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Live bacterial therapeutics for detection and treatment of colorectal cancer

Joanna Zhang, Jeff Hasty & Amir Zarrinpar

Live microorganisms can be manipulated and engineered for colorectal cancer detection and treatment through methods such as faecal microbiota transplantation, native bacteria engineering and synthetic circuit engineering. Although promising, substantial effort is required to translate these approaches for clinical use.

Despite advances in colorectal cancer (CRC) treatment and detection, CRC remains the second most common cause of cancer death in the USA alone. The stage of tumour progression at the time of treatment has an important role in prognosis. Early CRC detection remains a challenge because it lacks specific symptoms and relies on colonoscopies, which are invasive, costly, unacceptable to some patients and can potentially miss cancers, especially if patients do not undergo periodic screening and surveillance colonoscopies. Meanwhile, current anticancer therapies have many disadvantages, including adversely affecting normal cells and therefore causing systemic adverse effects, an inability to penetrate solid tumours and the development of drug resistance in some patients. Live bacterial therapeutics (LBTs), in which live microorganisms are used to treat human diseases, have become an emerging avenue for cancer detection and treatment due to bacteria's inherent ability to target and proliferate within solid tumours (Fig. 1). Furthermore, the ability to engineer bacteria for controlled release of payloads makes them an attractive chassis for continuous delivery of therapeutics such as immunomodulators, cytotoxic proteins and prodrug-converting enzymes. In this Comment, we discuss current advances in using LBTs for CRC treatment and detection.

Faecal microbiota transplantation (FMT), in which a healthy donor's stool is collected and introduced into the gastrointestinal system of a recipient with a disease, is currently approved by the FDA to treat *Clostridioides difficile* infections that are non-antibiotic-responsive. As certain gut microorganisms such as *Fusobacterium nucleatum, Bacteroides fragilis* and *Enterococcus faecalis* have been related to CRC development, FMT is being tested for treatment in patients with cancer[1]. A 2018 study reported the first case of treatment of checkpoint inhibitor-associated colitis with FMT[2]. A subsequent study demonstrated that FMT from patients responsive to programmed cell death protein 1 (PD1) blockade transferred into germ-free or antibiotic-treated mice improved the antitumour effects of PD1 blockade when compared with similar mice that received an FMT from patients who were poorly responsive[3]. However, the mechanism by which FMT improves response to anti-PD1 therapy is poorly understood and will require further testing and validation.

Tools in microbiology and synthetic biology provide another avenue in the use of LBTs for CRC as they can be applied to both native and non-native strains. For example, native *Escherichia coli* isolated from conventionally raised mice can be engineered to express genes such as bile salt hydrolase. Reintroduction of the strain showed perpetual engraftment in the intestine as well as improved insulin sensitivity and glucose tolerance in the host 3 months after administration[4]. This method effectively circumvents the problem wherein lab-engineered strains often have difficulty colonizing native physiological environments. Long-term engraftment with tumour or polyp-detecting native bacteria opens opportunities in which persistently colonizing bacteria can be used to monitor for advanced adenoma or CRC. Moreover, deconjugated bile acids could potentially suppress CRC formation in transgenic *Apc* mice through FXR signalling, although it is not yet clear whether bile salt hydrolase-containing engineered native bacteria can suppress polyp or CRC formation[5].

Another example of applying synthetic biology tools to cancer therapy involves the use of quorum sensing, whereby small molecules or oligopeptides are used for regulation of bacterial community behaviour. Using the *Vibrio fischeri* lux quorum-sensing system and its inducer acyl-homoserine lactone, researchers engineered a synchronized lysis circuit (SLC) into *Salmonella enterica* subsp. *enterica* serovar Typhimurium, a strain that specifically targets and proliferates within tumours. In these engineered bacteria, lysis of the entire population is triggered at a threshold population density. Following lysis, a small number of surviving cells reseed the population, thus repeating the cycle. In vivo applications of the SLC in mouse models of solid tumours demonstrated population oscillatory dynamics. Moreover, protein production was limited to tumours[6]. Thus, the SLC provides an attractive platform for delivery of bacterial cancer therapies due to its ability to release any protein produced by the host bacterium, including bacteria-based toxins and checkpoint inhibitor blockade nanobodies[7]. Moreover, the lysis system does not require additional circuitry to secrete therapeutic agents from the cells. Because of the increased tendency for mutations in the SLC from lysis-induced stress on the host bacterium, researchers have also made efforts to stabilize and improve inducibility of the SLC. These strategies involve incorporating genetic modifications into *E. coli* to improve SLC performance, such as integrating them into the *E. coli* genome, replacing non-functional components with functional strains using *E. coli*-targeting colicins to extend their functionality via a rock–paper–scissors mechanism, and creating a system that is responsive to an exogenous inducer molecule, such as p-coumaric acid[8].

Studies demonstrate that LBTs are useful in detecting cancer as engineered biosensors, particularly in the context of CRC. For example, *E. coli* Nissle can be engineered to produce salicylate. As *E. coli* Nissle preferentially engrafts onto neoplastic lesions in an $Apc^{min/+}$ mouse model of CRC, investigators could detect CRC by measuring urine levels of salicylate[9]. Harnessing bacterial methods for natural competence,

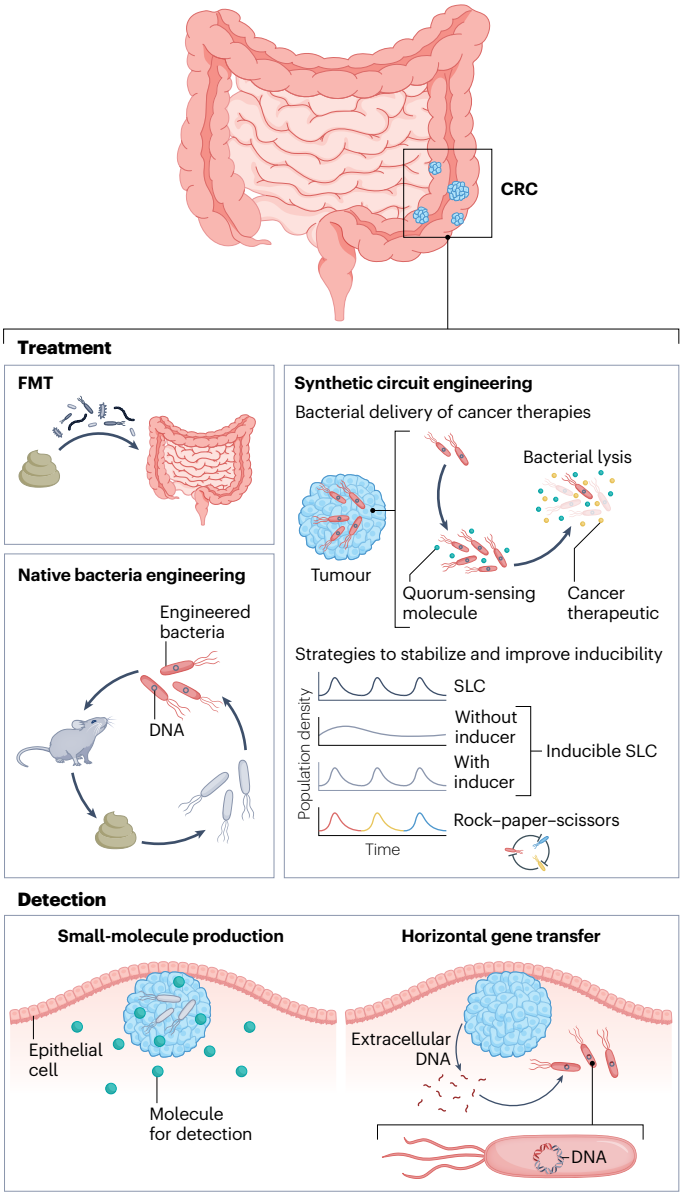

Fig. 1 | Applications of live bacterial therapeutics for colorectal cancer. Live bacterial therapeutics could play a crucial part in colorectal cancer (CRC) treatment through methods such as faecal microbiota transplantation (FMT), native bacteria engineering and synthetic circuit engineering. Bacteria can also be engineered for small-molecule production and horizontal gene transfer for CRC detection. SLC, synchronized lysis circuit.

investigators have also engineered *Acinetobacter baylyi* for CRC detection[10]. This biosensor takes advantage of *A. baylyi*'s natural ability for horizontal gene transfer, the process in which extracellular DNA is integrated into the bacterial genome via regions of homology. *A. baylyi* was engineered to specifically detect mammalian DNA. This DNA contains *KRAS*, an important oncogene, with the $KRAS^{G12D}$ mutation being an important driver for advanced colorectal adenomas. In combination with the *A. baylyi* endogenous CRISPR system, which is programmed to degrade wild-type *KRAS*, the biosensor only integrated sequences containing the $KRAS^{G12D}$ mutation. The integration then activates an output gene, which is modular and can be engineered to produce any arbitrary signal of interest. In this study, antibiotic resistance was used as an easily measured output. Crucially, this biosensor detected CRC both in an engineered orthotopic mouse CRC model in vivo as well as from natural CRC mammalian cells in vitro. As regions of homology and CRISPR spacers are modular in the biosensor genome, this engineering strategy can be applied to any target of interest for detection.

Substantial effort is still required for the translation of LBTs to clinical use in patients with cancer. Overall, preclinical evidence suggests that LBTs provide a useful platform for the detection and treatment of CRC as they possess both innate characteristics for beneficial remodelling of the gut microenvironment as well as synthetic engineering for both therapy production and tumour detection. Although the evidence provided here focuses on CRC, all aforementioned methods are also applicable to non-colorectal cancers, with ongoing efforts to treat cancer types including breast, liver and blood, among others.

Joanna Zhang[1,2,3], Jeff Hasty[1,2,3,4] & Amir Zarrinpar[3,4,5,6,7]
[1]Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA. [2]Biodynamics Laboratory, University of California, San Diego, La Jolla, CA, USA. [3]Synthetic Biology Institute, University of California, San Diego, La Jolla, CA, USA. [4]Center for Microbiome Innovation, University of California, San Diego, La Jolla, CA, USA. [5]Jennifer Moreno Department of Veterans Affairs, La Jolla, CA, USA. [6]Division of Gastroenterology, University of California, San Diego, La Jolla, CA, USA. [7]Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA.
e-mail: azarrinpar@ucsd.edu

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Competing interests

J.H. is a co-founder of GenCirq, which focuses on cancer therapeutics; he is on the Board of Directors and has equity in GenCirq. His spouse is employed part-time for bookkeeping and to support employees with Human Resources. A.Z. has a patent for PCT/US18/27998 pending and licensed to Endure Biotherapeutics and holds equity and is the acting Chief Medical Officer of Endure Biotherapeutics. J.Z. declares no competing interests.