Gut microbiota in host metabolism and cancer

From biological pathways to novel treatment strategies

de Clercq, N.C.

Publication date
2020

Document Version
Other version

License
Other

1

General Introduction and Thesis Outline

GENERAL INTRODUCTION

"All Disease Begins in the Gut"
Hippocrates (460-370 BC)

If Hippocrates was right, our gut is the key orchestrator of health and disease. Although Hippocrates is seen as the founder of Western medicine, his view on the extraordinary functions of the gut was not adopted by his medical disciples until the 20th century. In contrary, Eastern medicine has always recognized the gut -together with its inhabitants- as the core facilitator of health. For instance, Japanese refer to the intestines as *Onaka*, or "*Honoured Middle*",[1] and in the fourth-century, the Traditional Chinese medical practitioner Ge Hong even prescribed "yellow soup" (feces from a healthy person) to treat diarrhea.[2]

Despite the initial disgust for fecal matters in the West, in the past two decades a major paradigm shift occurred. Whereas initially bacteria were merely seen as a potential threat for human health, microorganisms are now being isolated for new treatment modalities. Particularly, the collection of microorganisms harboring in the intestines, the so called gut microbiota, is currently accepted to have a profound influence on human health and disease, including metabolic syndrome,[3] cancer,[4] and psychiatric conditions like depression[5] and anorexia nervosa.[6]

The gut microbiota not only consists of bacteria, but also of a vast number of diverse microorganisms including viruses, fungi and archaea.[7] The composition and function of these microbes are largely influenced by environmental factors (e.g. dietary intake, drugs), whereas host genetics only account for 2-8% of the variation in these intestinal microorganisms.[8] Collectively, these factors (in-)directly affect multiple microbial-host processes, including metabolism,[9] weight regulation[10], the immune system,[11,12] and effectiveness of drugs.[13,14]

As the findings in microbiota research seem to be promising, the publications in this field are growing exponentially. This outburst of studies contains papers with considerable heterogeneity and findings are often inconsistent or difficult to reproduce.[15] Nonetheless, the studies seem to share a common goal, which is answering the two most urgent questions in the field:

A.) what is a healthy gut microbiome composition, and
B.) can we prevent or cure disease by altering this composition?

Before we can answer these questions, we need to overcome some major hurdles. Regarding the first question, most microbiome studies are based on *associations*, meaning there is a difference in gut microbiota composition between two phenotypes,

but this does not imply that the gut microbiota actually *influences* the course of a disease or phenotype. To prove a causal relation between a microbe and a disease, the nineteenth century physicians Robert Koch and Friedrich Loeffler formulated the four postulates [16]. Since only a few diseases fulfill the original form of Koch's postulates, Evans and colleagues[17] proposed a modified version which is found to be more suitable to demonstrate causation between microorganism and diseases.

Based on these criteria, the following steps should be followed in order to prove a causal relation:

1. There must be an association between a phenotype or disease and a specific microbe, assessed with a cross-sectional or longitudinal study
2. Introduction or removal of the specific microbes causes a change in the phenotype or disease.
3. The causal relation between a specific microbe and disease should make biological or epidemiological sense

Once an association is proven and it is clear which microbe or group of microbes is "healthy" or "beneficial", step 2 can be considered: wat is the most effective method to manipulate or *engineer* the gut microbiota composition in order to prove causality and ultimately prevent or cure a specific disease. In fecal microbiota transplantation (FMT) an entire intestinal microbial ecosystem is transferred, using donor feces. In the past decades, this strategy is found to be a useful method to modify the gut microbiota composition and has even become treatment of choice in antibiotic resistant *Clostridioides difficile* (formerly *Clostridium difficile)* infections.[18] Furthermore, studies in humans have shown that metabolic traits can be transmitted via FMT, including glucose metabolism,[3] feeding behavior[19,20] and most remarkably body composition.[21] With FMT, an entire intestinal microbial ecosystem is transplanted, with the use of donor feces.

Other strategies to manipulate the gut microbiota composition, including dietary interventions, pre-and probiotics, are currently being investigated in many different diseases.

THESIS OUTLINE

In this thesis we explored the role of the gut microbiota in host metabolism and cancer (**Figure 1).** The term metabolism refers to all the chemical reactions accountable for numerous processes, such as harvesting energy from food, constructing proteins, lipids and others biomolecules. Specific conditions or diseases, such as undernutrition, metabolic syndrome (MetSyn) and cancer can lead to an imbalance in these processes.

In line with the modified instructions by Evans, we performed three observational studies to assess the association between gut microbiota composition and specific phenotypes. Furthermore, in an attempt to modify the gut microbiota and prove a causal relation between the intestinal microbes and a specific metabolic condition, we performed two intervention studies, using fecal microbiota transplantation. The ultimate aim of this thesis, was to identify specific microbial-host pathways involved in different metabolic conditions and effectiveness of anticancer treatments, which could guide future research into the development of new therapeutic options.

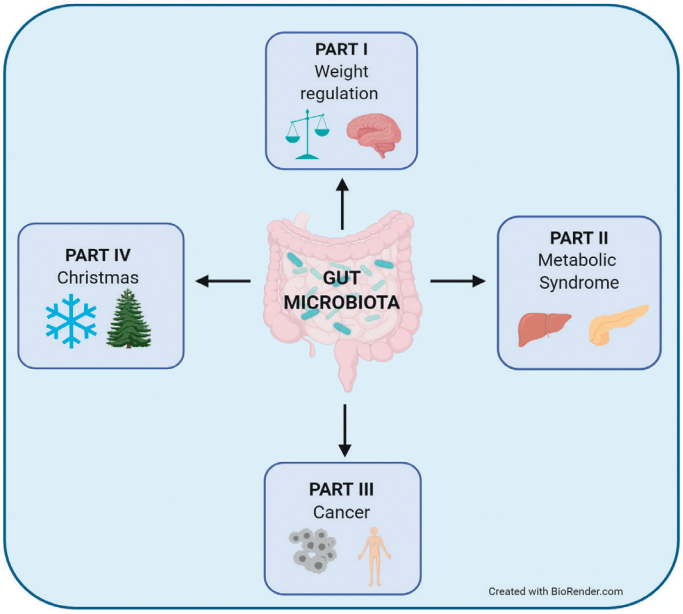

Figure 1. Thesis outline.

PART I. GUT MICROBIOTA & WEIGHT REGULATION

Part I focusses on the role of the gut microbiota in weight regulation, in particular in the pathophysiology of malnutrition. This complex metabolic condition, including both obesity and undernutrition, is believed to be the one of the major causes of death and disease worldwide.[22] In 2019, 1.9 billion people were overweight or obese and 462 million people were underweight.[23] Although these metabolic states seem completely opposing, they share important pathophysiological factors, such as a distorted nutritional intake and an imbalance of the gut microbiota composition.[24–29] Indeed, many observational studies have found distinct differences in gut microbiota composition between malnourished individuals and healthy controls.[24,30]

Chapter 2 provides an extensive overview on how the gut microbiota is involved in the pathophysiology of malnutrition, with a special focus on the gut-brain-axis[31,32]. This bi-directional axis refers to the interaction between the gut microbiota and the brain, which is found to influence both (feeding) behavior and host metabolism[31] and thus thought to be involved in the pathophysiology of malnutrition, including obesity and anorexia nervosa.

To explore a potential causal relation between gut microbiota composition and malnutrition, we next conducted a FMT from a healthy obese donor in a patient with persistent underweight (**Chapter 3**). Prior to the FMT, this patient was clinically recovered from anorexia nervosa and despite that she adhered to a high caloric diet, she remained underweight. In line with previous findings, we hypothesized that a FMT from an obese donor would increase the capacity to harvest energy from nutritional intake and consequently improve body weight.

PART II. GUT MICROBIOTA & METABOLIC SYNDROME

The global obesity pandemic mentioned in Part I has resulted in an alarming increase in the prevalence of metabolic syndrome and diabetes type 2.[33] The progression from obesity to metabolic syndrome is thought to be partly driven by the gut microbiota composition through the production of bio-active molecules, such as plasma metabolites. **Chapter 4** summarizes the current available literature on how the gut microbiota and their metabolites are involved in many metabolic functions, for example insulin sensitivity, energy expenditure and nutritional intake (appetite).

Some of these so called microbial-derived metabolites, such as short chain fatty acids (SCFAs)[34] and imidazole propionate[35], have been recognized as potential drivers for the development of insulin resistance in metabolic syndrome and diabetes type 2. However, only a few studies have dissected the molecular pathways through which these metabolites contribute to host metabolism and the development of insulin resistance. In an attempt to identify new microbial-metabolite pathways involved in insulin sensitivity, we conducted a cross-sectional study in 115 individuals with metabolic syndrome and explored the relation between the gut microbiota composition, plasma metabolites and insulin sensitivity (**Chapter 5**).

PART III. GUT MICROBIOTA & CANCER

In addition to malnutrition and metabolic syndrome, there is compelling evidence that the gut microbiota influences different aspects of cancer biology, including response to anti-tumor therapies and toxicity. In the past decades, bacterial and viral infections

have been found to potentially contribute to onset of cancer (carcinogenesis), such as *Helicobacter pylori*[36] in gastric cancer and *Fusobacterium nucleatum*[37] in colorectal cancer. Moreover, fecal and tumor microbes are associated with the response to chemotherapy[38] and immune checkpoint inhibitors (ICIs),[39,40] and manipulation of the gut microbiota can improve anti-tumor response in mice through enhancing host immune response. However, as stated previously, outcomes of these studies are heterogeneous and there is no consensus on how the "ideal" or "most beneficial" gut microbiota composition looks like and what the best strategies are to target the gut microbiota composition. These inconsistencies might also be driven by the fact that researchers have mainly analyzed fecal and tumor microbiota composition, even though the duodenal microbiome, and the metabolites they produce, play a crucial role in regulating the immune system.[41]

To further explore the role of the gut microbiota in therapeutic efficacy and toxicity we analyzed fecal, tumor and duodenal microbiome composition together with plasma metabolites in patients with resectable esophageal adenocarcinoma (rEAC) treated with neo-adjuvant chemoradiotherapy combined with atezolizumab, a PD-L1 antibody (**Chapter 6**). The ultimate goal of this exploratory study was to identify microbial-metabolite pathways involved in pathological response and/or immune-related adverse events.

Furthermore, the gut microbiota has been implicated in moderating different aspects of cancer cachexia. This multifactorial syndrome, characterized by reduced appetite (anorexia), increased energy expenditure and muscle loss, is associated with reduced response to anti-cancer therapies and poor survival rates. A major factor contributing to cancer cachexia is systemic inflammation mediated by both tumor and host factors, including the gut microbiota. Hence, in **Chapter 7** we explored the effect of FMT from healthy obese donors in cachectic patients with metastasized gastroesophageal cancer on metabolic outcomes. The ultimate aim of this study was to improve cancer cachexia and consequently oncological outcomes, including anti-tumor efficacy and survival rate.

PART IV. GUT MICROBIOTA & CHRISTMAS EFFECT

Chapter 2 and 4 provide a brief introduction in the gut-brain axis, in particular on how the gut communicates with the central nervous system (CNS) and thereby influences eating behavior and energy expenditure. However, beyond the metabolic functions of this bidirectional axis, the gut can also affect mental well-being through direct (vagus nerve) and indirect (immune system, metabolites and hormones) pathways.[42] For example, germ free mice exposed to a stressor resulted in a stress response and a change in gut microbiota composition compared to control mice. [43] Furthermore, the

stress response in these mice could partially be reversed through an FMT from the control group, providing evidence for a causal relation. However, so far, no study has been able to translate these findings in mice to the complex physiology in humans.

The Christmas season is a perfect supra-physiological circumstance to assess the consequence of a sudden exposure to a stressful condition, such as visiting your in-laws, on gut microbiota composition. Therefore, to remind ourselves that research should also be fun and entertaining, we investigated the effect of visiting in-laws during Christmas on the gut microbiota composition (**Chapter 8**). The aim of this study was to provide indispensable input to the annual marital discussions regarding the obligatory family visits during Christmas.

In summary, this thesis aims to further explore the role of the gut microbiota in the regulation of host metabolism and cancer treatment. The ultimate goal is to identify "beneficial" microbes or microbial-metabolites to improve body composition, insulin sensitivity and anti-cancer efficacy.

The research questions underlying this thesis are:

1. Can we change and improve host metabolism and oncological outcomes through modulating the gut microbiota using fecal microbiota transplantation?
2. Can we identify new microbial-metabolite pathways involved in insulin resistance and anticancer efficacy and toxicity?
3. Finally, is visiting our in-laws during Christmas detrimental for out gut microbiota composition and should we therefore avoid this annual obligatory visit?

REFERENCES

1 Yu F, Takahashi T, Moriya J, *et al.* Traditional Chinese medicine and kampo: A review from the distant past for the future. *J Int Med Res* 2006; 34: 231–9.

2 Yang Y, Owyang C, Wu GD. East Meets West: The Increasing Incidence of Inflammatory Bowel Disease in Asia as a Paradigm for Environmental Effects on the Pathogenesis of Immune-Mediated Disease. *Gastroenterology* 2016; 151: e1–5.

3 Vrieze A, Van Nood E, Holleman F, *et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; 143: 913-6.e7.

4 Routy B, Gopalakrishnan V, Daillère R, Zitvogel L, Wargo JA, Kroemer G. The gut microbiota influences anticancer immunosurveillance and general health. *Nat Rev Clin Oncol* 2018; 15: 382–96.

5 Bravo JA, Forsythe P, Chew M V, *et al.* Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 2011; 108: 16050–5.

6 Kleiman SC, Watson HJ, Bulik-Sullivan EC, *et al.* The Intestinal Microbiota in Acute Anorexia Nervosa and During Renourishment. *Psychosom Med* 2015; 77: 969–81.

7 Rowan-Nash AD, Korry BJ, Mylonakis E, Belenky P. Cross-Domain and Viral Interactions in the Microbiome. *Microbiol Mol Biol Rev* 2019; 83: 1–63.

8 Rothschild D, Weissbrod O, Barkan E, *et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018; 555: 210–5.

9 Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; 489: 242–9.

10 Bäckhed F, Ding H, Wang T, *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004; 101: 15718–23.

11 Belkaid Y and TH. Role of the Microbiota in Immunity and inflammation Yasmine. *Cell* 2015; 157: 121–41.

12 Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol* 2016; 16: 341–52.

13 Bashiardes S, Tuganbaev T, Federici S, Elinav E. The microbiome in anti-cancer therapy. *Semin Immunol* 2017; 32: 74–81.

14 Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat. Rev. Gastroenterol. Hepatol. 2017; 14: 356–65.

15 Schloss PD. Identifying and Overcoming Threats to Reproducibility, Replicability, Robustness, and Generalizability in Microbiome Research. *MBio* 2018; 9: 1–13.

16 Cohen J. The Evolution of Koch's Postulates. In: Infectious Disease (Fourth Edition). 2017: 1–3.

17 Evans AS. Causation and disease: the Henle-Koch postulates revisited. *Yale J Biol Med* 1976; 49: 175–95.

18 Van Nood E, Vrieze A, Nieuwdorp M, *et al.* Duodenal infusion of donor feces for recurrent clostridium difficile. *N Engl J Med* 2013; 368: 407–15.

19 Cai T, Shi X, Yuan L-Z, Tang D, Wang F. Fecal microbiota transplantation in an elderly patient with mental depression. *Int psychogeriatrics* 2019; 31: 1525–6.

20 Kao D, Roach B, Park H, *et al.* Fecal microbiota transplantation in the management of hepatic encephalopathy. *Hepatology* 2016; 63: 339–40.

21 Alang N, Kelly CR. Weight Gain After Fecal Microbiota Transplantation. *Open Forum Infect Dis* 2014. DOI:10.1093/o.

22 Malnutrition is a world health crisis. https://www.globalcause.co.uk/world-food-day/malnutrition-is-a-world-health-crisis-says-who-expert (accessed Jan 15, 2016).

23 WHO factsheet Malnutrition. https://www.who.int/news-room/fact-sheets/detail/malnutrition (accessed Feb 2, 2016).

24 Turnbaugh PJ, Ley RE, Mahowald M a, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; 444: 1027–31.

25 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; 444: 1022–3.

26 Tremaroli V, Kovatcheva-Datchary P, Bäckhed F. A role for the gut microbiota in energy harvesting? *Gut* 2010; 59: 1589–90.

27 Stenman LK, Burcelin R, Lahtinen S. Establishing a causal link between gut microbes, body weight gain and glucose metabolism in humans - towards treatment with probiotics. *Benef Microbes* 2015; 7: 1–12.

28 Claus SP. Fighting undernutrition: Don't forget the bugs. *Cell Host Microbe* 2013; 13: 239–40.

29 Smith, M.I., Yatsunenko T, Manary, M.J., Trehan, I. M, R., Cheng, J., Kau, A.L., Rich, S.S., Concannon P, Mychaleckyj, J.C. et al. Gut Microbiomes of Malawian Twin. *Science (80-)* 2013; 548. DOI:10.1126/science.1229000.

30 Smith MI, Yatsunenko T, Manary MJ, *et al.* Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science (80-)* 2013; 339: 548–54.

31 Reinehr T, Roth CL. The gut sensor as regulator of body weight. *Endocrine* 2014; 49: 35–50.

32 Dinan TG, Cryan JF. Gut–brain axis in 2016: Brain–gut–microbiota axis — mood, metabolism and behaviour. *Nat Rev Gastroenterol Hepatol* 2017; 14: 69–70.

33 NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet (London, England)* 2016; 387: 1513–30.

34 Gao Z, Yin J, Zhang J, *et al.* Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; 58: 1509–17.

35 Koh A, Molinaro A, Ståhlman M, *et al.* Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. *Cell* 2018; 175: 947-961.e17.

36 Peek RM, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; 2: 28–37.

37 Mima K, Sukawa Y, Nishihara R, *et al.* Fusobacterium nucleatum and T cells in colorectal carcinoma. *JAMA Oncol* 2015; 1: 653–61.

38 Viaud S, Saccheri F, Mignot G, *et al.* The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science (80-)* 2013; 342: 971–6.

39 Vétizou M, Pitt JM, Daillère R, *et al.* Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science (80-)* 2015; 350: 1079–84.

40 Routy B, Le Chatelier E, Derosa L, *et al.* Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science (80-)* 2018; 359: 91–7.

41 Santaolalla R, Fukata M, Abreau MT. Innate immunity in the small intestine. *Curr Opin Gastroenterol* 2011; 27: 123–31.

42 Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012; 13: 701–12.

43 Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011; 25: 397–407.