JOURNAL OF MICROBIOLOGY AND INFECTION
2024, VOL. 2, pp. 1-9
https://doi.org/10.61577/jmi.2024.100001

RESEAPRO JOURNALS

REVIEW

OPEN ACCESS

Future of engineered bacteria in cancer immunotherapy

Rohit Bhattacharjee and Devarshi Urvish Gajjar

The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

ABSTRACT

Background: Statistically, different kinds of cancers account for an average of more than 10 million people each year. It is very difficult to deliver anti-tumor agents or treat cancer because of abnormal vasculature. The adverse side effects of conventional strategies like chemotherapy, radiotherapy, and surgery highlight the urgent need for an alternative therapeutic option. Microbial therapy, which uses mainly bacteria, is being used as an alternative therapy to target tumor cells.

Mainbody: The failure of individual strategies resulted in the introduction of holistic approaches such as microbial therapy, which employs the use of facultative or obligate anaerobic bacteria, such as pathogenic and non-pathogenic bacteria, that naturally target and kill tumors. Bacterial-mediated cancer treatments (BMCTs) have gained popularity in recent decades as an alternative method of treating cancer tumors due to the inherent difficulties of conventional treatments. With advancements in genetic engineering and rDNA (recombinant DNA) technology, several strains of bacteria are introduced as cancer immunotherapy model systems. Emancipating from the concerns of cultural stigmas and toxicology, BMCT holds the potential to benefit cancer treatment. The genetic manipulation of a variety of pathogenic and non-pathogenic bacteria to elicit tumor regression, such as pathogenic strain attenuation, genetically engineering them and comprehending motility for better tumor targeting, modifying immunotoxins for cancer therapy, and novel strategies such as radiation mutation technology (RMT) are subjects of the review, which also includes a discussion of recent advancements, challenges, and prospects for bacteria in the context of the development of bacteria-mediated cancer therapy. Additionally, we discuss how tumor regression is caused by the colonization and proliferation of live bacteria in tumor microenvironments (TMEs).

Short Conclusion: Microbial therapy has the potential to become one of the most specific cancer treatments.

KEYWORDS

Bacteria; Anaerobic; Recombinant; Immunotoxins; Tumor microenvironment; Immunotherapy; Cell proliferation

ARTICLE HISTORY

Received 12 December 2023; Revised 19 January 2024; Accepted 27 January 2024

Background

Cancer is a growing health issue and a leading cause of mortality despite the lack of effective treatments. Annually, one in six people worldwide dies from cancer, resulting in an average of 10 million deaths. It is crucial to diagnose and treat cancer as soon as possible accurately. Chemotherapy, radiotherapy, and other alternative cancer treatments [1] side effects have presented numerous obstacles, such as toxicity to non-cancerous cells and the inefficacy of different kinds of drugs to target deep tumor tissue with the ongoing issue of tumor cells developing resistance to drugs. Surgical removal can be successful in some cancer types and stages of development. However, this approach has some inherent flaws, including the possibility of metastasis and cancer recurrence. Conventional approaches like radiotherapy and surgery alongside chemotherapy have different success rate degrees and unparalleled failure in the treatment of cancer, particularly far away tumor recurring and unfavorable effects. Cancer tumors, on the other hand, have necrotic centers and hypoxic core regions, rendering the majority of the cancer therapies ineffective due to deficiency of oxygen and also because of their abnormal vasculature. It is extremely difficult to deliver therapeutic agents because of the abnormal vascular architecture of the tumor region. The need for alternative strategies that are more effective and selective against tumor cells has grown as a result of these obstacles. As a result, holistic approaches [2] may produce subpar results even though a single strategy for treating cancer may not be effective. Listeria, Bifidobacterium, Clostridium, *Escherichia coli*, and Salmonella species are examples of facultative or obligate anaerobic bacteria that naturally target and kill tumors. There were reports two centuries ago that recovering from bacterial infections put cancer patients into remission. Between the 19th and 20th centuries, an American physician, William Coley, conducted many experiments to treat patients suffering from cancer with both heat-killed and live bacteria. Coley claimed that the complex cocktail he created could shrink cancerous tumors. Consistent results were, however, difficult to replicate due to an absence of progressive approaches and techniques and a poor comprehension of the way of action. Coley's heat-killed bacterial combination sustained in medical use for patients suffering from sarcoma and was known as "Coley's toxin"[3].

In 1976, it was reported that the Bacillus Calmette Guerin (BCG) bacteria could effectively help in treating superficial cancer of the bladder by stimulating the inflammatory response and thus activating the immune system. The clinical applications of the therapy are constantly evolving, and with

***Correspondence:** Prof. Devarshi Urvish Gajjar, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India, email: devarshimistry@yahoo.com

its continued usage, we can expect to see even more advancements in the field of medicine that could significantly improve patient outcomes. Due to the inherent difficulties of conventional methods of cancer therapy, bacteria-mediated cancer treatments [4] have gained prominence in recent decades as an alternative method of treating cancer tumors. Numerous bacterial strains have been developed as cancer immunotherapy model systems thanks to advancements in rDNA technology and genetic engineering. Research has primarily focused on molecular and biochemical strategies for manipulating bacteria in the fight against cancer due to technological advancements and our capacity to reduce pathogenic strains [5]. Bacteria are of great interest due to their remarkable ability to penetrate hypoxic tumor regions, proliferate within tumor cells, and escape the vasculature. Yazawa et al. reported in 2001 that systemic injection of the anaerobic and non-pathogenic strain of *Bifidobacterium longum* localized selectively to and thrived in induced rat mammary tumors by 7,12-dimethylbenzanthracene. Two reported strains, *Clostridium sordellii* and *Clostridium novyi*, were set up to have expansive tumor localization, particularly in inadequately vascularized areas, out of the 26 species of Clostridium, Lactobacillus, and Bifidobacterium tested. By removing the α-toxin, the *C. novyi* was reduced, resulting in the nontoxic strain *C. novyi-NT.* In animal tumor models, the remedial introduction of this strain in confluence with age-old methods such as chemotherapy, surgery, or radiotherapy was largely efficacious. Salmonella [6,7], along with many other facultative non-aerobic bacteria listed below, colonize both small and quiescent that is nonhypoxic and hypoxic tumors, respectively, as well as tumor regions that are metastatic and also are accessible to the circulatory system. In syngeneic 4T1 tumor-bearing mice (BALB/c), they examined infectious *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) strains SL1344 along with ATCC14028, E. coli strain 4608-58, uropathogenic strain CFT073, non-pathogenic *E. coli*, attenuated *Shigella flexneri* strain 2a SC602. The ability of these strains to colonize tumors was high. *E. coli* exhibited the strongest tumor-specific colonization among the tested strains, with minimal colonization of the spleen and liver.

In both immunocompetent and immunocompromised animal tumors, the colonization and amplification of the strain *Escherichia coli* Nissle 1917 were comparable. A live-attenuated *L. monocytogenes* (*Listeria monocytogenes*) [8] vaccine was used in an advanced phase I clinical trial for patients suffering from progressive cervix carcinoma who did not respond to any of the standard methods of cancer therapy like chemotherapy, radiotherapy, or surgery. In the context of recent advancements in BMCT (bacteria-mediated cancer therapy), approaches by which various non-infectious and infectious bacteria have been used to induce tumor shrinkage genetically is the subject of this review, which also includes a discussion of recent advancements, challenges, and prospects for bacteria. We also talk about how colonization and proliferation of live bacteria in tumor microenvironments (TMEs) cause tumor regression.

Types of Bacteria in Cancer Therapy

Pathogenic

Salmonella spp.

Among all the adaptable bacterial species known, *Salmonella enterica* serovar Typhimurium (ST) is said to be suitable for BMCT because it can thrive in both oxic and anoxic culture conditions [9]. As a result, it spreads easily throughout the body in animals exposed to high levels of oxygen and subsequently settles in anoxic tumor regions that are their preferred sites of colonization. The ability of Salmonella to colonize anoxic or hypoxic, metastatic, and necrotic tumors has been demonstrated to be tremendous [10]. As a result, it can work in conjunction with standard treatment methods. A significant barrier to the target specificity of cancer treatment has been removed by its preferential accumulation ratio in tumor areas being between 103 and 104 times higher than in normal body tissues. wherein experiments were conducted with engineered Salmonella by combining mutations in lipid and purine auxotrophy that attenuated the bacterial virulence by more than 10,000-fold and enhanced tumor targeting ability. These bacteria are genetically stable, safe in pigs, mice, and monkeys, and are presently in phase I clinical trials.

As a result, therapeutics can be delivered directly into the tumor regions using Salmonella as a carrier, shielding them from disintegration and potential immune system harm from the host [11]. In addition, Salmonella is adaptable in a variety of ways, including bacterial quorum sensing systems, target-oriented and lysis systems, protein secretion systems, bacterial ghost systems, and so on. As a result, it is adaptable to cancer treatment [12]. Utilizing RMT, Gao et al. 2020 developed an attenuated Salmonella strain (KST0650), which was oxygen-tolerant [13]. The findings demonstrated that the oxygen-tolerant strain had 20X more duplication activity in CT26 cancer cells and was less virulent than the wild-type. Additionally, KST0650 was able to penetrate the tumor tissues of mice successfully. The radiation-inducible recN promoter controlled the expression of the intracellular pro-apoptotic protein sATF6, which was also present on the plasmid of KST0650. In addition, in the murine tumor model, a synergistic anti-tumor effect with complete prevention of tumor development and protection against mouse mortality was demonstrated by treatment with radiation and KST0652. Its ease of production, affordability, and rapid mass production position it as a novel treatment alternative for cancer. Because of the natural induction of apoptosis and tumor cell death, Tumor necrosis-related apoptotic-induced ligand (TRAIL) is a desirable cytokine in cancer therapy. [14].

Additionally, *S. typhimurium* has been manipulated to produce a TRAIL, which is under the regulation of recA, a prokaryotic radiation-inducible promoter. This model's *in vivo* results have shown a significant increase in survival rates and a reduction in the growth of mammary tumors. *S. enterica* serovar has successfully expressed other genes, like cytolysin (HlyE). Under a promoter's control, Typhimurium can induce hypoxia [15]. When specifically targeted to hypoxic regions, it has been demonstrated that cytolysin, a pore-forming toxin, is effective against murine mammary tumors. There are several advantages to using Salmonella-mediated cancer therapy (SMCT). For instance, it has intrinsic anti-tumor properties, self-targeting tumor localization and proliferation, and other species-specific traits. It has many benefits over other bacterial species, such as the capacity to flourish in anoxic environments and the comparative simplicity of devitalization and subsequent gene alterations. It can coexist peacefully with a variety of humans as well as animals living on farms. The fact that it can be provided orally, activating immune responses both locally and

https://doi.org/10.61577/jmi.2024.100001

systemically, emphasizes its use as a model vector for cancer vaccine therapy.

Listeria spp.

One of the widely used vectors for treating cancers is the non-obligate, gram-positive, non-aerobic bacteria known as *Listeria monocytogenes*. Due to its association with foodborne illness, most people are aware of Listeria, still, numerous of the features that make Listeria infectious are also being designed to be used as delivery systems in cancer treatment [16]. To stay intracellularly agile and circulate from cell to cell [17], Listeria can commandeer the cytoskeleton machinery of the host cell. Due to Listeria's indigenous capability to dodge the phagolysosome and aid in releasing plasmid DNA into the cytoplasmic region, it has been hypothecated that the use of Listeria may make it possible for therapies to access deeper into tumors than they could with different microbe spp. [18]. To achieve this thing, Listeria has been manipulated in several distinctive fashions.

One illustration is the primitive study of *L. monocytogenes* coupled with nanoparticles that were set up to elicit GFP in solid human tumors [19] properly. *In vivo* tumors, where *L. monocytogenes* invaded and proliferated in tumors to ultimately deliver therapeutic genes, demonstrated their tumor-targeting properties. *L. monocytogenes*, then coupled with tumor-associated antigens (TAAs) for improved specificity, like Melanoma Antigen Gene-B (MAGE-B), that is specifically intriguing for breast cancer given its expression frequency in biopsies from patients suffering from breast cancers [20-21]. While Listeria has several characteristics that could be beneficial, the pore-forming protein listeriolysin O (LLO) is one of the most important features to note. LLO makes it easier for DNA molecules to get into the cytoplasm of cells of the target from endosomes. Diverse studies have been conducted to determine how well LLO works for drug delivery. In addition to condensed plasmid DNA containing modified polylysine and cationic polyethylene glycol (PEG), a neutral HER2-targeting liposome is attached to LLO. LLO can disrupt the integrity of an endosome when directed toward it, allowing plasmid DNA to be delivered and expressed in the cytoplasm. This results in increased expression in breast cancer cell lines that are positive for HER2.

Alternately, LLO has been combined with polylactic glycolic acid (PLGA) microspheres to enhance cytosolic release to cells of target and immune system presentation. It has been demonstrated that the combination of microspheres and LLO is readily taken up by phagocytic cells, resulting in an increment in the expression of peptide-MHC-I on the surface of cells. In addition, microspheres and LLO treatment of a T hybridoma cell line has resulted in the activation of cytotoxic T cells. Listeria is also investigated in the avenues of nanoparticle drug delivery. By starving self-assembling Listeria innocua DNA binding protein (LiDps) in cells, functional nanoparticles were produced with the incorporation of Gaussia princeps luciferase along with Zinc (Zinc (II)-protoporphyrin IX (ZnPP). It has been demonstrated that the Gluc-LiDps-ZnPP conjugate, which fights tumors by producing ROS through bioluminescence resonance energy transfer (BRET), is effective at being taken up by cells that are likely to cause tumors. Ultimately, this halted the relocation of the remaining SKBR3 breast cancer cells significantly. Listeria has surfaced as a favourite seeker for further fruitful treatment delivery systems as a consequence of enhancements in its manipulation.

Clostridium spp.

Among prokaryotic bacteria, one of the largest genera, Clostridium, is known to produce anaerobic spores. By producing endospores [22], the Clostridium bacterial group can withstand severe environmental conditions like increased temperatures and dehydration. Because it naturally thrives in low-oxygen environments like the absolute innermost region of the TME [23], Clostridium also introduces itself as an efficient delivery tool for cancer therapeutic drugs. In cancer immunotherapy, Clostridium and its spores have been extensively studied, along with drug delivery capability coming in second place [24]. Various Clostridium subtypes, such as *C. tetani*, *C. butyricum*, *C. histolyticum* [25-26], *C. beijerinckii* [27], and *C. acetobutylicum* [28], have been tested as anti-cancer agents. Studies have demonstrated the potential to effectively manipulate *Clostridium acetobutylicum* to deliver mouse TNF-, making it among the first organisms examined for its anti-cancer properties. Similar to this, it was shown that *C. acetobutylicum* could effectively release interleukin-2 (IL2), which is known to activate immune cells in the human body by encouraging the growth of T cells. [29]. Clostridium merits further investigation in this age of enhanced biotechnological approaches due to its reliable applications as an anoxic or hypoxia-targeted delivery system. Another niche of interest in which Clostridium is genetically altered or mutated to produce high-specificity antibodies is called CDAT (Clostridium-directed antibody therapy) [30]. *C. novyi-NT* can get into solid tumors in the hypoxic and necrotic regions, which are typically thought to be insensitive to other conventional therapies like radiation, surgery, and chemotherapy. Combination Bacteriolytic Therapy, or COBALT, is a procedure by which C. novyi-NT is treated along with other agents of chemotherapy or even radiation.

Escherichia coli.

In the treatment of cancer, *Escherichia coli* (*E. coli*) is manipulated as well as exploited. E. coli is capable of colonizing hypoxic tumor regions. Using biologically engineered *E. coli* strain K-12, cytolysin A (ClyA) is injected as a single intravenous therapy to CT26 mice with colon carcinoma, 4T1 metastasizing TNBC, alongside B16 melanoma tumors. It is known that *S. enterica* and *E. coli* produce the 34 KD hemolytic protein ClyA, which acts as a pore-forming protein and causes apoptosis. E. coli has been recently re-examined with cancer therapies in several breasts and other cancer models [32]. *E. coli* has been modified to deliver a nanobody with a unit domain that targets CD47 in the tumor. One of the many functions of the transmembrane protein CD47, which is also known as integrin-associated protein (IAP), is to assist in the elimination of aged or diseased cells. Many of the *in vivo* models of cancer, including B16 melanoma, 4T1 TNBC, as well as the A20 murine lymphoma, demonstrated that this therapy elevated the count of tumor-infiltrating T cells and subsequently slowed the rate of tumor progression [33].

Corynebacterium spp.

Diphtheria is brought on by the Gram-positive bacteria *Corynebacterium diphtheriae*. Both facultative anaerobic and aerobic growth modes are possible for Corynebacterium. Diphtheria toxin (DT) is a very potent toxin that spreads from

https://doi.org/10.61577/jmi.2024.100001

cell to cell and can cause harm. By rearranging the catalytic part with the target polypeptides and genetically altering (deleting) the cell receptor-binding domain, DT has been extensively studied as a treatment for cancer cells due to its high toxicity [35]. Together, these proteins bind to the targeted cancer cell surface [36]. Different kinds of cancer, including glioblastoma and pancreatic cancer, can be treated with DT-based immunotoxin (DTAT). The cell-penetrating protein BR2 and Treg cells receptor, CCR4 [37], DT386-BR2 [38], alongside DT-anti-CCR4 [39] are just a few of the various immunotoxins based on DT that have been studied [40].

Pseudomonas spp.

Gram-negative aerobic bacteria, *Pseudomonas aeruginosa*, can also thrive as a facultative non-aerobic bacterium under certain environmental conditions [41]. Phytotoxic factors, hydrocyanic acid, pigments, protein-degrading enzymes, endotoxins, and exotoxins are just a few of the many virulence factors that Pseudomonas is known to possess. Other virulence factors include toxins [42], which are essential to the pathogenesis of the organism. Pseudomonas exotoxin A (PE) is highly studied for its anti-tumor specificity by inhibiting Eef2 (eukaryotic elongation factor 2) activity [43]. It is one of this bacterium's fundamental poisonous virulence factors. PE has employed a variety of molecular tactics to kill the host cell successfully. With encouraging results, immunotoxins that are derived from PE have been examined against a plethora of hematologic and solid tumors in both preclinical and clinical studies. Pseudomonas species have also been altered on a genetic level to serve as delivery vehicles [44]. Mannose-sensitive fimbriae type 1 can attach to *Pseudomonas aeruginosa*-mannose sensitive hemagglutinin (PA-MSHA) surface. Malignant cells frequently have elevated levels of high-mannose glycans, which have been suggested as the foundation for alternative cancer treatments for some time. PA-MSHA significantly induced hepatocellular carcinoma (HCC), arresting the cell cycle process and also a halt to cell multiplication by increasing the levels of p21 and p27 and lowering the levels of CDK 2, cyclins E, cyclins D1, and CDK4 proliferating cell nuclear antigen (PCNA). Moreover, PA-MSHA hindered epithelial-mesenchymal transition progress (EMT), which kept HCCs from attacking, moving, and sticking to each other. PA-MSHA also restricted the EGFR/Akt/IB/NF-B pathway, but when NF-B was overexpressed, PA-MSHA significantly reduced EMT inhibition. Additionally, PA-MSHA's mannose-binding activity was significantly inhibited by D-mannose's competitive inhibition of PA-MSHA. In the in vivo study, PA-MSHA also significantly slowed tumor growth and stopped HCC from spreading to the lung. Cancer cell lines from the breast, cervical, colon, and pancreas have all been shown to be cytotoxic to this strain [45,46].

Non-pathogenic

Bifidobacterium spp.

The species of *Bifidobacterium* is an obligate, anaerobic, non-motile, and branched bacteria. It is one of the primitive bacteria that inhabit the human digestive tract. There are 50 known *Bifidobacterium spp*. Only 10 are found in humans in various environments. *Bifidobacterium* species have been used in numerous studies. for its ability to fight tumors [47]. *Bifidobacterium spp*. has been the subject of preliminary research as a significant vehicle for delivery that can be altered through bioengineering to express cancer immunotherapy genes of interest [48,49]. In mouse models, it was shown that biologically engineered *Bifidobacterium spp*. secreted enterolactone, which inhibits the growth of leukaemia by converting fatty acid chains to pectin oligosaccharides (POS) [50]. As it already led to the foundation that this bacterium could be utilized as a comparatively safe and competent tool for the delivery of treatment, studies evaluating particular cancer therapies have been carried out. Additionally, oral administration of bifidobacterium has demonstrated efficacy against solid tumors, making it particularly intriguing [51]. After oral administration and transfer to the GI (gastrointestinal tract), *B. breve* has been demonstrated to colonize solid B16 murine melanoma tumors efficiently. Xenographed human HER2-positive tumors have been significantly suppressed in mice by a genetically manipulated form of *B. longum* [52]. The genetic engineering in the bacterial strain was to express and secrete the trastuzumab scFv (single chain variable fragment). HER2-positive human cancer cells were stopped from growing in vitro by the recombinant scFv, which bound to HER2 at the cell surface. In addition, trastuzumab scFv was secreted when recombinant bacteria were injected intravenously and inhibited tumor growth in growing xenografted human HER2-positive tumors. This novel Bifidobacterium-based in situ transfer and system of producing trastuzumab scFv shows a promising path for cancer treatment in the future. By the fluorescent imaging of CdSeS quantum dots, it was also demonstrated in a mouse model that Bifidobacterium microbots can effectively deliver to solid tumors.

Lactic acid bacteria (*Lactobacillus plantarum and Lactobacillus casei*)

The rod-shaped, gram-positive bacteria in the microbiome of the intestine of humans and other classes of mammals belong to the genus Lactobacillus. The primary function of this bacterium, which is one of the most important probiotic bacteria in the intestine, is sharing fermentation of lactic acid with various bacteria and providing strength to the barriers of the intestine. *Lactobacillus plantarum* (*L. plantarum*) is the main topic of research into a plethora of clinical applications, including cancer therapy [53-55], for example, in a malignant melanoma model of a human, it has been demonstrated that the L-14 form of *L. plantarum* extract controls the gene expressions which are inculpated in migration and prevents A375 cells from moving. The consequences of *L. plantarum* L-14 extract on melanoma cells of humans were examined using A375 human melanoma cells. After the treatment, the location of cytochrome c and the molecular changes of genes related to migration and apoptosis were examined. The A375 cells' viability and migration were decreased, as well as the expression of migration-related genes by the L-14 extract. In addition, it was established that the L-14 extract sparked the intrinsic apoptosis of the A375 cells. This demonstrated that the L-14 extract protected A375 cells from cancer. Consequently, the data suggest that the L-14 extract ought to be looked into for melanoma drug development with LAB. The anti-tumor effects of *Lactobacillus casei* are mediated by the upregulation of caspases and inhibition of IL-22, which leads to apoptosis [56]. By producing bacteriocins that arrest the cell cycle phenomenon in the G2 phase and cause programmed cell death or apoptosis and cell proliferation, Lactobacillus targets malignant cells because LAB can reduce selenium ions to form

https://doi.org/10.61577/jmi.2024.100001

elemental selenium nanoparticles (SeNPs) and then drop the nanoparticles intracellularly, it has been shown to have beneficial antitumor effects. Selenium acts as an essential micronutrient that prevents cancer by preventing the activation of oncogenes, which prevents normal cells from becoming cancerous [57-58].

Magnetococcus spp.

Environmental microorganisms have been the subject of renewed interest in recent years for their potential therapeutic applications [59]. An anaerobic bacterial group, which is known to reposition in the direction of the earth's geomagnetic field, known as magnetotactic bacteria, was discovered in the sediment deep in the water as a potential drug delivery tool. The bacteria can efficiently show their motility by migrating to and dwelling in hypoxic regions [60] thanks to these properties, which are necessary for magnetotaxis to target tumors. Moreover, to their natural low-oxygen-seeking state, these bacteria's magnetic properties enable them to be magnetically guided to the tumor's location, making them useful for tumor targeting. The magnetotactic bacteria known as *Magnetococcus marinus* MC1 is, as of now, the one that has undergone the most research when it comes to the administration of medicines for cancer. Nanoliposomes containing drugs have been used to study this coccus that shows Gram-negative characteristics and that was discovered in the Atlantic Ocean. Based on their previous successes, these bacteria are a noteworthy development agent for microorganism-based drug delivery [61]. However, they still require additional research and application for more widespread in vivo testing of tumors.

Bacterial Mode of Action in Cancer

Bacteria utilize a variety of different mechanisms to focus on and target cancer cells. They include manipulating bacterial virulence agents, targeting the TME, secretion of cytotoxic molecules, and engineering bacterial vectors for the release of tumoricidal proteins and their subsequent expression.

Bacterial targeting of the TME

One of the prime reasons for extensively using bacterial-targeted delivery of drugs is said to be the potential of anoxic spp. to survive in very low-oxygen tumor core regions [62]. Oxygen concentrations below 10 mmHg of pressure [63] are a distinguishable feature of the TME. Tumors or neoplasms have a functionally abnormal architecture of blood vessel vasculature that results in abnormal and improper blood circulation throughout the entire tissue, subsequently causing oxygen concentration deprivation [64,65]. Tumors must adapt their genetic makeup to resist hypoxia-induced cell mortality as well as tissue necrosis as a result of the low oxygenic condition [66]. MDR1, A multidrug-resistant gene, along with the P glycoprotein gene, that is known for developing resistance to multidrug to several other anti-cancer drugs, is said to be more prevalent in the low oxygenic or hypoxic tumor region [67,68]. Nevertheless, the hypoxia brought on by these disorganized blood vessel vasculatures leads to the creation of a unique environment in which non-aerobic bacteria can thrive. By using microbes as gene and drug delivery systems, tumors that were previously not at all sensitive to conventional cancer therapy approaches such as chemotherapy can now be particularly targeted [69]. Bacteria's survival and motility mechanisms, as well as their oxygen dependence level, are crucial to their growth and survival in tumors [70]. For example, *Listeria spp.'s* mechanism of targeting tumors emphasizes the host immune system's involvement. Antigen-presenting cells (APC) like dendritic cells (DC), macrophages, and also myeloid-derived suppressor cells (MDSCs), which can then transport bacteria to TMEs, are directly infected by Listeria cells. Immune clearance is prevented from reaching Listeria cells in MDSCs; however, they are quickly eliminated in healthy tissue environments by this special method. Moreover, Clairmont et al. (2000) have found that the *S. typhimurium* VNP20009 strain accumulates 1000 times more in tumors than in the liver. The systemic circulation, liver, and spleen were cleared quickly of these attenuated strains, but tumor tissue proliferation lasted longer. Because of this, the host experiences less toxicity. The hypoxic and vascularized tumor environment is to blame for the selective tumor colonization and proliferation. It has been shown earlier that along with *Salmonella sp.*, the genus Clostridia targets and duplicates more often in the tumor's core non-aerobic regions [71,72]. As a result, the problem of specificity in cancer therapy drugs and gene delivery may be solved by bacteria.

Altering virulence factors of bacteria

Bacterial virulence factors are molecules, cellular structures, and systems of regulation that allow microbial infectious agents to enter and exit cells, extract nutrition from cancer cells, and attain growth and colonization inside the host, as well as evasion of the immune system and subsequent immunosuppression [73,74]. Consequently, normalizing bacterial virulence against the host immune system is crucial. Although the anti-tumor response may be influenced by certain virulence factors; as a result, the bacteria's anti-cancer effects may be diminished by deleting or altering these factors. As a result, it is essential to reduce strain while not compromising its anti-tumor activity. The cytotoxicity of *Listeria monocytogenes* can be manipulated by deleting the genes that are involved in invasive characteristics in cells. *Salmonella typhimurium* strain VNP20009 [75] and *Listeria monocytogenes* [76] have been widely examined for their anti-tumor specificity. *Clostridium spp*. Actin-specific ADP-ribosyl transferase, phospholipases, hemolysins along with some other pore-forming toxins [77] are just a few of the secreted toxins that infection causes to interfere with intracellular functions.

The bacterial secretion system

Bacteria transport virulence proteins through secretion systems that can be altered and utilized in novel cancer therapies. It involves fusing therapeutic moieties to signal molecules, which are required for bacterial secretion system delivery for highly effective and targeted drug delivery [78]. The type III secretion system (T3SS), which functions by directly administering the polypeptides present in the bacteria into the cytoplasmic region of the cell of the host [79], is one type of secretion system that is frequently utilized in cancer therapy. Numerous studies have focused on the effectiveness of T3SS for drug delivery, resulting in complete tumor regression by genetic fusion of T3SS with Survivin, a tumor-associated antigen [80-81]. Additionally, the elicitation and delivery of TAA/TSA from *Salmonella typhimurium* type 1 secretion systems (T1SS) have been investigated [82].

Bacterial minicells

It has been demonstrated that a plethora of rod-shaped Gram-negative and Gram-positive bacteria form minicells

https://doi.org/10.61577/jmi.2024.100001

through abnormalities in their cell division. The ribosomes, RNA, and protein of a normal cell membrane are present in these minicells, but they usually lack a proper bacterial chromosome [83]. Chemotherapeutic drugs have been loaded into genetically modified minicells by causing alterations or mutations in their machinery of cell division of usual rod-shaped bacteria like *Escherichia coli* and *Salmonella enterica* [84]. Since they are unable to multiply but retain the properties of virulence necessary for tumor targeting, minicells continue to represent an important potential advancement in drug delivery. Bacteria's capacity for delivering therapeutic drugs is largely due to their gene transfer properties [85]. *In vitro* as well as *in vivo* examinations have displayed that genes could be transferred to mammalian cells by intracellular bacteria. For their capability as gene delivery vectors, a variety of bacteria, including invasive *E. coli*, Listeria, Shigella, Salmonella, and Pseudomonas, have been studied and manipulated. Gene transfer takes place when attenuated bacteria release the DNA from the plasmid into the cytoplasmic regions of the host cells. All the species of bacteria used to deliver genes to professional and non-professional phagocytes are facultative intracellular pathogens designed to kill cells after invasion. The transfected genes are expressed in the cells as a result of the transfer of the plasmid DNA from the attenuated intracellular bacteria's cytoplasm to the nucleus. The host cells are invaded and survived by these intracellular bacteria in different ways. Shigella, for instance, multiplies and spreads throughout the cytoplasm of the cell and to adjacent cells after being taken up by host cells and lysed in the phagocytic vacuole. A 220-kb virulence plasmid that is responsible for entry, intracellular mobility, and cell-to-cell spread confers this invasive phenotype to *S. flexneri*. Despite the differences in their intracellular pathways, attenuated mutants from these bacterial genera have been shown to transfer functional DNA into mammalian cells. This otherwise extracellular bacterial species gains the ability to enter epithelial cells when the virulence plasmid of *S. flexneri* is transferred to *E. coli*. Scientists have demonstrated that bacteria that undergo lysis upon entry into mammalian cells can deliver plasmid DNA to their hosts using an invasive strain of E. coli that has been rendered auxotrophic, strain BM2710. This results in the cellular expression of transfected genes. RNA interference can be used to further target this so that genes that encourage tumor growth can be silenced. This encompassed the release of shRNAs (small hairpin RNAs) that the plasmid encodes. These shRNAs are then transfected into siRNAs (small interfering RNAs) in the cytoplasm, which then helps tumors break down the target mRNA. L. monocytogenes and S. enterica species have been the subject of some research into this process.

Challenges

Tumor-targeting bacteria are an appropriate tool for providing therapeutic loads, particularly for targeting cancers of several origins due to their unique characteristics, which include novel gene packaging mechanisms, targeting the low oxygenic region environment of the tumor, and tumor selectivity. However, despite the high therapeutic potential of engineered bacteria (modified and attenuated strains of Salmonella such as VNP20009, *E. coli*, *Bifidobacterium*, immunotoxins of *Corynebacterium spp.*, *Pseudomonas spp.*, etc) to target tumors, the huge non-homogeneity of cancers at the histologic as well as molecular levels may stop one anti-cancer moiety from providing a cure [86]. As a result, a promising cancer treatment may require a combinatorial approach. The bacterial toxicity as a result of associated toxins is one major factor. This can result in grave infections, significant side effects, or even can be lethal. As a result, scientists are overcoming these negative outcomes by employing genetically modified and attenuated strains. Genetic alterations could also alleviate the potential toxicity of bacterial therapy by lowering or eliminating particular virulence factors. While less attenuation is pathogenic, excessive attenuation reduces invasive potential. The widespread nature of bacterial vaccines is another major concern when using them because many of the BMCT-used bacteria, such as Listeria and Salmonella, are found in the surroundings and frequently lead to immunity to these pathogens upon pre-exposure. As a result, vaccine-induced or pre-existing vector-specific immunity may prevent the delivery of vaccines and therapeutic genes. Because certain kinds of chemotherapy may suppress the immunity in the system to the point where it is unable to adequately respond to bacterial colonization, one of the main limitations of BBCT is that it is not appropriate for patients who have previously received such chemotherapy. Additionally, live bacterial products can colonize foreign objects like implanted medical devices, artificial heart valves, and joint replacements, which could act as reservoirs for infection [87]. In addition, bacterial recombinant plasmids are susceptible to mutation, which alters the course of anti-tumor activity before the penetration of cancer cells. This can result in several risks, such as treatment failure, infection, or death. Multi-drug resistance that many bacteria are developing is a major threat to public health.

Thinking of the Future

The upcoming stride in making microbes an integral part of cancer treatment might be to manipulate them carefully. Because this novel mode of control could be used for a patient's uncommon tumor kind, diligent exploitation of these mechanisms for tumor-targeting characteristics suggests important uses as personalized treatments. The best possible microbial therapy would theoretically merge a species that is non-pathogenic but works well. This species would be made up of several strains chosen for their particular target of interest. In the end, these strains would be merged with efficacious conventional therapies to get accurate results. The remaining oxygen-rich tumor regions can be targeted by combining the hypoxia-honing abilities of microorganisms with other therapeutic approaches. The genetic adaptability of microorganisms may be their highest and utmost asset, making it possible to tailor individualized therapy to maximize cytotoxic effects precisely. Before it reaches the level of popularity of current mainstay therapies, the notion of cancer therapy by using microorganisms as delivery tools still has many avenues to tread. Cultural stigmas and toxicology concerns must be addressed before microorganisms can be entrusted to cancer treatment. More scientifically sound studies are required to overcome the side effects and current limitations of bacteriotherapy because the field of bacteria in cancer immunotherapy is still considered quite new [88].

However, numerous promising mechanisms can be altered to target tumors and enhance the outcomes of the patient, so the potential of bacteria in cancer therapy cannot be overlooked [89-92]. Although bacteria in cancer therapy have produced encouraging results both *in vivo* as well as *in vitro*, few of them have led to actual clinical trial phase. As a result, both the

https://doi.org/10.61577/jmi.2024.100001

clinical and scientific communities must immediately start designing extra clinical trials to examine and capitalize on the effectiveness of bacteria in cancer therapy. The bacterial capability to particularly colonize cancerous tissue and give out an antitumor response, as well as their capability as a targeted delivery vector system, altogether display a solid foundation for extremely potent cancer treatments. It is an excellent example of how therapeutic performance and quality can be significantly enhanced. From the early attempts to bring back Coley's strategy, significant advances were made not only in comprehending the procedure but also in genetically improving the bacteria. As a consequence of this, bacteria in cancer therapy will develop into a versatile option to standard treatments that are not confined to a specific kind of tumor. In point of fact, in addition to its capability for cancer prevention and biotechnological diagnostics, microbial therapy has the potential to become one of the most specific cancer treatments. As a result, bacteria in cancer therapy have the potential to aid in the end of cancer's curse on humanity.

Conclusions

Due to the inherent difficulties of conventional methods of cancer therapy, bacteria-mediated cancer treatments have gained prominence in recent decades as an alternative method of treating cancer tumors. Tumor-targeting bacteria are an appropriate tool for providing therapeutic loads, particularly for targeting cancers of several origins due to their unique characteristics. Listeria, Bifidobacterium, Clostridium, Escherichia coli, and Salmonella species are examples of bacteria that naturally target and kill tumors. Many bacterial strains have been developed as cancer immunotherapy model systems thanks to advancements in rDNA technology and genetic engineering. Nevertheless, bacterial toxicity as a result of associated toxins is one major factor that can have side effects or may be lethal. Multi-drug resistance that many bacteria are developing is a major threat to public health. Therefore, a promising cancer treatment may require a combinatorial approach. However, the best possible microbial therapy would theoretically merge a species that is non-pathogenic but works well. This species would be made up of several strains chosen for their particular target of interest. Cultural stigmas and toxicology concerns must be addressed before microorganisms can be entrusted to cancer treatment. More rigorous scientific research is needed to overcome the side effects and existing limitations of bacteriotherapy.

Acknowledgements

RB and DG thank DBT for funding for the Biotechnology teaching program - Phase II.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Keung EZ, Fairweather M, Raut CP. Surgical Management of Metastatic Disease. Surg Clin North Am. 2016;96:1175-1192. http://doi.org/10.1016/j.suc.2016.05.010
2. Dutt S, Ahmed MM, Loo BW Jr, Strober S. Novel Radiation Therapy Paradigms and Immunomodulation: Heresies and Hope. Semin Radiat Oncol. 2020;30(2):194-200. http://doi.org/10.1016/j.semradonc.2019.12.006
3. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. Lowa Orthop J. 2006;26:154-158.
4. Forbes NS. Engineering the perfect (bacterial) cancer therapy. Nat Rev Cancer. 2010;10(11):785-794. http://doi.org/10.1038/nrc2934
5. St Jean AT, Zhang M, Forbes NS. Bacterial therapies: Completing the cancer treatment toolbox. Curr Opin Biotechnol. 2008;19:511-517. http://doi.org/10.1016/j.copbio.2008.08.004
6. Luo X, Li Z, Lin S, Le T, Ittensohn M, Bermudes D, et al. Antitumor effect of VNP20009, an attenuated Salmonella, in murine tumor models. Oncol Res. 2001;12(11-12):501-508. http://doi.org/10.3727/096504001108747512
7. Clairmont C, Lee KC, Pike J, Ittensohn M, Low KB, Pawelek J, et al. Biodistribution and genetic stability of the novel antitumor agent VNP20009, a genetically modified strain of Salmonella typhimurium. J Infect Dis. 2000;181(6):1996-2002. http://doi.org/10.1086/315497
8. Wood LM, Guirnalda PD, Seavey MM, Paterson Y. Cancer immunotherapy using Listeria monocytogenes and listerial virulence factors. Immunol Res. 2008;42(1-3):233-245. http://doi.org/10.1007/s12026-008-8087-0
9. Akoachere JF, Tanih NF, Ndip LM, Ndip RN. Phenotypic characterization of Salmonella typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. J Health Popul Nutr. 2009;27(5):612-618. http://doi.org/10.3329/jhpn.v27i5.3637
10. Semenov AV, van Overbeek L, Termorshuizen AJ, van Bruggen AH. Influence of aerobic and anaerobic conditions on survival of Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium in Luria-Bertani broth, farm-yard manure and slurry. J Environ Manage. 2011;92(3):780-787. http://doi.org/10.1016/j.jenvman.2010.10.031
11. Mi Z, Feng ZC, Li C, Yang X, Ma MT, Rong PF. Salmonella-Mediated Cancer Therapy: An Innovative Therapeutic Strategy. J Cancer. 2019;10(20):4765-4776. http://doi.org/10.7150/jca.32650
12. Ganai S, Arenas RB, Forbes NS. Tumor-targeted delivery of TRAIL using Salmonella typhimurium enhances breast cancer survival in mice. Br J Cancer. 2009;101(10):1683-1691. http://doi.org/10.1038/sj.bjc.6605403
13. Gao S, Jung JH, Lin SM, Jang AY, Zhi Y, Bum Ahn K, et al. Development of Oxytolerant Salmonella typhimurium Using Radiation Mutation Technology (RMT) for Cancer Therapy. Sci Rep. 2020;10(1):3764. http://doi.org/10.1038/s41598-020-60396-6
14. Ganai S, Arenas RB, Forbes NS. Tumor-targeted delivery of TRAIL using Salmonella typhimurium enhances breast cancer survival in mice. Br J Cancer. 2009;101(10):1683-1691. http://doi.org/10.1038/sj.bjc.6605403
15. Paton AW, Morona R, Paton JC. Bioengineered microbes in disease therapy. Trends Mol Med. 2012;18(7):417-425. http://doi.org/10.1016/j.molmed.2012.05.006
16. Radoshevich L, Cossart P. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. Nat Rev Microbiol. 2018;16(1):32-46. http://doi.org/10.1038/nrmicro.2017.126
17. Wood LM, Paterson Y. Attenuated Listeria monocytogenes: a powerful and versatile vector for the future of tumor immunotherapy. Front Cell Infect Microbiol. 2014;4:51. http://doi.org/10.3389/fcimb.2014.00051
18. Hense M, Domann E, Krusch S, Wachholz P, Dittmar KE, Rohde M, et al. Eukaryotic expression plasmid transfer from the intracellular bacterium Listeria monocytogenes to host cells. Cell Microbiol. 2001;3(9):599-609. http://doi.org/10.1046/j.1462-5822.2001.00138.x
19. Van Pijkeren JP, Morrissey D, Monk IR, Cronin M, Rajendran S, O'Sullivan GC, et al. A novel Listeria monocytogenes-based DNA delivery system for cancer gene therapy. Hum Gene Ther. 2010;21(4):405-416. http://doi.org/10.1089/hum.2009.022
20. Kim SH, Castro F, Gonzalez D, Maciag PC, Paterson Y, Gravekamp C. Mage-b vaccine delivered by recombinant Listeria monocytogenes is highly effective against breast cancer metastases. Br J Cancer. 2008;99(5):741-749. http://doi.org/10.1038/sj.bjc.6604526
21. Kim YJ, Perumalsamy H, Markus J, Balusamy SR, Wang C, Ho Kang

https://doi.org/10.61577/jmi.2024.100001

S, et al. Development of Lactobacillus kimchicus DCY51T-mediated gold nanoparticles for delivery of ginsenoside compound K: in vitro photothermal effects and apoptosis detection in cancer cells. Artif Cells Nanomed Biotechnol. 2019;47(1):30-44. http://doi.org/10.1080/21691401.2018.1541900

22. Barbé S, Van Mellaert L, Anné J. The use of clostridial spores for cancer treatment. J Appl Microbiol. 2006;101(3):571-578. http://doi.org/10.1111/j.1365-2672.2006.02886.x
23. Ryan RM, Green J, Williams PJ, Tazzyman S, Hunt S, Harmey JH, et al. Bacterial delivery of a novel cytolysin to hypoxic areas of solid tumors. Gene Ther. 2009;16(3):329-339. http://doi.org/10.1038/gt.2008.188
24. Zhang Y, Ji W, He L, Chen Y, Ding X, Sun Y, et al. E. coli Nissle 1917-Derived Minicells for Targeted Delivery of Chemotherapeutic Drug to Hypoxic Regions for Cancer Therapy. Theranostics. 2018;8(6):1690-1705. http://doi.org/10.7150/thno.21575
25. Connell HC. The Study and Treatment of Cancer by Proteolytic Enzymes: Preliminary Report. Can Med Assoc J. 1935;33(4):364-370.
26. Parker RC, Plummer HC, Siebenmann CO, Chapman MG. Effect of histolyticus infection and toxin on transplantable mouse tumors. Proc Soc Exp Biol Med. 1947;66(2):461-467. http://doi.org/10.3181/00379727-66-16124
27. Fox ME, Lemmon MJ, Mauchline ML, Davis TO, Giaccia AJ, Minton NP, et al. Anaerobic bacteria as a delivery system for cancer gene therapy: in vitro activation of 5-fluorocytosine by genetically engineered clostridia. Gene Ther. 1996;3(8):741.
28. Theys J, Nuyts S, Landuyt W, Van Mellaert L, Dillen C, Bohringer M, et al. Stable Escherichia coli-Clostridium acetobutylicum shuttle vector for secretion of murine tumor necrosis factor alpha. Appl Environ Microbiol. 1999;65(10):4295-4300. http://doi.org/10.1128/AEM.65.10.4295-4300.1999
29. Barbé S, Van Mellaert L, Theys J, Geukens N, Lammertyn E, Lambin P, et al. Secretory production of biologically active rat interleukin-2 by Clostridium acetobutylicum DSM792 as a tool for anti-tumor treatment. FEMS Microbiol Lett. 2005;246(1):67-73. http://doi.org/10.1016/j.femsle.2005.03.037
30. Groot AJ, Verheesen P, Westerlaken EJ, Gort EH, Van Der Groep P, Bovenschen N, et al. Identification by phage display of single-domain antibody fragments specific for the ODD domain in hypoxia-inducible factor 1alpha. Lab Invest. 2006;86(4):345-356. http://doi.org/10.1038/labinvest.3700395
31. Jiang SN, Phan TX, Nam TK, Nguyen VH, Kim HS, Bom HS. Inhibition of tumor growth and metastasis by a combination of Escherichia coli-mediated cytolytic therapy and radiotherapy. Mol Ther. 2010;18(3):635-642. http://doi.org/10.1038/mt.2009.295
32. St Jean AT, Swofford CA, Panteli JT, Brentzel ZJ, Forbes NS. Bacterial delivery of Staphylococcus aureus α-hemolysin causes regression and necrosis in murine tumors. Mol Ther. 2014;22(7):1266-1274. http://doi.org/10.1038/mt.2014.36
33. Chowdhury S, Castro S, Coker C, Hinchliffe TE, Arpaia N, Danino T. Programmable bacteria induce durable tumor regression and systemic antitumor immunity. Nat Med. 2019;25(7):1057-1063. http://doi.org/10.1038/s41591-019-0498-z
34. Collier RJ. Diphtheria toxin: mode of action and structure. Bacteriol Rev. 1975;39(1):54-85. http://doi.org/10.1128/br.39.1.54-85.1975
35. Shafiee F, Aucoin MG, Jahanian-Najafabadi A. Targeted Diphtheria Toxin-Based Therapy: A Review Article. Front Microbiol. 2019;10:2340. http://doi.org/10.3389/fmicb.2019.02340
36. Shapira A, Benhar I. Toxin-based therapeutic approaches. Toxins (Basel). 2010;2(11):2519-2583. http://doi.org/10.3390/toxins2112519
37. Zheng Q, Wang Z, Zhang H, Huang Q, Madsen JC, Sachs DH, et al. Diphtheria toxin-based anti-human CD19 immunotoxin for targeting human CD19+ tumors. Mol Oncol. 2017;11(5):584-594. http://doi.org/10.1002/1878-0261.12056
38. Li YM, Hall WA. Targeted toxins in brain tumor therapy. Toxins (Basel). 2010;2(11):2645-2662. http://doi.org/10.3390/toxins2112645
39. Elsayad K, Kriz J, Moustakis C, Scobioala S, Reinartz G, Haverkamp U, et al. Total Skin Electron Beam for Primary Cutaneous T-cell Lymphoma. Int J Radiat Oncol Biol Phys. 2015;93(5):1077-1086. http://doi.org/10.1016/j.ijrobp.2015.08.041
40. Zahaf NI, Schmidt G. Bacterial Toxins for Cancer Therapy. Toxins (Basel). 2017;9(8):236. http://doi.org/10.3390/toxins9080236
41. Leshem Y, Pastan I. Pseudomonas Exotoxin Immunotoxins and Anti-Tumor Immunity: From Observations at the Patient's Bedside to Evaluation in Preclinical Models. Toxins (Basel). 2019;11(1):20. http://doi.org/10.3390/toxins11010020
42. Michalska M, Wolf P. Pseudomonas Exotoxin A: optimized by evolution for effective killing. Front Microbiol. 2015;6:963. http://doi.org/10.3389/fmicb.2015.00963
43. Iglewski BH, Liu PV, Kabat D. Mechanism of action of Pseudomonas aeruginosa exotoxin Aiadenosine diphosphate-ribosylation of mammalian elongation factor 2 in vitro and in vivo. Infect Immun. 1977;15(1):138-144. http://doi.org/10.1128/iai.15.1.138-144.1977
44. Kreitman RJ, Hassan R, Fitzgerald DJ, Pastan I. Phase I trial of continuous infusion anti-mesothelin recombinant immunotoxin SS1P. Clin Cancer Res. 2009;15(16):5274-5279. http://doi.org/10.1158/1078-0432.CCR-09-0062
45. Cheng X, Wang B, Jin Z, Ma D, Yang W, Zhao R, et al. Pseudomonas aeruginosa-mannose-sensitive hemagglutinin inhibits pancreatic cancer cell proliferation and induces apoptosis via the EGFR pathway and caspase signaling. Oncotarget. 2016;7(47):77916-77925. http://doi.org/10.18632/oncotarget.12844
46. Li T, Dong ZR, Guo ZY, Wang CH, Zhi XT, Zhou JW, et al. Mannose-mediated inhibitory effects of PA-MSHA on invasion and metastasis of hepatocellular carcinoma via EGFR/Akt/IκBβ/NF-κB pathway. Liver Int. 2015;35(4):1416-1429. http://doi.org/10.1111/liv.12644
47. O'Callaghan A, van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. Front Microbiol. 2016;7:925. http://doi.org/10.3389/fmicb.2016.00925
48. Ngo N, Choucair K, Creeden JF, Qaqish H, Bhavsar K, Murphy C, et al. Bifidobacterium spp: the promising Trojan Horse in the era of precision oncology. Future Oncol. 2019;15(33):3861-3876. http://doi.org/10.2217/fon-2019-0374
49. Wang L, Vuletic I, Deng D, Crielaard W, Xie Z, Zhou K, et al. Bifidobacterium breve as a delivery vector of IL-24 gene therapy for head and neck squamous cell carcinoma in vivo. Gene Ther. 2017;24(11):699-705. http://doi.org/10.1038/gt.2017.74
50. Wei C, Xun AY, Wei XX, Yao J, Wang JY, Shi RY, et al. Bifidobacteria Expressing Tumstatin Protein for Antitumor Therapy in Tumor-Bearing Mice. Technol Cancer Res Treat. 2016;15(3):498-508. http://doi.org/10.1177/1533034615581977
51. Li X, Fu GF, Fan YR, Liu WH, Liu XJ, Wang JJ, et al. Bifidobacterium adolescentis as a delivery system of endostatin for cancer gene therapy: selective inhibitor of angiogenesis and hypoxic tumor growth. Cancer Gene Ther. 2003;10(2):105-111. http://doi.org/10.1038/sj.cgt.7700530
52. Kikuchi T, Shimizu H, Akiyama Y, Taniguchi SI. In situ delivery and production system of trastuzumab scFv with Bifidobacterium. Biochem Biophys Res Commun. 2017;493:306-312.
53. van Geel-Schutten GH, Flesch F, Ten Brink B, Smith MR, Dijkhuizen LJ. Screening and characterization of Lactobacillus strains producing large amounts of exopolysaccharides. Appl Microbiol. 1998;50:697-703.
54. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008;6(11):e280. http://doi.org/10.1371/journal.pbio.0060280
55. JP Tamang. BIOCHEMICAL AND MODERN IDENTIFICATION TECHNIQUES Microfloras of Fermented Foods, Encyclopedia of Food Microbiology (Second Edition), Academic Press, UK, 2014:250-258. https://doi.org/10.1016/B978-0-12-384730-0.00038-0
56. Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E. Lactic acid bacteria: reviewing the potential of a promising delivery live vector

https://doi.org/10.61577/jmi.2024.100001

for biomedical purposes. Microb Cell Fact. 2015;14:137. http://doi.org/10.1186/s12934-015-0313-6
57. Chang WH, Liu JJ, Chen CH, Huang TS, Lu FJ. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by fermented soy milk. Nutr Cancer. 2002;43(2):214-226. http://doi.org/10.1207/S15327914NC432_12
58. Ohta T, Nakatsugi S, Watanabe K, Kawamori T, Ishikawa F, Morotomi M. Inhibitory effects of Bifidobacterium-fermented soy milk on 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine-induced rat mammary carcinogenesis, with a partial contribution of its component isoflavones. Carcinogenesis. 2000;21(5):937-941.
59. Bazylinski DA, Williams TJ, Lefevre CT, Berg RJ, Zhang CL, Bowser SS, et al. Magnetococcus marinus gen. nov., sp. nov., a marine, magnetotactic bacterium that represents a novel lineage (Magnetococcaceae fam. nov., Magnetococcales ord. nov.) at the base of the Alphaproteobacteria. Int J Syst Evol Microbiol. 2013;63(3):801-808. http://doi.org/10.1099/ijs.0.038927-0
60. Afkhami F, Taherkhani S, Mohammadi M, Martel S. Encapsulation of magnetotactic bacteria for targeted and controlled delivery of anticancer agents for tumor therapy. Annu Int Conf IEEE Eng Med Biol Soc. 2011;2011:6668-6671. http://doi.org/10.1109/IEMBS.2011.6091644
61. Felfoul O, Mohammadi M, Taherkhani S, De Lanauze D, Zhong Xu Y, Loghin D, et al. Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumor hypoxic regions. Nat Nanotechnol. 2016;11(11):941-947. http://doi.org/10.1038/nnano.2016.137
62. Yu B, Yang M, Shi L, Yao Y, Jiang Q, Li X, et al. Explicit hypoxia targeting with tumor suppression by creating an "obligate" anaerobic Salmonella Typhimurium strain. Sci Rep. 2012;2:436. http://doi.org/10.1038/srep00436
63. Wei MQ, Ellem KA, Dunn P, West MJ, Bai CX, Vogelstein B. Facultative or obligate anaerobic bacteria have the potential for multimodality therapy of solid tumors. Eur J Cancer. 2007;43(3):490-496. http://doi.org/10.1016/j.ejca.2006.10.005
64. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. Nat Rev Clin Oncol. 2017;14(2):113. http://doi.org/10.1038/nrclinonc.2017.1
65. Sedighi M, Zahedi BA, Hamblin MR, Ohadi E, Asadi A, Halajzadeh M, et al. Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. Cancer Med. 2019;8(6):3167-3181. http://doi.org/10.1002/cam4.2148
66. Jain RK, Forbes NS. Can engineered bacteria help control cancer?. Proc Natl Acad Sci USA. 2001;98(26):14748-14750. http://doi.org/10.1073/pnas.261606598
67. Carlisle R, Coussios CC. Mechanical approaches to oncological drug delivery. Ther Deliv. 2013;4(10):1213-1215. http://doi.org/10.4155/tde.13.94
68. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Res. 1998;58(7):1408-1416.
69. Cheong I, Zhou S. Tumor-specific liposomal drug release mediated by liposomase. Methods Enzymol. 2009;465:251-265. http://doi.org/10.1016/S0076-6879(09)65013-8
70. Nallar SC, Xu DQ, Kalvakolanu DV. Bacteria and genetically modified bacteria as cancer therapeutics: Current advances and challenges. Cytokine. 2017;89:160-172. http://doi.org/10.1016/j.cyto.2016.01.002
71. Yazawa K, Fujimori M, Amano J, Kano Y, Taniguchi S. Bifidobacterium longum as a delivery system for cancer gene therapy: selective localization and growth in hypoxic tumors. Cancer Gene Ther. 2000;7(2):269-274. http://doi.org/10.1038/sj.cgt.7700122
72. Chakrabarty AM. Microorganisms and cancer: quest for a therapy. J Bacteriol. 2003;185(9):2683-2686. http://doi.org/10.1128/JB.185.9.2683-2686.2003
73. Casadevall A, Pirofski LA. Virulence factors and their mechanisms of action: the view from a damage-response framework. J Water Health. 2009;7(Suppl 1):S2-S18. http://doi.org/10.2166/wh.2009.036
74. Cross AS. What is a virulence factor? Crit. Care. 2008;12:196.
75. Lee CH, Lin ST, Liu JJ, Chang WW, Hsieh JL, Wang WK. Salmonella induce autophagy in melanoma by the downregulation of AKT/mTOR pathway. Gene Ther. 2014;21(3):309-316. http://doi.org/10.1038/gt.2013.86
76. Glomski IJ, Gedde MM, Tsang AW, Swanson JA, Portnoy DA. The Listeria monocytogenes hemolysin has an acidic pH optimum to compartmentalize activity and prevent damage to infected host cells. J Cell Biol. 2002;156(6):1029-1038. http://doi.org/10.1083/jcb.200201081
77. Chagnon A, Hudon C, McSween G, Vinet G, Fredette V. Cytotoxicity and reduction of animal cell growth by Clostridium M-55 spores and their extracts. Cancer. 1972;29(2):431-434. https://doi.org/10.1002/1097-0142(197202)29:2%3C431::aid-cncr2820290226%3E3.0.co;2-z
78. Felgner S, Kocijancic D, Frahm M, Weiss S. Bacteria in Cancer Therapy: Renaissance of an Old Concept. Int J Microbiol. 2016;2016:8451728. http://doi.org/10.1155/2016/8451728
79. Fronzes R, Christie PJ, Waksman G. The structural biology of type IV secretion systems. Nat Rev Microbiol. 2009;7(10):703-714. http://doi.org/10.1038/nrmicro2218
80. Singer HM, Erhardt M, Steiner AM, Zhang MM, Yoshikami D, Bulaj G, et al. Selective purification of recombinant neuroactive peptides using the flagellar type III secretion system. mBio. 2012;3(3):e00115-12. http://doi.org/10.1128/mBio.00115-12
81. Farley MM, Hu B, Margolin W, Liu J. Minicells, Back in Fashion. J Bacteriol. 2016;198(8):1186-1195. http://doi.org/10.1128/JB.00901-15
82. Fensterle J, Bergmann B, Yone CL, Hotz C, Meyer SR, Spreng S, et al. Cancer immunotherapy based on recombinant Salmonella enterica serovar Typhimurium aroA strains secreting prostate-specific antigen and cholera toxin subunit B. Cancer Gene Ther. 2008;15(2):85-93. http://doi.org/10.1038/sj.cgt.7701109
83. Paton AW, Morona R, Paton JC. Bioengineered microbes in disease therapy. Trends Mol Med. 2012;18(7):417-425. http://doi.org/10.1016/j.molmed.2012.05.006
84. Grillot-Courvalin C, Goussard S, Courvalin P. Wild-type intracellular bacteria deliver DNA into mammalian cells. Cell Microbiol. 2002;4(3):177-186. http://doi.org/10.1046/j.1462-5822.2002.00184.x
85. Miyake K, Murata T, Murakami T, Zhao M, Kiyuna T, Kawaguchi K, et al. Arch Gynecol Obstet. 2019;299(6):1683-1690. http://doi.org/10.1007/s00404-019-05147-3
86. Duong MT, Qin Y, You SH, Min JJ. Bacteria-cancer interactions: bacteria-based cancer therapy. Exp Mol Med. 2019;51(12):1-15. http://doi.org/10.1038/s12276-019-0297-0
87. Rabie AM. RNA: The most attractive target in recent viral diseases. Chem Biol Drug Des. 2024;103(1):e14404. http://doi.org/10.1111/cbdd.14404
88. Rabie AM. Potent Inhibitory Activities of the Adenosine Analogue Cordycepin on SARS-CoV-2 Replication. ACS Omega. 2022;7(3):2960-2969. http://doi.org/10.1021/acsomega.1c05998
89. Rabie AM, Tantawy AS, Badr SMI. Design, Synthesis, and Biological Evaluation of Novel 5-Substituted-2-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazoles as Potent Antioxidants. Am J Org Chem. 2016;6(2):54-80. http://doi.org/10.5923/j.ajoc.20160602.02
90. Rabie A, Tantawy A, Badr S. Design, Synthesis, and Biological Evaluation of New 5- Substituted-1,3,4-thiadiazole-2-thiols as Potent Antioxidants. Eur Res. 2018;10:21-43. http://doi.org/10.7537/marsrsj100718.04
91. Nashaan FA, Al-Rawi MS, Alhammer AH, Rabie AM, Tomma JH. Tomma J. Synthesis, characterization, and cytotoxic activity of some imides from galloyl hydrazide. Eurasian Chem Commun. 2022;4(10):966-975. http://doi.org/10.22034/ecc.2022.340135.1453

https://doi.org/10.61577/jmi.2024.100001