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Systematic Review

Exploiting Anti-tumor Effects of *Salmonella typhimurium*: A Systematic Review

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ABSTRACT

Introduction: *Salmonella typhimurium* (*S. typhimurium*) has emerged as a promising agent for cancer therapy. This systematic review aimed to comprehensively analyze the existing literature regarding the utilization of *S. typhimurium* as a therapeutic strategy against cancer. The present systematic review aimed to evaluate the current state of knowledge regarding the anti-tumor properties of *S. typhimurium*, encompassing its tumor-targeting mechanisms, impact on tumor growth, modulation of the tumor microenvironment, and potential for combination therapies.
Materials and methods: A systematic literature search was conducted across major scientific databases, including PubMed, Web of Science, and Scopus, using predefined search terms. Studies published between 2000 and 2023 were included if they investigated the anti-tumor effects of *S. typhimurium in vivo*. Studies were independently screened, selected, and evaluated for quality by two experts in the field.
Results: The systematic review identified 152 relevant studies that met the inclusion criteria. These studies collectively demonstrated the ability of *S. typhimurium* to selectively target and colonize tumors, resulting in significant tumor growth inhibition in various cancer types. Mechanistic insights revealed that *S. typhimurium* can induce direct cytotoxicity, modulate the tumor microenvironment, and activate anti-tumor immune responses. Additionally, studies highlighted the potential of combining *S. typhimurium* with conventional therapies or immune checkpoint inhibitors to enhance therapeutic efficacy.
Conclusion: This systematic review underscores the promising potential of *S. typhimurium* as a novel and multifaceted approach to cancer therapy. The accumulated evidence suggests that *S. Typhimurium* possesses inherent tumor-targeting capabilities, exerts direct anti-tumor effects, and can synergize with other treatment modalities.

1. Introduction

One of the most challenging health problems to treat is cancer, which is a leading cause of death worldwide[1]. On average, 10 million people die from cancer each year. Detecting and treating cancer at an early stage is crucial. It

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is important to note that most conventional treatments can damage healthy tissues, even though they are lifesaving[2]. For example, chemotherapeutics, surgeons, and radiologists effectively save lives[3]. Surgical removal can be used to treat certain cancer types and stages. In addition, metastases and relapse are possible with this method[4]. The success of chemotherapy and radiotherapy in treating cancer varies accordingly, especially distant recurrences of tumors and adverse effects. The development of new cancer treatment ideas and strategies is crucial for improving the effectiveness in patients[5-7]. According to several studies, tumor treatment failure can be attributed to such regions[8,9]. Furthermore, tumor-specific therapeutic agents cannot be delivered to tumors because of abnormal vascular architecture. Various organisms, including fungi, and pathogens (parasitic worms [hydatid cyst protoscolex, *Trichinella spiralis*]) or even protozoan (*Trypanosoma cruzi*) offer potential anti-cancer properties through bioactive compounds and immunomodulation[10-14]. *Escherichia coli, Clostridium, Salmonella*, and *Bifidobacterium*, among others, contain inherent characteristics that make them tumor-targeting and tumor-killing bacteria[15].

It has long been recognized that *Salmonella typhimurium* (*S. typhimurium)* is a significant cause of foodborne illness in humans, which is a gram-negative bacterium from the *Enterobacteriaceae* family[16,17]. This pathogenic bacterium has recently been identified as a potential cancer therapeutic agent[18]. Investigations indicate *S. typhimurium* enters and selectively kills solid tumor tissues[19,20]. However, healthy cells were not harmed; a novel and potent tool for treating cancer has been discovered in *S. typhimurium* based on the intriguing discovery[21]. The ability of some bacteria to home tumors has been demonstrated while heterologous genes have been delivered intracellularly by other invasive species[22,23]. It is more advantageous to express target genes in *S. typhimurium* since they are highly replicating and invasive[24]. A tumor hypoxic zone promotes the survival of optional anaerobes that act as anti-tumor agents[25]. The therapeutic gene must be delivered effectively to the target tissues or cells for gene therapy to be successful[26]. *S. typhimurium* is one of the greatest pathogens for bacteria-mediated cancer treatments (BMCT). Animals with highly aerobic conditions can spread it systemically, and a hypoxic tumor region is its preferred colonization site, where it eventually settles. In addition to conventional therapies, *S. Typhimurium* can colonize hypoxic and necrotic and metastatic tumors[27,28].

The objective of this systematic review is to evaluate the current state of knowledge regarding the antitumor properties of *S. typhimurium*, encompassing its tumor-targeting mechanisms, impact on tumor growth, modulation of the tumor microenvironment, and potential for combination therapies in animal models. Salmonella's potential as a key microbial agent in cancer therapy will be investigated, as well as engineering methods that can be used to create *Salmonella*-based cancer treatments of the future.

2. Materials and Methods

The article presents a review of studies published between the years 2000 and 2022 in order to come up with a conclusion. All relevant studies on *S. typhimurium*'s anti-cancer properties are identified through systematic and exhaustive literature searches. A comprehensive search of multiple electronic databases, such as PubMed, Scopus, Web of Science, and Database, will begin the review process. The search is supplemented with manual searches of reference lists and relevant journals to minimize the risk of missing relevant studies. Searching keywords were *Salmonella typhimurium*, *Salmonella*-based therapy, *Salmonella*, cancer therapy, cancer, immunotherapy, gene therapy, and *in vivo*.

2.1. Inclusion and exclusion criteria

Upon retrieving the studies, screening was conducted in two stages. Reviewing the titles and abstracts of the papers was the first step in the screening process. Second, the quality of the articles was evaluated based on the full text of their studies. The title and abstract screening procedures involved studies with predefined inclusion, and the exclusion criteria led to the removal of irrelevant or duplicates. The full texts of studies were reviewed once they were available.

2.2. Quality assessment and data extraction

This study was developed based on a standardized data extraction form to extract relevant information from the selected studies. It included publication year, authors, sample size, and experimental model, which are the most important characteristics of the study. *Salmonella typhimurium* has a wide range of anti-tumor effects, including apoptosis induction, immune response modulation, tumor targeting, tumor growth inhibition, and other mechanisms. Several appropriate tools were used to assess the inclusion of studies, including the Cochrane Collaboration's Risk of Bias tool for randomized controlled trials for non-randomized studies. Upon the completion of independent quality assessments by both experts, the inconsistencies between the two were discussed.

2.3. Data analysis

Data synthesis and analysis were based on a narrative framework since the included studies are heterogeneous. A descriptive summary of the evidence aimed to provide a comprehensive overview. The results were arranged and presented according to the type of cancers investigated.

3. Results

Anti-tumor properties of *S. typhimurium* against cancer, and a systematic review of these studies were conducted. The review included 480 studies, which met the inclusion criteria, from 530 publications. Overall, 50 of them were duplicates and were excluded. Among these, 137 full-text articles were reviewed for eligibility. As a result of removing duplicates and screening the articles for eligibility, 24 articles were included in the quantitative synthesis (Figure 1).

3.1 Anti-tumor mechanism of Salmonella typhimurium

3.1.1. Hypoxic environment and tumor vasculature

A tumor's blood supply is unevenly distributed and chaotic, resulting in chronic and acute hypoxia[29]. As a result, oxygen delivery to the cells is diminished, and the proliferation of the cells is disrupted. Bacteria, such as *S. typhimurium*, thrive in hypoxic environments[30]. An abnormal vasculature can result from tumor angiogenesis that, in comparison with normal tissue, is more vascularized[31]. It is likely that *S. typhimurium* can invade such an unruly and highly vascularizing tumor microenvironment[32]. These facultative anaerobic bacteria are more likely to be localized. It begins to kill tumor cells so that they can survive and take up nutrients. In addition, chaotic vasculature contributes to the disorder[33]. A double-auxotrophic mutant *S. typhimurium* strain A1-R with tumor-targeting properties has been demonstrated to increase vascularity and destroy tumor blood vessels[34].

3.1.2. Abundant nutrients and competitive nature of Salmonella typhimurium

There are a variety of micronutrients in the tumor microenvironment[35]. In a study of lung and pancreatic adenocarcinomas in mice, nutrients positively influencing cancer cell metabolism differed between tumors and the circulation[36]. Cancers generate energy and biomass through metabolic adaptation, which accumulates metabolites. Moreover, it alters the expression of normal genes[37]. The tumor's microenvironment is immune-suppressive. As a result, by providing nutrients and protecting the bacteria from host immunosurveillance, these compounds could enable attenuated auxotrophic bacteria to survive and grow[38]. It has been found that prostate cancer and breast cancer can be treated in mice models by employing auxotrophic *Salmonella* for tryptophan, arginine, and leucine[39].

3.1.3. Tumor penetration

Salmonella typhimurium has been chosen for BMCT due to its potential for deep tumor penetration[40]. Passive transport is traditionally used to distribute chemotherapeutic drugs. *Salmonella typhimurium* invades deep tumor tissues by utilizing the abundant nutrients surrounding it. When *S. Typhimurium* has been administered systemically, apoptosis is induced by systemic subcutaneous administration[41]. In tumor tissue, bacterial motility influences the spatial distribution of bacteria; the motility of bacteria increases their penetration depth[42]. It has also been suggested that tumor migration of *S. typhimurium* is not dependent on motility or chemotaxis but a passive process. In order to study the

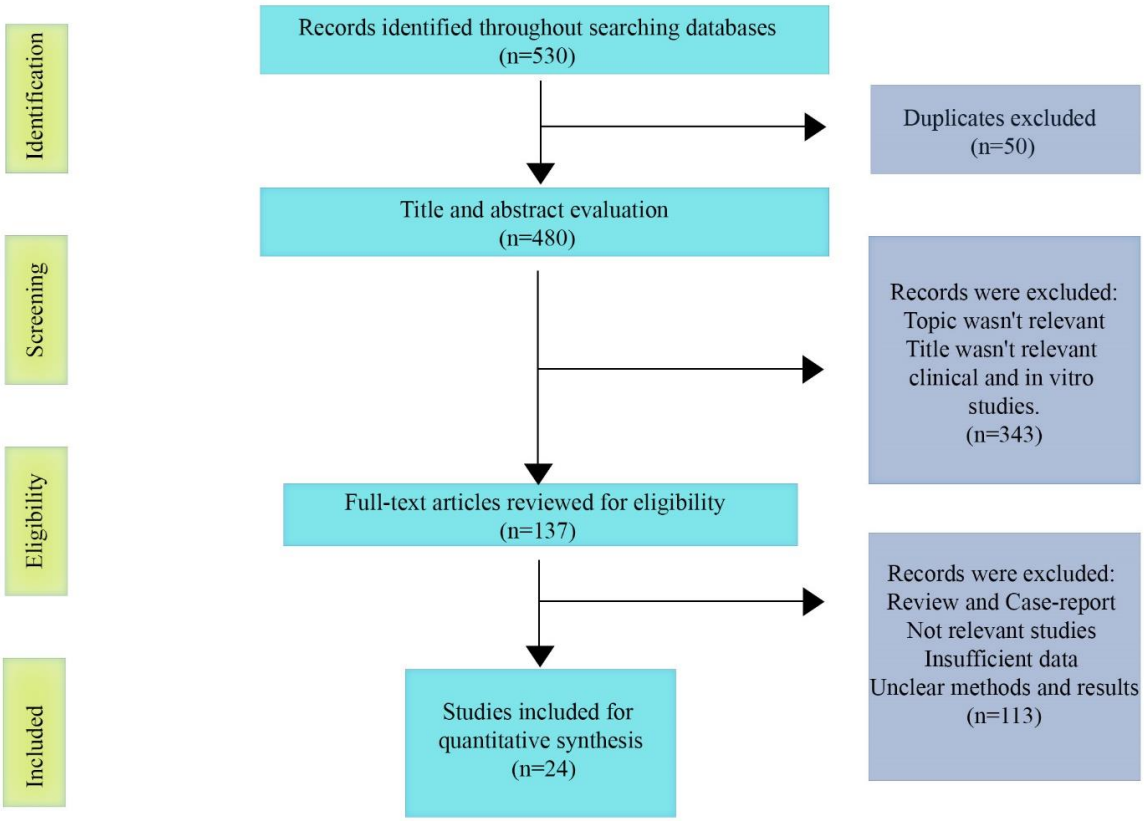

Figure 1. Methodology of the current study

events of tumor-colonization, different strains, and time points post-infection were used *in vivo*[43]. Tumor tissue is protected from bacteria by neutrophils; bacteria colonize intratumor more readily when their numbers are diminished[44].

3.1.4. Apoptosis and autophagy-inducing intrinsic anti-tumor action

Salmonella typhimurium has been shown to directly kill cancer cells in several *in vivo* studies[45,46]. Genetically engineered *S. typhimurium* strain A1-R infected cancer cells have grown into large colonies, as revealed by high-resolution multiphoton tomography images[47]. *Salmonella typhimurium* can induce both apoptosis and autophagy when it enters the cells of cancer cells[45,48]. There is no clear understanding of how *S. typhimurium* induces apoptosis. Bacterial toxins and competition for nutrients with cancer cells may cause apoptosis. In addition, autophagy may be induced; in tumor cells, scavengers are less active than in normal cells, meaning they are less active[49]. Phosph-Protein Kinase B (P-AKT) is downregulated in an AKT-dependent manner/ The Phosph-mammalian Targets Of Rapamycin (P-mTOR) pathway regulates cellular proliferation and survival[50]. Cellular physiology and homeostasis are influenced by the P-AKT / P-mTOR pathway. Matrix MetalloProteinase 9 (MMP-9) expression is reduced by the downregulation of this pathway, which is involved in metastasis; this oncoprotein plays an important role[51].

3.1.5. Inhibition of angiogenesis

It is essential to understand that angiogenesis plays a crucial role in tumor progression and development, HIF-1α and VEGF play a vital role in tumor angiogenesis[52]. The invasion of *S. typhimurium* in a tumor decreases the expression of VEGF and HIF-1α, inhibiting tumor angiogenesis by activating the P-AKT/P-mTOR pathway[53]. A recently identified tumor angiogenesis inhibitor protein is another mechanism for suppressing angiogenesis; through HIF-1, Connexin 43 (Cx43) inhibits VEGF expression besides interfering with its composition[54]. A tumor-targeting double-auxotrophic mutant *S. typhimurium* strain A1-R shows an association between a higher tumor vasculature and the destruction of tumor blood vessels[55].

3.1.6. Immunomodulation in tumor tissue

In animal models, *S. typhimurium* can manipulate the immune components of the tumor function to inhibit tumor growth by changing the tumor microenvironment from an immunosuppressive to an immunogenic state[56]. Evidence shows that *S. typhimurium* infection increases macrophage, natural killer (NK), CD4+ helper T cells, and CD8+ cytotoxic T cell infiltration[57]. Colony-Stimulating Factor 1 (CSF-1) and Chemokine C-C motif chemokine Ligand 2 (CCL-2) are chemo-attractants released by tumor cells in order to recruit monocytes that undergo differentiation into macrophages of the M2 subtype[58,59]. Tumor growth and malignancy are promoted by M2 macrophage polarization, which secretes immune-suppressive molecules to suppress antitumor immune responses in the host[60]. As a result of *S. typhimurium* invasion, these cytokines are released, including IL-10 and arginase 1 (Arg1)[61,62]. It has been demonstrated that TAMs activate M1 macrophages by releasing various activation markers, such as Sca-1 and MHC class II, in response to *S. typhimurium* invasion[63]. This is a paradigm shift from M2 to M1. The M1 macrophages orchestrate the anti-tumor immune responses by expressing nitric oxide synthase (NOS2) and TNF-α, which enhance the protective immune response to tumors *in vivo*[41].

A T cell that inhibits cytotoxic T-lymphocytes specific for tumor antigens mitigates antitumor immunity[64]. When injected into a colon cancer model, attenuated *S. typhimurium* reduces regulatory T (Treg) cell[65]. Cell surface molecule CD44, present on both cancer cells and Treg, is downregulated by *Salmonella* treatment[66]. Gap junction formation can be facilitated by *S. typhimurium* infection by upregulating CX43[67]. Tumor cells can present antigenic peptides to dendritic cells via gap junctions. Through antigenic presentation, cytolytic T cells can be activated against the tumor antigen, ultimately responsible for halting distant uninfected tumor growth *in vivo*[68].

3.1.7. Orchestration of tumor-associated macrophage function and polarization

The tumor-associated macrophage (TAM) is a constituent of the leukocytic infiltrate and has been used as a paradigm for cancer-related inflammation, tumor growth, invasion, metastasis, and drug resistance[69,70]. It is well-known that M1 macrophages have anti-tumor properties, whereas M2 macrophages promote tumor development, angiogenesis, and progression[71]. There are two sides to TAM, exhibiting pro- and anti-tumor activity, and this cell has high immunological reprogramming potential, especially in the presence of IFN-α or IFN-γ[72]. In a different approach, suppression of TAMs can be achieved by designing cancer vaccines against proteins which are overexpressed by TAMs, such as Legumain[73]. Legumain encodes an asparaginyl endopeptidase that is highly upregulated in murine and human tumor tissues[74].

3.1.8. Release of cytotoxic chemicals

During *S. typhimurium* treatment, cytotoxic compounds such as granzyme and perforin are released to kill tumor cells[75]. Immunomodulatory molecules, such as chemokines and cytokines, stimulate the immune system to eliminate tumors. Consequently, several cytotoxic agents, including Fas ligand (FASL), IL-18, TRAIL, IL-2, TNF-α, and cytolysin A, have been expressed in *S. typhimurium in vivo*[76-80]. Breast and colon tumors were inhibited by *S. typhimurium* engineered to express FASL, a proapoptotic cytokine [81-83]. Moreover, murine mammary tumors expressing the cytotoxic protein HlyE under hypoxic conditions showed increased necrosis and reduced growth when said with a

hypoxia-inducible promoter[84]. The RecA promoter is used in *S. typhimurium* to control the secretion of murine TRAIL, growth of mammary tumors delayed, activated apoptosis[85,86]. Similar results were achieved by hypoxia-induced nirB-regulated TRAIL for the suppression of melanoma *in vivo*[87].

3.1.9. Role of the type III secretion system

The virulence factor of *S. typhimurium* is well known, and it is encoded by *Salmonella* pathogenicity island 1 (SPI1) as the type III secretion system (T3SS)[88]. As a result, it is possible to inject effector proteins directly into host cells. Signaling pathways within cells are interfered with the cytoskeleton network in the host cell can be manipulated, and colonization is enabled[76]. They can produce and store most of the effector proteins secreted by the T3SS[89]. Injection of *Salmonella* pathogenicity island 2 (SPI2) effector molecules promotes cellular invasion promoted by SPI1[90]. *Salmonella* is resistant to most host defense mechanisms due to their ability to reconstruct endosomes into *Salmonella*-containing vacuoles to avoid host defense mechanisms and to enable intracellular survival[91,92]. Thus, SPI1 and SPI2 work together to facilitate the invasion of native forms or bacterial vectors[93]. It is, therefore, possible for *S. typhimurium* to induce apoptosis in established infection, and this has been confirmed by *S. typhimurium* effector expression causing apoptosis in tumor cells through activation of caspases 3 and 7, SpvB (an ADP-ribosyl transferase enzyme)[94].

P-glycoprotein is expressed in many cancers and is downregulated by *S. typhimurium* effector SipA such as breast, kidney, colon, and lymphoma[95-97]. Proteins of the T3SS family, such as inner rod proteins, needles, and flagellins inner rod, are perceived by nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family apoptosis inhibitory protein (NAIP) to activate the NAIP-NLR family caspase-associated recruitment domain-containing protein 4 (NLRC4)[98]. Caspases-1 and -8 are activated due to the assembly of the NLRC4 inflammasome[99]. As caspase-1 is activated, pyroptosis is induced, and caspase-8 stimulates caspase-3/7, resulting in the inflammatory death of the host cells, known as PANoptosis[100-102]. When effector proteins are internalized, the host immune response is modulated, cytokines are secreted, the cytoskeleton of the host cells is rearranged, and the invasion of bacteria is facilitated[76,103]. To develop cancer vaccines, we must improve antigen delivery; a promising strategy is *Salmonella* T3SS. Mice with tumors expressing NY-ESO-1 regressed when NY-ESO-1 tumor antigen was administered through T3SS[104]. Heterologous antigens like *Listeria monocytogenes*' MHC class I-peptide p60 were employed to translocate into host cells using T3SS from *Salmonella*, CD8 T cells induced by antigens, regression of tumors is significant[105] . Survivin, an antigen that is transported into antigen-presenting cells by SPI2, is an effector of immune responses induced by the SseF protein of SPI2[106]. In a mouse model, TAA was delivered via the SPI-2-regulated T3SS to stimulate anti-tumor activity (Figure 2)[107].

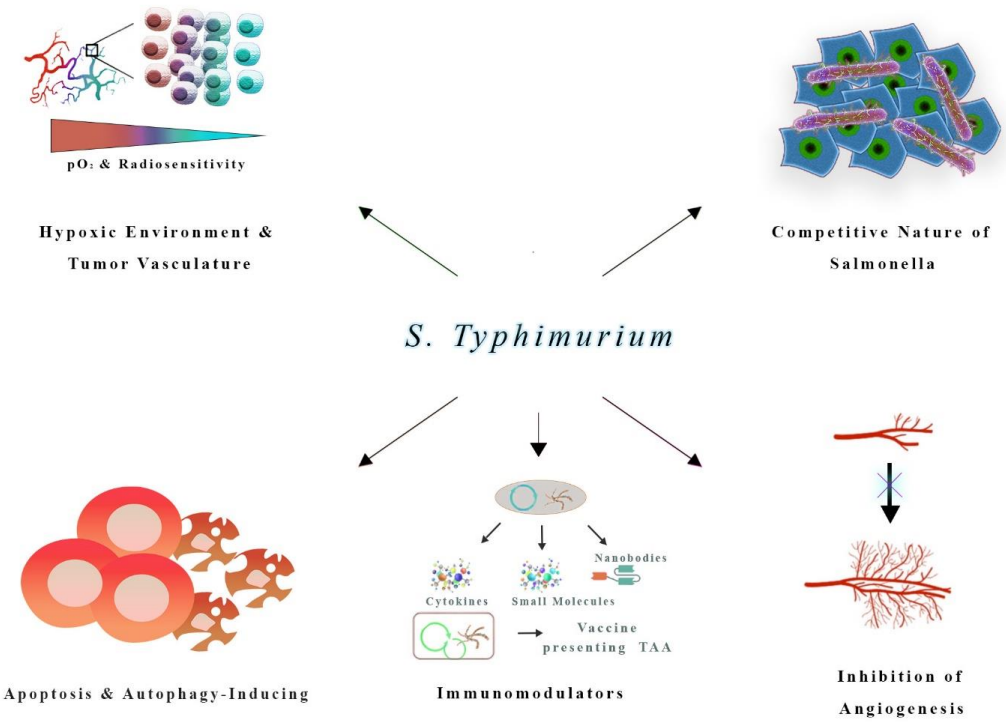

Figure 2. Anti-tumor mechanisms of *S. typhimurium*. Key strategies employed by *Salmonella typhimurium* for combating tumorsinclude its affinity for hypoxic tumor environments and chaotic vasculature, its competitive nature in utilizing abundant nutrients, its ability to induce apoptosis and autophagy within cancer cells, its inhibition of angiogenesis, and its role in immunomodulation in the tumor microenvironment. Together, these mechanisms illustrate how *S. typhimurium* exerts a multifaceted anti-tumor action, potentially impacting tumor vasculature, nutrient availability, and immune responses to facilitate tumor reduction.

4. *Salmonella* typhimurium's selective tumor targeting

Cancer researchers have been intrigued by *S. typhimurium*'s ability to specifically target tumor tissues[108]. Several intricate factors will be discussed in this subsection that *S. typhimurium* has the potential to benefit cancer patients over traditional cancer therapies because of its preferential tumor homing.

Various factors contribute to the unique conditions of the tumor microenvironment[109]. Healthy tissues do not have those characteristics. An inefficient or irregular blood supply causes low oxygen levels and vasculature leaks in tumors, providing an escape route for molecules and cells. *Salmonella* uses these proteins to selectively target tumors in both cases, leaky vasculature and hypoxia[110].

Chemotaxis is one of the most remarkable abilities of *Salmonella*; chemical gradients can be sensed and responded to by the body[111]. When it comes to targeting tumors, Salmonella can detect various chemotactic signals in the tumor microenvironment; bacteria are guided toward the tumor by these molecules[112]. Several signals are generated by necrotic and hypoxia areas inside the tumor, including metabolites, specific molecules, and gradients of nutrients[113].

A tumor is infected with *Salmonella* once reached; it can increase selectively within the tumor because of its nutrient-rich environment and relative protection[114]. During tumor microenvironment invasion, bacteria can take advantage of the weakened immune system to exploit available nutrients; the tumor can survive and reproduce more easily[115].

During tumor growth, the leaky vessels allow *Salmonella* to enter the tumor tissues and extravasate from the bloodstream[116]. *Salmonella* can access the tumor due to this vascular permeability, how it can be improved, produce anti-tumor effects, and replicate them[117]. The molecular interactions between *Salmonella* and tumor cells have been demonstrated in studies. Virulence factors are expressed by certain Salmonella strains; it allows them to attach to receptors on tumor cells that are expressed in a preferential manner, enabling cancer cells to be targeted and internalized[118].

Immune responses are triggered by *Salmonella*'s presence in the tumor microenvironment[119]. Immune cells can be activated by bacteria, like dendritic cells and macrophages, in the immune response against tumors; they play a critical role[120]. As a result of this immunomodulatory effect, the immune system may be able to respond more aggressively to Cancer and enhance anti-tumor activity.

5. Preclinical studies and animal models

Research involving *Salmonella*-based anti-tumor therapies has shown great potential for establishing *Salmonella*'s therapeutic potential for a variety of cancers[121]. Animal models were used in these studies, which provide invaluable insights into how *Salmonella* affects cancer progression and growth biologically and therapeutically. *Salmonella* has been shown to be effective in treating different kinds of cancer and in combination with existing therapies in some notable preclinical studies[24,122].

An animal model of breast cancer was used in recent studies[123]. Cancer cells were specifically targeted by *Salmonella* strains that carried cytotoxic genes[124]. As a result of the *S. typhimurium* being administered, there was a significant reduction in tumor size and an important tumor regression was observed in the mice treated with *Salmonella* administered intravenously, which led to prolonged survival[125,126]. Through this method, *S. typhimurium* was demonstrated to be capable of selectively targeting and inhibiting the growth of breast cancer cells *in vivo*[127].

Another preclinical study using *Salmonella* as a vehicle for gene therapy in mice with prostate cancer was conducted[128]. *Salmonella* engineered to infiltrate prostate tumors successfully activated immune responses that targeted prostate tumors[129,130]. A *Salmonella*-based therapy could benefit prostate cancer patients by boosting anti-tumor immune responses through enhanced reduced tumor growth and tumor cell killing *in vivo*[131].

A *Salmonella*-based therapy has been shown to reduce tumor burden in mouse models of colorectal cancer[132]. Anti-cancer agents expressed by *Salmonella*, such as tumor necrosis factor (TNF), to treat colorectal cancer, were used. As a result of the treatment, growth, and tumor size were significantly reduced.

In an *in vivo* study, ovarian cancer is modeled in mice, along with standard chemotherapy drugs, *Salmonella* was administered[133]. Chemotherapy alone had no anti-tumor effects, but combination treatment had enhanced outcomes. There was a significant reduction in tumor growth and increased survival rates with the combination treatment compared to chemotherapy alone. As a result of *Salmonella*'s synergistic action with chemotherapy, combination therapies can strengthen tumor regression[134]. It is important to note that immune checkpoint inhibitors have been studied in combination with *Salmonella*-based immunotherapies, which cancer-fighting drugs increase the immune system's ability to fight cancer[135]. Salmonella-based treatment and immune checkpoint inhibitors have been shown to significantly reduce tumor size and prolong survival in preclinical studies in mouse models of melanoma[136,137]. Immunotherapies can be enhanced and complemented by *Salmonella* in this way[138].

6. Challenges

Live-engineered bacteria represent a unique therapeutic opportunity that is accompanied by a number of challenges[139]. First, genes or motile genetic elements confer antibiotic resistance to living naturally modified bacteria. It is safer and more stable to engineer the expression cassette using chromosomal integration

without antibiotic selection markers[140]. Second, the manufacturing of GMP-grade test articles presents a specific challenge because live bacteria can neither be sterilized by heating nor filtered[141]. Furthermore, there would be no way to test for sterility by the conventional regulatory standard. In order to ensure "sterility," dedicated clean rooms with strict aseptic protocols and frequent monitoring of the process must be used during purification and production[142]. However, the final products need to be tested to ensure that no other diseases or conditions are present, even though they cannot be proven to be sterile. The third reason is that live bacteria proliferate in target tissues and thus spread[143]. It is not necessary that the administered dose corresponds to the effective dose (toxic or therapeutic). Target tissue "quality" influences the effective dose more than anything else which is determined by accessibility, whether there is hypoxia/tumor necrosis, and if pre-existing inflammatory cells have infiltrated the tumor[144]. Bacteria, at low doses, particularly when administered systemically, can harm the immune system[145]. It is less predictable and can take much longer for an infection to establish itself in the target tissue. Over time, the patients may become less vigilant, posing a greater risk. Fourth, oncolytic therapy involves turning a tumor into an infection that destroys the cancer; this could lead to severe consequences without proper management. It is essential to strike a carefully calculated balance between an infection's therapeutic benefits and toxic side effects. The practical difficulty of achieving this is great, antibiotics are effective only when administered on time, which would allow the infection to be eradicated before the anti-tumor effect was achieved[146]. A systemic inflammatory response risk during a late intervention is much higher. A multidisciplinary approach is needed to manage therapeutic infections, including oncologists Surgeons, interventional radiologists, or infectious disease specialists need to manage conditions requiring invasive treatment [142]. If an intratumoral infection is established, it is the team's responsibility to decide what to do and when to intervene. Fifth, in clinical settings, live biological agents are used. Public health and the environment are always concerns when it comes to its potential impacts[147].

7. Future and prospect

Researchers are working to better understand *Salmonella*'s mechanisms and address its weaknesses in order to develop future anti-tumor therapies based on *Salmonella*[123]. As a result of personalized medicine approaches, *salmonella*-based anti-tumor treatments may become a major part of the future treatment of cancer[141]. Personalized medicine incorporates genetics, molecular biology, and immunity into treatment planning. *Salmonella*'s genetic engineering capability allows precise therapeutic strategies. *Salmonella*, which can be genetically engineered to produce antigens and receptors specific to cancer cells, can be used to selectively kill cancer cells without harming healthy cells[139].

Research can optimize *Salmonella*'s anti-tumor properties by changing its genetic makeup; by enhancing its apoptotic capability, researchers could improve its immune-stimulating properties for different cancer types[117]. The presence of specific biomarkers can indicate the efficacy of *Salmonella*-based therapies. This approach selects patients based on their biomarkers to optimize treatment efficacy and minimize side effects[48,57].

When *Salmonella*-based treatments are combined with other immunotherapies, they can be more effective at treating cancer; they may produce a synergistic effect. This combination approach boosts the immune system and overcomes immunosuppressive microenvironments to prevent tumors from spreading. *Salmonella*-based therapies can combine immune checkpoint inhibitors with immune checkpoint inhibitors when the immune system encounters cancer cells, it attacks them[122]. *Salmonella* can enhance antitumor responses by activating immune responses and modulating the tumor microenvironment.

CAR-T cell therapy targets specific cancer antigens, with T cells engineered to target those antigens. It is more likely that CAR-T cells will enhance anti-cancer activity and tumor infiltration when delivered directly to tumors using *Salmonella*. A cancer vaccine is a personalized treatment because it incorporates tumor antigens into *Salmonella*[65]. Vaccines may help identify and destroy cancer cells by stimulating the immune system[104].

8. Conclusion

The current study has unveiled a groundbreaking frontier in the battle against cancer by harnessing the remarkable antitumor properties of *S. typhimurium*. Through a series of innovative *in vivo* studies, the current study has demonstrated the unprecedented potential of this bacterium as a novel therapeutic agent. This study not only shed light on the underlying mechanisms of *Salmonella*'s anti-cancer effects but also paved the way for the development of cutting-edge treatments that exploit this unique biological weapon. This exciting discovery holds promise for a future where *S. typhimurium* stands as a formidable ally in the fight against cancer, offering new hope and novel strategies for improving patient outcomes.

Declarations

Competing interests

The authors declare no conflict of interest.

Authors' contributions

The conceptualization of this project was spearheaded by Armin Batmani, who played a pivotal role in its development. The task of writing and preparing the original draft involved the dedicated efforts of Seyed Alireza Taheri, Mahsa Norouzi, Atefehsadat Monirvaghefi, Fatemeh Najafi, Abdolmahdi Asfaram Meshkinshahr, and

Sara Aghili. The project underwent a rigorous review and editing process, with contributions from Golnaz Behzad, Dorsa Mousavi Khatibi, and Bahare Kasaei. The project was self-funded, and it was executed under the supervision and guidance of Armin Batmani. All authors checked and approved the final version of the manuscript for publication in the present journal.

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Ethical considerations

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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