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MINI-REVIEW

Precision tumor treatment utilizing bacteria: principles and future perspectives

Zhaoyou Liu[1] · Lantian Wang[1] · Pengying Wu[1] · Lijun Yuan[1]

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Abstract

Bacteria-based tumor therapy, which releases therapeutic payloads or remodels the tumor's immune-suppressive microenvironment and directly kills tumor cells or initiates an anti-tumor immune response, is recently recognized as a promising strategy. Bacteria could be endowed with the capacities of tumor targeting, tumor cell killing, and anti-tumor immune activating by established gene engineering. Furthermore, the integration of synthetic biology and nanomedicine into these engineered bacteria could further enhance their efficacy and controllability. This comprehensive review systematically elucidates the classification and mechanisms of bacterial gene expression induction systems, as well as strategies for constructing bacterial-nanomaterial nanobiohybrids. The review concludes by highlighting the challenges associated with quality control and regulation of bacteria-based tumor therapy while also providing insights into the future prospects of this therapeutic technology.

Key points

- *A comprehensive overview of the current status of research on bacteria-based tumor therapy.*
- *The classification and mechanisms of bacterial gene expression induction systems are summarized.*
- *The challenges and perspectives in clinical translation.*

Keywords Bacteria-based tumor therapy · Synthetic biology · Gene circuits · Nanomedicine

Introduction

Bacteria-based tumor therapy, with the unique properties of bacteria and their excellent potential for modification, has emerged as a promising strategy for tumor treatment (Feng et al. 2023; Gurbatri et al. 2022; Kwon et al. 2024). The tumor's disordered vascular system, along with its hypoxic, acidic, and nutrient-rich microenvironment, facilitates the selective colonization of bacteria (Van Mellaert et al. 2006; Xie et al. 2022; Yu et al. 2012). Once inside the tumor, the immunosuppressive microenvironment enables bacteria to accumulate and proliferate (Chandra et al. 2017; Pawelek et al. 1997; Sznol et al. 2000). The inward-to-outward anti-tumor effects induced by bacteria-based tumor therapy can effectively address the limitations of traditional tumor treatments such as non-targeted distribution and limited tumor penetration (Jain 1998; Minchinton and Tannock 2006; Zinger et al. 2019). To enhance the efficacy of local tumor treatment while minimizing the risk of systemic toxicity, bacteria-based tumor therapy must be controlled. Precise regulation of the timing and location of bacterial therapeutic effects is critical for optimizing drug accumulation, resource utilization, and minimizing damage to normal tissues (Zhou et al. 2018).

To date, several techniques have been developed to induce bacterial gene expression (Fig. 1). Various prokaryotic expression regulatory elements have been integrated with exogenous genes to enhance the capacity of engineered bacteria to respond to both the internal and external signals (Cubillos-Ruiz et al. 2021; Omer et al. 2022). Furthermore, the combination of bacteria with nanomedicine offers complementary advantages, as the remarkable optical, magnetic, and acoustic properties of nanomaterials

✉ Pengying Wu
wutdyy@163.com

✉ Lijun Yuan
yuanlj@fmmu.edu.cn

Zhaoyou Liu
liuzhaoyoufmmu@yeah.net

[1] Department of Ultrasound Medicine, The Second Affiliated Hospital of Air Force Medical University, No.569 Xinsi Road, Xi'an 710038, Shaanxi, China

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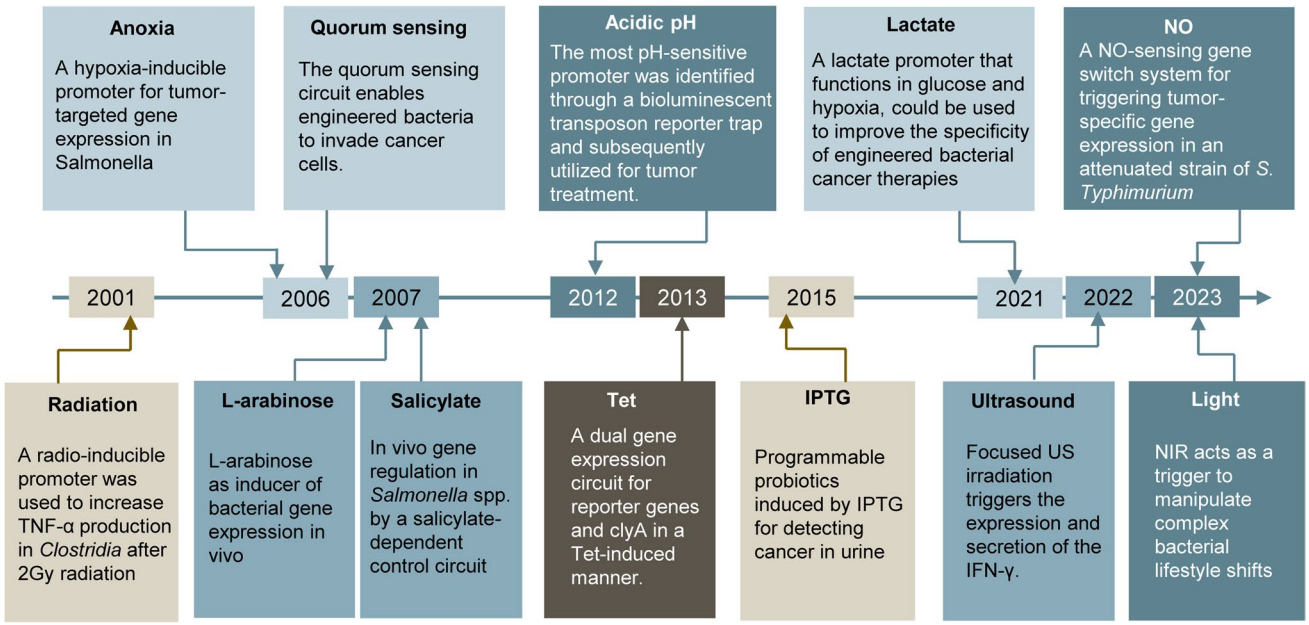

Fig. 1 The history of these techniques for regulating bacterial gene expression in bacteria-based tumor therapy

significantly enhance the effectiveness and controllability of bacteria-based tumor therapy (Lou et al. 2021; Sun et al. 2023; Zhou et al. 2024). These technologies can be utilized to manipulate bacterial targeting and colonization, interact with the dynamically changing environment, enable drug delivery within the appropriate therapeutic window, and facilitate post-treatment self-clearance, thereby achieving a balance of specificity, efficacy, and safety in the precision treatment of tumors.

This review is to summarize the engineering and modification of bacteria based on their tumor-targeting and colonization properties to enhance their anti-tumor effects through advanced genetic engineering and nanomedicine. Application of genetically engineered bacteria or bacteria-nanomaterial nanobiohybrids in precise tumor therapy are discussed. Additionally, the challenges related to quality control and regulation of bacterial therapy are summarized to provide insights for clinical translation (Fig. 2).

Bacteria target the TME and colonize

Tumors possess a microenvironment that is markedly different from that of most normal tissues (Jin and Jin 2020), characterized by hypoxia, acidity, and nutrient-rich conditions, which makes them a natural target for bacteria (Van Mellaert et al. 2006; Xie et al. 2022; Yu et al. 2012). The targeting of tumor tissue by bacteria is guided by specific receptors capable of identifying molecules released by dying tumor cells (Kasinskas and Forbes 2007). Bacteria in the bloodstream infiltrate tumor tissue through the disordered vascular system of the tumor (Leschner et al. 2009), where the lower oxygen levels provide an environment conducive to the proliferation of both obligate and facultative anaerobic bacteria (Duong et al. 2019; Lambin et al. 1998). Notably, a specific species of *Salmonella* has been shown to accumulate preferentially in tumors at ratios exceeding 10,000:1 (Lee et al. 2005). Their robust flagella and small size allow them to penetrate deeply into tumors (Chen et al. 2021a; Dang et al. 2001; Minamino and Imada 2015), while the necrotic areas provide ample nutrients for bacterial growth. Importantly, the immunosuppressive TME facilitates bacterial proliferation within the tumor while protecting it from clearance by the host immune system (Forbes et al. 2003; Zhang and Forbes 2015). Bacteria, due to their inherent immunogenicity, induce a significant influx of neutrophils into the tumor, which subsequently releases neutrophil extracellular traps composed of chromatin fibers and antimicrobial proteins, thereby confining the bacteria to the tumor area (Branzk et al. 2014; Leschner et al. 2009; Westphal et al. 2008).

Furthermore, a special type of bacteria known as magnetotactic bacteria can swim in a directed manner along the magnetic field lines (Faivre and Schuler 2008). This ability is facilitated by their remarkable flagellar propulsion, and a unique cellular organelle called the magnetosome,

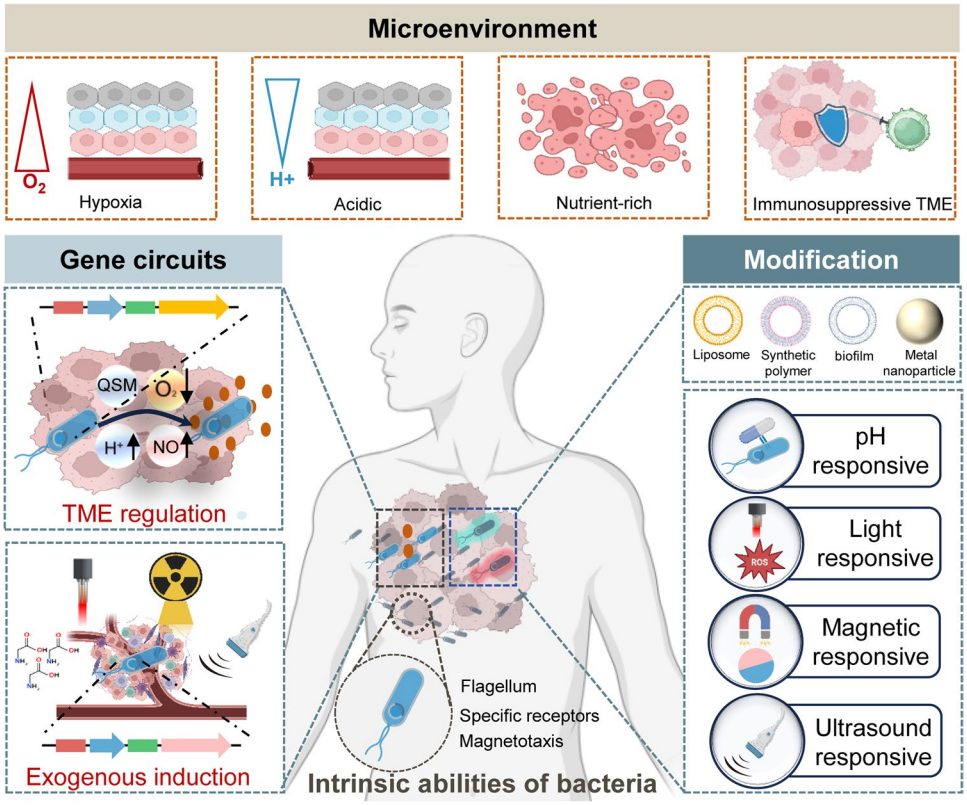

Fig. 2 Overview of bacteria-based tumor therapy. The tumor microenvironment (TME) is suitable for targeting and colonization by certain bacteria. On the other hand, some bacteria intrinsically can target the tumor region. Moreover, bacteria could be engineered with gene circuits and other chemical or nano-modifications to eradicate tumors with more accuracy and efficiency (created with BioRender.com)

which contains nanoscale magnetic iron mineral crystals that impart magnetic properties to the bacteria and function as a "biological compass" guiding their movement (Uebe and Schuler 2016). Driven by external magnetic fields, magnetotactic bacteria can navigate toward tumor sites (Kotakadi et al. 2022). Various magnetic field driving approaches have been developed, including rotating magnetic fields (Wu et al. 2022b), gradient magnetic fields, and oscillating magnetic fields. Due to the flexibility of magnetic fields, appropriate magnetic driving schemes can enhance bacterial infiltration within tumors (Gwisai et al. 2022; Mirkhani et al. 2024).

Internal signals induce bacterial gene expression

Based on the characteristics of bacteria that target and colonize the TME, the features of TME can be used to induce bacterial gene expression, thereby achieving drug enrichment and minimizing off-target effects. Figure 3 illustrates two strategies for bacterial expression of exogenous target genes (Boe 1996; Ganusov and Brilkov 2002). Internal signals inducing bacterial gene expression are divided into expression induced by TME characteristics and bacterial self-triggering (Table 1).

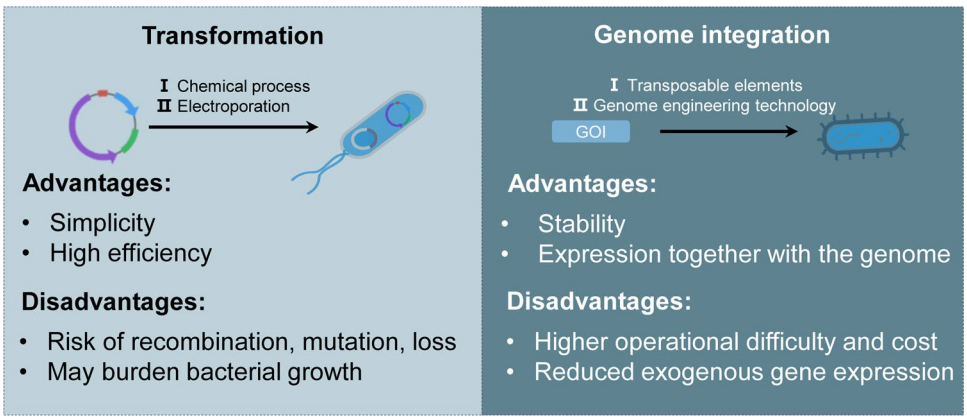

Fig. 3 Comparison of two strategies for bacteria expressing exogenous genes (created with BioRender.com)

Table 1 Internal signals induce bacterial gene expression

Induction signals	Species	Cancer	Working mechanism	Reference
Anoxia	Attenuated *S. typhimurium* strain (JRG4401)	4T1 breast tumors	Bacterial expression of the cytotoxic protein (HlyE) gene in hypoxic regions of mouse mammary tumors under the regulation of a highly hypoxia-inducible promoter (FF + 20)	Ryan et al. 2009
	VNP20009	U87mg glioma	Hypoxia-inducible promoter pflE regulates the expression of wild-type tumor suppressor proteins p53 and Azurin in glioma-hypoxic regions	Mehta et al. 2017
	Salmonella Typhimurium strain YB1	MDA-MB-231 breast tumors	Placing the bacterial essential gene asd under the regulation of the hypoxia-inducible initiator PpepT creates a "specialized" anaerobic *Salmonella typhimurium* strain to target tumor suppression	Yu et al. 2012
	E. coli Nissle 1917	B16F10 melanoma	Diadenylate cyclase (DacA), which generates the STING activator cyclic di-nucleotides, was placed under the regulation of the hypoxia-inducible promoter PfnrS	Leventhal et al. 2020
	E. coli BW25133	spontaneous mouse mammary tumor	A synthetic hypoxia-inducible promoter, nirB, preferentially and selectively induces cardiac peptide expression under hypoxic conditions for tumor suppression	Samadi et al. 2021
Acidic pH	*Salmonella Typhimurium* strain SB300A1	B16F10 melanoma, HCT116 colon cancer	Regulation of Shiga toxin 2 expression within the tumor microenvironment using the screened acidic pH-responsive promoter STM1787	Flentie et al. 2012
	E. coli MG1655	CT26 colon cancer	Cytolysin A (ClyA) protein-coupled to a green fluorescent protein (GFP) placed under the regulation of an acid-sensitive promoter (adiA) for therapeutic visualization	Qin et al. 2022
Lactate	*E. coli* Nissle 1917	SW480 colorectal cancer cell	A lactate promoter that functions in glucose and hypoxia could be used to improve the specificity of engineered bacterial cancer therapies	Zúñiga et al. 2021
		CT26 colon cancer	Integrated bacterial growth with genetic circuits that sense lactate can enhance the tropism of engineered bacteria, thereby regulating the expression of essential genes	Chien et al. 2022
NO	ΔppGpp *S. typhimurium*	/	Elevated NO at the tumor site binds NorR and recruits σ54, causing the expression of the DNA recombinase FimE. This catalyzes the permanent inversion of the DNA switch fimS, leading to sustainable expression of GOI	Qin et al. 2023
Self-triggering	*VNP200010*	4T1 breast tumors	*Salmonella* carrying the QS trigger system successfully initiates protein production inside tumors but not in healthy tissues	Swofford et al. 2015
	E. coli Nissle 1917	A20 lymphoma CT26 colon cancer	Bacteria are utilized as a delivery system for nano-antibodies and as time increases and the bacterial count reaches a lysis threshold, bacterial lysis releases the therapeutic proteins	Gurbatri et al. 2020
	VNP20009 with an additional deletion (Δasd)	4T1 breast tumors	*Salmonella* carrying gene circuits autonomously triggers lysis after invading cancer cells, thereby releasing protein drugs	Raman et al. 2021

TME regulates bacterial gene expression

Hypoxia-inducible bacterial expression systems primarily rely on the oxygen transcription factor fumarate and nitrate reduction regulatory protein (FNR) naturally present in *Salmonella* (Arrach et al. 2008; Leschner et al. 2012). This system functions through the binding and dissociation of FNR with $[4Fe\text{-}4S]^{2+}$. In hypoxic environments, FNR binds $[4Fe\text{-}4S]^{2+}$ to form a transcriptionally active homodimer that binds to specific DNA sequences in promoters, recruiting RNA polymerase to transcribe downstream genes (Chien et al. 2022; Ryan et al. 2009). Commonly used hypoxia-inducible promoters include pflE, ansB (Arrach et al. 2008), pepT (Strauch et al. 1985), as well as synthetic promoters like FF + 20 (Ryan et al. 2009), HIP-1 (Mengesha et al. 2006), and nirB (Nasr and Akbari Eidgahi 2014). An engineered hypoxia-inducible *Salmonella* strain placing the cytotoxic protein hemolysin E (HlyE) gene under the regulation of the hypoxia-inducible promoter FF + 20, can secrete HlyE in the hypoxic TME to kill tumor cells (Ryan et al. 2009). This strategy can also confine bacterial survival to the tumor, reducing systemic toxicity. The asd gene, which encodes a crucial enzyme involved in the synthesis of the bacterial cell wall, is regulated by a hypoxic promoter. Additionally, the asd gene is knocked out of the bacterium, ensuring it cannot survive in normal tissues (Yu et al. 2012).

The acidic microenvironment of tumors is another important feature. Indeed, due to the Warburg effect, even in an aerobic environment, the uptake of glucose by tumor cells is increased. High levels of aerobic glycolysis and glutaminolysis release large amounts of H^+ and lactate into the extracellular space, subsequently releasing them into the TME, maintaining a pH of 5.8–6.6 (Huber et al. 2017; Zhang et al. 2023). Exploiting this, Flentie et al. identified a tumor-specific acidic microenvironment inducible promoter STM1787 in *Salmonella* using a bioluminescent transposon reporter gene; 8 h after exposure to the tumor acidic microenvironment, gene expression increased 90-fold (Flentie et al. 2012). Additionally, acid-induced promoters adiA and Pasr from *E. coli* responsible for expressing arginine decarboxylase and acid shock RNA, respectively, were identified (Mallick and Das 2023; Qin et al. 2022; Stirling et al. 2020). By linking the cytolysin A (ClyA) with a green fluorescent protein (GFP) under the regulation of the acid-sensitive promoter adiA, visual therapeutics can be effectively achieved (Qin et al. 2022). Lactate in the TME is also used in gene expression systems based on the native lldPRD operon, in which the LldR repressor dimer further inhibits gene expression unless bound to lactate (Aguilera et al. 2008). The tropism of engineered bacteria can be enhanced by integrating bacterial growth with genetic circuits that sense oxygen, pH, or lactate, thereby regulating the expression of essential genes. Bacteria engineered with pH or oxygen sensors demonstrated preferential growth under physiologically relevant acidic or hypoxic conditions (Chien et al. 2022).

Salmonella enterica subsp. *enterica* serovar (*S. typhimurium*) colonizing tumor induces upregulation of inducible nitric oxide synthase (iNOS), leading to a 1000-fold increase in NO production at micromolar levels within the tumor site (Barak et al. 2010; Cinelli et al. 2020; Zheng et al. 2017). This enables the use of NO as an internal signal to induce bacterial gene expression. The NO-inducible system is based on NorR, a σ^{54}-dependent regulatory protein in *E. coli*, which senses and binds NO in the presence of NO, inducing σ^{54} binding and activating the Pnorv promoter (Tucker et al. 2008, 2010). To achieve permanent activation of NO-induced gene expression, the Pnorv promoter was utilized to regulate the expression of the DNA recombinase FimE, leading to permanent inversion of fimS and, thus, sustained expression of the target gene (Qin et al. 2023).

Self-triggering

Bacterial gene expression can also be regulated by communication between bacteria, which relies on quorum-sensing molecules (QSMs). QSMs are secreted externally, accumulate with increasing community density, and, after reaching a certain threshold, bind to receptors to regulate gene expression (Mukherjee and Bassler 2019). Bacterial QSMs include acyl homoserine lactone (AHL or AI-1), auto-inducible peptide (AIP), and autoinducer-2 (AI-2) (Zeng et al. 2023). The AHL is mainly secreted by Gram-negative bacteria, and the LuxI promoter regulates AHL production, followed by AHL binding to LuxR to turn on downstream gene expression (Din et al. 2016). The quorum-sensing phenomenon of bacteria can be utilized to design many interesting genetic circuits, such as the quorum-sensing lysis circuit, which provides new ideas for drug delivery (Chowdhury et al. 2019; Gurbatri et al. 2020; Raman et al. 2021). By expressing the lytic enzyme gene E of phage FX174 under the control of the LuxI promoter, when the bacteria are enriched in the tumor, and the AHL reaches a certain threshold, bacterial lysis is initiated, and therapeutic proteins pre-expressed constitutively in vivo are released (Din et al. 2016). This strategy enables the delivery of more nanobodies within the tumor, including anti-CD47 nanobodies (Chowdhury et al. 2019), anti-PD-L1/PD-1, and anti-CTLA4 nanobodies (Gurbatri et al. 2020).

External signals induce bacterial gene expression

In addition to TME-induced triggering, synthetic biology can also be used to design sophisticated, intelligent gene circuits in response to external signals. Compared with

TME-induced triggering, external signal triggering has better initiative and controllability. Depending on the external signals, they are categorized as follows (Fig. 4). The application of external signals to control bacterial expression of exogenous genes to achieve anti-tumor effects is summarized and displayed in Table 2.

Chemical inducers

Chemical induction was first applied to regulate bacterial gene expression, with common chemical inducers including isopropyl β-D-thiogalactoside (IPTG) (Danino et al. 2015; Fan et al. 2019; Harimoto et al. 2022), L-arabinose (Dai et al. 2013; Loessner et al. 2007; Nguyen et al. 2010; Stritzker et al. 2007; Wen et al. 2018), tetracycline (Jiang et al. 2013), and salicylic acid salts (Royo et al. 2007). The regulation of bacterial expression by chemical inducers is mostly based on the operon model, which is a directed DNA sequence that acts as a gene regulator to regulate bacterial gene expression (English et al. 2021).

IPTG is used as an inducer to bind the lac repressor protein in the lactose operon (Fig. 4A1), causing it to dissociate from the DNA-binding domain, thus turning on downstream gene expression (Lewis et al. 1996; Romanuka et al. 2023). Integration of the LuxCDABE gene cassette into an IPTG-induced expression vector and induction of fluorescent gene expression by intravenous injection of IPTG resulted in the detection of bacterial fluorescent signals in approximately 88% of metastases in a mouse colorectal cancer metastasis model (Danino et al. 2015). The expression of downstream target genes of the operon is closely related to the concentration of chemical inducers. Therefore, a tunable microbial surface engineering strategy based on IPTG is utilized to dynamically control the synthesis of bacterial capsular polysaccharides (CAP) to enhance bacterial interactions with their surrounding environment (Harimoto et al. 2022).

The L-arabinose-inducible pBAD promoter is derived from the arabinose operon. When L-arabinose is present, the AraC protein opens the gyratory structure. It acts as an activator to activate target gene transcription (Fig. 4A2). The induction of instantaneous bacterial bioluminescence by L-arabinose was sufficient to localize the bacteria within the tumor but freed the bacteria from the burden of continuous expression of luciferase or fluorescent proteins (Loessner et al. 2007). The induction/repression ratio using the L-arabinose control system can be up to 1200-fold (Guzman et al. 1995). However, recombinant proteins based on the pBAD vector system are expressed at lower levels, and some strains can catabolize L-arabinose.

The Tet-off and Tet-on, bacterial expression regulation systems, induced by tetracycline work in opposite ways. In the Tet-off system, the tTA transcription factor cannot bind to the DNA sequence of the promoter region in the presence of tetracycline, resulting in gene expression inhibition. Conversely, in the Tet-on system, the rtTA transcription factor binds to the DNA sequence and activates downstream gene expression (Fig. 4A3) (Gossen and Bujard 1992; Gossen et al. 1995). Compared to L-arabinose and IPTG, the Tet-on system induced by tetracycline has a lower baseline expression level of target genes while maintaining a higher induction efficiency. By constructing a bidirectional gene circuit, co-expression of the therapeutic gene and reporter gene can be achieved under the induction of doxycycline (or tetracycline) to meet therapeutic needs (Jiang et al. 2013).

Salicylate-induced systems are usually cascade regulatory circuits that achieve amplified expression of specific genes under the control of salicylic acid (Cebolla et al. 2001). The Pm promoter in the TOL operon from *Pseudomonas putida* catabolism of toluene and xylene can be activated by the xyIS protein, and the transcription of the xyIS gene is controlled by another regulator, XyIR, and the strength of Pm increases as more xyIS protein is expressed. The mutant of xyIS, xyIS2, can be activated by salicylic acid, thus placing the expression of xyIS2 under the control of the Psal promoter and its cognate regulator NahR, which is activated in the presence of salicylic acid to initiate the expression and activation of the xyIS2 protein. These two effects act together on the Pm promoter. This cascade reaction causes a massive amplification of the target gene (Fig. 4A). Placing cytosine deaminase (CD) in a regulatory circuit activated by acetylsalicylic acid (ASA) led to a 20- to 150-fold ex vivo induction and induction with ASA before 5-fluorocytosine administration significantly reduced tumor growth (Royo et al. 2007).

Light

Optogenetics integrates optics and genetics to control biological processes with a high degree of spatiotemporal precision using genetically encoded light-responsive proteins in response to light stimulus (Emiliani et al. 2022). In recent years, driven by synthetic biology, optogenetics has been introduced into bacteria to enable rapid, dynamic, and reversible regulation of bacterial gene expression (Lindner and Diepold 2022). Introducing genetically encoded light-responsive proteins into bacteria to precisely regulate bacterial expression in tumors by using light as an external trigger, the advantages brought by light as a signal are incomparable to chemical induction, even though changes in photoreceptor intensity induced by altering light intensity are similar to those by altering the concentration of a chemical inducer. The fact that light is not only controllable but also reversible and non-invasively transmits signals and that it is possible to restrict the activation region to a specific

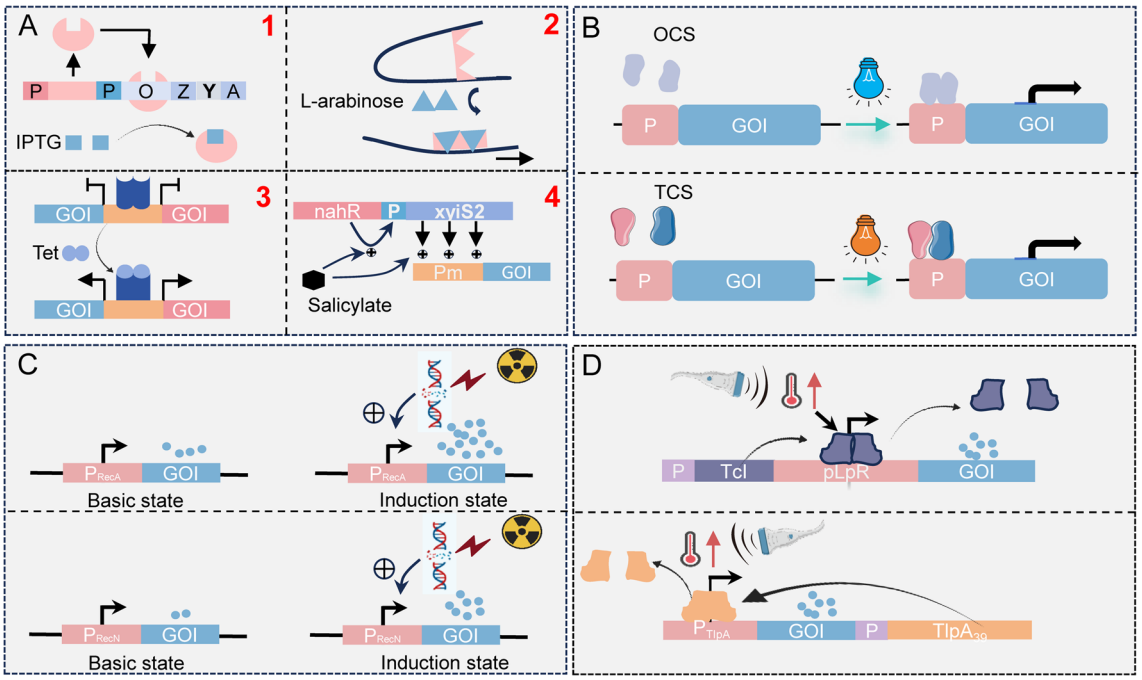

Fig. 4 External signals induce bacterial gene expression. **A** Four most commonly used chemical inducers to induce bacterial gene expression. **B** Optogenetic systems for inducing gene expression in bacteria. OCS, one-component systems; TCS, two-component systems. **C** Radiation-induced bacterial gene expression based on promoters RecN or RecA. **D** Induction of bacterial gene expression based on the thermal effect of ultrasound

place independent of diffusion greatly improves spatiotemporal controllability (Deisseroth 2011).

Optogenetic systems used to regulate gene expression in engineered bacteria can usually be categorized into the one-component system (OCS) and two-component system (TCS) (Lindner and Diepold 2022). OCS and TCS are distinguished based on the different mediators responding to light and inducing transcription. The OCS contains only a single protein, which assumes the role of a photoreceptor and is an inducer of transcription. The EL222 protein, first discovered in the marine bacterium *Erythrobacter litoralis*, is by far the most commonly used blue light-responsive transcription factor in the OCS (Motta-Mena et al. 2014). The TCS consists of a light-responsive kinase and a homologous transcriptional response regulator, and the YF1/FixJ-based pDawn system is one of the most common TCS in use today. OCS is simpler because it uses a single light-responsive protein to induce transcription and does not rely on the fully balanced expression of a second component. Its smaller size saves space for therapeutic genes, allowing for more personalized therapeutic delivery (Hoffman et al. 2022). OCS also have limitations; almost all of them rely on poorly penetrating blue light, but a recently discovered OCS that responds to near-infrared light, called iLight, is very promising (Kaberniuk et al. 2021).

TCS has a wide spectrum of responses, with the optogenetic tools CcaS/CcaR based on CBCRs activating downstream gene expression under 535 nm green light stimulation and inhibiting gene expression under 672 nm red light (Tabor et al. 2011); UirS/ UirR, enabling gene expression initiation in response to ultraviolet light and gene expression inhibition under 535 nm green light (Ramakrishnan and Tabor 2016). BphPs-based optogenetic tools have a faster response rate because BphP1 is activated under near-infrared light and activates the transcription of downstream genes by combining with the transcriptional repressor PpsR2, which does not require the signaling process of the phosphoryl group (Ong et al. 2018). BphS, a mutant of the bacterial photosensitive pigment BphG, is triggered by NIR light, leading to an elevation of c-di-GMP, thereby enhancing biofilm formation, preventing bacterial elimination by neutrophil-mediated killing effects and increasing the illumination power density of NIR triggering bacterial lysis and release of the antitumor toxin HlyE to induce tumor necrosis (Fu et al. 2023).

Since TCS is based on the interaction of two proteins, this has to take into account the sensitivity and balance of the host system to the expression of these two proteins. In addition, in non-blue light-activated systems, the hosts often do not endogenously express the chromophores required by the systems, such as biliverdin, phycocyanin, etc.. So they

Table 2 External signals induce bacterial gene expression

External signals		Species	Cancer	Working mechanism	Reference
Chemical inducers	IPTG	*E. coli* Nissle 1917	MC26 colon cancer	Integrate the LuxCDABE gene cassette into the IPTG-induced expression vector and induce fluorescent gene expression by intravenous injection of IPTG	Danino et al. 2015
			CT26 colon cancer	IPTG induced the expression of NDH-2 enzyme, which could increase the production of H_2O_2 at tumor sites. On this basis, magnetic Fe3O4 nanoparticles were covalently attached to bacteria to act as catalysts for Fenton-like reactions to convert H2O2 into toxic hydroxyl radicals (-OH) for tumor therapy	Fan et al. 2019
			4T1 breast tumors, CT26 colon cancer	IPTG induction enhances the synthesis of bacterial capsular polysaccharide (CAP) to improve in vivo microbial delivery, increasing the maximum tolerated dose tenfold and enhancing the anti-tumor efficacy in a mouse cancer model	Harimoto et al. 2022
	L-arabinose	*E. coli* Nissle 1917	4T1 breast tumors	The target gene was placed downstream of the pBAD promoter, and gene expression was selectively induced in vivo using non-toxic L-arabinose in mouse tumor tissues	Stritzker et al. 2007
		VNP20009	Human LS174T colon adenocarcinoma	The combination of the L-arabinose induction system and the bacterial communication system overcame the drawbacks of the induction system alone, prolonged the protein release time, and increased the protein yield	Dai et al. 2013
		ΔppGpp *S. typhimurium*	MC26 colon cancer	Under the induction of L-arabinose, the attenuated Salmonella typhimurium strain can express and secrete Vibrio vulnificus flagellin B (FlaB) within tumor tissues. This process leads to the phenotypic and functional activation of intratumoral macrophages, promoting an M1 phenotype while concurrently reducing the M2-like macrophages	Zheng et al. 2017
			CT26 colon cancer	A strain of *S. typhimurium* carrying cytotoxic proteins (cytolysin A) and expressing reporter genes that integrate monitoring and therapeutic functions successfully visualized the therapeutic process of these engineered bacteria in mice	Nguyen et al. 2010
			Glioma	L-arabinose-induced release of tissue inhibitor of matrix metalloproteinase-2 (TIMP2)	Wen et al. 2018
		SL7207 *S. typhimurium*	CT26 colon cancer	L-arabinose-induced bioluminescence enables noninvasive in vivo imaging, and the lysate gene E of phage FX174, placed under the control of PBAD for bacterial lysis induced by arabinose, can be engineered to release therapeutic loads	Loessner et al. 2007
	Tet	ΔppGpp *S. typhimurium*	CT26 colon cancer	Construction of a dual gene expression circuit for bilateral expression of bioluminescent reporter genes and clya in a tetracycline-induced manner	Jiang et al. 2013
	Salicylate	*Salmonella enterica* aroA	Fibrosarcoma	Acetylsalicylic acid (ASA) regulates cytosine deaminase (CD) cascade expression, and ASA induction prior to 5-fluorocytosine administration significantly reduces tumor growth	Royo et al. 2007
Light	NIR	*P. aeruginosa* strain ExoST	A549	NIR triggers complex bacterial lifestyle shifts through the optogenetic tool BphS for bacterial adhesion, colonization, and drug release	Fu et al. 2023

Table 2 (continued)

External signals	Species	Cancer	Working mechanism	Reference
Radiation	2gy γ radiation	*VNP20009*	4T1 breast tumors	Radiation-induced RecA promoter controls secretion of mouse TNF-related apoptosis-inducing ligand (TRAIL)	Ganai et al. 2009
		Attenuated *Salmonella* strain (KST0650)	CT26 colon cancer	Expression of the intracellular pro-apoptotic protein sATF6 is controlled by the radiation-induced recN promoter, and sATF6 is expressed and secreted under γ radiation	Gao et al. 2020
Ultrasound	Focused ultrasound	*E. coli* Nissle 1917	A20 lymphoma	Alteration of temperature-sensitive repressor protein conformation by the thermal effect of focused ultrasound activates the PLPR promoter to express immune checkpoint inhibitors against CTLA-4 and PDL1	Abedi et al. 2022
		E. coli MG1655	4T1 breast tumors	The PL and PR promoters tandemly regulate the IFN-γ gene, and focused ultrasound irradiation and heating trigger the expression and secretion of the IFN-γ gene	Chen et al. 2022
			4T1 breast tumors	Introducing an acoustic reporter gene (ARG1) into bacteria that produce gas vesicles (GVs), the IFN-γ gene is under the tandem regulation of the PL and PR promoters, and during ultrasound irradiation, the GVs are used as an ultrasound contrast agent for imaging, while IFN-γ is secreted for tumor treatment	Yang et al. 2024
		VNP20009	B16F10 melanoma	The temperature-sensitive transcriptional repressor TlpA39-based gene expression system (SINGER) expresses and secretes PD-L1nb and azurin upon focused ultrasound irradiation and heating	Gao et al. 2024

have to be artificially added or synthesized by the hosts, which will undoubtedly complicate the application of TCS. Nonetheless, TCS has developed into a relatively mature application system, and due to its robustness in regulating proteins, flexibility in module selection, and wide range of response spectra, TCS has unique advantages in regulating bacterial protein expression.

However, it must be recognized that the poor penetration of light into deep tissues has hindered the development of light-induced expression systems. For tumors located deep in the tissue, how to ensure that the light activates enough bacteria to produce effective therapeutic effects is an issue that has to be considered for light-induced expression systems. For specific tumors, such as those in the digestive tract, light-induced expression systems may be able to unite with gastrointestinal endoscope to increase the percentage of activated bacteria to enhance therapeutic efficacy.

Radiation

The current systems for controlling bacterial expression by radiation are all based on the RecA or RecN promoters in the SOS repair system of *Clostridium difficile*. Various physical, chemical, and biological effects caused by radiation result in DNA breaks and activation of RecA and RecN proteins, which play an important role in repairing damaged DNA, and it is in this process that the RecA and RecN promoters respond to radiation and initiate genes expression (Nuyts et al. 2001c). This induced expression is reproducible and does not differ between aerobic and anaerobic conditions, which is important for using radiation-induced expression promoters in anaerobic bacteria.

Radiation can efficiently penetrate tissues with little diffusion effect, and the release of the therapeutic load from the radiation-responsive promoters RecA or RecN can be precisely controlled in the tumor and avoid systemic toxicity. The reporter gene LacZ was placed under a radiation-responsive promoter, and after 2 Gy of irradiation, β-galactosidase activity was significantly increased by 20–30%, and expression could be induced repeatedly by repeated administration of radiation (Nuyts et al. 2001c). To improve the induction ratio of the radial expression system, after the addition of Cheo cassettes, the RecA promoter induction ratio can be increased tenfold (Nuyts et al. 2001a). Engineering of *Clostridium difficile* to express TNF-α gene under the regulation of the radiation-responsive promoter RecA successfully expressed TNF-α in situ under 2 Gy of radiation and prolonged the expression of TNF-α at the tumor site by repeated induction, thus enhancing the anti-tumor effect (Nuyts et al. 2001b).

Another radiation-responsive promoter RecN has a much lower basal activity than RecA although the induction ratio is not as high as RecA, which means that RecN is more

suitable for expressing proteins with toxicity because it hardly causes leakage (Fu et al. 2023). For example, the intracellular pro-apoptotic protein sATF6, under the regulation of RecN, can control the killing effect at the radiation-exposed site of the tumor while avoiding damage to healthy tissues (Gao et al. 2020).

In conclusion, bacterial expression systems based on the radiation-responsive promoters RecA and RecN show great potential for precise in situ delivery of drugs to tumors. However, radiation is potentially lethal to tissues, so it is important to make an informed choice between the appropriate dose of radiation and the best therapeutic effect that can be elicited and to select bacteria that are best suited to carry the radiation-responsive expression systems to achieve greater effects, including sensitization to radiation therapy, synergistic therapeutic effects, and protection of normal tissues from radiation damage.

Ultrasound

Ultrasound has a natural advantage as an external signal with its non-invasiveness, high penetrability, low attenuation, non-ionizing radiation, and excellent spatial accuracy (Peek et al. 2015; Qian et al. 2017). Currently, ultrasound regulation of expression in engineered bacteria relies mainly on thermal effect and temperature-responsive components, such as RNA thermometers (RNATs) (Neupert and Bock 2009), the PL&PR promoters, and various temperature-sensitive mutants of phage lambda protein "cI" (Valdez-Cruz et al. 2010). Temperature-sensitive mutants of phage lambda protein "cI" exist as monomers at low temperatures and block the PL and PR promoters, then dimerize when they reach their response temperature and release the promoter region, restoring transcriptional activity. In contrast, the temperature-controlled expression mediated by RNATs is at the translational level, where RNATs form stem-loops at body temperature that clamp the ribosome-binding sequences or translation initiation codons tightly by base pairing and only unclamp them when their response temperature is reached to allow ribosome binding, thus facilitating translation (Neupert and Bock 2009; Righetti and Narberhaus 2014).

The system for controlling bacterial expression by the thermal effect of ultrasound benefits from the remote tunability of ultrasound, and the selection of appropriate ultrasound parameters allows the temperature of the target zone to be maintained in the optimal range for initiating gene expression. A temperature-controlled gene expression circuit carrying interferon-γ (IFN-γ) constructed from the pR-pL promoter and cI protein was transferred into an ultrasound-responsive bacterium (URB), and IFN-γ was linked to the N-terminal end of the OmpA secretion signal peptide, and the temperature of the target zone was maintained at 45 ℃ under pulsed ultrasound irradiation to successfully secrete IFN-γ, the percentage of M1-type macrophages, $CD4^+$ T cells, $CD8^+$ T cells, and memory T cells increased significantly, and the immune response of the mice was successfully activated to inhibit tumor growth (Chen et al. 2022). In the clinical setting, where tumors are often treated over several weeks, a switch that is activated only upon ultrasound irradiation is not feasible, necessitating the introduction of a sustained expression function. Serine integrase Bxb1 is a class of enzymes that target DNA sequences at sites known as attP (attachment site of phage) and attB (attachment site of bacterial) and utilize these sites to mediate DNA sequence inversion (Xu et al. 2013). By placing Bxb1 under the regulation of the pR-pL promoter and the cI protein, Bxb1 was expressed when the tissue was heated by focused ultrasound and mediated the inversion of the otherwise inverted constitutive promoter, P7, resulting in sustained expression even when ultrasound was withdrawn (Abedi et al. 2022). Considering that 42–45 °C may cause thermal damage to normal tissues, an ideal system should be activatable in the range of 39–40 °C, with less leakage and a higher induction ratio. Recently, an ultrasound-responsive gene expression system based on the temperature-sensitive transcriptional repressor TlpA39 has been developed, and *VNP20009* carrying this system can precisely initiate the efficient expression of the therapeutic gene at 39 °C during ultrasound-mediated local warming. Additionally, the combination of the anticancer protein Azurin protein with the immune checkpoint inhibitor PD-L1nb has been shown to induce apoptosis in tumor cells while activating the host's immune system, leading to a dual anticancer effect (Gao et al. 2024).

Bacteria and nanomedicine unite in cancer therapy

Relying solely on genetically engineered bacteria has limitations, such as rapid clearance of bacteria by the immune system, inadequate drug loading, and difficulty in drug release, which may contribute to poor tumor treatment effects (Liu et al. 2022). With the development of synthetic biology and nanomedicine, it is possible to use a variety of materials such as synthetic polymers (Jackson et al. 2017), liposomes (Gao et al. 2014), and biofilms (Aryal et al. 2013) to modify bacteria, to realize the complementary advantages, and to further improve the safety, precision, and effectiveness of engineered bacteria as well as to improve the shortcomings of traditional nanomaterials, such as poor targeting and high systemic toxicity (Zhou et al. 2024).

Materials camouflage bacteria for tumor targeting and proliferation

Various biological, chemical, and physical strategies have been developed to modify bacteria to avoid phagocytosis and clearance by the reticuloendothelial system (Fig. 5A) (Liu et al. 2022), thus prolonging the duration in the host and enabling tumor targeting and proliferation.

Several biofilms, including macrophages (Thamphiwatana et al. 2017), erythrocytes (Liu et al. 2022), platelets (Xu et al. 2018), neutrophils (Zhang et al. 2018), and cancer cells (Wang et al. 2018) have been used for camouflage. A type of cell membrane encapsulated bacteria formed by encapsulating *Porphyromonas gingivalis* through erythrocyte membranes had virtually no effect on the intrinsic bioactivities of the bacteria. It reduced bacterial-induced inflammatory responses and side effects, prolonging the persistence of the bacteria in the body (Chen et al. 2021b). Biofilm camouflage of *ECN* (Cao et al. 2019) and *Listeria monocytogenes* (Liu et al. 2022) has also been accomplished for tumor therapy. *VNP20009* was encapsulated in macrophages by biomodification to form a "Trojan horse," macrophages were rapidly enriched at the tumor site due to tumor chemotaxis. Then, intracellular bacterial proliferation led to macrophage rupture. This delayed-release strategy avoided premature bacterial clearance and completed bacterial enrichment at the tumor site (Wu et al. 2022a).

Chemical modifications typically utilize chemical reactions, including dopamine co-deposition, chemical bonding covalent linkage, Michael addition, and intermolecular disulfide exchange to couple materials to the bacterial surface (Zhou et al. 2024). Simple co-deposition of *EcN* with dopamine and simultaneous coupling of a variety of functional small molecules and polymers under biocompatible conditions results in multifunctional coatings that increase the bioavailability of the bacteria by more than 30-fold (Pan et al. 2021a). To further enhance the tumor-targeting property, the tumor-targeting peptide RGD was coupled to the carboxyl group on the surface of *Magnetospirillum magneticum* AMB-1 by chemical modification to construct the tumor-targeting strain AMB-RGD (Wang et al. 2022).

Physical modifications included electrostatic interactions (Zhao et al. 2022), electroporation (Zoaby et al. 2017), and membrane coating (Wu et al. 2019). Therapeutic agents can be transported by bacteria, which may either be affixed to the external surface of engineered bacteria or loaded into the bacterial body. Electrostatic interactions play a crucial role in enhancing the versatility of nanobiohybrid construction. This is primarily due to the fact that the surface charges of both bacterial cells and various nanomaterials can be readily adjusted. A straightforward electrostatic adsorption method combines black phosphorus quantum dots (BPQD) with *E. coli* to create a novel hybrid system, referred to as BPQD/E coli (BE), designed to enhance photothermal

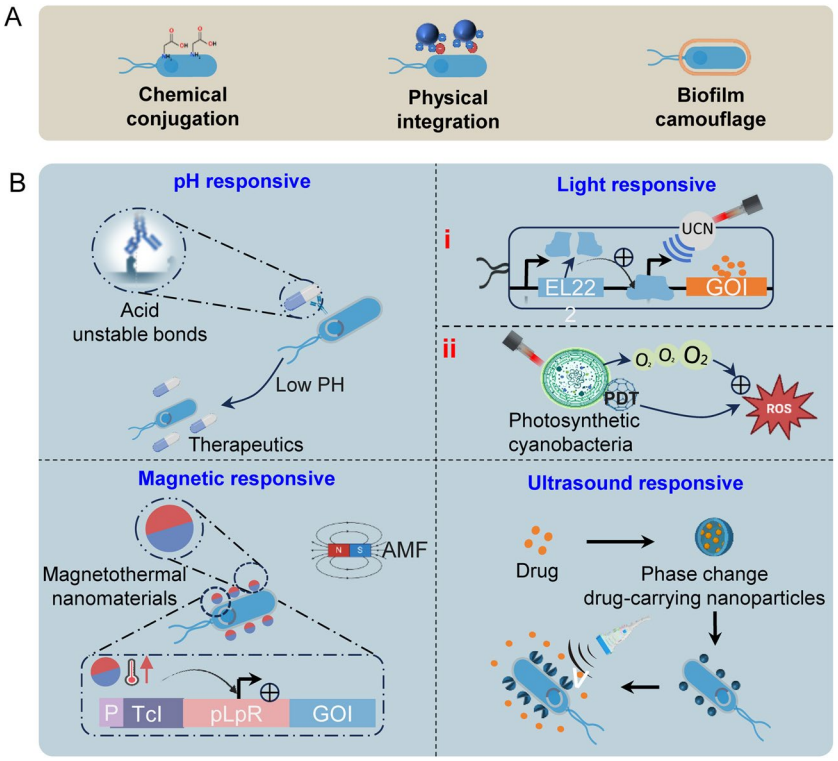

Fig. 5 Bacteria and nanomedicine unite in cancer therapy. **A** Integration strategies for bacteria and nanomedicine. **B** Nanomedicine-enhanced bacteria exert synergistic anti-tumor effects (created with BioRender.com)

therapy (PTT) for hypoxic tumors. The BE system effectively targets hypoxic tumors and facilitates the mediation of PTT achieving significant radiotherapy efficacy in response to lower doses of radiation, effectively and specifically damaging hypoxic tumor tissues to suppress tumor growth (Ji et al. 2023). Electroporation is a technique that involves the application of short bursts of electrical pulses to bacterial membranes, leading to the formation of temporary pores. This process effectively enhances the permeability of the membrane, allowing substances to enter the bacterial cells more easily. Through the use of electroporation, the presence of liposomes in 62% of the bacterial samples examined was identified (Zoaby et al. 2017). This demonstrates the effectiveness of electroporation in facilitating the introduction of liposomes into bacterial cells.

Bacterial loading nanomedicines to improve effectiveness and controllability

Bacteria can be greatly enhanced by loading nanomedicines for therapeutic effects; in fact, the ease of modification of nanomaterials opens up great possibilities for material-based bacterial therapies. The utilization of materials to encapsulate bacteria as drug factories for the production of therapeutic agents, along with the integration of nano-photosensitizers or acoustic sensitizers to achieve synergistic therapies, is a promising area of research. Furthermore, nanomaterials, characterized by their exceptional optical, magnetic, and acoustic properties, can precisely control the timing and location of the anti-tumor effects exerted by the material-modified bacteria. (Fig. 5B).

Immobilization of the chemotherapeutic drug Doxorubicin (DOX) onto *ECN* through the acid-unstable bond of cis-aconitic anhydride can respond to the acidic microenvironment of tumors (Xie et al. 2017). Upconversion nanoparticles (UCN) can respond to penetrating near-infrared (NIR) light and emit blue light (Cui et al. 2021), which can help to solve the limitation of OCS relying on poorly penetrating blue light. OCS-carrying *EcN* successfully delivered TNF-α locally to tumor using UCN to convert near-infrared light to blue light and activate the blue light-sensitive EL222 protein in tumor, thereby inhibiting tumor growth (Fig. 5B) (Pan et al. 2021b).

In treating tumors with PDT, the intratumoral hypoxic microenvironment inevitably leads to a significant reduction in the efficacy of type II photosensitizers, which are highly dependent on oxygen. Cyanobacteria can both target the tumor site and continuously produce oxygen by laser irradiation (Fig. 5B). Hence, a novel cyanobacteria-based bioreactor, Cyan@BPNSs, uses inorganic two-dimensional black phosphorus nanosheets (BPNSs) to modify cyanobacteria, significantly enhancing the killing effect of photodynamic therapy on tumor cells (Qi et al. 2021). There are many similar strategies, including the formation of photothermophilic bacteria PTB@ZIF-90/MB by surface biomineralization of palladium nanoparticles in combination with methylene blue (MB)-loaded ZIF-90 (Chen et al. 2020), *Salmonella typhimurium* YB1 in combination with photosensitizing indocyanine green (INPs) nanoparticles (Chen et al. 2019), and Bb@QDs formed by the electrostatic interaction between Bifidobacterium and Ag2S quantum dots (QDs) (Zhao et al. 2022).

Certain magnetic nanomaterials can convert magnetism into heat. Biological hybrid microrobots EcN@MNP with magnetic field, thermal, and hypoxic environment sensing are constructed by coupling nanomaterials with magnetic nanoparticles (MNP) prepared by zinc-iron hybridization with *EcN* (Ma et al. 2023). Moreover, the expression of mCherry was controlled using a temperature-controlled expression circuit as a dual reporter gene for thermal and localization signals for tumor treatment. According to the fluorescent protein imaging feedback, the microrobot combined with the effects of magnetothermal ablation and thermally expressed NDH-2 enzyme (respiratory streptokinase II) efficiently triggered apoptosis of cancer cells under the remote triggering of an alternating magnetic field (AMF).

Nanodroplet-transition microbubbles can be used as contrast agents to provide echo signals for ultrasound (Wang et al. 2012), and the microbubbles swell and collapse under continuous intensity ultrasound radiation. Based on this principle, phase-change drug-carrying nanoparticles were attached to bacteria to achieve precise drug release under ultrasound imaging via sonomechanical effects. An ultrasound-responsive biohybrid robot, SonoBacteriaBot (Du et al. 2023), encapsulated perfluoropentane (PFP) and the chemotherapeutic drug DOX in polylactic acid-glycolic acid (PLGA) to form DOX-PFP-PLGA nanodroplets, which were attached to the surface of *Escherichia coli* MG1655 called DOX-PFP-PLGA@EcM. The SonoBacteriaBot was tracked in real-time by the nanodroplet microbubbles, and under the continuous intensity of ultrasound radiation, the microbubbles expanded and collapsed to release the drug.

Summary and outlook

To date, plenty of intelligent and elegant gene circuits have been developed to precisely trigger anti-tumor effects in response to induced signals. In synergy with nanomedicine, bacteria-nanomaterial nanobiohybrids exert robust and effective multimodal synergistic tumor therapies (Zhou et al. 2024). Numerous experimental results have demonstrated that bacterial therapies under precise control systems successfully inhibited tumor growth and prevented systemic toxicity (Abedi et al. 2022; Chen et al. 2022; Fu et al. 2023). Although recent findings have shown the great potential of

bacterial therapies for oncology, there are still some challenges that need to be solved to improve their therapeutic efficacy and safety.

It is important to note that the immune function of patients with advanced cancers is low. Although *Clostridium* novyi-NT (Janku et al. 2021), *Listeria monocytogenes* (Hassan et al. 2019; Le et al. 2019, 2015), and *Salmonella monocytogenes* (Toso et al. 2002) have been proven to be safe in healthy people, the residual presence of bacteria in the body as well as the retention of virulence of bacteria may be a potential threat to cancer patients. Maintaining the stability and orthogonality of intelligent gene circuits and nano-biomaterials is very crucial and should be tumor specific regarding the varied TME. It is thus necessary to emphasize the temporal and spatial controllability of bacterial therapeutics by selecting the most appropriate control signals to trigger precise drug synthesis or release.

Anyway, bacterial therapy is a promising strategy in tumor treatment. The therapeutic potential of bacteria has been further amplified by synthetic biology and nanomedicine, which have endowed bacteria with more diversified functions in the complex TME. It is believed that with the steady development of synthetic biology and the deeper and deeper exploration of cancer, bacterial therapy will eventually become a powerful weapon for human beings to fight against tumors.

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Declarations

Conflict of interest The authors declare no competing interests.

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