Neural Influence on Colorectal Carcinogenesis

Impact

Colorectal cancer (CRC) is a major public health concern. According to the Global Cancer Observatory, it is the third most frequently diagnosed cancer and the second most deadly cancer globally, thus having a significant economic burden on the population and health care system, especially in the Western world. In Europe, CRC is the second most common cancer in men and the third most common cancer in women, with estimated new cases exceeding 500,000 each year (Sung, Ferlay et al. 2021). The healthcare costs for CRC patients reached about €19.1 billion in Europe and $22.3 billion in the United States, in 2015 (Mariotto, Enewold et al. 2020, Henderson, French et al. 2021). As the number of new cases worldwide is still expected to increase to 3.2 million, and 1.6 million deaths are expected per year, by 2040 (Morgan, Arnold et al. 2023), there is a need for a better understanding of colorectal carcinogenesis..

Over the last years, the involvement of the nervous system in carcinogenesis has become widely accepted, due to the emergence of landmark scientific papers describing the role of neural cells in various cancer types including brain, lung, prostate, pancreas, ovaries, breast, skin, gastrointestinal, and head and neck cancers (Peterson, Eberl et al. 2015, Zahalka, Arnal-Estape et al. 2017, Kamiya, Hayama et al. 2019, Mauffrey, Tchitchek et al. 2019, Venkataramani, Tanev et al. 2019, Venkatesh, Morishita et al. 2019, Amit, Takahashi et al. 2020, Balood, Ahmadi et al. 2022, Guillot, Dominici et al. 2022, Restaino, Walz et al. 2023, Savchuk, Gentry et al. 2023, Schmitt, Sakthivelu et al. 2023, Xue, Zhu et al. 2023). Clinical trials for drugs that target specific neurotransmitters and neurotrophic factor signaling are ongoing, as reviewed in **Chapter 4**. However, fundamental scientific knowledge of the role of the nervous system in the CRC field is still limited.

The findings outlined in this thesis significantly advance our understanding of the complex relationship between the Enteric Nervous System (ENS), the immune system and CRC. Here, we have effectively illustrated that a decreased density of enteric neurons can lead to significant changes in the immune microenvironment, within a CRC mouse model (Chapter 6). Specifically, we observed notable alterations in the expression of genes related to antibody production and identified a reduction in the population of B cells in the colon. We also intensively characterized enteric glia in the colon of human aged adults and uncovered specific transcriptomic alterations occurring in these cells during colorectal carcinogenesis (Chapter 7). In particular, we noted a heightened proportion of enteric glial cells exhibiting specialization in antigen presentation and inflammation. This finding suggests a potential role for enteric glia in modulating immune responses within the context of colorectal carcinogenesis. Together, these results indicate that enteric neurons and glia have the potential to mediate immune signaling in CRC. Given the growing focus on immunotherapies for the treatment of CRC, exploring interventions that target the ENS could be promising.

However, direct manipulation of enteric neurons and/or glia for therapeutic purposes is still in its early stages and requires further investigations before it can have a clinical application.

While the primary focus of this thesis is on CRC, its outcomes can have a significant impact beyond this specific type of cancer, as we have provided a rationale for further exploration of neural signaling pathways as an inherent concept in onco-biology. Up to date, a relatively limited number of drugs that modulate neural activities, such as Larotrectinib and Entrectinib, have been approved or are being under clinical trials. Our analysis in **Chapter 5** suggests that a vast array of neural signaling pathways are perturbed both at the level of DNA methylation and RNA expression, which are likely implicated in CRC, as well as various solid cancers. One of the significant contributions of our study is the compilation of comprehensive lists detailing the most affected neural signaling pathways across different cancer types. These lists serve as a valuable resource for identifying potential neural targets that could have a significant impact on CRC outcomes, when targeted therapeutically. By prioritizing these targets, future research can focus on investigating specific neural pathways that play pivotal roles in promoting or suppressing, tumor growth and progression. In addition, this step forward includes unraveling the mechanisms underlying these neural signaling alterations.
The results described in this thesis, specifically on the ENS might also be relevant to other fields. Research on the ENS is booming, but still much needs to be uncovered. To date, there is, for example, almost no comprehensive characterization or functional assessment of the enteric glia in healthy aged human colons. **Chapter 7** contributes, therefore, to our understanding of the aged ENS, which is a crucial regulator of gut homeostasis. This expanded understanding may shed light on the pathophysiology of various other gastrointestinal diseases and is thus of interest to a wide range of researchers, including neuroscientists, oncologists, and gastroenterologists.

As described in the introduction, models to study the bi-directional communication of neural cells and cancer cells are still missing. We reviewed various *in vitro* models, including protocols for the primary culture of mouse enteric neurons in **Chapter 3**, as well as protocols for colonic organotypic cultures with integrated or non-integrated ENS components (**Chapter 2**). We identified gaps in CRC organotypic models, including the lack of models that incorporate enteric neural cells.

In conclusion, this thesis contributes to bridging the gap between neurobiology and oncology, highlighting the connections between the enteric nervous system, the immune system, and colorectal carcinogenesis. By gaining more insights into these mechanisms, we started the essential groundwork for developing innovative neural-specific therapeutic interventions in the fight against CRC.

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