•综述•

基于靶向垂钓技术挖掘传统中药治疗四大慢病的作用机制研究进展

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摘要: 中药 (traditional Chinese medicines, TCMs) 在治疗慢性非传染性疾病方面拥有悠久的历史和显著的疗效, 包括癌症、心脑血管疾病、糖尿病及慢性呼吸系统疾病 (统称为“四大慢病”)。然而, 中药成分体系复杂, 其确切物质基础和作用机理的研究备受制约。近年来, 多种新兴靶向垂钓技术, 作为可从复杂样品体系中寻找生物分子间相互作用的手段, 为揭示TCMs的多成分、多靶点、多途径作用机制提供了有力支持。本文回顾了靶向垂钓技术的发展, 总结了基于靶标及配体垂钓技术在TCMs机制探索及活性成分确定中的典型模式, 并重点概述了近3年垂钓技术在TCMs治疗四大慢病机制研究及“成分−靶点”关系解析中的应用实例, 以深化对靶向垂钓技术的理解、推动其在中医药现代化研究中的应用, 并为相关领域的研究提供方法学借鉴。

关键词: 靶向垂钓; 中药; 靶标垂钓; 配体垂钓; 慢性非传染性疾病

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Target fishing technologies in traditional Chinese medicines: mechanisms and applications for four chronic diseases

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Abstract: Traditional Chinese medicines (TCMs), with its rich historical legacy and demonstrated efficacy in treating chronic non-communicable diseases, particularly in addressing cancer, cardiovascular diseases, diabetes, and chronic respiratory diseases, presents unique challenges for scientific investigation due to its complex chemical

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composition and poorly understood mechanisms of action. This review systematically examines the evolution of target fishing technology, evaluates established target identification technology and ligand fishing technology in TCMs research, and synthesizes recent advancements in applying these methodologies to elucidate the therapeutic mechanisms of TCMs for major chronic diseases, with special emphasis on component-target interactions. By providing a critical analysis of current applications and future directions, this work aims to advance the integration of target fishing technologies into modern TCMs research and establish a robust framework for mechanistic studies in this field.

Key words: target fishing; traditional Chinese medicine; target identification; ligand fishing; chronic non-communicable disease

随着全球人均预期寿命的不断提高, 慢性非传染性疾病 (chronic non-communicable diseases), 包括癌症、心脑血管疾病、糖尿病及慢性呼吸系统疾病 (统称为“四大慢病”), 已成为威胁人类健康的主要因素。近年来, 我国四大慢病的发病率逐年上升, 且患者群体呈年轻化趋势[1]。中药 (traditional Chinese medicines, TCMs) 凭借其整体观、多靶点、不良反应少且具有个体化精准治疗特点的优势, 在防治慢性疾病中显示出巨大的潜力。然而, TCMs因成分体系复杂且微量, 对其靶点及作用机制的确证研究进展相对缓慢。当前, 我国创新药物研发面临靶点枯竭困境, 90%以上的药物靶点源于国外文献报道, 而TCMs作为天然药物活性成分的重要宝库, 蕴含着大量尚未被充分挖掘的新型作用靶点。这些“隐性靶点”的发现将依赖于突破复杂化学结构的解析、活性成分的获取及功能机制的阐释等关键技术瓶颈, 将传统认知中的“不可成药”或未知靶点转化为具有成药性的有效靶点, 为创新药物研发开辟新的路径。

靶向垂钓技术 (target fishing technology) 是一种精准的生物分子筛选策略, 简称“钓靶”, 它通过设计特异性钓饵分子捕获和鉴定复杂体系中的目标物质或目的蛋白, 在解析和挖掘TCMs活性物质和作用靶点方面具有显著优势[2]。本文将综述“钓靶”技术及其发展, “钓靶”技术在TCMs研究中的经典应用模式及其应用于挖掘TCMs治疗四大慢病的“成分−靶点”关系研究实例。

1 靶向垂钓技术及其发展

鉴于网络药理学等计算方法在靶点预测中存在非特异性等局限性, 靶向垂钓技术作为连接“计算预测”与“实验验证”的关键手段, 在中药复方作用机制研究中展现出重要价值。

1.1 网络药理学与靶点预测工具

网络药理学发展逾20年, 已成为TCMs有效成分及靶点预测的关键手段。常用于筛选活性分子数据库有: 中药系统药理学数据库TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform,https://old.tcmsp-e.com/index.php)、中医药整合药理学研究平台TCMIP (Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine,http://www.tcmip.cn/TCMIP/index)[3-5], 亦可借助ChemMapper (https://lilab-ecust.cn/chemmapper/)、DrugBank (https://go.drugbank.com/)、Connectivity Map (CMAP,http://www.broadinstitute.org) 等数据库进行靶点预测。随着人工智能时代的到来, 机器学习技术已广泛应用于活性分子及药物靶点的发现, 有学者建立了一种深度学习模型, 即基于图卷积网络的药物“靶向”通路预测算法, 发现银杏素通过靶向干扰素基因刺激因子 (stimulator of interferon genes, STING) 缓解炎症和衰老[6]。然而, 网络药理学预测结果常包含非特异性信息且缺乏实验验证, 难以准确解析中药多成分、多靶点的作用机制。因此, “钓靶”技术应运而生, 通过高特异性筛选和验证, 为剖析TCMs复杂体系中“成分−靶点”关系提供精准工具与方法。

1.2 配体垂钓技术: 活性成分的识别与分离

“钓靶”技术包含配体垂钓技术 (ligand fishing technology) 和靶标垂钓技术 (target identification technology)[7]。配体垂钓技术主要用于从复杂体系中筛分出潜在的活性物质, 常运用靶标固定化技术及亲和色谱技术进行分离和分析。根据以上两种技术是否同时进行, 又可分为离线和在线模式。离线模式下, 需要将捕获的活性成分洗脱后分析, 如亲和超滤、磁珠或磁性纳米颗粒、平衡透析、纳米管等。相比之下, 在线模式能够实时分析、动态监测, 如生物层析、毛细管电泳、生物反应器等[8]。

1.3 靶标垂钓技术: 直接作用靶点的捕获与鉴定

靶标垂钓技术主要用于捕获并鉴定目标成分在复杂生物体系中的直接作用靶点。已有多种靶标垂钓技术广泛应用于TCMs的作用机制研究, 包括标记法和非标记法。

1.3.1 标记法: 基于探针的小分子靶点识别 标记法

较为常用, 通过合成修饰的小分子化学探针进行“钓靶”, 又分为以化合物为中心的化学蛋白质组学 (compound centric chemical proteomics, CCCP) 和以活性探针为基础的蛋白质谱分析 (activity-based protein profiling, ABPP)[9]。CCCP技术通过将TCMs活性小分子固定于载玻片、磁珠[10-12]或琼脂糖珠等捕获靶蛋白群, 是最早用于靶标蛋白鉴定的方法之一。但因TCMs成分复杂、修饰位点有限且多非特异性, 其应用受限。相比之下, ABPP利用活性导向的探针分子特异性标记靶标蛋白, 应用更广。随着生物正交化学技术的发展, 点击化学 (click chemistry, CC) 被引入ABPP探针设计中, 简称CC-ABPP, 可在活细胞中原位捕获靶标。然而, CC-ABPP无法实现体内药物–靶标相互作用的高分辨率空间成像。因此, 有学者又开发出透明化辅助组织点击化学 (clearing-assisted tissue click chemistry, CATCH)[13], 支持对完整哺乳动物组织中的药物靶标进行光学成像, 为小分子与靶点的体内相互作用提供可视化平台。在探针法的基础上, 研究者基于优化探针修饰基团开发了多种创新“钓靶”策略。如基于降解的蛋白质分析策略 (degradation-based protein profiling, DBPP)[14]及邻近诱导的蛋白质标记技术 (specific pupylation as IDEntity reporter, SPIDER)[15], 可识别弱结合蛋白。此外, 通过将探针法与人蛋白质组微阵列芯片[16-18]或具有均匀分布文库的mRNA展示 (mRNA display with a library of even distribution, md-LED)[19]等技术相结合, 显著提高了筛选效率, 并拓展了应用范围。

1.3.2 非标记法: 避免结构修饰的靶点发现技术 尽管标记法应用广泛, 但受限于有些小分子的活性易被改变或结构难以修饰, 非标记法随之兴起并得到广泛应用。早期常用的非标记法包括基于小分子药物与靶点结合后形成稳定的复合物对热变性敏感度降低的细胞热转移测定 (cellular thermal shift assay, CETSA)[20]、基于小分子药物与靶点结合后形成稳定的复合物对蛋白酶敏感度降低的药物亲和反应的靶点稳定性技术 (drug affinity responsive target stability, DARTS)[21]、基于非标记的靶点发现技术如基于氧化速率的蛋白质稳定方法 (stability of proteins from rates of oxidation, SPROX) [22]、表面等离子体共振 (surface plasmon resonance, SPR) [23]、微量热泳动技术 (microscale thermophoresis, MST)[24]等。随着技术的不断进步, 衍生出一些适用于复杂样品、具有高灵敏度、高通量的新方法。如将CETSA与质谱法的热蛋白组分析技术 (thermal proteome profiling, TPP) 相结合[25], 显著提高蛋白检测效率; 有限蛋白水解质谱技术 (limited proteolysis-mass spectrometry, LiP-MS)[20]可揭示药物与靶点的结合域; 以肽为中心的局部稳定性探测技术 (peptide-centric local stability assay, PELSA)[26]可提高灵敏度并提供结合区信息。此外, 基于不同化合物诱导的细胞表型变化, 有学者开发了一种基于细胞表型识别靶标的有效集成分析策略[27], 利用MorphoBase系统分析形态参数, 结合ChemProteoBase系统鉴定细胞蛋白质组学的整体变化, 为药物靶点发现提供了新思路。

2 多种靶向垂钓技术在传统中药作用机制研究中的应用方式

靶向垂钓技术为TCMs物质基础及作用机制研究提供了更为高效的分析手段。配体垂钓技术能够从复杂混合物中快速筛选活性物质[7], 特别适合于TCMs复方和制剂等样品的活性成分分析; 而靶标垂钓技术则专注于研究活性成分的作用机制, 揭示其与靶点的相互作用及调节机制。两者相辅相成, 共同推动TCMs的现代化研究进程。

近年来, 层出不穷的多种靶向垂钓技术为TCMs的“成分–靶点”的关系解析研究发现带来了重大突破。其模式性研究思路主要概括为三步走: 首先, 利用“钓靶”技术或网络药理学预测靶点; 其次, 体外验证TCMs成分与靶点的结合作用; 最后, 在体内外确认靶蛋白的功能及有效性或成分活性, 从而阐明TCMs中的具体成分通过确切靶点发挥治疗作用, 以阐释其物质基础及调控机制。基于中药单体、单味中药、复方及制剂在“靶点发现–结合验证–效应确证”中的研究进展, 本文总结了TCMs研究思路及所涉及的靶向垂钓技术方法 (表1)[6,14,15,19-21,23-61], 为后续实验提供参考。

3 基于靶向垂钓技术挖掘中药治疗四大慢病的“成分–靶点”关系研究实例

本部分总结了近3年运用“钓靶”技术解析TCMs的“成分–靶点”关系及作用机制的研究实例, 特别围绕TCMs治疗四大慢病的中药现代化研究进展进行系统梳理, 为后续推进TCMs的现代化进程及拓展其临床应用等提供科学依据和理论支持。

3.1 在癌症研究中的应用: 抗癌中药单体的靶点发现与机制阐释

靶向治疗作为癌症精准医疗的核心策略, 依赖于靶向垂钓技术对特异性靶点的识别与验证, 从而发现更多潜在治疗靶点, 实现对癌细胞的精准杀伤。基于ABPP技术, 有团队将其与人蛋白质组芯片、免疫共沉淀 (co-immunoprecipitation, Co-IP)、MST、点突变等技术结合, 发现蟾毒灵通过靶向热休克蛋白90 ATPase活性激活因子1 (activator of heat shock 90 kDa protein

Table 1 Research model of traditional Chinese medicines (TCMs): target discovery, validation and effect confirmation. BLI: Bio-layer interferometry; CD: Circular dichroism binding assay; CETSA: Cellular thermal shift assay; CMAP: Connectivity map; DARTS: Drug affinity responsive target stability; DBPP: Degradation-based protein profiling; FP: Fluorescence polarization; GDOP: Graph convolutional network - based drug "on - target" pathway prediction algorithm; HRMR-PM: High-resolution micro-confocal Raman spectroscopy photopatterned affinity microarray; ITC: Isothermal titration calorimetry; LiP-MS: Limited proteolysis-mass spectrometry; md-LED: mRNA display with a library of even distribution; MST: Microscale thermophoresis; PELSA: Peptide-centric local stability assay; SERS: Surface-enhanced raman spectroscopy; SIP: Solvent-induced protein precipitation; SPIDER: Specific pupylation as IDEntity reporter; SPR: Surface plasmon resonance; TPP: Thermal proteome profiling

TCMs type		Target identification	Binding validation	Functional verification
Monomer compound	Network pharmacology	ChemMapper, PharmMapper[28,29], CMAP[47], DrugBank[34], GDOP[6], KEGG, GO[34], SwissTargetPrediction[48], SuperPred[49], TCMSP, TCMIP[34], Matefisher[50]	BLI[30], CD[31], CETSA[20], DARTS[21], ECD[32], FP[33], ITC[34], MST[24], Pull down[35], SERS[36], SIP[37], SPR[23], differential scanning calorimetry[38], molecular docking[39], molecular dynamics simulation[40], grating-coupled interferometry[41], co-immunoprecipitation[42], fluorescence anisotropy assay[43]	Model organisms[44], disease animal models[45], cellular models[46]
	Target identification technology	CRISPR knockout screening[51], DBPP[14], Lip-MS[20], PELSA[26], SPIDER[15], photoaffinity-labeled microspheres[30], TPP[25], md-LED[19], MorphoBase + ChemProteoBase[27], supramolecular nanoprobes[52], human proteome microarray[16]		
Single herbal medicine	Network pharmacology	Cytoscape[29], TCMSP, TCMIP[29], drug target screening model[53]		
	Ligand fishing technology	Magnetic fluorescent nanobeads[54], target fishing using immobilized recombinant bacteria[55]		
Herbal formula	Network pharmacology	PubChem[56], SwissTargetPrediction[57]		
	Ligand fishing technology	Affinity ultrafiltration[58]		
	Target identification technology	HRMR-PM[59], benzophenone-based photo-crosslinking magnetic nanobeads[60,61]		

ATPase homolog 1, AHSA1) 的K137位点, 降低其与HSP90A的相互作用, 从而治疗多发性骨髓瘤[62]; 另有研究借助人蛋白质组芯片、Pull down、分子动力学模拟等技术证实, 冬凌草乙素通过靶向Kelch样ECH相关蛋白1 (Kelch like ECH associated protein 1, Keap1), 稳定Keap1-磷酸甘油酸变位酶5 (phosphoglycerate mutase family member 5, PGAM5) 复合物, 进而促进肝癌细胞线粒体凋亡[63]。此外, CC-ABPP技术也被广泛使用。研究人员将其与稳定同位素标记技术及CETSA、SPR、DARTS等验证手段联用, 发现原人参二醇通过靶向视网膜母细胞瘤结合蛋白4 (retinoblastoma binding protein 4, RBBP4), 调节H3K27me3和癌基因表达, 从而抑制结直肠癌细胞的增殖与迁移[64]; 同时, 另有团队结合CETSA、DARTS、SPR等多项技术发现, 四氢姜黄素通过靶向甲状腺激素受体相互作用因子13 (thyroid hormone receptor interactor 13, TRIP13), 破坏TRIP13/泛素特异性蛋白酶7 (ubiquitin-specific protease 7, USP7)/细胞性FLICE样抑制蛋白 (cellular FLICE-like inhibitory protein, c-FLIP) 复合物, 增强c-FLIP泛素化, 最终诱导三阴性乳腺癌细胞凋亡[65]。针对结直肠癌的耐药性问题, 结合CETSA与分子对接技术发现, 肉桂醛可通过靶向热休克蛋白家族D成员1 (heat shock 60 kDa protein 1, HSPD1), 阻滞细胞周期, 抑制结直肠癌细胞增殖, 且该化合物与临床常用药物联用可有效克服肿瘤耐药性[40]。此外, 科研人员通过DARTS、分子对接证实, 豆蔻酸可选择性抑制脂肪酸合酶 (fatty acid synthase, FAS), 干扰肿瘤细胞的脂肪酸合成, 进而发挥抗肿瘤作用[66]。

靶向治疗显著提高了抗癌疗效, 但肿瘤的免疫逃逸仍是当前治疗面临的重要挑战。免疫检查点疗法 (immune checkpoint therapy, ICT) 通过解除肿瘤对免疫系统的抑制, 重新激活抗肿瘤免疫, 为癌症治疗开辟新路径, 有学者[67]利用ABPP技术结合Pull down、点突变、CETSA、SPR、分子对接等, 发现竹节香附素A通过靶向反式激活应答DNA结合蛋白-43 (transactive responsive DNA-binding protein 43, TDP-43), 促进树突状细胞介导的抗原交叉呈递, 增强T细胞活化, 引发肿瘤细胞免疫源性死亡。去分化是癌症细胞对免疫治疗产生耐药性的主要原因, Lei等[68]应用DARTS技术, 发现吴茱萸次碱通过靶向延胡索酸水合酶 (fumarate hydratase, FH) 诱导三阴性乳腺癌细胞分化。此外, 关于凋亡和铁死亡的最新研究为ICT联合治疗提供了新的靶点与思路。有学者通过荧光偏振技术 (fluorescence polarization, FP) 发现类黄酮通过靶向RIPK3, 减少坏死性凋亡相关炎症, 抑制肿瘤进展[33]。此外, Liu

等[69]使用SPR、CETSA、等温滴定量热法 (isothermal titration calorimetry, ITC) 等, 发现野菊中的新型愈创木内酯二聚体通过减轻沉默信息调节因子1 (silent information regulator 1, SIRT1) 介导的脂质积累和铁死亡来缓解肝脂肪变性, 进而抑制潜在的肝细胞癌。这些药物的开发为ICT联合治疗提供了新策略, 推动癌症治疗向精准化、个体化迈进。

上述研究采用的靶向垂钓技术在癌症精准医疗领域中发现了多种重要的生物标志物和治疗靶点, 深化了对关键癌症靶标功能的理解, 为开发新型靶向抗癌药物奠定了重要基础, 为癌症的精准化和个体化治疗提供了新方向。

3.2 在糖尿病研究中的应用: 降糖中药活性成分与机制挖掘

为阐明TCMs降糖的药效物质基础, 研究者常采用配体垂钓技术挖掘其潜在活性成分。蛋白酪氨酸磷酸酶1B (protein tyrosine phosphatase 1B, PTP1B) 是胰岛素增敏的潜在靶点。有研究团队[55]通过在大肠杆菌表面表达PTP1B, 结合酶动力学实验和分子对接, 从丹参水提物中筛选出PTP1B的非竞争性抑制剂丹酚酸B。α-葡萄糖苷酶 (alpha-glucosidase, α-GC) 在控制餐后血糖方面发挥重要作用。有学者[59]采用三氟甲基苯基双吖丙啶探针结合基于高分辨率微共焦拉曼光谱仪的光亲和微阵列 (high-resolution micro-confocal Raman spectroscopy photopatterned affinity microarray, HRMR-PM), 鉴定出参芪降糖颗粒中的3种α-GC抑制剂, 即人参皂苷Mb、刺芒柄花素和戈米辛D。针对参芪降糖颗粒, Xie等[36]利用表面增强拉曼光谱 (surface-enhanced raman spectroscopy, SERS) 及分子探针磁性纳米粒子, 发现其中黄芪甲苷IV、人参皂苷Rh2、人参皂苷Rg3和人参皂苷Rh1展现出与α-GC的强结合力。此外, Hu等[54]应用配体垂钓、分子对接和分子动力学模拟, 筛选出栽秧花中10个新型α-GC抑制剂。

此外, 现有研究也常采用靶标垂钓技术探索天然小分子与靶蛋白的相互作用, 以解析TCMs抗糖尿病活性成分的作用机制。基于PROTAC技术, 研究人员[70]结合Co-IP、CETSA等方法, 从雷公藤红素中发现了2个新靶点——切除修复交叉互补族6-样 (excision repair cross-complementing group 6 like, ERCC6L) 和蛋白磷酸酶2A癌性抑制因子 (cancerous inhibitor of protein phosphatase 2A, CIP2A), 并开发了第一个糖苷酶降解剂ZH-011。针对参芪降糖颗粒的活性机制研究, 科研团队[30]借助ABPP技术鉴定出参芪降糖颗粒的关键靶点为GNAS, 该靶点参与调控胰岛素分泌及cAMP信号通路, 并通过生物膜层干涉技术 (bio-layer interferometry, BLI) 筛选出五味子甲素等7种与GNAS强结合活性成分。除单一靶点鉴定外, ABPP技术还被用于信号通路解析: 研究人员[71]将其与Pull down、CETSA、DARTS、SPR、MST等技术结合, 证实白术内酯II通过靶向二酰甘油激酶θ (diacylglycerol kinase theta, DGKQ), 激活DGKQ-腺苷单磷酸激活蛋白激酶 (adenosine monophosphate-activated protein kinase, AMPK)-过氧化物酶体增殖物激活受体γ辅激活因子1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PGC1α)-解偶联蛋白1 (uncoupling protein 1, UCP-1) 信号传导, 从而改善肥胖诱导的胰岛素抵抗。在成分与靶点结合的直接验证方面, 溶剂诱导蛋白沉淀 (solvent-induced protein precipitation, SIP) 技术展现出优势: 利用该技术发现, 刺芒柄花素可与Keap1结合以防止细胞内ROS过量产生, 进而改善四氧嘧啶诱导的1型糖尿病。而电子圆二色谱 (electronic circular dichroism, ECD) 技术则为结构–功能关系研究提供了支撑[37], 科研人员[32]通过该技术发现, 金丝桃中双环聚戊二烯酰基苯三酚相关硫萜类化合物与死亡相关蛋白激酶相关凋亡诱导激酶2 (death-associated protein kinase-related apoptosis-inducing kinase 2, DRAK2) 结合, 并促进原代胰岛葡萄糖刺激的胰岛素分泌。

通过整合配体垂钓技术与靶标垂钓技术, 上述研究鉴定了PTP1B、α-GC等关键靶点及其抑制剂, 揭示了中药降糖活性成分及作用机制, 为糖尿病精准药物研发提供了新方向。

3.3 在心脑血管疾病研究中的应用: 心功能保护的靶向干预机制探索

心脑血管疾病是我国居民首位死亡原因[72], 早期症状隐匿且缺乏特异性诊断指标。在早期诊断标志物挖掘方面, 研究者通过开发新型荧光探针[73,74], 实现了对半胱氨酸和硒代半胱氨酸的实时监测, 为确定早期诊断生物标志物提供了新策略。除监测手段外, 靶点筛选与通路分析也成为研究重点。基于药物靶向筛选模型[53], 结合随机森林和支持向量机算法[75], 研究团队从4种保护心血管TCMs中筛选出109个靶点, 并富集到核因子κB (nuclear factor kappa-B, NF-κB)、肿瘤坏死因子 (tumor necrosis factor, TNF) 等关键通路。Ye等[76]采用ABPP技术结合人蛋白质微阵列、SPR、分子对接等, 发现雷公藤红素通过靶向心肌细胞中信号转导和转录激活因子3 (signal transducer and activator of transcription 3, STAT3) 抑制其转录活性, 从而减轻血管紧张素II诱导的功能和结构性心脏缺陷。

由于心脑血管疾病患者个体差异较大, 药物治疗

需兼顾精准性和安全性。针对心脏功能调节的精准靶点探索, Zhang 等[77]应用人蛋白质组芯片、分子对接等, 发现草质素通过靶向血清和糖皮质激素调节激酶 1 (serum and glucocorticoid-regulated kinase 1, SGK1) 并降低其磷酸化水平, 降低下游氧化应激信号传导和钙释放水平, 从而调节心脏功能。聚焦低毒性抗炎药物的开发, 科研人员[16]采用 CC-ABPP 技术, 结合 Pull down、CETSA、SPR 等方法, 从天然产物改造衍生物中筛选出一种苯并恶烷类化合物, 并发现该化合物通过靶向丙酮酸激酶 M2 (pyruvate kinase M2, PKM2) 发挥抗炎作用且毒性较小, 具有成药潜力。在缺血性脑卒中的治疗研究中, Su 等[78]结合 CETSA、DARTS 等技术证实, 光轴苎叶蓝中的 N-肉桂酰吡咯类生物碱 PB-1 通过靶向真核翻译延伸因子 1A1 (eukaryotic translation elongation factor 1 alpha 1, eEF1A1) 抑制神经炎症和氧化应激, 从而改善缺血性脑卒中。

通过创新诊断技术和靶向治疗研究, 上述研究揭示了中药活性成分对血管及心肌细胞中 NF-κB、STAT3 等关键靶点的调控作用, 为心脑血管疾病早期诊断、精准干预及并发症防治提供了科学依据, 推动了心脑血管疾病治疗的个性化和安全性发展。

3.4 在慢性呼吸系统疾病研究中的应用: 基于网络药理学的多靶点解析

慢性呼吸系统疾病主要包括肺纤维化 (pulmonary fibrosis, PF)、慢性阻塞性肺疾病 (chronic obstructive pulmonary disease, COPD) 和支气管哮喘 (bronchial asthma, BA) 等, 这些疾病均以慢性进展性和症状反复发作为特征, 晚期可导致肺功能严重受损。特发性肺纤维化 (idiopathic pulmonary fibrosis, IPF) 是 PF 的一种特定类型, 具有病因不明、预后较差等特点。借助 SPR 配体垂钓技术结合分子对接发现, 紫丹参萜醚靶向转化生长因子-β (transforming growth factor-β, TGF-β), 抑制氧化应激、细胞凋亡和胶原蛋白沉积, 从而改善肺纤维化[79]。还有研究采用网络药理学构建“成分−靶点−疾病”相互作用网络, 结合分子对接预测与体内外实验验证的系统方法, 层层解析治疗机制: 獐牙菜苦苷靶向赖氨酸氧化酶 (lysyl oxidase, LOX)、V 型胶原 α2 链 (collagen type V alpha 2 chain, COL5A2) 和结缔组织生长因子 (connective tissue growth factor, CTGF) 调控 IPF 进展[80]; 另有研究证实[81], 五味子乙素可能通过抑制低氧诱导因子-1α (hypoxia inducible factor-1α, HIF-1α) 的表达改善 IPF 病理状态; 血府逐瘀汤的关键活性成分是槲皮素, 可通过调节 JUN 原癌基因 (jun proto-oncogene, JUN) 和前列腺素内过氧化物合酶 2 (prostaglandin G/H synthase 2, PTGS2) 发挥治疗 IPF 的作用[82]。这些研究为 IPF 的治疗提供了新思路。

COPD 和 BA 都表现出气道炎症和气流受限等特征, 常面临药物研发缓慢和患者依从性差的挑战。聚焦 COPD 的糖皮质激素耐药问题, 研究人员基于网络药理学、分子对接与体内外实验的多层次体系发现, 调补肺肾方通过下调磷脂酰肌醇 3-激酶 (phosphatidylinositol 3-kinase, PI3K)-蛋白激酶 B (protein kinase B, AKT) 信号通路、促进糖皮质激素受体 α (glucocorticoid receptor α, GRα) 表达改善耐药状况[39]; Shi 等[83]综合运用 SPR 技术, 发现瓜蒌皮中的主要活性成分是木犀草素-7-*O*-β-*D*-葡萄糖苷、槲皮素-3-*O*-β-芸香糖苷和芹菜素-7-*O*-葡萄糖苷, 通过抑制 PI3K/AKT 和表皮生长因子受体 (epidermal growth factor receptor, EGFR)/STAT3 信号通路, 进而改善 COPD。在 BA 治疗研究中, 科研人员发现, 咳喘六味合剂中的槲皮素、木犀草素、山柰酚和汉黄芪苷等活性成分, 可通过作用于炎症、T 细胞分化信号通路的 16 个关键靶点改善重症哮喘[84]; 还有研究证实, 汉黄芩素是玉屏风的关键活性分子, 并可能通过 PI3K/AKT、白介素 17 (interleukin, IL-17) 和 TNF-α 等多个分子靶点和信号通路调控哮喘炎症[85]。

尽管 TCMs 在慢性呼吸系统疾病治疗中展现出潜力, 但其机制研究水平仍相对有限, 目前多集中于网络药理学预测和活性成分的初步解析, 这可能与其依赖复方制剂的特点密切相关。因此, 亟需开发更先进的现代技术手段深入挖掘分子靶点, 为 TCMs 复方在慢性呼吸系统疾病治疗中的精准化、现代化和国际化提供科学依据。

4 结语与展望

在慢性非传染性疾病防治领域, TCMs 凭借其低毒副作用及整体调节的优势备受瞩目。然而, TCMs 多成分、多靶点的作用特性与现代医学单靶点药物研发模式存在本质差异, 导致其在临床应用中面临药效物质基础不明、作用机制不清等关键科学问题。随着分子生物学技术的突破, 基于“靶点发现−验证结合−效应确证”的系统研究策略, 为揭示 TCMs 复杂体系的科学内涵提供了新范式。

TCMs 治疗四大慢病的物质基础及靶点研究已形成多技术整合与机制创新的研究范式, 通过配体垂钓技术和靶标垂钓技术, 系统揭示了 TCMs 活性成分在肿瘤细胞凋亡调控、胰岛素信号通路激活、炎症网络抑制及纤维化通路干预等多个病理进程中的多靶点协同作用机制。本领域的研究突破主要体现在精准识别疾病关键调控靶点, 解析复方药物多成分−多通路整合

效应, 以及构建中西医结合的靶向治疗新策略。值得注意的是, TCMs复方的协同效应可能是多个靶点通过某种信号串联共同产生活性, 也可能是多成分在神经内分泌轴的多个靶点上产生作用, 如下丘脑–垂体–肾上腺轴和下丘脑–垂体–卵巢轴[86-88]。未来, 需进一步提升靶向垂钓技术的精准性与适用性, 深化TCMs多成分–多靶点相互作用研究, 突破复方成分复杂性解析瓶颈, 加速TCMs活性成分结构优化与新型制剂研发, 为慢病精准防控提供新型解决方案。

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