Old Herborn University Seminar Monograph 36: The microbiome and cancer.
A summary of the lectures given during the 36th Old Herborn University Seminar
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MICROBIOME AND THE HALLMARKS OF CANCER: COLIBACTIN-PRODUCING PKS-POSITIVE *ESCHERICHIA COLI*

Introduction

Dr. Janelle Arthur (Department of Microbiology and Immunology, Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, USA) presented data on the microbiome and the hallmarks of cancer, with special focus on colibactin-producing PKS-positive *Escherichia coli*. She started her presentation with the general statement that it is unlikely that resident microbes alone can cause colorectal cancer. Genetic susceptibility, mutagens, diet, and other environmental factors are also involved in this process. It is known that this disease is associated with dysbiosis, a disruption of the microbiome where the balance between 'good' and 'bad' microorganisms is disturbed, having an impact on health

Hallmarks of cancer

Multiple microbial mechanisms influence cancer, and it is not just "who is there?", but "what are they doing?". It is important to understand what those bacteria are actually doing. A few "bad bugs" have been identified as associated with and driving various aspects of colorectal cancer. These include enterotoxigenic *Bacteroides fragilis*, *Fusobacterium nucleatum*, and colibactin producing, also called PKS-positive, *E. coli*. PKS-positive *E. coli* bacteria carry the polyketide synthase (PKS) gene cluster. This gene cluster enables the production of colibactin, a small molecule genotoxin that can cause DNA damage to intestinal cells and plays a role in the development of colorectal cancer.

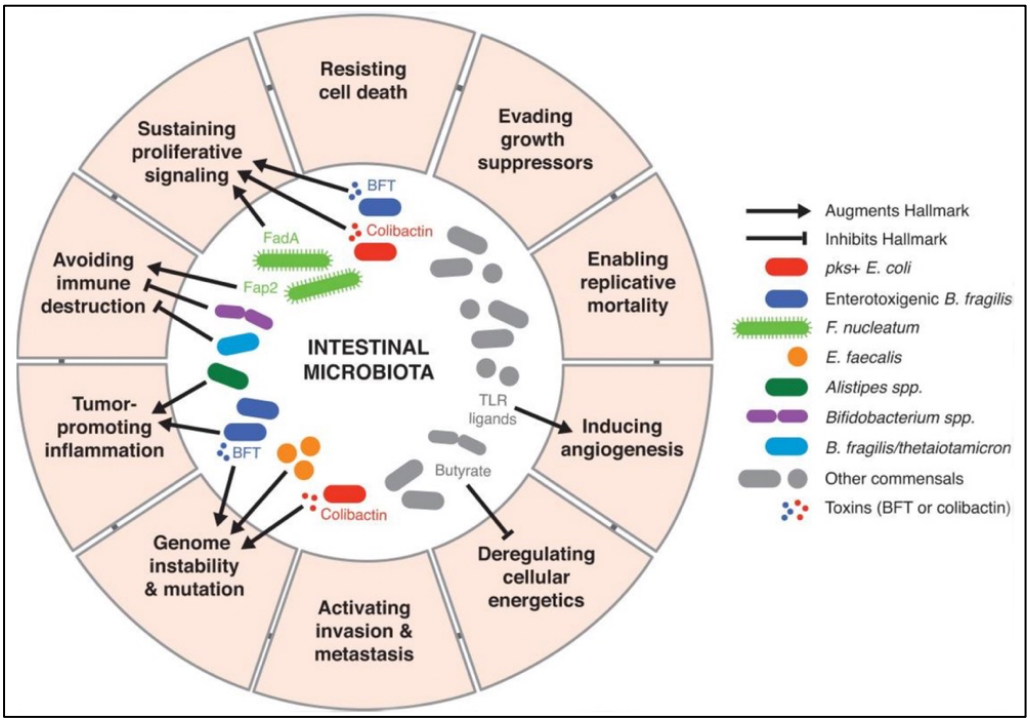

Figure 1: Microbial-derived signals modulate numerous hallmarks of cancer through diverse mechanisms. (Figure from *Fulbright* et al., 2017).

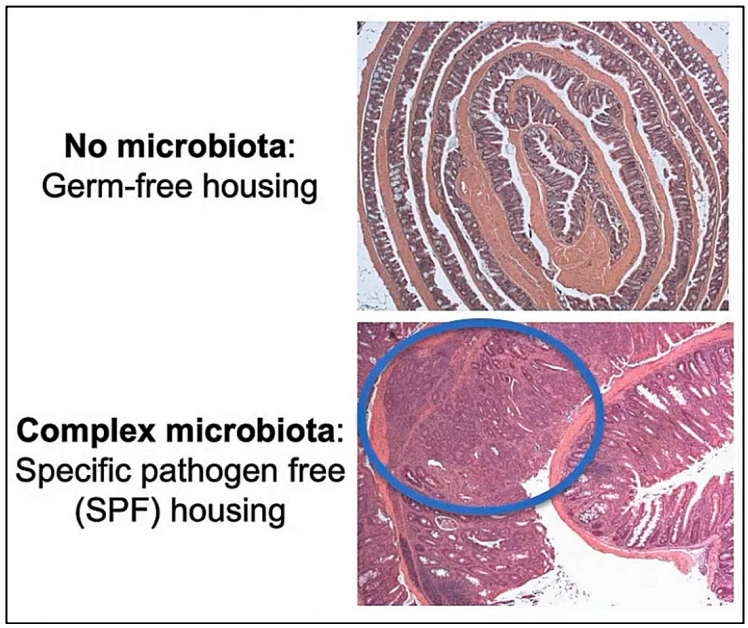

Figure 2: Histology of the colon (swiss roll technique) of IL-10$^{-/-}$ germfree (upper photo) and SPF (lower photo) mice.

Dr. Arthur and colleagues published in 2017 a drawing of how the microbiota influenced the hallmarks of cancer (Figure 1; *Fulbright* et al., 2017). The microbiota influences many of these hallmarks. In figure 1 there is at the 11 o'clock position a sustaining proliferative signalling influenced by the FadA adhesin from *Fusobacterium nucleatum* while the *B. fragilis* toxin and colibactin from PKS-positive *E. coli* influences proliferative signalling. Of the TLR ligands, inducing angiogenesis, and butyrate deregulating cellular energetics (at the 4 and 5 o'clock position) it can reasonably be assumed that they may influence cancer.

Microbiome, IL-10 and inflammation-associated colorectal cancer

Dr. Arthur presented a figure from a study performed in 2009, showing that resident microbes are required for inflammation-associated cancer in interleukin 10 (IL-10)-deficient (*Il10*$^{-/-}$) mice. (*Uronis* et al., 2009). IL-10 is an immunoregulatory cytokine that is required to dampen inflammation. When IL-10-deficient mice are raised under germ-free conditions they do not develop inflammation, but when they are colonized with a complex microbiota they develop robust inflammation in the gut, which is shown in figure 2. This figure shows “swiss rolls” of the colon. By this swiss roll technique (*Moolenbeek* and *Ruitenberg*, 1981), the entire colon of the mice is removed, flushed, and opened longitudinally and rolled with the mucosa inwards. After histological processing, microscopical examination of the entire length of the colon is then possible from the distal to the proximal colon. By cutting through there, a snapshot is obtained. But it is possible to look at the entirety of colon under microscopy to assess all inflammation and tumorigenesis. In figure 2, no inflammation can be seen in the upper histology image from germ-free mice, but robust inflammation (intestinal hyperplasia and thickening of the colon) and inflammatory infiltrates can be seen in the bottom histology image.

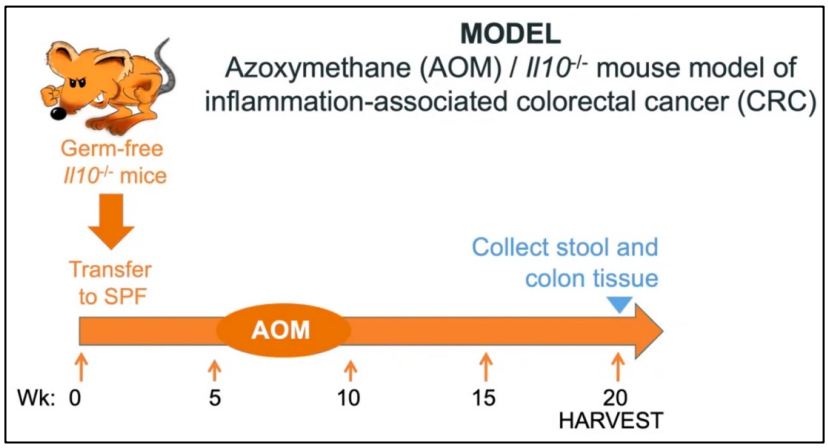

Figure 3: Mouse model of inflammation-associated colorectal cancer induction by azoxymethane in SPF IL-10 deficient mice.

After transfer of germfree *Il10*$^{-/-}$ mice to a specific pathogen free (SPF) environment or colonising them with the SPF microbiome by oral gavage of faecal material, they were injected i.p. with the colon specific carcinogen Azoxymethane (AOM) (Figure 3). AOM induces invasive colorectal tumours in the setting of *Il10*$^{-/-}$ inflammation. These tumours are flat, which is very similar to the colorectal cancers that inflammatory bowel disease (IBD) patients develop. IBD patients are at a very high risk for developing colorectal cancer and these colorectal cancers are difficult to spot by endoscope because they are flat. This mouse model is a very good model for inflammation-associated colorectal cancer and is very reproducible (*Arthur* et al., 2012).

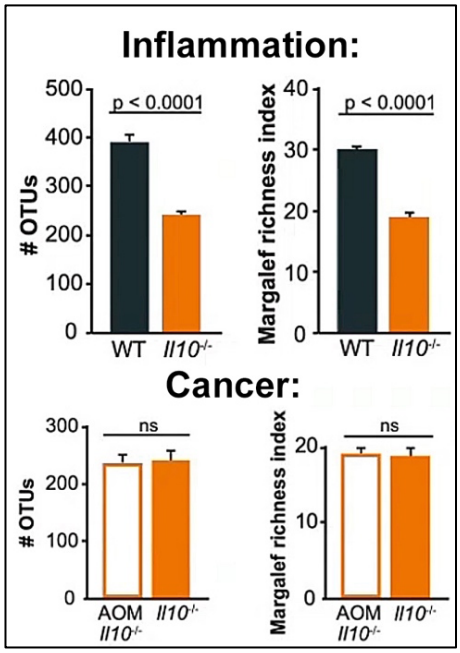

Figure 4: Alpha diversity in wild-type and *Il10*$^{-/-}$ mice. (OTU = operational taxonomic unit).

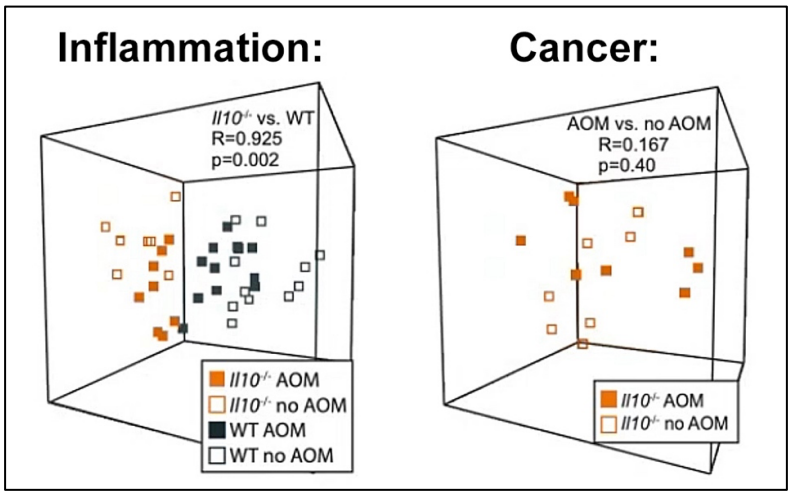

Figure 5: Beta diversity in the different groups of mice

After 18 to 20 weeks, stool samples and colon tissue were collected and 16S rRNA sequencing of the microbiome by Illumina HiSeq 2000 sequencing was used to determine what bacterial groups were present and which changed as inflammation and cancer developed. A major finding was that that chronic inflammation alters microbial community composition in the colon. Figure 4 shows that alpha diversity, a simple metric of how many bacterial groups are present, is reduced in the inflamed *Il10*$^{-/-}$ mice. Whether the *Il10*$^{-/-}$ mice received AOM (having robust inflammation and cancer) or had not (having robust inflammation and no cancer), the number of bacterial strains or species estimated was significantly reduced, which is similar to what is seen in human IBD patients.

Figure 5 shows the beta diversity, which measures dissimilarity in the composition of two communities. In this 3-dimensional plot the symbols represent the microbiota of an individual mouse. The distance between the symbols show the difference between these communities. One can clearly see that the symbols separate by mouse genotypes, the inflamed *Il10*$^{-/-}$ mice vs. WT uninflamed mice. From the figure it can be concluded that cancer is not driving the changes in the microbiome, but that inflammation is causing the robust changes.

From the experiments using this model it can be concluded that the microbiome is an important factor in the development of inflammation-associated colorectal cancer. The question is whether inflammation alters the microbiota and in this way select microbes that are associated with cancer. If this is true, even mild inflammation of the gut can alter the intestinal microbiome. The microbiome will become dysbiotic, more pro-inflammatory and thus more pro-carcinogenic which will act back and so induce more inflammation.

When looking at taxonomy in the colon microbiome, a hundredfold expansion of *E. coli* was observed in the *Il10*$^{-/-}$ mice. To find out whether *E. coli* could alter or even induce tumorigenesis, germfree wild-type and *Il10*$^{-/-}$ mice were mono-associated with either the Gram-negative *E. coli* NC101 or with the Gram-positive *Enterococcus faecalis* and injected with AOM to induce tumorigenesis in the *Il10*$^{-/-}$ mice. Both bacteria induce inflammation, but with different kinetics and different localization (*Kim et al, 2005).* As expected, both

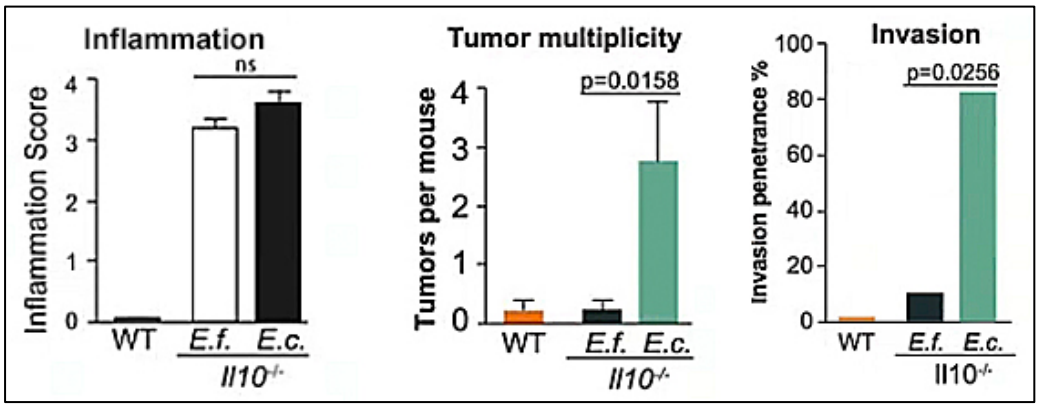

Figure 6: Inflammation, induction of tumours and invasion of tumours due to *Enterococcus faecalis* and *E. coli* NC101 in Il-10$^{-/-}$ and wild-type mic

bacteria induced robust inflammation in the *Il10*$^{-/-}$ mice, while the wild-type mice did not develop inflammation. Due to the absence of inflammation in the wild-type mice, these mono-associated wild-type mice do not develop tumours. Surprisingly though, the *Il10*$^{-/-}$ mice that were mono-associated with *Enterococcus faecalis* and developed robust inflammation did not develop tumours. In contrast, the *E. coli* mono-associated *Il10*$^{-/-}$ mice developed tumours, many of which were invasive (Figure 6). These data showed that inflammation and tumorigenesis are not always directly correlated and additional bacterial factors may influence the development of cancer.

As Figure 6 showed an increase of invasive tumours in the *E. coli* mono-associated *Il10*$^{-/-}$ mice, a potential bacterial driver of tumorigenesis was searched for. Searching the literature revealed a potential explanation in the "PKS pathogenicity island", a cluster of genes found in some *E. coli* strains that encodes enzymes for the biosynthesis of the small molecule genotoxin colibactin **(***Nougayrède* et al., 2006).

Colibactin is a polyketide-peptide that can cause double-strand breaks in DNA, and its presence is associated with colorectal cancer (CRC). This PKS pathogenicity island was present in the used *E. coli* NC101 strain, but not in a reference *E. coli* strain (*E. coli* K12) and also not in the used *Enterococcus faecalis* strain. Deletion of the PKS from the *E. coli* NC101 abrogated its ability to induce DNA damage in intestinal epithelial cells *in vitro.* Specially, flow cytometry analysis revealed that PKS+ *E. coli* induced cell cycle arrest, which indicates that the cells have stopped to repair their DNA damage, but PKS-deficient *E. coli* did not. An assay measuring γH2AX foci in the nucleas that form upon DNA damage repair also indicated that the PKS island is responsible for these phenotypes. When these strains are put back into the mice and ran through the same colitis-associated cancer model, the PKS positive *E. coli* enhanced tumorigenesis without impacting inflammation (Figure 7).

On the right-hand side of figure 7 it is shown that the inflammation score in mice mono-associated with either of these strains is the same. However, the tumour multiplicity (left on the figure) and importantly, the tumour invasion (centre of the figure) is significantly reduced when the PKS island is deleted from the strain. This suggests that the DNA damage from the product of this PKS island, the genotoxin colibactin, is driving the majority of the tumorigenesis in this model. Indeed, a higher

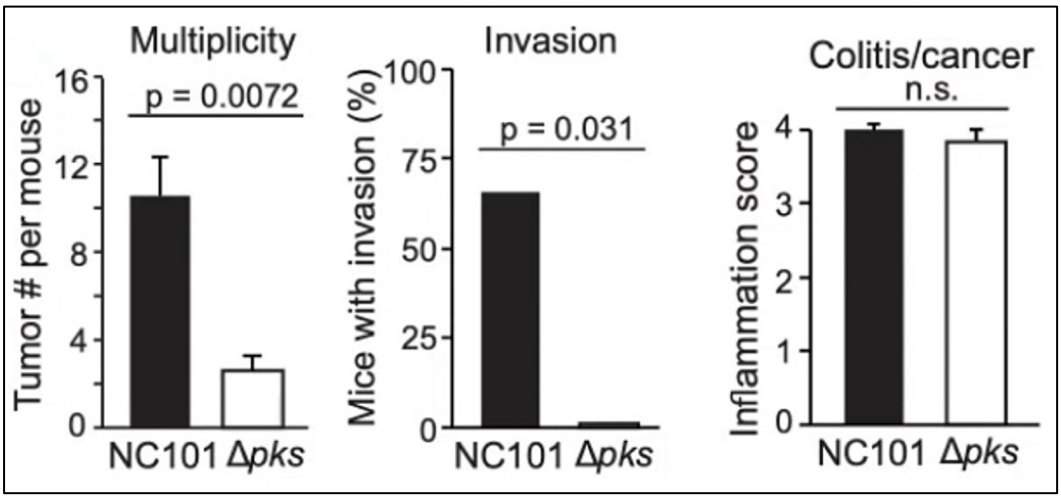

Figure 7: number of tumours, invasion and inflammation score in mice colonised with E. coli NC101 and the same strain with the PKS deletion.

proportion of patients suffering from colorectal cancer (CRC) or Inflammatory Bowel Disease (IBD) harboured PKS positive *E. coli*. From the CRC patients 67% harboured *E. coli* strains with the PKS island, 40% of the IBD patients did, and only 21% of healthy control persons harboured these *E. coli* strains (Table 1).

Colibactin and colorectal cancer

As mentioned earlier, colibactin is a genotoxic toxin that can cause DNA damage to intestinal cells and plays a role in the development of colorectal cancer. Colibactin is a small molecule, made as an inactive precursor (precolibactin) inside the *E. coli* cell. This is important because in bacteria, genetic material is not restricted to the nucleus as in eukaryotic cells; producing an active genotoxin in the bacterial cell cytoplasm would damage its own DNA. Instead, pre-colibactin is transported into the periplasm (the space in between the inner and outer membranes) and activated via cleavage of a pro-drug motif by the ClbP peptidase. Now in its active form, colibactin is released from the bacteria. It is not known how active colibactin is released from *E. coli*, how it enters the mammalian cell and reaches the nucleus, but cell-cell contact is required.

While the work of Dr. Arthur and that of others showed that *E. coli*-produced colibactin can promote colorectal cancer, chemists were focused on how enzymes of the PKS island synthesized colibactin, identified its active chemical form, and revealed details about its interactions with mammalian DNA and

Table 1: Human IBD and CRC patients harbour an abundance of PKS positive *E. coli*

Disease	Number of patients	% PKS positive	P value
CRC	21	66.87	< 0.001
IBD	35	40.0	<0.05
Control	24	20.8	

the specific types of DNA damage it inflicted (*Addington, Sandalli and Roe*, 2024). Most carcinogens have a known mutagenic signature – specific base pair substitutions or insertions/deletions – that results from exposure to the carcinogen. To link colibactin exposure to human cancer, researchers would have to determine colibactin's mutagenic signature. Two groups identified this mutagenic signature and found it in human colorectyal tumours and metatstases. The group led by Hans Clevers used human intestinal organoids (*Pleguezuelos-Manzano* et al., 2020). They exposed them repeatedly to *E. coli* with an intact PKS island or to an isogenic strain of which the PKS island was deleted. After this repeated exposure, they performed whole genome sequencing to identify carcinogenic signatures unique to the organoids exposed to PKS+ *E. coli*. They found a unique single base pair substitution and an insertion-deletion. These are called the SBS-PKS or the ID-PKS. They then looked in cancer genome databases and could find them in human colorectal tumours and metastases, often with SBS-PKS and ID-PKS evident in the same patient. This suggests that colibactin is inducing a carcinogenic signature that is found in human colorectal tumours and metastases. Its presence here demonstrates exposure to colibactin, and suggests colibactin may be contributing to colorectal cancer. In another study direct evidence was shown that colibactin contributes to mutational processes in humans, supporting its causal role in colorectal cancer (*Dziubańska-Kusibab* et al., 2020). Further work has revealed that 12% of colorectal cancers displayed a colibactin-induced mutational signature. Mutations were present in the adenomatous polyposis coli (APC) gene, a tumour suppressor gene that is one of the key mutations in colorectal cancer (*Rosendahl Huber* et al., 2024). Even more recently, evidence of colibactin exposure (presence of SBS-PKS and/or ID-PKS) was found in 21.1% of colorectal cancers across a global population (*Diaz-Gay*, 2025). Colibactin mutagenesis may be the cause of cancer or can contribute to causing cancer by stressing the DNA damage repair machinery at some point, most probably at the initiation stage. In sporadic cancer models it is shown that colibactin-producing *E. coli* can promote cancer. Thus, inflammation is not necessary, but inflammation (such as in IBD patients) alters the microbiota, including the bloom of potentially pro-carcinogenic microbes like PKS positive *E. coli*. Several questions remain that are currently investigated by the research team of Dr. Arthur. Since cell-cell contact is necessary for colibactin to induce its genotoxic effects, we must understand what features of intestinal *E. coli* allow it to stably colonize patients. This would permit it to reside in the mucosal microbiome and potentially adhere to epithelial cells and deliver colibactin to cause DNA damage. In addition, the molecular control of colibactin production is yet not well understood. We must understand what conditions in the gut induce colibactin production, especially those conserved across a broad range of PKS positive *E. coli* strains. Finally, future epidemiological studies will be needed for the research field to demonstrate if there is sufficient evidence to name colibactin as a carcinogen driving human colorectal cancer.

This paper was reviewed by Dr. Janelle Arthur before publishing.

LITERATURE

Addington, E., Sandalli, S., and Roe, A.J.: Current understandings of colibactin regulation. Microbiology (Reading) 170: 001427 (2024).

Arthur, J.C., Perez-Chanona, E., Mühlbauer, M., Tomkovich, S., Uronis, J.M., Fan, T.J., Campbell, B.J., Abujamel, T., Dogan, B., Rogers, A.B., Rhodes, J.M., Stintzi, A., Simpson, K.W., Hansen, J.J., Keku, T.O., Fodor, A.A., and Jobin, C.: Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338: 120-123 (2012).

Díaz-Gay, M., Dos Santos, W., Moody, S., Kazachkova, M., Abbasi, A., Steele, C.D., Vangara, R., Senkin, S., Wang, J., Fitzgerald, S., Bergstrom, E.N., Khandekar, A., Otlu, B., Abedi-Ardekani, B., de Carvalho, A.C., Cattiaux, T., Penha, R.C.C., Gaborieau, V., Chopard, P., Carreira, C., Cheema, S., Latimer, C., Teague, J.W., Mukeriya, A., Zaridze, D., Cox, R., Albert, M., Phouthavongsy, L., Gallinger, S., Malekzadeh, R., Niavarani, A., Miladinov, M., Erić, K., Milosavljevic, S., Sangrajrang, S., Curado, M.P., Aguiar, S., Reis, R.M., Reis, M.T., Romagnolo, L.G., Guimarães, D.P., Holcatova, I., Kalvach, J., Vaccaro, C.A., Piñero, T.A., Świątkowska, B., Lissowska, J., Roszkowska-Purska, K., Huertas-Salgado, A., Shibata, T., Shiba, S., Sangkhathat, S., Chitapanarux, T., Roshandel, G., Ashton-Prolla, P., Damin, D.C., de Oliveira, F.H., Humphreys, L., Lawley, T.D., Perdomo, S., Stratton, M.R., Brennan, P., and Alexandrov, L.B.: Geographic and age variations in mutational processes in colorectal cancer. Nature 643: 230-240 (2025).

Dziubańska-Kusibab, P.J., Berger, H., Battistini, F., Bouwman, B.A.M., Iftekhar, A., Katainen, R., Cajuso, T., Crosetto, N., Orozco, M., Aaltonen, L.A., and Meyer, T.F.: Colibactin DNA-damage signature indicates mutational impact in colorectal cancer. Nat. Med. 26: 1063-1069 (2020).

Fulbright, L.E., Ellermann, M., and Arthur, J.C.: The microbiome and the hallmarks of cancer. PLoS Pathog. 13: e1006480 (2017).

Kim, S.C., Tonkonogy, S.L., Albright, C.A., Tsang, J., Balish, E.J., Braun, J., Huycke, M.M., and Sartor, R.B.: Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology 128: 891-906 (2005).

Moolenbeek, C. and Ruitenberg, E.J.: The "Swiss roll": a simple technique for histological studies of the rodent intestine. Lab. Anim. 15: 57-59 (1981).

Nougayrède, J.P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., Buchrieser, C., Hacker, J., Dobrindt, U., and Oswald, E.: Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science 313: 848-851 (2006).

Pleguezuelos-Manzano, C., Puschhof, J., Rosendahl Huber, A., van Hoeck, A., Wood, H.M., Nomburg, J., Gurjao, C., Manders, F., Dalmasso, G., Stege, P.B., Paganelli, F.L., Geurts, M.H., Beumer, J., Mizutani, T., Miao, Y., van der Linden, R., van der Elst, S.; Genomics England Research Consortium; Garcia, K.C., Top, J., Willems, R.J.L., Giannakis, M., Bonnet, R., Quirke, P., Meyerson, M., Cuppen, E., van Boxtel, R., and Clevers, H.: Mutational signature in colorectal cancer caused by genotoxic pks^{+} E. coli. Nature 580: 269-273 (2020).

Rosendahl Huber, A., Pleguezuelos-Manzano, C., Puschhof, J., Ubels, J., Boot, C., Saftien, A., Verheul, M., Trabut, L.T., Groenen, N., van Roosmalen, M., Ouyang, K.S., Wood, H., Quirke, P., Meijer, G., Cuppen, E., Clevers, H., and van Boxtel, R.: Improved detection of colibactin-induced mutations by genotoxic E. coli in organoids and colorectal cancer. Cancer Cell 42: 487-496 (2024).

Uronis, J.M., Mühlbauer, M., Herfarth, H.H., Rubinas, T.C., Jones, G.S., and Jobin, C.: Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One 4: e6026 (200