Exploring available models to investigate the brain of the gut and its role in colorectal cancer

Impact

In 2020, almost two million new cases were reported for colorectal cancer globally, and in the same year over 900,000 people died of colorectal cancer[1]. In the Netherlands alone, approximately 4,600 patients died of colorectal cancer in 2020[2] and almost 13,000 new patients were diagnosed in 2021[3]. Family history of colorectal cancer and lifestyle-related factors, such as high body mass index, low physical activity, cigarette smoking, high consumption of red meat, and low consumption of fruit and vegetables can increase the risk for colorectal cancer[4]. Therefore, it is not surprising that the chance to develop colorectal cancer is up to 9 times higher in highly developed world regions, such as Europe, Northern America and Australia, compared to Africa and South Central Asia[1]. Despite the implementation of different screening programs and improved (personalized) treatment options, the health and economic burden of colorectal cancer is still immense, exceeding €500 million in medical costs in the Netherlands in 2019[5].

Different strategies are used to reduce the number of patients and health burden of colorectal cancer: I) education and health promotion to prevent colorectal cancer, II) implementation of screening programs for early detection of the disease, and III) the development of (personalized) treatments[6].

The tumor microenvironment, comprising all components in the proximity of tumor cells, has become increasingly recognized as an interesting therapeutic target for personalized medicine, which has already led to the successful implementation of immunotherapy for multiple types of cancer, including a subset of colorectal cancers[7]. Furthermore, a promising target in the tumor microenvironment that has recently gained attention, and which is also the focus of this thesis, is neurons. This is of particular interest in colorectal cancer, since the intestines harbor their own nervous system: the enteric nervous system.

Neuron-cancer interaction is an emerging research field combining neuroscience and oncology[8]. In many types of cancer, such as glioma[9], prostate[10,11], pancreatic[12,13], skin[14], and gastric[15,16] cancer, nerves are actively contributing to cancer development and progression. In this thesis, we investigated the role of the enteric nervous system, the brain of the gut, in colorectal cancer development and progression. We discovered that the enteric neuronal protein NDRG4, which is also a methylation biomarker for colorectal cancer, regulates the secretion of two proteins, fibulin-2 and nidogen-1, thereby affecting intestinal tumor growth (**Chapter 6**). Furthermore, we demonstrate that a lower density of enteric neurons results in an altered immune landscape in a mouse model of colorectal cancer (**Chapter 7**). More specifically,

antibody-related gene expression is altered in the tumor and the population of B cells, which are antibody-producing cells, is reduced in the colon. This shows that alterations in the enteric nervous system can affect colorectal cancer, by modulating the extracellular matrix and by influencing the immune system in the gut.

The results presented in this thesis contribute to the knowledge about enteric neuron-colorectal cancer interplay and provide a foundation for the future development of nerve-targeted therapies. So far, treatment options in clinical use that target neural-related signaling in cancer are limited. The FDA has recently approved two potent drugs, larotrectinib and entrectinib, that interfere with nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) signaling to Trk-receptors in Trk-positive cancers[17]. In addition, the NGF antibody tanezumab can be used to decrease bone metastasis-induced pain, but its effect on cancer progression has yet to be investigated[18]. The findings in this thesis suggest that (enteric) neurons can alter extracellular matrix composition and influence immune response, and cancer therapies targeting these processes could be of interest in the context of neuro-oncology. However, therapies to manipulate nerves directly are in their infancy and require multidisciplinary effort before they can be brought to the clinic[8].

Even though the data described in this thesis mainly focused on colorectal cancer, research on the enteric nervous system is gaining a lot of interest in general, due to its recognition as a crucial element of the microbiota-gut-brain axis and regulator of gut homeostasis. Moreover, a better understanding of the enteric nervous system can also be important for elucidating the pathophysiology of other gastrointestinal diseases and even for neurodegenerative disorders that primarily strike the central nervous system, such as Parkinson's and Alzheimer's disease. This thesis will therefore be of interest to a broad range of researchers (neuroscientists, oncologists, and gastroenterologists), clinicians, and patients and might help to create a bridge between previously unconnected fields.

Next to the work on colorectal cancer, several models and methodologies were explored in this thesis, such as protocols for the primary culture of mouse enteric neurons (**Chapter 3**), methods to assess gut motility (**Chapter 4**), and mouse models with an altered number of enteric neurons (**Chapter 7** and **Chapter 8**). The results presented in this thesis show that methodology to investigate the enteric nervous system can be improved in several ways. Current protocols for the primary culture of enteric neurons rely on similar approaches, but are carried out with tremendous variation in cellular isolation procedures and culture conditions.

Moreover, the lack of comparable outcome measurements (like cell viability and neuron quantity) hinders the assessment of optimal culture conditions. This emphasizes the need for a better validation and reproduction of study results in general, which is also why we optimized and validated a method for the assessment of gut motility (an indirect measure of enteric neuron function) in this thesis. This method can now be adopted by other researchers in the (neuro)gastroenterology field, provides more physiologically relevant results, and improves experimental animal welfare. In addition, we validated the reduced enteric neuron density in the *Hand2*$^{fl/+}$*;Wnt1-Cre2* mouse model, but could not reproduce an increased enteric neuron density in the *NSE-Noggin* mouse model, underscoring the reproducibility problems in biomedical research[19]. Model systems should be robust and consistent to enable high quality research and valorization, and thus not only concerns other researchers in the scientific community, but also funding agencies, companies, and the rest of the society.

Fellow researchers have been informed of this research through publications in open-access peer-reviewed journals. In addition, this work has been presented at local, national, and international conferences to provide knowledge transfer and enhance insight in the contribution of enteric neurons in colorectal cancer. To summarize, this thesis offers a new perspective on the role of neurons in colorectal cancer by investigating the regulating mechanisms of enteric neurons on the tumor microenvironment, knowledge which can also be translated to other cancer types.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2021;71(3):209-249.
2. Gommer AM, Dieren JMv, Poos MJJC. Dikkedarmkanker | Sterfte en overleving. https://www.vzinfo.nl/dikkedarmkanker/sterfte-en-overleving. Accessed December 31, 2022.
3. Gommer AM, Poos MJJC, Hendriks C, Dieren JMv, Hilderink HBM. Dikkedarmkanker | Leeftijd en geslacht. https://www.vzinfo.nl/dikkedarmkanker/leeftijd-en-geslacht. Accessed December 31, 2022.
4. Johnson CM, Wei C, Ensor JE, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes & Control. 2013;24(6):1207-1222.
5. Weerdt ACd, Gouwens S, Koopmanschap MA, Meer Avd, Kommer GJ. Dikkedarmkanker | Zorguitgaven. https://www.vzinfo.nl/dikkedarmkanker/zorguitgaven. Accessed January 3, 2023.
6. Binefa G, Rodríguez-Moranta F, Teule À, Medina-Hayas M. Colorectal cancer: from prevention to personalized medicine. World Journal of Gastroenterology: WJG. 2014;20(22):6786.
7. Ganesh K, Stadler ZK, Cercek A, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nature Reviews Gastroenterology & Hepatology. 2019;16(6):361-375.
8. Zahalka AH, Frenette PS. Nerves in cancer. Nature Reviews Cancer. 2020;20(3):143-157.
9. Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. Nature. 2019;573(7775):539-545.
10. Magnon C, Hall SJ, Lin J, et al. Autonomic nerve development contributes to prostate cancer progression. Science. 2013;341(6142):1236361.
11. Zahalka AH, Arnal-Estapé A, Maryanovich M, et al. Adrenergic nerves activate an angio-metabolic switch in prostate cancer. Science. 2017;358(6361):321-326.
12. Stopczynski RE, Normolle DP, Hartman DJ, et al. Neuroplastic changes occur early in the development of pancreatic ductal adenocarcinoma. Cancer Research. 2014;74(6):1718-1727.
13. Bressy C, Lac S, Nigri J, et al. LIF Drives Neural Remodeling in Pancreatic Cancer and Offers a New Candidate Biomarker. Cancer Research. 2018;78(4):909-921.
14. Peterson SC, Eberl M, Vagnozzi AN, et al. Basal cell carcinoma preferentially arises from stem cells within hair follicle and mechanosensory niches. Cell Stem Cell. 2015;16(4):400-412.
15. Zhao C-M, Hayakawa Y, Kodama Y, et al. Denervation suppresses gastric tumorigenesis. Science Translational Medicine. 2014;6(250):250ra115-250ra115.
16. Hayakawa Y, Sakitani K, Konishi M, et al. Nerve growth factor promotes gastric tumorigenesis through aberrant cholinergic signaling. Cancer Cell. 2017;31(1):21-34.
17. Vaes N, Idris M, Boesmans W, Alves MM, Melotte V. Nerves in gastrointestinal cancer: from mechanism to modulations. Nature Reviews Gastroenterology & Hepatology. 2022:1-17.
18. Sopata M, Katz N, Carey W, et al. Efficacy and safety of tanezumab in the treatment of pain from bone metastases. Pain. 2015;156(9):1703-1713.
19. Begley CG, Ioannidis JP. Reproducibility in science: improving the standard for basic and preclinical research. Circulation Research. 2015;116(1):116-126.