The deadly bite of *Salmonella* Typhi

Kaiyi Zhang¹, Mathias Hornef¹ & Marcus Fulde²

Pathogenic microorganisms overcome the host's innate and adaptive immune system and cause local or systemic infections, potentially leading to organ failure, sepsis, or even death. Some microorganisms can also directly or indirectly alter the differentiation and proliferation of host cells, promoting the development of tumors. A large number of oncogenic viruses have been identified and estimated to account for ~15% of human cancers. They do so by encoding oncogenes or through their intrinsic ability to manipulate the genomic stability of the host cell by integrating their own genetic elements. Also bacterial infections have been linked to carcinogenesis, although the underlying molecular mechanisms are less well understood. The best-studied example is *Helicobacter pylori*, which has been classified as a class I carcinogen by the World Health Organization due to its ability to promote stomach cancer after chronic infection, which causes tissue inflammation and atrophy of the gastric mucosa. In a recent issue of *Cell Host & Microbe*, the Neefjes laboratory explores the association between *Salmonella enterica* subsp. *enterica* sv. Typhi (*S. Typhi*)—which is the causative agent of human typhoid fever—and gallbladder carcinoma [1].

See also: T Scanu *et al* (June 2015)

Chronic carriage of *S. Typhi* following acute infection has been previously linked to cancer [2]. Although gallbladder carcinoma (GBC) is rare in western countries, there is a high incidence in countries with endemic *S. Typhi* infections such as South America and parts of Africa and Asia, particularly India and Pakistan [3,4]. *Salmonella*'s ability to persist within the gallbladder of a significant fraction of patients (2–5%) after systemic infection appears to provide the milieu for

its tumor-promoting effect. Initial experimental evidence in mice supports an association between *Salmonella* infection and GBC, as long-term histopathological follow-up of chronically infected mice revealed pre-malignant lesions with hyperplasia and metaplasia of the gallbladder [5]. Now, Scanu and colleagues provide insights into the molecular mechanisms underlying this association [1].

They start with the intriguing observation that GBC in the Netherlands, which has a very low incidence of *S. Typhi* infection, differs phenotypically from GBC in India, where the incidence of typhoid fever is very high. Furthermore, the authors detect increased expression of the proto-oncogene *c-Myc* in Indian tumor tissue and set out to test the effect of *Salmonella* infection in tumor-prone mice that express enhanced *c-Myc* levels, the so-called adenomatous polyposis coli (*Apc*)^{+/min} mouse model. Infection of *Apc*^{+/min} SV129Ola mice—which additionally express the lysosomal metal transporter *Nramp* and survive acute *Salmonella* infection—with the murine *S. Typhi* homolog *S. Typhimurium* induced signs of colon cancer-like anal bleeding. Subsequent histopathological analysis confirmed the presence of colorectal adenocarcinomas in addition to the small intestinal adenomas usually found in *Apc*^{+/min} mice. Of note, colorectal carcinoma was found following infection with wild-type *S. Typhimurium* but not after infection with an attenuated strain that is unable to translocate effector molecules through the plasma membrane into infected host cells. A similar tumor-promoting effect was confirmed using gallbladder epithelium-derived stem cell organoids or *c-Myc*-overexpressing mouse embryonic fibroblasts (MEFs). Neither model includes immune cells, indicating that the carcinogenic effect results from the direct interaction between *Salmonella* and epithelial or fibroblast cells. MEFs were subsequently used to analyze the

bacterial effector molecules and cellular signaling pathways involved. As elevated Akt and mitogen-activating protein kinase (MAPK) activities are frequently observed in human cancer tissue, the translocated bacterial effector molecules SopE, SopE2, SopB, and SptP, which are known to stimulate Akt and MAPK activities, were analyzed. Indeed, bacteria deficient in all four effector molecules or deficient only in SopB, or administration of Akt or MAPK inhibitors, prevented MEF transformation. Strikingly, even short-term infection (terminated by antibiotic treatment) readily promoted long-term transformation of MEFs and induced a defined alteration in the global gene expression pattern. Thus, *Salmonella*—through its effector molecules SopE, SopE2, SopB, and SptP—drives activation of Akt and MAPK in gallbladder epithelial cells, rendering existing pre-transformed (i.e. *c-Myc* high) cells into malignant tumor cells (Fig 1).

The established models now provide unique tools to further unravel the molecular mechanisms of bacteria-induced carcinogenesis. Similar to the clinical management of *H. pylori*, the results call for the development of strategies to prevent *S. Typhi* persistence in the attempt to not only stop the spread of this pathogen, but also prevent the development of GBC, a disease with poor clinical outcome at present. However, a number of questions remain to be addressed. For example, the authors assume that the *Salmonella*-mediated carcinogenesis is secondary to enhanced epithelial *c-Myc* expression. However, *Salmonella* has been shown to induce *c-Myc* expression [6,7] and thus might provide both signals. This might also explain why epidemiological studies associate only chronic infection with the emergence of gallbladder cancer, despite the fact that short-term exposure was sufficient to transform *c-Myc*^{high} epithelial cells [2]. A related question is how the deficiency of the examined *Salmonella* effector molecules

¹ Institute of Medical Microbiology, RWTH University Hospital Aachen, Aachen, Germany

² Institute for Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany. E-mail: marcus.fulde@tiho-hannover.de

DOI 10.15252/embr.201540748 | Published online 22 June 2015

Figure 1. The carcinogenic effect of *Salmonella*.

Salmonella pathogenicity island (SPI) 1 effector proteins and/or *Salmonella* invasion activate MAPK and Akt signaling. This, in turn, drives the expression of genes such as histone deacetylase (HDAC) 9, which are able to induce epigenetic modifications. In the pre-disposed host, such as one expressing enhanced levels of c-Myc, these changes may lead to tumor formation.

alters the overall bacteria–host cell interaction. The results presented by Scanu and colleagues suggest a major influence on bacterial invasion, intracellular survival, and proliferation, and thus, indirect downstream effects might contribute to the observed phenotype. For example, *Salmonella* translocates a second set of effector proteins

encoded by the so-called *Salmonella* pathogenicity island (SPI) 2 once it reaches the intracellular vacuole. Individual SPI2 effectors, such as SpiC, have also been shown to stimulate MAPK and might thus contribute to GBC development [8]. Finally, the precise molecular mechanisms that promote carcinogenesis downstream of Akt

and MAPK activation remain unclear. Constitutive MAPK signaling has been linked to epigenetic changes involving histone phosphorylation, which might explain the lasting carcinogenic effects of short-term bacterial exposure [9]. Consistently, the epigenetic modulator histone deacetylase 9 (HDAC9) was found to be up-regulated upon bacterial contact. Marked epigenetic differences have been noted between gallbladder tissues from patients from the US and Chile, countries of low and high incidence of *S. Typhi* infection, respectively [10].

The authors discuss the presence of similar epidemiological associations between cancer and other pathogenic bacteria, such as *Chlamydia* spp. and *Mycobacterium tuberculosis*, calling for detailed studies of the possible bacterial contribution to carcinogenesis. In addition, commensal bacteria might exert similar responses and should be examined for their potential to support the development of tumors, particularly within the gastrointestinal tract. Ongoing studies on the composition of the enteric microbiota in patients with malignancies might give a first hint on potential additional bacterial actors in this deadly play.

References

1. Scanu T, Spaapen RM, Bakker JM et al (2015) *Cell Host Microbe* 17: 763–774
2. Caygill CP, Hill MJ, Braddick M et al (1994) *Lancet* 343: 83–84
3. Nath G, Singh H, Shukla VK (1997) *Eur J Cancer Prev* 6: 557–559
4. el-Zayadi A, Ghoneim M, Kabil SM et al (1991) *Hepatogastroenterology* 38: 337–340
5. Gonzalez-Escobedo G, La Perle KM, Gunn JS (2013) *PLoS ONE* 8: e84058
6. Ye Z, Petrof EO, Boone D et al (2007) *Am J Pathol* 171: 882–892
7. Seong J, Piao HH, Ryu PY et al (2009) *J Microbiol* 47: 214–219
8. Uchiya K, Nikai T (2008) *Microbiology* 154: 3491–3502
9. Hamon MA, Cossart P (2008) *Cell Host Microbe* 4: 100–109
10. House MG, Wistuba II, Argani P et al (2003) *Ann Surg Oncol* 10: 882–889