Nerve fibers in the tumor microenvironment

Summary

The overall aim of this thesis is to study the role of nerve fiber in neurotropic cancer- pancreatic cancer (PDAC) and Cholangiocarcinoma (CCA), exploring a novel predictive, prognostic, and therapeutic target. This thesis provides clinical observation and studies focusing on functions of nerve fiber in PDAC, pCCA and iCCA patients undergoing surgery.

The role of nerve fibers in neurotropic cancer

Chapter 2 provides an overview of latest related theoretical knowledge of neural system in neurotropic cancer as well as the clinical studies that describe the clinical association between nerves and patients' oncological outcomes in PDAC and CCA. There are evident indications of a close relationship between nerves in TME and patients' survival in PDAC and CCA. The existed literatures show that perineural invasion is an independent prognostic factor of poor outcome in neurotropic cancer. This thesis focuses on the shared signaling pathways of the mechanisms behind perineural invasion in PDAC and CCA. Hereby we focus on signaling neurotransmitters and neuropeptides which may be a target for future cancer therapies. While the effect if nerve remodeling especially target the new outgrown small nerve fibers has not fully answered. It indicates that small nerve fibers crosstalk with other components among tumor microenvironment (TME) participating the interaction in cancer progress.

Chapter 2 propose our point of view in the potential mechanism of nerve fibers to be used as powerful biomarker for prognosis, as a tool to stratify patients for therapy or as a target in a (combination) therapy. Taking the presence of nerves into account can potentially impact the field of personalized care in PDAC and CCA.

The role of nerve fibers in PDAC

Chapter 3 presents our retrospective study about the influence of nerve fibers on PDAC progression. This study aims to explore whether NFD is associated with oncological outcomes of PDAC patients after surgery. In the study design, NFD is determined as the number of small nerve fibers, not to be confused by nerves which are invaded by tumor cells. We differ nerve fiber and perineural invasion through the size of neural trunk. Single immunohistochemistry is used to define specific immune cells which are co-localized with the nerve fibers. The results show high NFD is associated with a better survival in PDAC while NFD is not associated with tumor invasion. In addition, these small nerve fibers are located around lymphoid aggregates, most of which is CD20+.

Conclusion from **Chapter 3,** nerve fiber can be the potential biomarker to improve clinical outcome by a rational development of new therapeutic strategies. Though there are still need more studies to confirm this positive function of nerve fiber in PDAC, we expect that these results would provide reference for further studies, even be broadly applicable to other malignancies.

The role of nerve fibers in pCCA

Chapter 4 presents a retrospective study about the role of nerve fibers in CCA cancer progression. Based on the results of PDAC cohort that NFD is linked to oncological outcomes, we aim to investigate nerve fiber as a prognostic marker in a large European cohort of pCCA patients undergoing surgical resection. In this study design, extensive group comparisons between patients with high and low NFD were carried out. The associations of cancer-specific survival (CSS) and recurrence-free survival (RFS) with nerve fiber and other clinic pathological characteristics are assessed using univariate and multivariable cox regression models. Results suggest patients with high NFD have better CSS and RFS. Furthermore, this study conducts immunohistochemistry and identifies the small nerve fibers to be of parasympathetic origin. **Chapter 4** suggests that nerve fiber is identified as an important novel prognostic biomarker in pCCA patients, a high NFD is associated with a better survival outcome. Nerve fiber alone or in combination with nodal status in particular allows to stratify pCCA patients based on their risk for inferior oncological outcomes after curative-intent surgery.

Based on clinical observation and results from **Chapter 4**, we further aim to explore differences in immune cell populations and survival between high and low NFD patients. **Chapter 6** applied multiplex

immunofluorescence (mIF) on pCCA patients and investigated immune cell composition in the tumor microenvironment (TME) of high and low NFD. Group comparison and oncological outcome analysis was performed. Results show high CD8+PD-1 expression is related with better overall survival and RFS. Thus High CD8+PD-1 expression is further identified as an independent predictor of overall of pCCA patients.

The role of nerve fibers in iCCA

Chapter 5 aims to determine the role of nerve fiber in iCCA. Therefore, the impact of nerve fibers on long term survival is investigated in a large European cohort of patients with iCCA who were treated by curative-intent surgical resection. By univariate and multivariate statistics, the absence of nerve fibers is determined to be an independent predictor of impaired long-term survival. A group comparison between patients with and without nerve fibers show a better outcome in patients with nerve fibers compared patients without nerve fibers. **Chapter 5** suggests the presence of nerve fibers in the TME of iCCA might be as a novel and important prognostic biomarker in patients.

Abbreviations

CCA Cholangiocarcinoma

CSS Cancer-specific survival

ICCA Intre hepatic Cholangiocarcinomai

NFD Nerve fiber density

PCCA Perihilar Cholangiocarcinoma

PDAC Pancreatic cancer

RFS Recurrence-free survival

TME Tumor microenvironment