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Review

Salmonella infection acts as an environmental risk factor for human colon cancer

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ABSTRACT

Emerging evidence has demonstrated that perturbations of host-microbial interactions by pathogens can lead to an altered microenvironment that promotes tumorigenesis. A recent study provides new evidence and mechanisms on how repetitive exposure to non-Typhoidal *Salmonella* (NTS) increases the risk for colon cancer. This study integrated a serological and epidemiological approach with both *in vivo* and *in vitro* analyses, showed that the magnitude of exposure to NTS is associated with colonic tumorigenesis. *In vivo* exposure to repetitive low doses of NTS led to colonic tumors similar as a single high NTS dose in mice. Repetitive NTS infections significantly increase the proliferation of transformed cells in tissue cultures. The research results open new possibilities for the diagnosis, prevention, and treatment of colon cancer. The unanswered questions remain, including validation of the current findings in other cohorts, differences in lifestyle, and changes of gut microbiome after *Salmonella* infection. *Salmonellae* exposure can be limited by eating cooked meats and washing vegetables well. It is necessary to develop guidelines and criteria for screenings and follow-ups in people with exposure history to *Salmonella* and other cancer-associated pathogens.

1. Introduction

There are over 2600 *Salmonella* bacterial serotypes within the species *Salmonella enterica* (Cobo-Simon et al., 2023). Among them, *Salmonella enterica* subsp. *enterica* serovars Typhi and Paratyphi, the etiologic agents of Typhoid fever accounted for approximately 9 million cases and 110,000 deaths in 2019 alone (Typhoid, 2023). The non-typhoidal *Salmonella* (NTS) serovars Enteritidis and Typhimurium are the most common in clinical patients. NTS can also asymptomatically colonize certain animals, such as chickens, but can cause gastroenteritis in humans. Globally, NTS infections cause approximately 94 million cases of gastroenteritis annually including 150,000 deaths (Majowicz et al., 2010). In addition to the acute effects, *Salmonella* infection is known to increase the risk of chronic diseases, such as inflammatory bowel diseases (IBD), colon cancer, and gallbladder cancer (Scanu et al., 2015). However, the causal mechanisms leading to the development of these conditions are still not completely understood. The aim of this mini-review is to discuss the current understanding of the factors influencing the development of colon cancer with respect to the gut microbiome, host factors, and environmental influences, with an emphasis on NTS exposure.

2. Experimental models of NTS-linked tumorigenesis

In experimental models, researchers have tracked the development of colorectal cancer development in mice by observing the changes of the intestine during *Salmonella* infection (Lu et al., 2014), but not until recently has this been more directly studied in humans. In 2022, researchers from the Netherlands and the United States reported their collaborative study on how repetitive exposure to NTS increased the risk for human colon cancer, using human datasets, experimental models of *Salmonella* infection and colon cancer, and cell cultures with bacterial infection (van Elsland et al., 2022). They studied the serum antibody profiles against *Salmonella* from people that either did or did not later develop colon cancer. They found that those with serological evidence of prior *Salmonella* infection had a significantly increased risk of colon

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cancer when stratified by age, with increased risk if under the age of sixty (van Elsland et al., 2022).

To move from correlation to causality, experimental validation is critical. Therefore, researchers selected several special *Salmonella*e strains isolated from patients with colon cancer and infected mice with these strains to observe for evidence of tumor growth (van Elsland et al., 2022). Elsland et *al.* showed that *exposure of mice* to repetitive, low doses of NTS (10 CFU *Salmonella* exposures given once at 0, 4 and 8 weeks) led to the development of colonic tumors similarly to a single high NTS dose (a one time, 10,000 CFU inoculation at week 0) (van Elsland et al., 2022). These findings suggest that even a small exposure, perhaps an exposure resulting in a subclinical or asymptomatic infection, might predispose towards colon cancer development in humans. Furthermore, invasive Salmonellae were recovered from colonic tumors derived from Azoxymethane (*AOM*)/Dextran Sodium Sulfate (*DSS*)- treated mice, an established mouse model of inflammatory colorectal cancer (Lu et al., 2014; van Elsland et al., 2022).

In addition to the chemically-induced AOM/DSS mouse model, the impact of NTS infection on colon cancer development was also tested in a colonic epithelial, *APC* conditional deficiency ($APC^{\Delta CEC}$), sporadic cancer, mouse model (Robanus-Maandag et al., 2010). *Adenomatous polyposis coli* (*APC*) is a critical gene that suppresses tumor growth in the colon (for a recent review, see Abbott and Nathke (Abbott & Nathke, 2023)). If the APC gene is defective, it makes the host more susceptible to additional changes, *i.e.*, infection, inflammation, and altered microbiome, that may lead to colon and rectal cancers (Cheng et al., 2020). NTS infection was not found to increase the number of tumors formed, but instead was found to accelerate tumor growth in this sporadic cancer mouse model (van Elsland et al., 2022).

However, in the $APC^{\Delta CEC}$ mouse model, inflammatory modulators are unlikely to play an important role in NTS-accelerated tumor formation based on our observations. The enhanced tumors in the *Salmonella*-infected $APC^{\Delta CEC}$ mice are likely due to the activation of beta-catenin pathway that contributes to hyperprolifation of intestinal epithelial cells. Furthermore, in cell culture, *Salmonella*-infected cells progress to fully transformed cells, while multiple, mild infections increased the rate of colon cancer growth, similarly to exposure to a high *Salmonella* burden (van Elsland et al., 2022). All together, the experiments by Elsland et al. study suggest that *Salmonella* from the environment can increase the risk of colon cancer by simply contributing one step in the multistep process of cancer formation (van Elsland et al., 2022).

3. *Salmonella* effector proteins and malipulation of the key pathways in the host

The association between chronic *Salmonella* Typhi infections and gallbladder cancer was first noticed in India (Nath et al., 2008; Scanu et al., 2015). However, it has since been recognized that severe, NTS infections, such as those that require hospitalization, are also associated with an increased cancer risk (Mughini-Gras et al., 2018). In *S. enterica* serovar Typhimurium, an extensively studied NTS, *Salmonella* pathogenicity islands (SPIs), SPI-1 and SPI-2, each encoding a distinct type III secretion system (T3SS), have been shown to be critical for the secretion of over 40 effector proteins into host cells (see review by Pillay et al. (Pillay et al., 2023)). Once *Salmonella* effector proteins are injected into the host cell, various host processes are modulated to facilitate *Salmonella* survival and replication. One such effector protein, AvrA, for Anti-virulence agent A, was shown to activate the Wnt/β-catenin pathway to initiate hyperproliferation and tumorigenesis in the colon (Lu et al., 2014). More recently, AvrA was also demonstrated to inhibit autophagy via regulation of conversion of LC3-I to LC3 II, which in turn resulted in decreased levels of Beclin-1, a host protein important for autophagy (Jiao et al., 2020). By inhibiting autophagy in this manner, *Salmonella* is thought to promote intracellular survival in host cells consistent with the initial phenotype observed in an AvrA-deficient *Salmonella* mutant (Wu et al., 2012). The SPI-1 effector proteins SopE and SopE2 encode GEFs for Cdc42, a Rho family GTPase, which act to facilitate intracellular replication in the Salmonella CV (SCV) (Friebel et al., 2001; Stender et al., 2000). While reviewing all of the studied *Salmonella* effector proteins is out of the scope of this mini-review, please refer to the paper by Pillay et al. for a recent review of the known NTS effector proteins (Pillay et al., 2023).

4. Environmental factors and lifestyles related to *Salmonella* infection

Humans can be infected every few years with *Salmonella*, with symptoms ranging from severe to sub-clinical to asymptomatic, making it difficult to precisely quantify the number of prior infections. In an effort to overcome this challenge, Elsland et al. used a previously reported and validated method (Simonsen et al., 2008, 2009; Strid et al., 2007) to estimate the number of prior *Salmonella* infections based upon the serum levels of anti-*Salmonella* IgA, IgM, and IgG detected in the patient's serum. When stratified by age, younger patients with serological evidence of *Salmonella* infection (*i.e.* <60 years of age) were more likely to develop colon cancer compared to older patients (van Elsland et al., 2022).

The patient population included in the Elsland et al. study was from the Netherlands only (van Elsland et al., 2022). However, *Salmonella* infection rates vary among countries likely due to different environmental factors and lifestyles. For example, in western countries, *Salmonella* infections are often related to chicken products, meat, vegetables, and soil exposed to mice and other environmental factors. In China, most *Salmonella* outbreaks are linked to meat products, related to improper food handling, poor storage, and cross-contamination (He et al., 2023). From 2002 to 2017, China recorded 2815 outbreaks attributed to meat products, resulting in 52,122 illnesses, 25,361 hospitalizations, and 96 deaths (Zhao et al., 2022).

Conversely, in Japan, it's extremely rare to have *Salmonella* outbreaks. likely due to rigid farming and cleansing processes (Lee & Yoon, 2021). In India, *Salmonella* infections (e.g., enteric fever, gastroenteritis, localized infection, and symptomless carrier states) are quite common. Enteric fever is the most common manifestation with gastroenteritis next in frequency. The difference of *Salmonella* exposure is also due to the difference in eating habits between eastern and western countries (*i.e.* salads, well-cooked food, and drinking boiled water). Thus, *Salmonella* exposure can be limited by boiling eggs, eating cooked meats, and washing vegetables well. Efforts to minimize *Salmonella* exposure in the environment would likely lead to decreased infection rates and therefore subsequent long-term complications such as the development of cancer. The observation of human dataset in patients with colon cancer and *Salmonella* infection history were done in the Netherlands. *Salmonella* isolates were also identified in human colon cancer from The Netherlands. Validation of the current findings in cohorts from other countries could be challenging but necessary. Further studies on the various *Salmonella* strains isolated from human tumors are needed. We do not have the information on the timeline of *Salmonella* infection in the human organs. The time it takes for a pre-transformed cell to develop into a detectable cancer is undetermined but is generally thought to take years.

5. Gut microbiome after *Salmonella* infection

It is important to remember, however, the changes of the host signaling pathways by *Salmonella* infection tell only half of the story. Given the critical role of gut microbiota (Munoz et al., 2016) in human health, we should also aim to better understand the dynamic microbiome community within the colon (Sun, 2022; Sun & Xia, 2011). It is important to remember that the microbiome is not of a static composition and the abundancy profiles of certain taxa may not always be correlated to the significance of their role within the microbiome. In fact, the human colon microbiome consists of a dynamic population of microbes that respond

rapidly to the environment and secrete a plethora of chemical compounds, some of which are used as signaling molecules both among microbes, and between microbes and host (see Oliveira et al. (Oliveira et al., 2023), Su et al. (Su and Ding, 2023), and Markus et al. (Markus et al., 2023) for recent reviews). The recent progress in the field of metabolomics has emphasized the functional output of the microbial ecosystem and their metabolites. Metabolites from the bacteria contribute significantly to their protective effects in the colon. For example, short chain fatty acids produced by the microbiota are influenced by ecosystem structure, available nutrients, and infection (Munoz et al., 2016). Furthermore, a previous study demonstrated the importance of restoring the microbiome in *Salmonella*-infected colons to improve barrier function and suppress inflammation (Munoz et al., 2016). More studies are needed to better understand how *Salmonella* infection alters the human microbiome in the colon to affect the metabolome as well as inflammation, thus increasing the risk of tumorigenesis or other chronic diseases.

6. Molecular mechanisms for bacterial infection-associated cancers

The association between chronic *Salmonella* Typhi infections and gallbladder cancer was first noticed in India (Nath et al., 2008; Scanu et al., 2015). It has been recognized that severe, NTS infections, such as those that require hospitalization, are also associated with an increased cancer risk (Mughini-Gras et al., 2018). In western countries, such as the U.S.A. and Canada, there is a rising incidence of colon cancer in the younger population (Mauri et al., 2019). Understanding the depth of molecular mechanisms into the contribution of bacterial infections to colon cancer development has the potential to unravel novel, complex pathogenesis, as well as to provide insights into early detection and identification of targets for precision of therapy. Understanding the prognostic value of *Salmonella* infection will also provide a basis for the development of guidelines and criteria advocating certain strategies for screenings and follow-ups in people with exposure history to *Salmonella*.

Over the last few decades, significant progress has been made in understanding the host-bacterial interactions and the development of cancer (For a review, see Zha et al. (Zha et al., 2019)). This field of research was initially propelled by the observed association of *Helicobacter pylori* infection and the propensity to develop gastric carcinoma in multiple large patient cohorts (Nomura et al., 1991; Parsonnet et al., 1991). These observations were corroborated in a mouse model by Lee et al. which demonstrated that mice predisposed to gastric cancer formation (INS-GAS mice) and subjected to *H. pylori* infection had enhanced cancer development when compared to non-infected mice (Lee et al., 2008). Further human studies into this correlation have strongly linked *H. pylori* infection as a risk factor for the development of gastric cancer in humans and since 1994 *H. pylori* has been listed as a Group 1 carcinogen by the World Health Organization (International Agency For Research On Cancer, 1994; Uemura et al., 2001). Fortunately, recent studies have demonstrated an eventual protective effect in patients with *H. pyloric* infection who receive eradication therapy versus those who do not (Yan et al., 2022), further emphasizing the importance of identifying and treating gastrointestinal infections such as *H. pylori* (see review by Argueta and Moss (Argueta & Moss, 2021)).

With respect to colon cancer, multiple bacterial organisms have been implicated in the development of malignancy aside from *Salmonella,* including *Bacteroides fragilis*, *Escherichia coli* and *Clostridioides difficile* among others (see reviews (Dougherty & Jobin, 2023; Song et al., 2020)). For example, tumor slurries derived from patients with colon cancer were found to host toxigenic *C. difficile* strains. Furthermore, these strains were capable of driving tumorigenesis when transferred to a mouse model susceptible to tumor formation (Drewes et al., 2022).

7. Future perspectives

There are still many gaps in knowledge that prevent the application of these new findings linking bacterial infection and cancer to clinical practice. For example, in animal models, chronic infection with *Salmonella* alone in wild-type mice does not lead to tumor development (van Elsland et al., 2022). However, mice with a genetic APC-deficiency demonstrate enhanced tumor growth, and when exposed to further hits through chemical-induced mutations or inflammation demonstrated increased tumorigenesis (van Elsland et al., 2022). While these findings support the stepwise tumorigenesis model, it does not yet allow clinicians to predict who is at increased risk for colon cancer solely based on history of *Salmonella* infection alone.

Further, the Elsland et al. study included patients from the Netherlands only (van Elsland et al., 2022). However, while likely challenging, validation of the findings in cohorts from other countries should be pursued. Additionally, studies on the various *Salmonella* strains isolated from the human tumors should be done to further elucidate the mechanisms of tumorigenesis. Furthermore, we do not yet understand the timeline of *Salmonella* infection. For example, the timeline for the development of a pre-transformed cell to evolve into cancer is unknown.

Notably, in the study by Elsland et al. (van Elsland et al., 2022), no statistical difference in the rates of colon cancer development were observed between those with a history of *Salmonella* infection and those without. However, when stratified by age, there was a significant increase in the likelihood of developing colon cancer in those under the age of 60 with a prior history of *Salmonella* infection, as estimated by the *Salmonella* antibody serum profile (seroincidence) (van Elsland et al., 2022). This finding is suggestive of a contributory effect of *Salmonella* infection towards the development of young-onset colorectal cancer, a concerning trend seen worldwide (Siegel et al., 2019).

Recent meta-analyses and case-control studies have indicated that antibiotic exposure is associated with an increased risk of colon cancer (Aneke-Nash et al., 2021; McDowell et al., 2022). Interestingly, in the meta-analysis by Weng et al. (Weng et al., 2022) the authors not only observed an increased risk of developing colorectal cancer in patients with higher levels of antibiotic exposure, but that certain antibiotics such as cephalosporins and anti-anaerobic antibiotics were associated with a higher risk when compared to macrolides, tetracyclines, sulfonamides, and quinolones (Weng et al., 2022). Ceftriaxone, a third-generation cephalosporin and metronidazole, an anti-anaerobic antibiotic, are two of the most common antibiotics administered in a hospital setting to empirically treat gastrointestinal infections. It is not entirely clear whether increased exposure to antibiotics directly promotes tumorigenesis or if the indirect effect on microbiota composition, leading to dysbiosis, is the dominant factor to promote the development of colon cancer. In the experimental mouse model used in the studies by Elsland et al. mice were first treated with streptomycin to facilitate a productive *Salmonella* gastrointestinal tract infection (van Elsland et al., 2022). This may partially mimic what occurs in humans after antibiotic exposure. More studies are needed to elucidate further the temporal relationship between *Salmonella* infection and antibiotic exposure as they relate to the risk of developing colon cancer. However, regardless of whether antibiotics directly or indirectly affect the risk of colon cancer development, these studies further emphasize the responsible and judicious use of antibiotics.

8. Conclusion

Clearly, NTS have evolved a wide variety of mechanisms to efficiently and successfully infect humans, making it a prevalent foodborne pathogen worldwide. Unfortunately, it seems, a long-term consequence of NTS infection may lead to an increased risk of developing colon cancer. Multiple factors, including the host's genetic background (*ie* APC

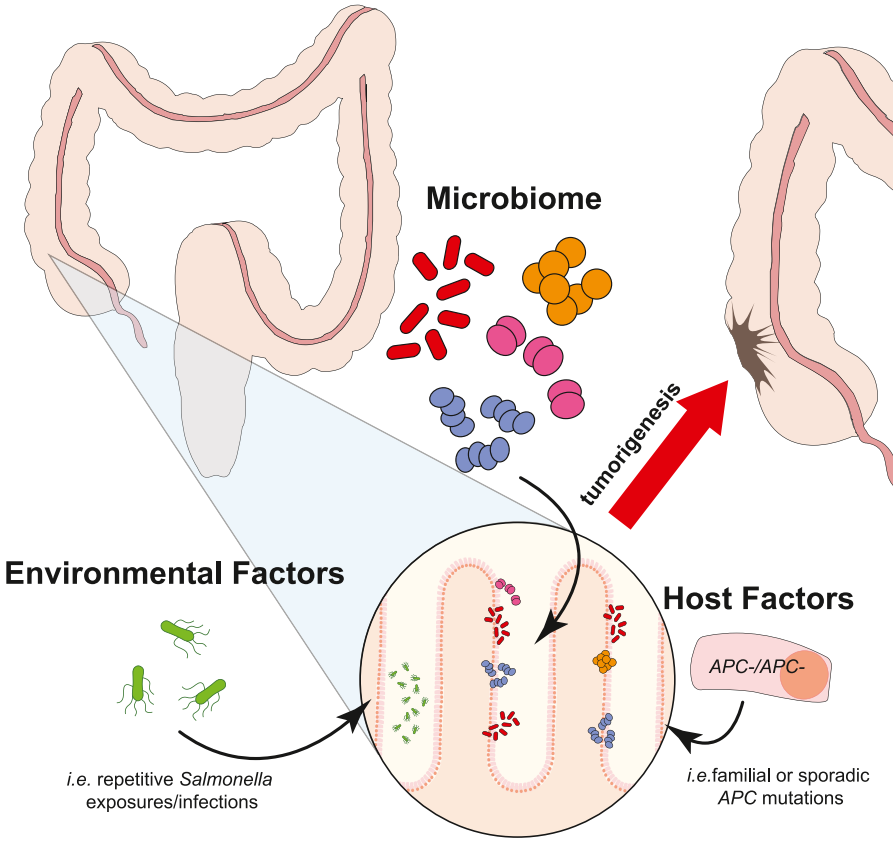

Fig. 1. Colon tumorigenesis is influenced by the gut microbiome, environmental factors, and host factors.

germline or sporadic mutations, etc.), history of past antibiotic exposure and the subsequent dysbiosis and redistribution of gut microbiota, host behaviors (*i.e.* personal hygienic practices) and environmental triggers (*ie* NTS exposure) (Fig. 1) should be considered and investigated for not only the development of clinical tools to predict malignancy risk but also to develop strategies to prevent and alleviate the effect of these factors on oncogenic potential. To move forward, the research on the mechanisms of pathogens in tumorigenesis will provide novel insights into the disease progression and help us better prevent the infection-related colon cancer and cancers in other organs.

Declaration of interests

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