Salmonella as a Promising Curative Tool against Cancer

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Bacteria-mediated cancer therapy has become a topic of interest under the broad umbrella of oncotherapy. Among many bacterial species, *Salmonella* remains at the forefront due to its ability to localize and proliferate inside tumor microenvironments and often suppress tumor growth. *Salmonella* Typhimurium is one of the most promising mediators, with engineering plasticity and cancer specificity. It can be used to deliver toxins that induce cell death in cancer cells specifically, and also as a cancer-specific instrument for immunotherapy by delivering tumor antigens and exposing the tumor environment to the host immune system.

Keywords: Salmonella ; Salmonella-mediated cancer therapy ; combination therapy

1. Introduction

Cancer is a leading cause of death worldwide and a burgeoning health burden with a limited number of successful therapeutics. On average, 10 million people worldwide lose their lives annually due to various cancers [1]. Every cancer requires an accurate diagnosis and prompt treatment at the earliest possibility. Even though most conventional treatment strategies such as surgery, chemotherapy, and radiotherapy remain major life savers, they have serious limitations that can damage healthy tissues [2][3]. Surgical removal of cancers can be effective in certain types and developmental stages of cancers; however, cancer relapse and the possibility of further spread due to metastasis are some of the inherent weaknesses of this method [4]. On the contrary, radiotherapy and chemotherapy provide varying degrees of success and inflict unprecedented failures in cancer treatment, especially distant tumor recurrences and undesirable effects [5][6]. Hence, to fill the gap, novel treatment concepts and strategies are essential as an ideal treatment for cancers. Cancer tumors consist of hypoxic core regions and necrotic centers, which make most cancer treatments incompetent due to lack of oxygen and abnormal vasculature. Studies have demonstrated that such regions are the key features of tumors that lead to treatment failure [7][8][9]. In addition, due to the abnormal vascular architecture, it is a huge challenge to deliver therapeutic agents to the tumor region. Hence, it is evident that a single treatment strategy may not be effective against cancer malignancies, but holistic approaches might bring suboptimal outcomes.

In recent decades, bacteria-mediated cancer treatments (BMCT) have garnered attention as an alternative strategy to treat cancer tumors due to the intrinsic challenges of conventional cancer treatment strategies. Advancements in genetic engineering and recombinant DNA technology had paved the path for developing numerous bacterial strains as model systems to be used in cancer immunotherapy. William Coley's controversial study, more than a century ago, revealed that some bacterial species may hold the key to creating targeted treatments for cancers that are challenging to cure. According to Coley, the complex cocktail he produced had the potential to shrink cancer tumors; however, the lack of progressive techniques and poor understanding of the mode of action made it difficult to reproduce consistent results. Revitalizing these early findings, scientists around the world have attempted to use novel bacterial species, such as *Bifidobacterium*, *Clostridium*, *Salmonella*, *Streptococcus*, and *Listeria monocytogenes* for tumor regression and have brought deep insight into their mode of action.

2. Bacterial Application for Cancer Therapy

Numerous studies have demonstrated the anti-tumor effects of several bacteria, either by directly killing or modulating immune components of the tumor microenvironment. The natural cytotoxic features of bacteria can result in substantial tumor regression. Therefore, many researchers have exploited non-pathogenic obligate anaerobes and facultative anaerobes which selectively infiltrate and replicate within solid tumors when administered systemically. The ability of bacteria to regress tumors came into the limelight in the early 1800s. BMCT started gaining momentum when Coley's toxin, a mixture of killed *Streptococcus pyogenes* and *Serratia marcescens* developed by Dr. William B. Coley, achieved

clinical responses for many malignant tumors [10]. Additionally, the anti-tumor characteristics of various bacteria have been documented, such as *Salmonella* [11], *Escherichia coli*, *Vibrio cholerae* and *Listeria monocytogenes* [12], *Clostridium welchii* [13], *Clostridium tetani* [14], *Bifidobacterium infantis* [15], *Streptococcus pyogenes* [16], and *Proteus mirabilis* [17]. *Salmonella* holds natural cytotoxicity that regresses tumors when injected in its native form [11]. Similarly, *Clostridium*, an obligate anaerobe, can regress tumors in mice [13][18][19].

The bacterial implication in cancer can function as a two-edged sword, since the association of certain pathogenic species has been linked to colon cancer. *E. coli* possesses a genomic island polyketide synthetase codes for the synthesis of colibactin that has been implicated in colorectal cancer [20]. In another scenario, *Clostridium* sps., especially *Clostridium perfringens* and *Clostridium septicum*, has been associated with colorectal cancer [21][22]. *Salmonella typhi/p*aratyphi produces a potent carcinogen N-nitroso compound and has been documented for hepatobiliary carcinoma [23].

3. Current Approach with Combination Therapy

Salmonella has been used with other therapeutic agents to enhance the efficacy of anti-cancer activities. It has been used in combination with chemotherapy, radiotherapy, immune checkpoint inhibitors, and immunomodulatory cytokines. The combined administration of *Salmonella* with chemotherapy reduces toxicity compared with individual therapy with bacteria or chemotherapeutics. For this, a murine melanoma model was treated with VNP20009 and cyclophosphamide, which induced a significant decrease in microvessel density and serum VEGF levels compared with either treatment alone. [24]. Similarly, the combination of anti-angiogenic agent HM-3 (a polypeptide inhibiting angiogenesis) and VNP20009 harboring expression plasmids for siRNA targeting *Sox2* demonstrated efficient treatment for lung cancer [25]. Another well known ST A1-R strain was implemented in combination with the chemotherapeutic drugs temozolomide, doxorubicin, and anti-angiogenic agents, which significantly suppressed the growth of tumors in patient-derived orthotopic xenograft models [26][27][28]. The co-administration of radiotherapy and BMCT produced prominent anti-tumor effects compared to either of the treatments alone. The combination of X-rays either with VNP20009 or ΔppGpp ST expressing cytolysin A (ClyA) or γ-radiation with *Salmonella* BRD509 induced a significant suppression of the tumor or delayed tumor growth [29][30][31]. In addition, the treatment with A1-R post-surgical excision of tumors significantly inhibited surgery-induced breast cancer metastasis [32]. In combination therapy, the use of prodrug strategy along with *Salmonella*-expressing, prodrug-activating enzymes such as HSV TK, carboxypeptidase G2 (CPG2), and cytosine deaminase have more promising tumor retardation capabilities compared to the use of the therapeutic strain alone [11][33][34].

4. Cancer Vaccines Delivered by *Salmonella*

Being an intracellular pathogen, with vast survival in different organs of the host, attenuated *Salmonella* has been widely used as a vaccine delivery system against various diseases [35]. As mentioned earlier, *Salmonella* is a multifaceted antagonist for cancer [36][37], apart from that, using auxotrophic *Salmonella* as a therapeutic and prophylactic vaccine delivery system is also an ideal strategy. Medina et al. have demonstrated the anti-cancer effect of auxotrophic ST (Δ*aroA*) as a vaccine delivery system that expresses β-gal as a model TAA against aggressive fibrosarcoma [38]. In another study, SPI-2 and T3SS of *Salmonella* were used to deliver survivin as a TAA into antigen-presenting cells, and the PsifB::sseJ promoter/effector combination was found to have an excellent anti-cancer immune response of CD8 infiltration in the tumor environment [39]. Similarly, elevated effector-memory CTL responses against CT26 colon cancer and orthotopic delayed brain tumor glioblastoma in mice were found after immunization with survivin, which was fused to the SseF effector protein and kept under the regulation of SsrB, the key regulator of SPI2 [40]. Heat shock protein 70, as an immuno-chaperone fused with SopE of *Salmonella* T3SS, has elicited a considerable CTL response against murine melanoma [41]. A multi-antigen DNA vaccine encoding fusion antigenic domains of tyrosine hydroxylase, survivin, and PHOX2B, delivered by auxotrophic ST *(*Δ*aroA*, Δ*guaAB*), has been demonstrated to exhibit significant elicitation of the CTL response, INFγ production, and excellent suppression of neuroblastoma in a mouse model [42]. In addition to the CTL responses, the elicitation of humoral response by a *Salmonella*-based oral DNA vaccine with a MG7-Ag mimotope against gastric cancer was confirmed [43]. An H_2O_2-inactivated *S*. Typhimurium RE88 *(*Δ*aroA*, Δ*dam*) has been established to induce anti-cancer immunity by using ovalbumin as a model antigen [44]. *Salmonella* has also been studied for the expression of oncogenic virus antigens. A recombinant ST that produced Human Papillomavirus Type 16 (HPV16) L1 Virus-like Particles (VLPs) induced the anti-tumor immune response in prophylactic as well as therapeutic contexts [45]. The same group has constructed a *Salmonella* that has expressed major capsid protein L1 of HPV16 virus via plasmid, and has shown the induction of anti-HPV16 neutralizing and humoral immune responses [46]. They have also demonstrated the intravaginal immunization of the HPV16-L1 *Salmonella* construct and its innate, adaptive, Th1, and Th2 mucosal immune responses [47]. Thus, *Salmonella* can be used to deliver oncogenic viral antigens for prophylactic vaccine development. In addition, *Salmonella* infection triggers the formation of gap junctions in melanoma that are

typically lacking in tumor cells. The transfer of tumor antigens to dendritic cells and the resultant induction of immune responses depend on these gap junctions [48]. Moreover, *Salmonella* has the ability to induce MHC class I and II immune responses by delivering cancer-related antigens via bacterial surface and translocating the antigen or its gene to the antigen-presenting cells, respectively. *Salmonella* has the virtue of being used as a delivery vehicle for extrinsic cancer antigens, oncogenic viral antigens, and to display the intrinsic antigens of the active tumor to achieve anti-cancer immunity based on these aspects.

5. Application of *Salmonella* in Tumor Targeting and Detection

Tumor targeting and accumulation phenotypes have made *Salmonella* the best player in the creation of genetically engineered strains for detecting tumors. Strains expressing fluorescent proteins are well studied for the purpose of visualizing and locating the tumor region in vivo [12]. Another approach for positioning tumor-specific *Salmonella* used positron emission tomography which locates the engineered ST VNP20009 strain in tumors by expressing the HSV1-TK reporter gene that can selectively phosphorylate radiolabeled 2′-Fluro-1-β-D-arabinofuranosyl-5-iodo-uracil [49].

Salmonella was engineered to express the fluorescent protein ZsGreen. It has a high sensitivity that can detect tumors 2600 times smaller than the current limit of tomographic techniques [50]. Since *Salmonella* preferentially accumulates in tumors and microscopic metastases, this approach would provide a method to detect a tumor, monitor treatment efficacy, and identify metastatic onset.

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