

丹参酮和丹酚酸类化合物的生物合成及其转录调控机制

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摘要: 丹参酮和丹酚酸类化合物是治疗冠心病、心肌梗死、高血压、高血脂、脑卒中等疾病的重要物质基础。近年来, 随着基因组学、转录组学、代谢组学和生物信息学的快速发展, 其生物合成途径和转录调控机制研究取得较大进展。文章在总结丹参酮和丹酚酸类化合物生物合成最新研究的基础上, 着重综述了转录因子对其生物合成的调控作用, 认为进一步厘清丹参酮和丹酚酸类化合物的生物合成途径 (尤其是下游合成途径的研究) 和不同家族转录因子的相互作用、信号响应和协同调控机制, 对于挖掘其合成、转运、调节、修饰相关的新基因及揭示调控网络的分子机制十分必要; 同时, 根据丹参活性成分生物合成规律, 人工设计并构建新的、具有特定生理功能的生物系统, 从而大幅度提高合成酶基因的表达及活性物质的产量, 是有待进一步深入研究的方向之一。

关键词: 丹参酮类化合物; 丹酚酸类化合物; 生物合成途径; 转录因子

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Biosynthesis and transcriptional regulation of tanshinones and salvianolic acids

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Abstract: Tanshinones and salvianolic acids are important materials in the treatment of coronary heart disease, myocardial infarction, hypertension, hyperlipidemia, stroke and others illnesses. In recent years, with the development of genomics, transcriptome, metabolomics and bioinformatics, many advances have been made in the biosynthesis and transcriptional regulation of tanshinones and salvianolic acids. This paper summarizes these advances and suggests further study on the downstream synthesis pathways and transcriptional regulatory mechanisms to reveal new molecular mechanism of synthesis, transport, regulation and modification. Additionally, we discuss the design and construction of new biological pathways to increase the expression of biosynthesis genes and the production of secondary components, is a newly developing research field.

Key words: tanshinone; salvianolic acid; biosynthesis pathway; transcription factor

中药丹参为唇形科 (Labiatae) 鼠尾草属 (*Salvia*) 植物丹参 (*Salvia miltiorrhiza* Bge.) 的干燥根及茎, 含有丰富的丹参酮类化合物, 如丹参酮I (tanshinone I)、丹参酮IIA (tanshinone IIA)、丹参酮IIB (tanshinone IIB)、二氢丹参酮I (dihydrotanshinone I)、隐丹参酮 (cryptotanshinone) 等 (图1) 和丹酚酸类化合物, 如丹参素 (3,4-

dihydroxyphenyllactic acid)、咖啡酸 (caffeic acid)、迷迭香酸 (rosmarinic acid)、丹酚酸A (salvianolic acid A) 和丹酚酸B (salvianolic acid B) 等 (图2), 具有活血化瘀、消肿止痛、养心安神之功效, 是治疗冠心病、心肌梗死、高血压、高血脂等疾病的主要物质基础^[1]。然而天然资源中丹参酮和丹酚酸类化合物含量极低, 且其累积与地理位置、生态环境及遗传背景有关, 也受诱导子、关键酶、调节基因或转录因子的调控。为充分利用生物技术方法, 有效提高丹参酮和丹酚酸等药效成分含

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量,近年来,测序和解析了丹参全基因组、叶绿体和线粒体^[2,3],发表了20多个来自丹参不同器官及生长阶段的转录组数据^[4,5],以及克隆与鉴定了大部分关键酶基因^[6,7],为阐明丹参酮和丹酚酸类化合物的生物合成途径,揭示转录因子单独或协同调控机制作出了极大贡献。前人已经对丹参酮和丹酚酸类化合物的生物合成、诱导子的诱导与调控等进行了详细的总结^[6-8],本文主要介绍该类化合物生物合成的最新研究,着重综述转录因子的调控作用。

1 丹参主要药效成分的生物合成研究

1.1 丹参酮类化合物的生物合成

丹参酮类化合物属于二萜类,主要在丹参根部周皮合成和积累,迄今已分离到50余种。其生物合成包括牻牛儿基牻牛儿基焦磷酸(GGPP)合成、GGPP环化和次丹参酮二烯修饰等3个阶段(图3)。GGPP的合成与其他天然二萜类基本一致,即甲羟戊酸途径(MVA)合成的异戊烯基焦磷酸(IPP)与甲基赤藓醇4-磷酸途径(MEP)合成的二甲基烯丙基焦磷酸(DMAPP)在法呢基焦磷酸合酶(SmFPPS)、牻牛儿基牻牛儿基焦磷酸环化酶(SmGGPPS)作用下生成GGPP。但不同于其他天然二萜类的是丹参酮生物合成中,GGPP的合成前体主要来源于MEP途径^[9]。GGPP环化阶段,先由柯巴基焦磷酸合酶(SmCPS)催化GGPP转化成柯巴基焦磷酸(CPP),再由类贝壳杉烯合酶(SmKSL)将CPP环化成次丹参

酮二烯(miltiradiene)^[10,11]。在此过程中,SmCPS的作用非常重要,直接决定次丹参酮二烯碳架结构的形成。抑制SmCPS1的表达,丹参酮IIA和丹参酮I的合成显著下降^[10]。而SmKSL之所以能催化CPP生成次丹参酮二烯,这一有别于裸子植物中紫杉醇、银杏内酯等生物合成过程的新功能,得益于其编码基因SmKSL在进化过程中缺失了‘internal/γ’结构域^[11]。次丹参酮二烯的修饰,主要包括细胞色素P450家族蛋白SmCYP76AH1催化次丹参酮二烯生成铁锈醇(ferruginol)^[12],SmCYP76AH3羟化铁锈醇的C-7和C-11位生成11-羟基铁锈醇(11-hydroxy ferruginol)、柳杉酚(sugiol)和11-羟基柳杉酚(11-hydroxyl sugiol),再由SmCYP76AK1羟化11-羟基铁锈醇和11-羟基柳杉酚的C-20生成11,20-二羟基铁锈醇(11,20-hydroxy ferruginol)和11,20-二羟基柳杉酚(11,20-hydroxyl sugiol),由于11,20-二羟基柳杉酚不稳定,可自氧化成10-羟甲基四氢丹参酮(10-hydroxymethyl tetrahydromiltirone)^[13],最后在其他CYP450氧化酶、脱羧酶、脱氢酶、还原酶的催化下形成丹参酮IIA、隐丹参酮等活性成分^[14]。CYP450s是萜类骨架物质氧化修饰的主要酶类,能将C-H键氧化,使之成为可进一步转化的羟基。前期通过cDNA芯片^[15]、基因组^[2]和转录组^[5]等筛选或预测到SmCYP450s 500余个,其中33个在丹参根周皮显著高表达,尤其是SmCYP76亚家族成员与丹参酮的下游合成途径

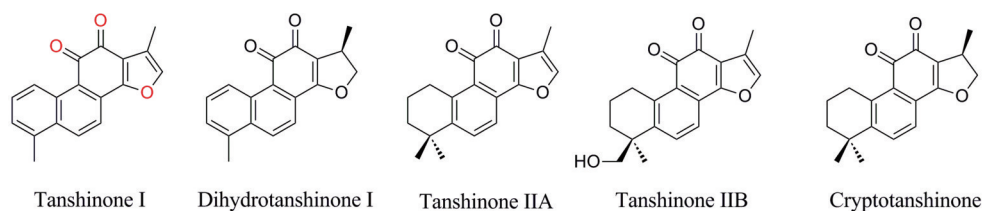


Figure 1 Representative compounds of tanshinones

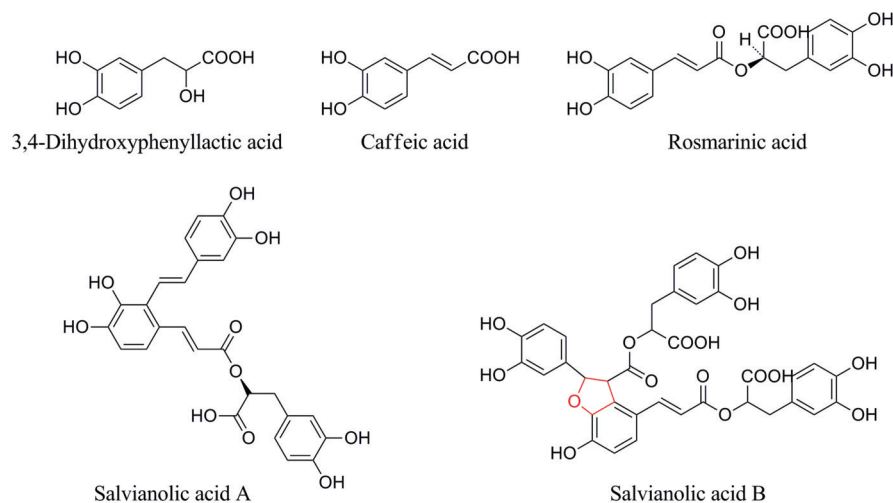


Figure 2 Representative compounds of phenolic acids

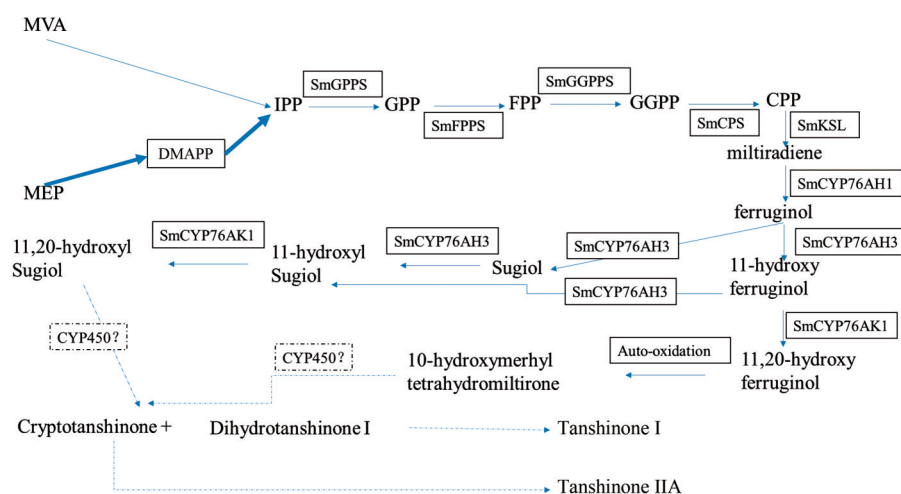


Figure 3 A schematic of the tanshinone biosynthetic pathway

关系密切^[16]。目前, *SmCYP76AH1*、*SmCYP76AH3*、*SmCYP76AK1* 已得到功能验证, 推测 *SmCYP71D375* 在丹参新酮合成的上游区域起作用, 分别从不同通路合成隐丹参酮和二氢丹参酮^[17]。

1.2 丹酚酸类化合物的生物合成 丹酚酸类化合物属于酚酸类, 主要在丹参的韧皮部和木质部中合成和积累, 迄今已分离到30余种。从结构上看, 大多数丹酚酸类化合物可视为咖啡酸的衍生物, 如迷迭香酸 (rosmarinic acid, RA) 为咖啡酸与丹参素的二聚物, 丹酚酸 B 为迷迭香酸二聚体, 丹酚酸 A 由1分子丹参素与2分子咖啡酸缩合而成 (图2)^[18]。因此, RA 可能是大多数丹酚酸类化合物生物合成的共同前体。目前认为, 丹参中 RA 的合成有3种可能, 但均起源于苯丙烷代谢途径的两条平行支路。传统认为, RA 由苯丙氨酸 (*L*-phe) 支路生成的4-香豆酰 CoA (4-coumaroyl-CoA) 和由酪氨酸 (*L*-tyr) 支路生成的4-羟基苯乳酸 (4-hydroxyphenyllactic acid) 在迷迭香酸合成酶 (*SmRAS*) 催化下生成2-氧-(4-香豆酰)-3-(4-羟基苯)-乳酸 (4-coumaroyl-4'-hydroxyphenyllactic acid), 然后由细胞色素 P450 蛋白 CYP98A14 催化生成 RA^[19]。但 Di 等^[20,21] 认为, 苯丙氨酸支路中4-香豆酸 (4-coumaric acid) 先被转化为咖啡酸 (caffeic acid), 然后咖啡酰 CoA (caffeoyl CoA) 与4-羟基苯乳酸在 *SmRAS* 催化下生成咖啡酰-4-羟基苯乳酸 (caffeoyl-4'-hydroxyphenyllactic acid), 再经 CYP98A14 转化成 RA, 才是 RA 合成的主要路线。而酪氨酸支路的4-羟基苯乳酸经丹参素与4-香豆酰 CoA 在 *SmRAS* 催化下生成4-香豆酰-3',4'-二羟基苯乳酸 (4-coumaroyl-3',4'-dihydroxyphenyllactic acid) 后, 由 CYP98A14 催化形成 RA 是一条衍生路线 (图4)。相对于 RA 的合成, 丹酚酸下游生源途径还没有完全明确。

Di 等^[21]、Li 等^[22,23] 认为丹酚酸 B 是由两分子 RA 在漆酶 (lactase) 等氧化酶的作用下生成苯环2位自由基和 α 位自由基, 然后二聚化反应直接生成或经丹酚酸 E (salvianolic acid E) 转化而成。然而, 综合 Ag^+ 和茉莉酸甲酯 (MeJA) 诱导后丹参毛状根中 RA 和丹酚酸 B 含量的变化情况^[24], 过表达 *SmRAS* 仅能提高迷迭香酸的含量却无法提高丹酚酸 B 的含量, 且丹酚酸 B 的主要降解产物是丹参素, 并测得合成过程中间产物—紫草酸 (lithospermic acid)^[25]; 以及 MeJA 和真菌提取物 (YE) 处理后酪氨酸支路上关键酶基因的响应与丹酚酸 B 合成的相关性等研究^[26,27] 表明, 丹酚酸 B 也有可能由咖啡酸与 RA 结合后生成紫草酸, 然后由紫草酸与丹参素自发酯化而成 (图4)。

2 丹参主要药效成分生物合成的转录调控研究

丹参酮和丹酚酸类化合物的生物合成受 YE、 Ag^+ 、MeJA、水杨酸 (SA)、脱落酸 (ABA)、赤霉素 (GA) 和乙烯 (Eth) 等生物及非生物诱导子的诱导^[1,28], 其机制之一就是转录因子激活或抑制合成途径关键酶基因的表达以调控次生代谢产物的合成。

2.1 JAZ 蛋白与 bHLH 转录因子家族 茉莉酸类物质 (JAs) 通过调控 JA 信号通路相应的转录因子进而调控丹参酮和丹酚酸类化合物的合成^[29], 其介导的转录调控核心模型是 SCF^{COI1}-JAZs-MYC2 复合体。即正常生长的植物内源 JAs 水平较低, JAZ (jasmonate-ZIM domain) 蛋白与 MYC2 等转录因子结合, 抑制茉莉酸早期响应基因的表达。当植物内源 JAs 或 JA-异亮氨酸复合物 (JA-Ile) 含量增高时, JAZ 蛋白与 E3 泛素连接酶 SCF^{COI1} 复合物相互作用, 并导致其自身被降解, 释放出 MYC2 等转录因子激活下游 JAs 应答基因的表达^[30]。

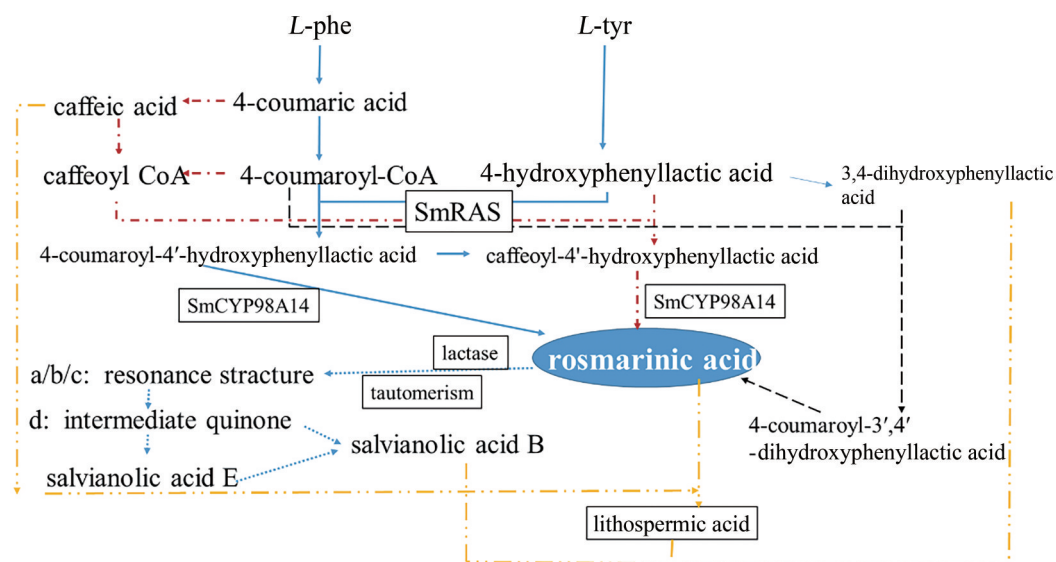


Figure 4 A schematic of the salvianolic acid biosynthetic pathway

从丹参中分离的9条 *SmJAZ* 基因均受 MeJA 诱导, 过表达 *SmJAZs*, 则 *SmRAS1*、*SmCYP98A14* 等基因的表达量下降, 负调控丹酚酸B的合成。与丹酚酸不同的是, 仅 *SmJAZ3/4/8* 负调控丹参酮的合成, 而 *SmJAZ1/2/5/6/9* 则起正调控作用, 其中 *SmJAZ2/4/9* 通过调控 *SmCPS1*、*SmCYP76AH1* 的表达来实现调控功能^[31]。*SmJAZs* 转录因子家族中, *SmJAZ8* 的作用较为特殊, 因其缺失 JAs 结构域而无法与 COI1 蛋白结合, 主要通过 N 端的两性抑制基序 (EAR) 起稳定抑制转录因子的活性, 抑制 *SmCPS1*、*SmCYP76AH1* 及处于 *SmCYP98A14* 下游酶基因的表达, 并参与 JAs 信号诱导的丹参初生代谢和次生代谢的负调控作用^[31]。过表达 *SmJAZ8*, 能显著抑制 *SmJAZ1/4/5/6/10* 的表达, 甚至产生对 JA 不敏感的丹参表型, 而 *SmJAZ8-RNAi* 则提升家族其他成员的表达量^[31], 是丹酚酸合成过程中较为重要的负调控因子。

SmJAZ 作为 JA 信号通路中负调控茉莉酸信号从 SCF^{COI1} 复合物向下游应答基因传导的核心蛋白, 其启动子区域含有能与 *SmMYC2* 转录因子结合的元件^[32,33]。*MYC2* 属于 bHLH (basic helix-loop-helix transcription factors) 转录因子家族, 含有高度保守的碱性螺旋-环-螺旋结构域, 其 N 端的碱性区域能识别 DNA 上的 E-box 和 G-box 位点, C 端 HLH 结构域形成的二聚体或四聚体能与靶基因的启动子结合, 是调控 JA 信号通路基因表达的另一核心成员。研究表明: 正常情况下, *SmMYC2a/2b* 与 *JAZ1/2* 结合, 调控功能被抑制。当内源 JAs 或 JA-Ile 含量升高时, 释放出的 *SmMYC2a* 激活 *SmHCT6*、*SmCYP14*; *SmMYC2b* 激活 *SmCYP14*; *SmMYC2* 激活 *SmTAT1*、*SmPAL1*、*SmC4H*

和 *SmCYP98A14* 等基因的转录表达^[34,35], 正向调控丹酚酸的生物合成。但在已注释的 131 条 *SmbHLH* 转录因子中^[36-38], 也有负调控丹酚酸合成的转录因子。如 *SmbHLH7* 能与 *JAZ3L* 结合, 负调控 *SmRAS1*、*SmCYP98A14*、*SmTAT1* 等基因表达^[38]; *SmbHLH37* 能与 *SmJAZ3/8* 结合, 负调控 *SmTAT1*、*SmPAL1* 基因^[39]; 而 *SmbHLH51* 与 R2R3-MYB 类转录因子 *AtPAP1* 形成调控转录复合体后, 正向调控丹酚酸类物质的合成 (图5)^[40]。

除调控丹酚酸合成外, bHLH 转录因子家族还对丹参酮的生物合成起调控作用。如 *SmMYC2* 可通过抑制 *SmbHLH74* 的转录, 削弱 *SmbHLH74* 对丹参酮合成关键酶基因 *SmHMGR1*、*SmGGPPS1*、*SmCYP76AH1* 的负调控^[41]。*JAMs* 在结构上比 *MYC2* 转录因子在 N 端少一个酸性激活结构域, 起负调控作用。拟南芥同源表达研究表明, *SmJAM3* 能通过负调控 *AtMYC2/3/4* 和正调控 *AtJAM1* 以进一步调控 *MYC2* 下游靶基因 *AtERF1*、*AtMYB75*、*AtPDF1.2*、*AtVSP2* 的表达。而在丹参中, *SmJAM3*、*SmMYC2*-likes 分别与 *SmJAZ3*、*SmJAZ9* 蛋白互作, 上调表达 *SmGGPPS* 等基因的转录, 促进丹参酮合成^[42]。此外, *SmbHLH7* 能正调控 *SmHMGS*、*SmGGPPS1*、*SmCYP76AH1*、*SmKSL1* 等丹参酮合成基因^[38], *SmbHLH10* 能与丹参酮合成关键酶 *SmDXS2*、*SmCPS1*、*SmCPS5* 基因启动子的 G-box 位点结合, 激活转录^[43], 而 *SmbHLH148* 几乎能激活所有丹酚酸和丹参酮生物合成途径上的基因表达 (图6)^[44]。

需要指出的是, *JAZs* 作为一种阻遏蛋白, 通常起抑制作用^[45]。但过表达 *SmJAZ1/2/5/6/9* 却显著提高转基因毛状根中丹参酮的含量, 且 *SmJAZs* 能与

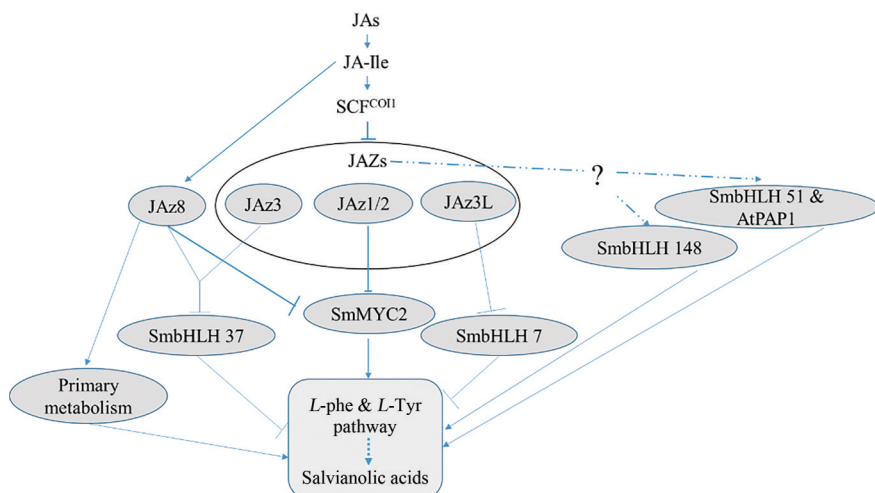


Figure 5 Transcription factor of SmbHLH regulation on salvianolic acid in JA signal pathway

SmMYC2a、SmMYB39、SmMYC2b、SmPAP1 等转录因子互作^[32,33], 促进 *SmMYC2a*、*SmMYC2b* 和 *SmPAP1* 表达, 抑制 *SmMYB39* 表达^[33]。因此, SmJAZs 蛋白在调控 JA 诱导的丹参次生代谢物合成中发挥中心枢纽作用, 存在复杂的共调控网络, 这种多个 SmJAZs 共同调控一种或一类次生代谢物合成的机制还有待进一步阐明。此外, 由于采样或命名等原因而导致的不同研究者研究同一名称的转录因子 (如 SmbHLH7) 却得到不一致结论的情况, 也应引起重视^[38,42]。

2.2 MYB 转录因子家族 MYB (v-myb avian myeloblastosis viral oncogene homolog) 是植物中最大的转录因子家族, 根据所含串联重复序列 (repeat, R) 数量, 可将 MYB 转录因子分为 4 大类。其中, 参与植物次生代谢物合成调控的转录因子主要来自 R2R3-MYB 亚家族, 可通过蛋白质互作、转录酶调控、氧化还原反应、ABA 信号系统或参与 JA 信号系统行使功能。

Li 等^[46]从丹参全基因组中筛选出 110 个 R2R3-

MYB 转录因子, 将其划分为 37 个亚族, 并预测 S3、S4、S5、S6、S7、S13、S20、S21 亚族对丹参酮和丹酚酸类化合物的合成具有调控作用。研究表明, S4 亚族的 R2R3-MYB 转录因子主要通过乙烯响应因子相关的 EAR 对苯丙烷代谢产物的合成起抑制作用^[47], 如 SmMYB4 通过抑制酚酸类上游苯丙烷代谢途径的 *SmPAL1* 和 *SmC4H* 基因和丹参酮合成途径下游关键酶基因 *SmGGPPS3*, 负调控两类化合物的积累^[48]; SmMYB39 作为负调控因子, 除直接抑制迷迭香酸代谢途径关键酶基因 *SmTAT*、*SmC4H* 和丹参酮代谢途径 *SmDXS2*、*SmDXR*、*SmHMGR1*、*SmGGPPS*、*SmKSL1* 的转录外, 还能通过抑制 SmbHLH7 转录或干扰 SmbHLH7 与其他转录因子形成正调控 MBW 复合体等机制行使负调控功能^[49,50]。S6 亚族转录因子 SmPAP1 与拟南芥中的 PAP1 蛋白的同源性高达 75%, 在丹参中过表达拟南芥 *AtPAP1*、*AtPAP2*, 能不同程度正向调控苯丙烷类代谢途径上 *SmPAL*、*SmC4H*、

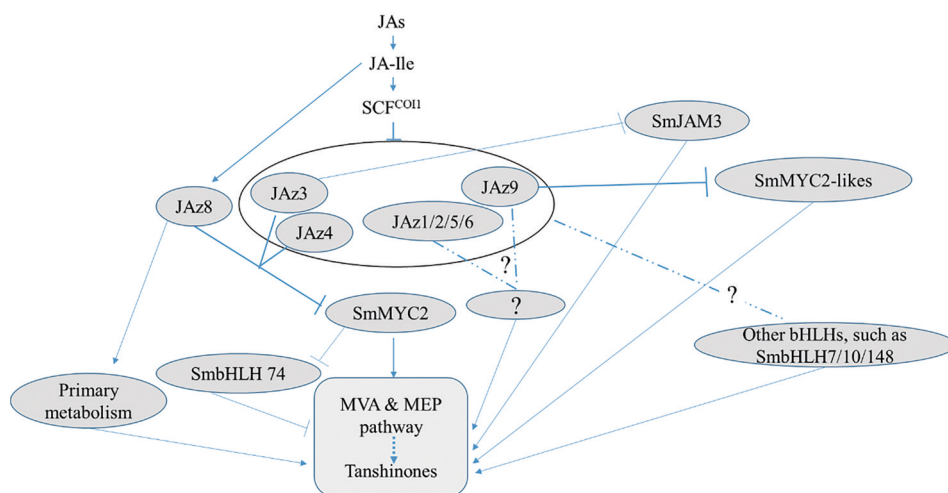


Figure 6 Transcription factor of SmbHLH regulation on tanshinones in JA signal pathway

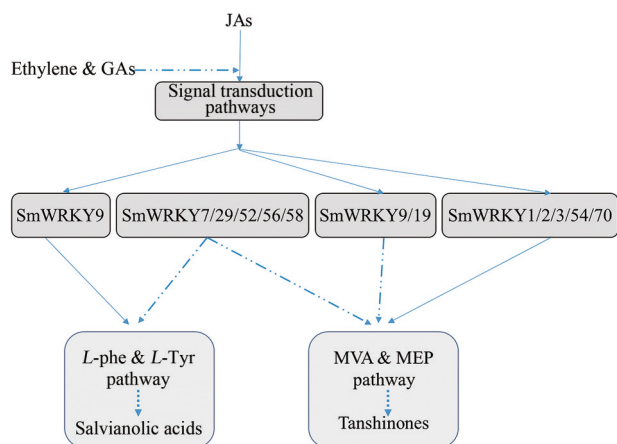


Figure 8 Transcription factor of SmWRKY regulation on secondary metabolism in *Salvia miltiorrhiza*

况下, 乙烯能促进 EIN3/EILs 的积累, 而 JAZ 蛋白可通过组蛋白去乙酰化酶 (HDA6) 直接抑制 EIN3/EILs 转录因子的活性, 当 JAs 与其受体 SCF^{CO11}-JAZ 结合后, JAZ 蛋白随之降解, 从而解除 HDA6 对 EIN3/EIL1 的抑制, 启动 EIN3/EIL1 调控的基因表达^[73]。丹参 SmEIN3 转录因子响应 ABA、YE、MeJA 和 SA 的诱导, 但 GA 抑制其表达。在转录水平上丹参 SmEIN3 不响应乙烯诱导, 乙烯调节的是转录后水平的 EIN3/EIL 蛋白及其活性, 以激活丹酚酸合成关键酶基因 *Sm4CL1*、*SmCYP98A14*、*SmHPPR*、*SmRAS* 和丹参酮合成关键酶基因 *SmDXSII*、*SmDXR*、*SmCYP76AH1*、*SmKSL*、*SmGGPPS*、*SmHMGS*、*SmAACT*、*SmHMGR*、*SmCPS* 的转录。从 SmEIN3 与 SmMYC2、SmJAZs 存在互作现象分析, 丹参酮和丹酚酸类化合物的生物合成调节中可能存在乙烯信号通路与 JA 信号通路的交流, 两者起共同调控作用 (图9)^[74]。

2.5 GRAS 转录因子家族 GA3 处理后, 能激活 *SmCPS1*、*SmKSL1*、*SmPAL* 和 *SmTAT* 等关键酶基因的表达, 促进丹参酮和丹酚酸类物质的合成^[75]。DELLA 是 GRAS 转录因子超家族中的重要成员, 其 N 端具有 GA 受体 GID1 结合域, 为 GA 信号途径的负调控因子。GA 可通过调控 SLEEPY1 等蛋白以促进 DELLA 降解, 而 DELLA 的降解则促进 JAs 的合成与 JAZ 降解, 从而进一步促进了 DELLA 的去抑制化^[76]。同时, DELLA 蛋白也可通过结合不同的转录因子介导 GA 信号调控下游靶基因^[77], 如以 DELLA-JAZ 蛋白互作的形式达到 GA-JA 途径调控网络的实现^[78]; 在缺氮时, DELLA 通过与 PAPI 蛋白相互作用, 增强 PAPI 对靶基因的调控 (图9)^[79]。Bai 等^[80]从丹参中克隆出 5 个能响应 GA 或乙烯诱导的 SmGRAS 转录因子, 通过激活 *SmCPS1* 和 *SmKSL1* 的转录, 正向调控丹参次生代谢的合成。Wang^[81]从丹参中筛选出同时响应 GA3、水杨酸 (SA)

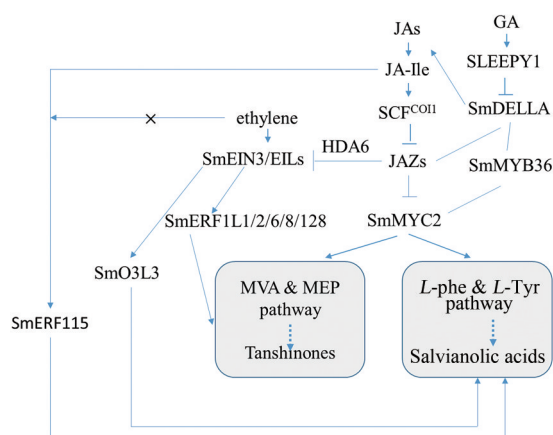


Figure 9 Transcription factor of SmEIN3/EILs, SmDELLA and SmERF regulation on secondary metabolism in *Salvia miltiorrhiza*

和 JAs 信号途径的 SmGRAS 转录因子 5 个, 并鉴定出 11 个亚家族共 34 个 SmGRAS 转录因子, 其中 4 个转录因子属于 SmDELLA 亚家族, 都能与 SmJAZ1、SmJAZ3、SmMYB36、SmMYC2 等蛋白互作, 推测对丹参次生代谢产物的合成具有调控作用。

2.6 ERF 类转录因子家族 ERF 类转录因子属于 AP2/ERF 家族成员, 在 AP2 结构域的 N-端具有特异识别顺式作用元件的 β -折叠结构, 而 C-端的 α -螺旋结构则用于与其他转录因子或 DNA 的 GCC-box 相互作用。AP2/ERF 类转录因子共有 5 个亚族, ERF (ethylene response factors) 亚族种类最多, 大多数成员响应乙烯信号通路 (也可响应 JA、SA 信号通路), 在植物生长、发育和次生代谢中扮演重要作用^[82]。Ji 等^[83]从丹参基因组数据中注释到 79 个 ERF 转录因子, 并根据丹参酮的分布、转录因子的表达模式及启动子顺式元件等推测 SmERF128/152 与丹参酮生物合成相关, SmERF8/166 与丹酚酸合成相关。SmERF2 和 SmO3L3 通过结合 (A/G/C) GCCGCC 型的 GCC-box, 分别激活丹参酮合成途径的 *SmDXR*、*SmDXS2*、*SmHMGS*、*SmKSL* 和丹酚酸合成途径的 *Sm4CL1*、*SmRAS* 等酶基因表达^[84]。SmERF6 和 SmERF8 可识别 *SmCPS1* 和 *SmKSL1* 启动子区的乙烯反应元件 GCC-box, 正向调控丹参酮的生物合成^[85,86]。SmERF115 受 MeJA 诱导, 但不受 YE、SA、ET 诱导, 能直接结合 *SmRAS1* 启动子激活表达 (可能也激活 *SmPAL3*、*SmTAT3*、*Sm4CL5* 等), 促进丹酚酸类化合物的生成^[87]。而 SmERF1L1 则对 MeJA、YE、SA、ET 的诱导均有响应, 能激活 *SmDXR*、*SmDXS2*、*SmHMGS*、*SmKSL* 的转录, 促进丹参酮的合成^[88]。SmERF128 可结合靶基因的 GCC 盒、CRTDREHBCF2 (CBF2) 基序和 RAV1AAT (RAA) 基序, 激活 *SmCPS1*、*SmKSL1* 和 *SmCYP76AH1* 的表达 (图9)^[89]。

2.7 LBD转录因子家族 LBD (lateral organ boundaries domain) 是一类植物特有的转录因子家族。具有完整亮氨酸拉链基序 (Class I) 的成员, 主要参与组织发育的调控, 而不具有完整亮氨酸拉链基序的成员 (Class II), 主要与花青素生物合成、氮代谢的调控相关。从丹参基因组中鉴定出 51 个 *SmLBD*, 90% 的 *SmLBD* 具有保守的 CX2CX6CX3C 结构, 74% 的 *SmLBD* 在叶片表达, 推测 *SmLBD9/13/21/50* 等与丹酚酸化合物的合成关系密切, *SmLBD44* 与丹参酮的合成调控相关^[90]。研究表明, LBD 转录因子的保守区段即 LOB 结构域可与 bHLH、MYB 转录因子相互作用, 参与 JA 信号下游途径调控^[91], 且同类 LBD 转录因子的功能相对保守, 但作用方式有所区别^[92]。如 *SmLBD16/23/50* 均能负向调控丹酚酸的合成, 且与 *SmJAZ1*、*SmMYB36/97*、*SmbHLH37* 和 *SmMYC2a/2b* 等转录因子存在蛋白互作, 但 *SmLBD16* 在调控苯丙烷途径关键酶基因表达时需 *SmPAP1* 参与, 而 *SmLBD23* 则不需要^[93]。

3 总结与展望

近年来, 丹参酮和丹酚酸类化合物的生物合成及其转录因子调控机制研究取得长足进展, 一方面得益于丹参全基因组图谱的完成, 不同组织、器官的转录组测序, 使其能在基因组和转录组层面整体分析与丹参酮和丹酚酸类化合物生物合成相关的差异基因和转录因子^[94], 另一方面得益于拟南芥、烟草、水稻等模式植物研究中所建立的高效成熟的转化系统。但是, 丹参作为药用模式植物, 其次生代谢产物的生物合成及调控机制研究, 还需继续深入, 主要体现在 4 个方面: ① 丹参酮和丹酚酸类化合物的生物合成途径还未被完全阐明, 急需明确参与丹参酮后期修饰及迷迭香酸下游途径的关键酶, 并进一步揭示其合成、转运、调节、修饰的新基因及作用机制。② 丹参中转录因子众多, 家族成员庞大, 同一家族 (亚家族) 成员功能相似等, 大大增加了转录因子调控机制研究的难度, 但是家族成员之间及不同家族转录因子之间的相互作用、响应的信号通路、参与的合成途径等错综复杂的调控关系, 仍是今后的研究重点。③ 部分转录因子的研究, 如 SPL (SQUAMOSA promoter binding protein-like) 转录因子^[95]、bZIP (basic region/leucine zipper motif) 转录因子^[96], 还停留在功能预测层面, 急需进一步深入。同时, 转录因子自身的转录也受到其他基因的调控, 如 microRNAs、ncRNAs 等^[97], 但这方面的研究较少。④ 人工设计并构建新的、具有特定生理功能的生物系统, 是一种极具潜力的丹参活性成分资源获取方法。如通过构建 *SmHMGR* 催化活性域-*SmGGPPS*, *SmFPS* 融合蛋白-*SmKSL*, *SmCPS* 融合蛋白酵母工程菌, 使次丹参酮

二烯的产量达到了 $365 \text{ mg} \cdot \text{L}^{-1}$ ^[98]; 组合改造 FPP 合成酶和 GGPP 合成酶, 将次丹参酮二烯的产量提高到 $488 \text{ mg} \cdot \text{L}^{-1}$ ^[99]; 引入 *CYP76A11*、*SmCPR1* 基因, 将铁锈醇的最高产量提高到 $10.5 \text{ mg} \cdot \text{L}^{-1}$ ^[12]。而转录因子能同时诱导一个或多个基因的协同表达, 调控效果优于单纯提高单个或多个结构基因的表达, 是另一大幅提高合成酶基因表达及活性物质产量的可能方法, 值得进一步深入研究。

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