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The Application of Oncolytic Bacteria for Cancer Treatment

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Abstract

THIS review discusses the anticancer activity of oncolytic bacteria against various types of cancer. Cancer is the most prevalent life-threatening disease globally. "Coley's toxins" are considered as the foundation for bacterial anticancer therapy. Due to the indiscriminate toxicity of numerous anti-cancer medications towards healthy cells, scientists are currently investigating bacterial metabolites in order to identify targeted and specific treatments for cancer that lessen undesired side effects. Bacterial- based cancer treatment has a notable impact, either through direct tumor cell destruction or by modulating cellular processes, resulting in the inhibition and regression of solid tumors. Different bacterial genera could be used for this purpose including *Listeria*, *Clostridium*, *Bacillus*, *Bifidobacterium*, *Salmonella*, *E. coli*, etc. which can function as obligatory or facultative anaerobes. These bacteria possess the capacity to specifically target and proliferate within the tumor tissues. This unique ability enables them to effectively eradicate tumor cells and stimulate an immune reaction against the tumor. Recent progress in molecular techniques has facilitated the development of genetically modified non-pathogenic bacteria for use in bacterial anticancer approaches. While promising outcomes have been observed, further research is necessary to ensure the effectiveness and safety of oncolytic bacteria as an alternative cancer treatment option.

Keywords: Cancer, Oncolytic bacteria, Bacterial toxin, Biofilm.

Introduction

Cancer is the most cause of life-threatening cases all over the globe [1]. According to the World Health Organization, cancer is considered as the second killer disease globally and is the cause for around 10 million deaths annually [1] . In 2050, the number of cancer diseased individuals expected to be 35 million, a 77% rise from the 20 million confirmed cases reported in 2022 (https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services accessed on December 4, 2024). The defining characteristics of cancer include uncontrolled and deregulated cell growth, invasive, immortalization, proliferative, and metastasis [2]. Cancer can occur in almost any organ or tissue however they are mostly encountered in the ovary, breast, prostate, stomach, pancreas, liver, brain, lung, and bone marrow. Due to the oncogenic signaling and genetic variation, tumors in different organs or tissues display distinct behaviors (https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services accessed on December 4,2024). Treatment options for cancer encompass immunotherapy, radiation therapy, conventional chemotherapy, surgical procedures. However, due to the resistance developed by many cancer cells, including those not confined to tumors, extensive research has been conducted over the past few decades to explore natural products derived from animals, plants, and microorganisms [3].

The utilization of bacteria and their metabolites as anti-cancer agents originated with the observations made by Busch. They noticed that certain cancers have undergone regression in hospitalized individuals who unintentionally contracted erysipelas infections caused by *Streptococcus pyogenes* [4]. In 1893, William Coley discovered that a sarcoma patient had completely recovered following an accidental erysipelas infection [5]. "Coley's toxins" which was developed by Coley [5], gained significant popularity, and was extensively employed for the treatment of carcinomas, sarcomas, lymphomas, melanomas, and myelomas [5]. The initial achievements of Coley's toxins played a pivotal role in the progression of this field.

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Microorganisms engage in diverse interactions, forming the human body microbiota, which contributes a fundamental role in maintaining the health of humans. The dominant microbiota, namely the oral cavity, vagina, gut, and skin, have been recognized as responsible for distinct aspects of human well-being [6]. Imbalance within these microbiotas is linked to persistent immune response activation and the generation of carcinogens derived from bacterial metabolites. This imbalance raises the likelihood of developing various conditions, such as inflammatory bowel disease (IBD), allergies, obesity, diabetes, cancer, and neurological illnesses [7]. The human body harbors commensal microbiota both externally and internally. Interestingly, bacteria have been found in certain regions previously believed to be sterile, such as tumors, placenta, breast milk, and blood. This discovery implies the possible presence of beneficial microbes (probiotics) or harmful organisms (pathogens) in these areas, offering insights that could aid in the diagnosis and curation of tumors [8].

During the early 19th century, researchers initially identified wild-type *Clostridium perfringens* (*C. perfringens*) in cancer patients, and they conducted experiments by introducing anaerobic bacteria (live) into animal models to investigate their ability to selectively destroy tumors [9]. Subsequently, culturable bacteria such as *Salmonella* Typhimurium (*S.* Typhimurium) and *Listeria monocytogenes* (*L. monocytogenes*) were engineered using advanced gene manipulation techniques. These modified bacteria were designed to specifically target and eliminate tumor cells through the expression of tumor-linked antigens, pro-drug-converting enzymes, or tumor-toxic agents [10]. As a result, investigating microbial makeup residing within tumors and understanding their capabilities such as oncolytic bacteria or those capable of producing proteins or other metabolites directly noxious to cancer cells has emerged as a fascinating area of research [11]. The aim of this review is to explore the role of oncolytic bacteria in cancer management and some of the mechanisms involved.

Short Overview of Cancer

Cancer, which ranks as the second most significant contributor to global mortality, has seen a rise in its occurrence. In the United States alone, around 1,665,540 individuals were affected by cancer, resulting in 585,720 fatalities in 2014 (https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2014.html) accessed on December 2024). Hence, cancer poses a grave concern to the well-being of human communities. Regrettably, its diverse nature at the tissue level presents a significant hurdle in accurately diagnosing the specific type of cancer, which further complicates the effectiveness of treatment options. Among males, the most prevalent types of cancer are observed in the prostate, rectum & colon, bronchus & lungs, and urinary bladder, in descending order of occurrence [12].

Among females, the most prevalent cancer types are found in the breast, lungs and bronchus, colon and rectum, uterine corpus, and thyroid, respectively. These findings highlight that prostate cancer and breast cancer are significant contributors to the overall cancer burden in men and women, respectively [13].

Chemical compounds play a clear role in the development of gene mutations and cancer cells. Notably, smoking exposes individuals to various carcinogenic chemicals, which significantly contribute to the occurrence of lung cancer. Intriguingly, environmental chemical substances possessing carcinogenic properties have a direct or indirect impact on the cytoplasm and nucleus of cells, resulting in genetic disorders and gene mutations [14].

Viruses, bacteria, and exposure to radiation are additional factors in the development of cancer, accounting for approximately 7% of all cancer cases. Generally, cancer disrupts the normal interactions between cells and impairs crucial genes. This disruption significantly affects the cell cycle, leading to abnormal and uncontrolled cell growth [15].

Proto-oncogenes play a crucial role in regulating cell division and growth in normal conditions. However, when subjected to genetic mutations, these proto-oncogenes can transform into oncogenes, posing a significant threat to the survival of cells. Furthermore, the absence or malfunction of tumor suppressor genes can result in uncontrolled cell division [16]. Genetic alterations responsible for the development of oncogenes and genetic disorders encompass various mechanisms, such as amplification (N-myc in neuroblastoma), chromosomal translocation (e.g., gene Bcr and oncogene Abl in chronic blood cancer), deletion (Erb-B gene in breast cancer), point mutation (Ras gene in colon cancer), and insertion activation (C-myc in acute blood cancer) [17]. Mutations in the p53 gene result in the production of an atypical protein that significantly disrupts the molecular processes associated with p53. These abnormalities in molecular and biological events contribute to the formation of cancer cells. As a result, the p53 gene has an intricate connection with cancer, and it has been observed that p53 abnormalities are present in approximately 60% of cancer cases [18].

Historical evidence and the role of bacteria as anticancer agent

The origins of bacterial based cancer therapy can be traced back to a significant milestone in 1813, whereby Vautier documented tumor regression in

individuals who had gas gangrene following infection with *C. perfringens* [9].

After some decades, William B. Coley, a pioneering surgeon from New York, devoted himself to finding a cure for cancer through immunotherapy. His motivation stemmed from the loss of his first cancer patient, a young girl afflicted with sarcoma in her right arm [19]. He discovered the most common association between infectious diseases and cancer involved sarcoma patients with erysipelas [19]. This serendipitous finding led to further studies on cancer treatment through direct injections of heat-killed Streptococci broth cultures, culminating in the development of Coley's toxin (a combination of heat-killed *Serratia marcescens* and *Streptococcus*) [19]. Over the following four decades, Coley administered immunotherapy to hundreds of diseased individuals with inoperable sarcomas [20]. These discoveries sparked curiosity and led to an exploration of the therapeutic potential of anaerobic bacteria present in the hypoxic regions of neoplastic tissues. Throughout time, numerous facultative anaerobic or obligate bacteria have undergone testing with the aim of targeting tumor cells and promoting tumor regression. These bacteria have been observed to exert their anti-tumor effects through various mechanisms. These include the administration of direct noxiousness to tumor cells via type III secretions, wherein cytotoxic compounds are introduced directly into the cytoplasm of the targeted cells [21]. Additionally, these bacteria facilitate non-specific immune responses, deplete crucial nutrients, and modify the tumor minienvironment through bacterial colonization. Furthermore, immunomodulatory properties such as the activation of dendritic cells or changes in T helper cell polarization may also contribute [22].

There have been previous instances where certain microorganisms were employed in the treatment of cancer, while others are currently undergoing clinical trials. The critical factors in creating bacterial therapy involve carefully choosing a specific type of bacteria and ensuring their slight harm to the host cell [23]. Bacteria, whether attenuated, live, or genetically modified non-pathogenic strains, can serve as immunotherapeutic weapon with direct anti-tumor effects (Table 1) [23]. Additionally, various attenuated bacteria are utilized in cancer therapy. The utilization of attenuated bacteria that can reproduce in necrotic tissues presents several benefits in radio and chemotherapy. *S.* Typhimurium and *L. monocytogenes* are among the commonly employed attenuated variants in cancer therapy [24]. In experiments conducted on mouse models, the attenuated strain VNP20009 of S. typhimurium demonstrated significant inhibitory activity on the proliferation of tumor and metastasis [25]. Moreover, recombinant *S*. Typhimurium accelerated *stx2* mediated tumor necrosis specifically in the acidic minienvironment of the tumor [25].

Microbes do not completely utilize all components of malignant areas, which highlights the necessity of combining therapy with chemotherapy. Hence, microbes can serve as sensitizing entities for drug-based treatment. Some bacterial preparations, like endotoxins, have been moderately explored for treatment of cancer. Bacterial toxins can be utilized to destroy tumors, and cancer vaccines can be developed based on immunotoxins derived from bacteria [26]. Bacteria can be utilized as carriers for delivering anticancer drugs and as vehicles for gene therapy. Anaerobic bacterial spores are particularly suitable for these approaches, as only spores that reach oxygen-deprived regions within a tumor will sprout, multiply, and become effective in their actions. Genetically engineered bacteria have demonstrated promising potential in selectively targeting and destroying tumors, as well as in bacterial gene-directed enzyme prodrug therapy. The following sections provide a comprehensive overview of these innovative approaches involving bacteria (Table 1).

Bacterial structures and products linked to anti-cancer activities

The mechanism of tumor suppression differs depending on the specific type of bacteria employed in the treatment (Fig. 1). The targeting of bacteria to tumors can occur via passive or active mechanisms [27].

At present, it is believed that bacteria evade the bloodstream and migrate towards the tumor location. Primarily, bacteria may introduce the tumor passively by becoming trapped within the disorganized tumor blood vessels, and subsequently, they are carried into the cancer cell due to the inflammation induced by a swift rise in TNF-α within the tumor vasculature [28]. Bacteria have the ability to utilize both pathways for targeting tumor sites. They can directly infect several immune cells such as dendritic cells (DCs) and others [29].

Active process can also include chemotaxis, where bacteria are drawn towards chemical constituents generated by dying cancer tissues. This process is particularly observed in hypoxic cancer cells that have minimal oxygen levels, attracting obligate anaerobic bacteria like *Clostridium* and *Bifidobacterium* [30].

To understand the gathering of *S*. Typhimurium in a tumor, a mathematical cylindroid model was created to investigate the chemotaxis process that directs the relative assistant of proliferation and chemotaxis. While the actual mechanism of action of selective homing is not yet fully comprehended, it appears that chemotaxis and hemodynamics are involved in this process [31].

The ability of bacteria to move is a crucial characteristic that allows them to internalize proficiently into tumor tissues. Bacteria can actively swim and propel themselves away from the blood vessels, enabling them to disperse and distribute throughout the tumor tissues [31].

Documented findings point out that *Salmonella* is capable of infiltrating tumor tissue and specifically targeting metastases in mouse models. Within three days of bacterial administration, *Salmonella* began to establish colonies in the tumor and disperse all over the tumor microenvironment [31]. Bacteria serve as immunotherapeutic tools based on the rationale that they induce an inflammatory reaction in diseased individuals. In 1867, Wilhelm Busch, a German physician, intentionally infected a cancer diseased individual with erysipelas caused by *Streptococcus pyogenes*, resulting in rapid tumor regression [4].

Anaerobic microorganisms possess a distinct capability to selectively thrive in the hypoxic, anaerobic area of hard tumors, which are often inaccessible to conventional drugs. Additionally, through molecular manipulation, modifications can be made to Salmonella and *Clostridium novyi*, rendering them capable of infecting solely with the hypoxic tumor tissues while remaining non-viable exterior the hypoxic cancer minienvironment [32]. Experimental investigations have revealed that bacteriocin and phenazine enzymes secreted by bacteria exhibit anticancer effects against various cancer cell lines [33]. Additionally, microbial products such as lipopolysaccharide (LPS)-based vaccines have demonstrated anticancer properties as well [34]. Research experiments have provided evidence that bacterial toxins can effectively hinder the growth of cancer cells [35]. Bacterial toxins exhibit dual functionality towards cancer cells, relying on their concentration. At higher concentrations, these toxins induce cancer cell death, while at lower concentrations, they modulate cellular processes by regulating cell apoptosis, proliferation, and cell differentiation [35].

Bacterial toxins could interfere with or facilitate the eukaryotic cell cycle, known as "cyclomodulins". Specifically, cell-cycle inhibiting factors (Cifs) and cytolethal distending toxins (CDTs) impede the process of mitosis and hinder the clonal development of lymphocytes [36]. The antitumor effects of these toxins can be classified into two categories: toxins conjugated to ligands and tumor surface antigens. For instance, enterotoxins like *Clostridium perfringens* enterotoxin (CPE) directly bind to receptors such as CLDN 3 and 4, inhibiting tumor growth by regulating these receptors [37]. Diphtheria toxin (DT) attaches to tumor-specific antigens present on the surface of cells via receptors and becomes activated. Some recombinant toxins could fuse with a ligand that specifically attaches to receptors found exclusively on the target cell [38]. The potent diphtheria toxin (DT) produced by *Corynebacterium diphtheriae* (*C. diphtheriae*) binds to receptors on the cell surface and subsequently triggers antitumor pathways [38]. Subsequently, the DT is adjusted into recombinant DT3895, which specifically attaches to the surface of different cancer cells and exhibits cytotoxic properties. DT3895 demonstrated anti-angiogenic action and displayed cytotoxic activities towards 18 human cancer cell lines, effectively inhibiting human, and animal tumors [39].

C. perfringens-produced CPE toxins swiftly induce cytolysis by directly binding to the upregulated CLDN3 and CLDN4 receptors found in tumor cells, thereby substantially impeding tumor growth [40]. Like DT, another toxin derived from *Pseudomonas aeruginosa* (*P. aeruginosa*), called exotoxin T, exhibited cytotoxic effects against various human and murine cancer cell lines [41]. In a mouse model, the cytotoxicity of *Pseudomonas* exotoxin A is documented to be efficient in cancer therapy, with a lethal dose of 0.3 μg [42].

Immunotoxins belong to a category of targeted cancer treatments where a toxin, such as Pseudomonas exotoxin A (PE), is correlated with cytokine or antibody. This linking allows the toxin to be directed specifically towards targets on cancer cells [43]. "Immunotoxin therapy" represents a promising strategy in the field of cancer treatment. Ongoing research aims to identify protein molecules that can be combined with immunotoxins, exhibiting minimal immunogenicity and high potency in selectively eliminating cancer cells [44]. By employing prodrug-converting enzymes, prodrugs can be transformed into cytotoxic agents within the tumor area. This approach can be accomplished by utilizing bacteria to enhance the success of cancer medication while minimizing the adverse effects typically linked with systemic introduction [44].

The enzyme cytosine deaminase (CD), which acts as a prodrug-translating enzyme, transforms the non-toxic 5-fluorocytosine (5-FC) into the chemotherapeutic agent 5-fluorouracil (5-FU). This conversion has been demonstrated to significantly reduce tumor growth [45].

The introduction of the attenuated *S.* Typhimurium strain (VNP20009) expressing *E. coli* cytosine deaminase (CD) and 5-fluorocytosine (5-FC) to patients resulted in the production of functional CD within the tumor area. This process is highly toxic as it disrupts RNA and DNA synthesis [46].

Peptides and other metabolic products, such as bacteriocins, refer to peptides and proteinaceous toxins that are released by bacteria [47]. These compounds demonstrate synergistic effects when used in combination with conventional cancer drugs. Bacteriocins exhibit a preference for binding to the

membrane of cancer cells rather than normal cells. This selective binding can be attributed to the negative charge present on the cancer cell membrane [48]. Among bacteriocins, colicins, which are extensively studied and formed by Enterobacteriaceae and *E. coli*, have exhibited anticancer potential in *in vitro* investigation [33].

In general, some of the detailed mechanisms of oncolytic bacteria are discussed below.

Biosurfactants

Biosurfactants are present on the surface of microorganisms and are composed of both hydrophobic and hydrophilic components [49]. They can be categorized into low and high molecular weight surfactants, because of their chemical structure and mechanism of action. Low molecular weight biosurfactants exhibit higher surface-active properties due to their simpler chemical structure. Rhamnolipids (glycolipids) and surfactin (lipopeptides) are among the most broadly investigated surfactants in this category [50]. Surfactin, a cyclic lipo-peptide developed by bacteria such as *Bacillus subtilis*, is a biodegradable biosurfactant derived from β-hydroxy fatty acids containing 13–15 carbon atoms [51]. It possesses a heptapeptide structure and exhibits lower toxicity compared to chemical surfactants. Surfactin has been utilized to demonstrate its effectiveness in inhibiting tumor growth and displaying anticancer activity. It can impede the proliferation of cancer cells and induce apoptosis, a non-inflammatory form of cell death [52].

Generally, the anti-cancer role of biosurfactants is associated with the ability of the agent to impede the growth of cancer cells[53], influence on differentiation [54], trigger apoptosis [55], and induce cytotoxicity [56], and halt the process of cell cycle [57].

Bacterial Biofilms

A biofilm refers to a dense collection of colonies that are tightly bound to biological or non-biological surfaces through an extracellular polymeric matrix. The formation of biofilms is controlled by a process called quorum sensing, which allows bacteria to survive and thrive in their host environment [58]. The biofilm of bacteria holds potential as an anticancer agent because it can effectively deliver therapeutic agents and confine the dispersion of tumors. The administration of anticancer drugs like doxorubicin and hydroxyurea, commonly employed in cancer treatment, has been found to stimulate and enhance the formation of biofilms in *P. aeruginosa* [59]. Bacterial cells that develop as biofilms on tumor cells are activated by the SOS response, which is a DNA repair system that can be induced. As a result, the bacteria acquire phenotypes that facilitate the invasion or penetration of cancer cells [60]. The essential bacterial macromolecules like DNA and proteins, required for the development of biofilms, form a protective coating on cancer cells to hinder metastasis [61]. Additionally, the release of polysaccharides by the bacterium *Streptococcus agalactiae* impedes the adherence of tumor cells to endothelial cells (Fig. 2) [62].

Bacteria-based Microrobot (Bacteriobot)

A bacteriobot is a novel drug delivery system known as a bacteriobot, which utilizes bacteria-encapsulated microbeads which are fabricated using a decomposable and environmentally friendly material and flagellated bacteria to enable the chemotaxis of the microrobot which is going to be attached to the microbeads. This system offers significant benefits like precise tumor targeting, bacteria-assisted tumor detection, and therapy [63].

This new innovative technique uses bacteria (attenuated) as microsensors and micro actuators to deliver microstructures for targeting and treating solid tumors [64]. The convergence of technologies from micro electromechanical systems with nano- and biotechnologies has led to the creation of various types of biomedical microrobots [65]. An effort to advance microrobot therapy involved the fabrication of a bacteria-based microrobot for targeted tumor treatment by [64]. They achieved this by encapsulating therapeutic agent (*S*. Typhimurium) in alginate microbeads, with additional flagellated bacteria (*S*. Typhimurium) attached to the microbeads. This encapsulated delivery system shields the bacteria from immune system attacks, making it safer compared to directly inoculating bacteria into the body [66].

Bacterial toxins

Natural and genetically engineered bacterial toxins possessing anti-tumor properties have gained acknowledgment as viable alternative agents for combatting advanced solid tumors in the field of cancer treatment [67]. The toxins produced by bacterial pathogens have displayed considerable therapeutic responses against cancer cells via a broad range of mechanisms of actions, including the induction of apoptosis and change to the human cell division cycle and differentiation. By targeting and disrupting critical pathways in cancerous cells, these toxins can obstruct the propagation and proliferation of cancer cells. For instance, diphtheria toxin, which demonstrates oncolytic characteristics by halting the function of heparin-binding epidermal growth factor, an essential element in the proliferation of cancer cells [68]. Likewise, *Clostridium difficile* toxins, particularly cytotoxins like TcdB, display cancer-inhibiting effects by triggering the synthesis of cytokines and proinflammatory chemokines, promoting necrosis, and promoting apoptosis in tumor cells [69]. These findings emphasize the potential of bacterial toxins as therapeutic agents,

leveraging their ability to interfere with cancerous mechanisms at a cellular level.

Moreover, other toxins secreted by bacterial cells have displayed considerable anticancer activity through distinct modes of action. *E. coli* Verotoxin 1 demonstrates inhibitory effect against cancer cell growth and protein synthesis by interfering with the S phase of the cancer cell cycle, thereby halting their proliferation [70]. Another toxin produced by P. aeruginosa, Exotoxin A, synthesized by similar interferes with the synthesis of protein but uniquely induces apoptosis specifically in cancer cells, illustrating its tailored oncolytic potential against different types of cancer [71].

Cancer cells frequently exhibit a substantial presence of tumor-specific antigens on their surfaces, which serve as binding sites for bacterial toxins. Once bound to these antigens, the bacterial toxins become activated. Among the bacterial toxins employed as cell-targeted agents are botulinum neurotoxin type A, exotoxin A, and listeriolysin LLO [72]. Toxins derived from bacterial cells could potentially offer an anti-tumor effect with fewer side effects compared to conventional tumor treatments. Bacterial toxins, either alone or in combination with anti-cancer drugs or radiation therapy, have the potential to enhance the effectiveness of cancer treatment [73].

Researchers have investigated the impact of PE on various murine and human cancer cell lines. This toxin possesses the capability to inhibit protein synthesis in mammalian cells, making it a subject of interest in cancer-related studies (Zahaf and Schmidt, 2017). To address HER2-overexpressing tumors, scientists have employed a therapeutic agent called HER2-Affitoxin, which is a combination of modified *P. aeruginosa* exotoxin A (PE 38) and HER2-specific Affibody. This approach has been utilized for the treatment of such tumors [74].

Due to its significant toxicity, DT obtained from *C. diphtheria* was chosen as the basis for creating the initial immunotoxin, known as denileukin diftitox (ONTAK) [75]. DT, a 535 amino acid exotoxin, attaches itself to the heparin-binding epidermal growth factor precursor (HB-EGF) present on the cell surface. It can be split into two primary fragments: DTA and DTB. The DTB fragment facilitates cell entry by binding to surface receptors and undergoing endocytosis to reach the cytoplasm. On the other hand, the DTA fragment is responsible for cytotoxic enzymatic activity, disrupting protein synthesis, and leading to cell death [76].

Clostridium perfringens type A strain, known for causing gastroenteritis, produces CPE. The C-terminal region of CPE is responsible for strongly binding to the CPE receptor (CPE-R), while the N-terminal region is believed to play a crucial role in its cytotoxic effects [77]. Research has shown that purified CPE exhibits a rapid cytotoxic impact on pancreatic cancer cells, leading to tumor necrosis and suppression of tumor growth in animal studies. Currently, investigations are underway to explore its potential application in treating colon, breast, and gastric cancers. However, before considering CPE for systemic cancer therapy, it is essential to demonstrate its long-term effectiveness and lack of toxicity *in vivo* [78, 79].

An alternative method involves creating genetically changed toxins. This is accomplished by removing the DNA responsible for the toxin's binding region and substituting it with different complementary DNA sequences encoding alternative cell-binding proteins. Through this process, chimeric toxins can be developed, capable of targeting cells based on the newly acquired binding activity. The capability to produce such chimeras holds significant potential in the design of future toxin-based therapies for cancer treatment. In one study, a novel treatment approach for glioblastoma involves the development of a recombinant interleukin-4-*Pseudomonas* exotoxin (IL4-PE). *In vivo* studies conducted on nude mice have shown that IL4-PE exhibits substantial antitumor effects against a human glioblastoma tumor model [80].

Clinical trials

According to documented reports the attenuated *S*. Typhimurium VNP20009 strain designed by Vion Pharmaceutics, Inc., was reported to be the first *Salmonella* strain to enter phase I human clinical trials. The therapeutic value of this strain was evaluated in 24 patients with metastatic melanoma and in one patient with metastatic renal carcinoma. Analysis of increasing doses (1×10^6 –1×10^9 CFU/m^2 delivered via intravenous injection) revealed that the maximum-tolerated dose) was 3.0×10^8 CFU/m^2. Despite rising concentrations of several proinflammatory cytokines (such as IL-6, TNF-α, IL-1β and IL-12) and tumour colonization were found in some patients, no significant regression of tumour was recorded, even in patients with colonized tumours [81]. Another clinical trial with *S*. Typhimurium VNP20009 was performed with four additional metastatic melanoma patients, but there was no significant tumour response, and only transient and minor side effects were displayed [81]. To enhance its oncolytic efficacy, VNP20009 was manipulated to express *E. coli* CD, which activates 5-FC to lethal 5-FU. Three increasing doses were administered via intratumoral injection for three patients experiencing oesophageal adenocarcinoma and neck and head squamous carcinoma (3×10^6, 1×10^7, or 3×10^7 CFU/m^2) for multiple cycles, 100 mg/kg/day 5-FC was delivered for three consecutive days orally for multiple cycles. Results demonstrated that two patients showed tumour colonization for at least 15 days after the initial exposure, with a plasma -to-tumour of 3:1, this ratio

was <1.0 in the noncolonized patient. It has been also realized that no significant drawbacks were observed after six treatment cycles. Lately, there have been four other unpublished and completed phase I clinical trials using *S*. Typhimurium (One clinical trial with *S*. Typhimurium χ4550 expressing IL-2 and three clinical trials with attenuated VNP20009). According to the results of these clinical trials discrepancies between the outcomes in preclinical animal models and human patients might be owing to differences in tumor structure and growth rates that could alter bacterial penetration, proliferation, and clearance within tumors as well as in the peripheral circulation. Another lesson learned from the clinical trials using *Salmonella* spp. is that TLR4-mediated signaling might be important for tumor colonization and antitumor activity, as a VNP20009 strain lacking lipid A function failed to colonize tumors sufficiently enough to suppress tumor growth.

The laboratory investigation performed by Möse and colleagues [83]indicated that the oncolytic Clostridium sporogenes ATCC 13732 (lately called *Clostridium butyricum* M-55) has entered phase I clinical trials with a large number of patients and showed promising results.

Apart from the above-mentioned reports the pre-clinical and clinical publications result of various oncolytic bacteria have been reported in different publications [84], [85].

Advantages and disadvantages of Bacteriolytic therapy

Bacteriotherapy, like other conventional therapeutic options, has several positive and negative consequences. Bacterial-based therapies offer the advantage of lower toxicity and fewer side effects on healthy cells. Besides, bacterial biofilms and biosurfactants demonstrate promising anticancer potential due to their selective targeting and bioactive properties [86].

One of the negative impacts of oncolytic therapy is that bacteria's pathogenicity leads to fatal infection in patients who received the therapeutic agent. Several investigations have exploited alerted, weakened, or genetically manipulated species to get rid of these restrictions. Another drawback of oncolytic therapy is the short half-life of bacterial proteins and peptides unstable-mutatable DNA [87]. Various studies have confirmed that utilizing genetic manipulation to improve bactericidal agents' effectiveness by research can enhance antitumor effects of the oncolytic method. For instance, in some investigation, scientists have consumed biochemical changes and reactions such as D-amino acid replacement, unstable amino acid replacement, etc., to enhance the shelf life and stability of bactericidal agent [88].

As mentioned, a tremendous obstacle to applying bacteria-derived medications as anticancer compounds is their low cytotoxicity at the dose required for therapeutic efficacy. Furthermore, systemic bacterial infections can be a significant risk factor for living organisms. Besides, even deletion of genes encoding the toxin and virulence factors could result in the death of about 15–45% in test mice [89]. Another significant challenge to applying bacteria for cancer treatment is incomplete tumor lysis. The oncolytic agent may not lyse the cancerous tissue and may result in efficient eradication. Hence, the administration of concomitant therapies such as bacteriotherapy coupled with chemotherapy is crucial to attain the required results. The other big barrier regarding the bacteria based oncolytic therapy is that it is challenging to treat small non-necrotic tumors from the metastasis of large tumors. Due to the physiological problems and communication knots with hypoxia in these cancerous tissues, it is difficult for bacteria to target these issues accurately. In bacterial therapy based on live bacteria, the main problem is the lack of accurate access of these bacteria to tumors since, in most cases, an intra-tumor infusion is required [90, 91]. Another primary risk of bacterial immunotherapy is the chance for modifications in nucleic acids and DNA mutations. Suppose the host body's bacterial DNA has a mutation or its genetic material changes through nucleic acid modification. This can lead to the bacterium losing its original anticancer function because of the conversion.

Conclusion

Bacterial-derived natural products have played a prominent role in the development of cancer drugs, leading to the creation of numerous clinically beneficial compounds. Several of these natural products have shown significant inhibitory effects on the growth of human cancer cell lines in laboratory settings, and specific compounds have demonstrated therapeutic advantages in *in- vivo* models of human cancer. Extensive research is required to further explore and expand their diverse and crucial role in contemporary medicine.

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Conflict of interest

All the authors declare no conflict of interest.

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TABLE 1. Anti-cancer activity of some bacteria strains

Bacterial species	Anticancer effects	References
Clostridium histolyticum	Significant reduction of the size of the tumor generated (diameter of 2 cm) following the direct introduction of the sterile filtrates of *C. histolyticum* into rat tumors.	[92]
Clostridium histolyticum	*C. histolyticus* produced proteolytic enzymes which displayed selective degradation of tumor cells while leaving healthy cells unaffected.	[93]
Clostridium tetani	Spores of *C. tetani* were intravenously introduced to tumors of mice and then germinated. Then, the germinated agent produced tetanus toxins within tumor tissues that caused the fatality of the tumor.	[94]
Attenuated *Listeria monocytogenes* (Lm^{at}-LLO)	*In vitro* Lmat-LLO causes reactive oxygen species production and introduced apoptotic killing of a broad range of melanoma cells.	[95]
Live-attenuated *Listeria monocytogenes* Δ*actA*/Δ*inlB* strain	Elicited specific antitumor immune responses to hepatobiliary cancer formation.	[96]
Bifidobacteria species	Down-regulation anti-apoptotic pathway and up-regulation of pro-apoptotic genes towards colon cancer.	[97]
Heat-killed *B. bifidum* MG731 (MG731)	Elicited stronger apoptosis on human gastric cancer MKN1 cells in vivo xenograft animal models as well as in vitro model.	[98]
Bifidobacterium metabolites	Induced anti-colon cancer effect on colon cancer cell line SW742.	[99]
Attenuated mutant strain of *Salmonella enterica* serovar Typhimurium (STMΔznuABC)	Displayed anti-proliferative and immuno-modulatory effect on four CMT cell lines.	[100]
Bacillus licheniformis	L-asparaginase (glutaminase free) produced from *Bacillus licheniformis* displayed considerable toxic activity against HepG-2 cells.	[101]
Bacillus subtilis SDNS	ϵ,-poly-L-lysine (ϵ,-PL) generated by *Bacillus subtilis* SDNS demonstrated an inhibitory effect toward Hela S3 cell line.	[102]
Bacillus subtilis lipopeptides	*Bacillus subtilis* produced by lipopeptide (iturin) has been found to have a potential inhibitory effect on renal carcinoma, breast cancer, colon adenocarcinoma, and alveolar adenocarcinoma.	[103]
Bacillus thuringiensis	Cytotoxic proteins produced from *Bacillus thuringiensis* demonstrated *in vitro* antitumor effect towards several cancer cell lines.	[104,105]

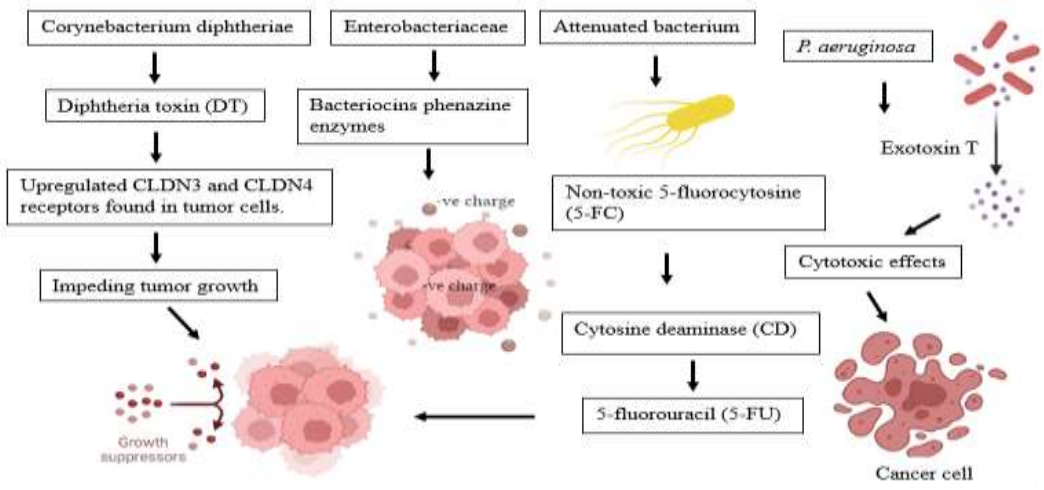

Fig. 1. Mechanism of action of oncolytic bacteria. Constructed using biorendor.com.

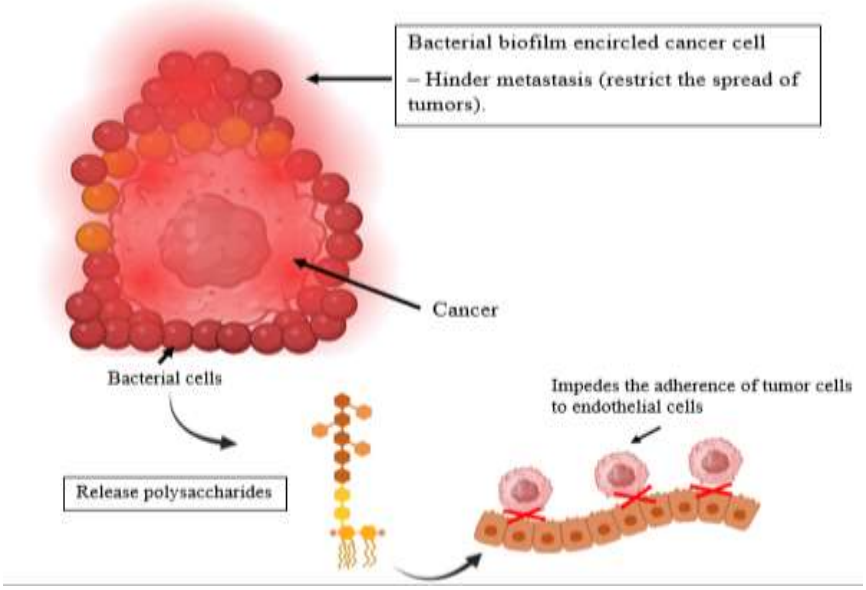

Fig. 2. Anti-cancer activity of biofilm. The bacterial biofilm encloses the cancer cell and prevents the spread (metastasis). Constructed using biorendor.com.

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تطبيق استخدام البكتيريا المحلله للاورام لعلاج السرطان

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[2] قسم علوم المختبرات الطبية، كلية العلوم الطبية التطبيقية ، جامعة الملك عبد العزيز ، جدة ، المملكة العربية السعودية 21589.

الملخص

السرطان هو أكثر الأمراض التي تهدد الحياة انتشاراً على مستوى العالم. وتعتبر سموم كولاي الأساس لعلاج السرطان البكتيري. ونظراً للسمية غير التمييزية للعديد من الأدوية المضادة للسرطان تجاه الخلايا السليمة، فإن العلماء يقومون حالياً بدراسة المستقلبات البكتيرية من أجل تحديد العلاجات المستهدفة والمحددة للسرطان والتي تقلل من الآثار الجانبية غير المرغوب فيها. إن العلاج القائم على البكتيريا للسرطان له تأثير ملحوظ، إما من خلال تدمير الخلايا السرطانية مباشرة أو من خلال تعديل العمليات الخلوية، مما يؤدي إلى تثبيط وتراجع الأورام الصلبة. يمكن استخدام أجناس بكتيرية مختلفة لهذا الغرض بما في ذلك الليستيريا، والكلوستريديوم، والعصيات، والبيفيدوباكتيريوم، والسالمونيلا، والإشريكية القولونية، وغيرها والتي يمكن أن تعمل كبكتيريا لاهوائية إلزامية أو اختيارية. تمتلك هذه البكتيريا القدرة على استهداف الأنسجة السرطانية والتكاثر فيها بشكل خاص. هذه القدرة الفريدة تمكنها من القضاء على الخلايا السرطانية بشكل فعال وتحفيز رد فعل مناعي ضد الورم. لقد سهّل التقدم الأخير في التقنيات الجزيئية تطوير البكتيريا المعدلة وراثيًا غير المسببة للأمراض لاستخدامها في الأساليب البكتيرية المضادة للسرطان. وفي حين تم ملاحظة نتائج واعدة، إلا أن هناك حاجة إلى مزيد من البحث لضمان فعالية وسلامة النهج العلاجي البكتيري المحلل للأورام كخيار بديل لعلاج السرطان. تناقش هذه المقالة النشاط المضاد للسرطان للبكتيريا المحللة للأورام ضد أنواع مختلفة من السرطان.

الكلمات الدالة: السرطان، المستقلبات، البكتيريا المحللة للأورام، السموم.