紫草素-中药植物天然产物: 生物合成、遗传调控、结构修饰与医药功能

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摘要 紫草素是来源于我国传统中药植物紫草科植物的一类具有萘醌结构的天然产物, 临床常用于主治疮疡和皮肤炎等病症。紫草素因具有多重生物学活性和较高的药用价值, 近年来一直被国内外关注, 并且随着生物合成、结构鉴定与修饰、遗传调控和组学等技术的迅速发展, 研究者对其生物合成功能基因挖掘、高效又低毒的分子结构修饰及其构效、作用机理等多个方面, 均开展了较为广泛的研究。为此, 本文系统地概括了近一个世纪以来对天然紫草素的结构鉴定及其在紫草科植物中的分布、生物合成、遗传调控、结构修饰、生物学活性及其医药功能的研究进展。其中, 参与香叶基氢醌和去氧紫草宁的羟化、紫草宁和阿卡宁酰基化途径的关键酶基因的鉴定, 促进完善了已知的紫草素生物合成途径; 特别是通过化学结构修饰所获得的大量半合成紫草素衍生物可显著提高其抗肿瘤靶向性, 以及紫草素主要通过抑制NF- κ B/STAT3等信号通路发挥抗肿瘤、抗炎、抗菌作用, 揭示了紫草素萘醌环和支链羟基修饰的有效性及其作用的分子机制。总之, 上述国内外的相关研究进展无疑极大地推动了紫草素的基础及应用研究的深入开展, 文末还展望了紫草素在新药创制方面的应用前景。

关键词 紫草素, 生物合成, 遗传调控, 结构修饰, 药理活性

药用植物作为我国传统中药材的重要来源, 长期以来一直被现代临床天然药物研发者重点关注。作为重要中药材之一, 紫草早在我国著名古书典籍《五十二病方》《神农本草经》和《本草纲目》等中就有明确的记载。现代版药典中, 《中国药典》(1963年版)第一部开始直至2010年版, 均以干燥根入选中药材的紫草包括紫草科软紫草属植物——软紫草(*Arnebia eu-*

chroma (Royle) Johnst)、内蒙紫草(*Arnebia guttata* Bunge)及紫草属植物——紫草(*Lithospermum erythrorhizon* Sieb. et Zucc.); 《中国药典》2015年和2020年两版则只收录了前两个物种。紫草素是紫草科植物根部富含的红色萘醌类化合物的统称, 它不仅是一种天然色素, 而且还具有极高的药用价值, 长期应用于中成药、化妆品以及食品工业等。为此, 本文将对天然紫

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草素的种类及分布、生物合成及调控、基因组学、结构优化及药理活性等方面的研究进展, 进行系统性的概括和总结, 以促进我国传统中药现代化的深入研发与应用。

1 紫草素及其在紫草科植物中的分布

1.1 紫草素的结构

紫草素母核为5,8-二羟基-1,4-萘醌(5,8-dihydroxy-1,4-naphthoquinone), 并具异己烯基侧链^[1]。根据其旋光性不同, 紫草素类化合物分为两种旋光异构体, 即左旋紫草素(阿卡宁, *S*型, alkannin)与右旋紫草素(紫草宁, *R*型, shikonin)(图1)^[1,2]。

(1) 紫草宁(*R*型)及其衍生物。目前, 从紫草科植物中分离得到的紫草宁类化合物总共有23种(图2, 表1)^[2~7], 分别是紫草宁(1)、乙酰紫草宁(2)、1-甲氧基乙酰紫草宁(3)、丙酰紫草宁(4)、异丁酰紫草宁(5)、异戊酰紫草宁(6)、 α -甲基丁酰紫草宁(7)、 β,β -二甲基丙烯酰紫草宁(8)、 α,β,β -三甲基丙烯酰紫草宁(9)、 α,β -二甲基丙烯酰紫草宁(10)、 β -羟基异戊酰紫草宁(11)、 β -乙酰氧基异戊酰紫草宁(12)、苯甲酰紫草宁(13)、 α -甲基- β -羟基异戊酰紫草宁(14)、丁酰紫草宁(15)、戊酰紫草宁(16)、5-乙酰氧基戊酰紫草宁(17)、 α,α -二甲基丙酰紫草宁(18)、 α -亚甲基丁酰紫草宁(19)、 β -乙酰氧基- α,β -二甲基丁酰紫草宁(20)、(*Z*)2-亚甲基-3-丁烯酰紫草宁(21)、(*E*)2-亚甲基-3-丁烯酰紫草宁(22)、4-羟基-2,4-二甲基戊烯酰紫草宁(23)。一般来说, 紫草宁类化合物的结构越简单, 植物来源越广泛, 例如, 紫草宁(1)、乙酰紫草宁(2)和 β,β -二甲基丙烯酰紫草宁(8); 结构相对复杂的化合物, 植物源就比较少, 例如, 1-甲氧基乙酰紫草宁(3)、 β -乙酰氧基异戊酰紫草宁(12)、苯甲酰紫草宁(13)、 α -甲基- β -羟基异戊酰紫草宁(14)、5-乙酰氧基戊酰紫草宁(17)、 β -乙酰氧基- α,β -二甲基丁酰紫草宁(20)、(*Z*)2-亚甲基-3-丁烯酰紫草宁(21)、(*E*)2-亚甲基-3-丁烯酰紫草宁(22)、4-羟基-2,4-二甲基戊烯酰紫草宁(23)。

(2) 阿卡宁(*S*型)及其衍生物。阿卡宁作为紫草宁的对映异构体, 从紫草科植物中分离得到的衍生物大约有10种(图2, 表1)^[2,7,8], 分别是阿卡宁(24)、乙酰阿卡宁(25)、异丁酰阿卡宁(26)、异戊酰阿卡宁(27)、 α -甲基丁酰阿卡宁(28)、 β,β -二甲基丙烯酰阿卡宁(29)、 α 、

图1 紫草宁和阿卡宁结构

Figure 1 Chemical structures of shikonin and alkannin

β,β -三甲基丙烯酰阿卡宁(30)、 α,β -二甲基丙烯酰阿卡宁(31)、 β -羟基异戊酰阿卡宁(32)、 β -乙酰氧基异戊酰阿卡宁(33)。从种类数量上来看, 阿卡宁类化合物要远远少于紫草宁类。

(3) 其他类型的天然产物。近年来, 除了上述常规的紫草宁和阿卡宁及其衍生物外, 紫草科植物中还有其他紫草宁和阿卡宁的类似物也陆续被鉴定(图2, 表1)^[2~4,6,9~12], 如去氧紫草素(34)、紫草烷(35)、去水紫草烯(36)、(*S*)-5,8-二羟基-2-(4-甲基-6-氧代-5,6-二氢-2*H*-吡喃-2-基)萘-1,4-二酮(37)、甲基紫草素(38)、羟基紫草烷(39)、乙酰羟基紫草烷(40)、 β,β -二甲基丙烯酰羟基紫草烷(41)、 β,β -二甲基丙烯酰乙酰氧基紫草烷(42)、lithoapermidin-A~F(43~48)、5,8-二甲氧基去氧阿卡宁(49)、甲氧基lithoapermidin(50~58)、紫草呋喃(59~65)、羟基紫草呋喃(66~74), 还有一些结构复杂的紫草代谢物(75~82)等, 约49种。

1.2 紫草素在紫草科植物中的分布

现代天然产物化学研究表明, 紫草科植物中某些属根部所产紫草素类化合物多为阿卡宁类; 而某些属主要合成紫草宁类化合物, 阿卡宁类含量甚少; 有些属植物则紫草宁类与阿卡宁类均能合成。从表2所列结果可看出, 紫草宁类化合物在大部分紫草科植物中均有分布, 但其主要分布在内蒙紫草、软紫草(*Arnebia euchroma*)和紫草中; 此外, 在红花琉璃草(*Cynoglossum officinale*)、蓝蓟(*Echium vulgare*)、远东齿缘草(*Eritrichium sichotense*)、钝叶齿缘草(*Eritrichium incanum*)、鹤虱(*Lappula echinata*)、新藏假紫草(*Macrotomia euchroma*)、滨紫草(*Mertensia maritima*)等植物中也均有分布^[2,3,6,11]。

阿卡宁类化合物在紫草科植物中的分布规律大致与紫草宁类化合物相似, 但与紫草宁类化合物相比, 它

图 2 紫草科植物中已鉴定结构的天然紫草素类化合物

Figure 2 Chemical structures of natural shikonins derived from Boraginaceae plants

表 1 紫草科植物中已鉴定结构的天然紫草素名称及其生物活性

Table 1 Structurally identified natural shikonins and their biological activities

化合物(No.)	生物活性	参考文献
紫草宁(shikonin)(1)	抗肿瘤、抗炎、抗菌、伤口修复、神经保护、抗血栓	[2~6]
乙酰紫草宁(acetylshikonin)(2)	抗肿瘤、抗炎、抗菌、伤口修复	[2,5,6]
1-甲氧基乙酰紫草宁(1-methoxyacetylshikonin)(3)		[2,3]
丙酰紫草宁(propionylshikonin)(4)		[2,3,5]
异丁酰紫草宁(isobutrylshikonin)(5)	抗肿瘤、抗炎、抗菌	[2,4~6]
异戊酰紫草宁(isovalerylshikonin) (6)	抗肿瘤、抗炎、抗菌	[2,3,5]
α -甲基丁酰紫草宁(α -methylbutylshikonin)(7)	抗肿瘤、抗炎、抗菌	[2,3,5,7]
β,β -二甲基丙烯酰紫草宁(β,β -dimethylacrylshikonin)(8)	抗肿瘤、抗炎、抗菌	[2~5,7]
α,β,β -三甲基丙烯酰紫草宁(teracrylshikonin)(9)		[2,3]
α,β -二甲基丙烯酰紫草宁(angelylshikonin)(10)		[2,3,5]
β -羟基异戊酰紫草宁(β -hydroxyisovalerylshikonin)(11)	抗肿瘤、抗炎、抗菌	[2,3,5,7]
β -乙酰氧基异戊酰紫草宁(β -acetoxisovalerylshikonin)(12)	抗肿瘤、抗炎、抗菌	[2,3]
苯甲酰紫草宁(benzoylshikonin)(13)		[2,3]
α -甲基- β -羟基异戊酰紫草宁(2'-methyl-hexa-1',3'-dienylshikonin)(14)		[2,3]
丁酰紫草宁(Butylshikonin)(15)		[2,3]
戊酰紫草宁(Valerylshikonin)(16)		[2,3]
5-乙酰氧基戊酰紫草宁(5-acetoxy-valerylshikonin)(17)		[2,3]
α,α -二甲基丙酰紫草宁(α,α -dimethyl propionylshikonin)(18)		[2,3]
α -亚甲基丁酰紫草宁(α -methylene-butenylshikonin)(19)		[2,3]
β -乙酰氧基- α,β -二甲基丁酰紫草宁(β -acetoxy- α,β -dimethylbutylshikonin)(20)		[2,3]
(Z)2-亚甲基-3-丁烯酰紫草宁((Z)2-Methylene-3-butenylshikonin)(21)		[2,3]
(E)2-亚甲基-3-丁烯酰紫草宁((E)2-Methylene-3-butenylshikonin)(22)		[2]
(E)4-羟基-2,4-二甲基戊烯酰紫草宁((E)4-hydroxy-2,4-dimethylpent-pentene acylshikonin)(23)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
阿卡宁(alkannin (or arnebin-4)) (24)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
乙酰阿卡宁(acetylalkannin (or arnebin-3)) (25)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
异丁酰阿卡宁(isobutrylalkannin)(26)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
异戊酰阿卡宁(isovalerylalkannin)(27)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
α -甲基丁酰阿卡宁(α -methylbutylalkannin)(28)	抗肿瘤、抗炎、抗菌、伤口修复	[2]
β,β -二甲基丙烯酰阿卡宁(β,β -dimethylacrylalkannin (or arnebin-1))(29)	抗肿瘤、抗炎、抗菌、伤口修复	[2,8]
α,β,β -三甲基丙烯酰阿卡宁(teracrylalkannin)(30)		[2]
α,β -二甲基丙烯酰阿卡宁(angelylalkannin)(31)		[2]
β -羟基异戊酰阿卡宁(β -hydroxyisovalerylalkannin)(32)		[2]
β -乙酰氧基异戊酰阿卡宁(β -acetoxisovalerylalkannin)(33)		[2,7]
去氧紫草素(deoxyalkannin, deoxyshikonin, or arnebin-7)(34)	抗肿瘤、抗炎、抗菌、伤口修复	[2,4,6]
紫草烷(alkannan)(35)		[2,6]
去水紫草烷(anhydroalkannin)(36)		[2]
(S)-5,8-二羟基-2-(4-甲基-6-氧代-5,6-二氢-2H-吡喃-2-基)萘-1,4-二酮((S)-5,8-dihydroxy-2-(4-methyl-6-oxo-5,6-dihydro-2H-pyran-2-yl)naphthalene-1,4-dione)(37)		[2]
甲基紫草素(5,8-dihydroxy-2-(1-methoxy-4-methyl-3-entenyl)-1,4-naphthalenedione)(38)		[4]
羟基紫草烷(arnebin-5)(39)		[2]
乙酰羟基紫草烷(arnebin-6)(40)		[2]

(表1续1)

化合物(No.)	生物活性	参考文献
β,β -二甲基丙烯酰羟基紫草烷(arnebin-2)(41)		[2]
β,β -二甲基丙烯酰乙酰氧基紫草烷(acetylarnabin-2)(42)		[2]
Lithoapermidin A (43)		[2,3]
Lithoapermidin B (44)		[2]
lithospermidin C (45)		[3]
lithospermidin D (46)		[3]
lithospermidin E (47)		[3]
lithospermidin F (48)		[3]
5,8-二甲氧基去氧阿卡宁 5,8- <i>O</i> -dimethyl-11-deoxyalkannin (49)		[4,11]
1-methoxylithospermidin C (50)		[3]
1-methoxylithospermidin D (51)		[3]
1-methoxylithospermidin H (52)		[3]
1-methoxylithospermidin F (53)		[3]
1-methoxylithospermidin I (54)		[3]
1-methoxylithospermidin J (55)		[3]
1-methoxylithospermidin E (56)		[3]
1-methoxylithospermidin A (57)		[3]
1-methoxylithospermidin B (58)		[3]
arnebifuranone (59)		[9]
shikonofuran A (60)		[3,11]
shikonofuran B (61)		[3,11]
shikonofuran C (62)		[3,11]
shikonofuran D (63)		[3]
shikonofuran E (64)		[3,11]
shikonofuran F (65)		[3]
hydroxyshikonofuran A (66)		[3]
hydroxyshikonofuran B (67)		[3]
hydroxyshikonofuran C (68)		[3]
hydroxyshikonofuran D (69)		[3]
hydroxyshikonofuran E (70)		[3]
hydroxyshikonofuran G (71)		[3]
hydroxyshikonofuran H (72)		[3]
hydroxyshikonofuran I (73)		[3]
hydroxyshikonofuran J (74)		[3]
arnebiabinone (75)		[12]
shikometabolin A (76)		[10]
shikometabolin E (77)		[10]
shikometabolin F (78)		[10]
6-(11'-deoxyalkannin)-alkannin/shikonin β -hydroxyisovaleryl (79)		[3]
6-(11'-deoxyalkannin)-alkannin/shikonin acetate (80)		[3]
6-(11'-deoxyalkannin)-alkannin/shikonin isobutyrylate (81)		[3]
6-(11'-deoxyalkannin)-alkannin/shikonin β,β -dimethylacrylate (82)		[3]

表 2 紫草宁和阿卡宁及其衍生物在不同紫草科植物中的分布

Table 2 Distribution of shikonin, alkannin, and their derivatives in Boraginaceae plants

属	种	天然紫草素		
		紫草宁及其衍生物	阿卡宁及其衍生物	其他类似物
软紫草属 (<i>Arnebia</i>)	黄花软紫草(<i>Arnebia guttata</i>)	1,2,4,8,9, 11,15,16,18,19	29	34,45,46,47,48,50,51,52,53, 54,55,56,57,58,60,61,62,63, 64,65,66,68,69,72,80, 81
	软紫草(<i>Arnebia euchroma</i>)	1,2,3,4,5,6,8,9,11,12,15,16, 17,18,19	24,25,29,32,33	34,35,36,45,46,47,49,50,51, 52,53,54,56,57,58,60,61,62, 63,64,65,66,69,71,72,73,75, 79,81
	<i>Arnebia hispidissima</i>	1,14	24,25,27, 32	34, 59
	<i>Arnebia tibetiana</i>	1,8		
	硬萼软紫草(<i>Arnebia decumbens</i>)	2,6		34
	<i>Arnebia nobilis</i>		24,25,29	34,39,40,41
	<i>Arnebia tinctoria</i>		24,27	
	<i>Arnebia densiflora</i>		30	
红根草属(<i>Alkanna</i>)	<i>Alkanna hirsutissima</i>	8,10		34
	紫朱牛舌草 (<i>Alkanna tinctoria</i>)		24,25,26,27,28,29,31,33	34,35
琉璃草属(<i>Cynoglossum</i>)	红花琉璃草 (<i>Cynoglossum officinale</i>)	1,2, 5,6,7,8		34,35, 36
蓝蓟属(<i>Echium</i>)	洋蓝蓟(<i>Echium lycopsis</i>)	1		
	<i>Echium rubrum</i>	1		
	蓝蓟(<i>Echium vulgare</i>)	1,2,5,6,7,8		34,35
	其他蓝蓟属	8		
齿缘草属(<i>Eritrichium</i>)	钝叶齿缘草	1,2,7,8		34, 35, 36
	远东齿缘草(<i>Eritrichium sichotenze</i>)	1,2,5,7,8		34,35,36
麻疯树属(<i>Jatropha</i>)	油芦子(<i>Jatropha glandulifera</i>)	1, 2, 8		
鹤虱属(<i>Lappula</i>)	<i>Lappula consanguinera</i>	1,2,5, 6, 7,8		34, 35,36
	鹤虱(<i>Lappula echinata</i>)	1,2,5, 6,7, 8		34,35,36
	<i>Lappula erythrorhizon</i>	7, 8		
紫草属(<i>Lithospermum</i>)	紫草(<i>Lithospermum erythrorhizon</i>)	1, 2,4,5, 6,9,10,11, 16, 18, 19,20, 21,22,23	29	34, 35, 36,38, 43,44,45,47, 48, 50,53,55,56,57,58, 60,61,62,63,64,66,67,68,69, 70,72,74,76,77,78, 80,82
	小花紫草(<i>Lithospermum officinale</i>)	1		
	田紫草(<i>Lithospermum arvense</i>)	2, 5,6, 11		
	<i>Lithospermum euchromum</i>	9, 11		
掸紫草属(<i>Macrotomia</i>)	<i>Macrotomia echioides</i>	1		
	<i>Macrotomia ugamensis</i>	1, 8		
	<i>Macrotomia cephalotes</i>		24, 25,27, 28, 29,32	34
滨紫草属(<i>Mertensia</i>)	滨紫草(<i>Mertensia maritima</i>)	1, 2,5, 7,8		34,35,36

(表2续1)

属	种	天然紫草素		
		紫草宁及其衍生物	阿卡宁及其衍生物	其他类似物
<i>Moltkiopsis</i>	<i>Moltkiopsis ciliata</i>	8,13	33	
	<i>Onosma caucasicum</i>	1		
	<i>Onosma conferitum</i>	1		
	密花滇紫草(<i>Onosma confertum</i>)	2,8		34
	昭苏滇紫草(<i>Onosma echioides</i>)		24	
	<i>Onosma heterophylla</i>		27,29,33	34,42
	细花滇紫草(<i>Onosma hookeri</i>)	1,2,8	29	
滇紫草属(<i>Onosma</i>)	<i>Onosma livanovii</i>	1		
	<i>Onosma polyphyllum</i>	1		
	<i>Onosma tauricum</i>	1		
	<i>Onosma sericum</i>	1		
	<i>Onosma setosum</i>	1		
	<i>Onosma visianii</i>	1		
	<i>Onosma zerizaminium</i>	1,8		
	滇紫草(<i>Onosma paniculatum</i>)	2, 8		
	<i>Onosma paniculata</i>		24,29	
<i>Plagiobotrys</i>	<i>Plagiobotrys arizonicus</i>		24	

们的植物源相对较少, 主要有紫朱牛舌草(*Alkanna tinctoria*)、*Macrotomia cephalotes*、软紫草、*Arnebia hispidissima*等。另有少量阿卡宁类化合物分布在*Arnebia nobilis*、*Arnebia tinctoria*等植物中^[2,7,8]。

此外, 近年来陆续鉴定和报道了大量其他的紫草宁/阿卡宁类似物结构。它们主要分布在内蒙紫草、软紫草、紫草中, 另在*Arnebia nobilis*、钝叶齿缘草、滨紫草等植物中也有分布^[2~4,6,9,11,12]。其中, 去氧紫草素(34)和紫草烷(35)的植物源也相对广泛, 原因可能是它们在紫草素的生物合成途径中作为紫草素合成的前体物质。

2 紫草素的生物合成及其遗传调控

2.1 紫草植物细胞培养生产紫草素及其理化调控因子研究

为了促进紫草素生物合成及其调控研究的开展, 开辟一条快速而高产紫草素的途径是必要的, 国内外

研究者自20世纪70年代初开始, 先后成功地建立了包括紫草、软紫草、滇紫草、车前叶蓝蓟(*Echium plantagineum*)等紫草科植物的两步法细胞培养或毛状根培养体系。Tabata等人^[13]最早报道了紫草愈伤细胞的诱导及其培养条件, Yazaki等人^[14]开发出使用农杆菌ATCC15834侵染紫草植株获得毛状根的方法。离体繁殖材料能否成功生产紫草素取决于为紫草素量身定制的培养基的开发。Fujita等人^[15]成功开发出可使紫草愈伤细胞和毛状根生产紫草素的M9培养基, 与紫草愈伤细胞和毛状根的生长培养基(如LS, MS等培养基)相比, M9培养基含有较低浓度的 NH_4^+ , 而 Cu^{2+} 的浓度高达10倍, 由此建立了先使用改良LS培养基在光照下进行愈伤细胞或毛状根的增殖培养, 再转接到M9培养基中黑暗培养以诱导紫草素产生的两步培养法。之后, 朱汝幸等人^[16]也使用两步法成功诱导了滇紫草的愈伤细胞并培养产生了高于原植株产率的紫草素。Haghbeen等人^[17]利用添加2,4-D和激动素的LS培养基对软紫草种子发芽后的根外植体进行诱导, 获得了大量可产紫草

素的软紫草愈伤细胞. 在此基础上, Fu等人^[18]对车前叶蓝蓟毛状根进行了诱导培养和培养体系的优化, 发现该毛状根在1/2 B5培养基中的培养生物量是M9培养基的5倍, 且同样能产生丰富的紫草素, 其中乙酰紫草宁的产量更是高达M9培养条件下的2倍.

在紫草科植物的快速繁殖材料培养体系建立后, 对刺激调控紫草素生物合成的物理因子、化学因子及生物因子等外界环境因素的研究取得了不少进展.

物理因素中, 光对紫草素的生物合成具有最明显但可逆的负调控作用, 这也与自然状态下紫草素在植物根部(即黑暗下)大量积累相吻合. 研究发现, 不同波长的光信号对紫草素的合成调控存在一定的差异, 在连续白光和蓝光照射下, 紫草素及其衍生物的合成被完全抑制, 而在连续红光照射下则被部分抑制^[19]. γ 射线处理也可以调控紫草培养细胞紫草素的合成, Zhang等人^[20]确定了促进紫草素合成的最佳剂量为1500 R, 辐照后可使愈伤组织中紫草素含量提高144.6%. 也有研究报道称, 低温可能促进软紫草细胞中紫草素的积累^[21].

调控紫草素类化合物产生的化学因子中, 植物激素和无机离子是紫草素生物合成的另两大调控因子. Liao等人^[22]发现, 生长素类激素中2,4-D的加入能够强烈抑制紫草素的生物合成, 而乙烯信号传导途径的阻断可能是紫草中2,4-D抑制紫草素生物合成的关键. 油菜素内酯、细胞分裂素类和IAA能够协同促进滇紫草中紫草素的合成, 其中细胞分裂素是紫草愈伤细胞合成紫草素所必需的^[23,24]. 茉莉酸甲酯和乙烯则可能通过促进紫草宁合成相关酶的表达与活性, 从而增加滇紫草、软紫草及硬紫草细胞中紫草素类化合物的产量^[25-27]. 此外, Sun等人^[28]发现, 0.1~5.0 mg/L的ABA能够抑制滇紫草细胞的生长, 并显著降低培养细胞中紫草素的合成. 对于无机离子, Wu等人^[29]通过使用一氧化氮(NO)合酶抑制剂处理滇紫草细胞, 发现内源性NO参与正向调控滇紫草细胞中紫草素的合成. Ca^{2+} 浓度和滇紫草细胞中的钙稳态被验证与紫草素的积累相关^[30,31]. 此外, NH_4^+ 能够抑制紫草LePGT酶的表达与活性, 从而对紫草素合成产生抑制作用, 相反, Cu^{2+} 能够促进紫草素的合成^[32].

真菌诱导子也是刺激调控紫草素类化合物合成的因素之一. Kim和Chang^[33]于1990年首先发现了青霉菌属真菌(*Penicillium* sp.)菌丝体的粗提物能够促进紫草素的生成. 随后, 宁文等人^[34-36]发现曲霉属真菌(*Asper-*

gillus sp.), 尤其是米曲霉(*Aspergillus oryzae*)菌丝体的粗提物和真菌诱导物的添加能使滇紫草愈伤细胞的紫草素总量和乙酰紫草宁的相对含量和产量提高一倍, 但其也会使细胞碳源消耗增多、胞内可溶蛋白合成量上升、细胞生长受到抑制, 因此其促进作用与添加浓度紧密相关. 刘长军等人^[37]也发现, 黑曲霉诱导子对软紫草悬浮培养细胞中紫草素的合成和外排具有高效促进作用. 除曲霉属真菌外, Brigham等人^[38]研究发现, 立枯丝核菌(*Rhizoctonia solani*)、瓜果腐霉(*Pythium aphanidermatum*)等菌丝与紫草根接触时也能诱导局部色素的产生.

2.2 紫草素的生物合成途径

紫草宁及其衍生物富产于部分紫草科植物的根部, 借助于紫草科植物愈伤细胞和毛状根培养体系的建立及多组学研究技术的发展, 其生物合成途径的框架目前已被研究得较为透彻, 整体以香叶基-对羟基苯甲酸(m-geranyl-p-hydroxybenzoic acid, GHB)的合成成为节点, 可分为上游分支部分与下游核心部分(图3).

首先甲羟戊酸(mevalonate pathway, MVA)/去氧木酮糖还原(2-C-methyl-D-erythritol-4-phosphate, MEP)和苯丙素(phenylpropanoid, PP)这两条上游分支途径分别合成焦磷酸香叶酯(geranylpyrophosphate, GPP)和对羟基苯甲酸(p-hydroxybenzoic acid, PHB), 之后这两个重要的前体物质在对羟基苯甲酸香叶基转移酶(p-hydroxybenzoate: geranyltransferase, PGT)的催化下汇聚合成开启下游反应的关键节点物质GHB, 再经环氧化等反应生成香叶基氢醌(geranyl hydroxyquinone, GHQ), 继而羟基化为3"-羟基香叶基氢醌(3"-hydroxy-geranyl hydroxyquinone, GHQ-3"-OH), 经一系列酶促反应最终在内质网中生成紫草宁及其衍生物, 并在胞外分泌作用下被运输到原生质体外的细胞壁中^[39-44].

自然界中, 紫草素类化合物合成的关键前体之一GPP主要来源于MVA和MEP这2种生物途径^[45]. Gaiser和Heide^[46]发现, 使用MVA途径中关键酶3-羟基-3-甲基戊二酸单酰辅酶A还原酶(3-hydroxy-3-methyl glutaryl coenzyme A reductase, HMGR)的抑制剂mevinolin处理硬紫草的悬浮细胞, 能使紫草素及其衍生物的合成减少98%, 所以目前普遍认为在紫草素生物合成途径中GPP主要来源于MVA途径. 随着RACE、转录组等研究技术的发展和成熟, 可合成紫草素类化合物的

图 3 紫草宁及其衍生物的生物合成途径概述。ACTH: 乙酰乙酰辅酶A硫解酶; C4H: 肉桂酸-4-羟化酶; 4-CL: 4-香豆酸辅酶A连接酶; CDPMEK: 4-(二磷酸胞苷-2-C-甲基-D-赤藓醇激酶; DXPS: 1-脱氧-D-木酮糖-5-磷酸合酶; DXR: 5-磷酸-去氧木酮糖还原酶; GDPS: 焦磷酸香叶酯合酶; GHQH: 香叶基氢醌3'-羟化酶; HDR: 1-羟基-2-甲基-2-(E)-丁烯基-4-焦磷酸还原酶; HDS: 1-羟基-2-甲基-2-(E)-丁烯基-4-二磷酸合酶; HMGR: 甲羟戊酸单酰辅酶A还原酶; HMGS: 3-羟基-3-甲基-戊二酰辅酶A合成酶; MCT: 2-C-甲基-D-赤藓糖醇4-磷酸胞苷转移酶; MECDPs: 2-C-甲基-D-赤藓糖醇2,4-环焦磷酸合成酶; MVAK: 甲羟戊酸5-磷酸激酶; MVDD: 甲羟戊酸5-焦磷酸脱羧酶; PAL: 苯丙氨酸氨解酶; PGT: 对羟基苯甲酸香叶基转移酶; PMVK: 磷酸甲羟戊二酸激酶; SAT: 紫草宁乙酰转移酶; DSH: 脱氧紫草宁羟化酶

Figure 3 Overview of various biosynthetic pathways of shikonin and its derivatives. ACTH: acetoacetyl-CoA thiolase; C4H: cinnamic acid 4-hydroxylase; 4-CL: 4-coumarate:CoA ligase; CDPMEK: 4-(cytidine 59-diphospho)-2-C-methyl-D-erythritol 2-phosphokinase; DXPS: 1-deoxy-D-xylulose-5-phosphate synthase; DXR: 5-phosphoric acid-deoxyxylulose reductase; GDPS: geranyldiphosphate synthase; GHQH: geranylhydroquinone 3'-hydroxylase; HDR: 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase; HDS: 1-hydroxy-2-methyl-2-(E)-butenyl-diphosphate synthase; HMGR: hydroxymethylglutaryl-coenzyme A reductase; HMGS: 3-hydroxy-3-methylglutaryl-CoA synthase; MCT: 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase; MECDPs: 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MVAK: mevalonate 5-phospho kinase; MVDD: mevalonate diphosphate decarboxylase; PAL: phenylalanine ammonia-lyase; PGT: p-hydroxybenzoate geranyltransferase; PMVK: 5-phosphomevalonate phosphokinase; SAT: shikonin acetyltransferase; DSH: deoxyshikonin hydroxylases

紫草科植物中的MVA途径相关酶基因已被陆续鉴定, 其中该途径相关的几乎所有酶基因序列在软紫草中均已明确^[47](<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=373122>), 紫草中香叶基香叶基焦磷酸合成酶(geranylgeranyl diphosphate synthase, GGPPS)、法尼基焦磷酸合成酶(farnesyl pyrophosphate synthase, FPPS)等酶相关基因也已鉴定^[48], 而该途径中最为关键且唯一的限速酶HMGR仅检测到一个433 bp序列, 至今尚无对其进行直接功能验证的报道^[49].

PHB是紫草素生物合成的另一重要前体, 研究认为, 其在紫草植物中主要来源于PP途径^[43]. 该途径中已知的肉桂酸-4-羟化酶(cinnamic acid 4-hydroxylase, C4H)、4-香豆酸辅酶A连接酶(4-coumarate:CoA ligase, 4CL)和苯丙氨酸解氨酶(phenylalanine ammonia-lyase, PAL)等相关基因序列在紫草和软紫草中均已被鉴定和克隆^[50,51]. 其中, PAL酶基因作为PP途径的起始酶基因被认为可能参与影响紫草素的合成.

在GPP和PHB均存在的情况下, 其可在关键酶PGT的催化作用下合二为一生成GHB, 开启下游紫草素类化合物的合成. PGT作为紫草素合成核心部分的关键起始酶, 在紫草与软紫草中均已被鉴定出是较大的基因家族, 具有14个成员不等, 可见其极其重要的限速作用^[40].

从GHB生成紫草宁及其衍生物中的一系列酶促反应的研究, 近些年也取得了较大的进展, 在紫草科植物中鉴定到了不同程度的相关酶基因. Wang等人^[44]通过软紫草愈伤细胞的转录组差异基因筛选和酶活分析, 发现软紫草中CYP76B74参与催化紫草素生物合成中的香叶基氢醌3"的羟基化, 合成GHQ-3"-OH. 车前叶蓝蓟和紫草中将GHQ催化为GHQ-3"-OH的细胞色素P450家族蛋白: EpGHQH1及CYP76B100和CYP76B101也通过不同组织转录组差异表达研究被揭示^[52,53]. 此外, Song等人^[54]还鉴定到紫草CYP82AR亚家族中的DSH1蛋白可以催化去氧紫草宁中类异戊二烯侧链的C-1'位置羟化生成紫草宁. Yamamoto等人^[55]则鉴定到紫草所特有的一种新的乙醇脱氢酶, 该酶可将GHQ-3"-OH的3"醇基氧化为醛基, 并伴随着C2'-C3'双键上的异构化, 从Z型转化为E型, 而这可能也是紫草宁及其衍生物合成中一种之前从未被发现的关键中间体. 此外, Oshikiri等人^[56]通过蛋白质组学鉴定到紫草中催化紫草宁生成乙酰紫草宁等衍生物的两个关键酶LeSAT1

和LeAAT1, 其能分别将乙酰基-CoA、异丁酰基-CoA和异戊酰基-CoA催化为乙酰紫草宁/阿卡宁、异丁酰紫草宁/阿卡宁和异戊酰紫草宁/阿卡宁.

2.3 紫草素生物合成的遗传调控及其转运

转录水平的调控是真核生物基因表达调控中的重要环节, 转录因子能识别并结合靶基因的DNA结合域, 激活或阻遏靶基因对RNA聚合酶的招募以调控基因的表达. miRNA也能通过与靶基因的3' UTR区互补配对, 指导miRNP复合体对靶基因mRNA进行切割或者翻译抑制, 从而起到调控基因表达的作用. 基于这一点, 对于紫草宁及其衍生物的合成受转录因子和miRNA调控的研究也取得了显著进展. Zou等人^[57]最先从紫草中克隆出根部高表达的两个转录因子LeEIL-1和LeERF-1, 并对其基因的启动子区域进行了分析. 之后, 研究人员进一步研究了LeEIL-1和LeERF-1的功能, 发现它们可能参与光信号和乙烯对紫草素的合成调控, 能够显著诱导紫草转基因毛状根中紫草宁及其衍生物的积累^[58,59]. Xie等人^[60]利用转录组从软紫草中预测到27个ERF转录因子. Zhao等人^[61,62]从紫草中克隆到1个MYB转录因子, 认为它们的功能可能也与紫草素合成有关.

降解组测序、RLM-Race等技术的发展为紫草科植物中miRNA的鉴定和功能验证提供了很好的机会. Zhao等人^[63]通过对不同光照培养下的紫草细胞进行高通量测序分析, 从中鉴定出18个在黑暗中低表达的miRNA, 分析了其靶基因, 并进一步通过RLM-Race验证了miR319是靶标TCP转录因子, 可能参与调控紫草宁的合成.

紫草宁及其衍生物的含量除了受到生物合成调控影响以外, 其在细胞中的运输也被转运蛋白所控制. 长期以来, ATP结合转运蛋白(ATP-binding cassette transporter, ABC)与次生代谢物质之间的关系一直是广泛关注的研究课题, Zhu等人^[64,65]从紫草中鉴定到两个ABC转运蛋白家族基因LeMDR3和LeMRP1, 并通过酵母缺陷型菌株转运实验验证了LeMDR3参与紫草宁的外向转运, 而定位于液泡膜上的LeMRP1可能参与紫草宁向液泡内转运, 在紫草宁的运输和积累的过程中发挥重要作用.

2.4 紫草素生物合成调控的组学解析

随着二代、三代高通量基因测序技术的日趋完善

以及测序成本的降低, 基因组学、转录组学、蛋白质组学、代谢组学等组学新技术不断涌现, 生命科学和医学的发展得到了极大的促进, 对多组学数据的整合分析, 也已经成为探索生命机制的新方向。

药用植物特别是中药植物作为植物种质资源中的重要部分, 其药用天然产物的生物合成途径的解析已愈发离不开多组学研究技术。近年来, 药用植物基因组的测序与研究进一步促进了对紫草科植物的鉴定、进化及其天然产物生物合成途径的探索。例如, Auber 等人^[40]通过基因组测序和分析获得了紫草共 367.41 Mb 的全基因组序列, 预测得到 27720 个蛋白编码基因, 并从中鉴定到 13 个香叶基转移酶的同源基因, 揭示了它们都起源于紫草科物种的共同祖先, 可能是由于异戊烯转移酶祖先基因的逆转录转座复制事件导致的。Tang 等人^[66]也通过对车前叶蓝蓟进行基因组测序和分析, 同时结合 7 个紫草目其他物种的转录组数据, 探讨了紫草目在唇形分支中的分类地位及其全基因组复制事件的发生, 认为紫草目与茄目和唇形目更为近缘, 且伽玛(γ)事件发生后, 紫草科物种可能至少经历了两轮 WGD (whole genome duplications), 并推测了可能参与紫草宁生物合成核心通路的香叶基环化酶、萜酚羟化酶和酰基转移酶等关键酶基因。

除基因组外, 转录组学、蛋白组学和代谢组学也被单独或整合用于完善紫草等药用植物中次生代谢产物合成及调控通路研究。例如, Wu 等人^[43]通过对软紫草、紫草和车前叶蓝蓟这三种紫草科植物的周皮、木质部、茎和叶等 6 个组织进行转录组学分析, 从中筛选出各物种在长期进化中被正向选择的基因, 同时还发现了两个蛋白 G10H 和 12OPR 可能与紫草素的生物合成相关。Yazaki 团队^[67]通过对繁殖和产紫草素两阶段的紫草细胞进行蛋白质组学差异分析, 结合转录组数据, 从中鉴定出乙酰紫草宁合成中必需的酰基化酶 LeSAT1。Xie 等人^[60]通过软紫草的转录组数据挖掘并分析了软紫草中 AP2/EFR 家族基因序列, 为紫草素类化合物生物合成的转录调控研究奠定了基础。

3 紫草素的化学修饰

天然紫草素(特别是紫草宁)还因具有多重药理学活性备受关注。根据前文所述, 紫草宁显著而广泛的抗肿瘤活性赋予了它“继喜树碱和紫杉醇之后又一极具

潜力的抗肿瘤天然产物”的盛名。然而, 由于其多重靶向而导致的毒副作用较大和水溶性较差等缺陷, 严重阻碍了其被发展成为临床应用的抗肿瘤药物。为了克服天然紫草素的以上缺陷, 国内外研究者殚精竭虑, 围绕紫草宁和阿卡宁两种结构, 进行了大量的结构修饰工作。

3.1 基于紫草宁的化学修饰

紫草宁和阿卡宁一样, 母核为 5,8-二羟基-1,4-萘醌, 并具异己烯基侧链结构, 母核萘醌环和支链羟基均是可修饰位点。

(1) 针对异己烯基侧链进行结构修饰。由于修饰位点基团的活性不同, 国内外研究者起初对紫草宁的结构修饰均是针对其异己烯基侧链上的活性羟基进行的(图4)。早在 1995 年, Ahn 等人^[68]率先合成了一系列乙酰紫草宁类似物(1a)作为 DNA 拓扑异构酶 I (topoisomerase-I) 抑制剂; 随后, Kim 等人^[69]合成了一系列苯乙酰紫草宁类似物(1b), 发现其可显著抑制人肺癌细胞 A549 的黏附能力并有效杀死肿瘤细胞。国内研究者对紫草宁的结构修饰工作最早开始于 2002 年, 当时一些研究者合成了靶向抑制人端粒酶(telomerase)的紫草宁环合衍生物和酰基衍生物(1c, 1d)^[70]; 随后又合成获得了一种呋喃甲酸紫草宁酯(SH-7, 1e), 其作为 DNA 拓扑异构酶抑制剂而发挥良好的抗肿瘤活性^[71]。基于此, 2009 年 Wang 等人^[72]合成了具有良好抗肿瘤活性的苯磺酰胺紫草宁类似物(1f), 并发现其可在缺氧条件下诱导人乳腺癌 MDA-MB-231 细胞中缺氧诱导因子(hypoxia inducible factor, HIF)-1 α 表达水平降低; Su 等人^[73]还合成了酰基- β -糖基紫草宁(1g), 其对耐药肿瘤细胞 K562/ADR, MCF-7/ADR, HL60/ADR 均具有明显的细胞毒性。

还有研究团队对紫草宁进行了较为系统的结构修饰工作, 先后合成获得了大量酰基紫草宁衍生物, 其中包括肉桂酰基(1h)^[74]、氟酰基(1i)^[75]、吡啶酰基(1j)^[76]、噻吩酰基(1k)^[76]、苯氧乙酰基(1l)^[77]、烷酰基(1m)^[78]、巯基糖基(1n)^[79]、苯丙氨酰基(1o)^[80]、氨基噻唑酰基(1p)^[81,82]、噻二唑环酰基(1q)^[83]、噻二唑环酰基紫草宁衍生物(1r)^[84]等, 均是靶向细胞骨架微管蛋白(tubulin)发挥抗肿瘤活性; 另外, 他们还针对其他抗肿瘤靶点设计了不少紫草宁衍生物, 例如, 下调 HIF-1 α 而诱发肿瘤发生凋亡的香豆素酰基紫草宁(1s)^[85],

图 4 针对紫草宁异己烯侧链的结构修饰代表性化合物

Figure 4 Some representative structural modification compounds of the isohexene side chain of shikonin

同时靶向微管蛋白(tubulin)和丙酮酸脱氢酶激酶1(pyruvate dehydrogenase kinase 1, PDK1)的硫辛酰基紫草宁(1t)^[86], 同时靶向抑制tubulin和人表皮生长因子受体(epithelial growth factor receptor, EGFR)的苯甲酰丙烯酰紫草宁(1u)^[87]、苯胺二羧酸紫草宁(1v)^[88]等. 值得一提的是, 其中吡啶酰基紫草宁衍生物(1j)不仅具有显著抗肿瘤活性, 还具有良好的抗病毒作用, 对人手足口病毒EV71和流感病毒H1N1均具有显著抑制作用^[89,90].

此外, Durchschein等人^[91,92]也先后合成两个系列含有环丙烷基的酰基紫草宁衍生物(1w), 均对黑色素瘤细胞具有显著的抑制活性; He等人^[93]合成的葡萄糖胺紫草宁糖苷(1x)可靶向端粒酶而发挥抗肿瘤功效; Shen等人^[94]合成的萘醌衍生物(Comp3-24, 1y)通过调节PI3K信号通路诱导肿瘤细胞凋亡和自噬; Li等人^[95]获得的紫草宁糖苷(1z)具有更好的水溶性和化学稳定性; Shao等人^[96]获得的苯并呋喃紫草宁衍生物(1aa)也是靶向微管蛋白从而阻滞细胞周期, 抑制肿瘤细胞生长和增殖; Rao等人^[97]获得的一系列 β -羟基异戊酰紫草宁类似物(1ab)对多重耐药肿瘤细胞株DU145和HeLa均表现出良好的抑制活性.

(2) 针对侧链和萘醌环进行结构修饰. 在针对紫草宁侧链和萘醌环的同时修饰方面, 国内研究团队也做了不少的工作(图5). 从2010~2014年, 研究者报道了对萘醌环上的羟基、羰基进行甲基化修饰, 并对支链羟基同时进行酰基化修饰(2a, 2b, 2c), 以提高紫草素的抗肿瘤活性^[98~100]; 从2014年开始, 研究者将针对萘醌环的结构修饰策略由原先简单的甲基化修饰变成羟肟结构修饰, 得到的紫草宁肟(2d)显著提高了紫草宁的肿瘤靶向性, 降低了毒副作用^[101], 继续不断对该结构进行优化, 得到的含硫的紫草宁肟(2e)具有更强的抗肿瘤活性和更低的毒副作用, 但具体的分子机制未见报道^[102~105].

此外, 一些研究者在同时针对侧链和萘醌环进行结构修饰方面, 也做了一些有意义的工作. 例如, Lin等人^[79]对紫草宁的糖基化修饰(2f), Li等人^[95]的紫草宁糖苷(2g), Zhao等人^[106]合成获得的四环萘醌衍生物(2h)以及He等人^[93]的葡萄糖胺紫草宁糖苷(2i)均可选择性杀伤肿瘤细胞.

(3) 针对萘醌环进行结构修饰. 与支链羟基相比, 萘醌环上的羟基活性不强, 因此单独针对紫草宁的萘醌环进行结构修饰的研究也比较少(图6). 比如, Zhang

等人^[107]合成的萘醌衍生物(3a)可通过靶向抑制硫氧还蛋白还原酶(thioredoxin reductase, TrxR)发挥抗肿瘤作用. Shen等人^[94]合成的萘醌衍生物(3b, 3c)可靶向抑制Akt发挥抗肿瘤活性.

3.2 基于阿卡宁的结构修饰

相比于紫草宁而言, 针对其对映异构体阿卡宁的结构修饰工作相对较少, 主要是我国学者报道的一些研究进展(图7). 为了进一步提高阿卡宁对肿瘤细胞作用的选择性, Wang等人^[100,101]对阿卡宁的萘醌环和支链羟基同时进行修饰, 得到了阿卡宁衍生物(4a), 随后又获得阿卡宁肟(4b). 为了进一步研究紫草宁/阿卡宁肟DMAKO的抗肿瘤活性靶点, Huang等人^[108]又针对阿卡宁肟进行了一系列乙酰化和泛素化修饰(4c). He等人^[93]为了提高阿卡宁对端粒酶的结合能, 对阿卡宁的支链羟基进行糖基化修饰获得了阿卡宁糖苷(4d).

4 紫草素的生物学活性及其医药功能

4.1 紫草素的抗肿瘤活性

紫草宁及其衍生物尽管具有多重生物活性, 受到广泛关注的仍首推其抗肿瘤活性. 研究表明, 紫草宁及其衍生物可通过激活miR-17-5p/PTEN/Akt, STAT3, PI3K/Akt, ROS等多种通路^[109,110]或途径抑制乳腺癌、肺癌、肝癌、结肠癌、胶质瘤等肿瘤的生长(表3). 以下分别针对全球发病率排序靠前的癌症——乳腺癌、肺癌、结肠直肠癌、胃癌、肝癌、甲状腺癌、胰腺癌、血癌、黑色素瘤及骨肉瘤, 简述近年来有关紫草宁抑制各类肿瘤增殖的研究进展.

(1) 紫草宁对乳腺癌的抗癌作用. 在抗乳腺癌增殖方面, 研究者发现, 紫草宁可以通过调节miR-17-5p/phosphatase and tensin homolog(PTEN)/protein kinase B(Akt)通路^[111]或抑制基质金属蛋白酶(matrix metalloproteinase, MMP)2/9^[112]的活化, 阻止乳腺癌细胞迁移和侵袭; 紫草宁还可以诱导小鼠乳腺癌细胞经p38依赖性途径发生凋亡^[113]; 可诱导雌激素受体(estrogen receptor, ER)阳性细胞MCF-7发生坏死和凋亡^[114]; 还可通过抑制丙酮酸激酶(pyruvate kinase, PK)M2的表达, 增强紫杉醇对三阴性乳腺癌的治疗效果^[115].

(2) 紫草宁对肺癌的抗癌作用. 2020年全球癌症死亡病例996万例, 其中肺癌死亡180万例, 远超其他癌症

图5 针对紫草宁多位点的结构修饰代表性化合物

Figure 5 Representative structural modification compounds for multiple sites of shikonin

图6 针对紫草宁萘醌环的结构修饰代表性化合物

Figure 6 Representative structural modification compounds for the naphthoquinone ring of shikonin

类型, 位居癌症死亡人数第一。研究发现, 将紫草宁与治疗肺癌的临床药物联用, 可以增强其疗效并克服耐药性, 以非小细胞肺癌(non-small cell lung cancer, NSCLC)为例, 紫草宁与吉非替尼联用具有协同抗肿瘤作用, 该作用主要是通过抑制PKM2/STAT3/cyclinD1通路或者通过抑制EGFR的磷酸化, 导致EGFR的降解^[116~118]。另有研究报道, 紫草宁以PI3K/Akt信号通路为靶点, 能克服阿法替尼治疗NSCLC的耐药性^[119]。Yeh等人^[118]的研究表明, 紫草宁通过p53介导的细胞凋亡途径对A549肺癌细胞具有潜在的和剂量相关的细胞毒作用, 为临床上治疗肺癌提供了一种有前途的辅助化疗候选药物。在基础机制研究方面, 紫草宁能激活活性氧(reactive oxygen species, ROS)介导的内质网应激反应, 从而使NSCLC细胞凋亡^[120]。另外, 紫草宁通过抑制整合素1表达和抑制细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)1/2信

图7 针对阿卡宁的结构修饰代表性化合物

Figure 7 Representative compounds for the structural modification of alkannin

号通路活性, 减弱NSCLC对细胞外基质的黏附和转移能力^[121~124]。

(3) 紫草宁对消化器官类恶性肿瘤的抗癌作用。结直肠癌(colorectal cancer, CRC)是世界上第三常见的恶性肿瘤。尽管在外科手术和治疗选择方面取得了进展, 结直肠癌的转移和侵袭仍然是癌症相关死亡的一个重要原因。紫草宁在抗结直肠癌增殖方面, 主要表现为可诱导细胞凋亡、阻滞细胞周期、抑制细胞侵袭和迁移以及诱导细胞自噬。紫草宁诱导细胞凋亡的机制主要是通过线粒体介导的通路, 增加ROS释放, 以及抑制丝裂原活化蛋白激酶(mitogen-activated protein kinase,

表 3 紫草素的生物活性及其药理机制

Table 3 Biological activities and pharmacological mechanisms of shikonin

生物学活性	模型	细胞系/菌株	作用机制	参考文献
抗肿瘤	乳腺癌	MDA-MB-231, BT549	通过miR-17-5p/PTEN/Akt途径抑制上皮细胞向间充质细胞转化抑制细胞迁移和侵袭	[111]
		4T1	通过p38依赖性凋亡途径发挥细胞抗肿瘤作用	[112]
		MCF-7	通过受体相互作用蛋白激酶引发细胞坏死和凋亡	[113]
		MDA-MB-231/468	抑制STAT3, FAK和Src介导的细胞转移	[114]
		MDA-MB-231	抑制ERK, Akt和p70S6激酶的活化	[115]
	肺癌	H1650 H1975	抑制PKM2/STAT3/cyclinD1信号通路抑制TrxR活性并激活EGFR蛋白酶体降解途径	[116~118]
		A549	诱导NSCLC坏死和自噬	[119]
		A549, H1299	激活凋亡信号通路, 抑制PI3K/Akt信号通路活性p53介导细胞凋亡激活ROS介导的内质网应激反应	[120,121]
		A549	抑制CCND1、上调caspase3和caspase7表达促进细胞凋亡抑制ERK1/2信号通路及整合素1的表达阻止肺癌细胞黏附与转移	[122~124]
	结直肠癌	SNU-407	抑制MAPK信号通路和内质网、线粒体介导的凋亡途径	[125]
		HCT116, HT29	诱导基于ROS线粒体介导的凋亡, 调控p53和Nrf2诱导细胞死亡	[126]
		HCT116, SW620	抑制缺氧诱导因子-1 α 信号通路抑制细胞增殖阻滞细胞周期靶向galectin-1/JNK信号轴诱导大肠癌细胞凋亡和自噬, 抑制YAP蛋白调控结肠癌细胞侵袭和自噬	[127,128]
	胃癌	AGS	抑制Egr1介导的p21基因表达阻滞人胃癌细胞周期	[129]
		BGC-823, SGC-7901, AGS	通过ROS释放诱导线粒体介导的细胞凋亡, 增加p38磷酸化, 抑制Akt磷酸化	[130]
		MGC-803	通过TLR2/NF- κ B途径抑制细胞侵袭和迁移	[131]
		HGC-27	线粒体介导的细胞凋亡	[132]
	甲状腺癌	TPC-1	下调DNMT1表达抑制甲状腺癌细胞的迁移和侵袭	[133]
		NPC	抑制PI3K/Akt信号通路活化抑制生长、侵袭和糖酵解	[134]
	胰腺癌	AsPC-1	调节RIP1和RIP3表达诱导不同胰腺癌细胞凋亡和坏死	[135]
		BxPC-3, PANC-1	通过抑制NF- κ B和NF- κ B调控的基因产物抑制癌细胞的生长	[136]
	黑色素瘤	A375SM	通过MAPKs通路介导的细胞凋亡抑制细胞增殖	[137]
		A375, A2058	调节STAT3活性抑制细胞存活、迁移和侵袭	[138]
		A431, B16F10	通过调节EGFR-NF- κ B信号通路引起细胞周期阻滞并诱导细胞凋亡	[139]
	骨肉瘤	143B	通过ROS/细胞外信号调节激酶途径诱导细胞凋亡	[140]
		OS	通过抑制MMP13抑制侵袭性	[141]
		小鼠软骨细胞	通过激活Nrf2/HO-1轴抑制NF- κ B活性, 发挥其抗炎和抑制ECM降解的作用	[142]
	血癌	BL	抑制C-MYC表达和PI3K/Akt/mTOR通路诱导细胞凋亡	[143]
		K562	提高PTEN表达水平和抑制PI3K/Akt信号通路诱导凋亡	[144]
		NB4	调节MAPKs和C-MYC表达抑制细胞增殖及诱导细胞凋亡	[145]
		HL-60	靶向抑制ERP57诱导细胞凋亡	[146]
		U937	抑制IGF1R-Akt-mTOR信号通路诱导细胞凋亡	[147]
	肝癌	SMMC-7721	激活caspases-3, caspases-8和caspases-9活性引起细胞凋亡	[148]
		HCC	下调波形蛋白、MMP-2和MMP-9的表达水平抑制癌细胞的迁移能力	[149]

(表3续1)

生物学活性	模型	细胞系/菌株	作用机制	参考文献
抗炎症	关节炎	CIA 鼠	下调PI3K/Akt和MAPKs信号通路发挥抗类风湿关节炎作用	[150]
		成纤维样滑膜细胞	抑制PKC-NF- κ B依赖的信号通路	[151]
		大鼠骨关节炎	调节PI3K/Akt信号通路抑制炎症和诱导软骨细胞凋亡	[152]
		兔软骨细胞	抑制NF- κ B p65的活化	[153]
	结肠炎	硫酸葡聚糖钠(dextran sulfate, DSS)诱导的急性UC	抑制NLRP3炎症小体和NF- κ B信号通路	[154]
		2,4,6-三硝基苯磺酸(2,4,6-trinitrobenzenesulfonic acid, TNBS)诱导的结肠炎	降低血清中TNF- α 和肿瘤坏死因子蛋白水平及其下调TNF- α 表达并降低MPO活性	[155]
		DSS诱导的急性UC	阻断NF- κ B和STAT-3的活化	[156]
	皮炎	特应性皮炎患者	抑制Derp2诱导的IL-6, IL-9和IL-17A的表达	[157]
		角质细胞	抑制JAK/STAT3依赖性途径	[158]
		诱导型调节性T细胞	抑制Akt/mTOR通路	[159]
		人永生化上皮细胞	通过激活线粒体信号通路和失活Akt和Erk通路诱导细胞凋亡, 阻断JAK2/STAT3通路抑制IL-17诱导的VEGF表达	[160]
抗病菌	真菌	银屑病	抑制IL-6, IL-12, p40, IL-23, IL-1 β 的表达和分泌	[161]
		土曲霉	ROS介导的抑菌作用	[162,163]
		红色毛癣菌	影响麦角甾醇生物合成和诱导凋亡	[164]
		白色念珠菌	通过HST3介导的H3K56ac直接发挥抗真菌活性 通过内源性NO积累, 抑制YHB1和CTA4的功能	[165,166]
	细菌	大肠杆菌, 沙门氏菌	抑制细菌增殖	[167]
		金黄色葡萄球菌	抗MRSA作用与紫草宁对肽聚糖的亲力和、细胞膜的通透性和ABC转运蛋白的活性有关	[168]
		嗜酸乳杆菌	抑制细菌增殖	[169]
		幽门螺杆菌	抑制2-氨基萘的N-乙酰化	[170]
		耐甲氧西林金黄色葡萄球菌, 耐万古霉素肠球菌	抑制细菌增殖	[171]
		新冠病毒	抑制Mpro水解酶活性	[172,173]
	病毒	丙型肝炎	上调Nur77表达及激活JNK	[174]
		乙型肝炎病毒X蛋白	下调自噬标志物LC3的表达	[175]
		3型腺病毒	抑制hexon蛋白表达	[176]
		甲型流感病毒	抑制神经氨酸酶的活性	[177]

MAPK)等通路^[125]。另有研究发现, 紫草宁也可通过调节p53, 核因子E2相关因子2(nuclear factor E2-related factor 2, *Nrf2*)以及ROS依赖性途径诱导细胞凋亡^[126]。细胞增殖是通过细胞周期完成的, 因此细胞周期的每个检验点对于细胞正常增殖非常重要, 许多抗癌药物也是通过阻滞细胞周期发挥其活性。研究发现, 紫草宁可通过抑制缺氧诱导因子阻滞结肠癌细胞周期, 从而抑制细胞增殖^[127]。在细胞自噬方面, 紫草宁可靶向调节galectin-1和c-Jun N-terminal kinase(JNK)信号通路或抑制Yes相关蛋白(Yes-associated protein, YAP)表

达, 诱导结肠癌自噬^[128]。此外, 紫草宁还表现出显著的抗胃癌活性, 例如, 紫草宁通过抑制早期生长反应1(early growth responsive gene-1, *Egr1*)介导p21基因表达诱导人胃癌细胞周期阻滞^[129]; 还可通过Toll样受体2(Toll-like receptor 2, TLR2)和核因子kappa-B(nuclear factor kappa-B, NF- κ B)介导的信号途径, 抑制胃癌细胞侵袭和迁移^[130-132]。

(4) 紫草宁对甲状腺癌和胰腺癌的抗癌作用。紫草宁抑制甲状腺癌和胰腺癌增殖的功能主要是通过诱导细胞凋亡和自噬以及抑制细胞迁移和侵袭而实现。其

中, 抗甲状腺癌机制是通过降低DNA甲基转移酶1 (DNA methyltransferase 1, DNMT1)或失活PI3K/Akt信号通路活性, 抑制甲状腺癌细胞的生长、迁移和侵袭^[133,134]; 紫草宁调节受体相互作用蛋白(receptor-interacting protein, RIP)1/3的表达, 诱导甲状腺癌细胞的凋亡和自噬. 抗细胞增殖则主要是抑制了其NF- κ B信号通路及相关基因表达^[135,136].

(5) 紫草宁抗黑色素瘤及骨肉瘤作用. 研究者发现, 紫草宁抑制MAPKs信号转导途径和激活转录因子3(signal transducer and activator of transcription 3, STAT3)以及EGFR/NF- κ B信号通路引起黑色素瘤细胞凋亡^[137~139]. 在抗骨肉瘤研究方面, 紫草宁可上调caspase-3和caspase-8活性, 或调节依赖于ROS/细胞外信号激酶等途径, 诱导骨肉瘤细胞凋亡^[140,141]. 紫草宁还可通过下调MMP13表达, 抑制骨肉瘤细胞侵袭能力. 此外, 还可通过激活Nrf2/HO-1轴抑制NF- κ B活性, 发挥其抗炎和抑制ECM降解的作用^[142].

(6) 紫草宁抗血癌作用. 紫草宁对血癌抑制作用主要体现在它可通过多种途径诱导细胞凋亡. 比如, 紫草宁可通过抑制C-MYC表达, 抑制PI3K/Akt/mTOR或IGF1R-Akt-mTOR信号通路的活性, 以及调节MAPKs和ERP57的蛋白表达, 诱导血癌细胞发生凋亡^[143~147].

(7) 紫草宁对肝癌的抗癌作用. 紫草宁在抗肝癌方面报道相对较少, 其机制主要体现在通过激活caspases-3, caspase-8和caspase-9引起肝细胞凋亡^[148]以及降低波形蛋白MMP-2和MMP-9的表达, 抑制细胞迁移能力^[149].

4.2 紫草素的抗炎活性

紫草宁及其衍生物在抗炎症方面的研究也一直备受关注(表3). 研究者发现, 紫草宁抑制PI3K/Akt和MAPKs以及PKC-NF- κ B信号通路的活性, 从而对类风湿关节炎血管生成产生影响^[150~152]. 另有研究发现, β -羟基异戊基紫草宁激活Nrf2/heme oxygenase-1(HO-1)轴来抑制NF- κ B通路的活性, 从而发挥抗炎和抑制细胞外基质降解的作用^[153]. 紫草宁在溃疡性及肠炎(ulcerative colitis, UC)治疗方面主要通过阻止NF- κ B和STAT3的活性, 降低血清中肿瘤坏死因子- α (tumor necrosis factor alpha, TNF- α)的表达和髓过氧化物酶(myeloperoxidase, MPO)的活性, 起到抗结肠炎效果^[109,154~156]. 在抗皮炎研究中, 紫草宁主要是调节

Janus激酶(Janus kinase, JAK)/STAT3、Akt/mTOR信号通路活性和ERK参与的线粒体途径的凋亡通路等, 发挥显著的抗皮炎作用^[157~160].

4.3 紫草素的抗菌和抗病毒活性

紫草宁具有显著的抗真菌、抗细菌和抗病毒活性(表3). 在抗真菌方面, 研究发现, 紫草宁可通过积累内源性NO, 抑制YHB1和CTA4的功能发挥抗真菌作用^[161~165]. 此外, 紫草宁及其衍生物还可通过HST3介导的H3K56ac(histone H3 on lysine 56 residue), 直接发挥抗真菌活性^[166]. 紫草宁及其衍生物抗细菌作用主要与其对肽聚糖的亲合力、细胞膜的通透性和ABC转运蛋白的活性有关^[167~171]. 紫草宁抗病毒方面, 研究显示紫草宁衍生物可通过抑制炎症因子interleukin-1 β (IL-1 β), interleukin-6/8(IL-6/8)和TNF- α 发挥抗肠道病毒EV71作用. 2019年, 新冠肺炎大流行之际, 研究者通过分子模拟筛选及蛋白活性实验发现, 紫草宁通过抑制Mpro水解酶活性可有效抑制病毒复制达到抗病毒效果^[172,173]. 此外, 研究还发现乙酰紫草宁可通过上调Nur77表达和JNK激活发挥抗乙型肝炎病毒作用^[174~177].

4.4 紫草素的其他生物学活性

紫草宁及其衍生物还可以愈合伤口, 保护急性肝肺损伤, 促进成骨细胞生成, 保护心肌细胞治疗动脉粥样硬化. 例如, 紫草宁可通过NF- κ B/MAPK途径, 减轻D-半乳糖诱导的记忆损伤、神经炎症和神经退行性变. 研究还发现去氧紫草宁能够促进糖尿病小鼠的伤口愈合^[178~182].

5 紫草素类新药的创制及其前景

正如传统医学记载, 作为根部含有紫草素的中药植物, 紫草在我国传统中药中具有独特的应用价值. 近数十年来, 紫草的生物学和生物技术、紫草素的合成生物学和药化药理, 不断取得新的进展或突破, 这为深入挖掘与创制植物源的现代天然产物新药, 提供了新思路、新策略和新方法. 关于紫草素类新药研发的开展, 就所涉及的生物学、生物技术与药学等多学科领域而言, 主要体现在以下四个方面. 一是紫草宁已被证明在体内外均能有效抑制肿瘤的增殖, 然而其

毒性尚不容忽视, 重视紫草宁及其衍生物的毒性特征, 确保紫草宁及其衍生物的安全性应用是临床治疗的先决条件。二是已有研究报道揭示了将目前临床抗癌药物与紫草宁联合使用, 可显著提高其抗癌疗效, 这无疑极大地提高了紫草宁作为综合疗法的组分应用到临床的可能性。三是众多研究显示, 紫草宁及衍生物可通过PI3K/Akt, STAT3, NF- κ B等多种信号途径发挥抗癌、抗炎、抗病毒作用, 这些机理的深入阐明有望产生良好的导向作用, 促进以紫草宁为基础的新型衍生

物及其生物技术的研发。四是不难预期, 基于基因组、转录组、代谢组等多组学融合研究, 明晰紫草宁及其衍生物合成调控通路中尚未发现的关键酶, 通过基因工程获得紫草宁及其衍生物的高产转基因株系, 以及借助工程菌株采用合成生物学的方法从多角度入手, 实现高效生产紫草宁及其衍生物, 能够为我国紫草素类新药的创制提供充足的原料, 同时无疑有望为我国中药植物生物技术引领国际植物天然药物的研究作出有力的贡献。

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Shikonins as natural products from traditional Chinese medicinal plants: their biosynthesis, genetic regulation, structural modifications, and pharmaceutical functions

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Shikonins are natural products having naphthoquinone structures used in clinical treatments for sores and dermatitis. These compounds are frequently extracted from a kind of important traditional Chinese medicinal plants, Boraginaceae. Due to their effective multiple biological activities and high medicinal values, shikonins have attracted increasing attentions from domestic and foreign researchers. Also, with the rapid development in gene identification, regulation and omics, researchers have extensively explored the functional gene mining of shikonins biosynthesis. Moreover, the structural modification of shikonins has been conducted at high efficiency and low toxicity, which influences their biological activity and mechanism. Thus, in this paper, we systematically summarized the structural identification of natural shikonins and their distributions in Boraginaceae in the past century. Meanwhile, we also clearly discussed the biosynthesis and genetic regulations, as well as the structural modifications and biological activities of shikonins. Among them, the identification of the key enzyme genes involved in the hydroxylation of geranyl hydroquinone and deoxyshikonin, as well as those involved in the acylation of shikonin and alkannin has improved the shikonin biosynthesis pathway greatly. Furthermore, several semisynthetic derivatives obtained through structural modifications have significantly improved the antitumor targeting of shikonins. As reported, the antitumor, anti-inflammatory, and antibacterial effects of shikonins acted mainly by inhibiting NF- κ B/STAT3 and other signaling pathways, which revealed the effectiveness of the modification on the naphthoquinone ring and the branched hydroxyl of shikonins, and further clarified the molecular mechanisms. Collectively, the above research progresses domestically and abroad have undoubtedly promoted the in-depth development of basic and applied research of shikonins. Finally, the prospect of shikonins as candidates was also briefly discussed for developing novel drugs.

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