MAK HILL Publications Journal of Animal and Veterinary Advances

Review Article
doi: 10.59218/makjava.2023.15.18

OPEN ACCESS

Key Words
Bacterial ghost, cancer therapy, delivery system, adjuvant

Corresponding Author
Banu Atalay,
Program of First Aid and Emergency, Vocational School of Healthy Services, Batman University, Batman, Turkey
banu.atalay@batman.edu.tr

Author Designation
Assistant Professor

Received: 19 February 2023
Accepted: 13 March 2023
Published: 19 March 2023

Citation: Banu Atalay, 2023. Use of Bacterial Ghosts as a Delivery System in Cancer Theraphy. J. Anim. Vet. Adv., 22: 15-18, doi: 10.59218/makjava.2023.15.18

Use of Bacterial Ghosts as a Delivery System in Cancer Theraphy

Banu Atalay
Program of First Aid and Emergency, Vocational School of Healthy Services, Batman University, Batman, Turkey

ABSTRACT
In cancer treatment, the main objectives are to concentrate the chemotherapeutically applied substance on the targeted tissue as specifically as possible and to minimize its effect on other cells until it reaches the target tissue. Different pharmaceutically developed drug delivery systems for this purpose have been tried for many years and are currently in use in practice. Bacterial ghosts (BG), which have been developed in recent years, are non-living bacterial structures that are usually obtained as a result of various processes of gram (-) bacteria, evacuated and consisting only of cell wall. The use of the emptied cytoplasmic compartments of ghost bacteria as a transport system by loading them with various pharmaceuticals seems to serve this purpose. Thus, it is expected that pharmaceuticals, which are mostly known to have cytotoxic properties, are transported to the cancerous area in a controlled and safe manner. In addition, since BGs do not completely lose their surface structures (pathogen-related patterns) they will have the ability to stimulate the immune system, a second expected benefit will be the adjuvant effect. In the present review, the information on the use of ghost bacteria systems as a transport system for chemotherapeutic agents used in cancer therapy is compiled.

INTRODUCTION

Cancer has been among the leading causes of death in the world for many years, due to the increase in predisposing factors, increased exposure and difficult treatment. It is important to develop chemotherapeutics that do not harm other tissues, especially for cancer formations that are difficult and risky to treat, and to develop transport systems that will cause as little damage as possible to living tissues other than the target tissue of existing chemotherapeutics.

Chemotherapy, which is one of the methods commonly used in cancer treatment, also causes significant damage to normal tissues and causes many off-target effects. Although, its use to treat human cancers provides short-term benefits, it has been reported that tumors eventually again recurrence[1]. An important step in the development of bacterial therapeutics is the identification of potential species and strains with minimal pathogenicity to the host. It has been known for years that bacteria are isolated in the environment of some cancers[2]. Various hemodynamic and chemotactic processes have been reported in bacterial invasion and growth in the tumor environment[3]. However, since the ghost bacteria (Bacterial Ghost, BG) we will talk about are not alive, an effect on metabolism is not expected.

Successful delivery of not only chemotherapeutic agents but also antigens and DNA used in gene therapy to the target region in cancer therapy is directly related to the success of the selected delivery/carrier system. The DNA vaccines used for this purpose consist of plasmids encoding the specific antigen, and regardless of the method in which the DNA is transferred, the transferred gene is expected to generate a strong immune response and change the behavior of the target cell. Various techniques, including viral vectors, attenuated bacteria, polycation DNA complexes, and cationic liposomes, are used to transport genetic materials to the target cell[4,5]. The BG system, which has been developed in recent years, constitutes a new platform for cancer vaccines. Since the diffusion of drugs used in cancer treatment into tumoral tissue, especially solid tumors, is an important issue, the inability of drugs to penetrate the tumor tissue sufficiently due to the long diffusion distance and interstitial pressure through extravascular tissues is an important issue[6]. At this point, the knowledge that motile flagellated bacteria have a driving force that allows them to migrate to distant vascular areas that are not commonly accessible for chemotherapeutic agents[7].

It is known that the tumor environment provides the necessary nutrients for bacterial growth and as a result, the bacterial growth rate in tumor tissues is higher than in normal tissues. It has also been reported that anaerobic bacteria such as Clostridium and Salmonella can only survive in the low oxygen environment of tumors[7]. The survival and growth of bacteria that settle into tumors appears to depend on oxygen requirements and other characteristics such as intracellular/extracellular survival mode and motility. These reasons may be important in terms of secretion of substances released from bacteria and slowing tumor growth, however, since ghost bacteria do not have metabolism and reproduction abilities, they should be considered only as a passive transport system.

In one of the studies on the transfer of antineoplastic drugs to the cancerous region in BGs, it was studied with doxorubicin in colon cancer[8].

In gene therapy, the aim is to deliver the genes encoding the appropriate proteins to the tumor cells, followed by restoring the cell's natural functions and potentially inducing an immune response. It has also been reported that transfection with genes encoding cytokines that attract professional antigen-presenting cells to the cancer site and activation and development of immune responses, including tumor cells, are also important[9,10].

Ghost bacteria production techniques: In general, ghost bacteria are obtained by controlled expiration of the E lysis gene from various gram (-) bacteria followed by evacuation of the cytoplasmic contents of the cell. The E gene used in the first technique, in which BGs were obtained by E-mediated lysis, is the first lethal gene for bacteria, is located on plasmids, and can be silenced. It has been reported that BG is obtained in gram (-) bacteria as a result of the expression of the E gene by phage in non-host bacteria, while gram (+) bacteria die without lysis[11]. The bacterium, whose cytoplasmic content has been emptied, remains in its natural form with its cell membrane antigenic properties. In other words, bacteria continue to theirs carry immunogenic structures such as lipopolysaccharide, peptidoglycan and lipid A.

Study examples on the use of ghost bacteria in various types of cancer: The targeting capacity of *E. coli* BGs encapsulated with 5-Fluorouracil (FU) to colon cancer cells was investigated in the study designed to demonstrate the carrier properties of BGs. From the obtained data, it has been reported that 5-FU loaded BGs show more apoptosis in cancer cells than free drug[12].

Kudela *et al*.[13] studied two BG systems prepared from *E. coli* NM522 and *Mannheimia haemolytica A23* strains in a melanoma cell line known to be capable of phagocytizing, processing and presenting antigens. In that study, they investigated the capacity of BGs to

target melanoma cells, their rate of adhesion to them, and the capacity of melanoma cells from 7 different lines to phagocytize BG. BGs in this study were obtained by conventional general phase lysis and the evacuated bacteria were loaded with plasmid encoding green fluorescent protein (pEGFP) to be visibly evaluated for the parameters studied. As a result of the study, it was determined that the panel of melanoma cell lines had a high capacity to bind and internalize BG, which was associated with the ability of BG to successfully deliver heterologous DNA to melanoma cells.

It is known that LPS on the BG cell surface of melanoma cells is recognized by phagocytes via the TLR-4 receptor, and this recognition eventually leads to IL-8 release[14]. It has also been shown that loading BG with DNA can be simplified using self-immobilizing plasmid (SIP) retained by the carrier envelope due to a specific interaction between cytoplasmic membrane-associated proteins and mini-circular DNA during and after protein E-mediated lysis. Thus, the safety profile has increased as the origin of replication and antibiotic resistance indicators that are not required for the vaccine have been removed[15].

Antigen-loaded BGs are readily phagocytosed by immature DCs, allowing their contents to be efficiently processed and presented via the MHC-I and MHC-II pathways within DCs[16,17]. BGs can thus serve as a combined adjuvant and antigen delivery system, enabling the generation of antigen-loaded mature DCs in a one-step procedure. Dobrovolskiene *et al*.[18] loaded tumor cell lysate (oncholysates) into BGs using melanoma, renal cell carcinoma (RCC) and glioblastoma tumor cell line lysates, and investigated the maturation of dendritic cells, and thus their ability to trigger Thelper 1-pole antitumor immune response.

The rationale for the use of a large number of tumor-associated antigens may be associated with the failure of therapeutic cancer vaccination, due to the possibility of loss of antigen in cancerous tissues in an escape logic during the immune response process[19].

It has been reported that the active substance of doxorubicin is not released from the lysoendosomal compartments and accumulates in the nucleus, and the viability of cancer cells is significantly reduced compared to the groups in which doxorubicin is administered freely[8].

Administration of liposomal paclitaxel conjugated with *S. typhimurium* showed greater cytotoxicity than liposomal paclitaxel. These results may be related to the chemotactic motility of bacteria carrying more liposomal paclitaxel to tumor cells[20].

CONCLUSION

The main reasons limiting the use of chemotherapy in cancer treatment are the development of resistance to anti-cancer drugs, the side effects of the drug, and the poor absorption of drugs by cancer cells. In order to overcome these disadvantages, the use of microorganisms and their derivatives in the treatment of cancer has been extensively discussed. As can be seen from the examples given above and the study results, there are several reasonable justifications for using BGs in cancer therapy. As shown by its evidence, the BG system can be used as a delivery system of chemotherapeutic drugs, as well as having the ability to stimulate the immune system due to its adjuvant property.

REFERENCES

1. Heppner, F. and J.R. Möse, 1978. The liquefaction (oncolysis) of malignant gliomas by a non pathogenic clostridium. Acta Neurochirurgica, 42: 123-125
2. Cummins, J. and M. Tangney, 2013. Bacteria and tumours: Causative agents or opportunistic inhabitants? Infect. Agents Cancer, Vol. 8, No. 11. 10.1186/1750-9378-8-11
3. Liu, F., L. Zhang, R.M. Hoffman and M. Zhao, 2010. Vessel destruction by tumor-targeting *Salmonella typhimurium* A1-R is enhanced by high tumor vascularity. Cell Cycle, 9: 4518-4524.
4. Gentschev, I., G. Dietrich, S. Spreng, A. Kolb-Mäurer and V. Brinkmann *et al*., 2001. Recombinant attenuated bacteria for the delivery of subunit vaccines. Vaccine, 19: 2621-2628.
5. Reschel, T., Č. Koňák, D. Oupický, L.W. Seymour and K. Ulbrich, 2002. Physical properties and *in vitro* transfection efficiency of gene delivery vectors based on complexes of DNA with synthetic polycations. J. Controlled Release, 81: 201-217.
6. Sahari, A., M.A. Traore, B.E. Scharf and B. Behkam, 2014. Directed transport of bacteria-based drug delivery vehicles: Bacterial chemotaxis dominates particle shape. Biomed. Microdevices, 16: 717-725.
7. Shirai, H. and K. Tsukada, 2020. Bacterial proteolytic activity improves drug delivery in tumors in a size, pharmacokinetic, and binding affinity dependent manner: A mechanistic understanding. J. Controlled Release, 321: 348-362.
8. Paukner, S., G. Kohl and W. Lubitz, 2004. Bacterial ghosts as novel advanced drug delivery systems: Antiproliferative activity of loaded doxorubicin in human Caco-2 cells. J. Controlled Release, 94: 63-74.
9. Kim, J.J., J.S. Yang, D.J. Lee, D.M. Wilson and L.K. Nottingham *et al*., 2000. Macrophage colony-stimulating factor can modulate immune responses and attract dendritic cells *in vivo*. Hum. Gene Ther., 11: 305-321.

10. Parmiani, G., L. Rivoltini, G. Andreola and M. Carrabba, 2000. Cytokines in cancer therapy. Immunol. Lett., 74: 41-44.
11. Hutchison, C.A. and R.L. Sinsheimer, 1966. The process of infection with bacteriophage ϕX174: Mutations in a phi-X Lysis gene. J. Mol. Biol., 18: 429-447.
12. Youssof, A.M.E., F.K. Alanazi, M.M. Salem-Bekhit, F. Shakeel and N. Haq, 2019. Bacterial ghosts carrying 5-fluorouracil: A novel biological carrier for targeting colorectal cancer. AAPS PharmSciTech, Vol. 20, No. 48. 10.1208/s12249-018-1249-z
13. Kudela, P., S. Paukner, U.B. Mayr, D. Cholujova and G. Kohl *et al*., 2008. Effective gene transfer to melanoma cells using bacterial ghosts. Cancer Lett., 262: 54-63.
14. Molteni, M., D. Marabella, C. Orlandi and C. Rossetti, 2006. Melanoma cell lines are responsive *in vitro* to lipopolysaccharide and express TLR-4. Cancer Lett., 235: 75-83.
15. Jechlinger, W., C.A. Tabrizi, W. Lubitz and P. Mayrhofer, 2004. Minicircle DNA immobilized in bacterial ghosts: *in vivo* production of safe non-viral DNA delivery vehicles. Microb. Physiol., 8: 222-231.
16. Cai, K., W. Tu, Y. Liu, T. Li and H. Wang, 2015. Novel fusion antigen displayed-bacterial ghosts vaccine candidate against infection of *Escherichia coli* O157:H7. Sci. Rep., Vol. 5. 10.1038/srep17479
17. Kraśko, J.A., K. Žilionytė, A. Darinskas, M. Strioga and S. Rjabceva *et al*., 2016. Bacterial ghosts as adjuvants in syngeneic tumour cell lysate-based anticancer vaccination in a murine lung carcinoma model. Oncol. Rep., 37: 171-178.
18. Dobrovolskienė, N., V. Pašukonienė, A. Darinskas, J.A. Kraśko and K. Žilionytė *et al*., 2018. Tumor lysate-loaded bacterial ghosts as a tool for optimized production of therapeutic dendritic cell-based cancer vaccines. Vaccine, 36: 4171-4180.
19. Nicholaou, T., W. Chen, I.D. Davis, H.M. Jackson and N. Dimopoulos *et al*., 2011. Immunoediting and persistence of antigen-specific immunity in patients who have previously been vaccinated with NY-ESO-1 protein formulated in ISCOMATRIX™. Cancer Immunol., Immunother., 60: 1625-1637.
20. Nguyen, V.D., J.W. Han, Y.J. Choi, S. Cho and S. Zheng *et al*., 2016. Active tumor-therapeutic liposomal bacteriobot combining a drug (paclitaxel)-encapsulated liposome with targeting bacteria (*Salmonella typhimurium*). Sens. Actuators B: Chem., 224: 217-224.