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Review article

The role of bacterial toxins and spores in cancer therapy

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ABSTRACT

Cancer is one of the leading causes of human death worldwide. Conventional anticancer therapies are ineffective in treating cancer patients due to various reasons. Thus, more effective and accessible alternative anticancer strategies have been evolved with time with high specificity towards tumor cells and with less or no adverse effects to normal cells. One such promising therapy is the use of bacterial toxins and spores to treat advanced solid tumors. Initially, Coley paved the way towards the bacterial anticancer therapy several decades ago and now it has emerged as a potential tool to eliminate tumor cells. Bacterial spores of obligate anaerobes exclusively germinate in the hypoxic/necrotic areas and not in the well-oxygenated areas of the body. This unique phenomenon has been exploited in using bacterial spores as a remedy for cancer. Bacterial toxins also play a significant role in either directly killing tumor cells or altering the cellular processes of the tumor cells which ultimately leads to the inhibition and regression of the solid tumor. With the advancement of molecular techniques, a number of genetically-modified non-pathogenic bacteria have been developed to use in bacterial anticancer strategies. Although promising results have shown so far, further investigations are required to ensure the efficacy and the safety of the bacterial spores and toxins in treating cancer.

1. Introduction

Cancer is one of the leading causes of mortality and morbidity among the worldwide human population. Cancer is a generic term for a group of diseases characterized by out-of-control cell growth that can affect any part of the body. There are > 200 different types of cancer, and each is classified by the type of cell that formed them or the associated organ/tissue. According to the World Health Organization (WHO), it is estimated to account for 9.6 million mortalities due to cancer in 2018 [59]. Globally, nearly 1 in 6 deaths is due to cancer. There are many types of cancer treatment. The types of treatment that a cancer patient receives will depend on the type of cancer and how advanced it is. However, conventional anti-cancer therapies such as chemotherapy and radiotherapy have numerous drawbacks. Thus, a number of novel treatment strategies have been developed as potential anti-cancer therapies to overcome those issues. Bacterial anti-cancer therapy is one such novel approach in cancer treatment [47].

2. Background

Bacteria have been recognized as an effective anticancer agent which led to the regression of certain cancers. Busch and Fehleisen observed that accidental attack of erysipelas (*Streptococcus pyogenes*) infections led to the regression of certain cancers in hospitalized patients [41]. In 1893, William Coley discovered that a patient with a sarcoma had recovered completely after the accidental erysipelas infection [8]. Coley seemed to comprehend that patients with severe streptococcal infection experienced arousal of an otherwise inexplicably quiescent but potentially active natural tumor defense mechanism. He started to use bacteria and their toxins to treat cancers in the final stages. A safer vaccine was developed by him in the late 1800's composed of two killed bacterial species, *Streptococcus pyogenes* and *Serratia marcescens* to simulate an infection with the accompanying fever, inflammation, chills without causing the actual infection [52,63]. This vaccine was known as 'Coley's toxins' [9] and was widely used to treat sarcomas, carcinomas, lymphomas, melanomas and myelomas [10,41,42]. The early success of Coley's toxins paved the way towards current advances in this field.

3. Overview of role of bacteria in cancer therapy

There are many different approaches for using bacteria in cancer therapy. Scientists discovered that certain anaerobic bacterial species (ex: *Clostridium)*, are viable in hypoxic cancerous tissues whereas they die in the tumor's well- oxygenated areas, implying that they are harmless to the rest of the normal tissues in the body [34]. These

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discoveries provided the rationale to use anaerobic bacteria as oncolytic agents. However, since, bacteria do not consume the entire malignant tissue, other therapies (e.g., chemotherapy) should combine with the bacterial anticancer therapy, thereby using bacteria as sensitizing agents for chemotherapy. Bacterial toxins are used in conjugation with either ligands or tumor surface antigens. Cancer vaccines can be based on bacterial immunotoxins [7]. Whole live, attenuated or genetically modified non-pathogenic bacteria can be used as immunotherapeutic agents, carriers of tumouricidal agents and to provide direct tumouricidal effects. Spores of anaerobic bacteria are used for the above strategies because spores only germinate, multiply and become active in hypoxic/necrotic areas. Recombinant bacteria for selective oncolysis, and bacterial gene-directed enzyme prodrug therapy (GDEPT) have shown beneficial effects. This review highlights the use of bacterial toxins and spores in cancer therapy.

4. Bacterial toxins as anti-cancer agents

Bacterial toxins have been tested to use in anticancer therapy. Various experimental evidences support that bacterial toxins are an effective means of inhibiting growth of cancerous cells. Bacterial toxins, depending on their concentration have exhibited dual activities on cancer cells. At high, lethal levels, they can kill the cells, whereas, in reduced levels, the toxins can change the cellular processes which control proliferation, apoptosis and differentiation [47]. These changes are associated with tumorigenesis and may either induce cellular abnormalities or inhibit normal cell controls. Cell-cycle inhibitors, including cytolethal distending toxins (CDTs) and the cycle inhibiting factor (Cif), block mitosis and are associated to compromise the immune system by inhibiting clonal expansion of lymphocytes. In contrast, cell-cycle stimulators such as the cytotoxic necrotizing factor (CNF) promote cellular proliferation and interfere with cell differentiation [43]. Bacterial toxins that block or stimulate the eukaryotic cell cycle are known as “cyclomodulins”. For instance, CNF is released by certain bacteria such as *E coli*. CNF stimulates the G1-S cycle transition and enhances DNA replication. However, the overall number of cells are not increased, and the cells become multinucleated instead, possibly through the capability of toxin to prevent cell differentiation and trigger cell apoptosis [44]. Cif is involved in enterohemorrhagic *E. coli* and enteropathogenic *E. coli*, whereas, CDTs are produced by several Gram-negative bacterial species, and *Salmonella typhi* and *Campylobacter jejuni.*

Bacterial toxins with the antitumor activity have been categorized into two classes; toxins which are conjugated to tumor surface antigens or toxins which are conjugated to ligands. Cancer cells often exhibit large number of tumor-specific antigens on the cell surface (sometimes as receptors) and certain toxins such as Diphtheria toxin (DT) bind to these cell surface receptors and later become activated. Bacterial toxins such as DT, *Clostridium perfringens* enterotoxin (CPE) and Pseudomonas exotoxin A (PE), from *Corynebacterium diphtheria*, *Clostridium perfringens* and *Pseudomonas aeruginosa*, respectively, have been used as cell-targeted toxins. These recombinant toxins comprised of a truncated bacterial toxin fuse to a ligand that binds selectively to a receptor on the target cell. The ligand– receptor complex becomes internalized and the toxin will induce cell death. Such recombinant toxins include LMB2, (anti-CD25 fused to PE) which showed the clinical activity in hairy cell leukemia and T cells neoplasms [30]. Other toxins, such as *Clostridium perfringens* enterotoxin (CPE) bind directly to receptors, CLDN3 and CLDN4; which are upregulated in tumor cells and can significantly inhibit tumor growth [29,37].

DT is a highly potent bacterial toxin. Even the entry of a single DT molecule into a cell could have lethal effects on the cell. Modified DT molecule was formed by deleting the cell receptor-binding domain of the protein and combining the remaining catalytic portion with targeting proteins that selectively bind to the surface of cancer cells. This resulted a “receptorless” recombinant DT385, which is highly cytotoxic to various cancer cell lines. Cellular toxicity was due to inhibition of protein synthesis and induction of apoptosis. In vivo studies, DT385 reduced angiogenesis and regressed tumor masses in CMA (chick chorioallantoic membrane) system. In mice, DT 385 therapy inhibited the subcutaneous growth of LLC (Lewis lung carcinomas), thus showing promising future prospects of using recombinant DT in targeted delivery of cytotoxic therapy [65].

Buzzi and coworkers [5] have also shown the efficacy of DT in a group of cancer patients who are refractory to conventional therapies. A group of advanced cancer patients were treated with a cross reacting material known as “CRM197”. CRM197 is a non-toxic mutant of DT, which possess the same immunological properties of the native DT and it has the ability to bind to heparin-binding epidermal growth factor (HB-EGF). HB-EGF is the specific cell membrane receptor for DT and this receptor is usually over-expressed in cancer. CRM197 was injected subcutaneously in the abdominal wall on alternate days for 6 days, and the results showed an increase in neutrophils and serum TNF-α, with minimal toxicity. Anti-tumor activity was observed to a certain extent by the mutant form of DT.

Prostate cancer is one of the leading causes of death in men, where surgery, radiation and androgen ablation therapy are some of the treatment protocols used. However, following the androgen ablation therapy, usually most of the patients developed androgen-independent carcinoma with multiple metastases. Thus, targeted killing of androgen-independent prostate cancer cells was essential to treat prostate cancer. These androgen-independent prostate cancer cells express high levels of BCL-2 proteins naturally. A novel gene therapy strategy was introduced to express DT-A (Diphtheria toxin) in androgen-independent prostate cancer cells and the results showed marked cytolysis of cancer cells and arrested development of recurrent tumors [48]. Another experimental study has revealed the ability of DT in killing ovarian cancer cells. Epithelial ovarian cancer is a type of common cancer in women over 60 years. In this study, a non-viral vector carrying the DT-A gene, driven by H19 regulatory sequences was constructed and was used in both ovarian cancer cell lines as well as in subcutaneous nude mice models for ovarian cancer. Subsequently, tumor growth inhibition and a significant killing potential in ovarian cancer cell lines were observed [39]. Moreover, ONTAK, a protein composed of DT fused to anti-IL-2 is used in the treatment of cutaneous T-cell lymphoma with promising results [17].

Clostridium perfringens enterotoxin (CPE), which is produced by *Clostridium perfringens* has been effectively used in anti-cancer therapy, especially to treat colorectal cancers. Colon carcinoma and other epithelial tumors exhibit high levels of claudin-3 and/or claudin-4 on the cell surface. CPE specifically binds to claudin-3 and/or claudin-4 and this complex is known as a ‘multi-protein membrane pore complex’, which leads to the loss of cellular osmotic equilibrium and further rapid cell lysis. The recombinant CPE (recCPE) was shown to be an effective anticancer agent both in in vitro and in vivo studies [45]. Furthermore, CPE has been effectively used in treating gastric cancer, where potent cytotoxicity and a significant inhibition of tumor growth in subcutaneous tumor xenograft models were observed [32]. CPE-based theranostic agents have been used in detecting ovarian cancer cells which express high levels of claudin-4 and claudin-3 [15]. However, before evaluating CPE for systemic cancer therapy, its long term efficiency and lack of toxicity in vivo need to be demonstrated [26,29,37].

Pseudomonas aeruginosa exotoxin T was shown to be inducing cytotoxicity against a wide variety of murine and human cancer cell lines [22]. Goldufsky and coworkers had explored ExoT's cytotoxicity against a variety of invasive and highly resistant tumor cell lines, including skin, breast, lung and ovarian, in order to determine ExoT's potential as a possible candidate for cancer therapy. According to the results of their study, ExoT was capable of causing potent cytotoxicity in all cell lines studied. Furthermore, they have demonstrated that ExoT was capable to induce cytotoxicity and to reduce tumor establishment and growth of B16 melanoma in vivo.

Botulinum neurotoxin (BoNT) is another emerging potential anticancer bacterial toxin. A recent study has demonstrated for the first time that BoNT briefly opens tumor vessels, allowing more effective destruction of cancer cells by radiotherapy and chemotherapy. It has been proposed that BoNTs act by an effect on the tumor microenvironment rather than by a direct cytotoxic effect on tumor cells [1]. Some bacterial toxins (alfa-toxin from *Stapylococcus aureus*, AC-toxin from *Bordetella pertussis*, shiga like toxins, and cholera toxin) are presently being studied on two cell lines, mesothelioma cells (P31) and small lung cancer cells (U-1690). AC-toxin showed increasing cytotoxicity with increasing dose of AC-toxin in both cell lines and the toxin significantly increased apoptosis. Nonetheless, cholera toxin did not induce apoptosis [43].

In addition to the aforementioned experiments, numerous research have been done with bacterial toxins and have gained positive results. Thus, bacterial toxins are emerging as a promising anti-cancer agent and these toxins can be effectively used in treating cancer patients.

5. Potential anti-cancer molecular mechanisms of bacterial toxins and the related signaling pathways

Several pathogenic microbes express and release specific protein toxins which suppress the immune response of the infected host. Generally, they catalyze the covalent alteration of specific proteins. Thus, they can inhibit the synthesis or release of antibodies and cytokines. Furthermore, these bacterial proteins inhibit macrophage migration and disturb the barrier function of epithelial cells. Often, these toxins are enzymes with high specificity against their cellular substrates, which are usually signaling molecules. These enzymatic toxins can alter their substrates inside the cytosol after bacteria enter the cells. A few toxins are able to alter the morphology and function of the cells, or sometimes even kill the host cells. Since many of these toxins have well-known chemical structures, molecular mechanisms, cellular receptors, and uptake pathways, they have been used to analyze or influence particular signaling pathways of mammalian cells [64]. Bacterial toxins are powerful cytotoxins produced by bacteria themselves. Cytolysin A (ClyA; also known as HlyE) is a bacterial enzymatic toxin, which forms pores in eukaryotic cell membranes, while, triggering caspase-mediated cell death. Several studies have demonstrated that treating mice with *S. typhimurium* or *E. coli* strains expressing the ClyA toxin inhibited tumor growth [27,53]. Three of the cytotoxins are categorized under the TNFα superfamily: TNF-related apoptosis-inducing ligand (TRAI-l), FAS ligand (FAS-l), and TNFA [16,19]. These proteins selectively cause apoptosis via death receptor pathways, activating the apoptotic mediators, caspase 3 and caspase 8 [19].

Bacterial toxins inhibit the protein synthesis by ADP ribosylation of elongation factor 2. These toxins may be ideal for the fusing with the ligands as these are expressed in the bacteria as single chains with binding domains that can be exchanged for a ligand in the tumor. This process is completed by intentionally eliminating the binding to the toxin receptors by conjugation with cell-binding proteins (monoclonal antibodies or growth factors) that can selectively kill cancer cells. A variety of ligands including IL-3, IL-4, G-CSF, transferrin, EGF, and vascular endothelial growth factor (VEGF) have been conjugated to DT to study the effect on the tumors. There have been clinical trials involving transferrin-DT conjugate (Tf-CRM) and DT-EGF inpatients of brain tumor and metastatic carcinomas, respectively. Similarly, various antibodies and ligands have been conjugated to PE. Conjugation of IL-13, IL-4, monoclonal antibody-recognizing a carbohydrate antigen Lewis Y, (Mab B3), and transforming growth factor (TGF-a) to PE has been studied in the clinical trials [54].

6. Bacterial spores to treat cancers

The anticancer agents, which are toxic to cancer cells, should not be toxic to normal tissues. Thus, the anticancer agents should be specifically tumor-targeted. In previous studies, the experiments have been performed with viral vectors to deliver such anticancer agents to tumor cells. However, there have been lot of limitations with viral vectors, including the lack of tumor specificity, inadequate transgene expression and low levels of distribution throughout the tumor mass [49,55].

The usage of bacteria in anti-cancer therapy is the initial treatment and most direct and effective method to destroy tumor cells. Clostridial spores are the main components in oncolytic therapy and have been thoroughly analyzed [3,20,38]. Clostridial spores have been used extensively as they germinate only inside the necrotic/ hypoxic core of a solid tumor [24,40], thus innocuous to the adjacent healthy tissues [58]. The growing bacteria secrete proteases inside the solid core of the tumor, rapidly digesting the tumor mass. This strategy is especially fascinating because it directly targets the hypoxic cells in poorly vascular regions which are refractory to traditional therapies. The majority of obligate anaerobes produce spores which are highly resistant; they can survive in well oxygenated areas without germination and when exposed to oxygen starved areas, they rapidly germinate and multiply. This unique feature of anaerobic bacterial spores has been exploited in various studies to achieve the targeted delivery of certain anticancer agents through bacterial spores selectively to the tumor.

Parker [46] demonstrated clostridial oncolysis and tumor regression in murine tumors by injecting a *Clostridium histolyticum* spore suspension directly into transplanted mouse sarcomas. The tumor-bearing mice were co-treated with penicillin and antitoxin followed by the spore suspension injection, and this group of mice survived longer than the control group, which did not receive the spore treatment.

Nevertheless, spore treatment with wild-type *Clostridium* was inadequate to completely eliminate solid tumors [14,62]. Thus, genetic modifications and repetitive screens are required to enhance the tumor oncolytic potential of *Clostridium*. M-55, which was isolated from a non-pathogenic *Clostridium oncolyticum* strain by Carey [6] broke this impasse. Since then, numerous genetically engineered *Clostridium* strains have been used in anticancer therapy. Among these, *C. histolyticium, C. tetani, C. oncolyticum, C. oncolyticum (sporogenes), C. beijerinckii (acetobutylicum) and C. novyi*-NT have been the widely investigated in various studies [36,61].

However, after numerous studies, it was revealed that direct intratumoral injection of a spore suspension was not required and the same therapeutic efficacy could be obtained via intravenous administration [34]. This significant finding was observed with the *Clostridium tetani* spores and the tumor-targeted specificity was invaluable in the cancer treatment. Following intravenous administration of the spore suspension, the tumor bearing mice showed signs of tetanus toxicity and died within 48 h, whereas the healthy controls were unaffected and suffered no adverse effects. This observation was due to the anaerobic nature of the bacterium; as *Clostridium* is an obligate anaerobe which selectively germinate only in hypoxic/necrotic regions of the tumor, thus leading to tetanus toxicity. In contrast, the healthy controls did not have anoxic/hypoxic areas in their bodies as healthy tissues are well oxygenated. However, the tumor regrowth was also observed with the clostridial spore treatment due to the fact that the outer viable rim of normal cells were unaffected by the vegetative cells of Clostridia.

Heap [24] demonstrated that spores of Clostridium engineered for clinical efficacy and safety, caused significant regression and cure of tumors in mouse xenograft tumor models in vivo. Human colorectal HCT116 carcinoma cells were administered subcutaneously into adult mice. When tumors reached a volume of $\sim 250\ mm^3$, 5×10^7 spores were injected. At the end of the follow-up period, colonization levels were determined in tumors and normal tissues and tumor growth delay was determined. A significant tumor regression was observed and tumor growth rate was decreased markedly. Furthermore, *C. novyi* spores has been investigated in combination with radiotherapy, radioimmunotherapy, and further chemotherapy in experimental tumor models [4,60].

Table 1
Advantages and disadvantages of bacterial toxins and spores in cancer therapy.

Advantages	Disadvantages
• High tumor-selectivity by obligate anaerobes	• Immunogenicity of bacterial cells results in an immune response of the host following infection, would prevent further administration of bacteria.
• Bacteria may be easily manipulated and engineered to overcome the limitations that hamper current cancer therapies - development of attenuated genetically modified bacterial strains	• Incomplete tumor lysis - Inability to colonize and multiply within small tumors, the viable rim of larger primary tumors and metastases [6].
• Bacteria are mobile and actively move away from the vasculature and penetrate deeply and accumulate in tumor tissue	• Induction of septic shock due to high immunogenecity
• Bacterial therapy achieves sufficient tissue penetration, which other treatments, including chemotherapy and radiation, do not. In combination with bacterial therapy, other conventional methods enhance their therapeutic potential [33].	• Intrinsic bacterial toxicity - toxicity at the dose required for therapeutic efficacy, whereas, reduced dose results in diminished efficacy [11].
• The vegetative cells germinated from bacterial spores can be eliminated by antibiotics later, thus no toxicity is occurred.	• Inaccessibility because most of the times, direct intra-tumoral injection is required [23].

Spores of genetically-engineered *C. novyi-NT*, devoid of the lethal toxin has been used to demonstrate the selectivity to tumor mass and the significant oncolysis of the solid tumor, with no systemic adverse effects. In another study, the effect of the mode of spore administration was compared. One group of mice were injected with *C. histolyticum* spores intra-tumorally, whereas the other group was given the spores of *C. sporogenes* intravenously, and the final outcomes were compared. Both studies ended up with similar outcomes, indicating that the spores are not necessarily be injected directly to the tumor. The spores/vegetative bacterial cells were not observed in normal healthy tissues as they are well oxygenated [57]. *C. novyi-NT* spores were rapidly cleared from the circulation by the reticuloendothelial system, hence no toxicity was seen in healthy mice. In tumor-bearing mice, toxicity was related to the tumor size and the spore dose [13]. Since the use of viral vectors in gene therapy showed many drawbacks, spores have become a potential candidate as a vector in gene therapy with multiple advantages. Thus, recently spores have been utilized as delivery vehicles for anticancer agents, cytotoxic peptides, therapeutic protein [47].

7. The immunological roles of bacterial toxins and spores in cancer immunotherapy

Cancer immunotherapy triggers a specific immune response in cancer patients to allow different types of host immune cells to attack the cancer cells. Once the host immune cells (mainly tumor antigen-specific cytotoxic and helper T lymphocytes) are activated, they recognize and destroy tumor cells. Bacterial infections (*C. novyi* infection) lead to the production of heat shock proteins (Hsp70) which are released from necrotic cells, and pathogenic bacteria possess pathogen-associated molecular patterns (PAMPs) [21]. Dendritic cells, which are the professional antigen-presenting cells are matured by Hsp70 and mature dendritic cells are required for effective antigen-specific immune responses. PAMPs bind to and activate toll-like receptors (TLR), stimulating up-regulation of costimulatory molecules (CD40) and pro-inflammatory cytokines (IL-12). Eventually, these chemical substances cause production of interferon gamma (IFN-γ) and a Th1-dependent cell-mediated response will commence, essentially mediated by cytotoxic T lymphocytes [28]. CD8+ T lymphocytes isolated from *C. novyi* NT-treated mice stimulate acquired immunity in the tumor-specific models. Furumoto and his co-workers, have demonstrated that chemical substances derived from bacteria (CpG oligonucleotides) can be utilized to activate dendritic cells and cause complete regression of B16F10 melanoma tumors, which are known to be highly immunosuppressive in mice [18]. Recombinant *Escherichia coli* strains are widely used for manufacturing recombinant proteins. These strains are used to deliver tumor antigens into dendritic cells. The simultaneous synthesis of listeriolysin O (LLO; a pore-forming cytolysin released from *Listeria monocytogenes*) together with ovalbumin (OVA; a model tumor antigen) in *E. coli* strains led to the MHC class I presentation of the OVA Kb-restricted epitope, SIINFEKL, after phagocytosis of the microbes by macrophages [25]. LLO is present as a bacterial cytoplasmic protein, and it is only released after the uptake of the L. *monocytogenes* by phagocytosis and degradation within the phagocytic vesicles. LLO punctures the phagosome, allowing its release into the cytosol along with co-expressed proteins, for processing and presentation by the MHC class I pathway. According to a recent study, a combination of PAMPs could eradicate solid tumors in cancer bearing mice [51].

8. Advantages and disadvantages of bacterial anticancer therapy

Bacterial anticancer therapy using bacterial toxins and spores is a novel strategy where either obligate anaerobes or facultative anaerobes are used to inhibit the growth of tumors, regress tumors and to prevent recurrence of tumors. However, this novel treatment method also has its own advantages [2] and disadvantages [11,35]. Certain human trials have shown that the flaws of bacterial therapy cannot be ignored [31,47,50]. Some of the advantages and drawbacks are mentioned in Table 1. The biosafety, instability of genes and the confounded interaction of the bacteria with treatment drugs, requires the more notable consideration regarding the use of bacterial spores and toxins in the cancer therapy. In some occasions, systemic infection of bacteria is rather inconvenient and carries a significant higher risk of obvious toxicity. Another major issue related to bacterial therapy is the potential for DNA mutations i.e. any loss of functionality due to mutations could lead to numerous problems such as failure of therapy or exaggerated infection. Although some of the safety concerns have been solved with the recombinant DNA technology yet demands further development [47].

9. Methods and tools to overcome the issues related to bacterial anticancer therapy

Certain drawbacks have been noticed during various studies using bacterial spores and toxins as anticancer agents. However, further experiments have been performed to avoid / prevent such drawbacks and to gain the maximum expected outcome in bacterial anticancer therapy.

One of the major obstacles in bacterial anticancer therapy is the incomplete oncolysis, leaving the viable rim, small tumors and metastases unaffected. Theys [56] demonstrated that a marked improvement of tumor colonization in very small tumors ($< 3\,cm^3$) could be achieved when the rats were injected with a vascular targeting compound, combretastatin A-4 phosphate (combreAp) after the administration of clostridial spores. This enhanced the distribution of bacteria throughout the vascular network and, specifically, in the tumors, before any possible tumor blood vessel damage. This study also revealed that eventually induced host immune response after a single or repeated administration of Clostridium, did not affect the tumor colonization. Moreover, in most of the rats, Clostridium-specific antibodies were not detectable in the bloodstream. Thus, no such induced immune response was observed. These results indicated that long-term production of therapeutic proteins from the recombinant Clostridium is possible in tumors.

Combination bacteriolytic therapy (COBALT) is one of the novel treatment methods for cancer, which is very effective and successful [58]. Bacterial treatment is combined with conventional chemotherapeutic agents such as, antivascular agents, chemotherapeutic drugs, heat shock proteins, and heavy metals to treat cancers in combined bacteriolytic therapy. The combination of specific bacterial species with low-dose radiotherapy lessened the tumor immune escape mechanism [12].

Thus, various methods have been investigated to overcome the issues related to bacterial anticancer therapy with bacterial toxins and spores and numerous studies are still underway to further improve this novel cancer therapy.

10. Conclusion

Cancer is one of the leading causes of death in humans. A number of novel treatment methods are emerging in the field of oncology. One such novel strategy is the use of bacterial toxins and spores as a means of treating cancers. Various studies such as in vitro, in vivo studies of tumor-bearing mice and rats, phase 1 clinical trials, have been performed to investigate the potential of bacterial spores and toxins in treating cancer. However, a number of advantages and disadvantages associated with bacterial therapy have been identified and further studies have been carried out to prevent adverse effects of bacterial usage and to enhance the therapeutic potential of bacterial strains. Since, cancer is a multifactorial disease, a single treatment protocol cannot eliminate it, thus, a combination of different therapies is required. Combined bacteriolytic therapy, where bacterial therapy is combined with conventional methods, has been successfully used to treat cancer patients with positive outcomes. Bacterial anticancer therapy using toxins and spores is a promising tool to save many lives of cancer patients. However, further investigations are required to enhance the efficacy of this novel treatment strategy in order to use in clinical practice without any adverse effects.

Declaration of competing interest

None declared.

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