Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/27/20240645

Bacteria mediated cancer treatment (BMCT) and quorum sensing system in BMCT

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Abstract. Many treatments against cancer already exist. For example, radiation therapy, chemical therapy and immunotherapy, combined the physical surgery, these treatments do make a remarkable effect in inhibiting the growth of the tumor and thus prolonging the patient's life. However, most of the treatments mentioned above would simultaneously damage normal cells and cause trauma or pain to the patients. In recent years, researchers have turned their attention to the remarkable natural abilities of certain bacteria such as Salmonella and E. coli, in suppressing malignant cells and solid tumors. Bacteria-mediated cancer treatment (BMCT) has emerged as a promising approach that offers the potential for reduced patient trauma and enhanced precision in targeting cancerous tissues. Furthermore, the integration of quorum sensing systems, initially utilized in the fermentation process holds promise in augmenting the navigational capabilities of bacteria. This advance opens the door to the development of highly location-sensitive bio-agents capable of delivering substantial quantities of specific therapeutic proteins directly to tumor sites. This review aims to comprehensively examine existing BMCT methodologies and explore the quorum sensing pathways that have been identified. Additionally, it contemplates the feasibility of synergizing these two systems to offer a novel perspective on cancer treatment.

Keywords: Quorum sensing, Salmonella, Bacteria-mediated cancer treatment, colorectal cancer

1. Introduction

Up to these days, people have discovered numerous effective methods for treating cancer, including but not limited to chemotherapy, radiation therapy, and surgical removal. However, these treatments often inflict significant damage on fragile patients. Much of the unnecessary harm arises from the inability of traditional treatment to target specific areas. While these 'scorched earth' approaches do effectively eliminate cancerous lesions, they also diminish patients' living quality, in some degrees contradicting the fundamental purpose of healthcare - to alleviate suffering. Therefore, humans have tirelessly sought more localized treatment methods. In recent years, a treatment called Bacterial-Mediated Cancer Therapy (BMCT) has come into view. BMCT leverages the ability of certain bacteria to locate tumors, along with other clinically advantageous characteristics of these bacteria. Coupled with emerging gene-editing technologies, it offers a more precise approach to treat cancer. However, there currently exists a dearth of comprehensive reviews on the underlying systems of BMCT. Thus, this review aims to cover as many of the systems and bacterial strains on which BMCT relies as

DOI: 10.54254/2753-8818/27/20240645

possible. Additionally, it will enumerate existing experimental progress and propose potential mechanisms for anti-cancer action. It is our hope that this review will provide future researchers with insights to achieve greater strides in BMCT research.

1.1. Introduction of bacteria-mediated cancer treatment (BMCT)

In the late 19th century, Dr. William Coley and his research team pioneered the utilization of bacteria in the treatment of cancer. A patient diagnosed with advanced bowel cancer was referred to the emergency room where medical professionals conveyed the grim prognosis of an imminent demise within a year, as conventional treatment appeared futile in addressing the tumor. However, a remarkable turn of events transpired after the patient was infected by acute erysipelas. This medical anomaly piqued the interest of Dr. Coley who then embarked on reviews on more patients who experienced similar recoveries and noticed a correlation between bacterial infection and tumor regression.

Subsequently, he thus formulated a therapeutic approach involving the development of what would later become known as “Coley toxins” which were concocted by blending inactivated Streptococcus pyogenes and Serratia marcescens

bacteria, and he then administered this toxin through direct injection into the tumors to treat more than a thousand cancer patients. Even though the five-year survival rate remarkably increased under this treatment, it was apparent that a majority of the treated individuals endured ongoing health challenges and faced a limited life expectancy.

After the invention of chemotherapy and radio treatment temporarily overshadowed the pursuit of BMCT, the study of using bacteria toxins to treat cancer was suspended. Nevertheless, as scientific understanding of immunology advanced over the years, Dr. Richardson and his coworkers revisited Doctor Coley’s pioneering work. They compared the effectiveness of Coley’s toxin with contemporary cancer therapies based on published results. It turns out that the Coley toxin could behave as the pathogen-associated molecular patterns (PAMPs), recruiting immune cells such as the dendritic cells, neutrophils, and the natural killer cells to the tumor sites, consequently inciting an inflammation that played a pivotal role in cancer resolution. [1-3]

However, the initial application of the bacterial toxins carried a significant risk of inducing severe infections. Fortunately, advancements in genetic engineering technology enabled the achievement of modified strains, producing toxicity-reduced bacterial toxins. Notable bacterial species, including BCG, ST., Clostridium, E.coli, Listerai, Monocytogens and Shigella have been harnessed in the production of cancer vaccines, offering promising prospects for the future of cancer immunotherapy. [2, 4]

BMCT presents distinct advantages in treating cancer juxtaposed with other traditional cancer therapies. Chemotherapy, a widely used and efficacious approach to cancer treatment, entails the systemic administration of therapeutic agents and therefore treats tumors distributed throughout the patient’s body. However, chemotherapy is associated with deleterious side effects such as alopecia due to follicle cell damage, gastrointestinal complications, and myelosuppression, leading to a diminished count of immune cells and platelets. One important reason contributing to the multifarious side effects is the passive diffusion-dependent nature of therapeutic chemicals. The chemicals lack the ability locating to nidus, necessitating the administration of substantial quantities of these chemistry agents to ensure the treatment efficacy. In stark contrast, anaerobic bacteria thriving exclusively within the hypoxic microenvironment at the central hollow part inside the tumors, offer an inherently site-specific modality for cancer intervention. [5]This inherent localization within the central hollow region of the tumor affords the unique advantage of mitigating risks to surrounding healthy tissues, thereby significantly reducing the potential for collateral damage in BMCT-based cancer treatment strategies. [2, 4]

Additionally, it is noteworthy that traditional therapies, reliant on systemic circulation for drug delivery, may encounter limitations in addressing early-stage tumors situated at a considerable distance from vessels. In contrast, as bacteria are relatively metabolic independent, they could infiltrate

DOI: 10.54254/2753-8818/27/20240645

malignant tissues deeply from vascular proximity, thereby extending their therapeutic reach within the context of cancer treatment.[2]

Similar to the pioneering work of Dr. Coley, bacteria exert their immunomodulatory influence by eliciting localized inflammation within tumor regions. A fundamental issue contributing to unbridled tumor growth resides in the phenomenon known as the tumor immune escape effect. Within this context, malignant cells turn down their expression of the major histocompatibility complex (MHC) molecules on their membranes and concurrently exhibit reduced level expression of the costimulatory proteins responsible for the secondary activation signal crucial for converting naive T cells into cytotoxic T cells. Consequently, this deceptive alteration leads the immune system to erroneously perceive tumor cells as normal tissue cells assisting the immune surveillance evasion of tumor cells.

Besides, certain tumor cells release immunosuppression molecules attracting regulatory T cells orchestrating the expulsion of other immune cell populations aimed at targeting the tumor. However, several structural components and unmethylated proteins present on the bacterial cell wall could send strong signals to the immune system informing a severe intrusion within the affected area. Then, the toll-like receptors on immune cells recognize these bacterial proteins, further activating the innate and adaptive immune response, which manifests through an augmented production of several inflammatory cytokines such as IL-12 [6].

1.2. Salmonella in BMCT

Among all bacteria, Salmonella exhibits an outstanding feature as a promising candidate for advanced tumor treatment, primarily owing to its amenability to genetic manipulation. The well-characterized expression systems in Salmonella have enabled the development of some ideally engineered strains such as the Salmonella Typhimurium VNP20009, A1-R (eu-/arg-), SHJ2037 (relA−/spoT−) [7-8].

As previously outlined in the 1.1 paragraph, Salmonella shared several advantages inherent to BMCT such as locating to tumor and providing site-specific treatment, triggering localized inflammation, penetrating into the tumor tissue, and operating independently off the circulatory system.[1-3, 5, 6, 9] In addition to these common attributes, Salmonella possesses some unique merits. It can directly eliminate cancer cells through mechanisms that remain not fully elucidated. The AI-1 that Salmonella produced in its quorum sensing system, for example, has been observed to induce tumor cell death within a remarkably short timeframe of around thirty minutes. Furthermore, when Salmonella competes for the nutrients with tumor cells, it simultaneously produces toxins that instigate the apoptosis process in cancer cells by promoting the degradation of long-lived cytoplasmic proteins.[10]By turning down the expression of mTOR and the protein kinase B, Salmonella can suppress the expression of the HIF-1α which results in the reduction of VEGF disrupting the tumor cells' capacity to recruit blood vessels [2, 7, 11-13].

Another notable feature of Salmonella is its effectiveness by oral administration, allowing it to carry much of the chemicals to the tumor when it is acting as a carrier [14]. Moreover, advancements in modifying Salmonella's quorum sensing system offer the prospect of finely regulating the timeline for production, release, and clearance, enhancing its therapeutic potential [7, 12, 13].

Possessing these adorable advantages in treating cancer, Salmonella has therefore already been used either to kill the tumor cells directly using the toxins it produced, or to induce inflammation, or to deliver therapeutic chemicals as carriers to tumor sites serving as a replacement for chemotherapy requiring injecting large amounts of the chemicals.[11] These applications have demonstrated significant potential in treating several types of cancers such as melanoma, liver cancer, breast cancer, prostate cancer, and colorectal cancer [2, 7, 12].

Even more, Salmonella is currently being investigated in clinical trials in treating pancreatic cancer. As an anaerobic bacterium, Salmonella exhibits better efficiency in addressing solid cancers. Yet, most clinical trials using Salmonella as cancer treatment are still in their first or second phases. The utilization of Salmonella as a therapeutic agent requires the patients not to have resistance to any kind of antibiotics since it is important to maintain control over the Salmonella population to prevent uncontrolled over-proliferation and the potential development of septicemia. Also, researchers face the

DOI: 10.54254/2753-8818/27/20240645

challenge of striking a delicate balance in the amount of natural toxins that Salmonella can carry. Excessive reduction of toxins may compromise its effectiveness in treating cancer, while retaining too many toxins could pose a risk of severe infection [7, 12].

However, Salmonella has not been discovered with the ability to completely eliminate all tumors in patients on its own. The BMCT based on Salmonella needs to cooperate with other conventional cancer treatments, such as administering effective medications or surgically removing some sizable tumors [7, 12].

In a phase I clinical trial report posted in 2002 by Toso et al., VNP20009 was used to treat patients with metastatic melanoma or renal cell carcinoma [15]. The deletion of the msbB and purI genes is conducted in order to reduce the overall toxicity of VNP2009 rendering it safe for intravenous infusion. The deletion of the msbB gene served to prevent the combination of the myristyl group with the lipid A domain of the lipopolysaccharides (LPS), which could reduce the toxicity of LPS of VNP20009, whereas the removal of the purI necessitated the provision of exogenous adenine which could contribute to its capacity to locate as well as reduce the possibility inducing bacteremia [6, 16].

The clinical trial incorporated a total of 24 patients who were intravenously injected with different doses of the VNP20009 strain. Bcterial concentrations were closely monitored in the patients' blood, urine, feces, and tumor sites. Since the VNP20009 strain did not carry additional chemicals, this trial's approach focused on inducing localized inflammation in tumor sites and thus facilitating the introduction of tumor necrosis factor-alpha (TNF-α) to trigger tumor auto-apoptosis [7].

However, the outcome of this clinical trial proved to be less than satisfactory. Approximately one-six of the patients had experienced prolonged low-concentration bacteremia without definitive indication of bacterial residence in the tumor site. Besides, the shrinkage of the tumor was not obviously observed. Therefore, even though, in many animal experiments, the concentration of VNP20009 in tumor sites, compared to its concentration in blood or other tissues, could be 1000 times higher, the bacteria's colonization ability in the human body remained uncertain. The research team also explored the maximum tolerated dose (MTD) by assessing the dose limiting toxicity (DLT) response, in which they found the original toxicity of Salmonella constrained the MTD, turning the antibiotics treatment a required addressing process after the experimental trial ended. In cases where four patients displayed symptoms indicative of low-concentration bacteremia, an immediate antibiotic injection was administered to eliminate any potential bacterial threats.

In another clinical trial conducted in 2008, advanced tumor patients were treated with a further modified VNP20009 strain which, in addition to the deletion of the msbB and purI genes, integrated the CD gene, a gene coding for an enzyme absent in mammalian cells [6, 16-18]. This enzyme had the unique ability to convert 5-FC protein into 5-FU protein. The product, 5-FU protein, acting as a cytotoxic antimetabolic agent within tumor sites, was released by Salmonella, and could theoretically curtail the growth of tumor cells. This treatment using the modified VNP20009 strain was further named TAPET-CD. Instead of intravenous injection, the research group experimented with shallow direct injections around the tumor to avoid the diffusion of the bacteria thereby reducing the risks of getting bacteremia. During the clinical trial, three patients were treated with bacteria that did not reach MTD, alongside highly concentrated 5-FC molecules.

Although one of the patients showed a notably high concentration of 5-FU in the tumor site, this patient had experienced a grade 3 toxic event prompting their withdrawal from the experiment prematurely, and sadly this patient passed away one year after participating in the trial. The other two patients exhibited responses to the injection, as evidence by an increasing concentration of 5-FU; nevertheless, there were no clear indications of tumor regression. These two patients passed away approximately 100 and 200 days, respectively, after the trial.

The ability of the modified Salmonella to locally thrive and release CD enzyme within the tumor did not perform as effectively in the human body compared to the results observed in mice experiments. Nonetheless, the modified Salmonella strain still displays considerable potential for cancer treatment, and thus more effective modifications need to be explored to improve the ability of the strain to grow and release proteins within tumors [18].

DOI: 10.54254/2753-8818/27/20240645

Three clinical trials are still progressing: (1) Dr. Garin Liu using SGN1 strain to treat advanced solid tumor patients (2) Dr. Gerald Batist employing Saltikva strain for Metastatic Pancreatic Cancer (3) Dr. Alexander and colleagues using oral-administrated Salmonella as a carrier to deliver DNA vaccine to treat neuroblastoma [19]. In these new clinical trials, researchers have begun exploring alternative administration methods, such as oral delivery, and have experimented with various modifications to increase the bacteria's reliance on exogenous nutrients to improve its localization ability. Additionally, they have investigated different tumor-suppressing pathways in order to maximize inhibitory effects.

2. Quorum Sensing System

2.1. Introduction of Quorum Sensing System

The concept of controlling bacterial populations through their intrinsic sensing systems traces its origins back to the fermentation process like brewing wines. In these processes, as the bacteria proliferate and thrive in the low-oxygen environment, they metabolize glucose into alcohol and carbon dioxide. Thus, alcohol accumulates, eventually reaching levels that prove lethal to the bacteria, culminating in the successful production of sterile and high-quality wine. Brief illustration of the common mechanism of all quorum sensing system can be seen in figure 1.

Quorum sensing represents a more sophisticated mechanism by which bacteria regulate their colony size. Generally speaking, quorum sensing system enables bacteria to gauge the density of their population and coordinate collective actions to enhance or ensure the efficacy of these actions. The pioneering work of Kenneth Nealson's team unveiled the quorum sensing system in a marine bacteria known as Vibrio Fischeri. This bacteria employs the quorum sensing system of the"Lux family" to regulate the expression of luciferase, an enzyme that could make the bacteria glow, which in other words is its bioluminescence. Typically, Fishceri exist individually, resembling zooplankton. However, rather than living separately, they prefer to colonize in the mental cavity of the Hawaiian bobtail squid where they could hide from predators and get nutrition more easily. Like paying the rent, during this mutualistic relationship, the bacteria have to benefit the squid in return, which is lighting the upper part of the squid and thus concealing the squid from predators when observed from both beneath and above during stary nights. The Fischri would only emit light when residing within the squid. In order to determine their presence, they continuously produce a specific autoinducer molecule which automatically exports through diffusion into their surroundings as soon as they are produced. LuxI is the gene responsible for encoding this autoinducer. Once the autoinducer reaches a critical threshold, detectable by membrane-bound proteins on the bacterium's surface, it enters the Fischeri cell via transport proteins and binds to LuxR. This interaction forms a complex that initiates operon activity, subsequently triggering the transcription of LuxI, LuxA, and LuxB. LuxI will further produce additional autoinducers, creating a positive feedback loop. Simultaneously, LuxA together with LuxB would collaborate in the synthesis of luciferase, the enzyme responsible for the emission of blue-green light [20-22].

DOI: 10.54254/2753-8818/27/20240645

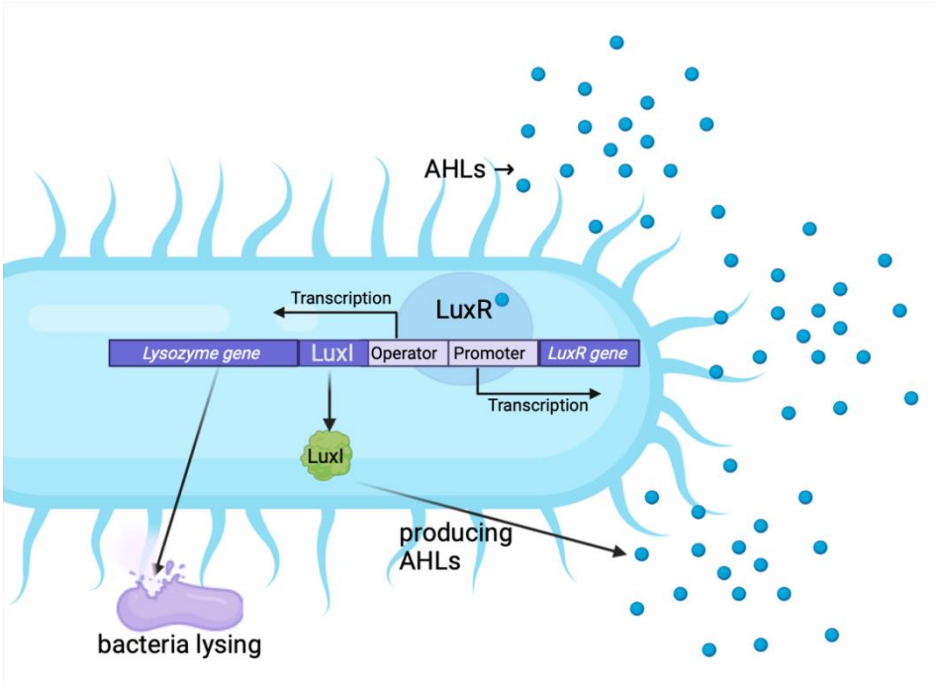

Figure1. The overall perspective of quorum sensing mechanism. Picture credit: original

The case of Fischri and the bobtail squid provides a quintessential illustration of how the quorum sensing system operates. In addition to this example, quorum sensing can lead to either expressing or inhibiting the expression of the downstream genes and therefore it could play many different roles in cell-mediated communication encompassing determining the appropriateness of launching a virulent attack, seizing opportunities to construct biofilms, and regulating motility, among other functions [23].

There exist numerous types of autoinducers in quorum sensing, and the associated pathways exhibit considerable diversity. It is accepted that the quorum sensing systems can be categorized into four primary groups based on the discernible autoinducers and their respective producing proteins: (1) LuxS, which produces AI-2; (2) LuxR/LuxI which produces AHL; (3) autoinducer peptides (AIPs); (4) AI-3/epinephrine/norepinephrine [12, 23-24]. However, there are also other quorum sensing systems that might exhibit similarities to or overlap with the aforementioned categories, and some have not been fully characterized, thus often omitted in conventional reviews of quorum sensing systems. Examples include the AI-1(CAI-1/LAI-1) system and its synthetase CqsA/LqsA, as well as the PAME (hydroxyl-palmitic acid methyl ester) and the DSF (methyl dodecenoic acid) [25-28].

2.2. Reviews on Four Main Quorum Sensing Systems

2.2.1. AI-2 and Enzyme LuxS System in Ecoli. The autoinducer-2 (AI-2) is produced in the methyl activation cycle (CAM) assisted by the LuxS enzyme. LuxS facilitates the conversion of its substrate, S-ribosylhomocysteine (SRH), culminating in the generation of homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which forms the chemical foundation of AI-2 as an array of isomeric variations. DPD itself is limitedly stable in aqueous environments like in the cellular cytoplasm, predisposing it to spontaneously cyclization into 4-hydroxy-5-methyl-3(2H)-furanone (HMF). Some cyclized isomers of HMF including S-DHMF and R-DHMF, assume roles akin to AI-2. These molecules may further undergo hydration processes, converting into other complexes like S-THP, S-THMF, and R-THMF. These complexes, too, contribute to the multifaceted dynamics of the AI-2 in the quorum sensing process [24, 27, 29].

DOI: 10.54254/2753-8818/27/20240645

To briefly summarize, the LuxS enzyme coming from the substrate SRH greatly helps the production of AI-2. The synthesis of SRH, in turn, emerges as a crucial component of the synthesis of AI-2 as well. Another enzyme, Pfs, additionally contributes to the transformation of SAH into SRH by catalyzing the cleavage of the glycosidic bond between MTA and SAH [23, 29].

After the generation of the AI-2, they could automatically permeate out of the bacteria rapidly and accumulate in the external environment. Conversely, the import of AI-2 into the cell is a regulated process, emphasizing the accumulation of the sufficient quantity of AI-2 for cellular uptake. In the periplasmic space of the Ecoli., AI-2 forms a complex with LsrB traversing the bacterial membrane via transporter LsrC and LsrD. During this transport, the enzyme LsrA hydrolyzes ATP into ADP and a molecule of the phosphate group, providing the requisite energy for this import process. Upon entry into the bacterial cytoplasm, AI-2 binds to LsrK, a kinase enzyme, leading to its phosphorylation into AI-2-P which would subsequently bind to LsrR forming a complex. The LsrR and AI-2-P complex downregulates the expression of the LsrACDEFG operon and thus decrease the bacteria's ability to import the AI-2 while governing its ability to degrade AI-2-P, a product primarily executed by the LsrEFG proteins [22, 29, 30].

2.2.2. AHL and LuxI/R system. In the introductory context of the quorum sensing system, the Vibrio fischeri bacterium serves as an illustrative example, utilizing this mechanism to initiate its bioluminescence system. In this system, LuxI acts as the synthase of AHL while LuxR functions as the receptor protein for AHL. AHL molecule typically consists of two components: the hydrophilic ring homoserine lactone ring (HSL) and one hydrophobic acyl side chain. Therefore, the overall hydrophobic or hydrophilic nature of AHL depends on the length of its acyl side chain. AHL, owing to its HSL structure, can diffuse through the membrane passively, without the expenditure of energy. Thus, the diffusion of the AHL hinges on the concentration difference between the interior and exterior of the bacterial cell. [21, 24]

When the bacteria density is low, AHL production outpaces its accumulation, allowing AHL molecules to readily exit the cell, which leaves LuxR unbound within the cell, precluding its binding to the PluxI promoter. Conversely, at high bacterial densities, exterior AHL concentrations rise, impeding its diffusion out of the cell. It hense binds to the LuxR protein, forming a complex binding to the PluxI promoter, which would initiate the expression of the downstream gene including the LuxR gene and the LuxICDABE gene, collectively constituting the Lux protein family. The region housing these Lux protein-coding genes is referred to as the Lux box, and the precise role and location of the Lux box remain subjects of ongoing investigation within the scientific community.[20, 21, 24]

It is worth noting that, one special category of LuxR, called the orphan LuxR or the solos LuxR, possesses the ability to recognize AHL molecules produced by other bacterial species. Interestingly, the AHL from other strains can also initiate the expression of downstream genes, which suggests that the AHL and LuxI/R system may serve as a commonly employed system among all gram-negative bacteria, thereby representing an inter-species communication platform within the bacteria realm [20, 21, 31].

Given the variability in acyl side chain composition, a diverse array of AHL molecules exists, each capable of forming complexes with derivatives of LuxR and thereby governing vital bacterial functions. For instance, in vibrio, the downstream LuxICDABE gene manages bioluminescence; In Pseudomonas aeruginosa, which utilizes two parallel AHL systems, distinct pairs of AHL bind to LasR or RhlR hence initiating the synthesis of hydrogen cyanide and alkaline protease; In Chromobacterium violaceum, AHL interacts with CviR, promoting the expression of the violacein pigment production. Importantly, when the side chain of the AHL exceeds a certain length, rendering the AHL no longer a micromolecule, the ability to diffuse through the membrane passively is deprived, converting the export into active transport mechanisms, which further expand the AHL and LuxI/R systems underscores the diversity and complexity inherent in these intercellular communication pathways [20, 21, 32].

DOI: 10.54254/2753-8818/27/20240645

2.2.3. Autoinducer Peptide (AIP) system. The AIP precursor is initially synthesized by the attached ribosomes. Subsequently, it undergoes posttranslational modifications, a process occurring during its excretion, to be activated and stabilized. During the excretion, the ATP-biding cassette transporter (ABC) will be responsible for facilitating the export of the AIP. Once modified, activated, and exported, AIP will accumulate in the extracellular environment. Similar to other quorum sensing systems, when the amount of the AIP reaches a physical critical concentration threshold, there is a reduction in further excretion.[28] Instead, it binds to a receptor on the outer membrane, causing the phosphorylation of the conserved histidine residue. This residue could further activate the receptor kinase which then transports a phosphoryl group to the aspartate residue of an intracellular response regulator which significantly influences the expression of various target genes, including the ABC transporter and others. The AIP system is a common feature observed in gram-positive bacteria.

2.2.4. AI-3/epinephrine/norepinephrine system. This system was reported in E. coli. where it regulates the toxins gene's expression. In a review written by Cristiano et al., they delve into the mechanism of AI-3 specifically in a type of E. coli. known as EHEC. In the human intestine, the resident flora produces AI-3 as signaling chemicals, while the human host produces epinephrine and norepinephrine to govern the intestinal activity. The QseC protein, located on the membrane of EHEC, could sense the accumulation of these chemicals and undergo autophosphorylate at precise intervals. Subsequently, it forms complexes with its response receptor, QseB, promoting QseB's phosphorylation. Another membrane-bound sensing protein in EHEC which is QseE interacts with epinephrine and norepinephrine and thus phosphorylates QseF protein. Alongside these Qse proteins, numerous other proteins are included in this phosphorylation cascade, ultimately resulting in the expression of LEE gene. The LEE gene is a significant gene for EHEC since it regulates the expression of the motility gene flhDC, the Shiga toxin gene StxAB, the effacing (AE) lesion formation, and the QseEF gene. Although AI-3 is not a direct regulator for the number of bacteria, it plays a crucial role in regulating the synthesis of toxins and orchestrating other meaningful collective activities, rendering it a component in quorum sensing systems the quorum sensing system demonstrating the significance of the system not only including the quantitative regulation but also encompassing various other facets of bacterial behavior [33, 34].

3. Prospective of Combining Quorum Sensing System with BMCT

3.1. Examples of Utilizing Quorum Sensing System in BMCT

Using anaerobic bacteria to release anti-cancer chemicals has l been a popular topic for long. However, the clinical study of using quorum sensing system to precisely control the turn-on and turn-off expression of the desired chemicals is still under progress.

One trial done at MIT and Harvard Medical School in 2015 used Lux system to replace the original quorum sensing system in Salmonella to harness the specificity of the Lux system in Fishiri which is a member of marine plankton [35]. The Lux in Fishiri does not share with other bacteria strains in mammals so it allows the quorum sensing system to function independently without interrupting normal function of other resident bacteria in mammals. Additionally, the study combined the LuxI with green fluorescent protein (GFP) which produce 3OC6HSL, a kind of HSL, that activates and binds to LuxR. The compound formed from 3OC6HSL and LuxR can be recognized by the operon of LuxI so that positive feedback of 3OC6HSLproduction is established. The team chose direct injection to deliver the system, and follow-up detection of the concentration of bacteria inboth tumor and the liver were conducted to monitor the bacteria diffusion. Another research where many genes are deleted to create a tryptophan auxotrophic Salmonella strain with reduced toxicity and improved invasion ability to immune system. These properties could facilitate bacteria to better locate into the tumor. The modified bacteria also carry vectors that code cytolysin-A (ClyA) which would induce apoptosis of tumor cells. Significant reduced tumor size indicates its promising perspective in treating human solid cancer [30].

DOI: 10.54254/2753-8818/27/20240645

3.2. Design of a novel potential BMCT strategy based on quorum sensing and Salmonella Typhimurium

3.2.1. Epidemiology and Necessity. Colorectal cancer (CRC) as a significant global health concern ranks third in terms of cancer incidence (6.1% of all cancer cases) and is the second leading cause of cancer-related mortality (9.2% of cancer deaths worldwide) According to data from 2020, the incidence of CRC even reached around 30% in Oceania and Europe, and the mortality rate has also risen to around 10% among all continents. The global burden for CRC is continuously growing, with approximately 2.2 million incidences and 1.1 million deaths. [36] As CRC are solid tumor that could be treated with the BMCT method by which Salmonella Typhimurium could be an ideal material. Through oral intake, the modified Salmonella could reach the nidus on the colorectal, reside there and proliferate.[37] After sensed sufficient amount of bacterial population through the quorum sensing system the bacteria would lyse and release the protein so the growth of the tumor would be inhibited. As the bacteria itself could be the pathogen that induce inflammation and recruit immune cells, the lysing process of the bacteria could also enhance the recognition of the immune cells to the tumor cells. Therefore, the ideal outcome for this treatment is to suppress the growth of the existing tumor and meanwhile improve the supervising ability of the immune system [14, 38]. A brief illustrative picture showing the design of the immune enhancer can be seen in the figure 2 below.

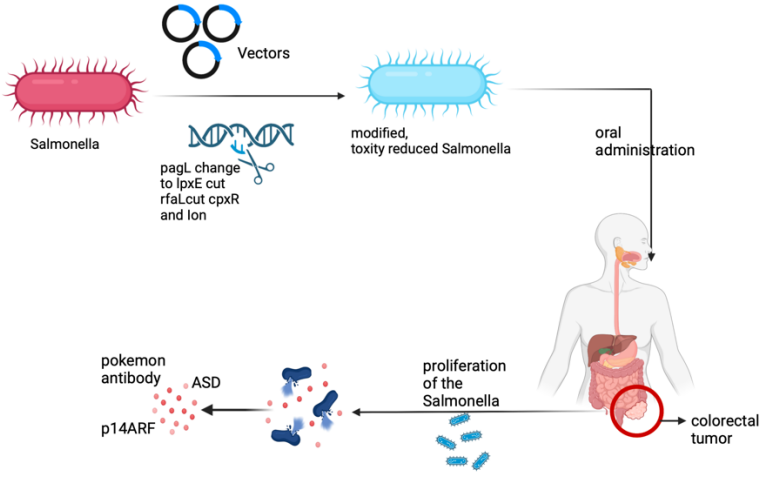

Figure 2. The outline of the design uses modified salmonella to treat colorectal cancer through oral administration. Picture credit: original.

3.2.2. Modification on Quorum Sensing System. The original quorum sensing system, the LsrR system, in Salmonella releases autoinducer 2 (AI2) in the environment and later absorbs them back into the bacteria through transport protein LsrA, LsrB, LsrC and LsrD while the AI2s reaches the threshold. However, instead of inhibiting the downstream gene, initiating a suicide gene would be more efficient. Therefore, here is where the LuxR quorum sensing system could step in. The LuxR quorum sensing system generates AHL as its autoinducer and the AHL-LuxR complex could initiate the expression of the downstream genes. The genes coding lysozymes are off when Salmonella density is low and would be turned on when the bacteria amount reaches the threshold [24, 39, 40].

3.2.3. Techniques and Establishment of the Vector. Vectors would be constructed through PCR and Gibson Assembly [41] to introduce desired genes into the Salmonella. The Pokemon antibody single chain variable fragment (scFv) gene, p14ARF, ASD and fragment from plasmid pET-N-His-TEV (from the Beyotime company) would be assembled to form the full vector which could be seen in figure 3.

DOI: 10.54254/2753-8818/27/20240645

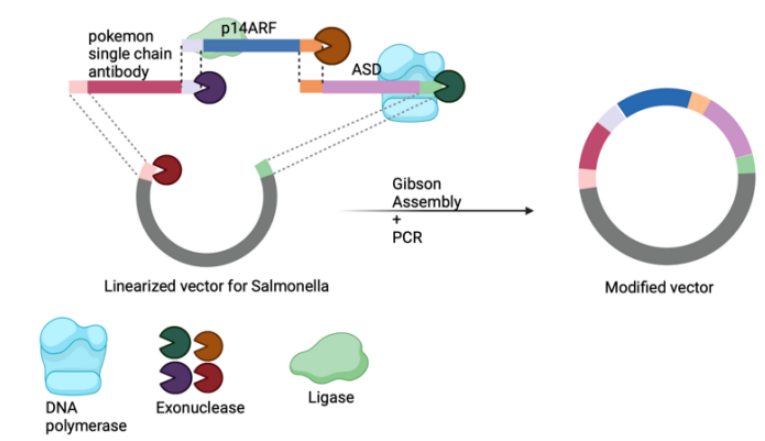

Figure 3. The establishment of the vector. Picture credit: original.

The rationale of using Pokemon antibody scFv and p14ARF gene is based on the discovery of the "Pokemon↑→ARF↓→MDM2→P53↓" pathway which has been shown to be responsible for the transition from benign colorectal tumor to a real cancerous malignancy. In order to invert the tumor growing process, high expression of P53 protein can be induced by binding Pokemon scFv with Pokemon protein to reduce its amount as well as increase the volume of p14ARF, so that cell cycle could suspend in the G1 phase. In detail, higher expression of p53 manifests in elevated levels of MDM2 and p21CIP1 with cell cycle arrest in both G1 and G2/M which are part of the interphase [42-43].

ASD gene codes for the enzyme ASD that produce DAP which is one of the most important components to form the cell wall of Salmonella. The insertion of ASD gene and the removal of the ASD gene from the strain genome puts Salmonella under certain survival pressure forcing them likely to retain the vector instead of lysing it. This modified Salmonella strain would be selected through cultivating them in agar plates without DAP [9, 37, 39].

Terminator, promoter and an operator integrated in the T7 expression system in Ecoli could ensure the self-replication of the vector and vectors be separated into two daughter cells during bacteria proliferation. To ensure the expression of the desired gene and the replication of the vector, the gene of the RNA polymerase called DE3 that could initiate the expression of the T7 promoter must be inserted into the genome of the bacteria [44].

To deliver the vector into the bacteria, positive charges need to be introduced since the negative phosphate group on the surface of the vector and the bacteria would repeal each other prohibiting the vector from getting into the bacteria. Calcium chloride solution is used to neutralize the negative charge. A temperature shock at 42 Celcius degree could open pores on the cell membrane, increase the cell permeability of the bacteria and allow vectors to enter. Cultivation of bacteria should be carried out at 37 Celcius degrees.

3.2.4. Animal Experiment. The hypothesis of the design for the animal experiment is as follows. The study with the animal model is divided into blank, control, and experiment groups. Mice in the blank group are without colorectal tumors and not injected with any vaccine or placebo.

The disease animal model for the control and experiment group is established by mutation of Apc, injection of DMH, and oral intake of the DSS. Apc is a crucial tumor suppressor gene within the Wnt pathway, and it plays an important role in the initiation and development of colorectal cancer. The 850th codon (TTG) of the Apc gene encoding leucine could be edited into a stop codon (TAG) to form the mutated gene [45]. Both positive and negative control groups would be established. For the positive control group, the disease model mice will be treated with Keytruda, a chemical shown to be effective in suppressing CRC tumor growth. Placebo will be provided to the negative control group. In

DOI: 10.54254/2753-8818/27/20240645

the experimental groups, aside from considering variations in bacterial concentrations within the BMCT or vaccine, it is essential to address the effective duration of this treatment.

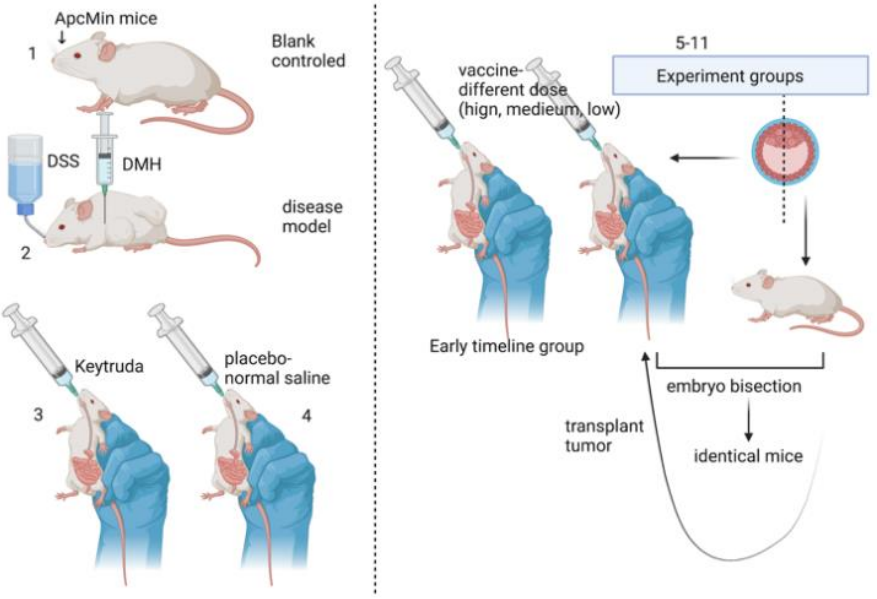

Figure 4. The group division and operation on mice in the animal experiment. Picture credit: original.

Consequently, the experimental groups can be categorized into two main groups: the typical experimental group and the lagging experimental group. Except for the different concentrations of the bacteria in the BMCT, the effective duration this treatment could induce is also an important concern. For the typical experiment group, we can gather data on the estimated time duration for the clearance of bacteria taken. Conversely, in the lagging group, a new tumor from the homologous mice could be transplanted to the experiment group after the elimination of bacteria to examine whether this treatment could stimulate an immune response or not. Designation of the animal experiment is illustrated in figure 4 above.

3.2.5. Medicine Delivery. Oral administration will be the optimal method to deliver bacteria to the target location primarily because it is the most prevalent way employed by previous researchers for administering the Salmonella Typhimurium vaccine carrier. Furthermore, there are additional reason that solidify our choice of this approach. In contrast to alternative methods of administration, the mice exhibit superior adaptability with less weight loss and extended average life span comparing to mice that are muscular injected or other delivery method. Besides, by this way of bacteria administration, most Salmonella will be absorbed in the jejunoileal vein within the small intestine and the bacteria are less likely to enter the brain, liver or other unrelated organs and tissues. These findings have been substantiated through histology analysis by Doctor Aganja and his research team [39-40].

4. Conclusion

In conclusion, this review begins with a brief overview of the development of BMCT, where doctor Coley is mentioned to explain that people initially noticed and harnessed the ability of bacteria to induce inflammation and thus to activate the immune reaction in the specific locations where tumors are. Subsequently, the review included and summarized more pros of bacteria in treating cancer and additionally emphasized the outstanding characteristics of Salmonella in cancer treatment. Several clinically ideal modified strains are also included in this review, which naturally transits the topics to the summary of clinical experiments using them to treat cancer. The second section of this passage mainly focuses on introducing the quorum sensing system which lays the firm foundation for making

DOI: 10.54254/2753-8818/27/20240645

BMCT a reality. It started with a case study where the marine bacteria, fischri, uses qs system to maintain its normal functions as well as the common management mode of the quorum sensing system. Next, according to the different types of autoinducer molecules and the enzyme responsible for the production of that autoinducer, the review details four systems while briefly summarizing other systems that have been discovered. In the third section, after introducing two distinct trials on mice indicating an ideal result on mice, a design, as an outlook of a possible treatment idea combining those two trials, is discussed in hopes of inspiring coming researchers in aspects that they could further their study.

Acknowledgement

To assist readers of this review in having a better understanding of the classification of autoinducers, the following reviews are highly recommended: the annual review from Wai-Leung Ng and Bonnie L. Bassler, the article “Emerging applications of bacteria as antitumor agents” from Vipin Chandra Kalia et al., and the article “Genetically engineered Salmonella Typhimurium: Recent advances in cancer therapy” by Liang et al.. Also, the concept of the sensing protein for the threshold that all autoinducers could achieve poses challenges in its clarity as the potential mechanisms remain unresolved, interpretation of these mechanisms in this review is limited to a macro-scale perspective without detailed chemistry. The potential and promise of employing quorum sensing systems in cancer treatment offer a less intrusive therapeutic approach. The author deeply appreciative of the invaluable support and contributions from Jiahua Ge, Yilin Yin, and Ruoxi Liu in shaping the design of the vaccine discussed in this review. Lastly, I extend my heartfelt gratitude to all the teachers who have provided invaluable guidance along my research journey. Their support has been instrumental in enabling me to conduct research, compile references, and write this review independently.

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