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# Bacteria in the cancer therapy

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**Abstract.** Human has been fighting with cancer for 130 years since the first tumor radical surgery in 1884. During this 130 years of cancer treatment history, there has developed mainly 4 types of treatment, chemotherapy, radiotherapy, surgery and immunotherapy. .However all of these treatment have their own side-effect, including the harm to normal cells, high cost, long developing time. And traditional cancer treatment are often failed to eliminate all the tumor tissue so few patients can be cure completely. Developing an effective cancer treatment is still a challenge. Luckily, a special approach is developing rapidly which is called bacteriotherapy. It can be combined with traditional treatment like chemotherapy and immunotherapy, use bacteria as a vector to deliver the drug or tumor suppressing gene to the tumor. In this way, the effect of harming normal cells can be reduce and the accuracy of attacking tumor can be improved. There are also tumor-specific bacteria found living in tumor which can be effective in cancer treatment. How to use bacteria well in cancer treatment is remain a challenge. This article reviewed the advantages of using bacteria for cancer treatment and the mechanism of bacteria in cancer treatment, giving example of the use of intestinal bacteria, involving the challenge and future of bacteriotherapy.

**Keywords:** bacteriotherapy, intestine bacteria, genetic modification, target, tumor microenvironment.

## 1. Introduction

Malignant tumor is a major disease in China, with a high incidence and death rate. The annual number of cancer cases has exceeded 2.5 million, resulting in over 1.4 million deaths. With the growth of number of cases of cancer with the incidence rate increased by 3%~5% every year, the 5-year survival rate of cancer in China is 40.5%, which means there is still 60% of cancer patients cannot get clinical cure [1]. Recurrence and metastasis are important reasons why cancer patients cannot be cured. This means the traditional cancer treatment cannot totally eliminate the cancer cells and successfully avoid the transfer of cancer cells. The traditional cancer treatment like chemotherapy, radiotherapy, surgery and immunotherapy each has the side-effect. For surgery, some minimal cancer cell population may remain as they are less possible to be detect by eyes or other examination. For chemotherapy, the use of toxic chemical may affect the normal function of the healthy cells as the chemicals are injected into the whole body rather than deliver the drugs direct to tumor. The development of resistance also lead to inefficiency of the treatment. A novel approach is the immunotherapy by the genetic modification of immune cells to detect the cancer cells. However, the process is long and expensive. What's more, immunotherapy is too specific which lead to the long process to genetic modified the tumor-specific

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gene. Nowadays, the bacteriotherapy has been proposed to reduce those side-effects of the traditional cancer treatment.

People live with bacteria, and not all of them are harmful to human. Instead, bacteria plays an important role for human survival. In human intestine, there are 500 to 1000 kinds of different bacteria like Escherichia coli [2]. The intestinal bacteria can affect the digestive function and it has been found that the diversity of glut microbiota is relative to the host's immunity. Moreover, research show that there are some bacteria living in the tumor, indicating that tumor tissue is a complex of interactions between the bacteria living in tumor, the host and the tumor cells [3]. It is meaningful to explore how to use the bacteria that lives in a specific tumor as a vector to deliver the drugs like those toxic chemicals used in chemotherapy into the tumor cells directly. Bacteria is also a good source for genetic modification, which means human can modify the gene of bacteria to make them carry the tumor suppressing gene, and deliver them into the tumor to avoid the tumor from growing which can finally eliminate the tumor cells from the body without destroying other normal cells. However, there remain lots of challenge for the development of this type of treatment. This paper covers the mechanism and advantages of using bacteria as a vector.

## 2. History of bacteriotherapy in cancer

The importance of bacteria in cancer treatment has been found in 19th century. In 1884, a German immigrant called Fred Stein was suffering from the inoperable tumor in his neck. To the doctors' surprise, the patient's tumor gradually vanished after getting erysipelas which is a skin infection cause by the bacterium Streptococcus pyogenes. A physician called William Coley tracked down the patient for 7 years and observed no cancer sign remained. During his research, he found 47 cases that can support the idea that bacterial infection is related to the cure of cancer. He believed that this miracle happened because the bacteria stimulate the immune system to fight off the cancer [4]. From 1891, Coley began to experiment on patients by injecting streptococcus which is a bacterium known to induce erysipelas attacks. The experiment result showed that the tumor once thought to be irreversible then began to dissolve and finally disappear in two weeks. But unfortunately, some of the patients got infected by the bacteria and finally died due to the infection. This indicate one of the drawbacks of the bacteriotherapy which is the unpredictable nature of infection. William Coley then decontaminated and inactivated the bacteria, the possibility of getting infected was then reduced. From then the well-known Coley toxin was produced. But there was still some unpredictable infection so the research was stopped by the doubts from other scientists.

The study by Old and his team in 1959 indicated the anti-tumor effects of the BCG bacteria in mice with bladder cancer. A trial base on the idea was then conducted to test the function of the Bacille Calmette-Guerin (BCG) which is the tuberculosis vaccine, in preventing the recurrence of bladder cancer. The interest of the research focusing on the relationship of bacterial infection raised again from 1976 [5]. The weakened living BCG bacteria were injected into the bladder of cancer patients directly and leave for some time for the bacteria to trigger the immune system to give immune response. The BCG therapy is not only shown to be effective in the activation of macrophages in tumor rejection, but also can support for the Coley's theory.

The development of immunotherapy base on bacteria keep thriving for decades. From 1980s the first cancer vaccine became available. Researchers tried to follow the way people treat inflammation cause by bacteria to treat the cancer for hundreds of years. However, the harmful toxin in bacteria bring the research to a challenge. Beside decontaminate or inactivate the bacteria, programming the bacteria to produce mutate bacterial strains for detoxification can limit the possibility of bacterial infection and also maintain the tumor targeting and reproduction ability of bacteria. From 2000s, the technology of genetic modification became more and more mature. In 2019, scientist began to programme the bacteria to safely deliver drugs directly to the tumors. This is also the main focus of a new filed—synthetic biology.

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## 3. Advantage of using bacteria to treat cancer

Microbes are everywhere, if there is life, there will be microbes exist. Bacteria is a member in the microbes family. There are about 5*10^30 species of bacteria on the Earth [6]. The bacteria exists in the food human eat and air human breathe. The human body is a habitat for a large number of bacteria, it can be found on the surface of the skin, mouth, nose, intestine and other part of the body.

In human body, the gut is a Leakey ecological niche in the human microbiota. There are approximately 30 trillion bacteria in human intestine, those can be divided into probiotics, conditional pathogens and pathogens.

10%～20%bacteria are probiotics which can synthesis necessary vitamins and amino acids for human body. One example is lactobacilli, bedside synthesizing essential amino acids and vitamins, it can suppress the growth of harmful bacteria in the intestine, also control the concentration of cholesterol in the blood. What's more, lactobacilli has immune regulatory effects which have advantage in cancer treatment.

60%~70% of the intestine bacteria is conditional pathogens, the main example is Escherichia coli. Whether this kind of bacteria has advantage on human depends on the immunity of the body, the attributes of these bacteria will determine by the strength of probiotics and pathogen.

20% of the bacteria will be pathogen. It mainly includes Proteus, pseudomonas, Salmonella and pathogenic Escherichia coli. Once these bacteria suddenly proliferate out of control in the intestinal tract, they will cause many problems, such as enteritis, diarrhea and other diseases. The rest part of the paper will review about the advantage, disadvantage and mechanism of bacteria in cancer treatment, focus on the intestinal bacteria.

### *3.1. Specific targeting to tumor tissues*

Tumor tissue have their specific microenvironment. As the tumor keep growing, the blood vessel web in normal tissues can no longer support the growth of tumor tissue, this stimulates the release of tumor growth factor and form new blood vessel web. The complex, messy and unstable blood vessel web cause insufficient supply of oxygen and nutrient. This cause the tumor tissue to do anaerobic respiration and glycolysis to release energy for its growth, but this will produce lactic acid causing the pH value decrease down to normal level in other tissue. The dense extracellular matrix and high cell density of solid tumor lead to the formation of high osmotic pressure inside the tumor which prevent the drug molecules to penetrate the solid tumor. Due to the special tumor microenvironment, human need to develop a specific cancer treatment. Bacteria therefore can be a good vector. Having specific tumor tissue targeting is the basic advantage of bacteria. In the nature, some of the bacteria can target to solid tumor which is because they are fit into the microenvironment in the tumor.

For example, they can overcome the low oxygen, low nutrient concentration and high osmotic pressure, make them able to penetrate the solid tumor and enter the necrotic area for reproduction and successfully survive in the tumor. For example, E.coli Nissle 1917 prefer living in low oxygen concentration environment which just fit into the micro environment in tumor tissue. After injection, the E.coli Nissle 1917 will automatically gather together into the tumor tissue and are able to reproduce inside the tumor. Experiment showed that after injecting E.coli Nissle 1917 inside the immune tolerance and weakened mice, the number of E.coli reached 106CFU/g in tumor after 25 days but completely disappeared in other normal tissue [7]. This show that E.coli Nissle 1917 is highly specific target to tumor tissue and won't harm other normal cells.

As a living organism, bacteria can overcome the high osmotic pressure in tumor tissue by the function of chemotactic factors and enhanced permeability and retention effect(EPR), make the bacteria able to penetrate the tumor tissue and reproduce. Some research show that some of the bacteria have a stable genetic material after several generation of variation wherever been reproduced intro or vitro. This can be the foundation of genetic modification of the bacteria to increase the ability of targeting tumor tissue.

Moreover, Bacteria can not only penetrate into the solid tumor but also can follow the metastasize with the cancer cells. An experiment injected salmonella typhimurium into mice. After the injection,

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the bacteria were observed to exist in every metastatic lesion of the tumor. Among them, 44.0% of bacteria are dispersed in the metastatic tissue, while only 0.5% of the bacteria exist in the normal tissue.

### *3.2. Activation of immune system*

Bacteria can also adjust the immune system. Some of the bacteria act as pathogen which will cause disease and there are specific antigen on the surface which can be recognized by the immune cells. So if bacteria enter the human body the immune system can be stimulated and the macrophage can be activated. At this time the ability of immune system to fight against cancer can be improve and make it easier for the lymphocytes to recognize and get rid of the cancer cells.

One example is the activation of macrophage by lactic acid bacteria. In THP-1 macrophages and human primary macrophages, some lactic acid bacteria strains can activate inflammatory transcription factor NF- κ B (but without activating IFN-I), some strains (IFN-I induced strains) can trigger the production of IFN-I ;IFN-I induced strains can activate the IFN-I response of macrophages by downregulating CD64 and upregulating CD40;Mechanically, IFN-I induces strains to be engulfed by macrophages and can be perceived by intracellular cytoplasmic receptors STING and MAVS; After receiving IFN-I induced strain stimulation, macrophages lacking STING showed reduced phosphorylation of TANK binding kinase-1 and IFN-I activation, as well as reduced expression of ISG [8].

Once the immune cells like CD8+ and CD4+ T lymphocytes have been activated by the inflammation cause by bacteria, they can recognize and destroy the tumor cells more efficiently. Bacterial infection can produce heat shock protein like HSP70 which is release in necrotic cell. After the level of HSP70 increase by the stimulate of inflammation, it can promote the mature of dendritic cells which is the cell responsible for presenting antigen essential for inducing effective antigen-specific immune response. Bacterial infection can also cause the release of pathogen-associated molecular pattern by bacteria. PAMPs combine and activate toll-like receptors, stimulating up-regulation of pro-inflammatory cytokines, and costimulatory molecules. As the result, these mediators lead to produce of interferon gamma (IFN-γ) and a Th1-dependent cell-mediated response will commence, essentially mediated by CD8+ effector cells. CD8+ lymphocytes isolated from C novyi NT-treated mice can in turn stimulate acquired immunity in a tumor-specific model [9].

### *3.3. Genetic modification*

Another advantage of using bacteria for cancer treatment is that bacteria can be a good resource for genetic modification, which allow human to insert tumor suppresive gene like TP53.So bacteria can act as an effective vector for gene therapy. These bacterial vectors can offer a strong therapy to specific cancer treatment by the production of specific protein in different tumor micro-environment. Bacteria can become a vehicle for delivering anti-cancer agents, cytotoxic peptides, therapeutic proteins or pro-drug converting enzymes to tumors. Two studies have described the production of Cytolysin A(ClyA), a pore-forming hemolytic protein in E.coli K-12 and S.typhimurium [10,11]. In the model of CT26 mice, this strategy can effectively kill hypoxia regions of tumors that resist radiotherapy. Another research includes the expression of hemolysis E(HlyE) which is another pore-forming toxin [12]. Fortunately, this treatment show effective increase in the necrosis of mammary tumors and decrease the growth of tumor in mice. , the E.coli strain χ6212 was programmed to deliver a Staphylococcus aureus derived a-hemolysin(SAH) [13]. Then, this engineered strain was able to regress tumors and expand tumor necrosis in 4T1 tumor-bearing mice [14].

As bacteria are very small organism with a high rate of reproduction, using bacteria to express a therapeutic gene or produce the drug for cancer treatment can have high efficiency and lower cost.

Also, bacteria share same genetic material with all the other organisms, so it can be an efficient tool for genetic modification in order to insert tumor suppresive gene in human or insert gene for protein formation exist in human. And there is something special in bacteria which is plasmid. Plasmid contains a loop of DNA. This form of DNA is easy to transform and modified.

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## 4. Limitation of the bacteriotherapy

One remarkable problem for bacteriotherapy is that the toxicity in the dose used for treatment is hard to control. If the toxicity in the dose required is too high, will lead to severe bacterial infection and cause the reduce of immunity in patient even cause death. However if the toxicity in the dose is lower than required level, the treatment may be lack of efficiency. Moreover, even though the toxin genes like in COBALT therapy has been removed, approximately 15~45% mortality in mice still happened [15]. Another major problem is there is possibility of incomplete tumor lysis which happens when bacteria don't consume all parts of the tumor tissue. To eliminate the whole tumor tissue, people can only combine bacteriotherapy with chemotherapy. This will make the treatment become more complex and increase the cost. Also, the aim for bacteriotherapy is to reduce the toxicity to other normal tissues by chemotherapy, increase the specificity of the treatment in order to reduce the pain of the patient, the combination of chemotherapy can still have those effect to human body. A more severe problem is the treatment on small non-necrotic metastases of large primary tumors. As the hypoxic region in some of the non-necrotic metastases is too small, it is hard for bacteria to target. Furthermore, the accessibility of the bacteria to tumor tissue is difficult because most of the times an intratumorally injection is required [16].

Another concern is the gene mutation happen in bacteria, any mutation happen in the tumor suppresive gene or protein formation gene inserted into the bacteria in gene therapy will cause severe effect on the micro-environment of the tumor tissue and may promote the growth and metastases of the tumor. Also, the technology of genetic modification is still under dispute. Luckily, most of these problems are now under researching and have made some progress. The development of bacteriotherapy that cure cancer in a safe and convenient way is promising.

## 5. Conclusion

Various application of bacteria have been investigated for use in cancer treatment. It is likely to use bacteria as immunotherapeutic agent to induce and stimulate immune response for cancer treatment, as well as programming bacteria as a vector to deliver drugs or tumor suppressive gene into the tumor due to its attribution of living in hypoxic region with low oxygen concentration which is specific in tumor microenvironment. All these strategies are under investigating and have made remarkable progress. In the study of using E.coli Nissle 1917 (EcN) as a targeted transport vector to deliver p53 and Tum-5 protein to tumor hypoxic region, results show that EcN is able to accumulate in the solid tumor areas of SMMC-7721 tumor-bearing BALB/c mice. And the data show that the programmed E.coli Nissle 1917 can inhibit the growth of human hepatoma SMMC-7721 cells. Moreover the experiment didn't show significant side-effect on the mice by EcN. The bacteriotherapy is the cooperation between bacteria and humans. Humans are living with bacteria, there are trillions of bacteria living in the guts of humans. Beside those harmful species, there are 30% bacteria are important for survival of human. Bacteriotherapy make people realize that all the organisms in the world have their own meaning. The research on bacteriotherapy act as a strong tool for human to fight against cancer in a better way with less side-effects and more specificity, giving hope to people who are suffering from cancer as well as their family members. At the same time, more in-depth research is needed on the targeting of bacteria to tumors, the interaction between bacteria and tumor microenvironment, and the dynamic interaction between bacteria and immune cells, which will provide a solid theoretical basis and new development direction for promoting bacterial treatment of tumors. The in-depth research of this "smart bacteria" is expected to become another powerful weapon for human beings to fight against cancer in the near future.

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