

· 综 述 ·

Bt Cry 毒素抗虫模拟物靶向创新设计

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摘 要: Bt Cry 毒素是当前研究最深入、应用最广的生物杀虫蛋白, 对农业害虫的绿色防治发挥了重大作用。然而, 随着其制剂和转基因抗虫作物的广泛应用, 由此驱动诱发的靶标害虫抗药性及潜在生态安全风险等问题日益凸显。探寻具备模拟 Bt Cry 毒素杀虫功能的新型杀虫蛋白材料, 不仅可为农作物持续健康生产保驾护航, 也能在一定程度上缓解靶标害虫对 Bt Cry 毒素的抗药性压力。近年来, 笔者团队以抗体“免疫网络学说(immune network theory)”中 Ab2 β 类型抗独特型抗体(anti-idiotypic antibody, Anti-Id)具备模拟抗原结构和功能的特性为理论依据, 借助噬菌体展示抗体库及特异性抗体高通量筛选与鉴定技术, 设计 Bt Cry 毒素抗体为包被靶点抗原, 从噬菌体抗体库中靶向筛选到了一系列具备模拟 Bt Cry 毒素杀虫功能的 Ab2 β 类型抗独特型抗体(即 Bt Cry 毒素杀虫模拟物), 其中活性最强的 Bt Cry 毒素杀虫模拟物对靶标害虫的致死率接近相应原 Bt Cry 毒素的 80%, 初步实现了 Bt Cry 毒素杀虫模拟物的靶向设计。本文从理论依据、技术条件、研究现状等方面进行系统概述, 并就相关技术发展趋势以及如何推进现有成果落地应用等展开深入探讨, 旨在为绿色杀虫材料创新研发提供全新思路和实例参考。

关键词: Bt Cry 毒素; 抗独特型抗体; 噬菌体展示抗体; 杀虫蛋白; 生物防治

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Targeted innovative design of Bt Cry toxin insecticidal mimics

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Abstract: Bt Cry toxin is the mostly studied and widely used biological insect resistance protein, which plays a leading role in the green control of agricultural pests worldwide. However, with the wide application of its preparations and transgenic insecticidal crops, the resistance to target pests and potential ecological risks induced by the drive are increasingly prominent and attracting much attention. The researchers seek to explore new insecticidal protein materials that can simulate the insecticidal function of Bt Cry toxin. This will help to escort the sustainable and healthy production of crops, and relieve the pressure of target pests' resistance to Bt Cry toxin to a certain extent. In recent years, the author's team has proposed that Ab2 β anti-idiotypic antibody has the property of mimicking antigen structure and function based on the "Immune network theory" of antibody. With the help of phage display antibody library and specific antibody high-throughput screening and identification technology, Bt Cry toxin antibody was designed as the coating target antigen, and a series of Ab2 β anti-idiotypic antibodies (namely Bt Cry toxin insecticidal mimics) were screened from the phage antibody library. Among them, the lethality of Bt Cry toxin insecticidal mimics with the strongest activity was close to 80% of the corresponding original Bt Cry toxin, showing great promise for the targeted design of Bt Cry toxin insecticidal mimics. This paper systematically summarized the theoretical basis, technical conditions, research status, and discussed the development trend of relevant technologies and how to promote the application of existing achievements, aiming to facilitate the research and development of green insect-resistant materials.

Keywords: Bt Cry toxin; anti-idiotypic antibody; phage-display antibody; insect-resistant protein; biological control

Bt Cry 毒素是革兰氏阳性细菌苏云金芽孢杆菌(*Bacillus thuringiensis*, 简称 Bt)在代谢过程中产生的一类伴孢晶体蛋白^[1], 其对鳞翅目[如小菜蛾(*Plutella xylostella*)^[2]、棉铃虫(*Helicoverpa armigera*)^[3]、稻纵卷叶螟(*Cnaphalocrocis medinalis*)^[4]]]、鞘翅目[如马铃薯甲虫(*Leptinotarsa decemlineata*)^[5]、玉米象(*Sitophilus zeamais*)^[6]]]等多种常见农业害虫具有特异性高致死毒杀作用, 而对人类和其他高等哺乳动物等非靶标生物, 目前尚无直接充分证据证明存在明显危害^[7-9]。

Bt Cry 毒素的杀虫机理目前尚未完全解析, 但在较为主流的观点中, 无论是“穿孔形成”模型假说还是“信号转导”模型假说, 都普遍认为 Bt Cry 毒素是通过与靶标害虫中肠受体如腺苷三磷酸结合盒转运蛋白(ATP-binding cassette transporters, ABC 转运蛋白)、钙黏蛋白(cadherin, CAD)、氨肽酶-N(aminopeptidase N, APN)、碱性磷酸酶(alkaline phosphatase, ALP)等特异性结合所诱发的一系列复杂的级联反应, 从而造成肠道穿孔, 最终导致虫体死亡^[10]。Bt Cry 毒

素制剂及其转基因抗虫作物现已被广泛应用于农业害虫绿色防治,根据国际农业生物技术应用服务组织(International Service for the Acquisition of Agri-Biotech Applications, ISAAA)权威统计,仅2019年全球种植的转 Bt Cry 毒素抗虫作物就超过 1 亿 hm^2 ,作物增产价值接近 190 亿美元^[11],在世界范围内产生了巨大的经济和生态环境效益。然而,由于 Bt Cry 毒素制剂及其转基因抗虫作物长期应用所诱发的驱动靶标害虫向抗药性快速突变进化的问题愈发严峻^[12],Bt Cry 毒素在农业害虫防控上的高效优势逐渐显得捉襟见肘。因此,探寻更为高效、安全的新型生物抗虫材料对保障农作物稳产乃至提质增产具有重要的科学意义和广阔的应用前景。

目前,苏云金芽孢杆菌仍然是挖掘新型抗虫蛋白材料的最主要渠道,近年来不断有包括 Bt Cry 毒素家族、Bt Cyt 毒素家族、Bt Vip 毒素家族在内的新型异种抗虫毒素蛋白被分离并获得认定。国际 Bt 数据权威网站(http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/)信息表明,截至 2022 年 7 月底,Bt Cry 毒素拥有 78 个基因家族(Cry1–Cry78),累计 818 个基因获得命名;Bt Cyt 毒素拥有 3 个基因家族(Cyt1–Cyt3),累计 40 个基因获得命名;而 Bt Vip 毒素拥有 4 个家族(Vip1–Vip4),累计 147 个基因获得命名。此外,动植物乃至微生物源凝集素(lectin)也是探寻和挖掘新型抗虫蛋白材料的重要渠道,如 Sadanandan 等^[13]分离获得的海绵凝集素、He 等^[14]分离获得的雪花莲凝集素、Bhagat 等^[15]分离获得的齐整小核菌凝集素分别对豇豆蚜(cowpea aphid)、小菜蛾、番茄根结线虫(*Meloidogyne incognita*)具有良好抗虫活性。但总的来说,就目前文献资料和应用实效来看,新发现或鉴定的具备抗虫功能的蛋白材料,无论是 Bt Cry/Cyt/Vip 毒素家族蛋白还是凝集素

蛋白,它们所展示出的抗虫活性和抗虫广谱性均无法与目前商业化开发应用最成熟的 Bt Cry 毒素(典型的有 Cry1Ab、Cry1Ac、Cry1B、Cry1C、Cry1F、Cry2A 等)相媲美。抛开靶标害虫日益进化的抗药性问题,Bt Cry 毒素目前仍然是抗虫效果最好、安全性最高的生物抗虫蛋白材料。基于此,笔者团队另辟蹊径,以抗体“免疫网络学说”中的 Ab2 β 类型抗独特型抗体具备模拟抗原结构和功能的特性^[16]为理论依据,并创造性结合近年兴起的噬菌体展示抗体技术路径,历经十余年探索,最终实现 Bt Cry 毒素抗虫模拟物靶向设计,并积累了一系列原创性科研成果。本文从 Bt Cry 毒素抗虫模拟物靶向设计的理论依据、技术条件以及研究现状等方面进行概述,并结合作者及所在团队在研究过程中遇到的技术瓶颈展开探讨,以期在推介最新研究成果的同时,也为同行提供潜在的启发思路和实例参考。

1 Bt Cry 毒素抗虫模拟物靶向设计的理论依据

1974 年,丹麦免疫学家 Niels K. Jerne 首次提出并阐述了抗体“免疫网络学说(Immune network theory)”理论^[17],认为抗原(antigen, Ag)物质进入动物机体刺激免疫系统产生抗体(antibody, Ab)诱发一系列免疫效应直至结束的整个过程是可逆向作用的级联免疫反应,其中应对抗原的一级抗体(Ab1)分子上的独特型抗原受体部位能反向刺激免疫系统,产生特异性应对该抗体独特位(anti-idiotypic, AI)的二级抗体(Ab2)——即抗独特型抗体(anti-idiotypic antibody, Anti-Id) (图 1)。而抗独特型抗体按其与其与靶标抗体独特型抗原受体部位结合的区域和功能划分,又能进一步细分为 Ab2 α 、Ab2 β 、Ab2 γ

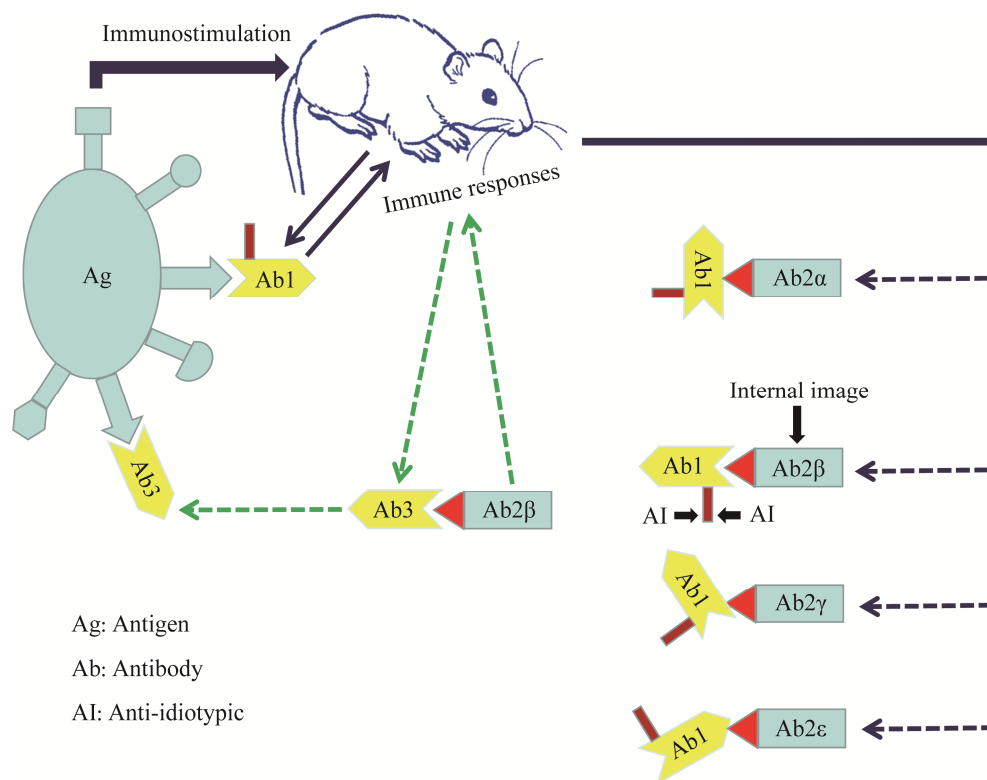


图1 机体级联免疫反应产生抗独特型抗体示意图

Figure 1 Schematic diagram of the cascade immune response to produce anti-idiotypic antibodies.

以及 Ab2ε 等 4 种主要亚型^[18]，它们分别对应抗体分子上的独特型抗原受体骨架区、互补决定区、靠近互补决定区以及远离互补决定区等部位逆向刺激免疫系统所诱导产生的抗独特型抗体类型。其中 Ab2β 类型的抗独特型抗体因直接由抗体分子上的独特型抗原受体互补决定区(即抗体独特位与抗原结合的关键核心区域)刺激免疫系统所诱导产生，具备抗原“内影像(internal image)”效应，其在三维构象上与抗原类似^[19]，因此 Ab2β 类型的抗独特型抗体具有模拟并替代抗原全部或部分关键结构乃至生物活性的功能，这为抗原模拟物的靶向设计提供了理论依据，并在生物医药和免疫检测领域相关研究中率先得到了印证^[20-21]。依据抗体“免疫网络学说”理论中的抗独特型抗体制备技术路

径，Hanoux 等^[22]、Lan 等^[23]、Wang 等^[24]均采用动物免疫的方式分别创制出了具备模拟人朊病毒蛋白(human prion protein)、催乳素(prolactin)、表皮生长因子(epidermal growth factors)关键受体结构，并能替代研究对象用于相应疾病治疗的 Ab2β 类型抗独特型抗体；而 Shi 等^[25]采用免疫动物获得了模拟玉米赤霉烯酮(zearalenone)关键特征性结构的 Ab2β 类型抗独特型单克隆抗体和 Shu 等^[26]采用体外筛选的方式分别获得了具备模拟伏马毒素 B₁(fumonisin B₁)关键特征性结构的 Ab2β 类型抗独特型纳米抗体，均能替代原毒素标准品用于建立针对相应毒素筛查分析的免疫学检测方法；此外，Xu 等^[27]采用体外筛选的方式从人源噬菌体抗体库中筛选到了初步具备模拟万古霉

素(vancomycin)抗菌功能的 Ab2 β 类型抗独特型人源重链单域抗体(single domain antibody), 其在食源性金黄色葡萄球菌(*Staphylococcus aureus*) 安全防控上具有潜在应用优势。Bt Cry 毒素发挥抗虫活性的活化态蛋白分子量在 60 kDa 左右, 属生物大分子物质, 具备天然免疫源性, 且其分区结构和相应功能研究得比较透彻^[28], 而抗体分子上含重轻链的完整抗原结合片段(Fab) 分子量在 50 kDa 左右, 其结构分区和相应功能也研究得比较透彻^[29]; 由于两者同属蛋白, 且抗体分子结合抗原的重链、轻链在结构和分子量大小上与 Bt Cry 毒素发挥抗虫功能的关键区域 domain II、domain III 具有一定可类比性, 因此相对上述小分子抗原模拟物创制成功的研究实例, 依托抗体“免疫网络学说”和抗独特型抗体技术路径, 创制具备模拟 Bt Cry 毒素结构和抗虫功能的 Ab2 β 类型抗独特型抗体更存在理论上的可行性和技术上的可操作性。

2 Bt Cry 毒素抗虫模拟物靶向设计的技术条件

依托抗体“免疫网络学说”理论中的 Ab2 β 抗独特型抗体技术路径创制具备模拟 Bt Cry 毒素抗虫功能的杀虫蛋白材料, 其本质归根到底是制备可模拟 Bt Cry 毒素抗虫功能的特异性抗体过程, 因此现有的抗体制备技术理论上都适用于 Bt Cry 毒素抗虫模拟物设计。目前抗体制备技术已经从传统的多克隆抗体、单克隆抗体发展到了全新的基因工程抗体(genetically engineered antibody, GEAb)人工定向设计阶段。传统多克隆抗体、单克隆抗体都依赖于免疫动物获得, 尽管在模拟抗原功能的 Ab2 β 类型抗独特型抗体创制上均有成功的实例^[22,25], 但多克隆抗体单次产量有限、单克隆抗体杂交瘤细胞容易传

代退化, 且它们制备过程均存在周期长、操作过程繁冗等缺陷^[30]; 此外依托活体动物开展实验研究, 在动物福利和动物伦理上也备受诟病, 与人类社会倡导文明发展、倡导人与自然和谐共处的大趋势相悖。新型基因工程抗体是建立在已解析抗体结构和功能的基础之上, 伴随着现代分子生物学快速发展而催生的在基因水平上进行人工改造或修饰的抗体新形式, 特别是依托噬菌体展示技术实现了抗体基因型与抗体蛋白表现型相统一(图 2), 这为抗原特异性抗体创制摆脱动物免疫及其长时且繁杂操作过程, 迈向体外人工靶向设计与高通量筛选的全新阶段提供了可能, 是抗体创制技术发展的巨大飞跃。噬菌体展示技术由美国科学家 George P. Smith 博士在 1985 年首次报道^[31], 如图 2 噬菌体展示抗体结构所示, 其原理是将外源多肽基因克隆到特异性修饰的噬菌粒载体上, 与 GIII 基因串联, 如此一来, 外源多肽基因随着噬菌粒载体转录表达和噬菌体衣壳蛋白自组装, 即可通过二硫键与噬菌体衣壳 GIII 蛋白串联, 由此展示到噬菌体衣壳蛋白表面。该技术一经报道就迅速与基因工程抗体相融合, 通过将人工抗体基因大量克隆到噬菌粒载体上, 并借助相应宿主菌实现大规模扩增形成噬菌体展示抗体库, 其即可充当类似动物“免疫系统”的角色。如图 2 淘筛流程所示, 通过多轮“投入-洗涤-洗脱-侵染-扩增”过程, 库中能与固相包被的抗原特异性结合的噬菌体展示抗体被逐渐“捕获”并得到富集, 由此在体外即可实现针对包被靶点抗原特异性结合的噬菌体展示抗体及其对应抗体基因的靶向高通量筛选。噬菌体展示基因工程抗体及其配套的抗原特异性高通量靶向筛选与鉴定技术, 有效地避免了传统多克隆抗体、单克隆抗体在制备过程中需要免疫动物乃至融

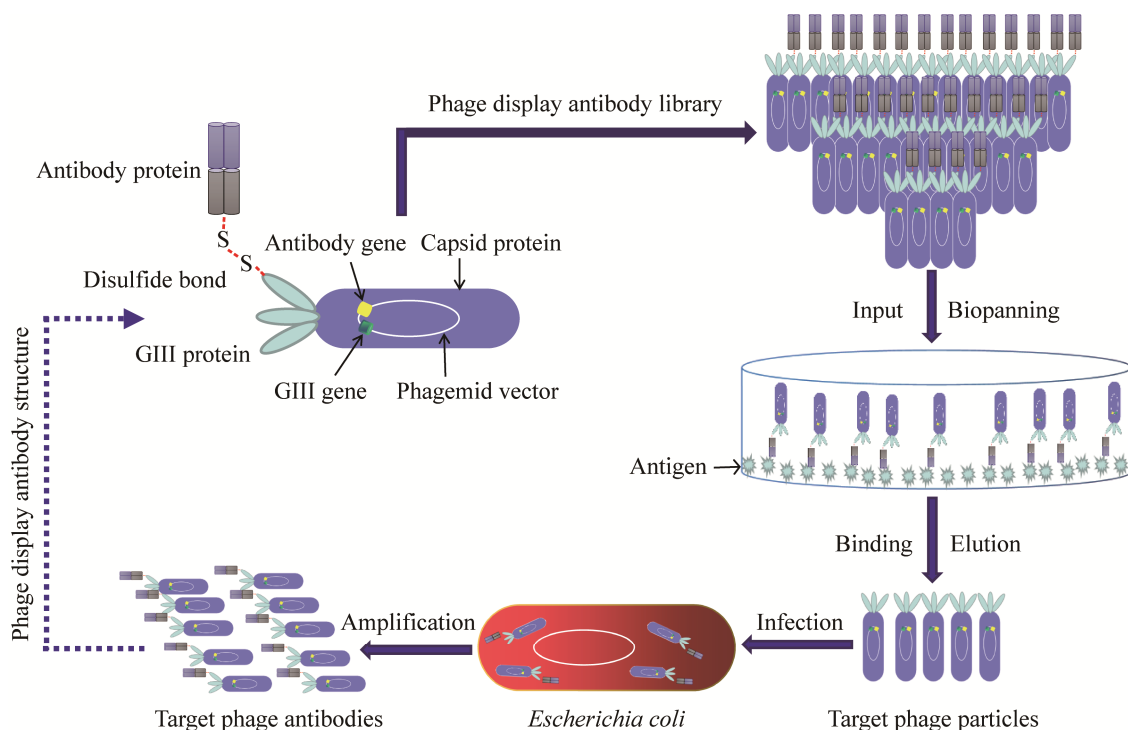


图2 噬菌体展示抗体及抗原特异性靶标噬菌体抗体库筛选流程示意图

Figure 2 Schematic diagram of phage display antibody and antigen-specific target phage display antibody library screening process.

合杂交瘤细胞等复杂繁冗操作,也摆脱了动物实验在伦理上的舆论压力,使抗原特异性抗体体外快速高效地靶向制备成为了可能^[32-33]。目前,噬菌体展示抗体技术在创制靶标抗独特型抗体方面已有相关报道,而设计的包被靶点抗原几乎涵盖现有抗体的所有形式:多克隆抗体、单克隆抗体、基因工程抗体,其中 Xu 等^[27]以万古霉素多克隆抗体 F(ab)₂ 片段为包被靶点抗原、Zhao 等^[34]以拟除虫菊酯类农药间苯氧基苯甲酸(3-phenoxybenzoic acid)单克隆抗体为包被靶点抗原,均从天然的噬菌体展示人源重链单域抗体库中分别成功靶向筛选获得了相应靶标的抗独特型抗体;而 Cai 等^[35]则以黄曲霉毒素(aflatoxin)单克隆抗体为包被靶点抗原,从一个自构建的经黄曲霉毒素单克隆抗体免疫的噬菌体展示驼源纳米抗体库中成功靶向筛选获得了

靶标抗独特型纳米抗体(nanobody),并作为毒素模拟物应用于免疫检测。显然,依托抗体“免疫网络学说”中的抗独特型抗体创制路径为指导,借助噬菌体展示基因工程抗体库及相应靶标抗体高通量筛选与鉴定技术,可以实现具备模拟 Bt Cry 毒素抗虫功能的 Ab2 β 类型抗独特型抗体体外高效靶向设计提供便捷的技术条件。

3 Bt Cry 毒素抗虫模拟物靶向设计的研究现状

依托抗体“免疫网络学说”中的抗独特型抗体创制路径,从噬菌体展示基因工程抗体库中靶向淘筛具备模拟 Bt Cry 毒素抗虫功能的 Ab2 β 类型抗独特型基因工程抗体,是目前 Bt Cry 毒素抗虫模拟物靶向设计的主体策略。该策略的前提是要获得高抗原结合活性和高纯度

的 Bt Cry 毒素抗体或抗体关键活性片段作为靶向的固相包被抗原,同时还需要有大容量的优质噬菌体展示基因工程抗体库作为靶向抗原的潜在靶标抗体资源,然后依托 Bt Cry 毒素抗体或抗体关键活性片段特异性靶向结合的噬菌体基因工程抗体高通量筛选与鉴定技术,初步大量获得 Bt Cry 毒素抗独特型基因工程抗体材料,再以 Bt Cry 毒素抗体或抗体关键活性片段以及 Bt Cry 毒素靶标害虫潜在受体,如刷状缘膜囊泡(*brush border membrane vesicles*, BBMV)或更为精细化的钙黏蛋白(CAD)、碱性磷酸酶(ALP)、腺苷三磷酸结合盒转运蛋白(ABC 转运蛋白)等^[10],作为双重竞争物,对这些材料进行分型鉴定,将其中具备 Ab2 β 类型特征的抗独特型基因工程抗体筛选出来,结合靶标害虫试药生测验证,从而获得具备模拟 Bt Cry 毒素抗虫功能的目标杀虫抗体蛋白及其基因材料。笔者团队依照该策略,前期依托从英国剑桥大学分子生物学与蛋白质基因工程中心引进的大容量噬菌体展示人源单链抗体库(Tomlinson I+J 库,库容量分别为 1.47×10^8 PFU/mL 和 1.37×10^8 PFU/mL)和人源重链单域抗体库(DAb 库, 3.0×10^9 PFU/mL)等优质基因工程抗体资源库,在国内外率先开展 Bt Cry 毒素抗虫模拟物靶向设计研究,并取得了一系列原创性科研成果,代表性研究实例见表 1。其中,徐重新等^[36]以制备的高质量 Bt Cry1C 毒素多克隆抗体为包被靶点抗原,从 Tomlinson I 库中成功筛选到了一个具备 Ab2 β 类型特征的 Bt Cry1C 毒素抗独特型单链抗体(*single chain variable fragment*, scFv),其(噬菌体展示形式,滴度为 1.2×10^8 PFU/mL)对供试靶标害虫小菜蛾(58.69%)和稻纵卷叶螟(52.82%)的致死率分别达到原毒素致死率的 77.87%和 73.21%;Dong 等^[37]以 Bt Cry1A 毒素 3 种亚型(Cry1Aa、Cry1Ab、Cry1Ac)的混合物

免疫获得的 Bt Cry1A 毒素广谱型多克隆抗体为包被靶点抗原,从 DAb 库中筛选到一个具备 Ab2 β 类型特征的 Bt Cry1A 毒素抗独特型人源重链单域抗体,其原核表达后的纯蛋白对供试靶标害虫小菜蛾的半致死浓度达到 $0.976 \mu\text{g}/\text{cm}^2$;而 Hao 等^[38]则以 Bt Cry1F 多克隆抗体 F(ab)2 片段为包被靶点,从商品化的噬菌体展示驼源纳米抗体库(库容量为 2.0×10^9 PFU/mL)中筛选获得一个具备 Ab2 β 类型特征的 Bt Cry1F 毒素抗独特型纳米抗体,其原核表达后的纯蛋白对供试靶标害虫玉米螟的致死率达到 23.3%。目前,这些成功的研究实例尽管已经具备初步模拟 Bt Cry 毒素的抗虫功能,但它们对靶标害虫的抗虫活性尚未达到相应原毒素的高致死水平(80%以上),因此进一步改进这些抗虫抗体蛋白的杀虫活性,对提升它们的应用价值极为关键。作者团队依托抗体体外亲和成熟技术对相关 Bt Cry 毒素抗虫模拟物的亲和成熟开展了一系列改造研究,也取得了良好效果。其中,Xie 等^[39]以多个 Ab2 β 类型的 Bt Cry1Ac 毒素抗独特型单链抗体为模板,通过轻链置换,创制的突变体对供试小菜蛾的致死率达到 83.3%,较模板提高了 25%左右;仲建锋等^[40]以 Ab2 β 类型的 Bt Cry1B 毒素抗独特型单链抗体为模板,采用定点突变技术对其重链可变区 CDR3 和轻链可变区 CDR2 进行定点饱和突变,创制的突变体对供试的稻纵卷叶螟致死率达到 56.67%,较模板提高了 34.23%;而 Lin 等^[41]以 Ab2 β 类型的 Bt Cry2Aa 毒素抗独特型单链抗体为模板,采用易错 PCR 技术对其进行随机突变,创制的突变体对供试的小菜蛾致死率达到 23.33%,其与靶标害虫 BBMV 受体的亲和力较模板提高了 2.2 倍。显然,依托抗体体外亲和成熟技术对改进这些 Bt Cry 毒素抗虫模拟物(Ab2 β 类型的 Bt Cry 毒素抗独特型基因工程抗体)的杀虫活性起到了

表 1 Bt Cry 毒素抗虫模拟物靶向设计研究代表性实例

Table 1 Representative examples of targeted design of Bt Cry toxin anti-insect mimics

Mimetic objects	Targeted antigen	Source of mimics	The effect of simulation	References
Bt Cry1C	Bt Cry 1C polyclonal antibodies	Phage display human scFv library	The corrected mortality of Bt Cry1C toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) for <i>Plutella xylostella</i> and <i>Cnaphalocrocis medinalis</i> were 77.87% and 73.21% of that of the original Cry1C toxin	[36]
Bt Cry1A	Bt Cry1A (a/b/c) mixed immuno-polyclonal antibodies	Phage display human heavy chain domain antibody library	The median lethal concentration (LC_{50}) of Bt Cry1A toxin insecticidal mimics (Ab2 β anti-idiotypic domain antibody) protein of which from prokaryotic expression to <i>Plutella xylostella</i> was 0.976 $\mu\text{g}/\text{cm}^2$	[37]
Bt Cry1F	Bt Cry1F polyclonal antibodies F(ab)2 fragments	Phage display camel nanobody library	The fatality rate of Bt Cry1F toxin insecticidal mimics (Ab2 β anti-idiotypic nanobody) protein of which from prokaryotic expression to <i>Pyrausta nubilalis</i> (Hubern) was 23.3%	[38]
Bt Cry1Ac	Bt Cry 1Ac polyclonal antibodies	Phage display human scFv library (chain displacement modification)	The fatality rate of Bt Cry1Ac toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) double light chain recombinant mutant protein of which from prokaryotic expression to <i>Plutella xylostella</i> was 83.3%	[39]
Bt Cry1B	Bt Cry 1B polyclonal antibodies	Phage display human scFv library (site-directed mutagenesis)	The corrected mortality of Bt Cry1B toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) mutant protein of which from prokaryotic expression to <i>Cnaphalocrocis medinalis</i> was 56.67%	[40]
Bt Cry2Aa	Bt Cry 2Aa polyclonal antibodies	Phage display human scFv library (error-prone PCR modification)	The fatality rate of Bt Cry2Aa toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) mutant protein of which from prokaryotic expression to <i>Plutella xylostella</i> was 23.33%	[41]
Bt Cry1Ab	Bt Cry 1Ab polyclonal antibodies	Phage display human scFv library	The corrected mortality of Bt Cry1Ab toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) for <i>Plutella xylostella</i> and <i>Cnaphalocrocis medinalis</i> were 72.65% and 81.11% of that of the original Cry1Ab toxin	[42]
Bt Cry1B	Bt Cry 1B polyclonal antibodies	Phage display human scFv library	The corrected mortality of Bt Cry1B toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) for <i>Plutella xylostella</i> and <i>Cnaphalocrocis medinalis</i> were 64.63% and 67.92% of that of the original Cry1B toxin	[43]
Bt Cry2Aa	Bt Cry 2Aa polyclonal antibodies	Phage display human scFv library	The corrected mortality of Bt Cry2Aa toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) for <i>Plutella xylostella</i> and <i>Cnaphalocrocis medinalis</i> were 78.57% and 71.42% of that of the original Cry2Aa toxin	[44]
Bt Cry2Aa	Bt Cry 2Aa polyclonal antibodies	Phage display human scFv library (chain displacement modification)	The affinity of phage displayed Bt Cry2Aa toxin insecticidal mimics (Ab2 β anti-idiotypic scFv) for <i>Plutella xylostella</i> BBMV receptor protein was reach up to 2.65×10^{-6} M	[45]

较为明显地促进作用,有助于进一步提升它们的应用价值。尽管作者所在团队现已率先初步实现 Bt Cry 毒素抗虫模拟物靶向设计与改造,但目前相关材料对靶标害虫的抗虫活性与原毒素相比还存在很大差距,整体上仍然处于室内研究的初级探索阶段,至于迈出实验室走向田间地头开展实测应用乃至产品化登记还为时尚早。

4 结语与展望

Bt Cry 毒素作为兼具高效广谱杀虫活性和对人类安全性极高的生物抗虫蛋白,可预见其在农业害虫绿色防治上的引领地位在短时间内几乎无法撼动,Bt Cry 毒素制剂及其转基因抗虫作物的市场需求具有长期性和广阔性,这也意味着其现已暴露出的诱发靶标害虫抗药性以及非靶标生物潜在安全风险等问题势必日益凸显。依托抗体“免疫网络学说”中的抗独特型抗体创制路径,从噬菌体展示基因工程抗体库中筛选具备模拟 Bt Cry 毒素抗虫功能的 Ab2 β 类型抗独特型基因工程抗体及其基因,实现了 Bt Cry 毒素抗虫模拟物靶向设计并可持续改造,这不仅是抗虫蛋白新材料创制思路的极大创新,更是抗虫蛋白材料迈向抗体化的全新突破。以抗体材料模拟 Bt Cry 毒素抗虫功能用于靶标害虫防治,不仅能缓解靶标害虫对 Bt Cry 毒素的抗药性压力,同时由于抗体源于高等动物的蛋白质属性,其在理论上对人类乃至非靶标生物相对更为安全,因此具有重要的科学研究意义和广阔的应用前景。不过就目前研究状况来看(表 1),采用 Ab2 β 类型抗独特型抗体创制路径从天然噬菌体展示基因工程抗体库中筛选获得的 Bt Cry 毒素抗虫功能模拟物对靶标害虫的杀虫活性普遍较低(致死率为 20%–60%),尚不具备商品化应用价值,这在库源材料选择、包被靶点抗原设计以及靶标抗虫抗体后期改造等

方面仍有很大改进空间。

在库源材料方面,前期主要依托天然的非免疫噬菌体展示基因工程抗体库为库源,尽管这些优质的库源材料库容量大、适用性广,但也意味着在其中捕获到高活性的靶标 Ab2 β 类型抗独特型抗体的概率也相对较小。通过构建靶点抗原免疫的噬菌体展示抗体库,再以该库为库源,能有效提升靶标抗体富集效率和捕获高抗原结合活性的噬菌体展示抗体的可能性。Xu 等^[46]和 Dong 等^[47]分别以自构建的微囊藻毒素免疫的兔源噬菌体展示单链抗体和 Bt Cry 毒素免疫的鼠源噬菌体展示单链抗体库为库源材料,均大幅度提升了高抗原结合活性的靶标抗体富集和筛选效率;因此,借鉴该思路,通过构建 Bt Cry 毒素免疫的噬菌体展示抗体库为库源材料,有望从中筛选获得更高抗虫活性的 Ab2 β 类型抗独特型抗体。

在包被靶点设计方面,以多克隆抗体或单克隆抗体蛋白作为包被靶点,容易导致供试库中噬菌体展示抗体与靶点抗体上的抗原受体独特位非特异性结合,这在富集淘筛过程中加大了靶标抗独特型抗体捕获的难度,也意味着获得具备模拟抗原结构和功能的 Ab2 β 类型抗独特型抗体更是难上加难。采用酶切方式,将抗体分子上的非抗原结合区(crystalisable fragment, Fc 片段)酶解,获得的抗原结合片段(antigen-binding fragment, Fab 片段)能在保持原有抗体活性的前提下实现抗体结合抗原的关键功能区域完整性的极小化^[48-49],因此以 Fab 片段作为包被靶点能提升从抗体库中捕获靶标抗独特型抗体的效率。Xu 等^[27]即以万古霉素多克隆抗体 F(ab)2 片段作为包被靶点,从噬菌体展示人源重链单域抗体库中高效获得了具备模拟万古霉素抗金黄色葡萄球菌的 Ab2 β 类型抗独特型抗体;Hao 等^[38]也是采用该方式,从噬菌体展示驼源纳米

抗体库中获得了具备模拟 Bt Cry1F 毒素抗玉米螟的 Ab2 β 类型抗独特型抗体,其捕获靶标抗独特型抗体的效率较包被完整抗体的类似研究有了明显提升。因此,在后期类似抗原功能模拟物靶向创制研究中,应当优先考虑设计其抗体 Fab 活性片段作为包被靶点,从而提升靶标模拟物筛选效率。

在靶标抗虫抗体改造方面,前期研究尽管依托链置换(strand displacement)、定点突变(site directed mutagenesis)以及易错 PCR (error PCR)等抗体亲和成熟技术对供试 Bt Cry 毒素抗虫模拟物(Ab2 β 类型抗独特型抗体)的抗虫活性有了一定提高(最高提升了 34.23%^[40]),但目前改进效果均不足以推进相应供试模拟物达到原毒素的高抗虫活性水平,显然这与 Barderas 等^[50]报道的借助定点突变技术对胃泌激素(gastrin TA4)单链抗体的亲和力提高 454 倍和 Boder 等^[51]报道的借助 DNA 改组与易错 PCR 组合技术对荧光素(fluorescein)单链抗体亲和力提高 208 倍等高水平相差甚远;因此在 Bt Cry 毒素抗虫模拟物(Ab2 β 类型抗独特型抗体)活性改造技术上仍有很大探究空间。

此外,当前 Bt Cry 毒素抗虫模拟物靶向设计策略,主要聚焦在依靠 Bt Cry 毒素抗体为包被靶点,采用抗独特型抗体技术路径获得具备模拟 Bt Cry 毒素抗虫功能的 Ab2 β 类型抗独特型抗体,而近年来随着对 Bt Cry 毒素抗虫机理不断深入解析,Bt Cry 毒素作用靶标害虫的关键受体也日渐清晰^[12],探索以靶标害虫毒素关键受体为包被靶点,从基因工程抗体库中靶向设计 Bt Cry 毒素抗虫模拟物也不失为一种潜在可行的路径。笔者团队近期分别以 Bt Cry 毒素靶标害虫棉铃虫类钙黏蛋白受体关键结合区蛋白(HaCadCR9-CR11)^[52]和靶标害虫小菜蛾受体 ABCC2 为包被靶点^[53],均从噬菌体展示人源重

链单域抗体库中靶向筛选获得了具备模拟 Bt Cry 毒素抗虫功能的抗体抗虫蛋白,证实采用靶标害虫毒素关键受体为包被靶点的对靶标筛选路径也能实现 Bt Cry 毒素抗虫模拟物靶向设计。

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