Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/23/20231047

Application of precision medicine based on synthetic biology in the prevention and treatment of chronic diseases

Yutong Su

Beijing University of Chemical Technology, No. 15 Beisanhuan Road East, Chaoyang District, Beijing, China

2803944723@qq.com

Abstract. Chronic non-communicable diseases have long been a significant factor affecting public health. If left unchecked, their protracted course and exorbitant treatment costs can inflict irreversible harm on patients' lives and finances. Chronic diseases often manifest insidiously and possess complex etiologies, posing substantial limitations to traditional treatment approaches, most of which merely provide symptomatic relief. Currently, synthetic biology has demonstrated substantial potential in the realms of chronic disease prevention, diagnosis, and treatment. This article focuses on the field of chronic disease prevention and treatment, using obesity, diabetes, hypertension, and inflammatory bowel disease as exemplars of typical chronic illnesses. It reviews advancements in research involving the application of synthetic biology techniques, including the construction of genetic circuits, gene control switches, and sensor systems, to provide a comprehensive overview of biologically-based methods for precise and controllable treatment of chronic diseases. The aim is to offer insights for the translation of fundamental synthetic biology research into clinical treatments for chronic diseases, thereby ushering in a new era of precision medicine guided by this principle in the field of chronic disease prevention and treatment.

Keywords: Synthetic Biology, Precision Medicine, Chronic Diseases.

1. Introduction

Chronic diseases, formally referred to as chronic non-communicable diseases, encompass common conditions such as obesity, diabetes, hypertension, and chronic respiratory diseases. Presently, chronic diseases have emerged as a paramount factor affecting human health, substantially diminishing patients' work capacity and quality of life, while imposing exorbitant treatment costs that burden both society and households.

Synthetic biology is a discipline that employs an engineering-based approach to design and construct genetic circuits, reprogram biological systems, and impart novel biological functions. It represents a rapidly evolving interdisciplinary field in recent years, enabling targeted design, modification, and even the de novo synthesis of living organisms to achieve functional control over biological entities.

To date, synthetic biology has made remarkable inroads into the realm of chronic diseases. Research teams worldwide have adopted a bottom-up approach, involving targeted design, pathway assembly, chassis integration, and more, to design life and create life, applying these advancements to the treatment of chronic diseases such as diabetes, hypertension, and inflammatory bowel disease. This has resulted

DOI: 10.54254/2753-8818/23/20231047

in treatments that are more potent and efficient than conventional therapeutic methods. It heralds the great potential of synthetic biology in precise treatment for chronic diseases and, indeed, the entire field of biomedicine. It is poised to lead the third wave of biotechnological revolution, carrying significant epoch-making implications.

2. Precision medicine and obesity

Obesity refers to the excessive total body fat content and/or abnormal distribution of fat, resulting from a combination of genetic and environmental factors, and is classified as a chronic metabolic disease [1]. With increasing living standards, obesity has become a global "epidemic," with the average Body Mass Index (BMI) of the global population gradually rising [2]. In 2016, over 1.9 billion adults aged 18 and above worldwide were overweight, with over 650 million individuals classified as obese. Among adults aged 18 and above, 39% were overweight, and 13% were obese. According to the "Report on the Nutrition and Chronic Disease Status of Chinese Residents (2020)," more than half of Chinese adults are overweight or obese, with overweight/obesity rates among children and adolescents aged 6-17 and those under 6 years old reaching 19.0% and 10.4%, respectively. Obesity is closely associated with the risk of comorbid diseases and mortality, making it a primary preventable cause of illness and disability [3].

Wang et al. constructed engineered bacteria, M-GLP-1, capable of producing GLP-1 (an effective blood sugar-lowering hormone). This engineered bacterium achieved anti-obesity effects by promoting fatty acid oxidation and increasing the gut microbial diversity in obese mice. M-GLP-1 not only demonstrated the potential for effectively treating obesity and related symptoms but also showed no significant side effects in experiments. This research provides valuable insights into the development of novel therapeutic strategies for obesity. The release of GLP-1 by engineered bacteria may become a potential option for future treatments of obesity and related metabolic disorders [4].

Previous studies have suggested that butyric acid (BA) may prevent or alleviate obesity induced by a high-fat diet (HFD) by altering metabolism. Bai et al. engineered Bacillus subtilis SCK6 by introducing the BCoAT gene, establishing a new pathway for BA synthesis, and knocking out the skfA gene. Compared to the SCK6 strain, the engineered bacterium BSS-RS06550 exhibited significantly enhanced BA production in an in vitro co-culture environment and had a higher growth rate [5].

In the human and mammalian body, fat can be categorized as white adipose tissue (WAT) and brown adipose tissue (BAT), each serving distinct functions. WAT primarily stores energy and can lead to obesity when high-calorie foods are consumed. BAT, on the other hand, burns excess fat and glucose to generate heat. Consequently, researchers aim to convert WAT into BAT to open up new avenues for treating obesity-related diseases. Existing studies have indicated that Panx1, when combined with the Gβγ subunit, becomes activated upon stimulation of β3AR. Inhibiting Panx1 channels suppresses β3AR-mediated lipolysis and reduces the expression of mitochondrial uncoupling protein-1 (UCP-1), leading to a decrease in cellular heat production capacity. Therefore, activating Panx1 channels may enhance the thermogenic capacity of cells in brown adipose tissue, offering potential applications in the treatment of obesity [6]. In related research on fat cell conversion, Wang CH et al. used the CRISPR-SAM system to modify human white pre-adipocytes, resulting in HUMBLE cells, which effectively activated the expression of the endogenous UCP1 gene, driving a brown-like phenotype. Obese mice receiving HUMBLE cell transplants exhibited improved glucose tolerance, insulin sensitivity, and increased energy expenditure. This study demonstrates the clinical potential of CRISPR-engineered HUMBLE cells in the prevention and treatment of obesity [7].

3. Precision medicine and diabetes

Diabetes mellitus (DM) is a common chronic disease globally characterized by metabolic disturbances resulting from either absolute or relative insufficient insulin secretion or insulin resistance. Type 1 and Type 2 are common classifications of diabetes, both falling within the category of chronic diseases. Type 1 diabetes is primarily caused by a severe deficiency in insulin secretion from pancreatic beta cells and is currently managed mainly through exogenous insulin therapy. Type 2 diabetes, on the other hand, is

DOI: 10.54254/2753-8818/23/20231047

characterized by insufficient sensitivity to insulin secretion, leading to insulin resistance, and is a more prevalent type, mainly treated with oral medications or injectable anti-diabetic drugs.

Han et al. designed a protein drug delivery system composed of engineered bacterial microcapsules, termed E@CS, and validated its durability and the diversity of drug release size concerning biological activity. To meet the long-term medication needs of diabetes patients, E@CS was engineered to produce exenatide (Ex-4). It was observed that E@CS could continuously generate and release Ex-4 for at least two weeks, leading to effective blood sugar reduction [8].

Chen et al. designed a non-coding amino acid (ncAA)-triggered therapy system known as NATS (non-coding amino acids-triggered therapeutic switch). By implanting NATS microcapsules in diabetic mice and administering ncAAs orally, they demonstrated rapid relief of high blood sugar within 90 minutes, accompanied by swift expression of therapeutic proteins. NATS exhibited excellent control characteristics, compatible with transcription switches, and enabled multi-level regulation. Compared to traditional transcription switches, NATS demonstrated faster response times, high sensitivity, dose-dependence, and reversibility. In terms of long-term blood sugar control, feeding diabetic mice implanted with NATS cells "cookies" containing ncAA achieved long-term blood sugar control, offering a new approach to treating chronic metabolic diseases, with diabetes as a representative [9].

The functional quality of insulin-secreting pancreatic beta cells plays a vital role in the clinical prognosis of diabetes transplantation. To optimize the functionality of pancreatic cells and enhance transplantation outcomes, Pim P. van Krieken et al. dissociated pancreatic islets into single cells and reassembled them into engineered "pseudo-islets" using adenovirus-mediated techniques. These pseudo-islets not only increased V1b receptor expression but also exhibited basic functions similar to intact natural islets in mouse experiments, showing similarities in glucose-induced intracellular signaling and insulin release. This research provides a new treatment option for diabetes patients and may improve the efficiency of clinical islet transplantation, potentially transforming the paradigm of diabetes treatment [10].

Yin et al. constructed a gene expression control switch regulated by protocatechuic acid (PCA). They reprogrammed the transcriptional repressor protein PcaV, which responds to PCA, the PcaV-responsive operator DNA sequence (OPcaV), and the transcriptional inhibitor KRAB. Researchers encapsulated PCA-regulated cells and transplanted them into Type 1 and Type 2 diabetes monkey models. In the monkeys, oral administration of PCA activated the control switch, prompting cells to release insulin or GLP-1, effectively lowering blood sugar levels. This study achieved precise drug delivery, applying green tea-regulated customized cell therapy to diabetes treatment [11].

Daniel Bojar et al. successfully developed a method for controlling Type 2 diabetes using caffeine-responsive functional cells. The research team utilized the extracellular domain of single-domain VHH camel antibodies (aCaffVHH) to sense caffeine signal molecules. By linking the extracellular domain of aCaffVHH to the EpoR receptor and coupling it with the JAK/STAT3 pathway, they allowed aCaffVHH camel antibodies to dimerize when caffeine was present, transmitting signals and inducing downstream GLP-1 expression. When encapsulated in microcapsules and transplanted into mice with Type 2 diabetes, these specialized functional cells (C-STAR) significantly improved blood sugar levels in response to caffeine induction [12].

Li CY et al. designed a cell system called GBOI capable of producing insulin under the control of a gate. GBOI consists of three key components: a glucose-sensitive trigger, a BL light-trigger, and a UAS promoter-driven insulin expression cassette. Under normal conditions, insulin is not expressed; the system is only triggered in the presence of high glucose and blue light. The glucose-sensitive GIP promoter induces the expression of the GI-Gal4 protein, which, in the presence of blue light, forms a complex with LOV-VP16. The GI-Gal4:LOV-VP16 complex subsequently promotes the expression of insulin driven by the UAS promoter. When engineered cells were implanted subcutaneously in Type 1 diabetes mice, they effectively improved blood sugar homeostasis [13].

A research group at the Swiss Federal Institute of Technology in Zurich devised a melanin-based light-controlled therapy system. Under blue light stimulation, chromophores isomerize retinaldehyde, prompting changes in connected melanin conformations. This activates downstream G proteins,

DOI: 10.54254/2753-8818/23/20231047

phospholipase C, and protein kinase C, leading to the opening of intracellular organelle (endoplasmic reticulum) calcium channels and cell membrane ion channel receptors, causing a significant influx of calcium ions [14]. T cell nuclear factors are activated and enter the cell nucleus, binding to specific promoters to induce GLP-1 expression. Encapsulating light-controlled functional cell microcapsules and transplanting them into Type 2 diabetes model mice, these microcapsules significantly improved mouse blood sugar levels through blue light induction [15].

Yu G et al. designed a novel cell called Human Islet-like Design (FAID) cells, which, when activated by far-infrared light (FRL), can selectively secrete insulin. Implanting FRL-activated FAID cells into diabetes model mice, the results showed that these cells effectively lowered mouse blood sugar levels to a safe range without causing hypoglycemia and reduced kidney damage in the mice. Furthermore, by adjusting the number of implanted FAID cells, duration of light exposure, and light intensity, the research team successfully controlled insulin expression levels, providing an effective means for long-term improvement of blood sugar control in diabetes model mice [16].

4. Precision medicine and hypertension

Hypertension is a chronic condition characterized by elevated arterial blood pressure, which can lead to functional or organic damage in organs such as the heart, brain, and kidneys. Hypertension is divided into primary (essential) hypertension and secondary hypertension, with primary hypertension accounting for approximately 90-95% of all cases. It is the most common chronic condition and a major risk factor for cardiovascular diseases.

Yang et al. introduced genes encoding tuna framework protein and elastin-like polypeptide into Lactobacillus plantarum NC8, enabling it to synthesize angiotensin-converting enzyme inhibitors. They found that the modified strain RLP, when administered orally, significantly reduced systolic blood pressure in spontaneously hypertensive rat models. During the treatment of hypertension, no significant side effects or bacterial translocation were observed, indicating the safety and effectiveness of RLP in treating hypertension [17].

Rössger et al. reprogrammed human cell lines and implanted them into mice. These reprogrammed cells were encapsulated in semi-permeable microcapsules. Engineered cells could respond to dopamine based on brain activity through remote control. The implants successfully produced sufficient levels of the blood pressure-lowering substance atrial natriuretic peptide (ANP), achieving the goal of reducing blood pressure in hypertensive mice to the normal physiological range [18].

5. Precision medicine and inflammatory bowel disease (IBD)

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the digestive system, including Ulcerative Colitis (UC) and Crohn's Disease (CD). While not typically life-threatening, IBD is characterized by recurrent flare-ups and is challenging to cure completely, significantly impacting patients' quality of life. Repeated damage to the intestinal mucosa increases the risk of cancer development. Previous studies have shown that fecal microbiota transplantation (FMT) has a significant effect on IBD, highlighting the close relationship between gut microbiota and IBD. Using synthetic biology techniques to engineer the microbiota has emerged as a safe and viable novel approach for IBD diagnosis and treatment.

Riglar et al. designed a genetically stable Escherichia coli-based colitis detection system. Engineered bacteria sense the concentration of the colonic inflammation marker thiocyanate and form a memory to detect the level of colonic inflammation. In both in vivo and in vitro experiments, when the thiocyanate concentration reached 1 micromolar per liter, Escherichia coli began to respond, corresponding to higher levels of inflammation in mice in an activated state. This model can stably exist in the colon for up to six months, eliminating the need for antibiotic selection and reducing interference with the existing microbiota. This non-invasive engineered bacterial tool for long-term monitoring of colonic inflammation levels offers a new approach to research on gut health and the diagnosis of related diseases [19].

DOI: 10.54254/2753-8818/23/20231047

A team led by Bang-Ce Ye from East China University of Science and Technology designed an engineered probiotic, i-ROBOT, that can non-invasively and in real-time monitor and record Inflammatory Bowel Disease (IBD). i-ROBOT, based on Nissle 1917, produces heritable genomic DNA sequences and fluorescence signals through the activation of a base editing system in response to the concentration of the inflammatory marker thiolated sulfate. i-ROBOT integrates three key modules: a fluorescence reporting module, a base editing record module, and a drug expression secretion module. It possesses the unique ability to simultaneously diagnose, record, and alleviate IBD in vivo. This intelligent system not only effectively improves IBD, including complications like intestinal fibrosis but also avoids side effects associated with excessive drug administration, significantly enhancing treatment precision and safety [20].

Interleukin-10 (IL-10) has shown promise in clinical trials for treating IBD. Lothar Steidler and colleagues used genetic engineering to create two mouse models, one for treating chronic colitis induced by 5% dextran sulfate sodium (DSS) and the other for preventing spontaneous colitis in IL-10-deficient mice. Both mouse models demonstrated that bacteria secreting this genetically engineered cytokine locally could reduce the therapeutic dose of IL-10. Gastric administration of Lactobacillus producing IL-10 reduced colitis in DSS-treated mice by 50% and prevented colitis in IL-10(-/-) mice [21].

The research team at Harvard University, led by Neel S. Joshi, used genetic engineering to enable Escherichia coli Nissle 1917 to produce fibrous matrices promoting intestinal epithelial integrity within the intestine. These matrices, consisting of three-leaf motifs (TFFs), were successfully secreted by engineered EcN both in vivo and in vitro, significantly enhancing intestinal barrier function and epithelial repair processes. This engineering significantly enhanced protection against colitis in mice and was closely associated with mucosal healing and immune regulation [22].

A research team from Soochow University developed an orally administered engineered probiotic specifically for treating IBD. Using genetic engineering techniques, they overexpressed hydrogen peroxide and superoxide dismutase in the well-established oral probiotic Escherichia coli Nissle 1917. The engineered probiotic was coated with chitosan and sodium alginate. Experimental results demonstrated that this engineered probiotic with the protective coating had a significant effect in a mouse model of IBD, effectively alleviating inflammation and restoring colonic epithelial barrier function. Additionally, the engineered probiotic regulated the gut microbiota composition, increasing the abundance of lactobacilli and bifidobacteria, which are crucial for maintaining intestinal homeostasis. This suggests that the engineered probiotic may promote intestinal health and protect against the development of inflammatory bowel diseases through multiple mechanisms [23].

Researchers from Massachusetts General Hospital and the Harvard T.H. Chan School of Public Health developed a drug delivery system, PROT3EcT, composed of Escherichia coli Nissle 1917, capable of secreting proteins into the surrounding environment. The system comprises three critical modules: a modified Shigella type III secretion system module, a related transcriptional regulator VirB expression module, and a therapeutic nanobody expression module. PROT3EcT can locally secrete drugs in the murine gut and stably colonize without affecting the balance of the gut microbiota. In a murine colitis model, a single prophylactic dose of PROT3EcT effectively reduced the levels of the pro-inflammatory cytokine TNF, preventing damage and inflammation. Furthermore, TNF-PROT3EcT showed the same effectiveness in inhibiting the development of colitis as anti-TNF-α monoclonal antibodies administered systemically [24].

6. Conclusion

Undoubtedly, there is still a long journey ahead, from foundational research in synthetic biology to clinical applications. The use of synthetic biology to modify living organisms inevitably raises concerns about biosecurity and faces challenges related to the relative instability of synthetic systems and their incompatibility with existing biological systems.

However, an increasing body of research indicates that in the field of chronic disease prevention and treatment, engineered organisms developed using synthetic biology techniques exhibit greater

robustness and controllability. These life forms, designed with specific therapeutic properties, offer the promise of more personalized and precise treatments.

Synthetic biology represents boundless creativity and holds great potential in the field of biomedical sciences. It is poised to usher in a new revolution in biology and medicine.

References

[1] Jin, X., Liu, B., Liu, H., et al. (2023). Exploring the Mechanism of the Therapeutic Effects of Canglian Baitu Powder on Obesity Based on Gut Microbiota. *Chinese Journal of Traditional Chinese Medicine, 38*(08), 1732-1738. DOI: 10.16368/j.issn.1674-8999.2023.08.281.

[2] Chinese Society of Overweight and Obesity Medicine and Nutrition. (2021). Chinese Guidelines for Medical Nutrition Therapy of Overweight and Obesity (2021). *Chinese Medical Frontiers Journal (Electronic Edition), 13*(11), 1-55.

[3] Chinese Nutrition and Chronic Disease Status Report (2020). *Acta Nutrimenta Sinica, 42*(06), 521.

[4] Wang, L., Chen, T., Wang, H., Wu, X., Cao, Q., Wen, K., Deng, K. Y., Xin, H. (2021). Engineered Bacteria of MG1363-pMG36e-GLP-1 Attenuated Obesity Induced by High Fat Diet in Mice. *Frontiers in Cellular and Infection Microbiology, 11*, 595575. doi: 10.3389/fcimb.2021.595575.

[5] Bai, L., Gao, M., Cheng, X., Kang, G., Cao, X., Huang, H. (2020). Engineered Butyrate-Producing Bacteria Prevent High-Fat Diet-Induced Obesity in Mice. *Microbial Cell Factories, 19*(1), 94. doi: 10.1186/s12934-020-01350-z.

[6] Senthivinayagam, S., Serbulea, V., Upchurch, C. M., Polanowska-Grabowska, R., Mendu, S. K., Sahu, S., Jayaguru, P., Aylor, K. W., Chordia, M. D., Steinberg, L., Oberholtzer, N., Uchiyama, S., Inada, N., Lorenz, U. M., Harris, T. E., Keller, S. R., Meher, A. K., Kadl, A., Desai, B. N., Kundu, B. K., Leitinger, N. (2021). Adaptive Thermogenesis in Brown Adipose Tissue Involves Activation of Pannexin-1 Channels. *Molecular Metabolism, 44*, 101130. doi: 10.1016/j.molmet.2020.101130.

[7] Wang, C. H., Lundh, M., Fu, A., Kriszt, R., Huang, T. L., Lynes, M. D., Leiria, L. O., Shamsi, F., Darcy, J., Greenwood, B. P., Narain, N. R., Tolstikov, V., Smith, K. L., Emanuelli, B., Chang, Y. T., Hagen, S., Danial, N. N., Kiebish, M. A., Tseng, Y. H. (2020). CRISPR-Engineered Human Brown-Like Adipocytes Prevent Diet-Induced Obesity and Ameliorate Metabolic Syndrome in Mice. *Science Translational Medicine, 12*(558), eaaz8664. doi: 10.1126/scitranslmed.aaz8664.

[8] Han, C., Zhang, X., Pang, G., Zhang, Y., Pan, H., Li, L., Cui, M., Liu, B., Kang, R., Xue, X., Sun, T., Liu, J., Chang, J., Zhao, P., Wang, H. (2022). Hydrogel Microcapsules Containing Engineered Bacteria for Sustained Production and Release of Protein Drugs. *Biomaterials, 287*, 121619. doi: 10.1016/j.biomaterials.2022.121619.

[9] Chen, C., Yu, G., Huang, Y., Cheng, W., Li, Y., Sun, Y., Ye, H., Liu, T. (2022). Genetic-Code-Expanded Cell-Based Therapy for Treating Diabetes in Mice. *Nature Chemical Biology, 18*(1), 47-55. doi: 10.1038/s41589-021-00899-z.

[10] van Krieken, P. P., Voznesenskaya, A., Dicker, A., Xiong, Y., Park, J. H., Lee, J. I., Ilegems, E., Berggren, P. O. (2019). Translational Assessment of a Genetic Engineering Methodology to Improve Islet Function for Transplantation. *EBioMedicine, 45*, 529-541. doi: 10.1016/j.ebiom.2019.06.045.

[11] Yin, J., Yang, L., Mou, L., Dong, K., Jiang, J., Xue, S., Xu, Y., Wang, X., Lu, Y., Ye, H. (2019). A Green Tea-Triggered Genetic Control System for Treating Diabetes in Mice and Monkeys. *Science Translational Medicine, 11*(515), eaav8826. doi: 10.1126/scitranslmed.aav8826.

[12] Bojar, D., Scheller, L., Hamri, G. C., Xie, M., Fussenegger, M. (2018). Caffeine-Inducible Gene Switches Controlling Experimental Diabetes. *Nature Communications, 9*(1), 2318. doi: 10.1038/s41467-018-04744-1.

[13] Li, C. Y., Wu, T., Zhao, X. J., Yu, C. P., Wang, Z. X., Zhou, X. F., Li, S. N., Li, J. D. (2023). A Glucose-Blue Light AND Gate-Controlled Chemi-Optogenetic Cell-Implanted Therapy for Treating Type-1 Diabetes in Mice. *Frontiers in Bioengineering and Biotechnology, 11*, 1052607. doi: 10.3389/fbioe.2023.1052607.

[14] Wu, X., Shao, J. W., Ye, H. F. (2019). Precise Design and Disease Diagnosis and Treatment of Functional Cells Driven by Synthetic Biology. *Biological Industry Technology, (01), 41-54.

[15] Ye, H., Daoud-El Baba, M., Peng, R. W., Fussenegger, M. (2011). A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice. *Science, 332*(6037), 1565-1568. doi: 10.1126/science.1203535.

[16] Yu, G., Zhang, M., Gao, L., Zhou, Y., Qiao, L., Yin, J., Wang, Y., Zhou, J., Ye, H. (2022). Far-Red Light-Activated Human Islet-Like Designer Cells Enable Sustained Fine-Tuned Secretion of Insulin for Glucose Control. *Molecular Therapy, 30*(1), 341-354. doi: 10.1016/j.ymthe.2021.09.004.

[17] Yang, G., Jiang, Y., Yang, W., Du, F., Yao, Y., Shi, C., Wang, C. (2015). Effective Treatment of Hypertension by Recombinant Lactobacillus plantarum Expressing Angiotensin-Converting Enzyme Inhibitory Peptide. *Microbial Cell Factories, 14*, 202. doi: 10.1186/s12934-015-0394-2.

[18] Rössger, K., Charpin-El Hamri, G., Fussenegger, M. (2013). Reward-Based Hypertension Control by a Synthetic Brain-Dopamine Interface. *Proceedings of the National Academy of Sciences of the United States of America, 110*(45), 18150-18155. doi: 10.1073/pnas.1312414110.

[19] Riglar, D. T., Giessen, T. W., Baym, M., Kerns, S. J., Niederhuber, M. J., Bronson, R. T., Kotula, J. W., Gerber, G. K., Way, J. C., Silver, P. A. (2017). Engineered Bacteria Can Function in the Mammalian Gut Long-Term as Live Diagnostics of Inflammation. *Nature Biotechnology, 35*(7), 653-658. doi: 10.1038/nbt.3879.

[20] Zou, Z. P., Du, Y., Fang, T. T., Zhou, Y., Ye, B. C. (2022). Biomarker-Responsive Engineered Probiotic Diagnoses, Records, and Ameliorates Inflammatory Bowel Disease in Mice. *Cell Host & Microbe, 31*(2), 199-212.e5. doi: 10.1016/j.chom.2022.12.004.

[21] Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., Fiers, W., Remaut, E. (2000). Treatment of Murine Colitis by Lactococcus lactis Secreting Interleukin-10. *Science, 289*(5483), 1352-1355. doi: 10.1126/science.289.5483.1352.

[22] Praveschotinunt, P., Duraj-Thatte, A. M., Gelfat, I., Bahl, F., Chou, D. B., Joshi, N. S. (2019). Engineered E. coli Nissle 1917 for the Delivery of Matrix-Tethered Therapeutic Domains to the Gut. *Nature Communications, 10*(1), 5580. doi: 10.1038/s41467-019-13336-6.

[23] Zhou, J., Li, M., Chen, Q., Li, X., Chen, L., Dong, Z., Zhu, W., Yang, Y., Liu, Z., Chen, Q. (2022). Programmable Probiotics Modulate Inflammation and Gut Microbiota for Inflammatory Bowel Disease Treatment After Effective Oral Delivery. *Nature Communications, 13*(1), 3432. doi: 10.1038/s41467-022-31171-0.

[24] Lynch, J. P., González-Prieto, C., Reeves, A. Z., Bae, S., Powale, U., Godbole, N. P., Tremblay, J. M., Schmidt, F. I., Ploegh, H. L., Kansra, V., Glickman, J. N., Leong, J. M., Shoemaker, C. B., Garrett, W. S., Lesser, C. F. (2023). Engineered Escherichia coli for the In Situ Secretion of Therapeutic Nanobodies in the Gut. *Cell Host & Microbe, 31*(4), 634-649.e8. doi: 10.1016/j.chom.2023.03.007.