NDRG4 When you get on the gut's nerves

ETCETERA

VALORIZATION

With an incidence of 10.2% of all cancers, colorectal cancer (CRC) ranks as the third most frequently diagnosed cancer, worldwide. The large extent of the global CRC burden is caused by the socioeconomic development/"Western lifestyle" adaptations, the aging population and the more frequently diagnosed condition in adults < 50 years. CRC incidence rates vary widely but tend to rise uniformly with an increasing income/human development index(HDI), while mortality rates are less variable because of a lower average mortality in developed regions.[1-6]

The newly accepted point-of-view that the tumor landscape is not just a simple cluster of tumor cells, but a complex tissue of tumor cells together with (non-) cellular components of the tumor microenvironment (TME)[7-11], has changed current perspectives on the classification of and treatment options for colorectal tumors. This has already led to great advancements in CRC treatments with the development of immunotherapy (e.g. Nivolumab, Atezolizumab or Ipilimumab). Nevertheless, the successful treatment of CRC-patients remains challenging due to (i) varying treatment efficacy depending on tumor-specific molecular features, (ii) the occurrence of severe side-effects (e.g. diarrhea, vomiting, ulceration) and (iii) the hindrance by drug resistance development[2, 12].

For the development of effective therapies with minimal toxicities, it is important to realize that different components within the TME may affect its pathogenesis. Besides the beneficial effects of targeting immune cells to treat CRC, another promising target of the TME comprises nerve cells, as recent advances have underscored the inevitable contribution of the nervous system and their neural-associated factors to (colorectal) carcinogenesis (summarized in **CHAPTER 3**)[13]. Moreover, owing to the discovery that NDRG4, one of the most accurate biomarkers for CRC, is specifically expressed within neurons of the enteric nervous system: "our brain in the gut", we have opened up the discussion concerning the contribution of the gut's intrinsic nervous system to colorectal carcinogenesis (**CHAPTER 2**). Even though caution is warranted, as ENS-derived messenger molecules are indispensable to preserve physiological (intestinal) functions[14, 15], ENS-targeted delivery of pharmacological agents may be favorable in the management of CRC. In fact, despite uncertainty whether differences in endogenous neuromodulator production is enough to affect CRC and whether antineurogenic therapies, e.g. targeting NGF, are as effective for CRC as for gastric cancer[16], both mechanisms seem highly likely since several studies agree on a differential neurotransmitter expression profile in the ENS in CRC compared to in healthy intestinal tissues[17-21]. Moreover, targeting enteric nerves and their fiber projections harbors great potential as (i) surgical and pharmacological denervation suppresses prostate[22], gastric[16, 23] and pancreatic[24-26] cancer development, (ii) patients with a megacolon, caused by decreased intrinsic innervation (comparable with Chagas disease), have a lower risk for developing CRC[27], (iii) intrinsic denervation

of the colon has protective effects already early in the development stages of colorectal carcinomas[28, 29] and because (iv) Duchalais *et al*[30] recently revealed that tumor epithelial cells have a preferential tendency to adhere to enteric neurons, thereby promoting perineural invasion. Finally, with the appreciation that not only CRC itself, but also the applied chemotherapy may induce severe gastrointestinal (GI) side effects that impact a patient's prognosis and quality of life, the ENS is emerging as a player in chemotherapy-induced GI-dysfunctions[31-33]. For example, *in vivo* studies comprising mice and rats have shown that Oxaliplatin[34-36] and Cisplatin[37] treatment disturb colonic motility, most likely by reducing the number of enteric neurons (and/or glial cells). Similarly, human myenteric neurons are functionally and structurally different in the colon of non-treated CRC-patients compared to those receiving chemotherapy[33]. Consequently, the ENS may represent a possible therapeutic target to alleviate chemotherapy-induced GI-dysfunctions.[31, 33-41]

Unfortunately, the development of these innovative therapeutic strategies is still in its infancy and current treatments only reduce CRC mortality with 12%, whereby CRC still accounts for 881 000 cancer-related deaths each year[1]. To further reduce the global social and economic burden of CRC, effective measures for the early detection of CRC and prevention of disease progression are urgently needed, as this will lead to effective treatment options, a better prognosis and a significant reduction in mortality. The successful implementation of screening programs in e.g. the USA (Cologuard®–*NDRG4*) and in different European countries (Fecal immunochemical test, FIT) partially explain the reduced mortality rates in developed areas. However, given that a positive FIT may also be indicative for benign conditions, the usage of biomarkers in bodily fluids, i.e. tumor-specific alterations in e.g. blood or stool, represents a more appealing strategy.

Interestingly, the differential expression of ENS-derived molecules (e.g. Substance P, neuropeptide Y) may represent a useful biomarker for the identification of CRC-patients or to discriminate non-responding from responding CRC-patients[13]. In accordance, *NDRG4* expression negatively correlates with metastasis and TNM staging (**CHAPTER 4**)[42]. In addition, it is suggested that differences in morphology/number of (enteric) nerve cells can serve as (prognostic) biomarkers. In fact, the presence of perineural invasion and neoneurogenesis is, despite limited evidence supporting CRC metastasis through enteric nerves[30] and ambiguity concerning nerve fiber sprouting from the ENS, associated with a poorer prognosis and reduced survival rate of CRC-patients (**CHAPTER 3**)[11, 43-49].

Together, these findings define the ENS (and its molecules) as an interesting, yet so-far neglected, target of the TME in colorectal carcinogenesis, which is supported by the booming interest in the contribution of nerve cells to different cancer types.[11, 14, 16, 22, 23, 25, 30, 43-52]

Finally, this thesis may also have some implications for the so-called intestinal/enteric neuropathies as it shows an association between the absence of *ndrg4* and a reduced number of distal enteric neurons and suboptimal motility (**CHAPTER 6**). Whereas the newest sequencing technologies have been able to identify a large number of the genetic factors associated with enteric neuropathies, their etiology is still not completely understood. However, it seems that enteric neuropathies share a common biological basis[53, 54]. *Brosens et al*[53] therefore proposed a seesaw model to explain the development of enteric neuropathies, where deficits in neuronal number/composition represents the common denominator, which can be affected by (epi-) genetic and environmental factors. The rearranged during transfection (RET) gene appears to be the fulcrum in neuropathies characterized by a numerical loss of neurons. Nevertheless, the reduced number of enteric neurons in the absence of *ndrg4* implies that *NDRG4* might represent (i) another denominator, which may also induce neuropathies (for which the genetic causes are unknown), or (ii) is an (epi-) genetic factor that shifts the seesaw to abnormal development, resulting in hypoganglionosis.

Importantly, the onset and severity of enteric neuropathies can highly vary depending on when, where and how a deficit occurs, and pathological features are not necessarily readily associated with clinical consequences[55-59]. It is therefore uncertain whether the seesaw model is actually able to explain the etiology of enteric neuropathies whose phenotypic features are rather diverse than clearly disease-specific, thereby indicating the need to further study these disorders[53]. Interestingly, *NDRG4* mRNA expression is significantly reduced in the aganglionic bowel of *Ret*$^{k-/k-}$ mice and Hirschsprung patients[60, 61]. It seems highly likely that this is attributed to the reduced number of enteric neurons, yet functional consequences of loss of *NDRG4* have not been investigated in this condition. Nonetheless, the discovery that absence of *ndrg4* mildly affects ENS development and functioning, suggest its contribution to enteric neuropathies with more subtle consequences. In addition, it may explain the presence of some phenotypic abnormalities that have not been associated with the identified "driver" genes for a certain neuropathy. Consequently, my research is another step in the understanding of the underlying mechanisms attributing to enteric neuropathies. Furthermore, it points out that future studies should investigate enteric neuronal genes/proteins, as this will provide new insights into the pathogenesis of a broad range of enteric neuropathies, which will improve current health care strategies.

Altogether, the knowledge acquired in this thesis is expected to have an impact on current screening/treatment strategies for intestinal disorders. The discovery of the specific expression of NDRG4 in the ENS pinpoints to a role for NDRG4 in ENS development and consequently in enteric neuropathies. In addition, the identification of the ENS as a

novel TME determinant in the pathogenesis of CRC, provides a potential new target and/or biomarker unit, which will improve the clinical management of CRC and the patient's quality of life. Moreover, because the ENS is located along the entire GI-tract, this newly identified target/biomarker unit may also be of importance for the successful care of other GI-cancers and/or (developmental/inflammatory) GI-related disorders.