关抗原4；PD-1：程序性死亡受体1；PD-L1：程序性死亡受体配体1；PAMP：病原体相关分子模式；CAR：嵌合抗原受体；CAR-T：嵌合抗原受体T细胞；IL-17：白介素-17；CCL19：趋化因子配体19。

究结果显示，13例GPC-3表达阳性的晚期肝癌患者接受自体CAR-T细胞输注后，其中2例患者获得部分缓解，6个月、1年、3年生存率分别为50.3%、42%和10.5%[117]，这项临床研究为CAR-T在肝癌患者中的治疗提供了希望。

5 总结与展望

目前肝癌的临床治疗手段和效果仍然存在较多局限，而肝癌免疫疗法为肝癌临床治疗提供了新策略。与手术治疗、放化疗等传统治疗手段相比，免疫治疗具有相对较高的特异性及较小的副作用等优势。肝癌免疫治疗效果与TIME密切相关，靶向肝癌TIME的研究为更深入地理解免疫治疗耐受机制及研发新的免疫治疗方案提供了新的思路。然而单一免疫疗法对改善肝癌患者预后效果并不理想，目前临床研究在积极探索双免疫检查点联合或与现有疗法相结合的综合治疗方案，将有望改善肝癌治疗局限性。因此，深入探究TIME的组成、特点及有效监测其在治疗过程中的动态变化，将为肝癌的治疗带来新的希望，使更多的肝癌患者从中受益。

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Microenvironment Remodeling and Immunotherapy of Hepatocellular Carcinoma*

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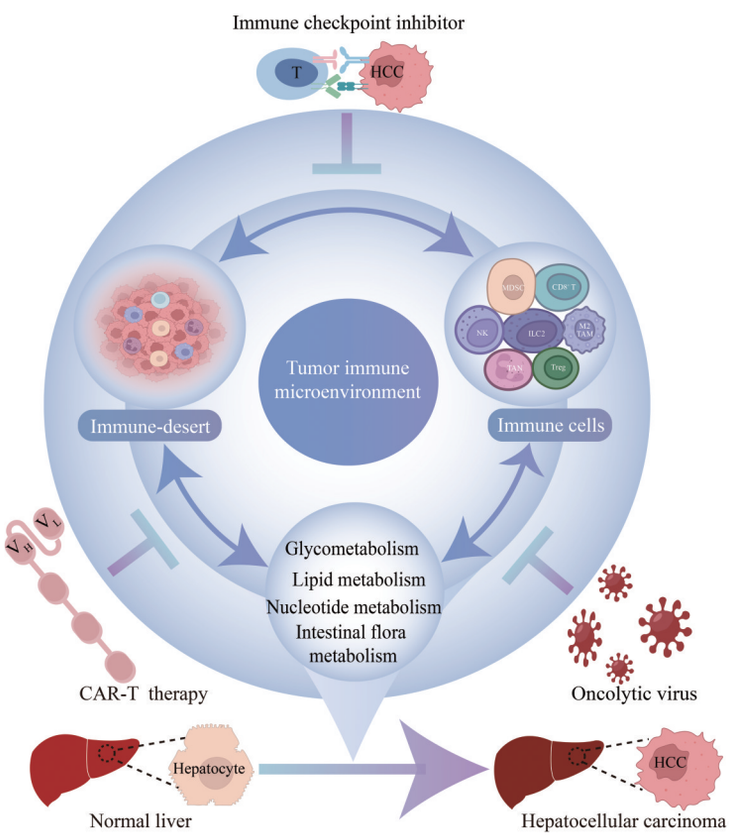
Graphical abstract



Abstract　Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the digestive tract system, which is induced by multiple factors, involving multiple genes and complicated mechanism. Its incidence and mortality rank fourth and second respectively in China, and accounting for more than 85% of primary liver cancers. Tumor immune microenvironment (TIME), plays a critical role in determining the tumor progression and treatment outcomes, making it become a hotspot in current studies. Summarising the previous studies, it is found that the progression of HCC is significantly influenced by the TIME and its complex interactions. TIME consists

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of various cellular and non-cellular components, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), regulatory T cells (Tregs), innate lymphoid cells (ILCs), as well as growth factors, proteolytic enzymes, and extracellular matrix proteins. Due to long-term exposure to bacterial components carried by the portal vein, food-derived antigens, and a large amount of foreign antigenic substances, the microenvironment of liver exhibits a certain degree of immune suppression to resist excessive inflammation caused by the non-pathogenic intestinal environment. Besides, the inhibitory immune microenvironment shaped by tumor cells which induces changes in the phenotype and function of immune cells, and attenuates the cytotoxic capabilities of immune system. Meanwhile, the regulation of immune cell metabolism is crucial for anti-tumor immune response. Abnormal metabolites of liver cancer microenvironment and intestinal flora metabolites regulate the remodeling of immune microenvironment and the progression in liver cancer. Normally, the cancer immune cycle functions effectively to remove tumor cells, while the immunosuppressive, exhausted T cells and metabolic disorders of the TIME leads to defects in the cancer immunity cycle and promotes to tumor progression. Furthermore, during the processes of rapid proliferation and differentiation, tumor cells alter their metabolic status through “metabolic reprogramming”, allowing them to compete with anti-tumor immune cells for vital nutrients including glucose, lipids, and nucleotides. At the same time, the abnormal consumption of metabolites leads to local hypoxia, lower pH levels, and the accumulation of metabolic products, which in turn suppress the proliferation and effector functions of immune cells, ultimately facilitating immune evasion and tumor progression. According to the above, local immune imbalance and metabolic disorders in the liver collectively shape the unique microenvironment of HCC, resulting in the accumulation of immunosuppressive cytokines, extracellular matrix and abnormal metabolites. These factors induce abnormal tumor angiogenesis, recruitment of immunosuppressive cells, reduce T-cell infiltration, and diminish anti-tumor function, which accelerates the progression of HCC and immune escape. Currently, there are still remarkable limitations in the clinical treatment methods and outcomes for HCC, while immunotherapy offers a new strategy. The advantages of immunotherapy demonstrate relatively higher specificity and fewer side effects compared to traditional treatment methods such as surgery, radiotherapy, and chemotherapy. Up to now, more and more evidence has been uncovered that liver cancer immunotherapy is closely related to TIME. Targeting the TIME of HCC provides a new perspective into a deeper understanding of the mechanisms of immunotherapy resistance and the development of new immunotherapy approaches. However, single immunotherapy has not shown satisfactory results in improving the prognosis of HCC patients. At present, dual immune checkpoint inhibitors or their combination with existing therapies are being widely explored in clinical studies, hoping to overcome the limitations of HCC therapy. Therefore, this review summarizes the composition of immunosuppressive microenvironment in liver cancer and metabolic regulation, and further discusses clinical therapeutic strategies by targeting microenvironment remodeling for the treatment of liver cancer, which provides new avenues for tumor immunotherapy.

Key words hepatocellular carcinoma, immune microenvironment, immune escape, immunometabolism, immunotherapy

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