Japanese Journal of Gastroenterology and Hepatology

Case Report

ISSN: 2435-1210 | Volume 10

Microbial Onco-Therapeutics

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Received: 06 Apr 2024
Accepted: 20 May 2024
Published: 25 May 2024
J Short Name: JJGH

Keywords:

Anti-cancer; Malignant; Viruses; Clostridium; Mycobacterium; Vaccine

Citation:

Khan NT. Microbial Onco-Therapeutics. J Gastro Hepato. 2024; V10(10): 1-5

1. Abstract

The reasonable development of anticancerous agents that specifically target malignant growth cells without influencing the sound cells or tissues, has prompted another novel interest in microbial-determined bioactive entities. Such essential arrangements might prepare to overcome the weaknesses of customary treatments and raise the potential and expectation for the fix of extensive variety of malignant growth in a particular way. This article plans to give an exhaustive synopsis of hostile to cancer-causing properties and basic instruments of bioactive particles of microbial origin, and examine the ongoing difficulties and powerful therapeutic utilization of combinatorial procedures to accomplish negligible systemic side-effect.

2. Introduction

Diseases therapeutics and malignant growth (cancer) diagnostics are critical n understanding the pathophysiology of cancers, requiring interdisciplinary collaboration from different fields [1]. The interaction between cancerous cells and microbial cells in human wellbeing considerably affect malignant growth risk [2]. The two sorts of cells require an enriched environment for their growth and progression [3]. The human stomach is a complicated biological system with parasite, microorganisms, infections, and archaea, with, Bacteroidetes, Proteobacteria, and Actinobacteria being the most well-known sorts of microscopic organisms [4]. The gastrointestinal hindrance, which keeps possibly risky life forms from entering the epithelium where they can harm or kill individuals, can be kept up with by microorganisms [5]. At present, it is felt that microorganisms increase the possibility for disease by changing the DNA in human cells. This changes the cell cycle, speeds up cell augmentation, and obstructs the normal cycles of controlled cell death, which monitor the body against abnormal cells [6].

3. Microbes in Cancer Treatment

Immunotherapy, which utilizes specific sorts of microorganisms, is one of the most novel areas of research. It is predicted that this sort of therapy will actuate the invulnerable framework to target and annihilate malignant growth cells [7].

3.1. Bacteria as Anticancer Agent

Microorganisms antitumor adequacy is unimaginably varied. Current anticancer bacterial microorganism arrangements have the situation with a treatment that is added on top of standard consideration, upgrading the probability that the patient will recuperate completely [8].

• **Mycobacterium Bovis BCG:** Mycobacterium bovis strain Bacillus Calmette-Guérin (BCG), made by Albert Calmett and Camille Guérin as a tuberculosis immunization, has been being used starting around 1921 [9]. A solitary intradermal portion of this immunization is given to kids in no less than 24 hours of birth in a few nations as a component of the necessary immunization schedule. An etiological agent of bovine tuberculosis is Mycobacterium bovis [10]. Be that as it may, it can likewise bring about tuberculosis side effects in people under unambiguous circumstances (like following utilization of untreated milk from a contaminated cow) [11]. Debilitating this

bacterium thus was required. There were a few associations between the beginning of TB and the reduction of malignant growth in the beginning of the twentieth 100 years [12]. The immunization was just approved as an extra bladder disease therapy after it was revealed in 1976 that utilizing BCG was related with a malignant growth relapse [13]. Utilizing urethral catheters, the microbial solution should be intravenously implanted during the M. bovis BCG strain's therapy of this specific malignant growth kind. To definitively eliminate the malignant growth cells and stop repeat, this treatment is normally used following resection [14].

• **Streptococcus Pyogenes:** Dr. William Coley was utilizing Streptococcus pyogenes to treat bone sarcoma [15]. Notwithstanding, for a long time utilizing this organism was forgotten because of the disclosure and improvement of elective disease treatments, including chemotherapy and radiation. Fortunately, utilizing S. pyogenes as an anticancer property, and the microscopic organisms are currently used to treat pediatric lymphangiomas [16]. Lymphangiomas are cancers made when lymphatic channel endothelial cells partition usually in the head and neck area [17]. Disabled lymph stream is predominantly liable for the unusual development of lymphatic vessels, which thus brings about cyst arrangement [18]. Medical procedure is the fundamental type of therapy, yet this is definitely not a straightforward methodology and is much of the time joined by unfortunate results, including mortality [19]. Sclerotherapy is an alternate, more secure methodology. Into lymphatic vessels that have gone through obsessive adjustment, Streptococcus pyogenes is infused into lymphatic vessels with pathogenic changes [20]. Starting around 1987, this bacterium has been utilized actually in Japan to treat pediatric lymphangiomas. As per studies, the strain is secure and reduces cyst volume by at least 50%. [21].

• **Clostridium Species and Salmonella Enterica:** Because of their affinity for development in hypoxic conditions, facultative and mandatory anaerobes can possibly be utilized in anticancer treatment [22]. Blood supply routes that for the most part enter the growth surface areas convey oxygen to the cells. Hypoxia and unfortunate oxygen dispersion into the cancer are the impacts of this [23]. The anaerobic environment encourages the development of anaerobic microorganisms, like Clostridium species, Salmonella species, Bifidobacterium species, or Listeria species [24]]. The fundamental advantage of using these microorganisms is that they focus on the growth straightforwardly, instead of chemotherapy, which spreads all through the body through blood and furthermore kills ordinary, sound cells [25]. Because of the anaerobic conditions, Clostridium is the most frequently involved sort of microscopic organisms with regards to hypoxia and the antineoplastic treatment [26]. Microscopic organisms fill in the necrotic parts of the growth and can straightforwardly damage tumour cells. The utilization of Clostridium in the fight against disease traces all the way back to 1935, when Connell composed a paper showing how the impact of Clostridium histolyticum chemicals made progressed malignant growth regress [27].Since then clinical investigations of the constricted type of Clostridium novyi-NT were fruitful and created amazingly reassuring results for the treatment of leiomyoma [28]. Albeit the specific component hidden Clostridium spp's. anticancer impact isn't yet settled, it is notable that the microorganisms might create specific compounds and poisons that kill disease cells. Furthermore, it creates specific proteins that can be combined with specific chemotherapeutics [29]. The cancers outside vascularization and inside hypoxia keep meds from precisely infiltrating it during standard therapy [30]. Salmonella enterica etiological agent, Typhimurium, looks like Clostridium apparently, likewise be found in areas of necrotic cancers and is to a great extent anaerobic [31]. Salmonella typhimurium VNP20009 is utilized in the therapy of malignant growth [32]. Starting in 2002, clinical examinations involving this bacterium as a melanoma treatment were directed. Also, stage I clinical preliminaries for the anticancer antibody VXM01, which depends on the weakened kind of Salmonella typhi, were finished effectively. This microorganism communicates VEGFR2 (the vascular endothelial development factor receptor-2) because of a plasmid. The inoculation keeps angiogenesis from happening. The blend was assessed in individuals with pancreatic disease [33].

• **Magntoccocus Marinus:** The latest anticancer systems utilize the accomplishments of different logical disciplines, for example, nanobiotechnology. Nanoparticles (nanocapsules), lipid vesicles with a chemotherapeutic medication inside, are the object of developing interest [34]. Nanoliposomes can convey the medication inside the growth. Nonetheless, they are not an ideal arrangement on the grounds that a significant number of the particles don't arrive at the target. As referenced before, the growth is just vascularized from an external perspective, which makes it unimaginable for chemotherapeutics to arrive at within the injury. Thus, conveying drugs straightforwardly to the growth with vectors/transporters would consider more exact focusing of the disease site [35]. Restricting the spread of the medication just to the cancer region would essentially lessen the unfavorable impacts of chemotherapy. For the referenced reasons, it was chosen to investigate extremely unique microorganisms named Magnetococcus marinus [36]. A Gram-negative coccus called Magnetococcus marinus MC1 was found in the Atlantic Sea near Rhode Island, USA. The cilia of this microorganism are coordinated in two groups at one shaft and permit the microscopic organisms to move. The presence of magnetosomes, impossible to miss parts made of magnetite particles (Fe3O4) encased by layers and shaping chains in the cytoplasm, recognizes this bacterial design from others [37]. Furthermore, this microorganism has negative aerotaxis limit, meaning it inclines toward an oxygen-unfortunate environment [38].

• **Toxoplasma Gondii:** The sexual period of microorganism happens in Felidae, or felines. Birds, creatures, including rodents and individuals, contaminated by dung that contain the parasite's oocytes. The parasite duplicates inside the living beings of the moderate hosts,

and growths foster in the muscles and mind. The resistant framework stops the development of the protozoa in healthy individuals [39]. It has been found that the protozoan and its lysate, known as TLA (Toxoplasma lysate antigen), which contains the microorganism's antigens, can be used to treat both disease and neurological ailments [40]. The disease, including melanoma, pancreatic malignant growth, cellular breakdown in the lungs, and ovarian malignant growth, are being treated with Toxoplasma gondii's (CPS) uracil auxotrophic carbamoyl phosphate synthase [41]. The conveyance of this strain made extra invulnerable cells become initiated and the level of the cytokine IL-12, which influences irritation, to rise. Also, IL-12 is responsible for forestalling angiogenesis, which brings about hypoxia and eased back cancer advancement [42]. Besides, improvement of veins is altogether restrained by the presence of Th1 cells and the diminished articulation of the angiogenesis marker CD31. Also, levels of CD4+, CD8+, and IFN are decisively raised [43]. Late examination in the mouse model proposes that T. gondii CPS treatment offers long term protection against repeat, which is connected with the development of immunological memory and the high titer of IgG perceiving the specific cancer's antigens [44].

4. Virotherapy and Cancer Vaccines

Most virus-based therapeutics incorporate numerous systems. These therapies work by introducing genes into the cancer host cells and are expected to lyse malignant growth cells or to either straightforwardly interact with the immune system [45]. Malignant growth immunizations and virotherapies are two distinct classes of infection therapeutics [46]. Antigens or epitopes well defined for disease are encoded and communicated in immunizations. Rather than encoding unfamiliar cancer antigens, virotherapies animate lytic processes that obliterate cells to uncover or set endogenous antigens free from contaminated cells [47]. "Oncolytic immunizations" are a crossover approach where a cancer antigen or neo-epitope is furthermore encoded by an oncolytic vector [48]. Both viruses that duplicate and viruses that don't can be utilized in viral treatment. Hypothetically portion intensifying infections are replication-competent since they can proceed to increase and disperse to local growth cells that aren't impacted. At last, growth cells contaminated with viruses die because of a second component of viability brought on by host anti-tumor immune responses sparked by the production of tumor antigens and inflammation brought on by infection. [49]. Together, these procedures might assist a host memory reaction with becoming prepared to kill both infused sores and uninfected metastatic disease. Recent trials for non-replicating-virus-mediated cancer gene therapy (Table 1).

Malignant growth immunizations have a downside in that, while cancer related antigens have been completely described, cancer explicit neoantigens, which are delivered by substantial transformations in a disease cell's genome, are normally private and spread the word about up a tiny part, all things considered [54]. Accordingly, strategies to perceive and set off an invulnerable reaction intended for growths are being sought after. Normal infections have been modified to communicate growth explicit antigens during the viral contamination cycle's cancer cell lysis deliberately work to deliver immunogenic viral vectors [55]. Antigen cross-show undoubtedly somewhat upholds this change, which prompts in situ "oncolytic immunization". A few oncolytic infections encode for immunostimulatory cytokines like Sort I interferons or GM-CSF to more readily animate the safe reaction against these host antigens [56]. Oncolytic viruses are remembered to work by consolidating two distinct procedures for particular killing: (1) cancer explicit cell lysis (oncolysis) and (2) immunogenic cell passing, immunostimulation from viral part acknowledgment, and arrival of both viral and growth antigens. Oncolytic infections can, as was at that point demonstrated, be adjusted to encode transgenes to help the host resistant reaction [57].

5. Conclusion

Thus, microbes possess innate potential to be utilized in suppressing cancerous malignant growth and is an effective tool in cancer therapy.

Table 1: Recent trials for non-replicating-virus-mediated cancer gene therapy

Biological Agent	Virus (gene)	Indication
AAV2hAQP1	AAV (Aquaporin-1)	Squamous cell Head and Neck cancer [50]
NP2	HSV-1 (NP2)	Cancer pain [51]
Ad5CMV-p53	Adenovirus (p53)	Liver cancer [52]
TK99UN	Adenovirus (HSV TK)	Hepatocellular carcinoma [53]

References

1. Dzutsev A, Badger JH, Perez-Chanona E, Roy S, Salcedo R, Smith CK, et al. Microbes and cancer. Annual review of immunology. 2017; 35: 199-228.
2. Sawant SS, Patil SM, Gupta V, Kunda NK. Microbes as medicines: harnessing the power of bacteria in advancing cancer treatment. International journal of molecular sciences. 2020; 21(20): 7575.
3. Azevedo MM, Pina-Vaz C, Baltazar F. Microbes and cancer: friends or faux? International journal of molecular sciences. 2020; 21(9): 3115.
4. Dolgin E. Fighting cancer with microbes. Nature. 2020; 577(7792): S16-S16.
5. Kaur T, Sharma D. Fundamentals of utilizing microbes in advanced cancer therapeutics: current understanding and potential applications. In Advances in Applied Microbiology 2023; 123: 91-131.

6. Whisner CM, Athena Aktipis C. The role of the microbiome in cancer initiation and progression: how microbes and cancer cells utilize excess energy and promote one another's growth. Current nutrition reports. 2019; 8: 42-51.
7. Gately S. Human microbiota and personalized cancer treatments: role of commensal microbes in treatment outcomes for cancer patients. Precision Medicine in Cancer Therapy. 2019; 253-64.
8. Jain KK. Use of bacteria as anticancer agents. Expert Opinion on Biological Therapy. 2001; 1(2): 291-300.
9. Holla S, Ghorpade DS, Singh V, Bansal K, Balaji KN. Mycobacterium bovis BCG promotes tumor cell survival from tumor necrosis factor-α-induced apoptosis. Molecular cancer. 2014; 13: 1-13.
10. Begnini KR, Buss JH, Collares T, Seixas FK. Recombinant Mycobacterium bovis BCG for immunotherapy in nonmuscle invasive bladder cancer. Applied microbiology and biotechnology. 2015; 99: 3741-54.
11. Benitez MLR, Bender CB, Oliveira TL, Schachtschneider KM, Collares T, Seixas FK. Mycobacterium bovis BCG in metastatic melanoma therapy. Applied microbiology and biotechnology. 2019; 103: 7903-16.
12. Minden P, McClatchy JK, Wainberg M, Weiss DW. Shared antigens between Mycobacterium bovis (BCG) and neoplastic cells. Journal of the National Cancer Institute. 1974; 53(5): 1325-31.
13. Lange C, Aaby P, Behr MA, Donald PR, Kaufmann SH, Netea MG, et al. 100 years of Mycobacterium bovis bacille Calmette-Guérin. The Lancet Infectious Diseases. 2022; 22(1): e2-e12.
14. Luo Y, Yamada H, Chen X, Ryan AA, Evanoff DP, Triccas JA, et al. Recombinant Mycobacterium bovis bacillus Calmette-Guerin (BCG) expressing mouse IL-18 augments Th1 immunity and macrophage cytotoxicity. Clinical & Experimental Immunology. 2004; 137(1): 24-34.
15. Maletzki C, Linnebacher M, Kreikemeyer B, Emmrich J. Pancreatic cancer regression by intratumoural injection of live Streptococcus pyogenes in a syngeneic mouse model. Gut. 2008; 57(4): 483-91.
16. Oliveira J, Brandão SF. Streptococcus pyogenes in tumor treatment: the past, present and future. 2020.
17. Marzhoseyni Z, Shojaie L, Tabatabaei SA, Movahedpour A, Safari M, Esmaeili D, et al. Streptococcal bacterial components in cancer therapy. Cancer Gene Therapy. 2022; 29(2): 141-55.
18. Kucerova P, Cervinkova M. Spontaneous regression of tumour and the role of microbial infection–possibilities for cancer treatment. Anti-cancer drugs. 2016; 27(4): 269-77.
19. Kanaoka M, Fukita Y, Taya K, Kawanaka C, Negoro T, Agui H. Antitumor activity of streptococcal acid glycoprotein produced by Streptococcus pyogenes Su. Japanese Journal of Cancer Research GANN. 1987; 78(12): 1409-14.
20. Łukasiewicz K, Fol M. Microorganisms in the treatment of cancer: advantages and limitations. Journal of immunology research. 2018; 2018: 2397808.
21. Siggins MK, Lynskey NN, Lamb LE, Johnson LA, Huse KK, Pearson M, et al. Extracellular bacterial lymphatic metastasis drives Streptococcus pyogenes systemic infection. Nature Communications. 2020; 11(1): 4697.
22. Yamamoto Y, Itoh N, Sugiyama T, Kurai H. Clinical features of Clostridium bacteremia in cancer patients: A case series review. Journal of Infection and Chemotherapy. 2020; 26(1): 92-4.
23. Yaghoubi A, Ghazvini K, Khazaei M, Hasanian SM, Avan A, Soleimanpour S. The use of Clostridium in cancer therapy: a promising way. Reviews and Research in Medical Microbiology. 2022; 33(2): 121-7.
24. Theys J, Lambin P. Clostridium to treat cancer: dream or reality?. Annals of translational medicine. 2015; 3(Suppl 1).
25. Van Mellaert L, Barbé S, Anné J. Clostridium spores as anti-tumour agents. TRENDS in Microbiology. 2006; 14(4): 190-6.
26. Kubiak AM, de Laak JV, Zhang Y, Bailey TS, Claessen L, Hittmeyer P, et al. Clostridia as live biotherapeutics: can modified Clostridium species enhance disease treatments? Future Microbiology. 2023; 18(7): 385-8.
27. Umer B, Good D, Anné J, Duan W, Wei MQ. Clostridial spores for cancer therapy: targeting solid tumour microenvironment. Journal of toxicology. 2012.
28. Burgos PMH, Nanjappa S, Snyder M, Bitetzakis C, Greene JN. Evaluation of Clostridium Bacteremia in the Cancer Population: A Case Series. Infectious Diseases in Clinical Practice. 2018; 26(6): 358-62.
29. Shah S, Hankenson J, Pabbathi S, Greene J, Nanjappa S. Clostridium tertium in neutropenic patients: case series at a cancer institute. International Journal of Infectious Diseases. 2016; 51: 44-6.
30. Theys J, Patterson AV, Mowday AM. Clostridium Bacteria: Harnessing Tumour Necrosis for Targeted Gene Delivery. Molecular Diagnosis & Therapy. 2024; 1-11.
31. Liang K, Liu Q, Li P, Luo H, Wang H, Kong Q. Genetically engineered Salmonella Typhimurium: Recent advances in cancer therapy. Cancer letters. 2019; 448: 168-81.
32. Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Hoffman RM. Cancer metastasis directly eradicated by targeted therapy with a modified Salmonella typhimurium. Journal of cellular biochemistry. 2009; 106(6), 992-998.
33. Broadway KM, Scharf BE. Salmonella typhimurium as an anticancer therapy: recent advances and perspectives. Current Clinical Microbiology Reports. 2019; 6: 225-239.
34. Fdez-Gubieda ML, Alonso J, García-Prieto A, García-Arribas A, Fernández Barquín L, Muela A. Magnetotactic bacteria for cancer therapy. Journal of applied physics. 2020; 128(7).
35. Kotakadi SM, Borelli DPR, Nannepaga JS. Therapeutic applications of magnetotactic bacteria and magnetosomes: a review emphasizing on the cancer treatment. Frontiers in Bioengineering and Biotechnology. 2022; 10: 789016.

36. Łukasiewicz K, Fol M. Microorganisms in the treatment of cancer: advantages and limitations. Journal of immunology research. 2018.
37. Valverde-Tercedor C, Montalbán-López M, Perez-Gonzalez T, Sanchez-Quesada MS, Prozorov T, Pineda-Molina E, et al. Size control of in vitro synthesized magnetite crystals by the MamC protein of Magnetococcus marinus strain MC-1. Applied microbiology and biotechnology. 2015; 99: 5109-21.
38. Alsharedeh R, Alshraiedeh N, Aljabali AA, Tambuwala MM. Magnetosomes as Potential Nanocarriers for Cancer Treatment. Current Drug Delivery. 2024; 21(8): 1073-81.
39. Alim M, Ozcelik S, Ozpinar N. Seroprevalence of Toxoplasma gondii in patients receiving cancer treatment. Cumhuriyet Medical Journal. 2018; 40(1): 19-24.
40. Ali MI, Abd El Wahab WM, Hamdy DA, Hassan A. Toxoplasma gondii in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. Journal of parasitic diseases. 2019; 43: 464-71.
41. Kim JS, Lee D, Kim D, Mun SJ, Cho E, Son W, et al. Toxoplasma gondii GRA8-derived peptide immunotherapy improves tumor targeting of colorectal cancer. Oncotarget. 2020; 11(1): 62.
42. Caner A. Toxoplasma gondii could have a possible role in the cancer mechanism by modulating the host's cell response. Acta Tropica. 2021; 220: 105966.
43. Zavareh FSE, Hadiipour M, Kalantari R, Mousavi S, Tavakolifard N, Darani HY. Effect of Toxoplasma gondii on colon cancer growth in mouse model. American Journal of BioMedicine. 2021; 9(2): 168-76.
44. Lotfalizadeh N, Sadr S, Morovati S, Lotfalizadeh M, Hajjafari A, Borji H. A potential cure for tumorssociated immunosuppression by Toxoplasma gondii. Cancer Reports. 2024; 7(2): e1963.
45. Harrington K, Freeman DJ, Kelly B, Harper J, Soria JC. Optimizing oncolytic virotherapy in cancer treatment. Nature reviews Drug discovery. 2019; 18(9): 689-706.
46. Roy DG, Geoffroy K, Marguerie M, Khan ST, Martin NT, Kmiecik J, et al. Adjuvant oncolytic virotherapy for personalized anti-cancer vaccination. Nature Communications. 2021; 12(1): 2626.
47. Koske I, Rössler A, Pipperger L, Petersson M, Barnstorf I, Kimpel J, et al. Oncolytic virotherapy enhances the efficacy of a cancer vaccine by modulating the tumor microenvironment. International journal of cancer. 2019; 145(7): 1958-69.
48. Workenhe ST, Mossman KL. Oncolytic virotherapy and immunogenic cancer cell death: sharpening the sword for improved cancer treatment strategies. Molecular Therapy. 2014; 22(2): 251-6.
49. Lawler SE, Speranza MC, Cho CF, Chiocca EA. Oncolytic viruses in cancer treatment: a review. JAMA oncology. 2017; 3(6): 841-9.
50. Ylösmäki E, Cerullo V. Design and application of oncolytic viruses for cancer immunotherapy. Current opinion in biotechnology. 2020; 65: 25-36.
51. Forbes NS, Coffin RS, Deng L, Evgin L, Fiering S, Giacalone M, et al. White paper on microbial anti-cancer therapy and prevention. Journal for immunotherapy of cancer. 2018; 6: 1-24.
52. Choi JW, Kang E, Kwon OJ, Yun TJ, Park HK, Kim PH, et al. Local sustained delivery of oncolytic adenovirus with injectable alginate gel for cancer virotherapy. Gene therapy, 2013; 20(9): 880-892.
53. Ponnazhagan S, Curiel DT, Shaw DR, Alvarez RD, Siegal GP. Adeno-associated virus for cancer gene therapy. Cancer research. 2001; 61(17): 6313-21.
54. Vähä-Koskela MJ, Heikkilä JE, Hinkkanen AE. Oncolytic viruses in cancer therapy. Cancer letters. 2007; 254(2), 178-216.
55. Zheng M, Huang J, Tong A, Yang H. Oncolytic viruses for cancer therapy: barriers and recent advances. Molecular Therapy-Oncolytics. 2019; 15: 234-47.
56. Wong HH, Lemoine NR, Wang Y. Oncolytic viruses for cancer therapy: overcoming the obstacles. Viruses. 2010; 2(1): 78-106.
57. Mondal M, Guo J, He P, Zhou D. Recent advances of oncolytic virus in cancer therapy. Human vaccines & immunotherapeutics. 2020; 16(10): 2389-402.