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Perspective

Engineering bacteria for cancer therapy

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The engineering of living cells and microbes is ushering in a new era of cancer therapy. Due to recent microbiome studies indicating the prevalence of bacteria within the human body and specifically in tumor tissue, bacteria have generated significant interest as potential targets for cancer therapy. Notably, a multitude of empirical studies over the past decades have demonstrated that administered bacteria home and grow in tumors due to reduced immune surveillance of tumor necrotic cores. Given their specificity for tumors, bacteria present a unique opportunity to be engineered as intelligent delivery vehicles for cancer therapy with synthetic biology techniques. In this review, we discuss the history, current state, and future challenges associated with using bacteria as a cancer therapy.

Introduction

Synthetic biology has produced numerous examples of genetic circuits that produce complex, dynamic behaviors in single-cells or across populations including switches, oscillators, sensors, filters, counters, and recorders [1–9]. While most genetic circuits have been engineered in a controlled laboratory environment as a proof of principle, a growing number of these circuits are being implemented for environmental, material, and medical applications. Because of the realization that bacteria exist in a multitude of tissue types previously thought to be sterile [10–12], an emerging focus of synthetic biology is to design bacteria to supplant natural niches to tackle various diseases including inflammation [13,14], infections [15–17], metabolic disorders [18,19], and cancer [20–22]. With synthetic biology's level of cellular control, bacteria can be programmed as living medicines that sense their environment and produce agents that diagnose and treat diseases.

Bacteria cancer therapy: a brief history

One of the earliest reports of using bacteria as a cancer therapy dates back to 1891, when Dr. William Coley started to inject live cultures of *Streptococcus* bacteria that led to remissions of inoperable bone and soft tissue sarcomas in patients [23]. Largely due to the dangers associated with infection from the live bacteria before the widespread availability of antibiotics, the clinical use of this therapeutic approach went undeveloped. The therapeutic effect generated by bacteria is thought to be mediated by two key principles. First, a multitude of studies has shown that many bacteria selectively colonize tumor cores through leaky vasculature upon systemic administration, primarily due to reduced immune surveillance in the tumor's hypoxic and acidic environment [24–32]. Second, while inside of tumors, bacteria compete with cancer cells for nutrients, produce toxins, and stimulate a local immune response [32,33]. Over the past century, several bacteria have been explored for cancer therapy such as studies using attenuated *Clostridium novyi* spores that demonstrated tumor regression in mice, dogs, and eventually a human patient [27,34]. While these studies have shown some promising results, further development in enhancing efficacy while retaining safety profiles are needed. To date, the only example of a common bacteria cancer therapy in clinics remains to be the use of *Mycobacterium bovis* (BCG therapy) for the treatment of high-risk non-muscle-invasive bladder cancer [35,36].

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Since finding a bacteria species with the ideal properties to treat cancers is challenging, the focus has shifted to genetic engineering of bacteria with enhanced safety and efficacy profiles for cancer therapy. *Escherichia coli* and *Salmonella typhimurium* are emerging as the leading and most frequently investigated engineered bacteria due to their ease in genetic manipulation. Importantly, various genetic engineering tools allow for genetic attenuations such as a reduction in strain virulence and endotoxicity [37–40]. For example, knockout of the *msbB gene,* involved in terminal myristoylation of lipid A, resulted in viable strains that reduce TNF-α induction 10^4-fold in *S. typhimurium*. Further knockouts of *purI and xyl* genes prevented metabolism of purine and xylose, reducing pathogenicity. A strain with all of these attenuations (VNP20009) was tested in a Phase I metastatic melanoma trial, where a maximum tolerated a dose of 3×10^8 intravenously administered bacteria was seen to cause no significant grade toxicities [41,42]. While safety was largely established, no efficacy was observed in these trials with bacteria alone, and thus engineering bacteria to locally produce therapeutics in tumors has now become the focal point of the bacteria cancer therapy approach.

Current research and future directions

One of the primary advantages of bacteria therapies is local therapeutic delivery (Figure 1), which allows for a reduction in toxicities associated with systemic drug delivery [43,44]. For example, chemotherapies including 5-fluorouracil for the treatment of advanced and metastatic colorectal cancer cause cytotoxicity to healthy cells, leading to dose reduction, delays and discontinuation of therapies which ultimately limits therapeutic efficacy [45–47]. Additionally, patient tumors can be resistant to standard of care treatments [48], necessitating alternative therapeutic approaches. To increase efficacy, combination cytotoxic therapy is employed as an effective approach such as fluorouracil, leucovorin, and irinotecan (FOLFIRI) and other combinations (FOLFOX, XELOX, FOLFOXIRI) [49–51]. However, systemic delivery of combination chemo- and immunotherapy lead to significantly higher toxicities than single agents alone [52,53]. Since bacteria selectively colonize tumors, this targeted and controlled approach can deliver therapeutics that can be toxic systemically, such as bacterial toxins, and improve efficacy and safety profiles. Various therapeutics can be made from bacteria to target a broad range of anti-tumor mechanisms such as membrane-damaging, apoptosis, protein synthesis inhibition, and innate/adaptive immune stimulation. Notably, cancer immunotherapy has witnessed a renaissance in the past decade with the development of novel engineered immune cell therapies and antibodies that target immune checkpoints to activate antitumor immunity [54]. Combining immunotherapy and microbial synthetic biology, bacteria have recently been engineered to deliver immunostimulants directly into the core of a growing tumor — locally priming antitumor immune cells to systemically attack primary tumor and disseminated metastases [55,56]. Delivery of such therapeutics by engineered bacteria *in situ* will enhance 'on-tumor' targeting specificity to elicit superior antitumor efficacy with less toxicity than current systemic anticancer treatment regimens.

The production of potentially toxic therapeutic cargo has necessitated further control over the expression and delivery of therapeutics. New synthetic biology approaches are being developed that enable bacteria to controllably sense and respond to tumor environments (Figure 1), which can be used for enhancing safety and efficacy. For example, genetic circuits were recently reported enabling bacteria to sense bacterial population density using quorum sensing to trigger gene expressions *in vivo* [15,57]. In addition, quorum sensing has been coupled with bacterial self-lysis, effectively releasing therapeutics and maintaining a low population in tumors [58]. This engineered strain increased survival in a liver metastasis model when orally delivered, while also improving safety profiles compared with constitutively producing therapeutic strains. The release of the therapeutic can be also achieved in many other ways such as secretion, passive cell death, or phagocytosis by host cells in the body [32]. Additionally, triggering of the production of therapeutics or release can be connected to an array of environmental cues that may mark a tumor microenvironment such as hypoxia or high lactate levels [19,21,59,60]. These sensing and actuating activities will affect surrounding cancer cells in a heterogeneous manner and can be used to further optimize therapeutic efficacy.

Although there has been significant development of engineered bacteria as a cancer therapy, the vast majority of studies incrementally study one bacteria strain, one therapeutic payload, one genetic circuit, in an animal model. However, the ability to create engineered therapies far outpaces the throughput of animal-based testing, thus creating a major bottleneck to the progress of engineered microbial and cell therapies [61,62]. Coculture of bacteria with mammalian cells provides a powerful platform to study the performance of engineered cells *in vitro* (Figure 1). Coculture systems have traditionally relied on physical compartmentalization of microbes and cells by porous membranes for a relatively short time scale to prevent bacterial overgrowth in culture [61]. This approach is simple to execute, but lacks spatial orientation and contact between the cells seen *in vivo*. The

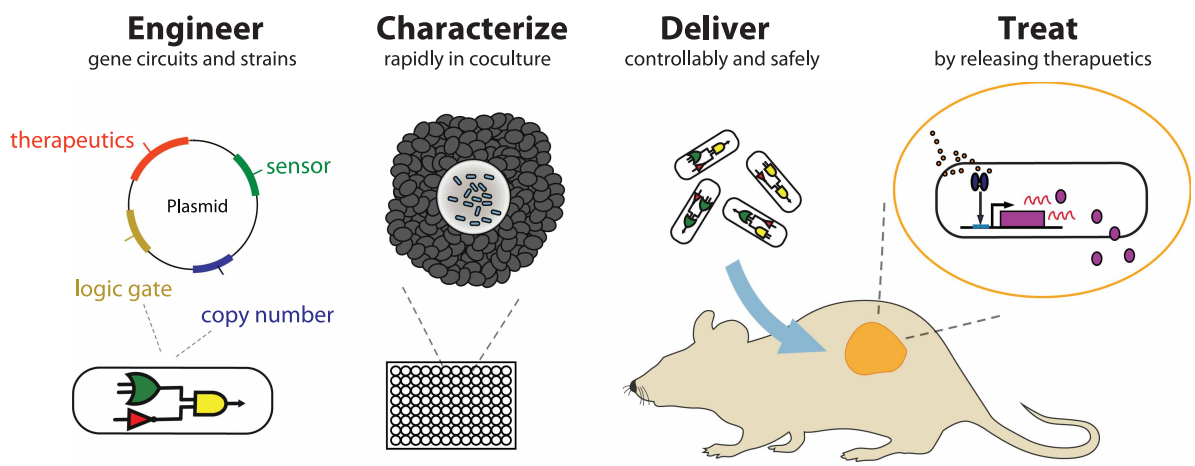

Figure 1. Approach for the development of bacterial cancer therapies.

Bacteria are engineered guided by synthetic biology design and introduction of plasmid or modifying genomes. *In vitro* coculture systems can be used to rapidly characterize and develop effective engineered bacteria. To safely deliver therapeutics to the tumor, bacteria can sense and respond to a tumor microenvironment using synthetic gene circuits. Therapeutics are locally released including potentially toxic molecules such as bacterial toxins and potent immune stimulators.

role of 3D interactions between cells has been shown to create strikingly distinct responses to drugs compared with traditional monolayers [63]. To this end, 3D models including the use of multicellular spheroids, tumor cylindroids, tumor-on-a-chip, and organoids have been introduced to recapitulate spatial orientation in relation to interaction with bacteria [64–67]. Microfluidics has also been employed to allow bacterial population control over a longer time period by washing out excess bacteria [58,66]. These approaches enabled quantitative measurement of bacterial penetration to tumor tissues in a controlled environment and provided valuable insights such as the role of chemotaxis, motility, and tumor heterogeneity [57,66,67]. The majority of these systems have tended to have low throughput and used over relatively short time windows due to complexity, population instability, and limitation to types of cells used in the platform. Furthermore, long-term analysis of bacteria circuit dynamics is not commonly assessed, which is important for creating new active sense and respond circuitry [61,68]. One example to address this challenge is a recent development of high-throughput coculture system that allows for long-term growth of bacteria in the necrotic core of tumor spheroids, recapitulating bacterial colonization and therapeutic outcomes of tumors *in vivo* [69]. Utilizing this system, therapeutic candidates exhibiting significant tumor reduction were identified that demonstrated superior efficacy to previously characterized therapeutics in animal models. Integration of such *in vitro* platforms will enable rapid characterization of novel therapeutics, gene circuits, strains, and cell lines for clinical translation of engineered bacterial therapy.

While there has been progress in the use of synthetic biology and coculture systems to develop bacteria for cancer therapy, the precise mechanism of tumor colonization is still not well understood. The role of bacterial motility has been suggested to contribute to an active colonization mechanism with contradicting results thus far. Some studies have shown overexpression of flagellum regulator *flhDC* can improve bacterial accumulation by up to five times compared with the wildtype bacteria *in vitro* [70], while others have demonstrated non-motile bacterial spores or non-motile cloning strains are also capable of localizing and germinating within the tumor [34,71]. Furthermore, the role of immune response to bacterial targeting to tumor tissue remains unclear. One report has demonstrated that pro-inflammatory cytokine production upon bacterial systemic introduction, specifically TNF-α, triggers an influx of blood into tumors that carries bacteria together and important for achieving effective colonization [26]. On the other hand, another study has shown that reduction in TNF-α by using msbB deleted *S. typhimurium* did not compromise its tumor-targeting capability [38]. These types of mechanistic studies on bacterial colonization are critical to better understand and design future bacteria therapies (Figure 1).

Comparison of colonization mechanism and therapeutic effect between bacterial species can accelerate the development of effective therapy. For example, BCG is delivered through the intravesical route and attaches to

the urothelium layer in the bladder [72], and seem to disappear quickly from the tissue without persistence [73]. *E. coli* has also been engineered to attach to the colorectal cancer epithelial cells after oral delivery [74]. *Listeria*, when injected intraperitoneally, can accumulate in tumors and are thought to be brought by infected myeloid-derived suppressor cells [75,76]. However, how these compare to the mechanism of selective colonization of bacteria in tumor necrotic cores is not well known. In addition, the extent of which tumors can be colonized via various delivery routes (intratumoral, intravenous, intraperitoneal, and oral) and which cancers can be accessed is still under exploration as well. One study reported more abundant growth of attenuated *Listeria monocytogenes* in metastasis compared with the primary pancreatic tumor upon intraperitoneal delivery [75]. Lastly, while it is widely believed that the inflammatory reaction to BCG mediates innate immune response, leading to antitumor adaptive immunity [77], the majority of attempts to improve upon the current BCG therapy have been unsuccessful for treatment of non-muscle-invasive bladder cancer [77]. Current research on combination treatment strategy with other standard-of-care therapies and understanding interpatient response variability may provide novel insight to improving bacteria cancer therapies [78–81].

Increasing knowledge of the microbiota that resides in the human body has led to a developing area of research in using microbes for therapy [82–88]. Future research may utilize and engineer these indigenous microbes for personalized therapy, by taking advantage of unique individual microbiota compositions [89]. To develop such next-generation microbial therapies, the novel design of engineered bacteria, development of platforms to study these microbes, and understanding mechanisms of bacterial colonization of various species can provide valuable insights towards clinical translation. With the fast-paced design of novel gene circuits to control bacteria's ability to sense and respond to physiological conditions, the diverse capabilities of engineered bacteria may offer new therapeutic paradigms to treating a wide range of diseases.

Summary

- Bacterial cancer therapy offers unique advantages over traditional approaches including localized drug delivery, and intelligent sensing and responding module controlled by synthetic biology technologies.
- Future efforts on enhancing safety and efficacy, developing characterization platforms, and understanding mechanisms of action, can accelerate clinical translation of bacterial therapies.

Abbreviation

FOLFIRI fluorouracil, leucovorin, and irinotecan

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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