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Bacterial cancer therapy: A turning point for new paradigms

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Cancer treatments have advanced considerably in recent years, appreciably enhancing the quality of life and survival of cancer patients. However, standard cancer treatments still have limitations that must be improved. In recent years, bacteria-based cancer therapy has gained much more attention owing to its unique properties that are unachievable with standard therapeutics. Bacteria species such as *Salmonella*, *Clostridium*, and *Listeria* have been shown to control tumor growth with improved prognosis in experimental animal models and clinical settings.

Keywords: Anticancer; Bacterial-mediated; Tumor targeting; Payload delivery; Engineered bacteria; Tumor microenvironment

Introduction: need for alternative cancer treatments

Cancer is one of the most common causes of human death worldwide. In 2018, 18.1 million new cancer cases and 9.6 million new cancer deaths were reported. According to the American Cancer Society, there will be ~17 million deaths from cancer annually by 2030. Although several therapeutic advances and cancer controls are still emerging, challenges among the tumor resistance to chemo- and radiotherapy need to be revised. Evidence suggests that the tumor microenvironment (TME) plays a crucial part in regulating the initial tumor response to treatments such as chemo-, immune- and radiotherapies, and surgery. Apart from the TME, tumor physiology, including its size, volume, location and metastasis, is another challenge in the treatment. Besides, emerging drug resistance against cancer cells can also occur owing to the poor efficacy in radiotherapy, chemotherapy and immunotherapy, resulting in an uncontrolled tumor, leading to fatality arising during the treatment.[1,2] These

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factors highlight the importance of seeking novel and more-successful therapies.[3,4]

Drug discovery against cancer results in several clinical drugs being produced at various stages of clinical drug development. Site-targeted drug delivery vehicles steer the compounds to their necessary target sites, which are detrimental to cancer cells. Such interesting properties always inspire the study of different delivery particles for cancer therapy.[5] Therapeutic bacteria are one such strategy that can resolve the barriers discussed previously. Bacterial-mediated tumor therapy presents an exciting avenue to treat various cancers. When genetically modified, bacteria can selectively reach the tumor, colonize within the TME, resulting in immune cell infiltration, and deliver payloads in the tumor-site-blocking disease pathways, leading to the complete elimination of the tumor cell population.[6]

Bacterial clearance by immune cells is limited, and available nutrients promote their growth in the TME. When compared with normal tissue, bacteria can harbor tumor sites in a 10 000:1 ratio. The bacterial flagella enable active transmission between cells and penetrate tumor sites far from the vasculature, which is unavailable for passive chemotherapy. This unique phenomenon can help the bacterial targeting of tumor sites after loading them with therapeutic drugs, resulting in high therapeutic efficacy without adverse effects. Apart from delivering chemo drugs, genetically modified bacteria such as *Escherichia coli* and *Staphylococcus aureus* can release cytotoxic proteins such as cytolysin A, α-hemolysin, immunomodulatory proteins, antigens, cytokines and prodrug-cleaving enzymes.[7–9] The fundamental advantages of using modified bacteria in bacterial-mediated cancer therapy are that it effectively reaches the hypoxic TME, populates and penetrates the tissue, and secretes tumor-cytotoxic enzymes such as lipases and proteases[10] as an essential tool for treating cancer. However, owing to the potent challenges against cancer therapy, the innovative attempts to harness the ability of bacteria to cure cancer are evolving. Over the past decades, significant numbers of reports on the utilization of bacterial anticancer therapies have emerged, and the application of bacteria as an anticancer paradigm is discussed in this review (Table 1).

Bacteria as tumoricidal agents

Advances in synthetic biology favor genetically modified bacteria for tumor therapy to overcome the disadvantages of conventional treatments and their pharmacokinetic failures. William B. Coley was the first to report a remarkable experimental success on a bacterial tumoricidal effect to cure patients with deadly cancers.[11] After his findings, researchers discovered that several facultatively anaerobic bacteria such as *Listeria*, *Bifidobacterium*, *Clostridium*, *Escherichia* and *Salmonella* spp. demonstrate natural tumor-targeting and killing potencies owing to their rapid penetration and proliferation in the hypoxic tumor site,[12] resulting in the emerging interest of investigating the specific anti-cancer therapeutic approach of utilizing facultatively anaerobic bacteria in the hypoxic areas of neoplastic tissues.[13] The mechanism of action can be exerted via direct toxicity to tumor cells, enabling a nonspecific immune response, the deterioration of essential nutrients required for tumor cells and the changes in the abundance of components of the microenvironment by bacteria colonization.[14]

Listeria monocytogenes is a Gram-positive bacteria that can directly infect antigen-presenting cells, dendritic cells (DCs), neutrophils or macrophages, and myeloid-derived suppressor cells (MDSCs), and has been widely employed in cancer immunotherapies as a delivery vehicle for tumor-specific antigens.[15] *Listeria* residing in MDSCs is protected from immune clearance through this unique mechanism, whereas *Listeria* cells in healthy tissue milieus are rapidly eliminated. *Listeria*-based immunotherapies are known to induce a robust immune response and memory with a capacity to stimulate innate and adaptive immunity. A novel immunotherapeutic agent called AXAL that uses *Listeria* for its construction is currently under clinical trials for treating metastatic cervical cancer.[16]

Recent reports on bacterial-mediated cancer therapy show attenuated bacterial strains to suppress tumor progression through colonization. Unlike non-uniform distribution and limited infiltration of a chemotherapeutic agent in solid tumors, bacteria are complex organisms that can draw energy from their environment; hence, their migration in deep tissue or solid tumors can be unlimited.[17] Besides, bacteria should proliferate and distribute evenly to exert optimal tumor therapy in tumor tissues, inducing tumor regression via a series of molecular mechanisms. *Salmonella typhimurium* A1-R or VNP20009 act by infection, colonizing and stimulating apoptosis or autophagy, such as toxin production or depletion of nutrients.[18] *L. monocytogenes* induces tumor suppression via the initiation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase because of increased intracellular populations, leading to high reactive oxygen species (ROS) levels.[19] After infection, *Clostridium* spp. can kill the tumor cells by producing bacterial toxins such as hemolysins and phospholipases, compromising the cellular membrane structure and intracellular functions. Likewise, infections by *Salmonella* spp., *Listeria* spp. and *Clostridium* spp. colonization can promote tumor elimination with the help of cytokines and chemokines.[20]

In addition, we cannot rule out the beneficial effects of the gut microbiome in cancer prevention. Probiotic bacteria such as *Lactobacillus*, *Bifidobacteria* and *Streptococcus* spp. found in natural foods or ingested fermented foods help with cancer prevention.[21] The probiotic live *Lactobacillus casei* BL23 bacteria was reported to have cell proliferation reduction and apoptosis induction when administered orally to C57BL6 6–8 week-old female mice. Jacouton *et al.* used this bacteria to modulate gut microbiota composition, which plays a pivotal part in the carcinogenesis of colorectal cancer. *L. casei* BL23 mediates immunomodulatory potential through interleukin (IL)-22 cytokine downregulation, and an antiproliferative property was mediated through Bik, caspase-7 and caspase-9 upregulation.[22] In another study, Kumar *et al.* reported that the probiotic bacteria *Lactobacillus plantarum* could dimmish a potent carcinogen: 1,2-dimethylhydrazine, in a 1,2-dimethylhydrazine-induced colon tumor in male albino Wistar rats, modulating the 1,2-dimethylhydrazine-free-radical-induced rat colon carcinogenesis development through the antioxidant-dependent mechanism.[23]

Genetically engineered bacteria for cancer imaging and therapy

Engineered bacteria can be tumor-specific therapy vectors by arming them with therapeutic proteins (Fig. 1). However, their

TABLE 1

Recent and ongoing clinical trials with live or engineered bacteria or their targeting components in cancer therapy.

Name of bacteria	Type of cancer	Mode of action	Phase	Refs
Salmonella typhimurium VNP20009	Cancer, neoplasm, neoplasm metastasis	Targeted tumor and cancer cell infection	I	[49]
S. typhimurium VNP20009	Metastatic melanoma; metastatic renal cell carcinoma	Target to tumor and inhibit tumor growth	I	[50]
S. typhimurium VNP20009	Melanoma	Targeted tumor and cancer cell infection	I	[51]
S. typhimurium VNP20009 expressing TAPET-CD (cytosine deaminase)	Head and neck or esophageal adenocarcinoma	Convert 5-FC to 5-FU and inhibit tumor growth	I	[52]
S. typhimurium expressing human IL-2	Liver cancer	Increase splenic, hepatic NK cell populations and inhibit metastatic tumor	I	https://www.clinicaltrials.gov/ct2/show/NCT01099631
S. typhimurium Ty21a VXM01	Pancreatic cancer	Through VEGFR2-specific T cell response	I	[53]
Clostridium novyi-NT	Colorectal cancer	Destruction of hypoxic and necrotic parts of the tumors	I	https://www.clinicaltrials.gov/ct2/show/NCT00358397
Listeria monocytogenes	Cervical cancer	Stimulation of innate and E7 antigen-specific adaptive immune responses	II	[16]
Dietary supplement: probiotic	Operable stage I–III breast adenocarcinoma tumors $\geq$ 1.0 cm	Inducing significant tumor reduction owing to the induction of the defense system	NA	https://www.clinicaltrials.gov/ct2/show/NCT03 35851 1
C. novyi-NT spores	Treatment refractory solid tumor malignancies	Infecting tumors and destroying them	I	[54]
MRx0518 a live biotherapeutic product	Resectable pancreatic cancer	Stimulating the immune function and improving the therapeutic effect of hypofractionated preoperative radiation	I	https://www.clinicaltrials.gov/ct2/show/NCT04 19390 4
ADXS11-001 (ADXS-HPV)	Head and neck cancer Squamous cell carcinoma of the head and neck HPV-positive oropharyngeal squamous cell carcinoma	Stimulating the body's immune system against HPV-positive oropharyngeal squamous cell carcinoma before transoral surgery	II	https://www.clinicaltrials.gov/ct2/show/NCT02 00218 2
APS001F	Advanced solid tumors Metastatic solid tumors	Targeting cancer cells and producing cytotoxic cytosine deaminase (CD)	I/II	https://www.clinicaltrials.gov/ct2/show/NCT01 56262 6
Clostridium butyricum CBM 588 probiotic strain	Hematopoietic and lymphoid cellneoplasm	Increasing gut bacteria biodiversity and preventing recurrent symptoms of gastrointestinal toxicity	I	[55]
Typhoid vaccine	Recurrent breast carcinoma	Stimulating the immune system to respond to a tumor	NA	https://www.clinicaltrials.gov/ct2/show/NCT02 41538 7
JNJ-64041809	Metastatic castration-resistant prostate cancer	Vaccines for cancer therapy	I	[56]
BacTRL-IL-12	Solid tumors	Colonizing solid tumor tissues and delivering genetic material encoding the proinflammatory transgene interleukin- 12 (IL-12)	I	https://www.clinicaltrials.gov/ct2/show/NCT04 02530 7

toxicity and targeting efficiency are variable depending on tumor types. To overcome this, it was found to be necessary to enhance the bacterial targeting efficiency through bacterial surface engineering to express specific desirable factors.[24] Genetically engineered bacteria can significantly increase tumors than in normal tissue and ensure an immunogenic anticancer response by expressing and releasing more reporter genes, anticancer agents, cytotoxic proteins and antigens while tailoring their metabolic pathways.[10,25] When administered in tumor-bearing mice, attenuated bacteria resulted in tumor regression, shrinkage and even complete elimination. For example, intracranially injected attenuated ΔppGpp *S. typhimurium* in orthotopic glioma mouse models acted as a vector for the targeted delivery of tissue inhibitor metalloproteinase (TIMP)-2-encoding plasmid. Genetically engineered, high proliferating bacteria applied at the tumor site resulted in *in situ* overexpression of TIMP-2, thus inhibiting matrix metalloproteinase (MMP)-2 in the TME.[26]

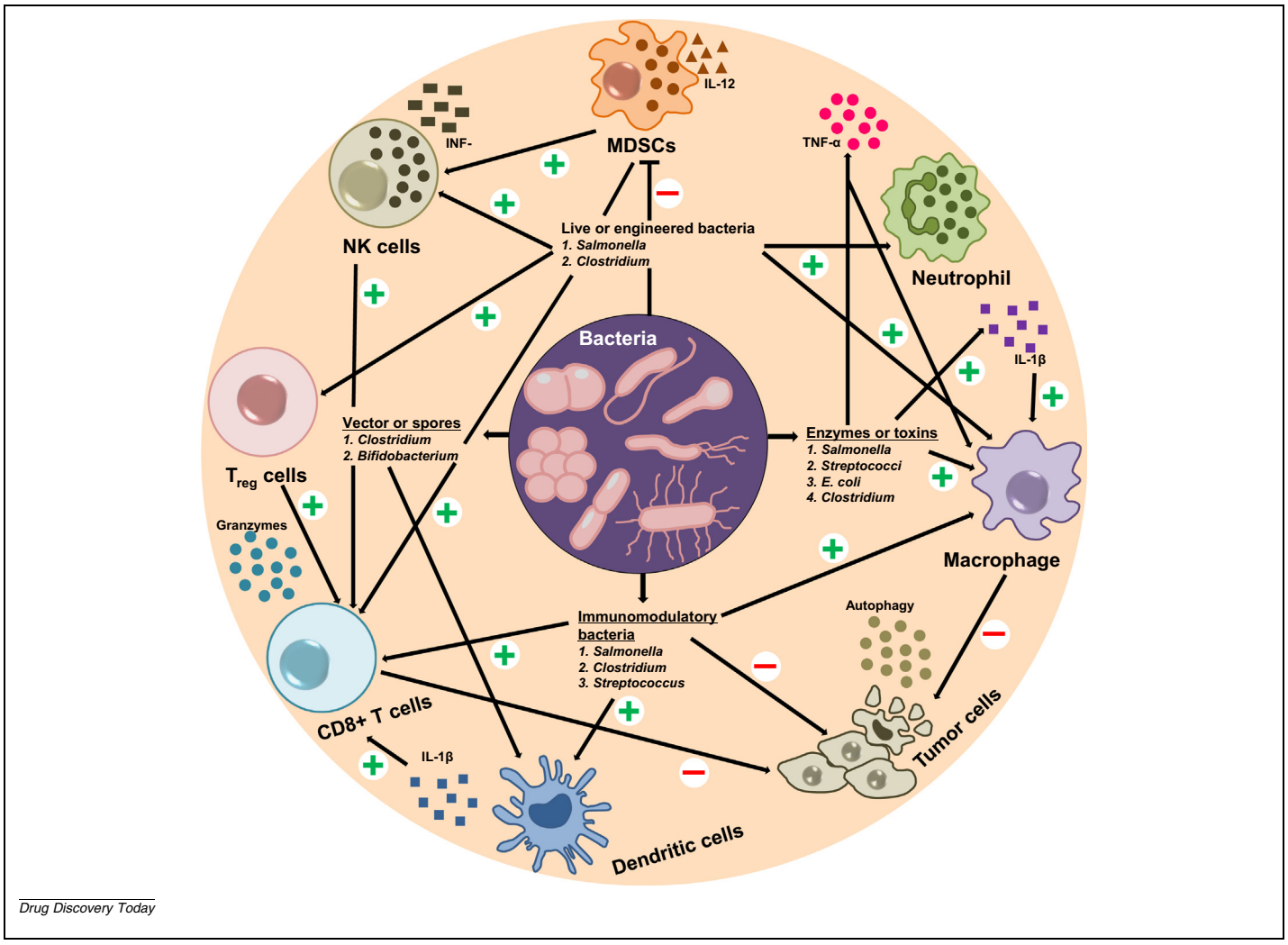

FIGURE 1
Schematic summary of the diverse bacteria, bacterial components and engineered bacteria utilized in cancer therapy.

However, an engineered *Clostridium* strain has effectively upregulated anticancer activity by converting prodrugs into active cytotoxic drugs, particularly at the tumor site, by expressing prodrug-converting enzymes.[13] Recent studies reveal that bacteria can produce antibodies that bind to important tumor development transcription factors [hypoxia-inducible factor (HIF)-1] to treat cancer. Clinical trials have also proved that genetically engineered *S. typhimurium* and *Clostridium novyi* can produce promoters such as HlyE or Stx2 and toxins such as recA. These can trigger the host immune system and induce cytokines such as IL-2, IL-4, IL-8 and CC chemokine-21, leading to tumor regression.[10] During *in vivo* imaging of tumor colonization, genetically engineered bacteria were actively studied as a new therapeutic approach for solid tumors. From a bacterial-mediated cancer therapy study, ^{18}F-FDS PET (fluorodeoxysorbitol positron emission tomography) can be a valuable tool to semi-quantitatively visualize the tumor-targeting bacteria with genetically engineered *E. coli* K-12 (MG1655).[27] Wu *et al.* also reported that live *S. aureus* effectively involved *in vivo* imaging of tumor colonization against CT26 cells injected in mouse.[9]

Bacteria as living microrobots to fight cancer

In recent years, scientists designed and developed microscopic and nanoscale systems as the earliest prototypes of nanorobots for more-effective *in vivo* diagnostic and therapeutic applications, especially in the context of cancer.[28] However, precise navigation to tumor sites remains the ideal goal of nanorobot R&D. Interestingly, bacteria can swim spontaneously through fluids, driven by a molecular motor that spins the cilia or flagella in a corkscrew-like manner. For example, researchers have invented helical and magnetic fluxes that a rotating magnetic field can carry forward. However, bacteria, especially in cancer treatment, are a model for efficient swimming; they can also sense biochemical references and adjust their metabolic pathways accordingly, much like board computation.[29] Genetically modified *Salmonella enterica* can lyse at a synchronous population density and release genetically encoded cargo. After quorum lysis, a small population of surviving bacteria reseeds the growing population and they multiply until they reach the critical threshold. This circuit could enable new drug delivery approaches via modulation of the population frequency and amplitude over time in *in vivo* environments.[30]

Bacteriobots containing *S. typhimurium* and Cy5.5-fluorescence-coated polystyrene (PS) microbeads have been reported to combat cancer. Fluorescence emission from Cy5.5 was measured only in the tumors from bacteriobot-injected mice but not detected in the control mice injected only with unstained bacteriobots or bacteria after the three days of intravenous injection in CT-26 tumor-induced mice. This bacteriorobot can also be a new paradigm in cancer therapy.[25] Zheng *et al.* reported the anticancer activity of novel bacteria microrobots prepared by attaching *S. enterica* to poly-L-lysine (PLL)-coated hyaluronic acid (HA).[31] HA acted as a motility steering factor, presenting a promising tumor-targeting therapeutic strategy for the future. The drug delivery system based on genetically modified *Modestobacter marinus* bacteria MC-1 can be used to deliver the SN-38 drug-loaded liposomes into the hypoxic regions of HCT116 colorectal xenografts in animals.[32]

Bacteria as immunotherapeutic agents to fight cancer

Cancer immunotherapy is a treatment that involves triggering a specific immune response in patients leading to different types of host immune systems attacking the tumor cells (Table 2).[33] The activated and stimulated host immune cells [mainly activated and induced by tumor-antigen-specific $CD8^+$ lymphocytes, $CD4^+$ lymphocytes, and natural killer (NK) cells] can recognize and eliminate tumor cells.[34] *C. novyi* infection can lead to the production of heat shock protein (Hsp)70, released from necrotic cells upon illness or tissue injury. Pathogen-associated molecular patterns (PAMPs) released from bacteria bind to and activate Toll-like receptor (TLR), which regulates the production of proinflammatory cytokines like IL-12 and other constitutive molecules such as CD40. Subsequently, these mediators produce interferon (IFN)-γ and initiate the Th1-dependent cell-mediated response, essentially mediated by $CD8^+$ effector cells.[35] It is well established that nonpathogenic-*C. novyi*-activated $CD8^+$ lymphocytes recognize and remove tumor cells in a mouse model by stimulating acquired immunity.[36] The use of live tumor-targeting bacteria as distribution vectors can exceed penetration limits and can increase the

TABLE 2

Current bacterial-mediated treatment strategies and related reports in cancer therapy.

Treatment process	Bacterial strain	Cancer type	Treatment approach	Evidence	Refs
Immunotherapeutic agents	*Salmonella typhimurium* A1-R	Mammary carcinoma	Induced brain 4T1 metastasis treated via tail vein bacteria injection	Inhibition of breast cancer brain metastasis with increased survival	57
	Bacillus Calmette-Guerin (BCG)	Bladder cancer	Vaccine	Internalization of BCG and stimulating the immune system	58,59
	Listeria monocytogenes	Breast cancer, melanoma, cervical cancer	*L. monocytogenes* vaccination	Regression growth all types of tumors	60
	Clostridium novyi-NT	Solid tumors	Single intratumoral injection	Local tumor destruction with tumor-specific immune response	61,15
	Salmonella enterica Serovar typhimurium	Melanoma cancer	Injection with triptolide	Enhanced bacteria infiltration caused antitumor effect	62
Bacterial toxins/ enzymes	*S. typhimurium* AR-1	Melanoma cancer, colorectal cancer, pancreatic cancer	Intravenous administration	Cell cycle arrest with recombinant L-methionine	63
	Corynebacterium diphtheria	Adrenocortical carcinoma, breast cancer	Direct inoculum to the cells	Inhibit the growth, reduce the angiogenesis	64
	Pseudomonas aeruginosa	Breast cancer, epithelial cancer, melanoma, leukemia	Locally administration	Immunotoxin induced anti-tumor response	65,66
	Escherichia coli DH5α	Colon carcinoma	Subcutaneous injection	Prodrug activation suppresses the tumor growth	67,68
	Streptomyces verticillus	Head and neck squamous cell carcinoma, ovarian cancer	Inoculated with HeLa cells	Influence oxygen and metal ion dependent cleaving of DNA	69
	Clostridium difficile	Breast cancer	Subcutaneous injection	Inhibit proliferation, induce necrosis and apoptosis	70
	Clostridium botulinum	Breast cancer	Direct inoculums to the cells	Receptor to neurotoxin interaction causes anticancer activity	71
	Fusarium culmorum ASP-87	Leukemia	T cell inoculated with L-asparaginase	Induction of apoptosis by cell cycle inhibitors	72
	Lactobacillus plantarum	Oral cancer	Human KB cells co-incubated with the probiotics	Inhibit the cancer by decreasing the MAPK gene expression	73
Bacterial vector/ spores	*S. typhimurium* ΔppGpp/pBAD-ClyA	Colon tumor	Intravenous injection	Combined radiation therapy inhibits tumor	74
	C. novyi-NT and S. typhimurium	Solid cancer	Intravenous injection	Targeting the most resistant regions in human solid cancer	75
	Bifidobacterium longum-C-CPE-PE23	Breast cancer	Subcutaneous injection	Suppress the tumor growth	76

anticancer activity of the chemotherapeutic drug while reducing systemic toxicity to the host. In support of this hypothesis, a previous study showed that IL-18-producing *S. Typhimurium* inhibits the growth of primary subcutaneous tumors and lung metastases by increased infiltration of leucocyte and NK and $CD4^+$ cell accumulation in immunocompromised mice without any direct toxicity to the normal tissues.[37] Wang *et al.* demonstrated that the administration of an IL-24-expressing genetically engineered *Bifidobacterium breve* strain enhances the antitumor activity in head and neck squamous cell carcinoma *in vivo,* mediating a mitochondrial control (Bcl2 and Bim) of caspase-mediated cell death.[38]

Several attenuated or genetically modified bacterial species have been explored for cancer therapy. A strong antiangiogenic effect, antitumor response induction and prevention of primary and metastatic tumors have been reported. Zheng *et al.* reported that the heterologous flagellin-expressing, genetically engineered, attenuated ΔppGpp *S. typhimurium* administration to colon-cancer-bearing mice showed an enhanced anticancer activity through the regulation of TLR4 and TLR5 metabolic pathways.[39] This approach also stimulated an M1 to M2 shift in macrophages and increased the cellular nitric oxide level in tumor regions. In a recent study, the pore-forming cytolysin of listeriolysin-O (LLO)-expressing *L. monocytogenes* was administered to cancer-bearing mice resulting in anticancer activity eradicating solid tumors via the MHC class I pathway.[36]

Bacterial toxins or enzymes in cancer therapy

Pathogenic bacteria producing toxins or enzymes can inhibit the immune system of the infected organism. The toxins can enter cells and change their substrates in the cytosol. Because many of those bacterial toxins have been studied in relevance to their design, cellular receptors, uptake pathways and molecular mechanisms are widely used to analyze cell-specific molecular signaling pathways in cancer therapy.[10] *S. enterica* producing cytotoxic protein cytolysin A (ClyA) can bind and form pores in the eukaryotic cell membrane, triggering caspase-mediated programmed cell death.[36]

Clostridium perfringens enterotoxin (CPE) binds directly to the receptors and inhibits tumor growth by upregulating CLDN3 and CLDN4 significantly. This CPE toxin also directly binds with claudin-3 and/or claudin-4 making a complex protein that leads to the loss of cellular osmosis and has been used in colorectal, gastric and ovarian cancer therapy.[40] Karpiński *et al.* demonstrated that *Clostridium botulinum* expressing botulinum neurotoxin A reduced cell proliferation by inducing a caspase-mediated apoptotic process in breast cancer (T47D).[41] *L. monocytogenes* spp. produce the toxin LLO, which binds to the cholesterol-binding receptors and stimulates pore formation in the cell membrane leading to cytolysis and apoptosis caused by a caspase-mediated signaling pathway in breast cancer cell lines MDA-MB-231 and MCF-7.[42]

Bacterial enzymes play a vital part in depriving the essential amino acids involved in the uncontrolled and rapid growth of tumor cells. A recent study reported that L-asparaginase from *E. coli* could cause toxicity to MCF-7, HepG2 and SK-LU-1 cell lines by activating asparagine hydrolysis and reducing its blood concentration to inhibit the progression of malignant cells.[43] Fiedler *et al.* studied that *Mycoplasma hominis* or *M. arginine* produce arginine deaminase enzyme, which hydrolyses the arginine in tumor cells, resulting in decreased tumor proliferation of glioblastoma *in vitro* and *in vivo*.[44] L-Asparaginase (EC 3.5.1.1) was the first bacterial enzyme approved for cancer treatment. This enzyme hydrolyzes the C–N bond of L-asparagine (ASN), resulting in aspartic acid and ammonia. L-Asparagine synthetase (ASNS) is present in normal cells and catalyzes the reaction between L-aspartate and L-glutamine to synthesize ASN. The absence or low expression of ASNS is the characteristic of these ASN auxotrophic tumors. L-Asparaginase targets ASN-deficient tumors because it reduces the plasmatic ASN levels, thereby starving the tumor cells and promoting their apoptosis. Asparaginase is currently used to treat acute lymphoblastic leukemia (ALL) in children worldwide.[45] Like other proteins, enzymes are produced inside cells by ribosomes, which link amino acids into chains. Although they are produced by bacteria, most enzymes are formed precisely as they are in human cells. Using bacterial enzymes in cancer therapy enables enzyme and/or protein engineering, the term used to modify an enzyme's structure and thus alter and improve its function by changing the catalytic activity of isolated enzymes to produce new metabolites. The poor pharmacokinetic and pharmacodynamic properties, high immunogenicity, proteolytic instability, short half-life, low substrate affinity (high K_m) and non-physiological temperature and pH optima are the significant challenges in the use of microbial enzymes as cancer drugs.[45] Furthermore, the considerable exploitation of bacterial-mediated therapy opens a window for developing a new and effective biocompatible personalized bacteriotherapy that can translate into clinical trials.

Challenges in bacterial cancer therapy

The significant adverse effects of fever, septic shock and death are the main problems associated with immunity when using bacteria against cancer. However, genetic engineering has led to the use of genetically modified bacteria, decreasing their pathogenicity hence can be applied in cancer therapy. There are no reports that have been found on bacterial-mediated complete inhibition of tumor growth through colonization alone. However, this demonstrates a vital prospect as an immunostimulator for cancer treatment or a vector for therapeutic components released inside a tumor.[46] Bacillus Calmette–Guerin (BCG) is the only FDA-approved live bacteria available and employed to treat superficial, non-muscle-invasive bladder cancer (NMIBC). However, the mechanism of bacterial therapy of cancer and toxicity *in vivo* is not yet clearly understood. Apart from the beneficial therapeutical potential, the main challenge is its potential acquisition of antibiotic resistance or mutations that would revert the bacteria attenuated phenotype, which could be a real risk.[47] The optimum dosage for therapeutic efficacy could lead to toxicity and therefore a reduced dose would be needed, resulting in diminished efficacy, which is the major problem when bacteria are applied as anticancer agents.[48] Live bacterial production is more complicated than making anti-

cancer drugs and would be the main challenge for producing GMP-grade test products. Cultivating, purifying and harvesting the live bacteria within strict aseptic protocols with real-time supervision are practical ways to ensure the quality of the final products.

Concluding remarks

Conventional therapies play a significant part in cancer treatment despite pathophysiological complications but still produce ineffective treatment results. Therapeutic bacteria can be considered for cancer therapy in line with its unique features publicized by the current research investigations. Bacterial agents can be a treatment option for cancer because bacteria can selectively target cancer cells and proliferate in hypoxic regions of tumors where inefficiency to withstand hypoxic conditions tends to be the primary cause of failed conventional therapies. In contrast with conventional treatment, bacterial cancer therapy has gained more advantages with post-administration control and direct and selective slaying of cancer cells that have fascinated scientists working on cancer.

Furthermore, most anticancer studies end up with *in vitro* studies and only limited research at the clinical trial phase. Hence, future research should be designed toward clinical trials to amend the anticancer activity of 'smart' bacteria and bacterial agents. However, bacterial-mediated tumor-targeting and treatment as a novel cancer therapy to extend patients survival are promising.

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