Bacteria as a vehicle in cancer therapy and drug delivery

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ABSTRACT

Although the conventional therapies have obviously improved the conditions of patients with cancer, some mechanisms of resistance have led scientists to use alternative agents that can penetrate in most solid tumors. Furthermore, the success of cancer therapies depends on limiting the uptake of toxins to normal tissues and their selectivity towards malignant cells. The involvement of natural and genetically modified non-pathogenic bacterial species, as potential antitumor agents, has led scientists to study bacteria and their products as an ideal vector for delivering therapeutic components to tumors. Moreover, bacterial ghosts, microbots and bactofection are the other strategies to destruct the malignant tissues. Although it has shown to achieve successful results in vivo, further investigations on the targeting mechanisms of the bacteria are needed to make it a complete therapeutic approach in cancer treatment.

Kewords: Bacteria; Cancer; Drug

INTRODUCTION

Cancer occurs when tumor cells grow, invade, and spread into the surrounding normal tissues uncontrollably; this process is called metastasis. Although treatment of cancer can involve several modalities such as resection, radiotherapy, and chemotherapy, other strategies have developed with the aim of improving cancer therapies. Experimental cancer treatments are medical therapies, including photodynamic therapy, Human Alphalactalbumin Made Lethal to Tumor Cells, gene therapy, telomerase therapy, hyperthermia therapy, dichloroacetate (DCA), non-invasive RF cancer treatment, complementary and alternative therapy, diet therapy, insulin potentiating therapy, and bacterial treatment, which have been considered as alternative treatments to replace conventional methods; yet, due to lack of evidence, efficacy, feasibility, availability, specificity, and selectivity, the prevalent use of these therapies in cancer therapy has become controversial [1]. Some microorganisms have been shown to selectively replicate in tumor cells such as many viruses, like vaccinia virus, Newcastle disease virus, reovirus, and adenovirus with an E1a deletion, which carry altered genes to cancer cells, find target cells in body, and destroy them. and yet, sometimes body procures neutralizing antibody against these microorganisms which leads to deactivation of their efficacy [2]. Some bacterial species are able to enter and then replicate within tumor cells, simultaneously carry and express multiple therapeutic proteins, and consequently be eliminated by antibiotics [3]. Furthermore, in many infectious diseases and cancer, to deliver genes, live attenuated strains of bacteria should be applied, which have different advantages, including low-cost preparation, intensive immune stimulation, tolerance, safety, and the major point of antigen entry into the Major Histocompability Complex class I pathway for the induction of cytotoxic T cells [4]. Therefore, the advent of advanced techniques, including bacterial drug delivery as bacterial vectors for genetic manipulation has created novel bioengineered microbes with great therapeutic efficacy in many therapeutic strategies including apoptosis induction, suicide gene therapy, immunotherapy, anti-angiogenesis therapy, and DNA vaccination [5]. The present review

highlights bacteria as a vehicle of new delivery system with no cytotoxicity and high efficiency, which can make it an alternative pathway to treat cancer cells.

Bacteria to Treat Infectious Diseases

The role of bacteria as anticancer agent has been recognized about a hundred years ago. The first observations of bacteria treating infectious diseases was reported in 1813 when Vautier understood that cancer patients who were suffering from a gas gangrene infection (often caused by bacteria called *Clostridium perfringens*), underwent tumor regression [6]. In the late 1800's, an American physician, William Coley, while examining his patient suffering from neck cancer could recover infection, for the first time, with live cultures of *S. pyogenes* and a few days later with killed extracts of *S. pyogenes* and *Serratia marcescens*, which were called Coley's toxins [7, 8]. Therefore, bioengineered bacteria based on Coley's toxins have been the basis for current advanced studies.

Bacteria in Cancer Therapy

The lack of selectivity towards tumor cells, despite making progress in tumor-targeting technologies, has led to limitations in the current cancer therapies. Some species of anaerobic bacteria, such as genus *Clostridium* (like *Clostridium beijerinckii, Clostridium novyi-Non Toxigene, C. histolyticum*), have a natural ability to target tumors, prosper, and consume oxygen-poor cancerous tissues; hence, they can colonize only within the necrotic and hypoxic areas of tumors, and as a result, microbial growth within the tumor can result in a strong cytolytic and oncolytic effects. *Clostridia* can express IL-2 and TNFa with the property of stimulating antitumor immunity and direct antitumor features by genetic modifications [9]. Moreover, these families can produce spores that reach an oxygen deprived area of a tumor where they germinate, multiply, and become active [10]. According to the strategy of "combination bacteriolytic therapy" (COBALT), *C. novyi-NT* spores are used in combination with several chemotherapeutic agents, such as docetaxel, vinorelbine, mitomycin C., and dolastatin-10 [11]. Making use of genetic engineering to increase the antitumor clostridial spore's activity, some prodrug converting enzymes such as cytosine deaminase with the ability to converting 5-fluorocytosine (5-FC) to 5-fluorouracil (5- FU) along with high local concentrations of cytotoxic drug can be used so as to cause less damage to healthy tissues [12]. Despite the lack of clinical toxicity, as delivery agents, bacterial spores are commonly ineffective against small metastases [13, 14]. Gram-positive anaerobes, such as *Bifidobacteria*, can reinforce induction of tumor-specific T cells and increase selective accumulation of antigen-specific CD8+ T cells in the tumor and thus destroy them [15].The antitumor properties of facultative anaerobes, including *L. lactis* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*, have profoundly been studied. To treat inflammatory bowel disease and moderate ulcerative colitis, natural non-pathogenic *L.lactis* as a therapeutic agent, which is administered orally, can produce IL-10 [16]. An attenuated *Salmonella typhimurium* strain has chromosomal deletion in two regions of its genes like *msbB* (reduction endotoxicity results in inducing mutation in the components of the lipopolysaccharide by preventing the addition of a terminal myristyl group to the lipid A domain) and *purI* (deletion creates a requirement for an external source of adenine). The mutation lowered the toxicity in mice by reducing the induction of pro-inflammatory cytokines and nitric oxide synthase. Thus, the organism is able to replicate in normal tissue such as the liver or spleen, but it is still capable of growing in tumors where available purines become essential to survival [17, 18]. Another property of Salmonella, in addition to invasion and induction of apoptosis in tumor cells, is the ability to penetrate within a tumor mass due to its motility and moving away from the vasculature of metastases [19]. Leucine and arginine released from tumors have been indicated to have high level of sensitivity and specificity between auxotrophic *Salmonella* strains in xenograft models of metastatic cancer in mice [20]. Systemic injection of engineered attenuated *S. Typhimurium* with TNF-Related Apoptosis-Inducing Ligand under the control of *recA* and also using γ irradiation are shown to

inhibit mammary carcinoma cell proliferation [21]. Furthermore, under the control of a Cytomegalovirus promoter, Fas ligand, hIL-12, h GM-CSF, mIL-12, m GM-CSF, IL-2 (which increases Natural killer cells) and immunomodulatory molecules like IL-18, chemokine CCL21 and LIGHT, a cytokine known to promote tumor rejection, have been cloned in an attenuated *S. Typhimurium*. As a result, angiogenesis is decreased and apoptosis or necrosis within the tumor tissue is increased [22-25]. The main advantage of using salmonella is its ability to grow rapidly under aerobic and anaerobic conditions. In anaerobic condition, *S. Typhimurium* carrying the gene for cytolysin (HlyE) under the control of a cell-specific promoter (hypoxia-inducible promoter), after systemic injection, could quickly migrate into hypoxia areas and diminish tumor growth [26]. In bactofection strategy, bacteria containing the naked plasmid DNA under the control of eukaryotic promoters can enter mammalian cells. Then, bacterial vectors may escape from phagosome to the cytosol of infected host cells to replicate and deliver the DNA directly into these cells [27, 28]. The recent studies have illustrated that some other bacteria like *Shigella flexneri* [27, 29, 30], *Salmonella spp* [27, 31], *E. coli* [27, 29, 30, 32], and *Yersinia enterocolitica* [33] can be used as transport molecules. Since the *Salmonella*, *Yersinia*, and *E. coli* stay inside the vacuolar phagosome of infected host cells, entering into the host cells' nuclei, where transcription of plasmid DNA occurs, is fundamentally unknown, yet according to different studies, releasing of the plasmid DNA happens either spontaneously or by antibiotics or application of auxotrophic mutants [29, 30]. A good example of using bacterial vectors is Lovaxin-C. This component is a recombinant live-attenuated *Listeria monocytogenes* which secretes the antigen HPV-16 E7 fused to a non-hemolytic listeriolysin O protein. In phase I of the study by Radulovic, promising specific T-cell and clinical response, with no serious adverse was detected in cervical cancer patients [34].

Bacterial toxins as the promising strategy to treat cancer

Bacterial toxins can be used for demolition of tumor cells or, at low concentrations; they alter cellular processes that control cell cycles, such as cell proliferation, apoptosis, and differentiation. These alterations could be shown in two conditions: firstly cell-cycle inhibitors, such as Cytolethal Distending Toxins, that cause cell block before entering into mitosis and the Cycle Inhibiting Factor (CIF) are injected to eukaryotic cell via a type III secretion system using pathogenic intracellular bacteria like *Burkholderia pseudomallei* cif (also known as CHBP) that converts glutamine 40 of NEDD8, which exerts important conformational control required for Cullin RING E3 ubiquitin Ligases (CRL) activity to glutamate (Q40E), which causes cytopathic effects and inhibits cell proliferation and secondly cell-cycle stimulators, such as the Cytotoxic Necrotizing Factor triggers G1–S transition to induce DNA replication. Then, not only has the number of cells do not increase, but the cells become multinucleated due to the toxin's ability to inhibit cell differentiation and apoptosis [35-37]. Certain bacterial toxins act through binding to antigens present on tumor surface, like Diphtheria toxin and Peudomonas exotoxin A, known to catalytically ribosylate EF-2 and lead to inhibition of protein synthesis accompanied by lysis cell and induction of apoptosis [38-40]. *Clostridium perfringens* enterotoxin (CPE) on type A strain is able to inhibit tumor growth *in vivo*. The C-terminal domain of toxin is responsible for high affinity binding to the CPE receptor and the N-terminal is supposed to be essential for cytotoxicity [41, 42]. Previous studies have indicated that the cytotoxic effects of CPE are thought to be useful as a novel therapeutic pathway on pancreatic cancer cells that led to tumor necrosis and inhibition of tumor growth [43]. Botulinum Neurotoxin is shown to have antitumor effect on the tumor microenvironment rather than affecting directly on tumor cells and can also grow in tumor blood vessels, making a window of opportunity for destruction of cancer cells by radiotherapy and chemotherapy [44]. Based on studies on some different bacterial toxins like alfa-toxin from *Stapylococcus aureus*, adenylate cyclase toxin

from *Bordetella pertussis*, shiga-like toxins, and cholera toxin on two cell lines, such as mesothelioma cells (P31) and small lung cancer cells (U-1690), adenylate cyclase toxin showed the potential to increase both cytotoxicity in both cell lines and to increase apoptosis, although cholera toxin did not induce apoptosis [35].

Nanoparticle-carrying bacteria

The cargo-carrying bacteria ('microbots') is a novel strategy to deliver specific therapeutic cargo for monitoring or altering gene expression and protein production, using an attenuated form of the intracellular bacteria *Listeria monocytogenes*. Three steps are necessary to make nanoparticles and bacteria hybrids. First, the bacteria are treated making use of a biotin-carrying antibody and thus will attach to the bacterial surface protein called muraminidase. Then, the treated bacteria are mixed by nanoparticles coated with streptavidin, a protein that binds strongly to biotin. Finally, the nanoparticle-loaded bacteria are mixed with plasmid DNA carrying biotin, which binds to the free strepdavidin sites on the surface of the nanoparticles and without any genetic manipulation, the microbots successfully enters tumor cells and releases nanoparticles, resulting in subsequent transcription and translation of the target proteins [45].

Nonliving bacteria

The Bacterial Ghost (BG) platform technology is a creative system for vaccine, drug or DNA delivery vectors. BGs are non-living, non-denatured empty cell envelops derived from gram-negative bacteria by controlled expression of the cloned lysis gene *E*. The role of gene *E* in the lysis of *Escherichia coli* was discovered in 1966 after infection with bacteriophage X174 [46]; following the development of genetic engineering technology, this hypothesis was confirmed [47]. *E* was the first lethal gene for bacteria which could be silenced on plasmids. Gene *E* codes for a 91-aa polypeptide and has no inherent enzymatic function and is able to produce a membrane protein with the ability to oligomerize into a transmembrane tunnel structure [48-50]. Based on the analysis of primary structure of protein E, it was revealed that a hydrophobic region at its N-terminal end is responsible to co-translational integration into the cytoplasmic membrane of *E. coli* [51, 52]. The analysis of the hydropathicity regions of protein E showed an E-specific lysis tunnel spanning the inner (IM) and outer membrane (OM), which is located at membrane adhesion sites within the host cell [53]. E-mediated lysis makes all cytoplasmic content release in to the environment while periplasmic components remain associated with the empty cell envelope [52]. The lysis tunnel diameter varies between 40 to 200 nm, does not indicate any regular structure, and the origin structure of the peptidoglycan remains intact [54]. A three-phase model for the process of E-mediated tunnel formation was described by Schön et al: (1) Integration of protein E into the IM with the C-terminal region, (2) Conformational change of protein E translocating the C-terminal domain to the Periplasmic Space accompanied by oligomerization, and (3) Fusion of IM and OM at membrane adhesion sites induced by exposition of the C-terminus of protein E to the cell surface [55]. The E-lysis processing could be illustrated in other gram-negative bacteria such as *Salmonella typhimurium, Klebsiella pneumoniae, Heliobacter pylori, Bordetella bronchiseptica, Vibrio cholerae,Haemophilus influenzae, Actinobacillus pleuropneumoniae, Mannheimia haemolytica, Pasteurella multocida, Pseudomonas putida, Pseudomonas aeruginosa, Pectobacterium cypripedii,* and *Ralstonia eutropha* [56, 57]. Furthermore, previous investigations have indicated that DNA loaded BGs are efficiently engulfed, ingested, and internalized by both APCs and tumor cells [58]. The BG system is safe due to nonliving bacteria, which retains all of the surface morphological, structural, and antigenic components of their living counterparts [59]. Hence, it is considered as an alternative method in vaccine development with a new efficient gene delivery platform. The inner space of BGs can be loaded with single components, or combinations of peptides, drugs, or DNA [60]. BGs are able to deliver the heterologous genes to monocyte-derived dendritic cells, macrophages, and melanoma. Cross-presentation of antigens (Ags)

delivered to DCs by BGs can activate both CD4+ and CD8+ T cells and stimulate the immune system to enhance the immune response. Bacterial LPS augments maturation of DCs, affects endosomal acidification of DCs, and modifies cross-presentation of Ags [61, 62]. For instance, to prevent trachomatous conjunctivitis and blindness, a *Chlamydia trachomatis* bacterial ghost vaccine was produced [63].

The obstacle of bacterial therapy

Using bacteria as a therapeutic vehicle may cause some problems. First, different side effects may be displayed by applying live, attenuated, or genetically modified form of bacteria. Second, systemic infection of bacteria is quite problematic and carries higher risk of apparent toxicity. Third, since the bacteria cannot consume all parts of the malignant tissue, it is necessary to combine bacterial therapy with chemotherapeutic treatments. Fourth, the major concern is mutations, which can result in losing their function.

CONCLUSION

The idea of using bacteria in cancer therapy has shown to be promising. The resistance of cancer cells to the drugs remains a significant problem. Thus, to solve this problem, bacterial therapy combined with cytotoxic agents has been proposed. Bacterial products such as toxins, spores, etc. are useful candidates to treat solid tumors. Furthermore, bacterial ghosts, microbots, and bactofection are the other strategies to destruct the malignant tissues. At last, further investigation and developments are being pursued to improve cancer treatment.

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