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·综述·

烟曲霉耐药机制及中药单体干预作用的研究进展*

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【摘要】 侵袭性曲霉病(invasive aspergillosis,IA)是感染曲霉菌引发的疾病,烟曲霉是常见的引起IA和过敏性疾病的曲霉属物种,主要通过掩蔽其表面分子、调节免疫反应等级制来逃避和适应宿主,临床大剂量使用抗真菌药物,导致耐药菌株数量在不断增加,给临床治疗带来困难。近年来,中药因其毒副作用小、耐药率低等原因,在抗真菌治疗方面有一定优势。因此研究烟曲霉菌的耐药机制并探讨中药单体干预作用,对寻找烟曲霉耐药机制相关靶点及中药抗真菌研究、减少耐药菌株形成具有重要意义。

【关键词】 烟曲霉;耐药机制;中药单体;综述

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Research progress on drug resistance mechanism of *Aspergillus fumigatus* and the intervention effect of herbal monomer

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【Abstract】 Invasive aspergillosis (IA) refers to a variety of diseases caused by *Aspergillus*, and *Aspergillus fumigatus* is the most common species of Aspergillus causing IA and allergic diseases. At the same time, the clinical use of large doses of antifungal drugs, resulting in the number of drug-resistant strains is increasing, bringing difficulties to clinical treatment. In recent years, traditional Chinese medicine has had some advantages in antifungal treatment because of its low toxic side effects and low drug resistance rate. Therefore, studying the drug resistance mechanism of *A. fumigatus* and exploring the effect of the monomeric intervention of traditional Chinese medicine is important for finding the targets related to the drug resistance mechanism of *A. fumigatus* and the antifungal research of traditional Chinese medicine and reducing the formation of drug-resistant strains.

【Key words】 *Aspergillus fumigatus*; drug resistance mechanism; monomer of traditional Chinese medicine; review

***曲霉属是一类分布广泛的真菌属,包括300多种不同的物种,曲霉感染称为侵袭性曲霉病(invasive aspergillosis,IA),包括导致过敏反应的感染,如过敏性曲霉鼻窦炎[1]或过敏性支气管肺曲霉病[2],皮肤感染导致皮肤曲霉病[3],曲菌瘤和慢性疾病如慢性肺曲菌病[4]。IA主要影响免疫功能低下患者,例如癌症患者、接受皮质醇治疗和器官移植者[3,5],但少数情况下亦可有免疫正常患者[6-7]。IA已成为现代真菌学的主要临床问题之一,其中烟曲霉是最常见的引起IA和过敏性疾病的曲霉属物种。近些年来,烟曲霉耐药菌株数量不断增加,抗真菌药物的疗效也随之降低,中药单体可以通过多途径抑制烟曲霉生物膜形成、减少烟曲霉毒力,改善病理损伤。现就烟曲霉耐药机制及中药单体干预相关研究予以综述,以期为烟曲霉耐药的中医药研究提供新途径。

1 烟曲霉耐药机制

近年来,随着免疫功能受损患者的增多,IA的发病率随之增加,尽管已经对该病早期诊断和治疗方面研究有所进展,但IA的死亡率仍然较高。未经治疗时的死亡率为80%~95%[8],用伏立康唑(VRC)或两性霉素B(Amphotericin B,AmB)治疗12周后的死亡率分别为29.2%和42.1%[9]。其原因包括宿主免疫缺陷难以抵御真菌、IA早期诊断困难以及耐药菌株的出现。烟曲霉作为最常引起IA的曲霉属菌,研究其耐药机制有助于临床治疗IA,降低患者死亡率。目前常用抗真菌药物有唑类、多烯类和棘白菌素类。

1.1 唑类 唑类通过抑制甾醇14α-去甲基酶(sterol 14α-demethylase,Cyp51)蛋白引起羊毛甾醇积聚及麦角甾醇缺乏从而发挥固有的抗真菌活性,在此基础上,烟曲霉主要耐药机制可分为、Cyp51蛋白突变、外排泵上调、细胞应激反应、HMG-CoA编码基因突变和生物膜形成。除此之外,细胞色素氧化酶也被认为是烟曲霉的耐药机制之一[10-11]。

1.1.1 Cyp51蛋白 唑类耐药机制的研究中,最常检测到的突变与靶蛋白Cyp51A有关。麦角甾醇是微生物细胞膜的重要组成成分,对确保细胞活力、膜的流动性、膜结合酶的活性、

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膜的完整性以及细胞物质运输等起着重要作用[12]，该酶参与麦角甾醇生物合成和甾醇代谢，在烟曲霉中起重要作用。Cyp51A拥有同源蛋白Cyp51B，这两种蛋白对于烟曲霉生长不是必须的[13-14]，但失活是是致命的[15]，唑类与Cyp51蛋白相互作用并抑制Cyp51蛋白[16]，反过来又降低麦角甾醇含量并破坏细胞中的甾醇代谢[17]，从而发挥杀菌作用。

1.1.1.1 Cyp51蛋白单点突变 Cyp51A基因由于蛋白内氨基酸置换所引起的单点突变是烟曲霉主要耐药机制，该突变会改变Cyp51的结构、稳定性和功能性，进而阻碍底物识别，最终导致唑耐药[16,18]。迄今为止，由G54、Y121、G138、P216、F219、M220、A284、Y431、G432、G434和G448位氨基酸取代组成的烟曲霉Cyp51A已被发现与产生唑耐药有关[19-33]。在没有Cyp51B的情况下，Cyp51A蛋白表达增加，反之亦然，表明一种蛋白质在蛋白质水平上补偿另一种蛋白质缺失的能力。在唑类耐药性背景下，Cyp51A对唑类敏感性的影响大于Cyp51B的影响[34]。Handelman等[35]研究发现在耐药临床分离株中鉴定出的G457S Cyp51B突变的引入会导致野生型受体菌株中对VRC产生唑耐药。

1.1.1.2 Cyp51过表达另一种被认为导致烟曲霉产生耐药性的机制是Cyp51的过表达。当转录因子和转录激活物接触到唑类化合物进而导致Cyp51转录上调。在烟曲霉Cyp51A启动子区存在串联重复序列(tandem repeats，TRs)：34 bp(TR34)、46 bp(TR46)或53 bp(TR53)的情况下，该基因的表达增强[36-39]。

由于甾醇生物合成受到高度调控，许多编码该途径酶的基因在其启动子区含有甾醇调节结合元件[40]。与烟曲霉Cyp51启动子结合并调节其表达的转录因子(transcription factors，TF)，如SrbA、HapE和AtrR。CCAAT结合复合物(CCAAT-binding complex，CBC)是重要的一般转录调节因子，CBC-HapX复合物是铁稳态的主要调节剂，Alexander Kühbacher等发现破坏铁调节剂HapX的结合会增加cyp51A表达[41]。此外，细胞色素b_5-CybE可调节烟曲霉Cyp51A的转录水平，CybE的缺失导致Cyp51A的代偿性上调[42]。

1.1.2 外排转运蛋白 多药物外排泵由跨膜蛋白组成，其介导抗菌分子或有毒化合物和内源性代谢物向细胞外空间的主动排出[43]。因此，外排活性是耐药性和真菌存活的决定性因素。目前有两种已知的外排转运蛋白超家族：ATP结合盒(ATP-binding cassette，ABC)和主要协同蛋白超家族(major facilitator superfamily，MFS)转运蛋白。烟曲霉基因组中鉴定出45个ABC和275个MFS转运蛋白[44]，但是只有少数被鉴定为药物蛋白。ATP结合盒是由两个跨膜结构域和两个细胞质核苷酸结合结构域组成，通过ATP水解产生的能量将底物排出，MFS超家族利用质膜上质子梯度动力排出药物[45-46]。

烟曲霉含有至少49个编码ABC转运蛋白的基因[47]。其中，Cdr1B被鉴定为在唑类耐药菌株中过表达，并且删除此类菌株中的Cdr1B基因导致对伊曲康唑的敏感性增加[48]，其他删除Cdr1B基因的菌株显示出唑类超敏反应[49]。关于MFS转运蛋白目前研究仅有mfsA、mfsB和mfsC被证实在烟曲霉唑类药物暴露期间上调[50]。

1.1.3 细胞应激反应 应激相关蛋白在应激条件下通过这些蛋白质控制基因表达从而产生耐唑性。热休克蛋白90(heat shock protein 90，Hsp90)是一种重要的分子伴侣蛋白，通过与其催化亚基相互作用来激活钙调磷酸酶，以调节应激反应，包括烟曲霉中唑诱导的应激。抑制Hsp90或钙调神经磷酸酶会增加烟曲霉对唑类的敏感性[51-52]。

损伤抗性蛋白家族(the damage resistance protein，Dap)同样会对唑类产生应激反应，该家族由DapA、DapB、DapC组成，有研究表明，DapA的缺失增加了唑的敏感性，因此其可能与唑抗性有关[53-54]。

Ca^{2+}作为第二信使在细胞功能调节中起重要作用，由Ca^{2+}通道蛋白、钙泵、Ca^{2+}转运蛋白和许多相关蛋白质组成的钙信号通路在真菌中起着调节作用。Crz1是钙信号通路下游的转录因子，参与调节细胞存活、离子稳态、感染结构发育、细胞壁完整性和毒力。Crz1的缺失会增加对唑类用药物的敏感性[55]。

1.1.4 HMG-CoA编码基因突变 研究发现3-羟基-3-甲基戊二酰辅酶A(HMG-CoA)还原酶编码基因Hmg1的突变导致对所有临床可用的唑类药物的耐药性显著增加。Gonzalez等[56]实验证明唑耐药与Cyp51B(G457S)和Hmg1(F390L)的修饰有关。在烟曲霉中，除了Hmg1外，还存在第二种HMG-CoA还原酶Hmg2，目前尚未有研究证明其与耐药有关，可能是未来研究的新方向[57]。

1.1.5 生物膜形成 生物膜指的是附在物体表面被细菌胞外大分子包裹的有组织的细菌群体。生物膜形成是烟曲霉耐药的重要机制，生物膜使得烟曲霉对抗真菌药物的敏感性降低。成熟生物膜中达到的细胞密度可能会阻碍药物渗透，因为它的疏水性与菌丝紧密结合[58]。

1.1.6 细胞色素氧化酶 研究发现一种新的细胞色素C氧化酶cox7c，其缺失或突变明确导致对唑类、多烯类和烯丙胺药物的耐药性[59]。

1.2 多烯类 多烯是第一种上市广谱抗真菌药物，但是由于其显著的肝肾毒性使得AmB等多烯类药物颇具争议。多烯抗真菌的关键是与麦角甾醇的结合[60]。该类药物与真菌细胞膜上的麦角甾醇结合，使细胞膜上形成微孔，改变细胞膜的通透性，从而引起细胞内小分子和离子外渗；随着其浓度的增高，大分子也可通过细胞膜外渗，导致细胞内成分不可逆的丢失，而致真菌死亡[61]。AmB暴露会诱导烟曲霉中细胞内活性氧(reactive oxygen species，ROS)的产生和积累，从而导致氧化损伤[62]。

多烯耐药性是由麦角甾醇生物合成基因的功能缺失突变引起的(特别是在曲霉属和念珠菌属)，在曲霉属中，只有土曲霉对AmB天然耐药[63]，其余发生耐药反应比较少见，而烟曲霉有43%～96.4%的概率产生AmB抗性[64-65]。烟曲霉多烯类耐药机制目前尚不明确，Fan等[66]研究确定了34个与AmB MIC差异相关的候选单核苷酸多态性(single-nucleotide polymorphisms，SNP)，包括18个基因间变异，14个错义变异，1个同义变异和1个非编码转录本变异，有助于快速筛选烟曲霉抗AMB基因。

1.3 棘白菌素类 真菌细胞壁成分包括1，3-β-D-葡聚糖、1，4-β-D-葡聚糖、1，6-β-D-葡聚糖、α-葡聚糖、甲壳素、甘露聚糖和多种糖蛋白，葡聚糖是细胞壁的重要结构成分，在保护环境、控制

渗透压、菌丝形态发生和宿主组织中的侵袭性方面起着至关重要的作用[67]。并且，1,3-β-D-葡聚糖在动物细胞中不存在，因此它是抗真菌抗生素的优选靶标[68]。1,3-β-D-葡聚糖合成酶是由至少两个亚基组成的跨膜异构糖基转移酶，其中 Fks1p 亚基(由 Fks1，Fks2 和 Fks3 基因编码)具有催化功能，而 Rho1p 亚基(属于 GTP 酶家族)具有调节功能。棘白菌素与该酶的 Fks1p 亚基非竞争性结合抑制其活性[3,69]，从而抑制 1,3-β-D-葡聚糖合成酶，影响烟曲霉细胞壁合成，使其生长过程中细胞壁葡聚糖缺乏、渗透压失常而最终导致细胞溶解[70]。在 3 大抗真菌药物类别中，棘白菌素对曲霉菌属药效最低，但是对比唑类和 AmB，棘白菌素的优势在于较少的药物相互作用和相关毒性。

1.3.1 葡聚糖合酶催化亚基突变 棘白菌素耐药性的研究涉及葡聚糖合酶(FKS)亚基的突变。编码该亚基的三个基因是已知的：FKS1、FKS2 和 FKS3。烟曲霉的突变发生在葡聚糖合酶的 *A. fumigatus* FKS1 基因中，随着几丁质产生的增加，烟曲霉对棘白菌素产生耐药性[71]。在 Fks1 中具有 S678Y 或 S678P 突变的实验室烟曲霉菌株具有与三种棘白菌素类药物相关表型的耐药性，从而证明其耐药[72-73]。

1.3.2 钙调磷酸酶途径 曲霉菌属在适应棘白菌素后会产生应激补偿机制，这种机制被称为矛盾效应(Paradoxical effect，PE)[74]，指的是真菌随着药物浓度升高敏感性下降，甚至产生耐药。热休克蛋白 90(Hsp90)和 70(Hsp70)是重要的分子伴侣，当卡泊芬净达到一定浓度作用于烟曲霉产生应激反应时，Hsp90 和 Hsp70 可能通过协同作用以控制钙调磷酸酶途径产生 PE。PE 的关键适应机制与 1,3-β-葡聚糖合酶活性的恢复有关。1,3-β-葡聚糖的合成通过 1,3-β-葡聚糖合酶复合物在细胞膜上进行，0.5 μg/mL 浓度下，Fks1 从菌丝尖端错误定位到液泡。然而，仅连续暴露于 4 μg/mL 卡泊芬净 48 h 就会使菌丝形态恢复正常，Fks1 重新定位到菌丝尖端。但是用两种浓度卡泊芬净处理后，Rho1 仍保留在菌丝尖端，用法尼醇处理烟曲霉会导致生长菌丝顶端的 Rho1 和 Rho3 定位错误，通过这种途径阻断细胞壁完整性[75]，说明 Rho1 对于 PE 是必需的[76]。

1.3.3 细胞壁完整性 烟曲霉生物膜内的菌丝含有细胞外基质(extracellular matrix，ECM)和多糖半乳糖氨基半乳聚糖(galactosaminogalactan，GAG)，GAG 是烟曲霉生物膜的主要粘附因子以及免疫调节化合物，当烟曲霉处于缺氧环境时，丙氨酸氨基转移酶(alanine aminotransferase，alaA)的 mRNA 水平大幅增加，alaA 的缺失导致 GAG 粘附功能的改变，进而导致细胞壁变化和生物膜对棘白菌素的敏感性增加[77]。卡泊芬净导致的细胞壁应激反应会诱导生物膜形成，且调节 GAG 和细胞壁多糖的生物合成的转录因子 SomA 下调会导致严重的生物膜形成缺陷和对细胞壁应激源的超敏反应[78]。

2 中药单体对烟曲霉的干预作用

目前中药单体抗真菌的研究备受关注，中药单体具有获取便利，耐药率低，毒副作用小等优点。对于治疗烟曲霉耐药具有独特优势，中药单体可以靶向烟曲霉生物膜，破坏细胞壁的连续性，减弱烟曲霉毒力，改善病理损伤等，还可与临床常用抗真菌药物联用，防止耐药。

2.1 肉桂醛 肉桂醛又叫桂醛，主要是从桂皮、桂叶和桂枝中提取出来的一种芳香醛类有机化合物，对细菌和真菌具有较强的抑制作用，并具有抗生物膜活性[79-80]，对临床分离的烟曲霉具有抗菌活性[81]，邬丽红等[82]研究表明肉桂醛作用于已经成熟的烟曲霉菌生物膜后，生物膜的致密结构变松散，菌丝表面及菌丝之间胞外基质被清除，且在高浓度时部分菌丝被裂解，提示肉桂醛防止烟曲霉生物膜的形成或者破坏已经形成的生物膜结构，使得药物能够克服生物膜屏障渗透到烟曲霉细胞内达到有效浓度而发挥抗菌作用。然而肉桂醛在水溶液中的溶解度差、不稳定和挥发性[83]。因此，应在未来的研究中进一步考虑使用不同的给药系统以解决这些局限性。

2.2 柠檬醛 柠檬醛是中药山苍子的主要成分，属萜类化合物，龙凯等[84]研究发现柠檬醛能够在体外有效抑制烟曲霉。罗闵丹等[85]研究发现中、高剂量柠檬醛能够有效阻止烟曲霉孢子在肺组织的萌发和菌丝生长，且与烟曲霉毒力密切相关的烟灰色色素明显减弱，进而改善 IPA 模型小鼠各器官的病理损伤。

2.3 大蒜素 大蒜素(二烯丙基硫代硫酸盐)是新鲜压碎的大蒜的活性化合物之一。大蒜素具有抗菌、抗炎、抗血栓、抗动脉粥样硬化、降血脂和抗癌等多种生物活性。大蒜提取物对新生隐球菌[86]、念珠菌和曲霉菌均具有体外抗菌活性[87]。Shadkchan 等[88]研究发现纯大蒜素可能是一种有效的体外杀菌剂，时间杀伤研究表明，纯大蒜素在给药后 2～12 h 内发挥杀菌活性，大蒜素治疗显著延长了感染小鼠的存活时间，以前的研究认为即使是最有效的大蒜素治疗也不如 AmB 治疗成功，但是通过化学合成的大蒜素在直接接触时，通过气相对选定对真菌具有体外杀菌活性，其浓度与临床使用的 AmB 相当[89]。大蒜素缺点主要是在血液、溶剂和模拟生理液中的半衰期很短(50min)[90]，且在培养过程中，大蒜素对哺乳动物的毒性明显高于曲霉属，因此需要进行更多的研究以找到最佳的治疗方式。

2.4 小檗碱 小檗碱是从几种草药中分离出来的生物碱，具有多种药理作用，包括抗菌、抗糖尿病和抗癌活性。高磊[91]研究发现小檗碱使菌丝和孢子形态发生畸变；透射电子显微镜观察可见小檗碱能破坏烟曲霉细胞壁的连续性，使细胞膜部分缺失，细胞器内容物减少，AmB 与小檗碱联合用药可以通过降低 AmB 药量的方式降低 AmB 毒副作用。

3 小结

本文探讨了烟曲霉的 3 大类抗菌药物耐药机制，以及中药单体干预作用。目前，由于其耐药机制复杂，还需对抗菌药物调节因子、多种抗真菌药物相互作用、生物膜形成机制、耐药靶点、转录因子等进行探索。生物膜形成是烟曲霉主要耐药原因之一。肉桂醛、柠檬醛能够调节生物膜形成，破坏细胞壁的连续性，增加烟曲霉对抗真菌药物敏感性，同时降低烟曲霉毒力，改善宿主病理损伤与抗真菌药物联用可以降低毒副作用。大蒜素可制成雾化剂与 AmB 联用治疗肺真菌病。中药单体主要的功能不是杀死烟曲霉而是抑制其产生耐药性和辅助抗真菌药物，可为研发治疗由耐药菌株引起的感染，以抑制耐药的发生的新药理论基础。

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