AUTONOME PROVINZ BOZEN – SÜDTIROL
PROVINCIA AUTONOMA DI BOLZANO – ALTO ADIGE
PROVINZIA AUTONOMA DE BULSAN – SUDTIROL

Südtiroler Sanitätsbetrieb
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Microbiota and Colon Cancer

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Viruses and Bacteria Can Cause Cancer in Humans…

Microorganism	Cancer
Epstein-Barr virus	Lymphomas, nasopharyngeal cancer, Kaposi sarcoma
Human herpesvirus 8	Kaposi sarcoma
H. pylori	Gastric adenocarcinoma, Malt lymphoma
Human papilloma virus	Oropharyngeal cancer, cervical cancer, anogenital cancer
Hepatitis B virus	Hepatocellular carcinoma
Hepatitis C virus	Hepatocellular carcinoma, lymphoma
Liver flukes	Cholangiocarcinoma
Merkel cell polyomavirus	Skin cancer
Schistosoma species	Bladder cancer
Salmonella typhi	Gall bladder cancer
Streptococcus gallolyticus	Colon cancer
Chlamydia pneumoniae	Lung cancer

Hold, GL. Dig Dis 2016;34:244–250.

Microbiota and Colon Cancer

- **colorectal cancer** (CRC), **one of the most common malignancies** in the western world, frequently causes death and is emerging worldwide
- it is expected that CRC **burden will** substantially **increase** in the next two decades consequent to adoption of a western lifestyle*
- It is well established that consumption of **foods and nutrients affect the risk** for developing CRC**
- dietary habits have substantially changed in the last decades especially in the western world and might affect colorectal carcinogenesis at various steps

colon cancer
sigmoid colon

*Arnold, M., Sierra, M.S., Laversanne, et al. Gut 2017; 66, 683–691.
**Song, M., Garrett, W.S., Chan, A.T. Gastroenterology 2015;148, 1244–1260.e16.

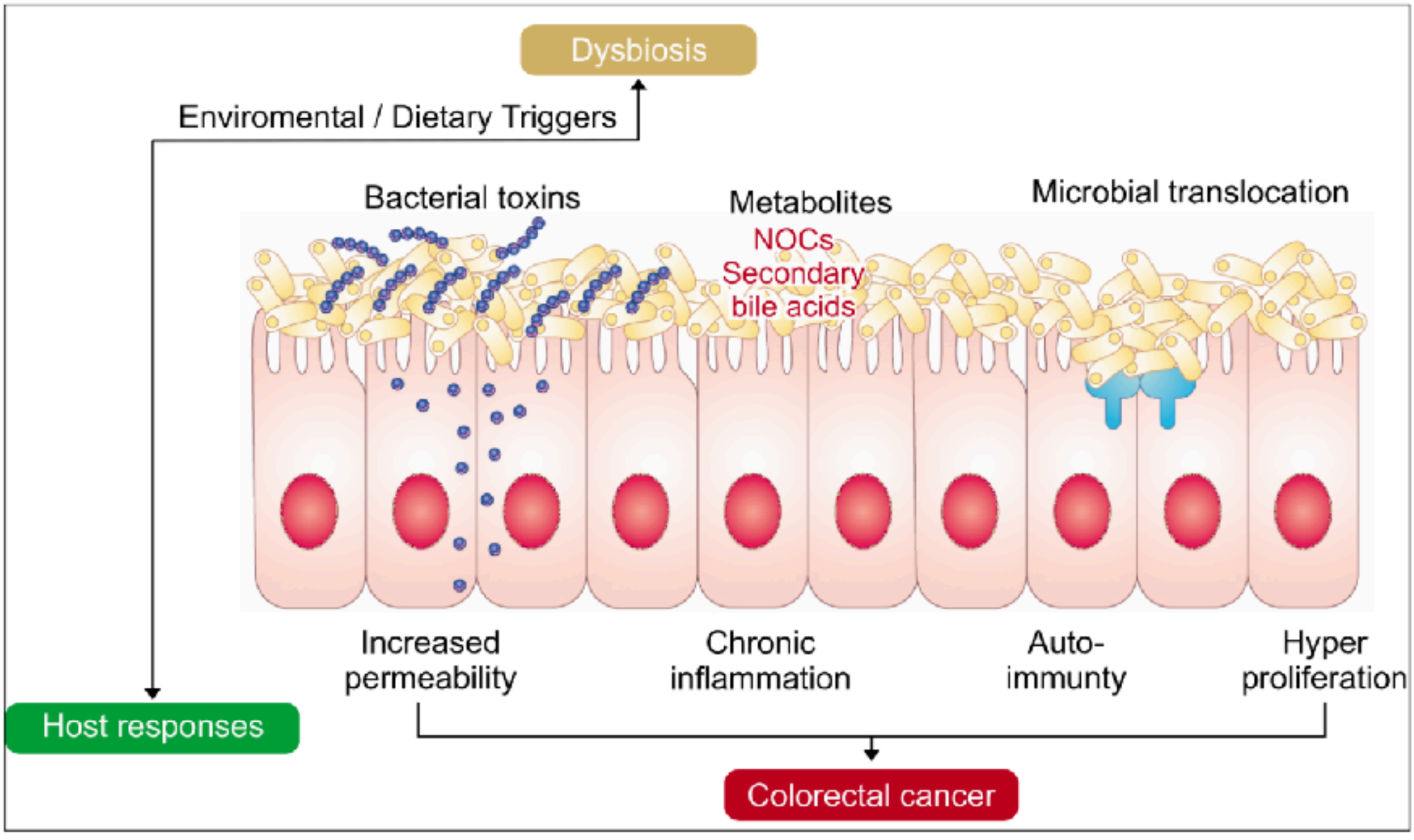
Microbiota and Colon Cancer

- **diet** may exhibit **effects on** the host's **immune response** and elicit inflammation
- in addition, dietary behavior tremendously **influences the composition of the intestinal microbiota**, which in turn impacts on the susceptibility to intestinal diseases*
- **exposure to antibiotics** early in life is associated with an increased risk for colorectal adenoma at the age of 60, which suggests that a dysbiotic microbiota is acquired and sustained over a longer period of time

colon cancer
indigo carmine

*Shanahan, F., van Sinderen, D., O'Toole, P.W., Stanton, C. Gut 2017. https://doi.org/10.1136/gutjnl-2017-313872
**Cao, Y., Wu, K., Mehta, R., et al. Gut 2017. https://doi.org/10.1136/gutjnl-2016- 313413.

General Mechanisms for Microbiota-related Colon Cancer



Yoon K, Kim N. J Cancer Prev 2018;23:117-125.

Colorectal Cancer and the Microbiota

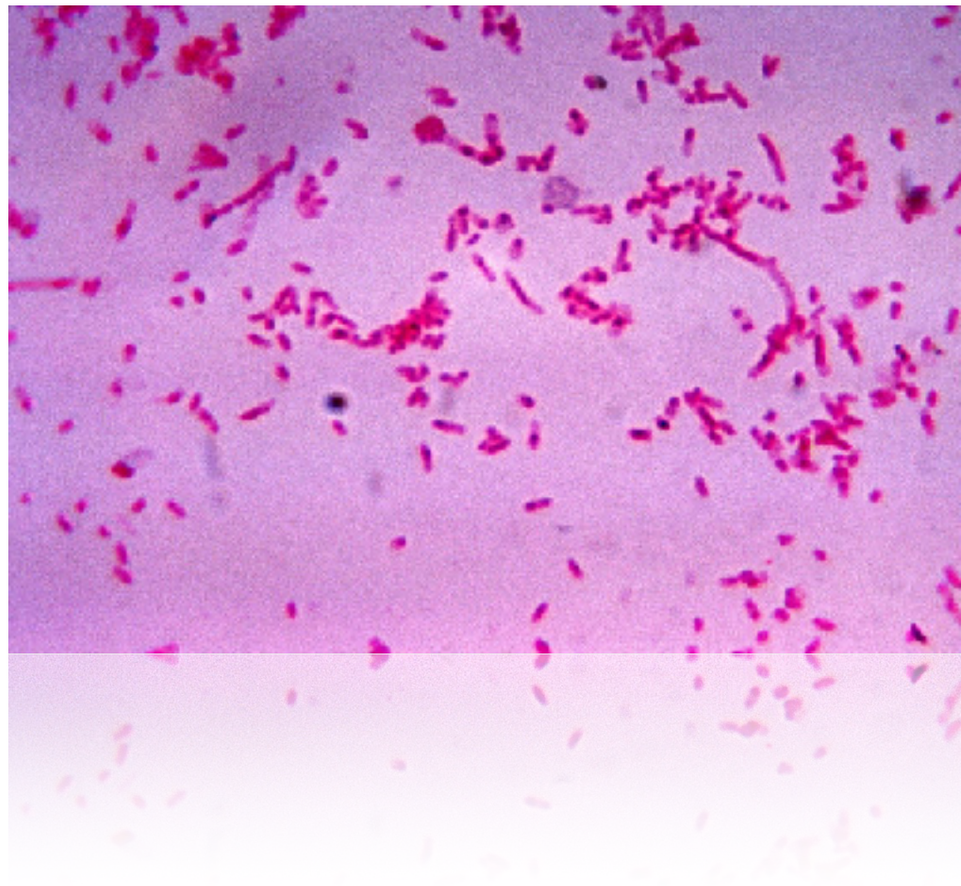
- CRC develops through a **series of genetic alterations**, which are thought to **drive** the **malignant progression** of normal mucosa to pre-malignant lesions, known as adenomatous polyps, with progression to malignancy occurring over a number of years – most likely decades
- this pathogenic progression is known as the **adenoma–carcinoma sequence**
- the **involvement of the gut microbiota** in CRC progression has been **clearly demonstrated** in numerous animal studies
- **germ-free animal studies** (animals free of microorganisms) have consistently demonstrated **lower tumor burdens** compared to conventionally raised counterparts
- similarly, **depletion of the gut microbiota** load through antibiotic usage has also been shown to **reduce CRC development***

*Grivennikov SI, Wang K, Mucida D, et al. Nature 2012;491:254–258.

Colorectal Cancer and the Microbiota

- there is particularly strong evidence for **specific organisms** that emerge as CRC susceptibility candidates
- **Fusobacterium nucleatum** is seen much more frequently in the gut microbiome of CRC patients (at the adenomatous polyp stage as well as adenocarcinoma) compared to healthy controls*
- other bacteria that have been implicated in CRC include **enterotoxigenic Bacteroides fragilis** (ETBF) and **Escherichia coli**, which have been shown to promote colon tumourigenesis **in colitis-associated cancer**, not sporadic CRC, **Streptococcus gallolyticus subsp. gallolyticus** and **Enterococcus faecalis**

Fusobacterium nucleatum



*Kostic AD, Chun E, Robertson, et al. Cell Host Microbe 2013; 14:207–215.

Colorectal Cancer and the Microbiota

- the colonic **mucosa** is constantly **exposed to the gut microbiota** and is considered to be in a state of **'continual or physiological inflammation'**
- the mucosa is single-cell thickness epithelium, which performs many functions including nutrient absorption/ secretion, physical barrier functions as well as immune regulation
- **crosstalk** between the epithelium and the gut microbiota **shapes the gut environment** with the microbiota having a profound influence on intestinal immune homeostasis

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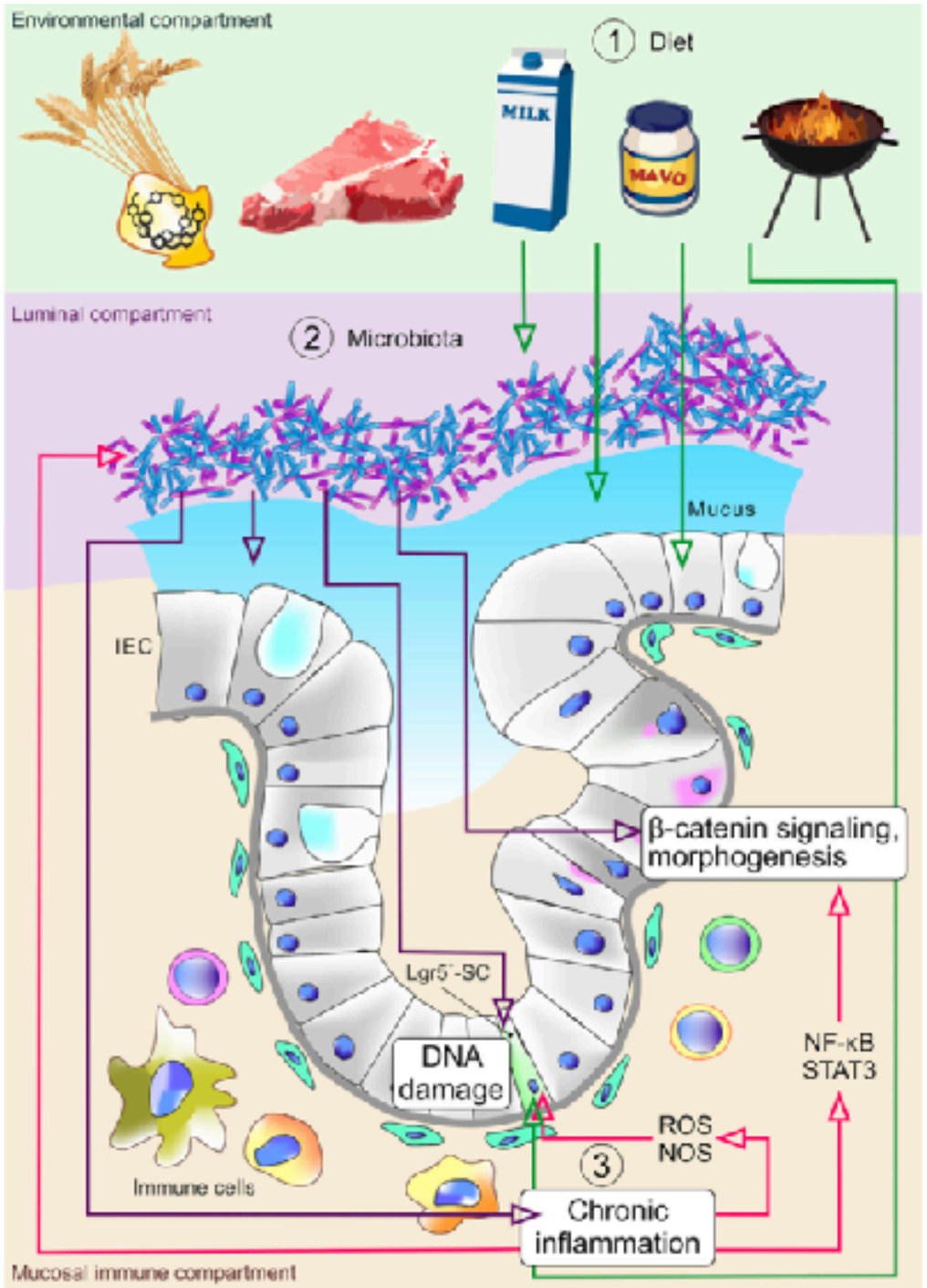


Tilg et al. Cancer Cell (2018), https://doi.org/ 10.1016/ j.ccell.2018.03.004

Colorectal Cancer and the Microbiota

- the epithelium has to contend with both microbial cells but also microbial metabolites that are produced predominantly by resident bacteria

- this **metabolomic exposure** can be **both pro and anti-carcinogenic** in nature with the net balance of protective and detrimental metabolites defining carcinogenic potential*

- microbial production of **short chain fatty acids** (SCFAs) including acetate, propionate and butyrate results from **fermentation of dietary fibre**, with diets rich in fibre generally considered to **reduce CRC risk**

*Louis P, Hold GL, Flint HJ. Nat Rev Microbiol 2014;12:661–672.

Short Chain Fatty Acids

- SCFAs are rapidly absorbed from the gut lumen but have a range of effects on different cell types including **anti-inflammatory effects** on myeloid cells* and colonic regulatory T cells that express the transcription factor FOXP3, which has **consequences for tumour-associated inflammation****
- **butyrate** is also the **preferred energy source for colonocytes**

*Maslowski KM, Vieira AT, Ng A, et al. Nature 2009; 461:1282–1286.
**Smith PM, Howitt MR, Panikov N, et al. Science 2013;341:569–573.

Short Chain Fatty Acids

- although it is recognised that SCFA effects on host cellular processes vary according to concentration and host genotype, it is also possible that **high butyrate levels may influence/limit immune responses towards the resident microbiota**, while conversely **low butyrate concentrations may trigger a pro- inflammatory state** resulting in gut microbiota remodelling to enhance levels of beneficial butyrate-producing species by suppressing potentially pathogenic organisms*

- the **effect of butyrate** on CRC development is, however, **controversial** with a number of studies demonstrating a pro-carcinogenic effect in different animal models**

*Chang PV, Hao L, Offermanns S, et al. Proc Natl Acad Sci U S A 2014;111:2247–2252.
**Belcheva A, Irrazabal T, Robertson SJ, et al. Cell 2014;158:288–299.

The Impact of Diet…

- dietary patterns high in **red meat** proteins, **animal fats** and **refined sugars** are considered **pro-carcinogenic and pro-inflammatory***
- high protein intake results in increased production of **branched-chain fatty acids** and **phenylacetic acid** which components of the gut microbiota, including several Bacteroides spp. and Firmicutes, metabolise to produce a number of **bioactive products including phenolic compounds, indoles and p-cresol****
- diets rich in **saturated fats** also **increase bile acid production** with gut bacteria known to contribute to bile acid metabolism

*Hold GL. Gut 2014; 63:5–6.

**Ou J, Carbonero F, Zoetendal EG, et al. Am J Clin Nutr 2013;98:111–120.

Secondary Bile Acids

- **production of** these **secondary bile acids** by 7-alpha-dehydroxylation, was also confirmed to be, at least partially, **bacterially driven**
- bile acids are known to exert **strong antimicrobial activities** through their ability to damage bacterial cell membranes
- both lithocholic and deoxycholic acid can be **proinflammatory**, eliciting reacive oxygen and nitrogen species production as well as **inducing NF-κB activation** in intestinal epithelial cells*
- both reactive oxygen and reactive nitrogen species cause **DNA damage including point mutations**, DNA breaks and protein-DNA crosslinking that **potentially contribute to chromosomal instability increasing the risk of CRC**

Bernstein H, Bernstein C, Payne CM, Dvorak K. World J Gastroenterol 2009;15:3329–3340.

Dietary Compounds and the Role of Microbiota in Colon Carcinogenesis

Yoon K, Kim N. J Cancer Prev 2018;23:117-125.

Dietary compounds	Microbial role	Known effect on host
Non-digestible carbohydrates	SCFAs production	Microbiota modulation; Antiinflammation; Tumor cell growth suppression vs. Promotion of polyp (?)
Protein	NOCs production	ROS production; genotoxicity
Heme	Facilitating Heme toxicity	Microbiota modulation; cytotoxicity; genotoxicity
Fat → bile acids	Secondary bile acid production	Microbiota modulation; cytotoxicity; genotoxicity

SCFAs, short-chain fatty acids; **NOC**s, N-nitroso compounds; **ROS**, re-active oxygen species

Targeted Cancer Treatments

- over the past thirty years, a number of **bacterial-based immunotherapies** have emerged
- synthetic biologists have engineered **bacteria capable of differentiating between diseased and healthy cells** in order that cancer cells can be identified, invaded and destroyed*
- studies exploiting this approach include **enabling cell-invasion in E. coli** by engineering expression of invasion adhesion protein from Yersinia pseudotuberculosis, which binds tightly to mammalian β1 integrin
- invasin expression was placed under the control of an anaerobically induced promoter, ensuring that bacteria only invaded cells in hypoxic environments**

*Ruder WC, Lu T, Collins JJ. Science 2011;333: 1248–1252.
**Anderson JC, Clarke EJ, Arkin AP, Voigt CA. J Mol Biol 2006;355:619–627.

Targeted Cancer Treatments

- a second investigation engineered cancer-invading **bacteria to target a specific tumourigenic pathway**
- the engineered bacteria generated short hairpin RNA segments that interfered with β-catenin, thus interrupting cancer development
- intravenous administration of the bacteria showed that **distal cancer sites could be targeted** as well as when locally administered
- this has a **huge potential impact** in terms of targeting CRC in vivo*

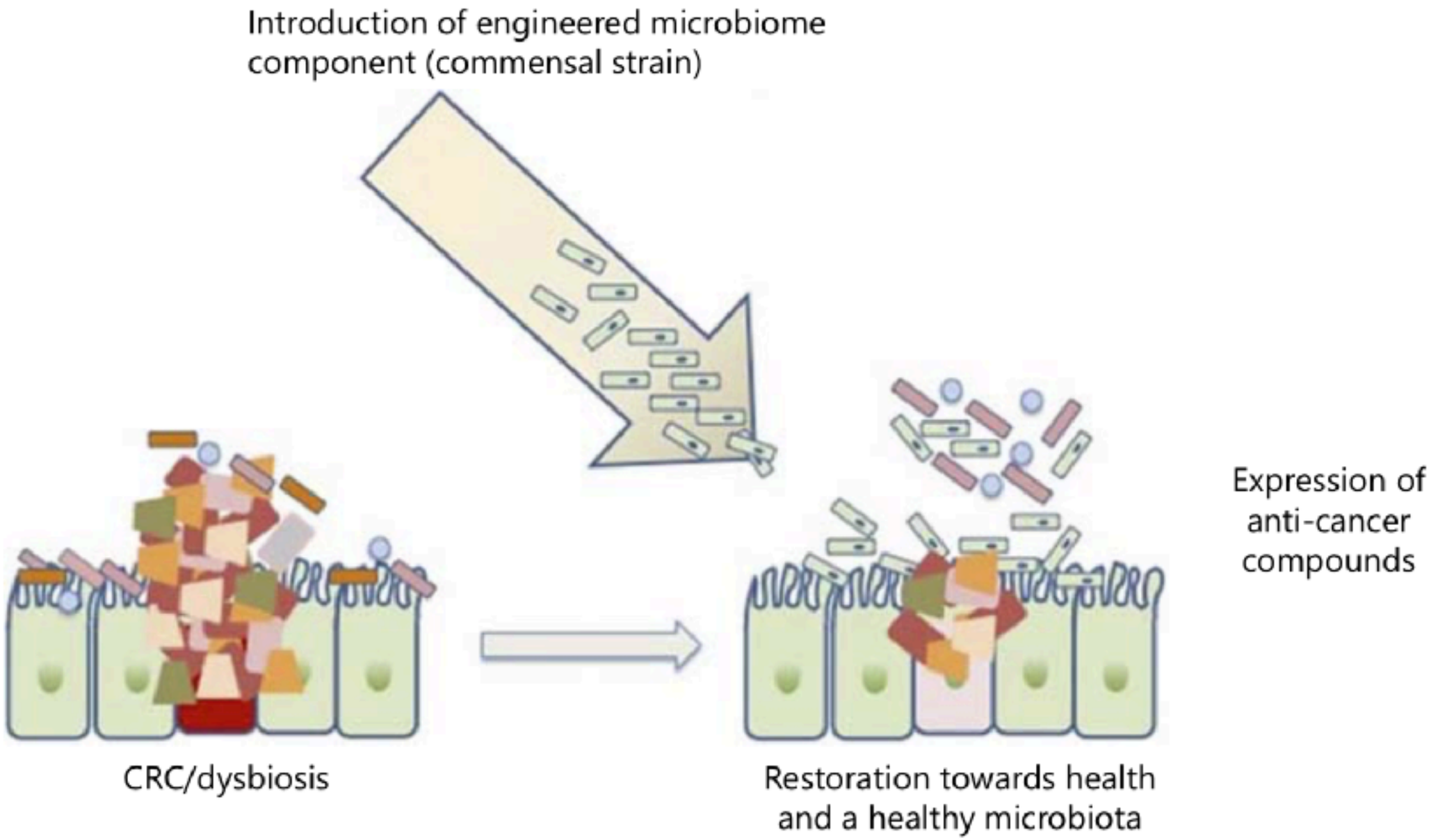
Xiang S, Fruehauf J, Li CJ. Nat Biotechnol 2006;24:697–702.

Microbiome Manipulation

- **microbiome engineering** has emerged as a **promising area of investigation** over the past decade
- in general, the **gut microbiome is well tolerated**; therefore, harnessing this congeniality to allow the **delivery of specific therapeutic molecules** holds promise
- **commensal bacterial** strains have been engineered to allow the **delivery** of **insulinotropic proteins** for diabetes, **HIV fusion inhibitors** and **interleukin-2**
- although development is **not currently CRC focused**, molecule delivery under the control of cell-based sensors that are triggered in response to specific pathological conditions offers a huge potential

Ruder WC, Lu T, Collins JJ. Science 2011;333: 1248–1252.

Microbiome Manipulation



Hold, GL. Dig Dis 2016;34:244–250.

Conclusions

- **understanding the gut microbiota** and how it contributes to health and disease is a **continually developing research field**
- in terms of CRC, more fully appreciating the contribution of the gut microbiota to CRC pathogenesis will allow us to open up **new avenues for prevention and treatment**
- however, a number of **important questions** remain **unanswered**
  - **clarification** between whether CRC development is due to the **carcinogenic activity of the gut microbiota or** whether an **altered microbiota results from the tumour environment** remains a major stumbling block. It is likely that in reality it is a combination of both phenomena
  - further understanding of **biofilm communities** and how their presence **influences microbial metabolism** in the context of gut inflammation and CRC is needed
  - further delineation of how microbes and their metabolites influence CRC progression will play a **major part in influencing how we treat** and try to prevent this disease in the years to come