靶向CD47/SIRP-α轴在卵巢癌治疗中的研究进展

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摘要 作为天然免疫检查点, CD47高表达于卵巢癌细胞表面, 与巨噬细胞表面的SIRP-α结合后可传递“Don’t eat me”信号, 从而实现肿瘤细胞的免疫逃逸。研究表明, CD47的高表达是卵巢癌不良预后并产生铂耐药的独立危险因素。目前, 通过靶向CD47/SIRP-α轴治疗卵巢癌的体内外研究以及临床试验已取得显著成果, 表明针对该轴的治疗策略具有广泛的应用前景。该文就靶向CD47/SIRP-α轴在卵巢癌中的研究进展进行了综述, 为卵巢癌的临床治疗提供了新思路。

关键词 卵巢癌; CD47; SIRP-α; 单克隆抗体; 双特异性抗体

Research Progress of Targeting the CD47/SIRP-α Axis in Ovarian Cancer Treatment

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Abstract CD47, an innate immune checkpoint, which is highly expressed on ovarian cancer cells, can transmit the “Don’t eat me” signal when combined with SIRP-α on the surface of macrophages, thereby enabling tumors to escape from the immune system. Studies have suggested that high expression of CD47 on ovarian cancer cells is an independent risk factor for poor prognosis and platinum resistance. So far, both researches *in vitro* and *in vivo* and clinical trials about ovarian cancer treatment-related strategies of targeting the CD47/SIRP-α axis have shown significant results with broad application prospects. This article reviews the progress of targeting the CD47/SIRP-α axis in ovarian cancer, providing new strategies for the clinical treatment of ovarian cancer.

Keywords ovarian cancer; CD47; SIRP-α; monoclonal antibody; bispecific antibody

卵巢癌是妇科常见的恶性肿瘤之一, 其死亡率居妇科恶性肿瘤之首[1], 国内卵巢癌患者5年生存率只有40%[2], 卵巢癌易发生转移并容易诱导化疗后的耐药。化疗是目前治疗卵巢癌的主要手段之一, 研究表明靶向治疗能显著提高卵巢癌患者的生存率[3], 且较化疗具有更低的不良反应发生率[4]。作为经典的免疫检查点, CD47/SIRP-α轴抑制剂在多种肿瘤靶向治疗的试验中已经进行了有效的探索, 本文将介绍这些抑制剂在卵巢癌治疗中的研究进展。

1 CD47/SIRP-α轴的结构及功能

CD47又名整合素相关蛋白(integrin-related protein, IAP), 是一种在正常和病变组织中广泛表达的高度糖基化跨膜蛋白, 属于免疫球蛋白超家族成员,

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其分子结构包括1个N-端胞外可变区、5个跨膜片段构成的跨膜区和1个C-端胞质尾区[5]。依据CD47分子的结构差异可将其分为4种亚型[6]。1型主要分布于角质细胞, 2型是分布最广泛的亚型, 主要分布于造血细胞、上皮细胞和血管内皮细胞等, 而3型和4型主要在神经元细胞、睾丸细胞和肠黏膜细胞中表达[7]。人体的正常细胞表面表达有CD47分子, 其被认为通过向巨噬细胞传达“Don’t eat me”信号来避免正常细胞被吞噬[8], 但CD47也过度表达于多种肿瘤细胞表面, 其最早于1986年在卵巢癌细胞表面被发现并报道[9]。

CD47的受体包括血小板反应蛋白1(thrombospondin 1, TSP-1)、整合素(integrin)和信号调节蛋白α(signal regulated protein alpha, SIRP-α), 其中SIRP-α又被称为含SH2结构域的蛋白酪氨酸磷酸酶底物1(SH2 domain-containing protein tyrosine phosphatase substrate-1, SHPS-1), 其在巨噬细胞、粒细胞等髓系细胞膜上呈高表达[10-11], 与CD47结合后可通过细胞内的一系列级联反应抑制细胞的吞噬作用, 从而抑制固有免疫反应, 产生“Don’t eat me”的效果[12]。

CD47/SIRP-α相互作用在体内免疫细胞的自我识别中起重要作用, 具体表现在其对体内衰老细胞的清除和体内肿瘤细胞的免疫逃逸两方面。其一, CD47/SIRP-α相互作用可协助清理体内衰老的红细胞, 正常红细胞表面CD47含量较高, 可与巨噬细胞表面的SIRP-α结合传递“Don’t eat me”信号从而避免自身被吞噬, 当红细胞衰老后, 其CD47表达下调, CD47/SIRP-α相互作用减少, 从而被巨噬细胞吞噬[13-14]; 其二, 已有研究表明, CD47/SIRP-α相互作用可能是肿瘤细胞免疫逃逸的机制之一[15]。越来越多的证据表明, CD47在多种血液系统肿瘤[16-19]、消化系统肿瘤[15,20-22]、生殖系统肿瘤[23-25]等肿瘤中均高表达, 且CD47的表达水平与大多数实体瘤患者的不良预后密切相关[22,26]。许多研究显示, 通过阻断CD47/SIRP-α的相互作用, 可激活巨噬细胞对肿瘤细胞的吞噬功能, 从而对肿瘤表现出显著的杀伤作用[27-29], 为肿瘤的免疫治疗提供了新的思路。

2 抗CD47单克隆抗体靶向策略

抗CD47单克隆抗体靶向策略是阻断CD47/SIRP-α轴的重要策略之一。然而, 已有前期临床试验表明抗CD47单克隆抗体尚存在易产生耐药、导致贫血等亟待解决的安全性问题[30-31]。为此, 研究人员提出了一系列改良方案, 包括: ①以Hu5F9-G4(magrolimab, M)为代表的抗CD47单克隆抗体所包含的Fc端为IgG2型或IgG4型, 因此不易引发强烈的抗体依赖性细胞介导的细胞毒作用(antibody-dependent cell-mediated cytotoxicity, ADCC), 从而减少了此类抗体对红细胞和血小板的影响; ②通过构建亲和力高、特异性强和分子量小的纳米抗体(nanobody, Nb), 克服了传统单克隆抗体分子量大、组织穿透力差的缺点[32]; ③以溶瘤病毒为载体的单克隆抗体能够靶向肿瘤微环境, 激活局部免疫反应, 进而在提高药效的同时减少不良反应的发生[33]。

在SIKIC等报道[34]的抗CD47抗体Hu5F9-G4的I期临床试验中, 研究人员对包括13例卵巢癌患者在内的癌症晚期患者注射Hu5F9-G4, 5周后对1例转移性卵巢癌患者的腋下淋巴结进行活检, 发现有高浓度的Hu5F9-G4聚集, 提示该抗体在肿瘤微环境中有良好的渗透作用。由于该抗体不会显著激活免疫系统对正常细胞的ADCC, 研究者尚未在参与临床试验的卵巢癌患者中观察到明显的贫血等不良反应, 表明人体对Hu5F9-G4的耐受性尚可, 这为后续的临床试验奠定了良好的基础。

PURO等[35]成功研制出了与CD47特异性结合的人源化抗体AO-176, 其优先与肿瘤细胞结合, 对人类的红细胞、初始T细胞和活化T细胞的结合力较已知的高亲和力抗CD47抗体AO-104显著降低, 即使在高剂量(200 μg/mL)应用的情况下也几乎不与红细胞结合, 不会引起溶血等不良反应。作为一种IgG2抗体, AO-176与Fc受体的亲和力有限, 因此其发挥促进OV90卵巢癌细胞被吞噬的作用, 并非通过ADCC途径, 而是通过阻断“Don’t eat me”信号通路来实现的[36-37]。与MATEO等[38]之前报道的几乎仅有阻断效应的抗CD47单克隆抗体B6H12相比, AO-176在发挥阻断作用的同时还能杀伤肿瘤细胞。此外, 有研究发现对实验组OV90卵巢癌移植瘤小鼠模型注射AO-176后, 肿瘤生长抑制率(tumor growth inhibition, TGI)平均值为52%, 提示该抗体在体内具有良好的抗肿瘤活性, 但能否改善患者预后、延长患者生存时间仍有待进一步研究[35]。

上皮性卵巢癌(epithelial ovarian cancer, EOC)对以铂为基础的辅助化疗应答率较低, 这与肿瘤细胞上CD47的高表达有关[25]。LIU等[39]证实人源

化抗CD47抗体SRF231显著提高了标准化疗在铂耐药PDX小鼠模型中的抗肿瘤作用。在体外实验中, SRF231与奥沙利铂或阿霉素联用时能促进肿瘤细胞死亡。研究者进一步对SKOV3以及OVCAR3异种移植小鼠模型进行体内实验, 发现单剂量注射SRF231即能发挥治疗作用, 且与阿霉素联用能增强小鼠免疫系统的抗肿瘤活性[39]。上述研究对优化卵巢癌的治疗方案具有一定的指导意义。

目前, 将单克隆抗体(monoclonal antibody, mAb)或耦联药物的mAb直接注射入体内等传统治疗方法存在如下缺点: ①mAb在肿瘤组织中分布有限; ②由于肿瘤外靶向效应以及Fc受体介导的固有免疫反应而产生全身毒性[40-41]。MA等[42]研制出以纳米抗体(nanobody, Nb)为基础的人源性重链抗体HuNb1-IgG4, 其同时具备Nb和人Fc结构域的优点, 成功克服了传统单克隆抗体由于体积大而对肿瘤渗透性差的缺点[43-44]。与Hu5F9-G4单克隆抗体相比, HuNb1-IgG4诱导的吞噬作用和体内抗卵巢癌的作用更加强大, 而且其对红细胞的亲和力显著降低, 且显示出良好的安全性。

TIAN等[45]通过体外研究发现αCD47-G1抗体一方面通过促进巨噬细胞M1标志物的基因如*IL6*、*IL10*、*NOS2*、*TNF*和*IL12A*转录, 增强了巨噬细胞对A2780卵巢癌细胞的吞噬作用; 另一方面能够显著上调NK细胞颗粒酶B以及细胞上活性标志物CD69等的表达, 增强了NK细胞的ADCC。溶瘤病毒(oncolytic virus, OV)作为一种理想载体, 能特异性地将药物靶向肿瘤微环境, 极大地激活局部免疫反应, 从而在降低毒副作用的同时提高疗效[33]。基于该特点, 研究团队构建表达了抗CD47抗体的溶瘤病毒OV-αCD47-G1, 在卵巢癌小鼠模型中进行瘤内注射, 显著抑制了卵巢癌的进展并延长了小鼠的寿命。研究者进一步利用小鼠抗CD47抗体序列设计出了OV-αCD47-G1的对应物OV-αCD47-G2b, 证明了后者能够促进巨噬细胞向M1型极化和NK细胞活化, 在免疫活性卵巢癌转移小鼠模型中发挥强大的抗肿瘤活性, 效果优于单用αCD47-G2b, 而且与抗PD-L1抗体联用效果更佳[45]。

3 双特异性抗体靶向策略

由于CD47普遍表达于以血液系统细胞为代表的许多正常组织细胞上, 单一靶向CD47的治疗策略可能会受到“抗原沉默(antigen sink)”的影响, 导致抗体的生物利用度降低, 并增加靶标毒性(如贫血)的风险[31]。为了解决这一问题, 有研究者将目光转向了在正常组织中表达水平更少的SIRP-α, 通过设计靶向SIRP-α的抗体来阻断CD47/SIRP-α通路; 其他的替代方案还包括同时靶向CD47和其他肿瘤相关抗原(tumor-associated antigen, TAA)的双特异性抗体(bispecific antibody, BsAb)或者联合应用多种抗体的治疗策略[46-47]。

作为一种人工合成的非自然抗体, 双特异性抗体能同时特异性地结合两个不同的抗原或同一抗原的两个不同抗原表位[48], 该特点决定了BsAb在抗肿瘤治疗方面具有治疗效果显著、副作用较小等一系列优势[49]。基于上述特性, 研究人员目前已研制出卡妥索单抗(catumaxomab)、博纳吐单抗(blinatumomab)和埃万妥单抗(amivantamab)等用于治疗癌症的BsAb, 另有近百种多特异性抗体(multi-specific antibody)也已处于临床研发阶段(表1)[50-52]。

FISCHER等[53]设计了一种抗CD47/TAA的双靶向双特异性抗体(dual-targeting bispecific antibodies, biAbs)即κλ小体, 利用过表达CD47分子的卵巢癌细胞株OVCAR-3进行体外抗体依赖性细胞吞噬(antibody-dependent cellular phagocytosis, ADCP)实验, 证明了抗CD47/MSLN biAb相较抗CD47单价抗体显著增强了巨噬细胞对肿瘤细胞的吞噬作用。进一步的全血结合实验表明该双特异性抗体与人血细胞的结合相当微弱, 展现出了良好的抗体耐受性。LAKHANI等[54]设计了一种双功能融合蛋白SL-175154(SIRPα-Fc-CD40L), 成功地将固有免疫应答和适应性免疫应答进行联合, 通过对14名耐铂卵巢癌、输卵管癌和原发性腹膜癌患者开展I期临床试验, 表明了SL-175154抗体在剂量达到3.0 mg/kg时仍能被人体耐受, 且尚未观察到贫血、血小板减少、肝功能受损或细胞因子释放综合征等不良反应。

4 联合抗体靶向策略

两种或多种不同类型靶向药物的联用能通过桥接先天性和适应性免疫系统, 增强患者体内的吞噬作用、抗原呈递作用和T细胞致敏作用, 从而实现抗肿瘤治疗效果的提升。作为经典的适应性免疫检查点, PD1/PDL1相互作用可通过干扰T细胞受体信号传递来抑制T细胞应答[55]。多项研究已经证实, 阻

表1 靶向CD47/SIRP-α轴治疗卵巢癌的临床研究

Table 1 The clinical trials of targeting the CD47/SIRP-α axis in the treatment of ovarian cancer

药物名称 Drug	药物形式 Design	研发公司 Sponsor	用药方式 Interventions	试验状态 Status	试验阶段 Progress	适应症 Indications	临床试验编号 Clinical study No.
AO-176	CD47 blocker	Arch Oncology, Inc.	AO-176; AO-176 + paclitaxel; AO-176 + pembrolizumab	Recruiting	Phase 1/2	Solid tumor	NCT03834948
Hu5F9-G4	CD47 blocker-Fc	Gilead Sciences, Inc.	Hu5F9-G4 only	Completed	Phase 1	Solid tumor	NCT02216409
		Gilead Sciences, Inc.	Hu5F9 + cetuximab	Completed	Phase 1/2	Solid tumor; colorectal cancer	NCT02953782
		Gilead Sciences, Inc.	Hu5F9 + avelumab	Completed	Phase 1	Ovarian cancer	NCT03558139
SRF231	CD47 blocker	Surface Oncology, Inc.	SRF231 only	Completed	Phase 1	Advanced solid cancers; hematologic cancers	NCT03512340
SL-172154	Bispecific antibody: CD47/TAA	Shattuck Labs, Inc.	SL-172154 only	Recruiting	Phase 1	Ovarian cancer; fallopian tube cancer; primary peritoneal carcinoma	NCT04406623

断CD47/SIRP-α轴可促进抗PDL1抗体等靶向治疗药物的抗癌活性, 从而发挥固有免疫联合适应性免疫治疗方案的优势[56-58]。

LAKHANI等[59]设计了抗CD47抗体Hu5F9-G4和PD-L1抑制剂阿维鲁单抗(avelumab, A)联用治疗卵巢癌和其他实体瘤患者的方案, 发现其中1例实体瘤患者的病情得到部分缓解(partial response, PR), 且试验中全部13例卵巢癌患者的疾病稳定率(stable disease rate, SD rate)达到56%, 初步证明了上述两种抗体联合应用具有良好的有效性和耐受性。由于研究者仅在1例伴肿瘤细胞PD-L1阳性表达的卵巢癌患者中观察到其肿瘤缩小, 因此抗体联合治疗的策略对此类患者的作用还有待进一步研究。

类似地, FISHER等[60]研究了联用抗CD47抗体Hu5F9-G4和抗EGFR抗体西妥昔单抗(cetuximab, C)对实体瘤患者的作用。肿瘤组织活检显示经过治疗的实体瘤患者中巨噬细胞及基线T细胞的浸润增加, 且该效应与患者较长的总生存期(overall survival, OS)相关联, 提示上述两种抗体存在一定的协同抗肿瘤作用。

5 结语与展望

作为卵巢癌的新型免疫检查点, CD47作为药物靶点具有很好的开发前景。目前已有的靶向干预CD47/SIRP-α轴的研究方法包括CD47和SIRP-α单克隆抗体[28]、CD47/TAA双特异性抗体和联合抗体[61]等, 它们在卵巢癌的体内外研究中取得了较大进展, 具体表现为可以显著抑制卵巢癌小鼠模型中肿瘤的进展、延长小鼠寿命, 其对铂耐药的肿瘤也有强大的作用, 并可进一步克服溶血、Fc受体介导的全身毒性等生物安全问题。然而, 部分抗体的研究还未进入临床试验阶段, 已进入临床试验阶段的研究亦存在样本量小、受试者肿瘤分期单一、安全性有待观察等不足。相信随着对CD47/SIRP-α轴研究的不断深入, 新型靶向抗体药物的研发将为众多卵巢癌患者带来更可观的益处。

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