Plant Biotechnology Persa

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The role of bacteria in the treatment of cancer: A comprehensive review

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Abstract

Cancer is an important public health issue worldwide and is the main cause of death in the developed countries and the second cause of death in the developing countries. There are several treatments for cancer such as photodynamic therapy, surgery, chemotherapy, hormonal therapy, radiotherapy and immunotherapy. Current cancer treatments have various side effects, including the gradual resistance of cancer cells to treatment. The era of targeted cancer therapy has brought about new clinical approaches such as antibodies, small molecules, antiangiogenics, and antivirals. Yet even these strategies remain limited in their ability to accumulate in tumors and tumor penetration, which are the main obstacles in the treatment of cancer. Historic efforts to harness living organisms to fight cancer have recently been revived in the field of synthetic biology. Certain circulating bacteria can intrinsically home in on tumors, and can be engineered to controllably induce local cytotoxicity while remaining unobtrusive to the host system. Due to the ineffectiveness of conventional treatments such as chemotherapy and radiation therapy in advanced tumor stages, resistance to treatment and non-specificity of these treatments, with the advancement of studies in this field, it is hoped that bacterial therapy will add a new dimension to cancer treatment.

Introduction

Cancer can affect everyone, but the prevalence of this disease is different across local, national, and regional boundaries. Cancer is an important public health issue worldwide and is the main cause of death in the developed countries and the second cause of death in the developing countries. Cancer is a genetic and epigenetic disease, resulting from mutations in cells. Some mutations cause inactivation of genes which usually prevent abnormal cell proliferation. These genes are classically called tumor suppressor genes. Some mutations lead to the production of proteins which have oncogenic functions and play important roles in cell growth stimulation, and finally result in the transformation of normal cells into cancer cells and, as a result, uncontrolled cell proliferation occurs, because of some changes in biological pathways [1-6]. Cancer occurs in different organs such as lung, breast, colon and prostate [7]. Many studies have shown that the immune system reacts to tumors and tries to eliminate them [8]. Some cancer-related factors that influence survival include stage, tumor grade and histology, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) status. Cancer survivorship depends on hormone receptor status, tumor grade and histology and human epidermal growth factor receptor 2 (HER2) status [9]. According to world health organization, smoking, being overweight or obese, eating an unhealthy diet, and being infected with the sexually transmitted HPV are the main factors that increase the chances of developing cancer [10]. Smoking is responsible for 80% of the worldwide lung cancer burden in males and at least 50% of the burden in females [11,12] and some infections such as the human papilloma virus (HPV) and hepatitis are the causes of up to 25% of cancer cases in low- and middle-income countries [13]. In Korea as a developed country, the survival rate of cancer patients between 2009 and 2013 was 69.4% [14]. It is estimated that the incidence of cancer will rise in 2030 and the number of patients suffering from cancer will be 2,135,000 in the United States [15]. Costs of treating cancer are rising and significant. The total annual economic cost of cancer in 2010 was estimated at almost US$ 1.16 trillion [7].

It was estimated that there were 14.1 million new cases in the world in 2012 and cancer caused 8.2 million deaths, approximately 70% of deaths from cancer occurring in low- and middle-income countries [10, 16]. Most cancers happen in the less developed countries in the world, with 60% of cancers and 70% of deaths from cancer occurring in Africa, Asia, and Central and South America [17]. The most commonly affected body systems and organs include the respiratory system, breast, and the genitourinary system. In 2016, 1,685,210 new cancer diagnoses and 595,690 cancer deaths occurred in the United States. Both the number of estimated new cases and estimated deaths from cancer are higher among men than among women in the US. The most common cancers in men are prostate (21%), lung and bronchus (14%), colon and rectum (8%) and urinary bladder (7%), but the highest number of deaths are from the cancers of lung and bronchus (27%), prostate (8%), colon and rectum (8%), pancreas (7%). In women, the most common estimated new cases are the cancers of breast (29%), lung and bronchus (13%), colon and rectum (8%), and uterine corpus (7%), but most deaths are due to the cancers of lung and bronchus (26%), breast (14%), colon and rectum (8%) and pancreas (7%) [18]. There is a strong relationship between cancer and age, prevalence in the youngest age group (0–14 years) are about 10 per 100 000, increasing to 150 per 100 000 by 40–44 years and to more than 500 per 100 000 by 60–64 years (7). Liver cancer has the fastest growing rate among all cancer sites among both men and women [19].

There are several treatment methods for cancer based on the type, location and stage of the cancer [20, 21] such as photodynamic therapy [22], surgery [23], chemotherapy [24], hormonal therapy [25], and immunotherapy [26]. Current cancer treatments have various side effects such as the gradual resistance of cancer cells against treatment [27], skin and hypersensitivity reactions [28], diarrhea, dyspnea, dysuria, fatigue, hot flashes [29], nausea, and neutropenia [30]. Sexual dysfunction and infertility are some of the late effects of cancer treatment [31] and they can also happen in both males and females who had been treated for cancer in their childhood [32, 33]. Many of the treatments we use for cancer can influence the reproductive hormones, which are important for the maintenance of normal bone remodeling, leading to bone loss [34]. For example, chemotherapy for breast cancer with taxanes, anthracyclines and HER-2–targeted aromatase inhibitors often result in neuropathy [35], cardiomyopathy, congestive heart failure [36], and osteoporosis, respectively [37] and lung cancer treatment with EGFR inhibitors can cause severe acneiform rash and immunotherapy drugs lead to immune mediated toxicities, including pneumonitis, colitis and nephritis [38]. It has been shown that liposomal doxorubicin which is used for ovarian cancer treatment has various side effects such as stomatitis, rash, mucositis and vomiting [39].

Chemotherapy can induce drug tolerance, nausea, fatigue, loss of appetite, uterine bleeding, dyspareunia, vaginal dryness or discharge, hot flushes, loss of libido, optical difficulties (cataract), deep venous thrombosis, venous thromboembolism, embolism, and pulmonary, cardiovascular, ischemic, cerebrovascular and sexual problems. Medications used in chemotherapy can also act as carcinogens [49-53]. Psychological issues consist of depression, anxiety, guilt, fear of recurrence, body image difficulties, loneliness and anger [54]. Chemotherapy is responsible for insomnia in cancer patients because of its role in the occurrence of headaches, digestive symptoms, and nausea [55]. Chemother-

apy appears to be most harmful for patients who have a good performance condition [60]. Early effects of radiotherapy can be nausea, skin erythema, diarrhea, and dry or moist desquamation of the skin, while the late effects are vascular damage, neural damage, radiation-induced fibrosis and a range of endocrine and growth-related effects and atrophy [56]. It has been shown that radiotherapy can be a cause of secondary malignancies [57]. Immunotherapy induces inflammation of the colon and the pituitary gland [58]. Hormonal therapy may result in thromboembolic events, peripheral edema, and increased appetite [59].

Bacterial therapy can be used for some diseases. Wild type or recombinant lactic acid bacteria (LAB) can be used as an alternative treatment for inflammatory bowel disease (IBD) and mucositis. It has been shown that probiotic LAB especially recombinant L. lactis could be a sufficient treatment agent for irritable bowel syndrome (IBS), because of their ability to produce anti-inflammatory peptides such as defensins, cathelicidins and histatins [40]. Furthermore, LAB can produce anti-inflammatory vitamins and can be used for IBD treatment and as a source of essential nutrients of which IBD patients often do not get enough [43]. In another study engineered lactic acid bacterium NCDO 2118 was used to produce oxidative enzyme, 15-lipoxygenase-1 (15-LOX-1) which acts as a catalyst in the production of several anti-inflammatory agents, such as protectins and lipoxins [41]. Bifidobacteria can play a role in the improvement of the clinical symptoms of IBD [32]. Various bacteria such as Escherichia coli, Bifidobacterium bifidum, Bifidobacterium lactis and Lactococcus lactis have been used to cure eczema patients because of their ability to produce Th2 cytokines and to stimulate IL-10 [42-46]. Lactobacillus plantarum, Propionibacterium freudenreichii and Enterococcus faecium M-74 are used for the treatment of hypercholesterolemia and cardiovascular diseases due to their ability to reduce serum cholesterol levels by affecting the expression of a gene encoding cholesterol oxidase [47, 48].

The era of targeted cancer therapy has brought new clinical approaches such as the use of antibodies, small molecules, anti-angiogenics, and antivirals. Yet even these strategies remain limited in their tumor penetration and their abilities to accumulate in tumors, which are prevailing obstacles in the treatment of cancer. Historic efforts to harness living organisms to fight cancer have recently been revived by synthetic biology [61, 62]. Certain circulating bacteria can intrinsically home in on tumors, and can be engineered to controllably induce local cytotoxicity while remaining unobtrusive to the host system. The observation that bacteria accumulate preferentially in tumors has prompted the investigation of the use of a number of strains for cancer therapy, including E. coli, and S. typhimurium, both of which have exhibited safety and tolerance in human clinical trials. S. typhimurium was initially shown to mediate anti-tumor effects through recruitment of the host immune system and by competing with cancer cells for nutrients [63-65]. Subsequently, the engineered production of therapeutic cargo was achieved through simple genetic modifications. As a next step, synthetic biology seeks to add controlled and dynamic production of cargo by utilizing genetic circuits that have sophisticated sensing and delivery capabilities, such that bacteria can sense tumor-specific stimuli and self-regulate cargo production as necessary [66, 67].

While these fast-paced advances have shown great potential, the main challenges in engineering bacteria for tumor therapy are the need for better control of inherent overgrowth and the

limited ability of bacteria to penetrate tumor environments, despite their enhanced accumulat compared to passively circulating agents [68].

Gene therapy vectors:

Gene transfer systems can be divided into non-biological groups (such as chemicals and physical methods making plasmid DNA transfer to mammalian cells possible) and biological (viruses and bacteria). Like viruses, the inherent biological properties of bacteria allow the efficient delivery of DNA to cells or tissues, but in terms of safety, it is preferable to viral infections. Today, there are two broad methods for using bacteria as vectors [69-71].

1. Bacterial replication

Ideal antitumor treatment is selective tumor removal with minimal negative effect on normal cells. To reach this goal, features of cancerous cells that distinguish them from normal tissues should be well known. At first, the specificity of the tumor is specific to the nature of the hypoxia of solid tumors. Necrotic areas provide nutrients such as purines and suitable conditions for the growth of anaerobic bacteria. The chemotaxis of bacteria to materials in necrotic areas such as aspartate, serine, citrate, ribosomes and galactose produced by cancer cells has been observed [72, 73].

2. Transfer of intracellular plasmid

Transmission of plasmid DNA into mammalian cells by bacteria is a powerful tool for expressing heterologous proteins in different cells. Transmission of the genetic material is achieved through the complete penetration of the bacteria into target cells. Various bacteria including Salmonella, Listeria and Escherichia coli have been studied. The bacterial species used are classified according to their location in the host cell: a group in the cytoplasm (Listeria, Shigella), a group in the vacuole (Salmonella, Yersinia) and another group in the extracellular space (Agrobacterium). On the other hand, one of the traditional methods of using bacteria as vectors is targeted gene expression in cancer cells through the targeted setting of gene expression. Gene expression is regulated at different levels, but, typically, is more prevalent in the transcription level. SLPI is a serine protease inhibitor whose specific tissue expression is highly regulated at the transcriptional level. The promoter of this gene can be a good candidate for targeting gene expression in lung cancer through the use of bacterial plasmids [74-77].

Bacterial toxins and cancer therapy

The spectrum of toxins produced by bacteria is vast, among which the tetanus, botulinum and diphtheria toxins can be mentioned. In the treatment of cancer, toxins can interfere with proliferation and at reduced levels with the control of apoptosis, differentiation and duplication. Bacterial toxins that overturn the cell cycle are classified as cyclomodulins. Bacterial toxins act in two ways on cancer cells [78, 79].

1. Bacterial toxins bind to tumor surface antigens

Diphtheria toxin (DT) is attached to the surface of the cells expressing the HB-EGF (Heparin-binding epidermal growth factor) precursor and through the endothelium mediated by clathrin and after several changes after translation; it catalytically activates and ultimately results in the inhibition of protein synthesis, the lyse of cells, and the induction of apoptosis. Another example is endotoxin CPE produced by strain A of Clostridium perfringens, the cause of diarrhea and vomiting, in which the end domain of C is responsible for its ability to bind to the receptor and the end domain of N has toxic effects. Studies have shown that, in pancreatic cells, pure CPE leads to tumor necrosis and inhibits tumor growth in the living creature. The effect of this toxin has been investigated on gastric, lung, and colon cancers, however, it is necessary to consider the long-term treatment effectiveness and lack of toxicity in living creatures [80-82].

Bacterial toxins conjugate with ligands

Protein toxins like pseudomonas exotoxin, diphtheria and ricin toxin can play a role in the treatment of very lethal cancers. However, these toxins require a special place on the surface of cancerous cells for effective treatment. This problem can be solved by removing the binding site to the toxin receptor and conjugating it with monoclonal antibodies and growth factors attached to cancer cells [83].

Cytolethal distending toxin is a bacterial toxin produced by gram-negative bacteria. Its mechanism of action is unique in that it enters the eukaryotic cell and breaks the double-stranded DNA; this causes the activation of DNA damage mechanisms and stops the cell at the G2/M stage. The affected cell enlarges and eventually apoptosis occurs. The enzyme component of this toxin can conjugate to the ligand and can be considered as an appropriate therapeutic target [84].

Bacterial spores in the treatment of cancer

Most anaerobic bacteria survive as spores in oxygen-rich conditions although they do not have the ability to grow and proliferate, however, when they are in suitable conditions, such as the dead areas of the tumor, spores sprout and bacteria grow. This feature allows for targeting cancer [85].

Spores of genetically modified strains of C. novyi NT lack deadly toxin and have targeted function on normal cells without side effects. A remarkable lysis of tumor tissue has been observed in mice receiving intra-tumor injection of Clostridium histolithium spores, and in intravenous injection of Clostridium perfringens spores. In addition, Clostridium was found only in the tumors of the mice receiving intravenous injections of the bacterium [86, 87].

Bacteria as immune agents

The use of the immune system in cancer treatment is a promising approach. In this therapeutic system, stimulation of the immune system is used to destroy cancer cells. The most important problem with this method is the ability of the tumor to escape the immune system and cause weak tolerance and immunogenicity. In some cases, the body considers a cancerous antigen to be a self-antigen; therefore, the bacteria are used to enhance the immunogenicity of the cancerous cells [88, 89].

Recombinant anaerobic bacteria

Brown et al. first showed that the necrotic areas in human solid tumors could be used to target cancer treatment to tumors

by using a genetically engineered non-pathogenic strain of the bacterial genus Clostridium. This genus contains a great and heterogeneous group of spore-forming, gram-positive bacteria that become vegetative and grow only in the lack of (or at very low levels) of oxygen [90, 91]. Malmgren et al. were the first to reveal this phenomenon by detecting that tumor-bearing mice died of tetanus within 48 hours of the intravenous injection of Clostridium tetani spores, while non-tumor-bearing animals were unaffected. Möse et al. later reported that a nonpathogenic clostridial strain, (C. butyricum M-55), localized and developed in solid Ehrlich tumors in mice, causing broad lysis without any associated effect on usual tissues. Similar reports were soon presented and extended by several researchers by using tumors in mice, rats, hamsters and rabbits, and with clinical trial studies with patients having cancer [92, 93]. While the anaerobic bacteria did not meaningfully alter tumor control or eradication, these important clinical reports confirmed that spores of nonpathogenic strains of clostridia could be administered without harm, that the spores sprout in the necrotic areas of the tumors, and that lysis in these tumor areas can occur. This is an important difference over a similar approach using genetically modified, live weakened Salmonella, which, even though producing outstanding colonization of transplanted tumors in mice, formed only marginal colonization of human tumors in a Phase I clinical trial [94, 95]. The causes for the difference between the rodent and human tumors in colonization by Salmonella are unknown. But, colonization by clostridia is different from that of the Salmonella bacterium in being dependent on hypoxic necrotic areas, which are similarly common in human and rodent tumors. Furthermore, as noted above, brilliant colonization of human tumors has been observed subsequent to intravenous injection of clostridial spores. The Clostridium used in clinical trials was a strain of C. sporogenes that was renamed C. oncolyticum to reflect the lysis that occurs in human tumors. This strain has been genetically changed to express an enzyme of E. coli, cytosine deaminase, which can alter the non-toxic 5-fluorocytosine to the toxic anticancer drug 5-fluorouracil. Animal interventions have shown the effectiveness of this method and clinical trials are planned. Furthermore, other enzymatic prodrug systems for arming clostridia are in progress, including CB 1954 (BOX 2), which, once activated by E. coli nitroreductase, kills non-cycling cells efficiently and is therefore expected to have superior activity against cells in hypoxic areas [96-99].

Genetically modified recombinant toxins

The manufacture of immunotoxins by chemical systems is expensive since it needs huge amounts of toxins and antibodies. Also, the chemical conjugation methods used yield heterogeneous products, and chemical derivatization often affects antigen binding. It is possible to overcome these problems and to make cytotoxic factors by genetic modification. The PE and DT have been used to produce modified toxins in E. coli. Ricin-derived molecules have been hard to manufacture perhaps because the A chain of the plant toxin must be attached to the recognition domain of the cell via a disulfide bond, and subunits that are linked to disulfide are hard to create in bacteria [100,101]. Adding of a proteolytic cleavage arrangement might help to overcome this problem. PE x-ray crystallographic construction has been used for the synthesis of genetically modified recombinant toxins. The particular binding of PE to target cells happens through an interaction of cellular PE receptors with domain I. The connection of domain II with domain I happens between Gly253and Glu252. Therefore, in making recombinant toxins, domain I was deleted, and the COOH-terminal-amino acid of different growth factors and other targeting molecules were bonded straight to Gly253 of PE (a few added amino acids have been added as a link between the COGH-terminus of the growth factor and Gly253 of PE occasionally, to make cloning more viable) [102-104]. One of the widely studied molecules is TGF-at-PE4O, which was built by replacing transforming growth factor alpha with domain I of PE (TGF-ot). In this chimeric toxin, the 23-kD domain I is changed by the 6-kD growth factor, to create a chimeric toxin that selectively attaches to and kills cells with receptors of epidermal growth factor (EGF). The expression vector used for the making of TGF-ot-PE40 and other PE-based chimeric toxins in E. coli encloses the promoter of bacteriophage T7, an effective ribosome binding site and an Nde I site (CATATG), which encrypts a methionine initiation codon where targeting ligands can be simply inserted. The gene encoding the phage T7 polymerase is inserted into the E. coli chromosome following a lac promoter so that it can be induced by the addition of isopropylthiogalactoside (IPTG). TGF-c-PE40, like other chimeric toxins made in E. coli, is stored in huge amounts within the cell in insoluble aggregates (inclusion bodies). Inclusion bodies are easily isolated and can contain up to 90% recombinant protein in an insoluble form after cell disruption. The protein is then dissolved in a strong denaturant such as 7 M guanidine-HCl, renatured, and can be purified to near homogeneity in two or three steps by conventional column chromatographic ways. TGF-a-PE40 binds to EGF receptor-containing cells with about the same affinity as TGF-c, and its toxicity on these cells is directly related to the number of receptors present [105-108].

Tumor-targeting bacteria

One of the strong points of bacterial therapy is the ability to specifically target tumor sites. In 1964, a series of reports described the use of nonpathogenic Clostridia in experimental tumor models. The rationale for using Clostridium is that it is an obligate anaerobic bacterium. Therefore, when injected into a body, the spores replicate and develop only in hypoxic regions. In hosts with advanced cancer, these hypoxic regions can be found and Clostridium is presumed to develop and proliferate in these oxygen-poor areas while being absent from well-oxygenated healthy tissues [109, 110]. Moreover, this selective targeting is associated with the death of tumor cells. Not all spore-forming bacteria are effective, and the spore-forming microorganisms Bacillus mesentericus and Bacillus subtilis do not produce oncolysis. These results indicate that although the obligate anaerobic phenotype of Clostridium is probably the basis for their ability to specifically target necrotic areas of tumors, other factors may be involved. Although promising, the strategy shows major limitations. First, the oncolytic effect is restricted to large, well-established tumors but is undetectable in smaller metastatic nodules, probably because these lesions lack hypoxic regions [111,112]. Second, Clostridium-dependent lysis is found in the center of large tumors, leaving the liquid necrotic center surrounded by a better-oxygenated layer of malignant cells that constitute the seed for the re-growth of the tumor [113]. Finally, some toxicity is observed in preclinical mouse models. Fox et al.

placed the Escherichia coli cytosine deaminase gene into Clostridium beijerinckii by using it as the Clostridium expression vector and produced an increased cytosine deaminase activity in the extracts of the transformed bacteria. Recent in vivo studies of the use of Clostridia as tumor vectors have focused on their potential in gene therapy and controlled gene expression by use of radio-inducible promoters. Another group investigating Clostridium in combination with chemotherapy has reported significant antitumor activity (Dang et al. 2001). Many years after the first injection of Clostridium spores into tumors, various advances have shown promise for Clostridium as a tumor-targeting therapeutic vector [114].

Discussion and conclusion

In 1868, German doctors W. Busch and F. Fehleisen separately observed that some hospitalized cancer patients have accidentally recovered after an infection with the Streptococcus pyogenes in the form of Erysipelas. In 1890, William B Colley, a physician at New York Memorial Hospital, for the first time, defined bacteria as an anticancer agent. He observed tumor regression several times after infection with a pathogenic bacterium. In 1976, Bruce, Morales et al. reported successful treatment of bladder cancer with Bacillus Calmette–Guérin (BCG). Numerous scientists from then on, and even today, selectively targeted cancer cells with weakened, engineered, and alive microorganisms, such as mycobacterium, bifidobacterium, bacillus, salmonella, and listeria [115,116].

Cancer treatment encounters major challenges, including the specificity of treatment. Perfect therapy will be able to eliminate selected tumor cells with minimal side effects for normal body cells. Furthermore, chemotherapy and radiotherapy produce significant problems. Resistance to cancer treatment in patients with advanced tumors has led to the need for alternative cancer treatments [117,118].

When bacteria are injected systemically, the bacteria accumulate in tumors and in the areas far from vessels which are hypoxic and necrotic areas. When bacteria begin to produce therapeutic molecules, they spread to living tissues. The concentration of bacterial molecules in the distal region of tumors is greatest and as long as the expression of proteins continues, it stays constant. Systemic injection of chemotherapy molecules is spread into the blood vessels of the tumor. The highest molecular concentration of chemotherapy is in the bloodstream [119, 120].

After the first general observations of Colly, scientists have made use of specific species of anaerobic bacteria that grow in the tumor's hypoxic and necrotic tissue and die when in contact with the oxygenated parts around the tumor and are not harmful for other parts of the body. These findings suggest that bacteria can be used as oncolytic agents. However, bacteria do not consume all of the malignant tissue, resulting in the need for a combination of chemotherapy treatment. Therefore, bacteria can be used as chemotherapeutic sensitive agents [121,122].

Bacterial products, such as endotoxins, have been partially tested for cancer treatment. Bacterial toxins can be used to kill tumors and as cancer vaccines. Bacteria can be used as agents for the transfer of anticancer drugs and serve as vectors for delivering therapeutic genes. Spores of anaerobic bacteria are also used for the mentioned strategies because they sprout, activate and multiply in the hypoxic regions of tumors [123].

Compared with targeted cancer treatments, bacteria have

a special place, which is associated with three unique bacterial properties. Almost all tumors have low oxygen or hypoxia, and anaerobic bacteria prefer such environments. In addition, bacteria can be easily manipulated and can overcome the limitations of conventional cancer treatments. Also, unlike other therapies, such as radiation therapy, bacterial therapy has a good penetration into tumor tissues. However, the problems of bacterial treatment related to the toxic nature of bacteria and their genetic instability cannot be ignored. Significant efforts have been made to overcome these problems including the use of engineered and weakened bacteria, recombinant DNA technology, and also the simultaneous use of this treatment with other therapies, such as chemotherapy, heat shock proteins, heavy metals and radiation [124, 125].

Various applications of bacteria, including live and weakened bacteria as anticancer agents and vector carriers of genes, spores as factors compatible with the environment and tumor conditions, and bacterial toxins for destroying cancer cells, have been studied so far. These results indicate that the use of bacteria is a promising treatment for cancer. In addition, a variety of bacterial approaches to treating cancer especially in Phase 1 clinical trials of cancer patients based on basic knowledge of cancer have become possible. Due to the inability of conventional treatments such as chemotherapy and radiation therapy in advanced tumor stages, resistance to treatment and non-specificity of these treatments, with the advancement of studies in this field, it is hoped that bacterial therapy can add a new dimension to the treatment of cancer.

Conflict of interest

The authors declared that they have no conflicts of interest.

Ethics approval

Not required

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