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Review article

Bacteria and Archaea: A new era of cancer therapy

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ABSTRACT

Cancer is one of the most important mortality in the world. The major drawbacks of chemotherapy are the poor absorption of drugs into tumor tissues and development of resistance against anti-cancer agents. To overcome these limitations, the use of microorganisms has been extensively considered in the treatment of cancer. Microorganisms (bacteria/Archaea) secrete different bioactive compounds that can efficiently inhibit cancer cells growth. Biological nanocarriers derived from microorganisms including outer membrane vesicles (OMVs), bacterial ghosts (BGs) and archaeosomes have also been considered as drug delivery systems. Conjugation of drug loaded nanocarriers to bacteria strongly kills the cancer cells after internalization through the bacteria. Merging of microbiology and nanotechnology may provide versatile microbial nano-hybrids for promising treatment of cancer. This strategy causes more amount of drug to enter into cancer cells. In this review, we present evidence that microorganism, their derivatives as well as their intervention with nanotechnology can be a powerful vehicle for eradication cancer.

1. Introduction

Despite remarkable advances in the treatment of cancer, it remains one of the major mortality in the world [1]. The diffusion of anti-cancer drugs into solid tumors is a significant issue in the effectiveness of drugs. Long extravascular diffusion distances and existing of interstitial pressure impede the diffusion of drugs into tumor tissues [2]. In this regards, development of novel anti-cancer agents from natural sources has been extensively considered [1]. Microorganisms belonging to the bacteria and Archaea kingdom have been received extensive attention as important resources for exploring natural agents [1,3]. Bacteria and Archaea can secrete proteins, toxins and enzymes that efficiently inhibit tumor cells growth [4,5]. Tumor environment supplies nutrients required for the bacterial proliferation; consequently, bacterial growth rate in the tumor tissues is more than normal tissues. Anaerobic bacteria such as *Clostridium* and *Salmonella* can survival only in the low-oxygen environment of tumors [6]. Furthermore, the tumor hypoxic microenvironment inhibits the action of macrophages and neutrophils and as a result bacteria cannot easily remove from tumor tissues [7]. Hence, bacteria can be employed as promising strategy for targeting tumor tissues.

Biological nanocarriers derived from Archaea and bacteria have also been considered as drug delivery system due to biodegradable, fusing with the target cells, enhancing cellular uptake and longer circulation time *via* escaping the immune system [8,9]. Outer membrane vesicles (OMVs) and bacterial ghosts (BGs) are cellular derivatives of Gram negative bacteria that contain bacterial membrane proteins [8,10]. Archaeosomes are liposomes prepared from polar lipids extracted from Archaea [11]. OMVs, BGs and archaeosomes can be explored as biological drug delivery systems for different therapeutic agents. Furthermore, they can stimulate the immune responses to enhance the effect of anti-cancer agents [9,12].

In recent years, several nanocarriers have been used as drug delivery system including, liposomes [13,14], solid lipid nanoparticles (SLNs) [15], polymeric nanoparticles [16], dendrimer [17] and micelles [18]. However, a small percentage of nanocarriers reach to tumor tissues due to their dependence on the systemic circulation, nonexistence of a propelling force to enter into tumor tissues and the lack of sensory-based

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movement ability for targeting the hypoxic regions of tumor tissues [19]. Motile bacteria can penetrate into distal areas of blood vessel, which make them to be considered as appropriate vehicle for carrying of nanocarriers to cancerous tissues [6]. Moreover, some special nutrients that are released in the tumor microenvironment attract the bacteria [20]. Therefore, merging of nano-formulations and microorganisms can give raise a novel and promising approaches to fight the cancer. For example, *E. coli* moves toward cancer cells under a concentration gradient of L-aspartate [21]. *S. typhimurium* can target tumor tissues using its flagella and chemotactic receptors [22]. The aim of the review article was to present the role of microorganism and their derivatives as well as their intervention with nanotechnology in the cancer treatment.

2. Bacteria/Archaea as savior for eradication of cancer

Non-pathogenic and attenuated strains of microorganisms can be actively targeted cancer cells. Moving flagellate bacteria possess a driving force that makes them able to migrate to the distant vascular areas where are not commonly accessible for chemotherapeutic agents [6]. *Clostridium difficile* toxin B recombinant protein (rcdtB) was considerably inhibited tumor growth and triggered apoptosis *via* reducing Bcl-2 expression, inducing inflammatory responses and increasing cyclooxygenase-2 and erythroblastic leukemia viral oncogene homolog 2 expression in mouse with breast cancer [23].

It has been shown that tumor endothelial cells overexpress vascular endothelial growth factor receptor 2 (VEGFR-2). Therefore, VEGF can be employed as ligand for delivery of drugs to tumor vessels *via* VEGFR-2 in order to antiangiogenic therapy. Shiga-like toxin 1 (SLT-1) produced by *E. coli* O157:H7 can be able to damage endothelial cells. Conjugation of SLT to VEGF (SLT-VEGF) can internalize *via* VEGFR-2 mediated endocytosis and exhibit its cytotoxicity on cancer cells with VEGFR-2 overexpressed. Immunohistochemical analysis showed that SLT-VEGF selectively reduced VEGFR-2 overexpressing $CD31^{+}$ endothelial cells in tumor vasculature. SLT-VEGF considerably decreased microvessel density (MVD) in pancreatic cancer cells. In addition, combination of SLT-VEGF with gemcitabine significantly inhibited tumor spread in mouse [24].

Carotenoids are pigments that produced by the most haloarchaeal species. It was observed that carotenoids inhibit the growth of cancer cells through inducing apoptosis, increasing gap-junctional communication and suppressing the cell cycle [5]. Carotenoids of *Natrialba* sp. M6 showed significantly cytotoxic effect than 5-fuorouracil (5-FU) on cancer cells (Caco-2, MCF-7, HepG-2 and HeLa cells). Moreover, these pigments triggered more apoptosis than 5-FU in tumor cells. Interestingly, it was found that extracted pigment was considerably safer than 5-FU on normal cells. These results confirm that carotenoids extracted from Archaea can present a novel prospect for cancer therapy without any side effects on normal cells [25]. These findings is in agreement with Abbes et al. results which showed that carotenoids extract from *Halobacteria* displayed significant anti-cancer activity against HepG2 cell lines [5].

Supernatant metabolites derived from *Halobacterium salinarum* triggered apoptosis through overexpression of caspase3 gene, decreased SOX2 (SRY-Box Transcription Factor 2) gene expression (as a pluripotency gene) in cancer cells and reduced the tumor volume in mouse model [26].

Cationic anti-microbial peptides (CAMPs) are short peptides with positive charge and amphipathic structure that can inhibit the growth of Gram negative and positive bacteria, protozoa and fungi. VLL-28 as CAMPs is a transcription factor Stf76 encoded by pSSVx (a hybrid plasmid-virus) from *Sulfolobus islandicus* that inhibit the growth of Gram negative and positive bacteria as well as *Candida albicans* [27]. Moreover, VLL-28 exhibited stronger cytotoxicity activity against murine and human cancer cells *via* activation of caspase-3 and inducing of apoptosis pathway [3]. Therefore, VLL-28 can be considered for development of novel anti-cancer agents.

The anti-cancer agents from microorganism and their mechanism of action are shown in Table 1.

It has been reported that intratumor bacteria cause drug resistance in cancerous tissues through metabolizing anti-cancer drugs. It was observed that bacteria (*Gammaproteobacteria*) metabolize gemcitabine (2′,2′-difluorodeoxycytidine) into 2′,2′-difluorodeoxyuridine (inactive form) which result in resistance to the chemotherapy agents [32]. *Fusobacterium nucleatum* promotes colorectal cancer resistance to 5-FU and oxaliplatin through activation of autophagy pathway [33]. Doxorubicin is also degraded by *Raoultella planticola* to metabolites, 7-deoxydoxorubicinol and 7-deoxydoxorubicinolone which leads to reduction of the efficacy of the drug and increasing of side effects [34]. The efficacy of chemotherapy may be enhanced by co-treatment with antibiotics. Nevertheless, antibiotic agents may inhibit the growth of other bacterial species that are beneficial for the body. There are also some concerns about the use of bacteria as anti-cancer agents including probability of occurrence of mutations, side effects and limited targeting efficiency. Using genetic engineering, anti-cancer activity of bacteria has improved and their virulence against normal cells has been minimized. However, further studies are needed to approve the use of bacteria/Archaea as anti-cancer agents for treatment of cancer in clinical cases.

3. Bacteria/Archaea: As biological nanocarriers for anti-cancer agents

Gram negative bacteria naturally secrete nanoscale OMVs. These nanocarriers contain proteases, sulfatases and adhesins [10] which allow them to bypass the process of phagocytosis, facilitate the interaction of them with host epithelial cells and allow to enter cells *via* different routes such as lipid raft, clathrin-dependent endocytosis and

Table 1
anti-cancer agents from microorganism and their mechanism of action.

Microorganism	Anti-cancer agent	Mechanism of action	Ref.
Clostridium perfringens	Enterotoxin (CPE)	Cytolysis of proteins CLDN3 and CLDN4 on the cell surface of prostate cancer cells	[28]
Pseudomonas aeruginosa	Exotoxin T (ExoT)	Reduce the growth and inhibit cell migration of B16 melanoma	[29]
Clostridium difficile	Toxin B recombinant protein (rcdtB)	Trigger apoptosis *via* reducing Bcl-2 expression, inducing inflammatory responses and increasing cyclooxygenase-2 and erythroblastic leukemia viral oncogene homolog 2 expression in mouse with breast cancer	[23]
Nocardiopsis sp.	Androsamide	Suppressing the motility of Caco2 cells caused by epithelial mesenchymal transition	[30]
Pseudomonas aeruginosa	Azurin	Enhancing the intracellular levels of p53 and Bax, triggering the release of mitochondrial cytochrome *c* into the cytosol, activating the caspase cascade and inducing apoptotic pathway	[31]
Halobacterium halobium	Aarotenoids	Inhibition of HepG2 cell viability through oxidative stress induction	[5]
Natrialba sp. M6	Aarotenoids	Induction of apoptosis-dependent cell death cancer cells (Caco-2, MCF-7, HepG-2 and HeLa cells)	[25]
Halobacterium salinarum	Metabolites	Trigger apoptosis through overexpression of caspase3 gene and decreased SOX2 gene expression on prostate cancer cell lines	[26]
Sulfolobus islandicus	VLL-28 (as CAMPs)	Inhibition the growth of cancer cells *via* activation of caspase-3 and inducing of apoptosis pathway	[3]

micropinocytosis [9,10]. Due to small size, OMVs can also enter to tumor cells by enhanced permeability and retention (EPR) effect which facilitates the entrance of their cargos into cancer cells [9]. Since OMVs are rich in lipopolysaccharide (LPS), cytoplasmic protein, outer membrane lipid as well as some genomic material (such as DNA and RNA), they have been considered as adjuvant or as vaccine and antigen carrier in anti-bacterial and anti-cancer therapies [35,36]. OMVs merge into membrane of host cells and transport their cargos into cells [9]. Single intravenous injection of OMVs derived from *S. typhimurium* (at a low dose) induced immune system through increasing the secretion of cytokines. Furthermore, OMVs led to extravasation of red blood cells (RBCs) in the tumor mainly due to the effect of LPS on the OMVs (Schwarzman response). In the near infrared (NIR) region, hemoglobin within RBCs possesses absorbance and may be considered as endogenous photothermal factor for photothermal therapy. According to the findings, RBCs extravasation within the tumor led to accumulation of hemoglobin in the tumor, increasing the optical absorbance in the NIR region and finally destruction of tumor [35]. OMVs derived from *Klebsiella pneumonia* were used for delivery of doxorubicin (DOX) into lung cancer cells. DOX-OMVs induced extensive apoptotic and necrosis pathway in tumor tissues and considerably inhibited tumor growth in the mice. Moreover, these nanocarriers elicited appropriate immune responses which resulted increasing the anti-tumor effect of DOX [9]. According to the results of various studies, it can be concluded that OMVs not only improve drug delivery into tumor cells, but also stimulate the immune responses to increase the effect of anti-cancer drugs.

BGs are nonliving cell envelopes of Gram negative bacteria that produced by controlled expression of the cloned gene E. BGs contain all cell membrane structures, such as LPS, peptidoglycan and adhesins without any infectious threats [37,38]. BGs exhibit high loading capacity that can be exploited as carrier for vaccine and drug delivery [36]. Due to existence of LPS, BGs active macrophages to secrete cytokines including interleukin-6 (IL-6) and TNF-α which lead to trigger humoral and cellular immune response [8]. *E. coli* BGs encapsulated with 5-FU have been investigated to target colon cancer cells. According to the results, 5-FU loaded BGs exhibited more apoptosis than free drug in cancer cells [39].

Liposomes, as drug delivery systems are spherical vesicles that can be made from natural and synthetic lipids. However, liposomes can also be prepared from lipid extracted from bacteria owing to the membrane of bacteria have a high proportion of phosphatidylethanolamine [40]. Bacteria are able to grown in different environments and can be easily stored [41]; consequently, microorganism lipids can be appropriate materials for liposomes preparation. In our previous research, we observed that carboxyfluorescein (*CF*, as a drug model)-loaded liposomes prepared by lipids extracted from *E. coli* enhanced the internalization of *CF* to cancer cells through the endocytic pathway [42]. Hence, it can be concluded that bacterial liposome can provide a promising new avenue for vaccine and drug delivery.

Archaeosomes are liposomes that prepared from natural or synthetic archaeal lipids [43]. The Archaea possess distinct ether lipids in their membrane which allow them to live in harsh condition [44]. As a result, archaeosomes exhibit tight membrane packing, low permeability and high stability against extreme pH and temperature than conventional liposomes [43,45,46]. Oral administration of peptide and protein are limited due to their instability in gastrointestinal (GI) tract [47]. As mentioned, archaeosomes are more resistant to acidic pH than liposomes [12]. In a study, archaeosomes prepared from polar lipid fraction E (PLFE) extracted from *Sulfolobus acidocaldarius* were evaluated for oral delivery of insulin. It was found that archaeosomes were more stable in simulated GI tract conditions than conventional liposomes (composed of egg phosphatidylcholine and cholesterol). In addition, archaeosomes considerably decreased the blood glucose levels more than conventional liposomes [48]. Results showed that simultaneous utilization of human papilloma virus L1/E6/E7 genes in combination therapy with archaeosomes led to inducing the T-cell immune responses and strong cytotoxic activity against cancer cells [49]. DOX containing archaeosomes prepared from PLFE of *S. acidocaldarius* and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (tetraether-diester hybrid) significantly increased the cytotoxicity effect of the drug. Moreover, these nanocarriers were thermo-sensitive which can provide a promising platform for hyperthermia treatment of tumors [50]. In our previous work, we prepared archaeosomes from *S. acidocaldarius* and methylene blue and *CF* were used as drug models. These archaeosomes exhibited monodispersity, prolonged release and high uptake in cancer cells (Fig. 1) [42,51].

Drug delivery systems derived from bacteria and Archaea are shown in Table 2.

4. Nanotechnology and microorganisms intervention for cancer treatment

Since the active delivery of nanocarriers to the target tissue depends on the blood flow traffic, adding a motive force can be improved the delivery [20]. Moreover, nano-drug delivery system should be capable of escaping from lysosomal enzymatic degradation. Bacteria have natural endosomal escaping ability, which make them as suitable carriers for delivery of nano-drug delivery system [7,57]. Moreover, since collagen content of extracellular matrix (ECM) of tumors is high and diffusivity of larger molecules such as antibody or liposomes is negatively correlated to collagen content of tumors, penetration of such structures is inhibited by ECM of collagen of tumors [6]. It was shown that the combination of therapeutic bacteria and conventional chemotherapy may lead to dramatically large synergetic effect [6].

In vivo studies have been shown that bacteria are absorbed to the cancer cells through secretion of nutrients in the tumor microenvironment, such as glucose, aspartate, ribose, arginine, serine and leucine. Moreover, bacterial motility is pH dependent. The *Salmonella* loaded with DOX-containing liposomes were developed to swim autonomously toward tumor cells. In the presence of ammonia (secreted by tumor cells), imbalances the osmotic gradient led to the release of DOX from liposomes. Due to simultaneous antibiotic and anti-cancer activities of DOX, the drug destroyed the bacterial carrier and inhibited the growth of cancer upon releasing from liposomes [20].

As mentioned, penetration of nanocarriers in tumors is inhibited by ECM of collagen due to higher collagen content of ECM in tumors. Employing of anaerobic bacteria can overcome this limitation. The amount of oxygen in the tumors is very low owing to rapid oxygen consumption by cancer cells; therefore, anaerobic bacteria such as *C. novyi-NT* can only grow under hypoxia regions in tumors. Moreover, these bacteria can move to farther blood vessels by their flagella, where anti-tumor agents usually cannot enter. It was observed that *C. novyi-NT* improved the Doxil (PEGylated liposomal DOX) delivery and increased the drug concentration in tumor tissues due to decomposition of collagen ECM *via* bacterial proteolytic activity. The phenomenon that in turn decreases interstitial fluid pressure, decompresses blood vessels and enhances vessel density in tumors [6].

Previous studies reported that *Salmonella* infects cancer cells through type-III secretion system (TTSS) expression and employs the membrane lipopolysaccharide (LPS) to trigger production of nitric oxide and inflammatory cytokines. Conjugation of *Salmonella* with low temperature sensitive liposomes (LTSL) can exhibit extra effects. Combination of DOX containing thermosensitive liposome with *Salmonella* through Biotin-Streptavidin bindings and tumor heating with high intensity focused ultrasound could increase localization of drug and induce expression of inflammatory cytokine in colon cancer cells [58]. These findings are in line with other studies. Liposomal paclitaxel conjugated with *S. Typhimurium* showed more cytotoxicity than liposomal paclitaxel. These results may be associated with chemotactic motility of bacteria which transported more liposomal paclitaxel to the tumor cells [22]. However, the anti-cancer efficacy of *Salmonella* (especially strain VNP20009) is limited due to trigger autophagy in tumor cells, which

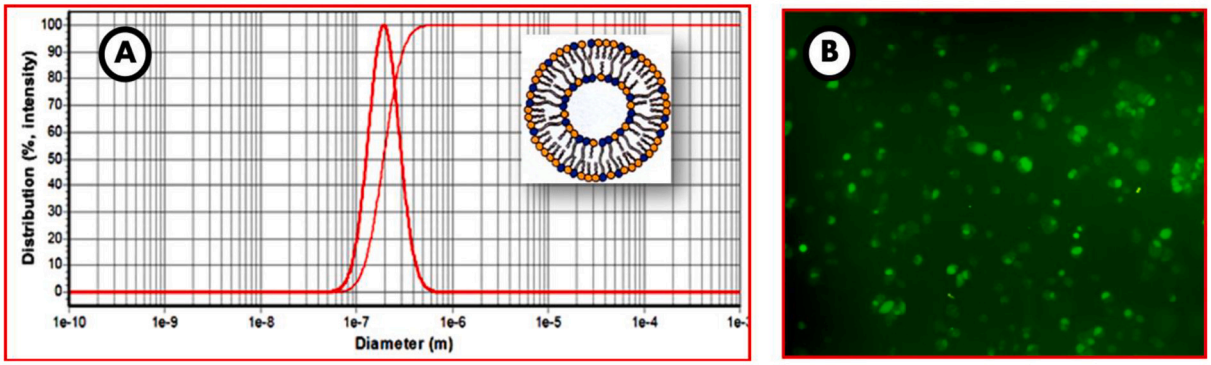

Fig. 1. Prepared archaeosomes from *S. acidocaldarius* A) particle size distribution and B) cellular uptake in cancer cells.

Table 2
Drug delivery systems derived from bacteria and Archaea.

Drug delivery system	Microorganism	Payload	Applications	Outcome	Ref.
OMVs	*Klebsiella pneumonia*	DOX	Drug delivery	Inducing apoptotic and necrosis pathway in tumor tissues	[9]
OMVs	*S. typhimurium*	-	Adjuvant	Inducing immune system through increasing the secretion of cytokines	[35]
OMVs	*E. coli*	-	Immunotherapy	Suppress tumor by interferon-γ-mediated anti-tumor response	[52]
OMVs	*E. coli*	Decorated with EGFRvIII and B16-M30	Immunotherapy	Induction of high anti-EGFRvIII antibody titers, M30-specific T cells and infiltration of $CD4^+$and $CD8^+$T cells at the tumor site	[53]
BGs	*E. coli*	5-FU	Drug delivery	Inducing apoptosis in colon cancer cells	[39]
BGs	*Salmonella*	DOX	Drug delivery	Higher anti-proliferative and apoptosis activities over the free DOX in the HepG2 cell line	[54]
BGs	*E. coli*	Oxaliplatin	Adjuvant	Induction of immunogenic cell death and activation of T-cell response, leading to long-term anti-tumor memory effects	[55]
Liposome	*E. coli*	*CF* (as drug model)	Drug delivery	Internalization of *CF* to cancer cells through the endocytic pathway	[42]
Archaeosome	*S. acidocaldarius*	DOX	Drug delivery	Increasing the cytotoxicity effect of the drug in cancer cells	[50]
Archaeosome	*S. acidocaldarius*	*CF* (as drug model)	Drug delivery	High uptake in cancer cells	[51]
Archaeosome	*Methanobrevibacter smithii*	Ovalbumin (OVA)	Tumor antigen delivery	Promoting the infiltration of dendritic and natural killer cells to the tumor site	[56]
Archaeosome	*Halobacterium salinarum*	pDNA	Adjuvant	Inducing apoptosis in the tumor cells	[49]

eliminating the bacteria; therefore, decrease their anti-cancer activity. To overcome this drawback, *Salmonella* was co-administered with hydroxychloroquine (HCQ, as autophagy inhibitor) loaded liposomes. Liposomes were employed for reducing toxicity of HCQ on normal cells as well as targeted tumor cells through the EPR effect. Results showed that co-treatment led to increase apoptosis in tumor cells, induce up-regulation of IFN-γ and TNF-α in serum, reduce tumor growth and increase survival. This approach provides more efficacies of bacteria in cancer treatment without side effects related to HCQ and VNP20009 [59].

The hypoxic microenvironment in the solid tumors decreases their vulnerability to radiotherapy and anti-cancer agents. Nevertheless, the reduced surrounding oxygen provides a suitable environment for proliferating of anaerobic bacteria such as *Bifidobacterium longum* (*B. longum*). According to the previous study, *Bifidobacterium* (as non-pathogenic bacteria) grow only in the tumor area, which indicating the hypoxic microenvironment of tumor is appropriate for its localization and proliferation. Moreover, it has been reported that vascular pores of tumors are between 1 and 20 μm and length and width of *B. longum* are approximately 1–4 and 0.3–0.4 μm, respectively. Therefore, even if the surface of bacteria is conjugated with nanocarriers, its width does not exceed 1 μm, which leads to nanocarriers-bacteria freely penetrate through vasculature of tumors into solid tumors. In a study, perfluorohexane (PFH)/ poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles conjugated with *B. longum* was employed for targeting solid tumors to attain accuracy imaging and high efficacy of treatment. According to the obtained results, PFH/PLGA-*B. longum* displayed more anti-cancer effect, longer residence time and provided precision imaging than PFH/PLGA NPs [60].

Paclitaxel containing liposomes conjugated *E. coli* were employed for treatment of lung cancer. According to the findings, liposomal drug-loaded bacteria exhibited higher anti-cancer effect on lung cancer cells through downregulation of VEGF, hypoxia-inducible factor (HIF-1α) and inducing apoptosis in cancer cells. This formulation considerably increased the expressions of IFN-γ, TNF-α and interleukin-4 (IL-4) in rats. In addition, the number of blood immune cells did not alter by unconjugated liposomal paclitaxel, while they were significantly enhanced by conjugated liposomal paclitaxel. No significant adverse effect and hypersensitivity were observed in rats [7].

E. coli Nissle 1917 (EcN) was employed as a bacterial carrier for acid-labile release of hybrid micelles (containing DOX and α-tocopheryl succinate) to improve the synergistic anti-tumor efficacy. Results showed that nanocarriers released from the bacteria in the response to the acidic pH of tumor cells and then, micelles led to increase the cellular uptake and selective accumulation extend drugs residence time in tumor tissues [61].

RBCs have been considered as natural carrier for drug and imaging agents delivery in the body due to their abundance, high biocompatibility, low toxicity and improving the solubility of drugs [62,63]. RBCs-based bacterial microswimmers (*E. coli*) were designed for delivery of DOX and superparamagnetic iron oxide nanoparticles (SPIONs). This strategy alters RBCs from passive carriers into active drug deliveries. These bio-hybrid microswimmer displayed a pH-dependent release with significantly increased release at acidic tumor environment, owing to the osmotically RBCs swelling and finally their hemolysis and potential cargo delivery to cancer cells [62].

According to the results of various studies, integration of nanotechnology and microbiology can be provided a promising perspective for

treatment of cancer in future.

The role of microorganisms in the treatment of cancer is presented schematically in Fig. 2.

5. Innovation in the genetic of bacteria for using as vaccine and for antibody production

Insufficient or imperfect activity of intrinsic immunity may be one of the main reasons for escaping tumor from immune system, which in turn inhibits generation of protective adaptive responses. Bacteria based vaccines have been extensively considered for cancer immunotherapy due to provoking of strong immune responses. Several of microorganisms such as *Lactobacillus*, *Salmonella* and *Bifidobacterium* have been evaluated as vaccine vectors for the treatment of cancer [64–66]. Oral *Salmonella*-based vaccine (strain CVD 915) efficiently inhibited liver metastases through triggering of tumor-specific T-cell. According to the reported results, attenuated *Salmonella*-based vaccine can be employed as a neoadjuvant for preventing the development of liver metastases [67]. A WT1 (Wilms' tumor 1) oral cancer vaccine, recombinant *Bifidobacterium longum* displaying a partial WT1 protein, was designed for the treatment of castration-resistant prostate cancer (CRPC). This vaccine triggered higher cellular immune responses against cancer cells than Db126 peptide vaccine. In addition, tumor growth was inhibited by the vaccine and survival rate in TRAMP-C2 mice CRPC model was raised in a dose-dependent manner. These results indicate that WT1 oral cancer vaccine can be used as novel immuno-oncology drug for the CRPC treatment [65]. In a phase I clinical trial, a mixed bacterial vaccine (Coley's toxins, a mixture of heat-killed *Streptococcus pyogenes* and *Serratia marcescens*) was evaluated in patients with NY-ESO-1 (New York esophageal squamous cell carcinoma 1) expressing cancers. According to the findings, this vaccine increased immunoregulatory cytokines that are involved in inducing of tumor regressions [68]. JNJ-64041757 (JNJ-757) is a live, attenuated, double-deleted *Listeria monocytogenes*-based immunotherapy that expresses human mesothelin (a tumor-associated antigen), was evaluated as monotherapy and in combination with nivolumab in patients with advanced non-small-cell lung carcinoma (NSCLC). Results showed that JNJ-757 potentially induced cellular immunity against mesothelin expressing cancer cells and showed synergistically effect with nivolumab [69]. ADXS-NEO (a bacteria-based vaccine) is a L. *monocytogenes*-based immunotherapy that designed for targeting of patient's mutation-derived tumor-specific neoantigens in patients with head and neck cancer, NSCLC and colorectal cancer [70,71]. A phase I clinical study is currently underway to evaluate its effectiveness.

Bacteria are also considered for production of monoclonal antibody due to easy genetic manipulation, fast growth and cost-effectiveness of antibody production [72,73]. Human epidermal growth factor receptor 2 (HER2) is overexpressed in patients with ovarian, bladder, lung, gastric and breast cancers. A HER2 bispecific antibody was expressed in *E. coli* and efficiently triggered T cell-mediated HER2-specific cytotoxicity *in vitro* and *in vivo* [74]. A recombinant antibody to target cells expressing epithelial cell adhesion molecule (EpCAM) and vascular endothelial growth factor receptor 2 (VEGFR2) was expressed in *E. coli* for inhibition of growth and metastasis in breast cancer cells. It was shown that the recombinant antibody decreased the Akt/Nf-Kβ signaling pathway in cancer cells [75].

6. Conclusion

The major limitations of chemotherapy are development of resistance against anti-cancer agents, side effects and poor absorption of drugs into cancer cells. To overcome these drawbacks, the employing of microorganisms and their derivatives has been extensively considered in the cancer therapy. OMVs, BGs and archaeosomes can also be considered as potential biological drug delivery systems. Merging of microbiology and nanotechnology can lead to alteration of nanocarriers transfer mechanism from passive to the targeted delivery. Moreover, combination of therapeutic bacteria and nanomedicine can be led to considerably high synergetic effect and considerable inhibition of cancer cells growth. Despite the attractiveness of bacterial therapy, it should be mentioned that its efficiency and safety have always been disputed with concerns of gene stability, bacterial dose-dependent side effects and immune responses. Further and deep studies are needed to evaluate its efficacy and toxicity for treatment of cancer.

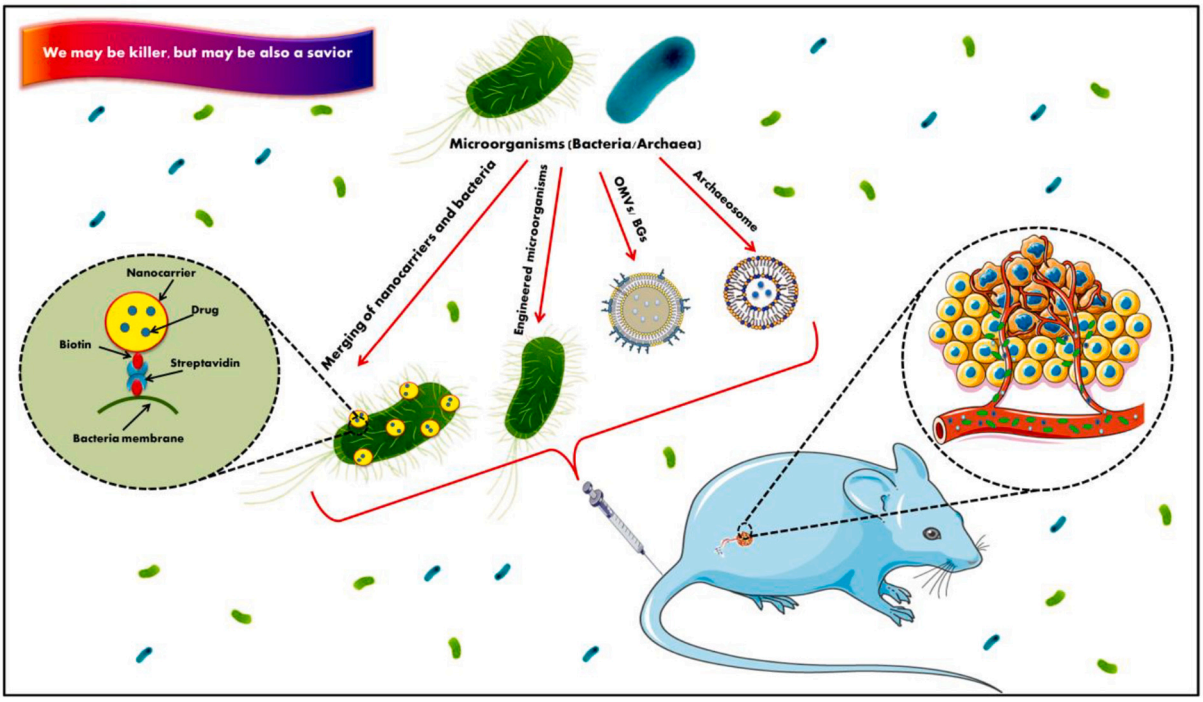

Fig. 2. Schematic representation of anti-tumor activity of microorganisms in cancer cells.

Declaration of Competing Interest

The authors declare no competing interests.

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