·生物工程与大健康·

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改造肠道微生物在疾病诊断与治疗中的应用

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摘 要: 肠道微生物是近年来新兴的热门研究领域,它与人类疾病健康存在着密切的关系。伴随高通量测序技术的发展,研究者们发现了肠道微生物在疾病的诊断与治疗中的潜力。合成生物学通过设计编辑工具以及反馈回路,可以构建具有诊断疾病或者靶向治疗疾病的肠道微生物工程菌株。这些工程菌能够对环境进行感知、计算和反馈。本文概述了改造后的肠道微生物在疾病诊断与治疗中的应用,同时阐述了目前改造后肠道微生物的临床应用现状,并对“工具短缺”以及目前改造后肠道微生物所存在的安全性等问题进行了讨论。

关键词: 肠道微生物,合成生物学,工程菌,疾病诊断与治疗,临床应用

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Applications of engineered intestinal bacteria in disease diagnosis and treatment

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Abstract: Intestinal bacteria interact closely with human health and diseases. With the development of high-throughput sequencing technologies, researchers have discovered the potential of intestinal bacteria in the diagnosis and treatment of diseases. Meanwhile, synthetic biology strategies are applied to engineer these bacteria for clinical applications. These engineered intestinal microbial are constructed by designing editing tools and feedback loops to gain functions of diagnose or targeted therapy. Consequently, these engineered bacteria are capable of sensing, calculating and responding to the environment. In this review, we summarize the recent advances in engineered intestinal bacteria in disease diagnosis and treatment. Furthermore, we also discuss the current status and future prospect of the engineered intestinal bacteria regarding their clinical applications, market, and safety issues.

Keywords: intestinal bacteria, synthetic biology, engineered bacteria, disease diagnosis and treatment, clinical application

人体肠道内微生物具有丰富的多样性，种类包括了细菌、病毒、真菌和原生动物等[1]。其中仅细菌数量就高达 100 万亿个，因此肠道微生物也被称作人类的第二个基因组[2]。随着宏基因组测序与生物信息学的发展，越来越多的数据表明，肠道内微生物与宿主之间存在着密不可分的关系。宿主通过饮食等调节肠道内微生物群的平衡[3]，肠道微生物从肠道内未分解的物质中获取所需能量，供其生长，同时也参与宿主体内的物质代谢、营养吸收、生理和免疫防御过程[4]。在此基础上，人类开启了人类微生物组计划，旨在揭示两者之间的关系[5]。伴随着研究的深入，人类健康和疾病与肠道微生物间的关系逐渐被探讨以及研究。合成生物学在此基础上利用基因合成、编辑以及调控等手段结合工程学来定向改造细菌，构建新的人工系统。合成生物学为肠道微生物改造提供“工具”，同时通过设计基因线路构建以肠道微生物为载体，靶向分泌表达治疗因子的工程菌，以及能够响应环境并且对其进行调控的“智能微生物”。改造后的肠道微生物也被称为工程菌，工程菌具有能够在肠道内定植、稳定持续给药等优点，近年来，在医学应用方面从实验室到临床阶段不断有新的成果涌现。但随之也出现菌株的安全性、工具的短缺以及基因线路的复杂性等问题。本文从肠道微生物与疾病之间的关系出发，阐述合成生物学工具构建以及基因回路设计，总结目前利用合成生物学设计和改造肠道微生物在疾病诊断与治疗中的应用，并对现有的问题加以讨论并且提出展望。

1 肠道微生物与疾病之间关系的研究现状

系统生物学整合了基因组学、转录组学、蛋白组学以及代谢组学等高通量组学，把研究目标从单个分子或细胞转移至整个细胞或生物体内的基因调控以及整体与体外环境间相互作用和信号转导[6]。近年来，系统生物学的发展逐步揭示出肠道微生物与疾病之间的关系。研究表明，健康状态下我们的肠道菌群处于平衡状态，但当人体处于疾病状态时，如肠道炎症、代谢疾病甚至精神疾病等，肠道内菌群的组成也会改变。肠道微生物通过参与人体代谢影响健康，其自身能够帮助人类代谢人体内无法完全消化的纤维素[7]，能够为宿主代谢提供酶，如肠道微生物中存在尿酸降解酶，可替代人体内缺乏的尿酸氧化酶，将尿酸代谢成二氧化碳和水，从而避免因尿酸升高导致的高尿酸血症[8]，肠道微生物还通过合成宿主自身不能合成的维生素 B 和维生素 K 等为宿主提

供营养，其产生的短链脂肪酸对人体也有诸多益处[9]。人们将这一类对人体有益的菌称为益生菌，目前大多数的工程菌都是基于益生菌进行改造的。

肠道微生物参与疾病治疗最初是直接通过粪便移植的方式来进行的，这种方法是将健康人群的肠道微生物菌群移植到非健康人群中来进行疾病治疗。最早记录利用粪便治疗肠炎和腹泻的方法是中国东晋时期的《肘后备急方》一书[10]。国外最早的记录是 1958 年，美国科罗拉多大学医学院外科医生 Eiseman 利用健康人群粪便治愈肠炎患者[11]。尽管利用健康患者粪便治疗疾病的方式出现已久，但由于粪便移植治疗不具有靶向性，并且其作用机制尚不明确，粪便中的病毒和杂菌较多，使得治疗效果因人而异，因此也限制了该治疗方式的推广[12-13]。

合成生物学利用天然或合成的生物元件来设计基因线路，从而实现程序化的细胞行为。它利用肠道微生物定植在肠道内与宿主相互作用这一特点，对微生物进行定向改造，为研究微生物群之间的结构-功能关系、设计新的生物疗法提供了新思路[14]，同时也使微生物靶向治疗疾病成为可能。近年来，改造后的细菌已广泛地用于治疗肥胖、糖尿病和结肠炎等疾病的研究中[15-17]。除单纯的治疗外，科学家还将研究重点放到疾病的预防和诊断上。通过设计能够感知肠道微环境相关变化的回路，对人体内健康状态进行检测和诊断[18](图 1)。

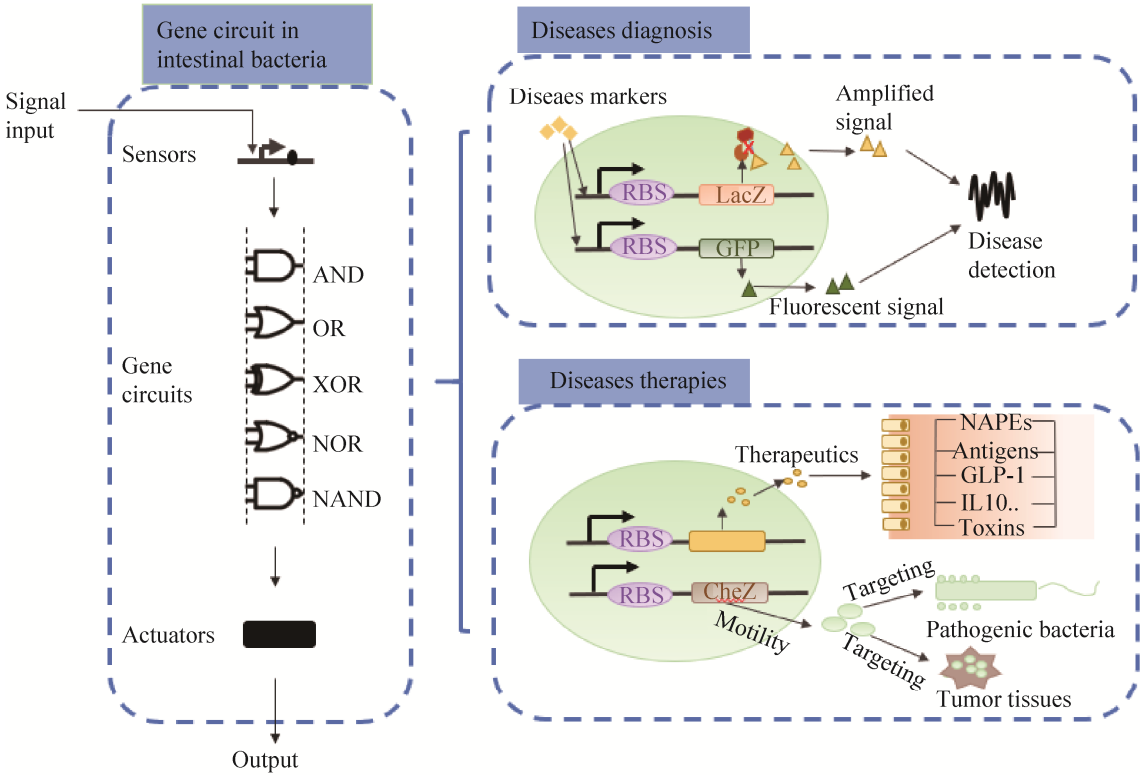

图 1　改造肠道微生物对疾病进行诊断与治疗(工程菌内基因回路由感应元件、基因元件以及报告元件三部分组成。在接收到体外信号后，通过体内基因回路计算操控工程菌的行为，表达报告因子用以诊断，治疗因子用以治疗，毒性因子用以杀死病原菌)

Fig. 1　Engineering of Intestinal microbiota for the diagnosis and treatment of diseases. The circle in engineered bacteria is composed of sensors, gene circuits, and actuators. After receiving *in vitro* signals, by modulating the behavior of engineered bacteria through *in vivo* genetic loop calculation, the engineered bacteria would express reporters for diagnosis, therapeutics for diseases treatment, and toxics for killing cancer cells or pathogens. NAPEs: N-acylphosphatidylethanolamines; GLP-1: glucagon-like peptide; IL10: interleukin10; RBS: ribosome binding site; LacZ: LacZ; GFP: green fluorescent protein; CheZ: a motivity protein that drives the bacteria.

2 合成生物学在改造肠道微生物中的研究应用

合成生物学为改造肠道微生物提供手段，一方面为处于开发初级阶段的微生物提供载体质粒和用于编辑改造的工具。另一方面结合电路学设计更为复杂的调控路径来改造和调控肠道微生物。

2.1 编辑工具

肠道菌群种类丰富，但大部分还有待研究，因此需要构建合适的系统以及工具对其进行编辑和改造。目前大多数改造对象为益生菌，或者通过合成生物学构建“合成益生菌”。合成生物学的作用是提供工具，用于编辑微生物。每一种菌都含有特定的“工具箱”，用以在体内进行基因编辑。常见的肠道微生物有大肠杆菌、乳酸杆菌、拟杆菌、双歧杆菌以及枯草芽胞杆菌等，它们均已有较为成熟的编辑工具[14,19]。大肠杆菌作为基础的底盘细胞，已经有大量的可供选择的操作系统用于基因的表达和编辑。乳酸杆菌中以 pWV01、pSH71、pAMβ-1 等穿梭质粒为主，可进行治疗因子的表达与调控[14,20]。其他几种菌的研究目前都还处于起步阶段，拥有为数不多的操作系统。例如 pNBU2 质粒适用于拟杆菌[21]，而双歧杆菌中应用较多的是大肠杆菌-双歧杆菌穿梭质粒，如 pBV220 以及 pBBAD/Xs[20,22]，pDG1730 质粒则应用于枯草芽胞杆菌[19]。基因的表达通过设计启动子实现，一方面可以通过设计不同强度的启动子库实现不同强度物质表达，另一方面则是设计诱导型启动子，利用单组分或双组分信号转导调节系统诱导下游基因的表达。最常见的就是乳酸杆菌中的 NICE 和 sppIP/IP-673 诱导系统[23-24]，而大肠杆菌以化学物诱导为主[14]。

2.2 回路设计

合成生物学能够对工具进行复杂的“组装”与利用。结合电路学，设计回路用以调控物质在微生物体内的表达。简单的线性回路设计单纯以分泌治疗物质为主，为使得物质的表达和分泌更为可控，工程菌体内的回路设计从原来的单一线路，被设计成能对肠道疾病信号作出反应的智能微生物，在其体内形成可调控的细胞信号转导网络[25]。工程微生物的信号网络主要由感应元件、计算元件以及报告元件 3 个模块组成。感应元件感应标记物并以此激活下游开关，目前应用的感应物有一氧化氮 (Nitric oxide，NO)、海藻糖 (Fucose)、硫代硫酸盐 (Thiosulfate)、连四硫酸盐 (Tetrathionate)、乳糖 (Lactose)、无水四环素 (Anhydrotetracycline，ATC)、阿拉伯半乳糖 (Arabinogalactan)、异丙基硫代半乳糖苷 (Isopropyl β-D-thiogalactoside，IPTG) 和鼠李糖 (Rhamnose) 等[18]。逻辑线路在信号网络系统中充当计算元件，对接收的信号进行计算与处理，以控制细菌的行为。报告元件主要为下游蛋白的表达，包括治疗因子的分泌——疾病治疗、荧光蛋白的表达——疾病诊断以及驱动蛋白的表达——控制细菌行为等。Din 等在合成路线中添加了正负反馈调节路线，使得细菌具有鲁棒振荡动力学特征。通过添加转录因子 (LuxR) 和 N-酰基高丝氨酸内酯 (AHL) 调控噬菌体裂解基因的合成，当 AHL 达到一定阈值后，细菌裂解释放药物，细菌死亡数量下降，等待下一轮扩增裂解给药。这种振荡式的给药方式减轻了工程菌的负担[26]。在靶向性调控方面，科学家通过改造肠道微生物的趋向性，让菌能够在特定部位表达药物或细胞因子[27]。振荡器利用模块化的部件和基因电路对细菌进行编程设计，以精确控制治疗物质的响应、表达和传递，有实验表明综合对细胞振荡性给药以及趋向性调控行为两种手段对肠道微生物进行改造，改造后的肠道菌有 80%能够靶向肿瘤细胞，并且分泌治疗性多肽[28-29]。疾病的检测信号弱会影响诊断结果，Bonnet 实验室通过前期摸索，利

用细菌的无限复制与表达从而扩大分子检测的敏感性，构建了用于检测疾病的全细胞传感器，为解决这一问题提供了新的思路[30]。除前面提到的感应元件，该实验室前期还探索出通过化学诱导二聚体(CID) 系统来研究和控制生物系统的方法[31]，为疾病检测提供新的入手点。

3 改造肠道微生物参与疾病的诊断

微生物具有无限复制的特点，能够将检测到的信号无限放大，有利于增大疾病监测的敏感性。同时肠道微生物可以直接通过粪便提取，具有不会对人体造成二次伤害的优势。基于这两点优势，肠道微生物在疾病的检测与诊断中具有良好的应用前景。

3.1 炎症诊断

炎症性肠病是一种慢性炎症肠道疾病，分为克罗恩病 (Crohn's disease，CD) 和溃疡性结肠炎(Ulcerative Colitis，UC)，临床症状为腹痛和腹胀。与腹泻和结肠炎的症状相似，需要用结肠镜取病理组织来对其进行检测，该病检测操作麻烦且对病人造成不适感。连四硫酸盐 Tetrathionate 是一类炎症的标志物，Kristina 实验室建立了一种基于口服给药和流式细胞检测的方法无创检测结肠炎症 (结肠炎)。该实验室在大肠杆菌 Nissle1917 (*Escherichia coli* Nissle1917，EcN) 中构建了硫代硫酸盐传感器，在结肠炎状态下，激活肠炎小鼠的硫代硫酸盐传感器，刺激下游表达荧光蛋白，取粪便样品对粪便进行流式细胞检测，通过荧光强度来反映肠道健康状态[32]。Pamela 实验室前期构建了能够在肠道内感知、记忆并作出相应报告的大肠杆菌遗传记忆系统，该系统由触发元件和记忆元件两部分组成。触发元件的 λCro 基因受四环素诱导型启动子调控，而记忆元件则来源于噬菌体中的 cI/Cro 区域，在四环素的调控下，使细胞处于不同状态[33]，从而记录肠道疾病状态。在此基础上，作者还将该系统应用于改造 *E. coli* strain PAS638，使其可以感应肠道内连四硫酸盐，并检测体内炎症状态[25]。但是目前可供检测的信号分子仍然有限，Bonnet 实验室设计的新受体，通过单结构域抗体的二聚化来激活开关，该实验室证明了单域 VHH 抗体与咖啡因结合后会形成二聚体，导致转录抑制因子 LexA 的单体 DNA 结合域(DBD)被激活，通过表达荧光蛋白来反映激活状态[34-35]。通过抗体或者特定设计配体蛋白这一方法为生物感应器提供了新的方向。

3.2 霍乱检测与诊断

霍乱由胃肠道感染霍乱弧菌引起，情况严重可致死。Holowko 等将霍乱弧菌中用于感应自身群体的蛋白异源表达至大肠杆菌中，同时通过计算器模拟其体内行为系统，从而达到诊断霍乱的目的[36]。该系统也成功应用于抑制肠道内其他有害菌 (如沙门氏菌) 的生长[37]。Mao 等设计了一株乳酸乳杆菌，在检测肠道中霍乱弧菌的群体感应信号的同时，触发报告基因的表达，使其更易在粪便样本中检测，方便了疾病的监测[38]。改造益生菌还被用于干扰细菌间的通讯以抑制霍乱弧菌的毒性[39]，从而预防霍乱。

3.3 癌症

癌细胞生长和繁殖速度很快，需要大量的葡萄糖提供能量。葡萄糖通过影响肿瘤的生长及代谢从而影响肿瘤的趋向性[40]。葡萄糖的浓度代表肿瘤的生存能力。癌细胞选择有氧糖酵解作为葡萄糖代谢的主要方式[41]。Panteli 等设计了一个葡萄糖敏感细菌，细菌体内含 Trz1 葡萄糖受体，该受体与肿瘤微环境中的糖结合时，触发体内绿色荧光蛋白 (Green fluorescent protein，GFP) 的表达[42](图 2)，利用荧光信号来实时反映出肿瘤的生存能力。该实验将 Trz1 受体的葡萄糖传感能力与细菌的肿瘤靶向能力结合起来，进一步还可以通过不同的局部微环境将重组蛋白药物直接递送到

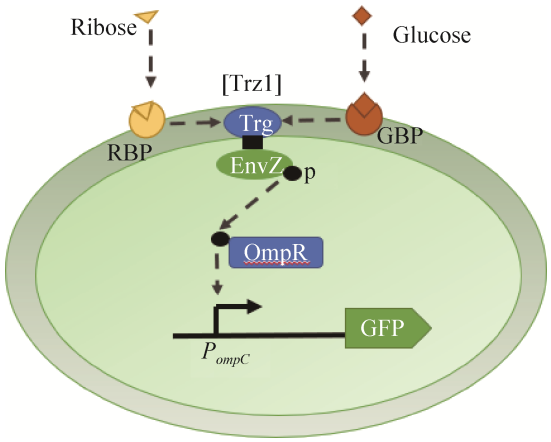

图 2 改造菌检测实体肿瘤细胞环境中葡萄糖浓度的空间分布情况 (融合受体蛋白 Trz1 结合趋化受体 Trg 的核糖/葡萄糖/半乳糖的识别域和信号转导域 EnvZ，将糖转化为光信号输出。核糖和葡萄糖分子在膜内分别与核糖结合蛋白 (Ribose binding protein，RBP) 或葡萄糖/半乳糖结合蛋白 (Galactose-binding protein，GBP) 结合。在结合配体与 Trz1 间的物质相互作用，导致 Trz1 内膜 (Inner membrane，IM) 构象的改变，使磷转移到细胞质渗透孔蛋白调节剂 (Osmoporin regulator，OmpR) 中，磷酸化的 OmpR 作为转录激活子作用于启动子 P_{OmpC}，从而激活绿色荧光蛋白 (GFP) 的表达)

Fig. 2 Spatial distribution detection of glucose concentration in solid tumor using engineered bacteria. The Trz1 fusion receptor combines the ribose/glucose/galactose sensing domain of Trg chemotaxis receptor and the signal transduction domain of EnvZ osmoporin, which transduce sugar into fluorescence signal. Ribose and glucose molecules bind to the ribose binding protein (RBP) and the glucose/galactose binding protein (GBP), respectively, in the membrane. The ligand-binding protein complex then interacts with the periplasmic portion of the Trz1 receptor, which causes conformational change in Trz1 across the inner-membrane (IM) and phosphorus transfer to cytoplasmic osmoporin regulator, OmpR. The phosphorylated OmpR then acts as a transcriptional activator on the osmoporin promoter, P_{ompC}, to activate the expression of the green fluorescent protein (GFP).

肿瘤的不同区域，达到靶向治疗的效果。Bonnet 等利用类似原理检测到了糖尿病人尿液中的糖类成分，用于糖尿病检测[30]。细菌具有定植于肿瘤的倾向性，Bhatia 实验室开发了一种利用细菌对肝转移进行检测的改造肠道微生物；他们证实了构建的微生物 EcN 能够在肿瘤内富集，其细菌浓度远远大于正常细胞，改造后的 EcN 在肿瘤细胞定植后，会大量表达 LacZ 蛋白，该蛋白可将一个底物切割成可以在尿液中检测到的小分子，从而达到无创检测肿瘤的目的[43]。

合成生物学还能够为疾病的检测提供记录装置。哥伦比亚大学 Wang 实验室结合 CRISPR/Cas 编辑技术，通过 CRISPR 在不同时间段的 spacer 插入位点来记录这些细菌与肠道内环境发生相互作用的时间[44]。这一系统可用来监测炎症、感染以及癌症等疾病的发展，同时可提高疾病检测的时效性。在检测技术的改进方面，卢冠达实验室通过结合传感器构建了“细菌药丸”，细菌在检测到胃部出血时会发出荧光信号，传感器接受到荧光信号后，通过无线信号传输到电子设备上，从而实现在不取出粪便的情况下实时检测胃肠道内的疾病[45]，极大地降低了疾病检测的难度。这些工具也为后续研究利用肠道微生物对疾病检测和诊断的技术奠定了基础。

4 改造肠道微生物参与疾病的治疗

改造肠道微生物不仅能够对疾病进行诊断，也能够参与疾病治疗。改造肠道微生物参与疾病治疗的研究主要从两个方面入手，一方面通过细胞分泌治疗因子用于靶向疾病组织发挥作用或者参与宿主代谢，另一方面通过运输将宿主内毒性因子运送至工程菌体内将其代谢成无毒物质。目前参与的疾病研究包括全身性代谢疾病 (肥胖和糖尿病等)、炎症、细菌性感染以及免疫类疾病等。

4.1 慢性全身性代谢性疾病

肥胖和糖尿病之间存在密切关系，过度肥胖还会引起 2 型糖尿病以及心血管疾病。N-acylethanolamides (NAEs) 是一类进食时，由小肠中脂质衍生的厌食性信号分子，

N-acylphosphatidylethanolamines (NAPEs) 是它的前体物质。Sean 实验室分别在 2014 年和 2018 年证明将表达 NAPEs 的工程菌 EcN 添加到高脂肪饮食小鼠的饮用水中，肥胖小鼠模型的体重增加明显受到抑制[46-47]。后期的实验结果表明，Sean 实验室构建的工程 EcN 还有降低肝脏炎症和缓解纤维化早期症状的作用。胰高血糖素样肽-1 (GLP-1) 是由肠细胞产生的肠促胰岛素激素，在葡萄糖存在下刺激肠上皮细胞转化为胰岛素分泌细胞，从而促进胰岛素分泌。研究证明，改造乳酸菌分泌 GLP-1，将肠道细胞重新编程为葡萄糖诱导性胰岛素分泌细胞可以改善糖尿病大鼠模型中的高血糖症状[48]。改造干酪乳杆菌分泌 GLP-1 还能使大鼠中血清低密度脂蛋白胆固醇、甘油三酯和富含甘油三酯的脂蛋白胆固醇显著降低[49]。这些数据表明，微生物分泌重组促胰岛素能够有效缓解胆固醇代谢和饮食引起的血脂异常以及胰岛素敏感性代谢功能障碍。组织中积累的吡咯并喹啉醌 (Pyrroloquinoline quinone，PQQ) 可防止肝脏和全身氧化损伤，联合短链脂肪酸 (Short chain fatty acid，SCFAs) 可以降低高脂血症[50]。PQQ 的浓度随着 SCFAs 而变化。果糖脱氢酶 (Fructose dehydrogenase，fdh) 能够将果糖转化为 5-酮-d-果糖，甘露醇-2-脱氢酶 (Mannitol-2-dehydrogenase，mtlK) 可以将果糖转化为甘露醇。Chaudhari 等构建可以表达 mtlK 和 fdh 酶的工程 EcN，发现小鼠体内的 SCFAs 和 PQQ 均出现上升，体重和血液内葡萄糖浓度均有所降低。该方法提高了 PQQ 和 SCFAs 的产量，既能改善肥胖症状又可以辅助增强二甲双胍的血糖控制和胃肠的耐受性[51]。

4.2 炎症性疾病

IL-12、IL-10、IL-27、IL35 等是常见用于抑制炎症的因子。改造肠道微生物，使其分泌抗炎因子是常见的肠道微生物改造方式之一。Kotula 等利用重组乳酸乳球菌 *Lactococcus lactis* 在小鼠模型[28]中治疗 IBD。在该研究中，使用表达重组 IL-10 的 *L. lactis* 灌胃 IL-10 (-/-) 小鼠可以成功预防该小鼠的结肠炎，使结肠炎的发生率降低了 50%。Miyoshi 实验室在前期构建了在乳酸乳球菌中分泌和表达 IL-10 的两种质粒系统，第一个系统基于应激诱导控制表达系统(SICE)，用于在粘膜表面产生和传递蛋白，第二个系统则是改造菌株使菌株分泌纤连蛋白结合蛋白 A (Fibronectin binding protein A，FnBPA)，该蛋白能使菌株具有黏附功能，通过黏附细胞直接向宿主细胞传递 IL-10 的 cDNA 盒，该盒位于真核 DNA 表达载体 (pValac) 中[52-53]。除乳酸乳球菌外，双歧杆菌也用作安全载体分泌 IL-10，用以治疗小鼠结肠炎[22]。类似的各种炎症因子也相继被应用到工程菌中，Hanson 等改造乳酸菌在肠道内粘膜层分泌 IL-27 减轻结肠炎[54]。双歧杆菌作为 IL-12 的口服载体也用于研究治疗 *Balb/c* 小鼠心肌炎[55]。乳酸乳球菌工程菌 *L. lactis*-IL35 分泌 IL-35 也能有效降低慢性阻塞性肺疾病的发生率和严重程度[56]，NO 是克罗恩病的肠道内标志物，Bentley 通过设计运动回路以及添加驱动蛋白来改造益生菌 EcN，改造后的 EcN 能够在 NO 升高处富集并表达粒细胞巨噬细胞集落刺激因子(Macrophage colony stimulating factor，GMCSF)。GMCSF 首先进入胞浆，然后通过 TolAⅢ形成的孔释放到细胞外。激活成熟粒细胞及单核巨噬细胞从而提高抗感染和免疫功能，缓解克罗恩病炎症症状[57] (图 3)，该实验方法也为改造肠道微生物的临床设计做了铺垫。

4.3 细菌感染性疾病

志贺氏菌病是一种急性肠侵袭性疾病，全世界有数百万人感染。以乳酸乳球菌作为疫苗载体，传递保守的抗原蛋白粘膜外膜蛋白 A (OmpA)，能够有效诱导全身以及粘膜免疫[58]。Paton 等设计

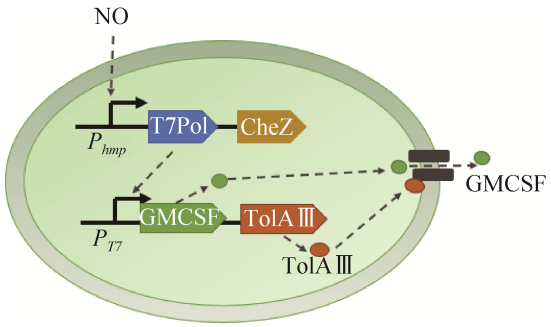

图 3　检测肠道炎症标志物一氧化氮 NO 用于治疗炎症性肠病 IBD (NO 存在下，CheZ 蛋白表达驱动非运动型细胞定向移动，T7pol 蛋白表达诱导 T7 启动子，激活下游粒细胞巨噬细胞集落刺激因子 GMCSF 和成孔蛋白 TolA Ⅲ的表达，TolA Ⅲ在细胞膜表面形成孔，帮助 GMCSF 排出至细胞外行使功能)

Fig. 3　Detection of intestinal inflammatory marker NO for the IBD treatment. In the presence of NO, CheZ protein drives the directional movement of non-sporty cells. T7pol protein induces the T7 promoter, thereby activating the expression of downstream GMCSF and pore forming protein TolA Ⅲ. TolA Ⅲ forms pores on the surface of the cell membrane and helps GMCSF move to extracellular regions.

了可与毒素结合的益生菌以预防和治疗肠毒素大肠杆菌引起的腹泻[59]。类似的益生菌也可与肠道受体竞争，结合霍乱毒素从而预防感染霍乱[60]。利用细菌的群体感应分子使工程化细菌识别并抑制致病菌是目前的研究热点。绿脓杆菌是常见的条件致病菌，Gupta 和 Hwang 先后改造 *Escherichia coli* 用以消灭绿脓杆菌。前者在大肠杆菌中设计了一个感应绿脓杆菌分泌 AHL 蛋白的回路，当该蛋白浓度达到一定量时即可启动工程菌表达 Copy 蛋白从而抑制绿脓杆菌的生长。后者在此基础上添加了动力蛋白 CheZ，使得大肠杆菌在感应到 AHL 蛋白过量表达时便迁移至绿脓杆菌附近，杀死绿脓杆菌[61-62]。Jayaraman 等改造工程化大肠杆菌的群体感应分子 CAI-1，使其特异性地检测霍乱弧菌，并通过表达裂解蛋白 YebF-Art-085 进行响应，从而自我裂解以释放杀伤蛋白 Art-085 达到杀死霍乱弧菌的目的[63]。

4.4　免疫类疾病

4.4.1　HIV

Cyanovirin-N (CV-N) 是一种独特的氨基酸蛋白，在低纳摩尔浓度下可杀灭艾滋病毒 HIV-1 和 HIV-2、猿猴免疫缺陷病毒和猫免疫缺陷病毒，其对物理化学降解具有极强的抵抗力。尽管 CV-N 灭活 HIV 的确切机制尚未完全阐明，但已有研究证实 CV-N 可以干扰病毒与其进入细胞必需的靶细胞受体间相互作用。在链球菌 *Streptococcus* 中表达强效的 HIV 灭活蛋白 CV-N 并分泌于菌体表面，作为一种可能的途径局部递送 CV-N，可有效捕获 HIV 病毒粒子以防止其性传播[64]。高度定植的大肠杆菌菌株 EcN，可用以表达 HIV-gp41-溶血素 A，这种杂合肽可以阻断 HIV 与靶细胞融合并进入靶细胞，发挥抗 HIV 作用[65]。

4.4.2　癌症

肿瘤的异质性使癌症难以治疗，许多小分子癌症药物能够靶向肿瘤周围并快速裂解细胞，但难以深入肿瘤区域。在前期检测的基础上，Adachi 等开发出可特异性分泌药物或抗性因子的微生物载体，乳酸菌工程菌 GLBL101c 表达分泌的 HPV16-E7 能够引起宫颈瘤 CIN3 的消退，通过将点突变插入核糖体结合位点以修饰 HPV16 衍生的 E7 基因，构建表达分泌该基因的 IGMKK16E7 菌株，强化了 E7 的表达[66-67]。内皮抑素基因是一种抗血管生成基因，南京大学 Li 等在益生菌中构建了将内皮抑素基因转运至实体瘤的递送系统，仅在肿瘤部位表达该基因，成功抑制了肿瘤的生长，同时将肿瘤相关抗原递送至抗原呈递细胞以引发抗肿瘤免疫[68]。Anderson 等为细菌配备群体感应 (Qs) 开关，只有当细菌数量达到阈值密度时才会激活效应基因表达，达到在肿瘤部位进行治疗的目的[69]。由于工程益生菌的易操控性以及低毒性，许多研究将其作为传递小分子物质

的载体[70-71]，再加上其特殊的定植能力，常被用作后续抗肿瘤药物的载体[72]。

5 现状与发展

以上所有研究表明，随着对宿主-肠道微生物之间的相互作用机制以及合成生物基因电路设计的不断深入理解，有望集成各种方法以系统、精确的方式发展工程微生物疗法。可操作菌株以及设计工具的发展将使我们能够针对更多人类疾病作出相应调整，本文将目前参与到疾病诊断与治疗中的改造肠道微生物作了一个总结，如表 1 所示。

研究发现肠道微生物在人体健康中占据着重要的位置，各个国家也相继开发出用肠道微生物(益生菌) 来进行疾病治疗的方案。Rebiotix 公司利用粪便移植来治疗艰难梭状菌感染。加拿大政府批准了一种治疗住院病人艰难梭菌感染的益生菌专利配方[73]。这都为微生物制剂的市场应用打开了大门。许多利用微生物组治疗疾病的方案都已进入临床阶段。Assembly 公司是发现和开发口服活微生物生物治疗产品的临床阶段领导者，利用肠道细菌的自然进化功能，在疾病治疗领域提供临床效益。该公司开发的 ABI-M201 由一组确定的肠道共生菌群组成，用于治疗溃疡性结肠炎，目前在临床一期阶段。相较于肠道微生物组，利用合成生物学改造肠道微生物治疗疾病才起步不久，Synlogic 是较早将合成生物学利用到微生物中，使其行使治疗功能的公司。该公司设计改造的 SYNB1020 可用于治疗由肝硬化和尿素循环障碍 (UCD) 引起的高血氨症，此项研究目前处于 1b/2a 期临床试验。用于治疗苯丙酮尿症的 SYN1618 现已获得美国食品与药物管理局 (FDA) 治疗苯丙酮尿症 (PKU) 的快速通道[74-75]。Synlogic 公司还与 Abbvie 公司合作，准备研发一系列治疗肠道炎症等的工程菌。Intrexon 的 ActoBio 系列药物通过对乳酸菌进行改造用于治疗糖尿病以及口腔疾病，改造菌株如表 2 所示。同时 ActoBio 也将研究目标放在代谢疾病以及肠道炎症疾病的治疗上。除以上两家公司，陆续也有公司尝试利用合成生物学改造肠道微生物来治疗疾病。Ernest Pharmaceuticals 公司着重于改造能够在肿瘤中定植的沙门氏菌，用于靶向治疗癌症。Trayer Biotherapeutics 公司通过改造乳酸菌，使其能够治疗苯丙氨酸代谢紊乱。ActoGenix 与 Intrexon 合作通过改造乳酸链球菌使其生产出一种或多种治疗性的肽和蛋白质[76]。Blue Turtle Bio 公司通过改造细菌来治疗癌症[77]。总体来说，利用合成生物学制造具有治疗疾病效果的菌株药物目前在医学领域初露锋芒[78]，吸引着越来越多的公司加入其中。

6 讨论

癫痫、自闭症、抑郁症等与脑神经相关的疾病也与肠道微生物相关，而合成生物学的快速发展也推动了疾病与肠道微生物之间具体关系的探究[79-81]。但是微生物与肠道内环境之间相互作用机制尚不明确，阻碍了工程菌的推广与应用。由于应激源和病原体的反应复杂，合成生物系统启动的反应必须考虑这种复杂性和细微差别，才能发挥作用，因此需要开发对刺激作出多方面反应的系统。Wang 等将基因电路作为研究重点，通过设计组装不同的逻辑门元件形成基因逻辑门电路，从而使合成生物能够根据多种输入组合而非单一输入启动生物编程[82-83]。然而许多肠道菌目前还缺乏成熟有效的编辑工具。面对更复杂的治疗系统时，还须考虑工程菌的稳定性和安全性。改造后的微生物具有较大的环境压力，负担体内合成线路运作的同时还需同肠道内原生菌株竞争营养，“抢夺”生态地位，以保证改造微生物在肠道内的治疗时间[84]。此外，工程菌达到治疗效果后，如何从体内及环境中去除也是亟待解决的问题。改造后的菌株仍属

表 1 工程微生物在疾病中的应用

Table 1 The application of engineered microorganisms in diseases

Strains	Applications	Purposes	Mechanism	References
E. coli Niss-le 1917 (EcN)	Obesity	T	Expressing NAPE,fructose dehydrogenase (fdh)	[46,51,86]
EcN	Phenylketonuria	T	Degrading Phe in serum	[74]
EcN	Hyperammonemia human	T	Producing l-arg and consumed NH_3 in an *in vitro* system	[75]
EcN	Immunodeficiency virus (HIV)	T	Secreting an HIV fusion inhibitor peptide	[65]
EcN	Cancer	D	Generating a high-contrast urine signal	[43]
EcN	Diabetes	D	A genetic switch to detect pathological biomarkers	[30]
EcN	Inflammatory ethanol-induced	D	Thiosulfate and tetrathionate sensor	[65]
EcN	Oxidative damage & hyperlipidemia	T	Secreting pyrroloquinoline quinone (PQQ)	[50]
EcN	Cholerae	T	Expressing the autoinducer-molecule cholera autoinducer 1 (CAI-1)	[39]
EcN	Infection-*Salmonella*	T	Expressing and secreting the antimicrobial peptide, Microcin J25	[87]
EcN	Nutritions	P	Expressing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),	[88]
E. coli	Cholerae	T	Inhibiting virulence of Vibrio cholerae	[39]
E. coli	Inflammatory bowel disease (IBD)	D&T	Co-expressing the pore forming protein TolAIII with the biologic, granulocyte macrophage-colony stimulating factor (GM-CSF), and recording an inflammatory response	[57]
E. coli strain JE3513	Infection-*Pseudomonas aeruginosa*	D&T	Sensing and killing pathogen	[61-62]
Lb. casei	HIV	T	Secreting single-chain variable fragment (scFv) and other HIV-1 antigen	[89]
Lb. casei	Infection	T	Displaying albumin-binding domain variants against Shiga toxin 1 B subunit	[90]
Lb. casei	IBD	T	Expressing superoxide dismutase (SOD)	[91]
Lb. plantarum	Immune	T	Expressing the highly immunogenic tetanus toxin C-terminal Fragment (TTFC) as antigen	[92]
Lb. plantarum	Respiratory allergies	T	Expressing a major Japanese cedar pollen allergen Cry j 1 suppressed nasal clinical symptoms	[93]
Lb. plantarum	Inflammation	T	Expressing interleukin 10 (IL-10)	[94]
Lactobacillus	*Infection-s. pneumoniae*	T	Producing the intracellular antigen PspA	[95]
Lactococcus lactis	Immune	T	Expressing rotavirus VP8	[96]
L. lactis	Inflammations	T	Expressing IL2, IL6	[97]
L. lactis	Immune	T	Expressing BLG protein—cow β-lactoglobulin	[98]
L. lactis	Avian flu	T	Expressing chicken 2 (chIL-2) together with avian influenza hemagglutinin (H5)	[98-99]
L. lactis	Infection-*Leishmania major*	T	Expressing the protective Leishmania antigen, LACK (LAC) and IL-12.	[100-101]
L. lactis	Diarrhea	T	Expressing dendritic cell-Targeting peptide fused with porcine epidemic diarrhea virus (PEDV) COE antigen	[102]

待续

续表 1

L. lactis	Infection-*Clostridium perfringens*	T	Expressing the toxoid of *C. perfringens* α-toxin	[103]
L. lactis	IBD	T	Expressing anti-tumor necrosis factor α (TNFα) antibodies, IL-17, IL-23，IL12 and IL-10	[104]
L. lactis	IBD	T	Binding different chemokines and neutralize CXCL8 production	[105]
L. lactis	IBD	T	Secreting biologically active heme oxygenase-1 (HO-1)	[106]
L. lactis	Diabetes type I	T	Expressing fusion protein HSP65-6P277 or glutamic acid decarboxylase or tyrosine phosphatase-like protein	[107-109]
L. lactis	Cancer	T	Producing catalase	[110]
L. lactis	Cancer	T	Expressing human papillomavirus 16 antigen E7 (HPV16 E7)	[111]
L. lactis	Cancer	D	Expressing attachment to breast cancer MDAMB232 cells	[112]
L. lactis	Infection	D&T	Expressing anti-enterococcal peptides	[113]
L. lactis	Cholesterol	T	Overexpressing bile salt hydrolase	[114]
L. lactis	Glucose tolerance	T	Expressing glucagon-like peptide-1 (GLP-1), insulin	[115-116]
L. acidophilus	Influenza	T	Expressing the highly pathogenic avian influenza virus protein hemagglutinin (HA)-1	[117]
L. paracasei	Diabetes type I	T	Expressing GLP-2	[115]
Bifidobacterium longum	Cancer	T	Production of Tumstatin, inhibiting proliferation &inducing apoptosis of tumorous vascular endothelial cells	[118]
Bifidobacterium longum	Cancer	T	Production of an enzyme to convert pro-drug 5-fluorocytosine to the toxic 5-FU within tumors	[119]
Bifidobacterium longum	Infection	T	Displaying *Salmonella*-antigen	[118]
Bifidobacterium longum	Ulcerative colitis(UC)	T	Expressing alpha-melanocyte-stimulating hormone, manganese superoxide dismutase (rhMnSOD)	[120-121]
Bacillus subtilis	Infection-*Helicobacterpylori*	T	Display of *H. pylori* urease B protein on spore coat	[122]
Bacillus subtilis	IBD	T	Producing 4,4′-diaponeurosporene	[123]

T: therapies; D: diagnostics; P: prophylaxis.

表 2　临床研究阶段的工程微生物

Table 2　Engineered microorganisms at clinical stage

Strains	Applications	Clinic phase	Companies
EcN	Hyperammonemia-Urea cycle disorders	Phase Ⅰ/Ⅱ	Synlogic
EcN	PKU	Phase Ⅰ	Synlogic
L. lactis	Type I diabetes	Phase Ⅰb/Ⅱa	Intrexon
L. lactis	Oral mucositis	Phase Ⅰ clinical trial	Intrexon
Salmonella	Liver cancer	Phase Ⅰ	Ernest Pharmaceuticals
Salmonella	Breast cancer	Phase Ⅰ	Ernest Pharmaceuticals

于活菌，可能引起环境中的基因污染或转移的安全性问题。针对这一问题，Synlogic 公司开发的工程菌只能存在于低氧环境，且依赖于胸苷营养物而生存，一旦该物质浓度较低，工程菌便会自动死亡，此方法提高了工程菌的安全性[75]。Chan 等设计的“Deadman”和“Passcode”杀毒开关也采用了类似方法，这一方法需要输入一个或连续输入多个分子配体维持对毒素的抑制，但其序列的

不稳定性也容易导致自毁序列的过早激活[85]。由于肠道微生物的特殊性，还需开发更稳定的合成工具用以解决菌株及其工具的安全性问题。

7 展望

合成生物学和肠道微生物都是近年来快速发展的领域，肠道微生物治疗疾病具有定向给药、可复制、操作简单灵活等优点，合成生物学也可以为遗传学家、生态学家、计算生物学家和临床医生提供强大的“工具箱”，为实现微生物疗法提供多样化的工具和技术手段。合成生物学与肠道微生物的结合，为人类的疾病治疗开创了新思路。随着科学技术的发展，合成生物学有望形成一套系统性、强调控性的细菌控制回路，通过精准的设计和模块的构建使肠道微生物改造朝着更具有靶向性、安全性的方向前进。

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