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REVIEW ARTICLE

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The potential use of theranostic bacteria in cancer

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Abstract

Conventional chemotherapy approaches have not been fully successful in the treatment of cancer, due to limitations imposed by the pathophysiology of solid tumors, leading to nonspecific drug uptake by healthy cells, poor bioavailability, and toxicity. Thus, novel therapeutic modalities for more efficient cancer treatment are urgently required. Living bacteria can be used as a theranostic approach for the simultaneous diagnosis and therapy of tumors. Herein, we summarize the currently available literature focused on the advantages and challenges for the use of theranostic bacteria in cancer therapy.

KEYWORDS

cancer, diagnostics, theranostics bacteria, therapeutics, tumoricidal agents

1 | INTRODUCTION

Cancer is a disease involving the uncontrolled growth of cells in any part of the body, and remains a major public health problem with more than ten million new cases every year (Eaton, 2003). The World Health Organization estimated that in 2018, approximately 18.1 million new cases were diagnosed, with 9.6 million cancer deaths (Bray et al., 2018). The increased incidence and mortality of cancer is due to the overall aging of the population, as well as changes in the prevalence and distribution of the main risk factors linked to social and economic development (Lin et al., 2019). The most common types of cancer treatment strategies available today, are chemotherapy, surgery, radiotherapy, and immunotherapy, or a combination of these (Abbas & Rehman, 2018). These strategies have not been completely successful so far and have many limitations, including their nonspecific effects on healthy cells, poor bioavailability, toxicity, rapid clearance, and lack of effects on metastasis (Agnoletto et al., 2019; Mukherjee & Patra, 2016). Novel therapeutic modalities are urgently needed for more effective treatment of cancer.

To overcome the problems with chemotherapy, various types of drug delivery systems have been developed to increase the antitumor efficacy, while minimizing adverse side effects (Jain et al., 2015). Research over the last few decades has clearly shown that the use of bacterial-mediated cancer therapy (BMCT) could offer benefits compared to conventional methods of drug administration, although this approach is still at an early stage (Mi et al., 2019; Shrivastava, 2020). Therapeutic approaches using live bacteria may be particularly effective at targeting different regions of the tumor,

depending on oxygen concentration. Bacteria can selectively proliferate within the tumor, where they can cause various alterations in immune cells to increase infiltration into the tumor, release numerous chemokines or cytokines, which further facilitate tumor destruction (Gun et al., 2019; Zhou et al., 2018).

The main problem for the use of live bacteria at a dose resulting in therapeutic efficacy, has always been associated with toxicity problems and other deleterious effects, which can lead to reducing the dose resulting in decreased effectiveness (Patyar et al., 2010). Some new approaches have involved the laboratory engineering of bacteria to attenuate their virulence for safety reasons, or to further increase their antitumor activities (Felgner et al., 2016). Therefore, focus has shifted towards using molecular biology to design specific bacteria for sensing, imaging or treating human diseases (Qu et al., 2018). In the past decade, studies on the use of bacteria have significantly increased as a class of biological vectors for cancer theranostics (Park et al., 2013; Patyar et al., 2010; Tietze et al., 2015).

Theranostics describes the integration of both therapy and diagnosis into a single integrated system that can carry out imaging and therapy at the same time, and can also be used for monitoring the therapeutic response and detecting recurrence (Kelkar & Reineke, 2011). Real-time imaging can allow clinicians to monitor the patient's response to different treatment regimens and can guide decisions whether to continue or change their personalized treatment regimen (Turner, 2018). Nanomedicine and nanotechnology have been often used for theranostics (Walia & Acharya, 2016). Nanomedicine employs materials with one or more dimensions of the order of 100 nm or less, and is being investigated for treating cancer, bacterial infections, as carriers for targeted drug delivery, magnetic resonance imaging (MRI), and so forth (Madamsetty et al., 2019; Walia & Acharya, 2016).

Bacteria are a perfect option for synthesizing nanoparticles for biomedical applications. They are nontoxic, eco-friendly, allow easy genetic manipulation, possess motility, can undergo chemotaxis towards tumors, so they can function as drug/gene delivery vehicles (Kojima et al., 2016; Sharma et al., 2019). The chemotactic property of the bacteria allows scientists to construct fluorescent or bioluminescent bacteria, or bacteria-bead conjugates to follow the pattern of bacterial accumulation and proliferation within tumor cells by optical imaging, MRI or PET (Kojima et al., 2016). In a recent investigation by Kang and coworkers, *Escherichia coli* (MG1655) was introduced as suitable tool for visualization of tumors (Kang et al., 2020). The absorbed F-fluorodeoxysorbitol (FDS) by *E. coli* investigates by positron emission tomography (PET) in tumoral tissues. The results showed that tumoral F-FDS uptake was correlated with the number of *E. coli* in tumors. Therefore, *E. coli* is a tool for semi-quantitative visualization of tumors. Effective bacterial theranostic systems have advantages such as high efficiency, real-time monitoring capability, and low induction of drug resistance (S. Chen et al., 2018). In this review, we provide an overview of some current research reports describing bacteria-based anticancer approaches, as well as the unique aspects of tumor-targeting bacteria as theranostic agents.

2 | BACTERIA-MEDIATED CANCER THERAPY (BMCT)

The choice of the most appropriate cancer treatment for each tumor type and patient remains a challenge for oncologists and researchers. The major limitations of conventional cancer therapy, including the failure of the drug to penetrate the tumor tissue, inducing drug resistance, lack of specificity, and harming normal cells, have prompted a search for alternative approaches (Mitra et al., 2015). The ability of bacteria to act as antitumor agents was recognized as early as the 19th century, when clinicians tried to cure cancers by the direct injection of live bacteria (*Streptococci* and *Clostridia*; Coley, 1891; Nauts et al., 1946). At the end of the 19th century, William Coley, a pioneering New York surgeon, injected a mixture of heat-killed *Streptococcus* and *Serratia marcescens* into cancer patients, now known as Coley's toxins (Hoffman, 2016). Although this therapy could successfully reduce the tumor volume and improved the survival of patients by activating the host immune system, it was never submitted to the Food and Drug Administration for approval (Nauts et al., 1946).

These observations have continued to evoke interest in exploring additional microbes and their interaction with the host tumor microenvironment for the battle against cancer. Bacteria can be used in antitumor therapy based on different strategies. One of the main advantages of bacterial therapy for cancer is the inherent ability of certain bacterial species to target tumors by unique mechanisms. The strains of bacteria that can accumulate in the tumor microenvironment depends on their oxygen tolerance, and can be divided into two groups, obligate anaerobes (*Bifidobacterium* and *Clostridium* spp.) and facultative anaerobes (*Escherichia*, *Listeria*, and *Salmonella* spp.; Duong et al., 2019). Due to the lack of anoxic tissues within the normal human body, obligate anaerobes can only survive in the anoxic region of the tumor (Lambin et al., 1998). This feature plays an important role in specificity of tumor targeting, resulting in minimal side effects from bacteria proliferating in normal tissues. In contrast, facultative anaerobes can invade and proliferate in both oxygenated and non-oxygenated tumor tissue, causing some cytotoxic effects to the normal tissues (Zhou et al., 2018). Using genetic manipulation, tumor-targeting facultative anaerobic bacteria can be designed with a lower toxicity, or to improve their efficacy without enhancing toxicity. They can then accumulate in a tumor using several mechanisms (Bereta et al., 2007; Piñero-Lambea et al., 2015). They are able to preferentially proliferate within the large hypoxic regions of tumors, and thereby deplete the nutrients required for cancer cell metabolism (Forbes, 2010; Song et al., 2018). The use of genetic engineering approaches can improve bacterial tumor targeting, and has allowed bacteria to control tumor proliferation and exert antitumor effects in those tumors that are resistant to chemotherapy due to having an insufficient blood supply (Qu et al., 2018). Bacterial motility is a key feature that allows them to penetrate deeply into tumor tissue (Toley & Forbes, 2012). Bacteria are complex living microorganisms that can use their flagellae to actively move from the vasculature and disperse themselves throughout the tumor tissue in regions distant from the

vasculature, following systemic administration (Duong et al., 2019). This motility can allow bacteria to access regions that are currently untreatable with conventional chemotherapy drugs relying on passive diffusion (M. Zhang & Forbes, 2015). Following targeting and penetration of tumors, live bacteria can undergo vigorous proliferation. The introduction of specific nutrient-dependent mutations into bacteria can improve their tumor-specific proliferation. For instance, one study showed that the *S. typhimurium* strain A1-R which is auxotrophic for leucine and arginine, when injected into tumor-bearing mice selectively propagated in the tumor, with a tumor to liver CFU ratio > 1000:1. Moreover, these bacteria had completely cleared from the normal tissue after 15 days (Zhao et al., 2005).

Another approach is to use genetic engineering to design bacteria that can secrete antitumor agents after tumor colonization. Bacteria can employ type III secretion mechanisms to directly inject genetically encoded antitumor proteins into the cytoplasm of the host cells (Galán et al., 2014). These secreted proteins can be divided into three categories according to their mechanism of action: (a) cytotoxic compounds that directly kill tumor cells; (b) proteins that target cancer cell signaling pathways and induce programmed cell death pathways; and (c) immune regulatory proteins that activate the immune system. Some cytotoxic compounds such as toxins are inherent to the bacterial physiology and can be used for tumor eradication, and can also act as cancer vaccines (Zahaf & Schmidt, 2017). Local release of toxins inside the tumor is important for the anticancer activity of bacterial toxins. As toxins are not specific for cancer cells, their use requires a tightly regulated system with low basal expression (Van Dessel et al., 2015). Cancer signaling pathways play a role important in maintaining cancer cell proliferation (Sever & Brugge, 2015). Bacteria can target tumor cell receptors and trigger the expression of tumor necrosis factor-α (TNF-α) family members (Forbes, 2010), bacteriocin, and other secreted mediators leading to modulation of signaling pathways and induction of apoptosis in tumor cells (Chikindas et al., 2018).

The inherent ability of bacteria to elicit an innate immune response is another advantage for cancer therapy. Tumors can escape the host immune response using multiple mechanisms, including downregulating the expression of tumor antigens, downregulation or loss of histocompatibility complex (MHC) class I molecules on their cell surface, and the production of tumor-derived immunosuppressive factors (TGF-β and IL-10; Rodríguez, 2017; Wiguna & Walden, 2015). Also, tumors tend to gradually change over time to reduce their antigenicity and increase their aggressiveness (Agnoletto et al., 2019). The idea of using bacteria to increases the antigenicity of cancer cells is an interesting idea. Bacteria can induce the production of many cytokines, increase inflammatory responses, stimulate phagocytic activity, and increase oxidative stress, all of which can result in increased tumor antigenicity (Qu et al., 2018). Tumor-targeting bacteria have been modified to express tumor-specific antigens or immunoregulatory factors (Wood & Paterson, 2014). For example, prostate-specific antigen (PSA) which is upregulated in prostate tumors, was fused to the cholera toxin subunit B (CtxB; a subunit of *Vibrio cholerae* toxin) as an adjuvant to induce an effective cellular immune response (Fensterle et al., 2008). Bacteria can be modified to improve immune responses by expressing and secreting monoclonal antibodies that can inhibit the proteins necessary for tumor cell function or block ligand binding (Levitzki, 2012). For example, *Clostridium novyi* was engineered to express a monoclonal antibody that targeted an epitope of hypoxia-inducible transcription factor-1 (HIF-1α; Groot et al., 2007).

Bacteria can serve as delivery vehicles for various therapeutic payloads and effector proteins using several different mechanisms depending on the type of bacteria (Zhou et al., 2018). Care should be taken when using bacteria as anticancer agents, due to possible toxicity occurring at high doses. Studies have shown that live attenuated bacteria can be uses as vectors or vehicles for gene transfer to allow direct expression of tumor suppressor genes, antiangiogenic genes, cytotoxic agents, cytokines, tumor antigens, RNA interference sequences, etc (Forbes, 2010). Also, engineered bacteria can stably express therapeutic protein cassettes (Wong & Slavcev, 2015). The rapid specific replication of bacteria within tumors can lead to continuous expression of therapeutic proteins inside tumors in a cost-effective manner (Wong & Slavcev, 2015). Activation of prodrugs is a common tactic, whereby endogenous metabolic enzymes of bacteria convert nontoxic chemical compounds that can be safely administered systemically, into cytotoxic drugs to confine the effects to the tumor tissue (Malekshah et al., 2016). Different inherent bacterial features that are specific to certain strains can trigger antitumor responses (Kim et al., 2015). The combination of these inherent bacterial properties with additional genetic engineering can serve as advanced diagnostic and therapeutic agents. Recently bacteria have been used to capture microbeads loaded with therapeutic drugs as an effective vehicle for targeted drug delivery (DDS; Cho et al., 2015).

One type of theranostic approach (combining diagnosis and targeted therapy) for detecting and attacking tumors was based on engineered bacteria called "Bacteriobots" (bacteria-based microrobots; Park et al., 2013). This approach allowed the monitoring of migration patterns and tracking the proliferation of these bacteria, using an MRI or positron emission tomography (PET; Jiang et al., 2010). This integration of diagnostics and biotechnology could be a new step forward in anticancer therapy.

As stated above, bacteria have been empirically applied in tumor therapy for more than 100 years (Elkodous et al., 2019) and is now known as BMCT (Phan et al., 2015). Due to advances in biotechnology and the genetic manipulation of bacteria, the application of microorganisms for both cancer detection and treatment is a growing field. Recently, studies have focused on engineered bacterial species like *Clostridium*, *Salmonella*, and *Escherichia* (especially *Escherichia coli* Nissle 1917 [EcN]) to detect tumors or to deliver anticancer agents (Zheng et al., 2017). Different genetically programmed bacteria that can express luciferase have been widely used for real-time in vivo imaging of tumors. Among these bacteria, tumor therapy using *Salmonella* and EcN have shown promising results (X. Yu et al., 2020).

Zhu et al. (2018) used EcN as a vehicle to deliver epothilones (a class of anticancer drugs that target microtubules) to tumor cells.

Microtubules are composed of αβ-tubulin dimmers, and play a vital role in cell division in eukaryotic cells. Microtubules can be blocked by administration of epothilones (J. J. Lee & Swain, 2008). Epothilone B is a macrolide compound isolated from the *myxobacterium Sorangium cellulosum*, which stabilizes microtubules by binding to β-tubulin. This interaction leads to a change in the microtubule dynamics, formation of abnormal spindles during mitosis, and triggers apoptotic cell death (J. J. Lee & Swain, 2008). Previous studies demonstrated the anticancer activity of epothilone B against different tumor types such as, colon, breast and lung cancer (Oehler et al., 2011). However, systemic application of epothilone B is associated with side effects, thus site-specific targeting by encapsulation or delivery vehicles is an important requirement (Diaz-Padilla & Oza, 2011).

Zhu et al evaluated the antiproliferative effects of EcN-Epothilone B against HeLa cells. They used EcN bacterial ghosts (BGs) as a vehicle in a drug delivery system (Zhu et al., 2018). The BGs are empty nonliving bacterial shells with the same surface characteristics as the Gram-negative bacteria from which they are derived. Recently, this new approach has been introduced as a vehicle for delivery of drugs or antigens, which have either been loaded into the periplasmic space or else are expressed on the surface of the bacteria (Hajam et al., 2017). In contrast to PEG-b-PLA micelles, Epothilone B was not conjugated, but simply diffused into EcN BGs. Zhu showed that the loading capacity of EcN was higher than PEG-b-PLA micelles, the EcN ghosts were target specific to the HeLa cells, and EcN-Epothilone B had a strong antiproliferative effect (Zhu et al., 2018).

Li and colleagues in 2018 reported another study that used EcN as an antitumor approach (Elkodous et al., 2019). In their study, EcN was manipulated to express and release colibactin, a type of cyclomodulin. Cyclomodulins are bacterial toxins with effects on the chromosomes of eukaryotic cells. Colibactin causes DNA double-strand breaks, cell cycle arrest in the G2/M phase, and apoptosis of eukaryotic cells (Faïs et al., 2018). The results of Li and colleagues demonstrated that the application of colibactin-expressing EcN had some advantages including, safety, specific tumor-targeting and potent tumor suppressor activity (Elkodous et al., 2019).

Salmonella is another Gram-negative bacterial species that has been utilized in drug delivery and cancer immunotherapy (Y. Guo et al., 2020). The application of *Salmonella* in cancer therapy is due to specific tumor-targeting and induction of cancer cell death. It was previously found that the accumulation of *Salmonella* bacteria in the tumor region was higher than the normal tissue (Kim et al., 2015; Pangilinan & Lee, 2019). However, the colonization of pathogenic *Salmonella* in normal tissue could cause some side effects. However nowadays, scientists have tried to overcome this challenge using genetic engineering. For example, Yu and colleagues engineered an "obligate" anaerobic *Salmonella typhimurium* strain. In their strain, the *asd* gene (critical gene for synthesis of diaminopimelic acid and the bacterial cell wall) was only expressed in a hypoxic environment. In aerobic conditions, this gene is silent, thus the cell wall synthesis in normal oxygenated tissue was inhibited and the bacteria died. Therefore, the hypoxic microenvironment of a solid tumor was an ideal condition for replication and growth of these bacteria (B. Yu et al., 2012).

Immunotherapy is a promising innovative treatment for cancer patients that stimulates or boosts the body's natural defenses to fight cancer. The tendency of the tumor to evade from the body's immune response due to the development of tolerance (via modification of surface antigens and modulation of the surrounding environment) is the main concern among researchers. The idea of usage of bacteria to target tumors in the treatment of cancer is a novel treatment strategy that employs bacteria to enhance the antigenicity of tumor cells (Patyar et al., 2010). Tumor cells are not directly destroyed by bacteria. Initial bacterial accumulation leads to the activation of immune mechanisms (innate and adaptive immune response) and intensification of pro-inflammatory cytokine production by cell-wall components lipopolysaccharide and peptidoglycan (Agrawal et al., 2004; Avogadri et al., 2005; Dougan & Dougan, 2019; Igney & Krammer, 2002). This structural component bacteria is recognized by intratumoral innate immune cells (via PRRs) and significantly triggers the infiltration of immune cells and subsequent secretion of pro-inflammatory cytokines such as IL-1β and TNF-α, which results in drastic tumor growth suppression (Kany et al., 2019). For example, Phan et al. (2015) showed that the ΔppGpp *Salmonella typhimurium* strain can induce activation of inflammasome pathways by both lipopolysaccharides (LPS) and damaged cancer cells (Phan et al., 2015). Bacteria can be genetically attenuated and reprogrammed to produce and deliver anticancer agents such as various tumor suppressor cytokines such as interleukin-18 (IL-18; Loeffler et al., 2008), LIGHT (Loeffler et al., 2007), or CCL21 (Loeffler et al., 2009), which would suppress tumor growth. These antitumor activities required the migration of dendritic, antibody-producing B cells, CD8+ CTL, CD4+ helper T cells, and natural killer T (NKT) cell (Loeffler et al., 2009). Intravenous administration of an IL-18-expressing attenuated *S. Typhimurium* strain in several murine trials showed local bacterial expression inhibited primary tumor growth in mice, triggered leukocyte infiltration (mainly granulocytes), and recruitment of NK and $CD4^+$ T cell. Also, this phenomenon significantly enhanced cytokine production in the tumor region, including that of IFN-γ, IL-1β, TNF-α, and GMCSF (Loeffler et al., 2009). Zheng et al.(2017) employed heterologous flagellin, which evoke the innate immune system via TLR5 and Naip5/6, as a potent immunoregulatory adjuvant (Zheng et al., 2017). In this study, an attenuated ΔppGpp S. Typhimurium strain expressing Vibrio vulnificus flagellin B displayed markedly promoted antitumor immunity via two-step activation of the TLR4 and TLR5 signaling pathways through a MyD88 dependent pathway, resulting in massive tumor infiltration of macrophages and neutrophils and increases interleukin 1β (IL-1β), TNF-α, and nitric oxide (NO) in tumors. A number of engineered tumor-targeting bacteria act as vectors for the expression of cytotoxic proteins (Jiang et al., 2010; V. H. Nguyen et al., 2010), antigens and antibodies (Nishikawa et al., 2006), or genetic materials, such as short hairpin RNA (Grillot-Courvalin et al., 1998) to the tumor microenvironment.

Today, immunotherapy using immune-checkpoint inhibitors is a new strategy for cancer therapy. The local delivery of immune

checkpoint inhibitors molecules to tumor sites by bacterial vehicles can attenuate immune-mediated adverse effects. For example, Gubatri et al. (2020) programmed an engineered *E. coli* Nissle 1917 system, for the controlled production and intratumor release of nanobodies targeting programmed cell death-ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein-4 (CTLA-4), and granulocyte-macrophage colony-stimulating factor (GM-CSF). In the system, after injection into syngeneic mice, these bacteria reduce both the primary tumor and distant metastases by enhancing systemic immune response and T cell activation (Gurbatri et al., 2020).

Chowdhury et al. (2019) designed an approach for specific targeting of CD47 and its ligands on tumor cells using drug delivery vehicles such as quorum-sensing bacteria. In the study, a secreted nanobody from tumor-colonizing bacteria blocks the phagocyte inhibitory ligand CD47 (CD47nb) in tumors and subsequently increased activation of tumor-infiltrating T cells, resulting in tumor reduction and suppression of metastases (Chowdhury et al., 2019).

The accumulation of *S. typhimurium* in the tumor region led to the infiltration of immune cells (such as macrophages and neutrophils) into the target site. The infiltration of immune cells was probably due to the inflammatory responses and IFN-γ produced by the bacteria. Moreover, IL-2 production and natural killer cell activation are the other effects of *Salmonella* bacteria (C.-H. Lee, 2012).

Attenuated modified *Salmonella* (defective in the synthesis of guanosine 5′-diphosphate-3′-diphosphate [ppGpp]) was tested against tumor growth in mice by Kim et al (Kim et al., 2015). ΔppGpp *Salmonella* demonstrated good colonization in the tumor leading to infiltration by immune cells, including macrophages, $CD8^+$ T cells, and B cells. In mice treated with this modified *Salmonella*, in addition to immune cell infiltration, IL-1β and TNF-α secretion was increased and the tumor growth was inhibited (Kim et al., 2015).

It has been previously demonstrated that bacterial flagellin can act as an adjuvant due to its interaction with TLR5 in host cells (C. T. Nguyen et al., 2013). Some studies have suggested that the immune response induced by flagellin can suppress tumor growth (Burdelya et al., 2008; Rhee et al., 2008; Sfondrini et al., 2006). Zheng and colleagues engineered a genetically modified ΔppGpp *S. typhimurium* species that expressed and secreted the flagellin B (FlaB) molecule originating from *Vibrio vulnificus*, and tested it for cancer therapy in mice (Zheng et al., 2017). This FlaB-secreting bacterial species demonstrated a potent tumor-suppressive effect associated with TLR4 and TLR5 activation. Site-specific accumulation of *Salmonella* led to TLR4 and TLR5 activation and increased the tumor infiltration by macrophages and neutrophils (Zheng et al., 2017). These immune cells produced cytotoxic mediators capable of shrinking of the tumor.

3 | THERANOSTIC BACTERIA IN CANCER THERAPY

Theranostic approaches for the simultaneous diagnosis/imaging and therapy of cancer has become a very active field of research. Theranostic materials should be biodegradable, and removed from the body at a short time after administration. Theranostic systems often employ imaging modalities, such as fluorescence, bioluminescence, MRI, PET or ultrasound (US; Zavaleta et al., 2018). In general, theranostic agents are capable of specific accumulation at target sites (Daniel et al., 2013). Not only does the tumor specific accumulation permit in vivo imaging of the tumor location and size but also allows the specific delivery of the therapeutic agent, helping to reduce side effects and increase the efficacy. Therefore, a suitable theranostic system should possess certain characteristics, including safety for normal surrounding tissue, specificity for the target site, traceable within the body, destructive effect on tumor cells or infectious agents, and rapid clearance from the body (Kouhsari et al., 2018). Figure 1 demonstrates a schematic view of the bacterial theranostic approach.

Although a wide array of nanomedicines and nanoparticles have been investigated in theranostics research, the use of live microorganisms, especially bacteria, is an interesting alternative. In previous studies, some engineered bacteria have been employed in imaging and delivery systems at the same time (Sedighi et al., 2019).

The use of bacteria in theranostic systems has several advantages. The genetic manipulation of bacteria is relatively easy, so researchers can rationally design bacterial cells to express the imaging agent (diagnostic) and the cytotoxic agent (therapeutic) at the same time (Sedighi et al., 2019). For example, in some studies, infrared fluorescent proteins (IFP1.4 and IRFP) have been introduced into the bacteria used for diagnosis. The IFP1.4 and IRFP proteins can absorb and emit fluorescence in the infrared region where light transmission through tissue is maximal (Filonov et al., 2011; Sedighi et al., 2019). In other studies, genetically manipulated *Lactobacillus*, *E. coli*, and magnetotactic bacteria have been investigated for imaging, drug delivery, and therapy (C. Chen et al., 2016; Daniel et al., 2013; Y. Zavaleta et al., 2018).

One of the most amazing applications of the bacterial utilization in BMCT is toxin delivery by the genetically engineered bacteria. As stated above, attenuated *Salmonella* (ΔppGpp) demonstrated good colonization in the tumor tissue. Therefore, in some studies, this strain was utilized as a vector for bacterial toxin delivery (cytolysin A) in tumor tissues. For example, in one interesting study, Nguyen and coworkers designed a non-pathogenic *S. typhimurium* species. They used cytolysin A (*ClyA*), a pore-forming bacterial toxin, for the killing of the tumor cells and the bacterial luciferase gene *(lux)* to generate an in vivo bioluminescence imaging signal. The expression of the ClyA protein was dependent on the L-arabinose concentration. The concentration of L-arabinose in tumor regions is generally higher than normal tissue. Thereby, the production of the cytotoxic agent was limited to the tumor region and not produced in normal tissue. Their results showed promising tumor shrinkage and also the *lux* gene expression allowed bioluminescence imaging of the tumor location (V. H. Nguyen et al., 2010).

Also, in another example, Jiang et al. engineered safe *Salmonella* (ΔppGpp) as theranostic agent (Jiang et al., 2013). These strains are transformed by a plasmid (pJL87)-encoding therapeutic (*cly A*), and reporter (luciferase) agents. The expression of ClyA and luciferase

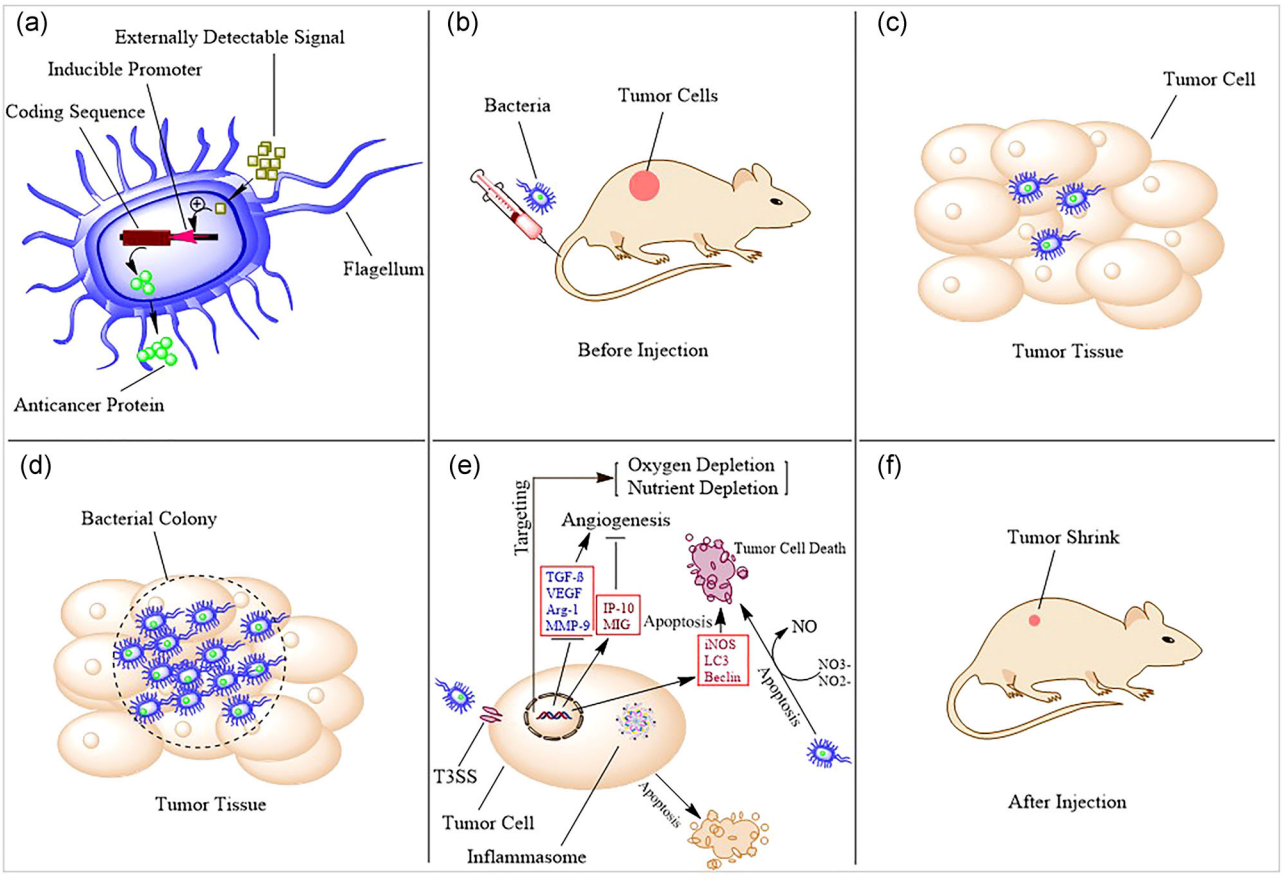

FIGURE 1 Schematic of bacterial theranostic approach. (a) Produce genetically manipulated-bacteria. (b) Injection of bacteria into mouse. (c) Specific access to tumor region. (d) Proliferation and accumulation of genetically manipulated-bacteria in tumor region. (e) Expression of traceable and anticancer agents, consumption of oxygen and nutrient elements by bacteria. (f) Growth inhibition or tumor-shrinking

were under control of *tetA* and *tetR* promoters, respectively. The immunoblot and luminescence assay demonstrated that Cly A and luciferase expressed only in presence of doxycycline. The results indicated that these theranostic bacteria have significant power to suppress both primary and metastatic tumors.

In another study, Friedrich et al introduced the gene encoding for an anticalin (an artificial antibody like molecule) that recognized Hsp70 into bacterial cells. In some circumstances, Hsp70 is located on the surface of tumor cells. The fluorescently-labeled anticalin specifically bound to Hsp70 as shown by fluorescence microscopy. Moreover, anticalin demonstrated a high level of cytotoxicity in vitro, therefore this system had potential as a theranostic agent (Friedrich et al., 2018).

4 | THERANOSTIC NANOPARTICLE PRODUCING BACTERIA

Nanoparticles (NPs) are one of the most common approaches in the cancer therapy/diagnostic field (Kaplowitz, 2005; Valsalam et al., 2019). The sizes of nanoparticles are from 1 to 100 nm and they can be divided into organic (polymeric NPs) and inorganic (non-polymeric NPs) sub-types (Duong et al., 2019). NPs associated with bacteria are categorized as inorganic nanoparticles. Suitable theranostic NPs should have the ability to access and accumulate at the tumor site, produce an imaging signal, and at the same time kill the tumor cells. In recent years, the use of bacteria like *E. coli*, and *Staphylococcus*, and their derivatives have been introduced as effective theranostic approaches. However, the magnetotactic bacterium, *Magnetospirillum*, is possibly the most favorable species in the theranostics approach (L. Guo et al., 2012; Lippert, 2008). By using an external magnetic field, the bacterial drug carrier can be directed towards the target site, thereby increasing the effective concentration of drugs in the tumor (L. Guo et al., 2012). Moreover, MRI can be used to delineate the tumor locations, and therapeutic hyperthermia can be triggered by applying an alternating magnetic field.

Bacterial magnetosomes (BMs) are intracellular nano-sized magnetic organelles containing crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4). The magnetic crystals are enclosed by a membrane containing phospholipids and proteins. BMs can be extracted from magnetotactic bacteria like *Magnetospirillum magneticum* and *Magnetospirillum gryphiswaldense* with several advantages including safety, high specific absorption rate, suitable contrast in MRI, and good biocompatibility. In addition, BMs are capable of transforming the energy from external alternating magnetic fields (AMFs) into heat, which can carry out localized hyperthermia (Dai et al., 2017; Mannucci et al., 2018).

Guo et al. demonstrated the role of bifunctional bacterial magnetic nanoparticles (BBMPs) on HepG2 cells. The BBMPs (about 75 nm) were constructed from BMs, with an added galactosyl ligand and loaded with doxorubicin. In this complex nanocomposite, the galactosyl ligand bound to the asialoglycoprotein receptors (ASGP-R) on the hepatocellular carcinomas cells, and doxorubicin was used as an anticancer drug. They reported that the drug loading capacity was higher than other methods such as polymeric lipids and micelle carriers. Due to the high release rate of doxorubicin at acidic pH conditions (pH < 3.5 in endosomal compartments), tumor specific cell killing was obtained (L. Guo et al., 2012).

Another theranostic approach has been described to detect and treat glioblastoma. Glioblastoma is one of the most severe types of brain tumors, with a mean survival of less than 15 months. In a report, Mannucci and coworkers in 2018 described a method based on extracted magnetosomes from *Magnetospirillum gryphiswaldense* for theranostics of glioblastoma in mice. They injected BMs into the target site and exposed the mice to an AMF. This exposure caused a temperature increase within the tumor cells. They demonstrated with the use of MRI monitoring that the injected MNs remained in the target site during cancer progression, but there was an inhomogeneous distribution throughout the tumor. Nevertheless tumor growth was decreased by repeated application of the AMF (Mannucci et al., 2018).

Tumor hypoxia is a well-known factor that limits the effectiveness of cancer treatment using chemotherapy drugs and ionizing radiation (Y. Zavaleta et al., 2018). The hypoxic regions within solid tumors lead to a reduction in the susceptibility of tumor cells to anticancer drugs. Also, the radiotherapy of these regions is difficult (C.-H. Luo et al., 2016). However, anaerobic bacteria tend to colonize within the hypoxic regions. Thus, scientists have employed anaerobic bacteria such as *Clostridium* and *Bifidobacterium* to overcome these problems (C.-H. Luo et al., 2016; Y. Luo et al., 2019). *Bifidobacterium* is a safe species because it is acts as a probiotic bacterium in the gut of humans and other mammalian animals. A previous study demonstrated that *Bifidobacterium* cells could localize within targeted tumor sites (Kimura et al., 1980). They showed that 96 h after injection, *Bifidobacterium* could be found in the hypoxic regions of solid tumor tissue, and were not found in normal organs such as blood, liver, or kidneys. Y. Luo et al. (2019) designed a *Bifidobacterium longum* preparation conjugated with poly(lactic-co-glycolic acid; PLGA) and perfluorohexane (PFH) nanoparticles. In this study, *B. longum* was injected intravenously and used as a vehicle for transporting the PFH/PLGA NPs into solid tumors. The mean diameter of the PFH/PLGA NPs was 213 nm. PLGA has been shown to be a good agent for drug delivery in many studies (Wang et al., 2018). Furthermore, PHF is a well-known agent for producing ultrasound-induced cavitation bubbles and thereby killing tumor cells. The PFH/PLGA was labeled with DiR fluorescent dye and used as a probe for tumor imaging with a fluorescence system (748 and 780 nm), and allowed tracking of the conjugated bacteria within the body. Their results showed that PFH/PLGA-*B. longum* was a good theranostic agent for simultaneous diagnosis (fluorescent up to 168 h after injection) and after application of ultrasound could induce necrosis in the breast cancer model (Y. Luo et al., 2019).

Traore et al. constructed a "Nanoscale Bacteria-Enabled Autonomous Drug Delivery System" (NanoBEADS) as a theranostic approach. In the NanoBEADS system, a bacterium was attached to a nano-polymeric compound for therapy or/and diagnosis. They used bacteria such as *S. typhimurium* or *E. coli* with the nanoscale particles. In this method, the bacteria were decorated with a biotinylated antibody that was attached to streptavidin-conjugated nanoparticles. This NanoBEADS system was tested in cancer models and is suggested to be an ideal theranostic agent (Traore et al., 2018).

5 | THERANOSTIC GAS PRODUCING BACTERIA

Recently, some theranostic approaches for the diagnosis and therapy of cancer, have been described that are based on gas-filled bubbles or gas vesicles (Xiaowei Walia & Acharya, 2016). Carbon dioxide or hydrogen sulfide are the main gases that have been explored as gas-filled bubbles in medical research (J. Lee et al., 2016; Liu et al., 2017). These bubbles and vesicles can be used as ultrasound (US) contrast agents (J. Lee et al., 2016; Shapiro et al., 2014). The US method is a safe and low-cost method imaging modality, based on backscattering or reflection of sound waves above 20 kHz. Thus, diagnosis using US is practicable due to differences in the acoustic characteristics between the hollow bubbles and the surrounding tissue or plasma (Min et al., 2015).

Some gas vesicles are stable, protein-shelled units with diameters from 45 to 250 nm. Shapiro et al used bacterial-derived gas vesicles as a unique approach for the noninvasive detection of cancer. These gas vesicles were extracted from *Anabaena flosaquae* and *Halobacterium* NRC-1 bacterial cells. They confirmed that gas vesicles were potent ultrasound contrast agents and could be used for imaging (Shapiro et al., 2014). Future studies could investigate the addition or conjugation of these bacterial bubbles with anticancer genes or drugs, for theranostic purposes.

However the bacterial bubbles do not have any inherent targeting ability, as compared to their parent bacterial cells. Researchers could overcome this problem by attaching surface ligands, to specifically recognize the target cells (Klibanov, 2006).

For example, Lee and coworkers described a theranostics approach employing poly(D,L-lactide-*co*-glycolide) (PLG) nanoparticles that encapsulated calcium carbonate ($CaCO_3$) decorated with rabies virus glycoprotein (RVG) peptides. The nicotinic acetylcholine receptor (nAChR) is a molecular marker on the cell surface, which is highly expressed in neuroblastoma, and the RVG peptides specifically attached to nAChR in the tumor site. Also, carbon dioxide bubbles were generated from the encapsulated calcium carbonate under the acidic conditions that were present in the cancer cells, and US imaging could detect the location of the tumor. Moreover, the remaining calcium ions and bicarbonate were cytotoxic at the concentration

produced, and could be used in cancer therapy. Therefore, they showed that these gas-generating nanoparticles allowed detection by US, caused necrotic cell death, and inhibited the tumor growth (J. Lee et al., 2016).

6 | CONCLUSIONS AND PERSPECTIVES

Nowadays, theranostic approaches have heralded a fantastic new era for researchers. Recently, the use of engineered bacteria and probiotic bacteria in theranostic systems has been explored. Bacterial systems have some advantages; the use of engineered bacteria is capable of reducing costs because these bacteria can be viewed as living factories that continuously produce useful products. Also, the use of these bacteria is a safe and noninvasive method. Due to these advantages, the application of theranostic bacteria in the future is expected to continue to advance.

However, there are numerous challenges still remaining. The design and engineering of these multifunctional bacteria containing targeting ligands, and anticancer payloads combined with imaging agents is complicated. Also, these agents should be activated only within the targeted tumor tissue. Due to the ongoing advances and developments in genetic engineering techniques that have emerged in recent decades, it seems it will be possible to carry out these endeavors. The replacement of many conventional methods of separate treatment and imaging by combined theranostic agents is expected in the near future.

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CONFLICT OF INTERESTS

Michael R. Hamblin declares the following potential conflicts of interest. Scientific Advisory Boards: Transdermal Cap Inc, Cleveland, OH; BeWell Global Inc, Wan Chai, Hong Kong; Hologenix Inc. Santa Monica, CA; LumiThera Inc, Poulsbo, WA; Vielight, Toronto, Canada; Bright Photomedicine, Sao Paulo, Brazil; Quantum Dynamics LLC, Cambridge, MA; Global Photon Inc, Bee Cave, TX; Medical Coherence, Boston MA; NeuroThera, Newark DE; JOOVV Inc, Minneapolis-St. Paul MN; AIRx Medical, Pleasanton CA; FIR Industries, Inc. Ramsey, NJ; UVLRx Therapeutics, Oldsmar, FL; Ultralux UV Inc, Lansing MI; Illumiheal & Pettherа, Shoreline, WA; MB Lasertherapy, Houston, TX; ARRC LED, San Clemente, CA; Varuna Biomedical Corp. Incline Village, NV; Niraxx Light Therapeutics, Inc, Boston, MA. Consulting; Lexington Int, Boca Raton, FL; USHIO Corp, Japan; Merck KGaA, Darmstadt, Germany; Philips Electronics Nederland B.V. Eindhoven, Netherlands; Johnson & Johnson Inc, Philadelphia, PA; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany. Stockholdings: Global Photon Inc, Bee Cave, TX; Mitonix, Newark, DE.

AUTHOR CONTRIBUTIONS

Khalil Azizian, Inna Pustokhina, Roya Ghanavati participated in the design of the study and acquisition of data and drafted the manuscript. Abolfazl Amini, Ebrahim Kouhsari, Michael R. Hamblin participated in the interpretation of data and in drafting, reviewing and revising the manuscript. All of the authors read and approved the final draft of the manuscript. Michael R. Hamblin was supported by US NIH Grants R01AI050875 and R21AI121700.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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