P450s介导远志皂苷等齐墩果烷型植物三萜生物合成的研究进展

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摘要: 植物三萜是一大类结构多样、具有广泛工业及药用价值的天然产物。齐墩果烷型三萜因拥有良好的药理/生物活性而被广泛熟知, 其生物合成历经了前体供应、骨架合成、萜类合成等3个阶段。在齐墩果烷型三萜骨架糖基化之前, 细胞色素P450单加氧酶 (cytochrome P450 monooxygenase, P450s) 会先行对该骨架进行许多结构修饰, 而这些修饰对三萜骨架的结构多样性与功能性至关重要。本文综述了齐墩果烷型三萜皂苷生物合成中P450s对*β*-香树脂醇 (*β*-amyrin)、齐墩果酸 (oleanolic acid) 的催化作用, 探讨了远志皂苷的主要苷元母核——原远志皂苷元的可能生物合成途径, 并简要概述了远志 (*Polygala tenuifolia*) 中CYP716A249的发现, 为齐墩果烷型植物三萜的生物合成途径解析提供一些借鉴。

关键词: 细胞色素P450单加氧酶; 植物三萜; 齐墩果烷型三萜皂苷; 远志皂苷; 生物合成

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Advance in biosynthesis of plant-derived oleanane type triterpenoids such as Polygala saponins with catalysis by cytochrome P450s

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Abstract: Plant-derived triterpenoids constitute a large and structurally diverse class of natural products with various implications in industrial and pharmaceutical uses. The oleanane type triterpenoids are widely known for their pharmacological and/or biological activities. The biosynthesis pathway of oleanane triterpenoids is divided into three stages: precursor supply, skeleton synthesis, and terpenoids synthesis. Plant cytochrome P450 monooxygenases enzymes (P450s) are involved in the synthesis and diversification of natural products, and are responsible for other modifications of terpenoids, such as formation of triterpenoids. P450s-catalyzed structural modification prior to glycosylation is crucial for diversification and functionalization of triterpenoid scaffolds. In this paper, the catalyses of P450s on *β*-amyrin and oleanolic acid in oleanane type triterpenoid saponins biosynthesis were reviewed. Presenegenin is a major aglycon of Polygala saponins. The CYP716A249 in *Polygala tenuifolia* was used as an example to other P450s participating in the possible biosynthetic pathways of presenegenin. These results provide references for elucidation of the biosynthesis pathways of plant-derived oleanane type triterpenoids.

Key words: cytochrome P450 monooxygenase; plant-derived triterpenoid; oleanane type triterpenoid saponin; Polygala saponin; biosynthesis

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三萜是一大类结构多样、具有广泛工业及药用价值的天然产物[1]。植物通常会合成三萜来回应自身的发育信号和来自外部环境的刺激[2-5]。一方面, 三萜可以在植物的防御反应中发挥作用, 如燕麦植物根部所蓄积的抗菌类三萜糖苷 (avenacins), 可防御根部感染病原真菌[6]。另一方面, 也有研究表明部分三萜与植物生长及器官发育密切相关, 如羽扇豆醇 (lupeol) 参与结瘤形成[7], β-香树脂醇 (β-amyrin) 参与结瘤形成[8]、根发育[9], thalianol 参与植物生长与发育[10], β-amyrin/二氢-羽扇豆醇 (dihydro-lupeol) 参与根生长和开花[11], marneral 参与发芽及根发育、开花、胚胎形成[12]等。

由于潜在的生物活性, 三萜已被广泛用于药物、食品、化妆品等领域[13-16], 如一些植物来源的三萜已被用于膳食补充剂、OTC 非处方药物, 而另一些半合成的三萜衍生物则正在进行临床试验研究[16,17]。基于上述原因, 植物三萜的生物合成和蓄积过程正被广泛研究, 力求生产出一种替代资源, 以便于可持续的工业化生产[16,18-21]。植物三萜的生物合成历经前体供应、骨架合成、萜类合成等三个阶段 (图 1), 起源于异戊二烯 (C5), 形成共同的底物 2,3-环氧角鲨烯 (C30), 经由氧化角鲨烯环化酶催化, 环化成不同的三萜骨架 (C30)[22]。三萜骨架可被多种功能基团, 诸如羟基、羰基、羧基、环氧、烷基、酰基、丙二酰基以及糖基等修饰[23-25], 而上述修饰最终决定了三萜结构的多样性。迄今为止, 已从天然资源中发现了超过 23 000 多种的三萜结构, 包含从无环到六环的 100 多种结构骨架[1]; 而四环和五环结构在三萜中则占主要构成比例[26]。

三萜皂苷由三萜皂苷苷元与糖苷配基组成, 是三萜中的一类重要化合物, 具有抗炎[27,28]、抗癌[29]、抗病毒[30,31]等许多重要生物学特性[32-34], 其中尤以齐墩果烷型三萜皂苷的生物活性被广泛熟知。在三萜骨架糖基化之前, P450s 会先行对该骨架进行许多结构修饰[35]。本文综述了齐墩果烷型三萜皂苷生物合成中 P450s 对 β-amyrin、oleanolic acid 的催化作用, 探讨了远志皂苷的主要苷元母核——原远志皂苷元的可能生物合成途径, 并简要概述了远志 (*Polygala tenuifolia*) 中 OAS (oleanolic acid synthase, 齐墩果酸合成酶) (PtOAS): CYP716A249 的发现, 为齐墩果烷型植物三萜的生物合成途径解析提供一些借鉴。

1 植物三萜生物合成相关 P450s 的研究进展

P450s 是植物新陈代谢相关酶中最大的一类酶家族, 其编码蛋白的基因约占植物基因组的 1%[36]。P450s 是一类包含亚铁血红素 b 的酶, 可以催化氧分子进行还原性分离, 其中一个氧原子被加入到底物中, 另一个氧原子则被变成了水。P450s 在自然界中广泛分布, 可以催化的底物范围较广, 包括萜类、抗生素、脂肪酸、维生素、烷烃类等多种底物[37,38]。在植物中, P450s 通常经由 N 端锚定结构结合在细胞膜上, 并与细胞膜上锚定的细胞色素 P450 还原酶 (cytochrome P450 reductase, CPR) 相互作用。CPR 可以为 P450s 提供来源于烟酰胺腺嘌呤二核苷酸 (NAD) 辅酶因子的电子, 而这些电子是 P450s 发挥氧化催化反应所必需的。

P450s 通常依据基因的序列同源性和系统发育标准, 分为不同的家族与亚家族。该命名原则依据 *P450s* 基因序列提交到命名委员会 (David Nelson: dnelson@uthsc.edu) 的时间先后次序 (年月日) 而定, 规定: 氨基酸序列同源性>40% 的 P450s, 命名为家族; >55% 的 P450s, 则命名为亚家族。目前, 已从植物中发现了 127 个 P450s 家族, 而从脊椎动物、昆虫、细菌、真菌中则分别发现了 19、67、333 和 399 个 P450s 家族[39]。陆生植

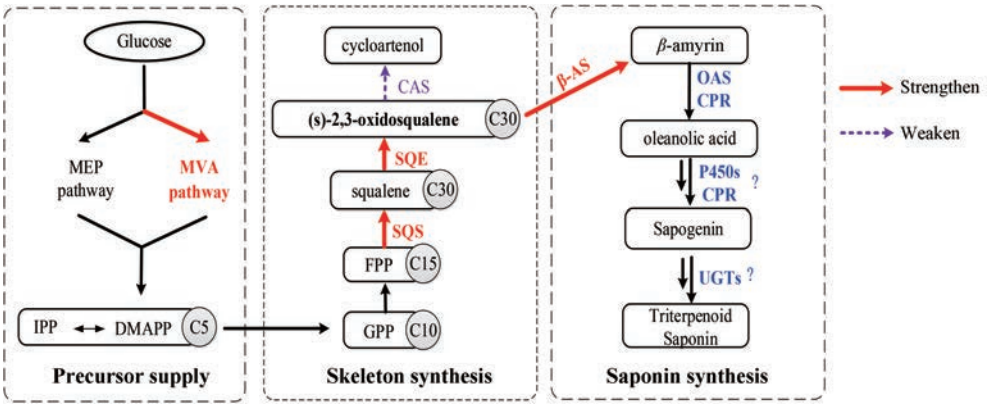

Figure 1 Biosynthesis framework of oleanane type triterpene saponins. MVA: Mevalonic acid; IPP: Isopentenyl pyrophosphate; DMAPP: Dimethylallyl pyrophosphate; GPP: Geranylpyrophosphate; FPP: Farnesyl pyrophosphate; SQS: Squalene synthetase; SQE: Squalene epoxidase; CAS: Cycloartenol synthase; β-AS: β-Amyrin synthase; OAS: Oleanolic acid synthase; CPR: Cytochrome P450 reductase; P450s: Cytochrome P450 monooxygenase; UGT: UDP-glucosyltransferase

物的P450s家族在系统发生树上分为11个具有明显进化枝的簇[36]; 其中CYP51、CYP74、CYP97、CYP710、CYP711、CYP727、CYP746等簇只包含单一的P450s家族, 而CYP71、CYP72、CYP85、CYP86等簇则包括了多个P450s家族。

P450s的催化作用对三萜的结构多样性与功能性至关重要。迄今为止, 在70个不同种属植物中, 共发现了271个参与三萜生物合成的酶[40], 包括分属于37个不同种属植物的87个已知功能的P450s (TriForC Database, publicly available pathway database of known and novel high - value triterpenes, http://bioinformatics.psb.ugent.be/triforc/#/home, 只记录了84个)。CYP51 (成员: CYP51H), CYP71 (成员: CYP71A、CYP71D、CYP81Q、CYP93E、CYP705A), CYP72 (成员: CYP72A) 以及CYP85 (成员: CYP87D、CYP88D、CYP88L、CYP708A、CYP716A、CYP716C、CYP716E、CYP716S、CYP716U、CYP716Y) 被发现与三萜的结构修饰有关[41]。特别的是, CYP81Q59分别存在于3种不同的植物 [黄瓜 *Cucumis sativus* L.、甜瓜 *Cucumis melo* L.、西瓜 *Citrullus lanatus* (Thunb.) Matsum. et Nakai] 中[42]。

2 P450s介导齐墩果烷型植物三萜生物合成的研究进展

在上述87个P450s中, 参与齐墩果烷型三萜生物合成的有47个 (截止投稿日), 分别归属于CYP85、CYP51、CYP72、CYP71等4个簇[40,41], 包括可催化*β*-香树脂醇的42个P450s和可催化齐墩果酸的7个P450s (其中2个P450s可分别催化*β*-香树脂醇与齐墩果酸)。

2.1 催化*β*-香树脂醇的P450s *β*-香树脂醇是一个重要的五环三萜类化合物, 也是齐墩果酸生物合成途径中一个重要的前体物质。有研究表明, *β*-香树脂醇的衍生物: triazolyl-napthyl derivative of *β*-amyrin (TNB) 可促进鼻咽癌HK-1细胞凋亡, 且TNB对放化疗有增敏作用, 联合放化疗可更好地抑制肿瘤细胞增殖[43]。迄今为止, 发现可以介导*β*-香树脂醇发生催化反应的P450s共有42个, 分属于26个不同种属植物 (表1), 可分别对*β*-香树脂醇骨架上的C-3、C-6、C-11、C-12,13、C-16、C-22、C-24、C-28、C-30等进行催化, 并继而生成各种*β*-香树脂醇的衍生物和齐墩果酸等中间产物[40,41]。

表1中, 有2个P450s (CYP716S5、CYP716A141) 可分别催化*β*-香树脂醇与齐墩果酸; 有4个P450s (CYP87D16、CYP716A11、CYP93E2、CYP93E7) 仅以*β*-香树脂醇为底物; 有26个P450s仅以*β*-香树脂醇及其衍生物为底物, 而无法催化*α*-香树脂醇; 有21个P450s可直接氧化催化*β*-香树脂醇生成齐墩果酸, 属C-28氧化酶。在这21个C-28氧化酶中, 有相同催化底物的P450s分别为: ① CYP716A17、CYP716A52v2、CYP716A75、CYP716A78、CYP716A79、CYP716A110、CYP716A244, ② CYP716A83、CYP716A86、CYP716A252、CYP716A253, ③ CYP716A44、CYP716A46, ④ CYP716A80、CYP716A81等; 除CYP716A244外, 上述P450s均属于CYP85簇[41]。另, CYP716A154与CYP716AL1是同一个P450。

在使用TriForC数据库[40]查询相关P450s功能时, 建议在初步明确了目标P450s的描述 (description) 后, 应进一步结合区域专一性 (regiospecificity)、酶作用底物 (substrates)、反应 (reaction)、通路 (pathways) 等细节综合判断。如表1所示, CYP51H10、CYP716A179可分别对*β*-香树脂醇骨架上的2个C进行催化, 而CYP716A141则可分别对3个C发生催化反应; CYP716A80、CYP716A81、CYP716A1除了可对C-28进行催化外, 还可能发生一些未知的催化反应。上述研究结果也从侧面反映了发现新P450s并验证其功能的复杂性及困难程度。此外, 需要说明的是, 表1中的CYP716E26、CYP716A44、CYP716A46在TriForC数据库中没有找到, 为笔者参照文献[41]后添加。

2.2 催化齐墩果酸的P450s 齐墩果酸以游离或结合成苷的形式, 广泛存在于许多种植物中, 据不完全统计, 已在120余种植物中发现含有齐墩果酸[64]。由于齐墩果酸结构复杂, 人工合成困难, 目前国内均从植物中提取获得。有研究表明, 齐墩果酸具有抗心肌缺血[65]、抗动脉粥样硬化和抗血栓[66,67]、保护脑组织[68,69]、抗炎[70]等抗心脑血管疾病作用[71]。此外, 齐墩果酸还具有抗病毒、抗变态反应、抗氧化应激、促进肝糖原合成及肝细胞再生等作用, 其相关制剂已在临床应用于肝脏保护[72,73]。由于齐墩果酸的溶解度较差, 严重影响了

Table 1 The type of P450s catalyze *β*-amyrin and its derivatives[40,41]

Regiospecificity	Description	Substrates	P450s	Plant	Genbank	Ref.
C-3	Pentacyclic triterpenoid C3-oxidase	*α*-Amyrin, *β*-amyrin, lupeol	CYP716A14v2	*Artemisia annua*	KF309251	[5]
C-6*β*	Amyrin C-6*β*-hydroxylase	*α*-Amyrin, *β*-amyrin	CYP716E26	*Solanum lycopersicum*	XM_004241773	[44]
C-11	Oleanane C-11-oxidase	*β*-Amyrin, 30-hydroxy-*β*-amyrin, 11*α*-hydroxy-*β*-amyrin	CYP88D6	*Glycyrrhiza uralensis*	AB433179	[45]

Continued

Regiospecificity	Description	Substrates	P450s	Plant	Genbank	Ref.
C-12,13*α*	*β*-Amyrin C12,13*α*-epoxidase	*β*-Amyrin, oleanolic acid	CYP716S5	*Platycodon grandiflorus*	KU878856	[46]
C-16*β*, C-12, 13*β*	*β*-Amyrin C12,13*β*-epoxidase	*β*-Amyrin, 16*β*-hydroxy-*β*-amyrin	CYP51H10	*Avena strigosa*	DQ680852	[47]
C-16*α*	*β*-Amyrin C-16*α*-hydroxylase	*β*-Amyrin	CYP87D16	*Maesa lanceolata*	KF318735	[4]
		α-Amyrin, *β*-amyrin	CYP716Y1	*Bupleurum falcatum*	KC963423	[48]
C-16*β*	*β*-Amyrin C16*β*-hydroxylase	*β*-Amyrin	CYP716A111	*Aquilegia coerulea*	KY047600	[46]
C-16*β*, C-28		*β*-Amyrin, erythrodiol, oleanolic acid	CYP716A141	*P. grandiflorus*	KU878855	[46]
C-16*β*, C-22*α*, C-28	*β*-Amyrin C-22*α*-hydroxylase	*α*-Amyrin, *β*-amyrin, lupeol	CYP716A2	*Arabidopsis thaliana*	LC106013	[49]
C-22*α*, C-28	Pentacyclic triterpenoid C28-oxidase	*α*-Amyrin, uvaol, *β*-Amyrin, erythrodiol, lupeol, betulin	CYP716A179	*G. uralensis*	LC157867	[50]
C-24	Oleanane C-24-oxidase	*β*-Amyrin, 24-hydroxy-*β*-amyrin, sophoradiol	CYP93E1	*Glycine max*	AF135485	[51,52]
	β-Amyrin C-24-oxidase	*β*-Amyrin	CYP93E2	*Medicago truncatula*	DQ335790	[53]
		β-Amyrin, 24-hydroxy-*β*-amyrin	CYP93E3	*G. uralensis*	AB437320	[45,51]
			CYP93E4	*Arachis hypogaea*	KF906535	[51]
			CYP93E5	*Cicer arietinum*	KF906536	[51]
			CYP93E6	*Glycyrrhiza glabra*	KF906537	[51]
			CYP93E7	*Lens culinaris*	KF906538	[51]
			CYP93E8	*Pisum sativum*	KF906539	[51]
			CYP93E9	*Phaseolus vulgaris*	KF906540	[51]
C-28	Pentacyclic triterpenoid C28-oxidase	*α*-Amyrin, uvaol, *β*-amyrin, erythrodiol, lupeol, betulin, oleanolic aldehyde, ursolic aldehyde	CYP716A12	*M. truncatula*	DQ335781	[53,54]
		α-Amyrin, uvaol, *β*-amyrin, erythrodiol, lupeol, betulin	CYP716A15	*Vitis vinifera*	AB619802	[53]
		β-Amyrin, erythrodiol	CYP716A17	*V. vinifera*	AB619803	[53]
		α-Amyrin, uvaol, *β*-amyrin, erythrodiol	CYP716A83	*Centella asiatica*	KU878849	[46]
			CYP716A86	*C. asiatica*	KU878848	[46]
		β-Amyrin, erythrodiol, 16*β*-hydroxy-*β*-amyrin, 12,13*α*-epoxy-*β*-amyrin, 12,13*α*-epoxyoleanolic acid	CYP716A140	*P. grandiflorus*	KU878853	[46]
	β-Amyrin C28-oxidase	*β*-Amyrin, erythrodiol	CYP716A52v2	*Panax ginseng*	JX036032	[55]
			CYP716A75	*Maesa lanceolata*	KF318733	[4]
			CYP716A78	*Chenopodium quinoa*	KX343075	[56]
			CYP716A79	*C. quinoa*	KX343076	[56]
			CYP716A110	*A. coerulea*	KU878864	[46]
			CYP716A244	*Eleutherococcus senticosus*	KX354739	[57]
		α-Amyrin, uvaol, *β*-amyrin, erythrodiol	CYP716A252	*Ocimum basilicum*	JQ958967	[58]
			CYP716A253	*O. basilicum*	JQ958968	[58]
		α-Amyrin, *β*-amyrin, lupeol	CYP716A175	*Malus x domestica*	XM_008392874	[59]
	Amyrin C28-oxidase	*α*-Amyrin, uvaol, ursolic aldehyde, *β*-amyrin, erythrodiol, oleanolic aldehyde	CYP716A44	*S. lycopersicum*	AK329870	[44]
			CYP716A46	*S. lycopersicum*	XM_004243858	[44]
	β-Amyrin C28-oxidase (CYP716AL1)	*α*-Amyrin, uvaol, *β*-amyrin, erythrodiol, lupeol, betulin	CYP716A154	*Catharanthus roseus*	JN565975	[60]
C-28, unknown	Pentacyclic triterpenoid C28-oxidase	*β*-Amyrin, erythrodiol, lupeol, betulin	CYP716A80	*Barbarea vulgaris*	KP795926	[61]
			CYP716A81	*B. vulgaris*	KP795925	[61]
	Triterpenoid oxidase	Tirucalla-7,24-dien-3*β*-ol, *α*-amyrin, uvaol, *β*-amyrin, erythrodiol, lupeol	CYP716A1	*A. thaliana*	NM_123002	[49,62]
C-30	*β*-Amyrin C30-oxidase	*β*-amyrin, 30-hydroxy-*β*-amyrin	CYP72A63	*M. truncatula*	AB558146	[63]

其制剂的进一步发展。若采用生物合成的方法, 对齐墩果酸骨架进行一定的人工结构修饰或改造, 则是解决其生物利用度低、增强其临床疗效的重要途径之一。

目前, 在积雪草 *C. asiatica*、苜蓿 *M. truncatula*、桔梗 *P. grandiflorus* 等 3 个不同种属植物中共发现了 7 个 P450s (表 1, 2), 分属于 CYP85、CYP72 等 2 个簇[41]。这些 P450s 可分别对齐墩果酸骨架上的 C-2、C-6、C-23 (表 2) 和 C-12,13、C-16 (表 1) 等进行催化, 并继而生成常春藤皂苷元 (hederagenin)、苜蓿酸 (medicagenic acid) 等中间代谢产物。

C-2*α* 羟化酶、C-6*β* 羟化酶 2017 年, Alain Goossens 小组[46]从已公开的积雪草 *C. asiatica* 转录组数据库中挖掘出了 6 条编码 *CYP716* 基因, 经分析后发现上述基因与已知 CYP716 蛋白有 46%～78% 的氨基酸同源性; 经对 5 条基因的全长序列进行克隆并随后提交到 P450s 命名委员会以及 Genbank, 最终获得了如下基因: *CYP716A86*、*CYP716A83*、*CYP716D36*、*CYP716E41* 和 *CYP716C11*; 将上述 P450s 分别与 *G. glabra* 中的 *β*-AS (GgbAS)、*M. truncatula* 中的 CPR (KU878869) 共转化入酿酒酵母 (*Saccharomyces cerevisiae*) 中, 经对发酵产物进行测定后, 发现: 齐墩果酸①经由 CYP716C11 催化, 可生成 maslinic acid, 再经由 CYP716E41 催化, 生成 6*β*-hydroxy-maslinic acid; ②或先经由 CYP716E41 催化, 生成 6*β*-hydroxy-oleanolic acid, 再由 CYP716C11

Table 2 The type of P450s catalyze oleanolic acid and its derivatives[40,41]

Regiospecificity	Description	Substrates	P450s	Plant	Genbank	Ref.
C-2*α*	Oleanolic acid C2*α*-hydroxylase	Ursolic acid, oleanolic acid, 6*β*-hydroxy-oleanolic acid	CYP716C11	*C. asiatica*	KU878852	[46]
C-2*β*	Oleanolic acid C-2*β*-hydroxylase	Oleanolic acid, hederagenin, gypsogenin, gypsogenic acid, echinocystic acid, caulophyllogenin	CYP72A67	*M. truncatula*	DQ335780	[74]
C-6*β*	Maslinic acid C6*β*-hydroxylase	Ursolic acid, oleanolic acid, maslinic acid	CYP716E41	*C. asiatica*	KU878851	[46]
C-23	Oleanane C-23-oxidase	Oleanolic acid, hederagenin, bayogenin, 2*β*-hydroxyoleanolic acid	CYP72A68v1 CYP72A68v2	*M. truncatula*	XM_013608494 DQ335782	[74,75]

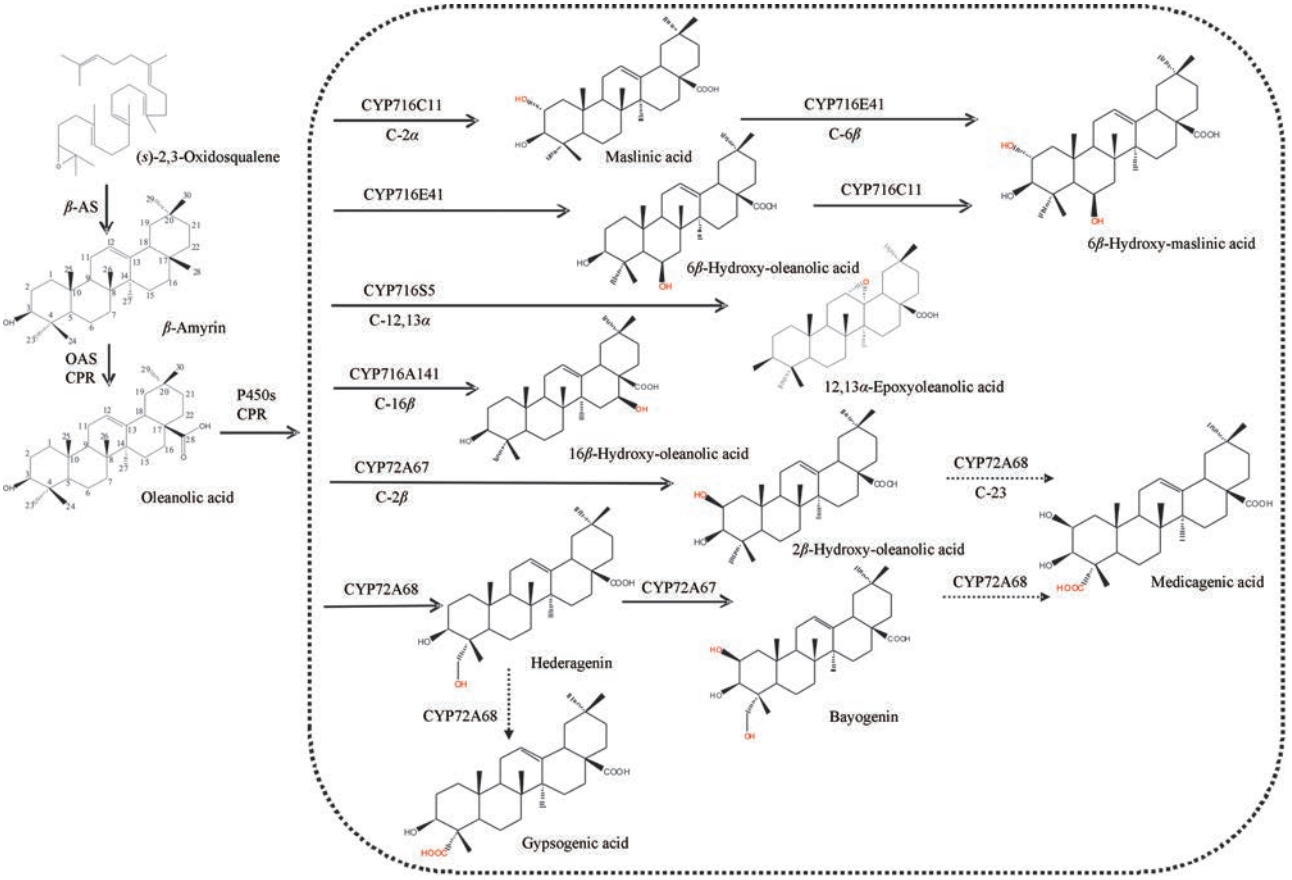

Figure 2 The catalysis of P450s to oleanolic acid (·····▸: Multistep catalytic reaction)

催化, 生成6β-hydroxy-maslinic acid (图2)。

C-12,13α氧化酶、C-16β羟化酶 Alain Goossens小组[46]采用BLAST软件从“The Compositae Genome Project”(http://compgenomics.ucdavis.edu/) 数据库中挖掘出了6条*CYP716s*基因; 采用cDNA末端快速扩增技术 (rapid amplification of cDNA ends, RACE), 以桔梗*P. grandiflorus*幼苗的cDNA为模板, 对上述5条*CYP716s*基因的全长开放阅读框 (full-length open reading frame, FL-ORF) 进行克隆; 相关基因序列提交到P450s命名委员会以及GenBank, 最终获得了如下基因: *CYP716A140*、*CYP716A141*、*CYP716S4*、*CYP716S5*、*CYP716S6*。经由酿酒酵母*S. cerevisiae*体内进行功能研究后, 发现: 齐墩果酸 ① 经由CYP716S5氧化催化, 生成12,13α-epoxy-oleanolic acid; ② 还可经由CYP716A141催化, 生成16β-hydroxy-oleanolic acid (图2)。

C-2β羟化酶、C-23氧化酶 2015年, Carla Scotti小组[54]发现, 苜蓿*M. truncatula*中的CYP72A67是催化该属植物中溶血性皂苷生物合成的关键氧化酶, 而CYP72A68则是生物合成苜蓿酸 (medicagenic acid) 的关键酶。齐墩果酸①经由CYP72A67催化, 生成2β-hydroxy-oleanolic acid, 再经由CYP72A68多步催化, 生成苜蓿酸; ②也可经由CYP72A68催化, 生成常春藤皂苷元 (hederagenin), 再经由CYP72A68多步催化, 生成丝石竹酸 (gypsogenic acid); 或者常春藤皂苷元先经由CYP72A67催化生成贝萼皂苷元 (bayogenin) 后, 再经由CYP72A68多步催化生成苜蓿酸 (图2)。

3 P450s介导远志皂苷生物合成的研究进展

从目前已发现的可以催化齐墩果酸的P450s个数及所归属的簇来看, 有关齐墩果烷型植物三萜生物合成途径解析的研究依然进展缓慢, 已严重制约了上述三萜的合成生物学研究, 进而也限制了该类物质药理/生物活性的深入研究。本课题组长期从事远志等山西道地药材的资源评价与次生代谢产物研究, 近年来又逐步开展了远志皂苷生物合成途径解析的相关研究。本文探讨了远志皂苷的主要苷元母核——原远志皂苷元的可能生物合成途径现, 并对远志皂苷生物合成相关CYP716A249 (*Polygala tenuifolia* OAS, PtOAS) 的发现过程作一简要概述。

远志, 是我国重要的大宗药材之一, 具有安神益智、交通心肾、祛痰、消肿之功效; 始载于《神农本草经》, 列为上品, 视为养命要药; 也是目前临床益智药处方中使用频率名列前3位的单味中草药[76]。远志皂苷, 属齐墩果烷型的五环三萜类化合物。目前, 从远志属植物中共发现了120余种远志皂苷, 分属于11种皂苷苷元母核, 包括原远志皂苷元、常春藤皂苷元、瓜子金皂苷元等 (图3)。在诸多不同构型的远志皂苷中, 仅

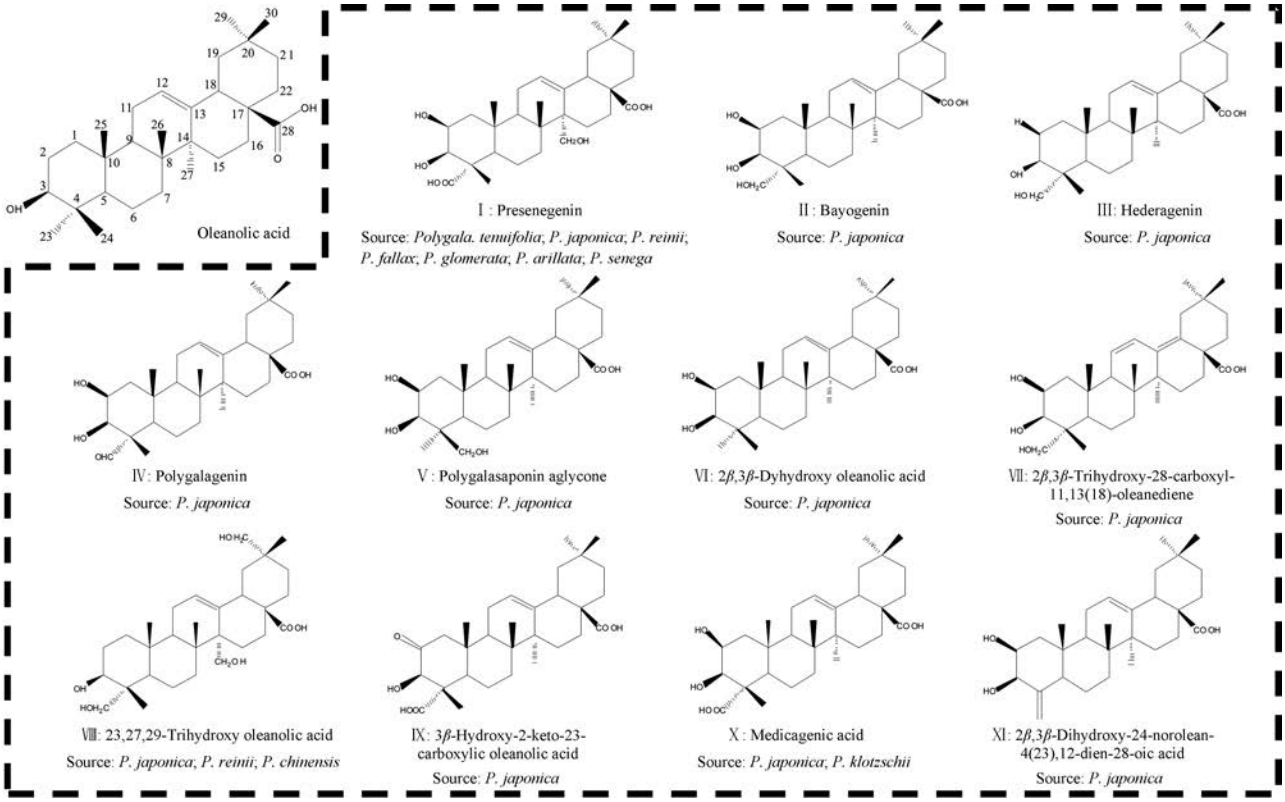

Figure 3 The type of triterpene saponins aglycone from polygala

以原远志皂苷元为母核衍生而出的就有50余种之多[77]。细叶远志皂苷, 作为远志总皂苷碱水解后的次级皂苷成分, 被发现有抗阿尔茨海默病 (Alzheimer's disease)[78]、促睡眠[79]等作用。区别于原远志皂苷元, 细叶远志皂苷在C-3位连接了一个葡萄糖。

由于齐墩果酸的溶解度差, 生物利用度较低, 限制了其临床的进一步使用; 而原远志皂苷元的碳骨架则在C-2、C-23、C-27等多个C上拥有羟基、羧基、羟甲基等极性基团 (图3), 会增强该皂苷元的溶解性, 也势必会进一步增加该皂苷元及相应远志皂苷的生物利用度。因此, 如能进一步解析远志皂苷的生物合成途径, 则可为后续远志皂苷及其苷元的合成生物学研究, 以及今后远志的创新药物研发奠定坚实的基础。

3.1 远志皂苷可能的生物合成途径 P450s是原远志皂苷元生物合成的关键酶 (图1, 图4)。原远志皂苷元是在齐墩果酸骨架上, 历经多个P450s的一步及/或多步催化生成, 其可能的生物合成途径至少有以下几种 (图4): 齐墩果酸 ① 经由C-27α羟化酶→C-23α氧化酶 (一步及/或多步)→C-2β催化生成; 或经由C-27α羟化酶→→C-2β→C-23α氧化酶 (一步及/或多步) 催化生成; ② 经由C-2β羟化酶→C-23α氧化酶 (一步及/或多步)→C-27α羟化酶催化生成; ③ 经由C-2β、C-27α羟化酶→C-23α氧化酶 (一步及/或多步) 催化生成; ④ 经由C-23α氧化酶 (一步及/或多步)→C-2β、C-27α羟化酶催化生成等。

目前, 在TriForC数据库中只查到了一个远志皂苷合成酶 (*Polygala tenuifolia* β-AS, PtbAS)[80], 而催化齐墩果酸涉及到的多个P450s (图4) 至今尚未报道。C-2β羟化酶、C-23氧化酶也仅在苜蓿*M. truncatula*中被发现 (表2), 而C-27α羟化酶则尚未被发现 (TriForC数据库)。上述研究现状也综合反映了原远志皂苷元结构的复杂性和解析该类三萜骨架生物合成途径的困难程度。

3.2 远志皂苷生物合成相关P450: CYP716A249 (PtOAS)的发现 本课题组在GenBank数据库中查询到与远志相关的有编码序列 (coding sequence, CDS) 全长的10条P450s序列 (2016年, 由Kim小组上传), 采用实时荧光定量PCR (Quantitative Real-time PCR, qRT-PCR) 技术, 对这些P450s在不同生长年限 (1、2、3年生) 和不同组织 (根、茎、叶、花) 栽培远志 (山西汾阳产) 中的mRNA表达水平进行分析, 发现CYP716A249 (GenBank: KY385302.1) 在根中的mRNA表达水平明显高于在其余3个组织中, 且在2年生远志中的mRNA表达水平较高。

将CYP716A249的氨基酸序列与其他不同植物来源 (诸如: 拟南芥*A. thaliana*、铁皮石斛*Dendrobium catenatum*、甘草*G. uralenses*、核桃*Juglans regia*、烟草*Nicotiana tabacum*、人参*P. ginseng*、救荒野豌豆*Vicia sativa*等) 的P450s序列构建系统进化树, 发现CYP716A249和核桃*J. regia*的β-amyrin 28-oxidase-like聚为一类。之后分别构建PtbAS、CYP716A249、CPR (Genbank: AB433810) 的基因表达元件, 并依次转

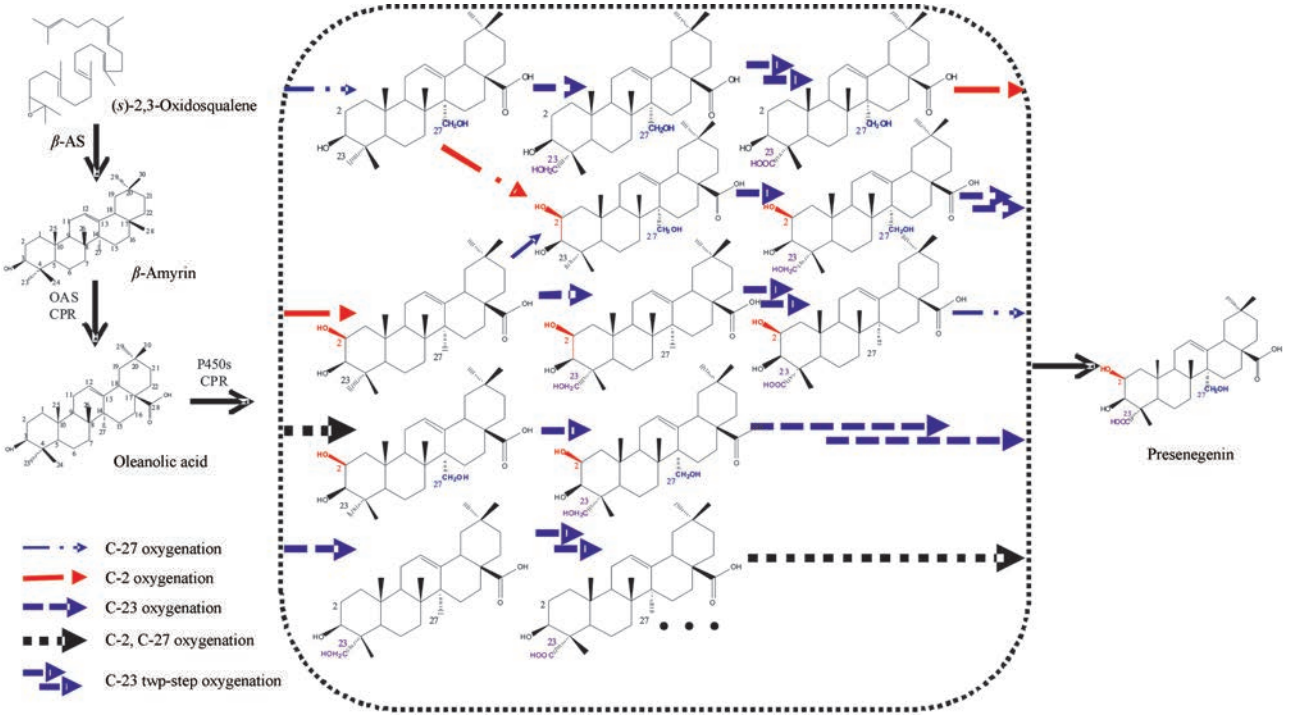

Figure 4 Possible biosynthetic pathway of the presenegenin

化入酿酒酵母*S. cerevisiae*中, 经由气相色谱–质谱(gas chromatography-mass spectrometer, GC-MS)联用技术、气相色谱–质谱(liquid chromatography-mass spectrometer, LC-MS)联用技术、核磁共振(nuclear magnetic resonance, NMR)技术等对发酵代谢产物检测后, 确证CYP716A249可催化β-香树脂醇生成齐墩果酸, 属于C-28氧化酶[81]。

4 展望

2018年2月, 韩国的Kim小组又上传了49条与远志相关的P450s序列(有CDS全长)至GenBank数据库。而本课题组也于2018年12月完成了不同生长年限(1、2、3年)栽培远志(山西汾阳产)根、茎叶(混合样本)的IsoSeq高通量转录组测序(pacbio平台, 20G clean data), 以期获得更多拥有CDS全长的P450s序列, 并在此基础上综合分析前期栽培远志根、茎叶的DGE数据, 筛选出与远志皂苷生物合成紧密相关的P450s。

以上述拥有CDS全长的P450s为候选研究对象, 可以进行后续的功能鉴定, 如① 底物饲喂法: 以齐墩果酸、常春藤皂苷元、苜蓿酸等为底物, 饲喂表达候选P450s的酿酒酵母*S. cerevisiae*, 采用GC-MS、LC-MS、NMR等检测发酵代谢物中是否有新的/目标代谢物, 进而初步鉴定远志的P450s功能; ② 也可分别构建苜蓿*M. truncatula*的C-2β羟化酶、C-23氧化酶(表2)基因表达盒, 并依次转化产齐墩果酸的酿酒酵母*S. cerevisiae*[81], 构建产苜蓿酸的酿酒酵母*S. cerevisiae*菌株, 之后再构建候选P450s的基因表达盒并转入上述菌株中, 经对发酵代谢物进行GC-MS、LC-MS、NMR等检测, 如发现原远志皂苷元代谢物, 则可初步筛选出远志的C-27羟化酶。

随着新P450s陆续被发现, 对于不同种属植物中的三萜类物质, 尤其是齐墩果烷型三萜的生物合成途径有了更深入的认识。同时, 也应意识到这些已知功能的P450s不仅可作为开展三萜类物质合成生物学研究的理想工具, 如借助于混合搭配(mix-and-match)组合生物化学进行三萜的模块化生物合成等, 也可作为完成新P450s功能鉴定的潜在基因资源, 如苜蓿*M. truncatula*中的CYP72A67、CYP72A68v1/v2等。

References

[1] Hill RA, Connolly JD. Triterpenoids [J]. Nat Prod Rep, 2017, 34: 90-122.

[2] Phillips DR, Rasbery JM, Bartel B, et al. Biosynthetic diversity in plant triterpene cyclization [J]. Curr Opin Plant Biol, 2006, 9: 305-314.

[3] Misra RC, Maiti P, Chanotiya CS, et al. Methyl jasmonate-elicited transcriptional responses and pentacyclic triterpene biosynthesis in sweet basil [J]. Plant Physiology, 2014, 164: 1028-1044.

[4] Moses T, Pollier J, Faizal A, et al. Unraveling the triterpenoid saponin biosynthesis of the African shrub *Maesa lanceolata* [J]. Mol Plant, 2015, 8: 122-135.

[5] Moses T, Pollier J, Shen Q, et al. OSC2 and CYP716A14v2 catalyze the biosynthesis of triterpenoids for the cuticle of aerial organs of *Artemisia annua* [J]. Plant Cell Online, 2015, 27: 286-301.

[6] Papadopoulou K, Melton RE, Leggett M, et al. Compromised disease resistance in saponin-deficient plants [J]. Proc Natl Acad Sci U S A, 1999, 96: 12923-12928.

[7] Delis C, Krokida A, Georgiou S, et al. Role of lupeol synthase in *Lotus japonicus* nodule formation [J]. New Phytol, 2011, 189: 335-346.

[8] Confalonieri M, Cammareri M, Biazzi E, et al. Enhanced triterpene saponin biosynthesis and root nodulation in transgenic barrel medic (*Medicago truncatula* Gaertn.) expressing a novel β-amyrin synthase (AsOXA1) gene [J]. Plant Biotechnol J, 2009, 7: 172-182.

[9] Kemen AC, Honkanen S, Melton RE, et al. Investigation of triterpene synthesis and regulation in oats reveals a role for β-amyrin in determining root epidermal cell patterning [J]. Proc Natl Acad Sci U S A, 2014, 111: 8679-8684.

[10] Ben F, Osbourn AE. Metabolic diversification--independent assembly of operon-like gene clusters in different plants [J]. Science, 2008, 320: 543-547.

[11] Krokida A, Delis C, Geisler K, et al. A metabolic gene cluster in *Lotus japonicus* discloses novel enzyme functions and products in triterpene biosynthesis [J]. New Phytol, 2013, 200: 675-690.

[12] Go YS, Lee SB, Kim HJ, et al. Identification of marneral synthase, which is critical for growth and development in Arabidopsis [J]. Plant J, 2012, 72: 791-804.

[13] Laszczyk MN. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy [J]. Planta Med, 2009, 75: 1549-1560.

[14] Sawai S, Saito K. Triterpenoid biosynthesis and engineering in plants [J]. Front Plant Sci, 2011, 2: 25.

[15] Salvador JAR, Moreira VM, Gonçalves BMF, et al. Ursane-type pentacyclic triterpenoids as useful platforms to discover anticancer drugs [J]. Nat Prod Rep, 2012, 29: 1463-1479.

[16] Moses T, Pollier J, Thevelein JM, et al. Bioengineering of plant (tri)terpenoids: from metabolic engineering of plants to synthetic biology *in vivo* and *in vitro* [J]. New Phytol, 2013, 200: 27-43.

[17] Sheng H, Sun H. Synthesis, biology and clinical significance of pentacyclic triterpenes: a multi-target approach to prevention and treatment of metabolic and vascular diseases [J]. Cheminform, 2011, 28: 543-593.

[18] Dai Z, Wang B, Liu Y, et al. Producing aglycons of ginsenosides in bakers' yeast [J]. Sci Rep, 2014, 4: 3698.

[19] Luo Y, Li BZ, Liu D, et al. Engineered biosynthesis of natural products in heterologous hosts [J]. Chem Soc Rev, 2015, 46: 5265-5290.

[20] Arendt P, Miettinen K, Pollier J, et al. An endoplasmic reticulum-engineered yeast platform for overproduction of triterpenoids [J]. Metab Eng, 2017, 40: 165-175.

[21] Reed J, Stephenson MJ, Miettinen K, et al. A translational synthetic biology platform for rapid access to gram-scale quantities of novel drug-like molecules [J]. Metab Eng, 2017, 42: 185-193.

[22] Xu XS, Zhang FS, Qin XM. Research advances on triterpenoid saponins biosynthesis and its key enzymes [J]. World Sci Technol/Mod Tradit Chin Med Mater Med (世界科学技术—中医药现代化), 2014, 16: 2440-2448.

[23] Osbourn A, Goss RJM, Field RA. The saponins-polar isoprenoids with important and diverse biological activities [J]. Nat Prod Rep, 2011, 28: 1261-1268.

[24] Moses T, Papadopoulou KK, Osbourn A. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives [J]. Crit Rev Biochem Mol Biol, 2014, 49: 439-462.

[25] Thimmappa R, Geisler K, Louveau T, et al. Triterpene biosynthesis in plants [J]. Ann Rev Plant Biol, 2014, 65: 225-257.

[26] Ghosh S. Biosynthesis of structurally diverse triterpenes in plants: the role of oxidosqualene cyclases [J]. Proc Indian Natl Sci Acad. 2016, 82: 1189-1210.

[27] Matsui S, Matsumoto H, Sonoda Y, et al. Glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 in a human lung fibroblast cell line [J]. Int Immunopharmacol, 2004, 4: 1633-1644.

[28] Sun SX, Li YM, Fang WR, et al. Effect and mechanism of AR-6 in experimental rheumatoid arthritis [J]. Clin Exp Med, 2010, 10: 113-121.

[29] Man S, Gao W, Zhang Y, et al. Chemical study and medical application of saponins as anti-cancer agents [J]. Fitoterapia, 2010, 81: 703-714.

[30] Cinatl J, Morgenstern B, Bauer G, et al. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus [J]. Lancet, 2003, 361: 2045-2046.

[31] Zhao YL, Cai GM, Hong X, et al. Antihepatitis B virus activities of triterpenoid saponin compound from *Potentilla anserine* L [J]. Phytomedicine, 2008, 15: 253-258.

[32] Suzuki H, Achnine L, Xu R, et al. A genomics approach to the early stages of triterpene saponin biosynthesis in *Medicago truncatula* [J]. Plant J, 2010, 32: 1033-1048.

[33] Sparg SG, Light ME, Staden JV. Biological activities and distribution of plant saponins [J]. J Ethnopharmacol, 2004, 94: 219-243.

[34] Huhman DV, Berhow MA, Sumner LW. Quantification of saponins in aerial and subterranean tissues of *Medicago truncatula* [J]. J Agric Food Chem, 2005, 53: 1914-1920.

[35] Buchanan B, Gruissem W, Jones R. Biochemistry and Molecular Biology of Plants [M]. Beijing: Science Press, 2000: 1250-1318.

[36] Nelson D, Werckreichhart D. A P450-centric view of plant evolution [J]. Plant J, 2011, 66: 194-211.

[37] Urlacher VB, Girhard M. Cytochrome P450 monooxygenases: an update on perspectives for synthetic application [J]. Trends Biotechnol, 2012, 30: 26-36.

[38] Bernhardt R. Cytochromes P450 as versatile biocatalysts [J]. J Biotechnol, 2006, 124: 128-145.

[39] Nelson DR. Progress in tracing the evolutionary paths of cytochrome P450 [J]. Biochim Biophys Acta, 2011, 1814: 14-18.

[40] Miettinen K, Iñigo Sabrina, Kreft L, et al. The TriForC database: a comprehensive up-to-date resource of plant triterpene biosynthesis [J]. Nucleic Acids Res, 2017, 46: 586-594.

[41] Ghosh S. Triterpene structural diversification by plant cytochrome P450 enzymes [J]. Front Plant Sci, 2017, 8: 1886.

[42] Zhou Y, Ma Y, Zeng J, et al. Convergence and divergence of bitterness biosynthesis and regulation in Cucurbitaceae [J]. Nat Plants, 2016, 2: 16183.

[43] Li Z. Research on Mechanisms underlying the Anti-cancer Activities of Triazolyl-napthyl Derivative of β-Amyrin in Nasopharyngeal Carcinoma (β-香树醇酯奈基三唑衍生物对人鼻咽癌抗癌作用机制的研究) [D]. Shandong: Shandong University, 2018.

[44] Shuhei Y, Hikaru S, Yuko S, et al. Functional characterization of CYP716 family P450 enzymes in triterpenoid biosynthesis in tomato [J]. Front Plant Sci, 2017, 8: 21.

[45] Seki H, Ohyama K, Sawai S, et al. Licorice beta-amyrin 11-oxidase, a cytochrome P450 with a key role in the biosynthesis of the triterpene sweetener glycyrrhizin [J]. Proc Natl Acad Sci U S A, 2008, 105: 14204-14209.

[46] Miettinen K, Pollier J, Buyst D, et al. The ancient CYP716 family is a major contributor to the diversification of eudicot triterpenoid biosynthesis [J]. Nat Commun, 2017, 8: 14153.

[47] Geisler K, Hughes RK, Sainsbury F, et al. Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants [J]. Proc Natl Acad Sci U S A, 2013, 110: 3360-3367.

[48] Moses T, Pollier J, Almagro L, et al. Combinatorial biosynthesis of sapogenins and saponins in *Saccharomyces cerevisiae* using a C-16 hydroxylase from *Bupleurum falcatum* [J]. Proc Natl Acad Sci U S A, 2014, 111: 1634-1639.

[49] Yasumoto S, Fukushima EO, Seki H, et al. Novel triterpene oxidizing activity of *Arabidopsis thaliana* CYP716A subfamily enzymes [J]. FEBS Lett, 2016, 590: 533-540.

[50] Tamura K, Seki H, Suzuki H, et al. CYP716A179 functions as a triterpene C-28 oxidase in tissue-cultured stolons of *Glycyrrhiza uralensis* [J]. Plant Cell Rep, 2017, 36: 437-445.

[51] Moses T, Thevelein JM, Goossens A, et al. Comparative analysis of CYP93E proteins for improved microbial synthesis of plant triterpenoids [J]. Phytochemistry, 2014, 108: 47-56.

[52] Shibuya M, Hoshino M, Katsube Y, et al. Identification of β-

amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay [J]. FEBS J, 2006, 273: 948-959.

[53] Fukushima EO, Seki H, Ohyama K, et al. CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis [J]. Plant Cell Physiol, 2011, 52: 2050-2061.

[54] Carelli M, Biazzi E, Panara F, et al. *Medicago truncatula* CYP716A12 is a multifunctional oxidase involved in the biosynthesis of hemolytic saponins [J]. Plant Cell, 2011, 23: 3070-3081.

[55] Han JY, Kim MJ, Ban YW, et al. The involvement of *β*-amyrin 28-oxidase (CYP716A52v2) in oleanane-type ginsenoside biosynthesis in *Panax ginseng* [J]. Plant Cell Physiol, 2013, 54: 2034-2046.

[56] Fiallos-Jurado J, Pollier J, Moses T, et al. Saponin determination, expression analysis and functional characterization of saponin biosynthetic genes in *Chenopodium quinoa* leaves [J]. Plant Sci, 2016, 250: 188-197.

[57] Jo HJ, Han JY, Hwang HS, et al. *β*-Amyrin synthase (EsBAS) and *β*-amyrin 28-oxidase (CYP716A244) in oleanane-type triterpene saponin biosynthesis in *Eleutherococcus senticosus* [J]. Phytochemistry, 2017, 135: 53-63.

[58] Misra RC, Sharma S, Sandeep, et al. Two CYP716A subfamily cytochrome P450 monooxygenases of sweet basil play similar but nonredundant roles in ursane- and oleanane-type pentacyclic triterpene biosynthesis [J]. New Phytol, 2017, 214: 706-720.

[59] Andre CM, Legay S, Deleruelle, et al. Multifunctional oxidosqualene cyclases and cytochrome P450 involved in the biosynthesis of apple fruit triterpenic acids [J]. New Phytol, 2016, 211: 1279-1294.

[60] Huang L, Li J, Ye H, et al. Note added in proof to: molecular characterization of the pentacyclic triterpenoid biosynthetic pathway in *Catharanthus roseus* [J]. Planta, 2012, 236: 1571-1581.

[61] Khakimov B, Kuzina V, Erthmann PØ, et al. Identification and genome organization of saponin pathway genes from a wild crucifer, and their use for transient production of saponins in *Nicotiana benthamiana* [J]. Plant J, 2015, 84: 478-490.

[62] Boutanaev AM, Moses T, Zi J, et al. Investigation of terpene diversification across multiple sequenced plant genomes [J]. Proc Natl Acad Sci U S A, 2015, 112: 81-88.

[63] Seki H, Sawai S, Ohyama K, et al. Triterpene functional genomics in licorice for identification of CYP72A154 involved in the biosynthesis of glycyrrhizin [J]. Plant Cell, 2011, 23: 4112-4123.

[64] Tian LT, Ma L, Du NS. Survey of pharmacology of aleanolic acid [J]. China J Chin Mater Med (中国中药杂志), 2002, 27: 11-26.

[65] Kim HY, Cho KW, Kang DG, et al. Oleanolic acid increases plasma ANP levels via an accentuation of cardiac ANP synthesis and secretion in rats [J]. Eur J Pharmacol, 2013, 710: 73-79.

[66] Pan Y, Zhou F, Song Z, et al. Oleanolic acid protects against pathogenesis of atherosclerosis, possibly *via* FXR-mediated angiotensin (Ang)-(1-7) upregulation [J]. Biomed Pharmacother, 2018, 97: 1694-1700.

[67] Niels HB, Nicolaj CH, Rosalia RR, et al. Antiatherogenic effects of oleanolic acid in apolipoprotein E knockout mice [J]. Eur J Pharmacol, 2011, 670: 519-526.

[68] Martín R, Carvalho-Tavares J, Hernández M, et al. Beneficial actions of oleanolic acid in an experimental model of multiple sclerosis: a potential therapeutic role [J]. Biochem Pharmacol, 2010, 79: 198-208.

[69] Liu XJ, Han YW, Li XM. Effects of oleanolic acid on early brain injury following subarachnoid hemorrhage in rats by inhibiting NF-*κ*B / ICAM-1 signaling pathway [J]. Chin J Mod Appl Pharm (中国现代应用药学), 2017, 34: 1225-1228.

[70] Martín R, Cordova C, San Román JA, et al. Oleanolic acid modulates the immune-inflammatory response in mice with experimental autoimmune myocarditis and protects from cardiac injury. Therapeutic implications for the human disease [J]. J Mol Cell Cardiol, 2014, 72: 250-262.

[71] Bai X, Wang X, Nan ML, et al. Research progress on natural pentacyclic triterpenoids and derivatives in anti-cardiovascular and cerebrovascular diseases [J]. Chin Tradit Herb Drugs (中草药), 2019, 50: 745-752.

[72] Pollier J, Goossens A. Oleanolic acid [J]. Phytochemistry, 2012, 77: 10-15.

[73] Wei J, Liu H, Liu M, et al. Oleanolic acid potentiates the antitumor activity of 5-fluorouracil in pancreatic cancer cells [J]. Oncol Rep, 2012, 28: 1339-1345.

[74] Biazzi E, Carelli M, Tava A, et al. CYP72A67 catalyzes a key oxidative step in *Medicago truncatula* hemolytic saponin biosynthesis [J]. Mol Plant, 2015, 8: 1493-1506.

[75] Fukushima EO, Seki H, Sawai S, et al. Combinatorial biosynthesis of legume natural and rare triterpenoids in engineered yeast [J]. Plant Cell Physiol, 2013, 54: 740-749.

[76] Lin ZH, Gu J, Xiu J, et al. Traditional Chinese medicine for senile dementia [J]. Evid-Based Compl Alt, 2012 (1741-427X): 692621.

[77] Li CJ. Studies on Chemical Constituents and Biological Activities of *Polygala tenuifolia* Willdenow and *Polygala glomerata* Lour (远志和华南远志的化学成分及其生物活性研究) [D]. Beijing: Peking Union Medical College, 2008.

[78] Wang L, Jin GF, Yu HH, et al. Protective effect of tenuifolin against Alzheimer's disease [J]. Neurosci Lett, 2019, 705: 195-201.

[79] Cao Q, Jiang Y, Cui SY, et al. Tenuifolin, a saponin derived from Radix Polygalae, exhibits sleep-enhancing effects in mice [J]. Phytomedicine, 2016, 23: 1797-1805.

[80] Jin ML, Lee DY, Um Y, et al. Isolation and characterization of an oxidosqualene cyclase gene encoding a *β*-amyrin synthase involved in *Polygala tenuifolia* Willd. saponin biosynthesis [J]. Plant Cell Rep, 2014, 33: 511-519.

[81] Zhang FS, Wang QY, Pu YJ, et al. *PtOAS* and its applications (远志齐墩果酸合酶基因PtOAS及其应用): CH, CN109234291A [P]. 2019-01-18.