Cancer Immunotherapy 2

Markus Moehler
Friedrich Foerster *Editors*

Immune Strategies for Gastrointestinal Cancer

Springer

Cancer Immunotherapy

Volume 2

Series Editor
Matthias Theobald
III Department of Internal Medicine
Johannes Gutenberg University of Mainz
Mainz, Germany

Each volume of the series Cancer Immunotherapy will focus on one specific cancer covering scientific aspects as well as clinical applications including clinical trials and new drugs once immunotherapy for these cancers has reached the clinic.

Markus Moehler • Friedrich Foerster
Editors

Immune Strategies for Gastrointestinal Cancer

Springer

Editors
Markus Moehler
First Department of Medicine, Research Center for Immunotherapy (FZI)
University Cancer Center Mainz, University Medical Center of the Johannes Gutenberg University Mainz
Mainz, Germany

Friedrich Foerster
First Department of Medicine
University Cancer Center Mainz, University Medical Center of the Johannes Gutenberg University Mainz
Mainz, Germany

ISSN 2662-8384 ISSN 2662-8392 (electronic)
Cancer Immunotherapy

ISBN 978-3-031-39943-5 ISBN 978-3-031-39944-2 (eBook)
https://doi.org/10.1007/978-3-031-39944-2

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Paper in this product is recyclable.

Immunotherapy in GI Cancers: Introduction

In the twenty-first century, the coming-of-age of immunotherapy has been the most relevant development for the field of cancer medicine. Particularly the class of immune checkpoint inhibitors has produced astonishing results in clinical trials in various (neo-)adjuvant or metastatic settings across numerous cancer entities. Consequently, the Nobel Assembly at Karolinska Institutet awarded the 2018 Nobel Prize in Physiology or Medicine to James P. Allison and Tasuku Honjo for their "discovery of cancer therapy by inhibition of negative immune regulation." The most prominent checkpoint inhibitors are atezolizumab, durvalumab, ipilimumab, nivolumab, and pembrolizumab, which have been commercially highly successful, generating more than 20 billion USD in sales in 2022.

While immunotherapy has produced impressive results in cancers such as non-small-cell lung cancer and melanoma, it has unfortunately been less effective in gastrointestinal (GI) cancers. In fact, initial results of clinical trials testing checkpoint inhibitors as monotherapy or in combination with chemotherapy in unselected patients with metastatic GI cancers were disappointing. However, it has become clear that selected patient populations present excellent responses, such as metastatic colon cancer patients with microsatellite instability which has led to the approval of pembrolizumab by the U.S. Food and Drug Administration and the European Medicines Agency in the first-line setting and of nivolumab and ipilimumab in the second-line setting. Many indications have followed since, giving patients with biliary tract, esophageal, gastroesophageal, gastric cancer, and hepatocellular carcinoma access to immunotherapy. This has ensued changes in the treatment landscape of these diseases at an unprecedented scale and speed.

Current and future clinical trials are exploring different kinds of immunotherapy in various settings both alone and in combination with other treatments, which will have a strong impact on the management of patients with GI cancers in the future. Their success will depend on patient selection and study design. Innovative combinations will most likely create the greatest value.

This book aims to provide the reader with an overview of the current standard-of-care of patients with GI cancers paying particular attention to the current role of immunotherapy. In addition, the perspective chapters offer the reader an outlook on the future of immunotherapy for the different entities including forms of treatment that are currently at an early stage of clinical development.

In the rapidly developing world of cancer immunotherapy, it may seem inadequate to use the medium of a book, which can only capture the knowledge and ideas as at the time of writing. However, given the multitude of therapies in clinical development for the different GI cancer entities, we deemed it overdue to assemble a book devoted to the current roles and future potentials of immunotherapy in the treatment of GI cancers.

We have been very fortunate to gather a circle of highly distinguished authors who share their expert knowledge and experience in the following chapters. We owe them a great debt of gratitude for their contributions and their commitment in bringing this book to life.

Cancer immunotherapy is currently one of the most exciting topics in medicine and will most likely remain so in the coming years. We are confident it will deliver on its promise of improving the treatment options and the prognosis of GI cancer patients. Our book has attempted to capture this enthusiasm for making it an insightful and worthwhile read. We truly hope that you, the readers, will be inspired by it and that it will help to strengthen your motivation for clinical research and daily practice.

First Department of Medicine
University Medical Center of the
Johannes Gutenberg University Mainz
Mainz, Germany

Friedrich Foerster
Markus Moehler

Contents

Part I

Role of the Tumor Microenvironment

An Overview of the Tumor Microenvironment and Response to Immunotherapy in Gastrointestinal Malignancies

Cameron J. Herting and Gregory B. Lesinski

Contents

Abstract

The activity of the immune system under homeostasis and disease states is governed by complex interactions of cells both through direct cell–cell contact and the secretion of soluble immunomodulatory factors. In cancer, the tumor microenvironment is increasingly being recognized as a key mediator of these interactions. The tumor microenvironment consists of a diverse milieu of malignant and stromal cells that typically constitute the tissue, as well as both tissue-resident immune cells and those that infiltrate from the circulation. It is now clear that

C. J. Herting · G. B. Lesinski (✉)
Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, USA
e-mail: gregory.b.lesinski@emory.edu

Cancer Immunotherapy (2021)
https://doi.org/10.1007/13905_2021_1,
Published Online: 14 August 2021

defining factors that govern the balance between heterogeneous cell populations within the microenvironment can advance our understanding of disease progression across different malignancies. In addition, this knowledge can be leveraged to improve clinical outcomes in patients with tumors that are traditionally refractory to immunotherapy. As the development of immunotherapeutic anticancer modalities has accelerated over the past decade, it has become abundantly clear that we must investigate and understand these interactions to rationally design effective treatment regimens. In this chapter, we will outline the major players within the tumor microenvironment across prevalent gastrointestinal malignancies. Emphasis will be placed on cell types, such as fibroblasts, that play a substantial role in shaping the dynamic microenvironment of gastrointestinal cancers. We will also describe the differential response of selected gastrointestinal cancers to immunotherapy and illustrate how the microenvironment influences these responses. This chapter will provide the reader with a fundamental overview of the tumor microenvironment and immunotherapy in gastrointestinal malignancies.

Keywords

Cancer · Fibroblast · Gastrointestinal · Immunotherapy · Inflammation · Macrophage · Microenvironment · T cell

1 An Overview of Gastrointestinal Malignancies

Cancer is ubiquitously present within all tissues of the human body. Depending on the location of origin, tumors present with variable severity and frequency. This chapter will offer an introductory journey through the GI tract to highlight the types of tumors that are frequently found at each location. Next, we will discuss in greater detail the cellular players present within the tumor microenvironment and their relevance to immunotherapy.

The gastrointestinal tract is a series of organs that form a non-interrupted pathway from the mouth to the anus (Fig. 1). Epithelial cells line these organs and are subject to constant exposure to environmental stimuli that pass through the body via the natural digestive process. While these cells are typically well-equipped through DNA repair or apoptotic mechanisms to deal with various molecular insults, they are also susceptible to transformation, which can be further fueled by inflammatory conditions [1–3]. Organs such as the liver, gallbladder, and pancreas are also considered GI organs as they secrete enzymes into the GI tract and actively participate in digestion. Each of these organs can be afflicted with primary tumors and further can be common sites of metastatic spread of tumors from various anatomic origins. In this introduction, we will discuss the primary tumors present at each location.

The upper part of the GI tract can be defined as the mouth, esophagus, stomach, and the duodenum. For the purposes of this chapter, we will refrain from discussing

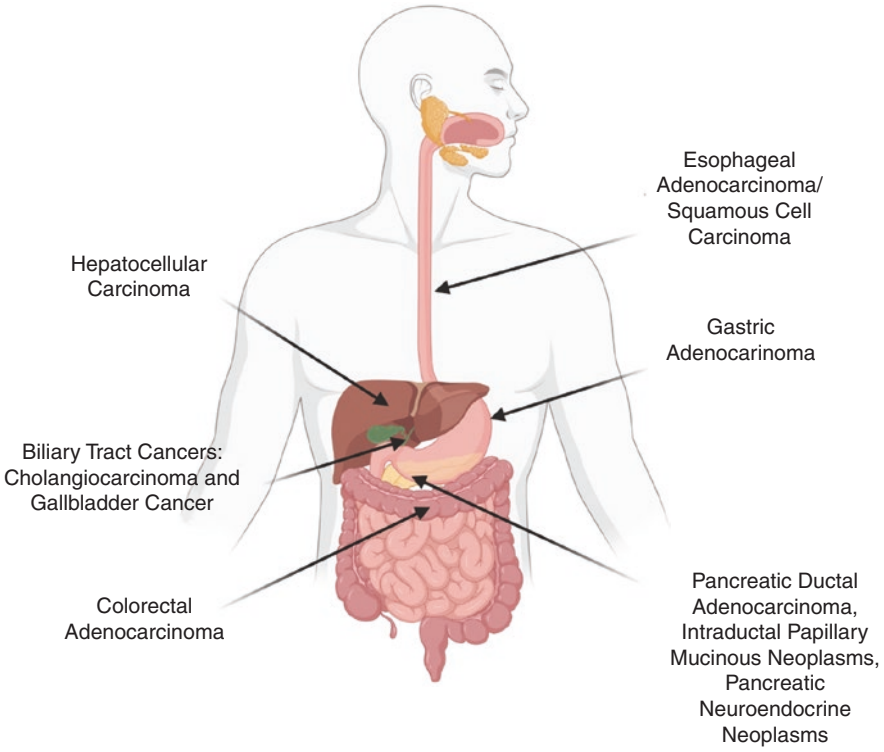

Fig. 1 Common sites of cancer development in the gastrointestinal tract. Common sites of cancer development are listed with arrows directed towards where the tumors are found. This list is meant to diagram the most frequently occurring lesions and is not a comprehensive list

cancers of the mouth. The esophagus, the first organ of the GI tract, is afflicted primarily with esophageal cancer (EC), a term that encompasses both squamous cell carcinoma and adenocarcinoma, diseases with a relatively poor 5-year survival rate of 15–20% [4]. The next location in the GI tract that is a common site for primary tumors is the stomach, where gastric cancer (GC) can arise. This disease presents a notable global health burden, landing in the top five cancers with respect to both disease incidence and cancer-related deaths [5]. Proximal to the stomach are the liver and pancreas. These organs are intimately connected to the GI tract and can also give rise to multiple individual types of cancer. Within the liver, there are two main types of primary tumors evident. First, hepatocellular carcinoma (HCC) is a cancer originating from hepatocytes, and can be driven by several factors including chronic liver inflammation from viral infection, typically with hepatitis B virus or hepatitis C virus, non-alcoholic fatty liver disease, or chronic alcohol and/or drug use [6–8]. Second, are biliary tract cancers (BTCs) which encompass cholangiocarcinoma (CCA) and gallbladder cancer, which arise in epithelial cells that constitute the biliary tree [9]. Based on anatomic location, CCA can be further divided into intrahepatic, extrahepatic, and hilar tumors. Similar to the liver, the pancreas also

functions as an exocrine organ responsible for delivering digestive enzymes into the duodenum that facilitate the breakdown of food as it exits the stomach and enters the lower GI tract. The pancreas is the site of pancreatic ductal adenocarcinoma (PDAC), a cancer primarily afflicting the acinar cells responsible for producing the pancreatic digestive enzymes, intraductal papillary mucinous neoplasms (IPMN), and rare pancreatic neuroendocrine neoplasms (pNEN) that compromise only 1–2% of total pancreatic tumors [10–12]. The next location within the GI tract is the small intestine, where over forty different subtypes of tumors arise although they only account for 3–6% of total intestinal tumors. Typically these tumors are managed by operative resection with survival rate dependent on disease subtype and metastatic spread [13]. Due to the large variety of tumor subtypes, relatively small incidence, and limited information regarding tumor microenvironment and immunotherapy, we will not discuss these tumors further. The distal portion of the GI tract harbors the colon and rectum, which are frequent sites of tumorigenesis. In particular, colorectal cancer (CRC) has emerged with increasing frequency attributable to modifiable risk factors, primarily in the western world, and contributes substantially to cancer-associated mortality with near to 1 million deaths per year [14].

Cancers within the GI tract are often difficult to detect due to an absence of symptoms until late-stage disease, limited biomarkers of early stage neoplasms, and invasive as well as expensive screening tools [15]. The high mortality rates of GI malignancies, in particular PDAC, and BTC can be largely attributed to lack of detection until late-stage disease [16, 17]. By and large, surgical resection remains the only curative approach to treating these malignancies. Late-stage tumors where metastatic disease is present negate the possibility of surgical resection, thereby significantly reducing the likelihood of curing the patient. There is, however, a bit of gray area where chemotherapy can be leveraged to allow for surgical approaches to be employed. For example, in locally advanced and borderline resectable PDAC, neoadjuvant FOLFIRINOX can decrease tumor burden to a point suitable for surgical resection [18]. In total, the optimal treatment regimen for GI tumors is likely to be one that combines pharmacological and surgical therapeutic approaches. The coming years will elucidate the role that microenvironment-targeted therapies and immunotherapies play in GI oncology.

2 Key Players in the Tumor Microenvironment

The establishment of a tumor-supportive cellular microenvironment is a major factor that influences the progression and therapeutic response of GI neoplasms. The tumor microenvironment (TME) can be defined as the combination of various cell types that constitute the tumor mass. The composition of the TME varies based on tumor type and location, differs between patients, and even within different areas of single tumors. Cross-talk between tumor and stromal cells influences the development and progression of GI tumors. An intuitive way to conceptualize this was put forth by Dvorak with his description of cancer as “a wound that never heals” [19]. In essence, this description captures the dynamic environment within a tumor,

where stromal cells and immune cells are constantly interacting in a manner that prevents further cell death and tissue damage. At early stages of tumor development, tumor cells secrete damage-associated molecular patterns (DAMPs), for example, interleukin-1α (IL-1α), that initiate a wound healing response [20, 21]. By late-stage disease, it is common to find a tumor microenvironment characterized by abundant immunosuppressive cell populations, extensive deposition of extracellular matrix proteins, and limited infiltration of immune cell populations with cytotoxic potential against tumors [22, 23]. This microenvironment centers around the activities of numerous stromal cell, tissue-resident immune cell, and infiltrating immune cell populations will be described herein (Fig. 2).

Perhaps the most widely studied, appreciated, and therapeutically targeted cell type within the tumor microenvironment is the cancerous cells. In tumors, the malignant cells are those with acquired mutations that drive aberrant replication [24]. These mutations can be those that activate oncogenic signaling pathways, inactivate or downregulate tumor suppressor genes, alter the epigenetic landscape of the tumor cells, and more [25]. Within the tumor microenvironment, cancer cells are initially responsible for dictating how the surrounding tissue responds. In essence, the cancer cells secrete factors that drive how the nearby cells within the tissue, and immune cells from the periphery, initially respond. For example, in PDAC cancer cells produce factors including transforming growth factor-β (TGF-β), interleukin-1α/β (IL-1α/β), fibroblast growth factor 2 (FGF2), and platelet-derived

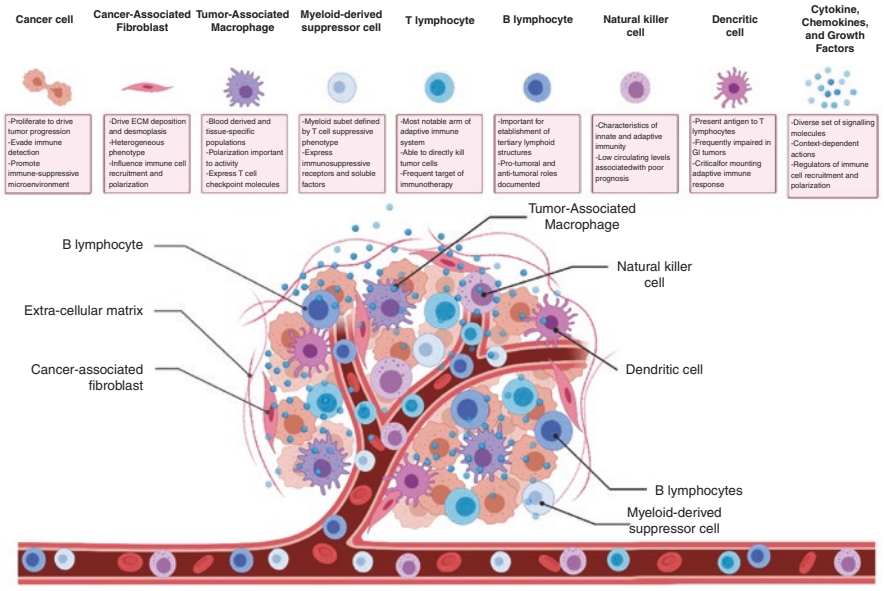

Fig. 2 Major cellular and soluble components of the tumor microenvironment in gastrointestinal malignancies. Here, the most common and well-studied cellular components of the gastrointestinal tumor microenvironment are listed. This figure is not exhaustive, and excludes cellular subsets, such as eosinophils, innate lymphoid cells, and mast cells, that may also play a role

growth factor (PDGF) that cause quiescent pancreatic stellate cells (PSCs) to activate into cancer-associated fibroblasts (CAFs) that produce substantial extracellular matrix proteins and drive the formation of PDAC-associated desmoplasia [26–29]. Further, as the tumor microenvironment is established, the stromal compartments begin secreting factors that modulate the behavior of the cancer cells. Again, considering PDAC as an example, CAFs also regulate cancer cell metabolism under conditions of glucose deprivation and secrete factors such as interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) that drive cancer cell invasion as well as differentiation of myeloid cells into immunosuppressive phenotypes [30–34]. Taken together, there is a clear cross-talk between cancer cells with the stromal and immune components of tumors throughout all stages of tumor development that mold the composition and behavior of the TME.

3 Cancer and Stromal Cell Cross-Talk: Role of Epithelial to Mesenchymal Transition

In addition to cancer and stromal cell cross-talk, there are intrinsic characteristics of tumor cells that are important to consider. For example, during tumor progression, it is common for cancer cells to activate transcription factors such as SNAIL and TWIST that cause the cells to undergo the epithelial to mesenchymal transition (EMT), a process that is typically observed during embryogenesis [35]. It should be noted that this is a dynamic and reversible process and cells within tumors are commonly observed on a spectrum between the two cell phenotypes [36, 37]. Specific to GI cancers, this EMT has been shown to facilitate the ability of malignant cells to resist treatment and metastasize in PDAC and CRC [38, 39]. As a result, EMT has become a notable target in a variety of malignancies [40]. An important factor that dictates the overall cancer cell phenotype and behavior is the cell of origin from which the cancer arises. In both PDAC and CRC, there exists some debate on the identity of the cell of origin and it is perhaps possible that it varies from patient to patient [41, 42]. For example, in CRC, genetic models suggest that the intestinal stem cells within the crypts of the intestinal villi are the cell of origin [43–45]. However, it has also been shown that non-stem cells within the intestine can acquire stem-like properties and serve as the cell of origin in subsets of CRC [46, 47]. In PDAC, the debate revolves around whether the ductal or acinar cells are the cell of origin. Conventional wisdom defined ductal cells as the cell of origin largely due to the morphology of tumor specimens [48, 49]. Recent work with genetically engineered mouse models, however, has shown that either cell type can be the cell of origin and that ductal-cell and acinar-cell derived tumors display distinct disease progression and morphological characteristics [50]. Future work will more conclusively characterize the roles that EMT and cell of origin play in disease progression and treatment response. It is, however, already clear that both factors play an important role in cancer cell biology.

One stromal cell type that plays a particularly important role across GI malignancies is fibroblasts. Fibroblasts are cells that make up connective tissue and are

primarily responsible for producing extracellular matrix proteins under homeostatic conditions, as well as injury [51]. Activated fibroblasts in cancer are known to modulate resistance to chemotherapy, deposit large amounts of extracellular matrix proteins, drive angiogenesis, and regulate tumor progression [52]. Murine studies have established a prominent and multifaceted role for fibroblasts in the progression of both PDAC and CRC [53–55]. The relationship is more complicated than a simple model whereby CAFs support tumor growth. In fact, prior studies have shown that depletion of α-smooth muscle actin (α-SMA)-positive CAFs, or blockade of CAF-activating sonic hedgehog (SHH) signaling, leads to accelerated tumor progression in PDAC and CRC, respectively [56–58]. The role of CAFs in GI cancers is further muddled when one considers their heterogeneity. Recent work in PDAC has identified multiple different subtypes of CAFs with distinct roles and actions within the tumor microenvironment [59, 60]. The two largest phenotypically defined populations of CAFs appear to be those polarized by IL-1α and TGF-β and they appear to be associated with immune cell chemotaxis and ECM deposition, respectively [61]. Characterization of CAF heterogeneity has arguably advanced the furthest in PDAC, however is likely relevant to tumors of other anatomic origins. In addition to their cross-talk with cancer cells, CAFs also communicate with immune cell populations that are prominent in GI malignancies. For example, IL-6 produced by CAFs is known to prevent infiltration of anti-tumoral T cells in PDAC and CAF-derived IL-8 promotes accumulation and polarization of pro-tumoral M2 macrophages in CRC [62, 63]. Further, CAF-derived TGF-β has been shown to attenuate the cytotoxic potential of $CD8^+$ T cells as well as suppress natural killer (NK) cell function [63–65]. In total, CAFs are a diverse component of the tumor microenvironment in GI malignancies. They represent a heterogeneous population with both pro- and anti-tumoral properties. Ongoing work will illuminate strategies that target CAFs to enhance the efficacy of anti-tumoral immunotherapy.

4 Tumor Microenvironment: The Role of Tumor-Associated Macrophages (TAM)

Another prominent component of the tumor microenvironment in GI malignancies is tumor-associated macrophages (TAMs). TAMs can be derived from both circulating monocytes and tissue-resident macrophage populations. For example, in HCC, TAMs both infiltrate from the circulation and arise from liver-resident Kupffer cells [66, 67]. Further, tissue-resident TAMs derived from embryonic hematopoiesis play a distinct role in the progression of PDAC [68]. That is not to say that circulating TAMs do not play a role as $CCR2^+$ macrophages recruited to PDAC as well as liver metastases of CRC have been shown to contribute to resistance to chemotherapy and confer an overall poor prognosis [69, 70]. TAMs are also a heterogeneous population of cells with respect to their gene expression profile and phenotype. TAMs are often described as polarized to either M1 or M2 phenotypes, with the former considered anti-tumoral and the later pro-tumoral [71, 72]. It should be noted, however, that this polarization scheme was derived from early *in vitro* experiments comparing

the response of macrophages to specific stimuli as well as their metabolic profiles in different murine strains. As a result, the polarization of macrophages in complex *in vivo* situations such as a tumor is likely much more complex than a simple M1 to M2 spectrum [73–75]. This polarization scheme, while imperfect, still offers a valuable conceptual framework considering the multifaceted role of macrophages in GI malignancies. TAMs can promote angiogenesis, drive a metastatic tumor cell phenotype, and suppress anti-tumoral T cells through expression of checkpoint molecules such as PD-L1 in CRC [76–78]. Other studies, however, have shown that increased infiltration of macrophages in CRC is associated with improved patient outcomes [79]. Transcriptional analysis of these macrophages indicates that increased levels of M1-like cells, particularly those that express high levels of immune checkpoints like PD-L1, LAG-3, and TIM-3 are responsible for this positive correlation [80, 81]. Agonism of CD11b has also been investigated as a strategy to sensitize PDAC to immunotherapy through reprogramming of innate immunity and repolarization of TAMs [82]. In total, the role of TAMs in GI cancers depends substantially on the phenotype of the cells. Considering their highly plastic nature, therapies that aim to re-educate or re-polarize TAMs as opposed to depleting them are more likely to have beneficial therapeutic effects.

5 New Interest in Cancer Immunology: Myeloid-Derived Suppressor Cells (MDSCs)

In addition to TAMs, other myeloid cell population that are of interest in cancer immunology are myeloid-derived suppressor cells (MDSCs) that, as the name suggests, are largely immunosuppressive. MDSCs were initially defined as cells sharing the CD11b and Gr-1 (Ly6C and Ly6G in mice) surface epitopes [83, 84]. The terminology MDSC arose from the observation that these cells are of myeloid origin, systemically expand in the context of cancer, and exert immune-suppressive function and were simply meant to describe the phenotype of these cells, not define a novel or distinct cell population [85]. It is now apparent that the MDSC cell subsets encompass at least two main populations, polymorphonuclear MDSCs (PMN-MDSC) and monocytic MDSCs (M-MDSC) with distinct cellular characteristics, but overlapping functions [86]. MDSCs are pathologically activated myeloid cells that arise from situations of chronic inflammation as observed in tumors [87]. Due to the aberrant and chronic activation of inflammatory pathways that are otherwise transient in nature, MDSCs mature into cells that are incapable of carrying out the normal phagocytic functions of myeloid cells, but are potent suppressors of T and NK cell-mediated cell killing [88–90]. It therefore comes as no surprise that a major role for MDSCs in the tumor microenvironment is suppression of anti-tumoral T cell immunity. MDSCs are recruited to tumors through similar chemotactic pathways to those that recruit monocyte-derived TAMs, including CCL2 and CCL5 [91, 92]. Within the tumor, MDSCs upregulate various immunosuppressive pathways and receptors through activation of HIF-1α [93]. Further, in CRC it has been demonstrated that MDSCs produce large amounts of reactive oxygen species (ROS) and

nitric oxide (NO), both of which are immunosuppressive [94, 95]. MDSC can also exert immune suppression by a number of functional mediators. For example, in addition to the production of NO and ROS, or expression of inhibitory checkpoint ligands (i.e. PD-L1), they can also modulate the metabolic features of tumors through secretion of arginase, and deprivation of cysteine needed for optimal T cell function and survival [96–98]. Further, they are a prominent source of cytokines including IL-10 that can modulate dendritic cell function and antigen presentation, or IL-6 and TGF-β that modulate T cell function. In total, MDSCs are a major immunosuppressive cell type in the tumor microenvironment when considering their impact on activity of T lymphocytes and NK cells. It comes as no surprise then, that the presence of MDSCs is considered tumor-supportive in both CRC and PDAC [99, 100]. Therefore, strategies to deplete their presence, inhibit their individual functional mediators, or block their infiltration into the tumor microenvironment are suitable therapeutic avenues to target this population.

6 Adaptive Immunity in Cancer Immunotherapy

Among the most prominent cell type of relevance to mechanisms of cancer immunotherapy are T lymphocytes. T cells, so named due to their origin in the thymus, can differentiate into a multitude of phenotypic and functional subsets and represent the largest constituent of the adaptive immune system [101]. Similar to the other cell types discussed in this section, T lymphocytes are highly plastic cells with a variety of activation states governed by engagement of the T cell antigen receptor (TCR), co-stimulatory receptors, and immune checkpoint receptors [102]. All T lymphocytes are characterized by expression of the CD3 receptor [103]. This receptor is responsible for associating with the TCR and transmitting its signal via intracellular cascades within the T cell [104]. In addition to CD3, T lymphocytes can be further sub-divided by their expression of the CD4 and CD8 surface receptors that classify the cells as helper T cells and cytotoxic T cells, respectively [105, 106]. During development, progenitors from the bone marrow enter the thymus lacking expression of CD4 and CD8. They then undergo TCR rearrangement and emerge as $CD4^+CD8^+$ double-positive thymocytes that further differentiate into $CD4^+$ or $CD8^+$ cells following selection [101]. In general, $CD8^+$ T cells are those tasked with cytotoxic function while $CD4^+$ T cells facilitate this action by supporting $CD8^+$ T cell engagement with antigen-presenting cells and producing cytokines [107–109]. The process of T cell engagement and activation can be considered to be a three-step process [110]. First, the TCR engages an antigen-major histocompatibility complex (MHC) conjugate on the surface of antigen-presenting cells, leading to conformational change of the CD3 complex and thymocyte activation [111]. For complete activation, the T cell must then be co-stimulated by receptors such as CD28 [112, 113]. Following co-stimulation, T cells express cytokines such as interleukin-2 (IL-2) that drive cell proliferation and expansion [114, 115]. These general steps underlie essentially all T cell-mediated immunity in both $CD4^+$ and $CD8^+$ cells. A subset of $CD4^+$ T cells, T regulatory (T_{reg}) cells are also responsible for resolving the

cytotoxic T cell response and managing T cell memory formation, largely through regulating IL-2 levels [116, 117]. T cell activity is also regulated by a variety of immune checkpoint receptors (e.g. PD-1) that are upregulated upon activation and prevent excessive adaptive immune responses [118]. These receptors will be discussed in detail in the next section discussing immunotherapy in GI malignancies.

T cell infiltration into tumors is prognostic in a variety of GI malignancies including HCC, gastric cancer, PDAC, and CRC [119–122]. By and large, these studies have shown that high amounts of T lymphocytes within tumors are associated with improved patient outcomes. Conversely, it is recognized that immunologically "cold" tumors, characterized by very limited T cell infiltration, have universally poor prognoses. This is exemplified by PDAC which is considered an immune "desert" with T cell infiltration largely limited to the tumor border and a 5-year survival rate of <10% [123]. Conversely, esophageal cancer (EC) is known to harbor larger amounts of tumor-infiltrating T cells and these have also been shown to be specific to antigens expressed by EC tumor cells including MAGE-A3 and NY-ESO-1 [124, 125]. This raises another significant point, for successful anti-tumoral T cell immunity, T cells must be able to infiltrate the tissue, they must be able to recognize a suitable tumor-associated antigen, and they must not be sufficiently restrained by checkpoint molecules or other mediators. In total, T cells represent a major tumor microenvironmental component that cancer immunotherapy often aims to manipulate.

In addition to T lymphocytes, B lymphocytes are also present in the tumor microenvironment from several GI malignancies, albeit at relatively low frequency. Their activity within the microenvironment remains somewhat controversial and incompletely defined [126–128]. For example, classical studies indicated that mice lacking B cells exhibited enhanced anti-tumor immunity when compared to those with intact B cells [129]. Further studies, including those utilizing the MC38 model of CRC, illustrated that cytotoxic T cell responses and T_H1 cytokine profiles were increased in mice lacking B cells [130]. In fact, it was even illustrated that mice lacking mature B cells were less likely to develop inflammation-associated cancer [131]. In total, these results were interpreted as evidence that B lymphocytes are largely tumor-promoting. Recent work in GI malignancies, however, is questioning these results. In patients with gastric and esophageal adenocarcinomas, immunohistochemical analysis of IGKC, a marker of a B cell subset called plasma cells, indicated that patients with increased infiltration of plasma cells had prolonged overall survival following surgical resection [132]. Similar results were obtained in CRC where a higher density of $CD20^+$ B cells and $IGKC^+$ plasma cells were both associated with improved overall survival [133]. These conflicting results beg the question, what exactly do B cells do in the tumor microenvironment?

B cells play three major roles within the tumor microenvironment. First, they serve as a subset of antigen-presenting cells, thereby facilitating T cell differentiation, expansion, and activation [134, 135]. Second, B lymphocytes in cancer immunity participate in the formation of tertiary lymphoid organs. These structures form at peripheral sites of inflammation and are characterized by separated T and B cell locations, activated dendritic cells, and generation of lymphatic vessels [136, 137].

These tertiary lymphoid structures seem to serve similar purposes to secondary lymphoid structures, such as lymph nodes, as removal of the latter does not impair formation of a memory T cell response [138]. Specific to CRC, it has been demonstrated that formation of tertiary lymphoid structures directly correlates with T cell infiltration and predicts better prognosis of early stage disease [139]. Based on these data, it might be concluded that B cells in the tumor microenvironment support antitumoral T cell immunity through formation of tertiary lymphoid structures. However, in PDAC, it has also been shown that particular B cell subsets expressing interleukin-35 (IL-35) contribute to tumor growth through polarization of TAMs to a tumor-supportive phenotype [140, 141]. This data illustrates the final role for B cells in the tumor microenvironment which is production of chemokines and cytokines. Further, the secretion of IL-35 by B cells may complement tumor-derived IL-35 that has also been noted in PDAC tumors [142]. Overall, B cells have seemingly diverse and context dependent roles within the tumor microenvironment. Further work must define these roles with greater certainty to allow for effective pharmacological targeting of this cell subset for cancer immunotherapy.

7 Natural Killer (NK) Cells as a Novel Frontier in Cancer Immunotherapy

Natural killer (NK) cells are a cell type known to bridge the adaptive and innate immune response. These large granular lymphocytes lack the ability to rearrange T cell receptor or immunoglobulin domains, like T and B cells, respectively, and instead are able to "naturally kill" target cells without priming or engagement of MHC molecules [143]. NK cells are able to recognize and kill adjacent cells that express surface makers associated with oncogenic transformation [144]. As such, NK cells are sentinel mediators of cancer immunosurveillance [145]. One example is the capability of NK cells to eliminate oncogenically transformed cells that have downregulated or eliminated expression of MHC class I. This molecule typically prevents NK cell activation through engagement of the killer cell immunoglobulin-like receptors [146, 147]. Furthermore, individuals with high levels of NK cell cytotoxicity are at reduced risk of cancer and conversely, those with defects in NK cell activity display higher rates of cancer [148, 149]. In the setting of CRC, higher levels of circulating NK cells are associated with a positive prognosis, as assessed by overall survival [150]. This epidemiological evidence strongly implies that NK cells play a pivotal role in surveillance for and elimination of malignant cells. As a result, NK cells have become a notable substrate for cell-based therapy in PDAC [151]. The common NK cell-based therapeutic modalities typically involve extracting cells, expanding them *ex vivo*, and reinfusing them into the patient. Relevant to this strategy, low levels of circulating NK cells have been tied to a lack of CXCR2 expression and low levels of *ex vivo* NK cell expansion are associated with reduced overall survival [152]. Within the tumor microenvironment, NK cells are thought to serve as a link between the innate and adaptive immune system. They secrete cytokines that modulate the activities of both dendritic cells and T cells [153, 154].

Considering the scarcity of NK cells within tumors, in some cases they constitute less than 1% of total cells, it has been challenging to study these cells and solidify the specific actions they perform and roles they play within the tumor microenvironment. Future work, likely leveraging cutting-edge single-cell technologies will further delineate the niche these cells occupy and illuminate how they can be leveraged therapeutically across GI malignancies.

8 Dendritic Cells (DCs) Link the Innate and Adaptive Immune System

Dendritic cells (DCs) play an important role as a focal point for communication between the innate and adaptive immune systems. DCs are notable for their ability to present antigen to naïve T cells and induce their activation [155]. They achieve this by continuously sampling their environment, capturing and processing foreign antigens or irregular self-antigens, and displaying these antigens to T cells with MHC molecules [156]. Further, upon exposure to a "danger" signal, for example, a peptide associated with a foreign microbe, DCs will migrate to the lymph node where they induce the expansion of T lymphocytes via engagement of co-stimulatory molecules and polarize them into either T_H1 or T_H2 cells via cytokine signals [157]. In addition to their roles in disease, DCs play a key role in tolerance through the deletion of self-reactive T cells [158]. In the context of gastrointestinal tumors, DCs appear functionally impaired, and unable to effectively induce an adaptive immune response [159, 160]. For example, in patients with both CRC and PDAC, circulating DCs are decreased compared to patients without tumors [161, 162]. In the PDAC cohort, higher levels of circulating and tumor-infiltrating DCs were associated with prolonged survival following surgical resection. Moreover, it has been shown that CRC tumor cells express immunosuppressive factors, such as 2, 3 indolamine dioxygenase (IDO), that actively dampen the ability of DCs to cross-present antigen to T cells [163]. It is clear that DCs play a critical role in establishing a sustained antitumoral adaptive immune response. By and large, however, tumors develop the capabilities to suppress DC maturation and activities, thereby enhancing their ability to escape immune detection and elimination. Future therapies should target these mechanisms to enhance DC activity within tumors, particularly in situations when T cell-targeted immunotherapy is employed.

9 Targeting the Tumor Microenvironment to Enhance Clinical Immunotherapy

The tumor microenvironment is a complex mixture of different cell types, both neoplastic and non-neoplastic as well as immune and non-immune, that largely communicate through the secretion of soluble factors. The most prominent of these factors are chemokines, cytokines, and growth factors that mediate immune cell recruitment, differentiation, and activity. There are hundreds of these factors that act in concert to

coordinate these actions, a thorough discussion of which is beyond the scope of this introductory chapter. There are a few, however, that have come to prominence as key mediators of the tumor microenvironment in gastrointestinal malignancies. For example, in PDAC, interleukin-6 (IL-6) has emerged as a factor that can be secreted from stromal cells, tumor cells, or infiltrating myeloid cells [164–166]. This cytokine drives the desmoplastic stromal response and suppresses the activity of cytotoxic T cells [165, 167, 168]. Moreover, high levels of circulating IL-6 in PDAC are a predictor of poor prognosis, and IL-6 neutralization enhances the response to both chemotherapy and immunotherapy in preclinical PDAC models [62, 169, 170]. This evidence has led to the initiation of clinical trials studying the combination of IL-6 neutralizing therapy with both chemotherapy (EudraCT: 2016–000643-13) and immune checkpoint blockade (NCT04191421). Furthermore, IL-6 derived from hepatic stellate cells can drive progression of HCC and induce expansion of MDSCs [171, 172]. In addition to IL-6, the chemokine CCL2 has been identified as a key regulator of the tumor microenvironment in gastrointestinal malignancies, largely through its actions as a myeloid cell chemoattractant protein. The role of these recruited myeloid cells, however, appears to be somewhat context dependent. For example, CCL2 secreted by PDAC tumor cells following radiation therapy recruits myeloid cells to the tumor. Subsequently, these cells promote tumor proliferation and neovascularization, largely tumor-supportive actions [173]. Following CD40 agonism, however, CCL2 and interferon-gamma (IFNγ) cooperate to recruit myeloid cells that degrade the fibrotic extracellular matrix and sensitize tumors to chemotherapy [174]. Both CCL2 neutralization (NCT02732938) and CD40 agonism (NCT04130854) have been investigated in clinical trials for GI malignancies. Overall, there are a plethora of chemokines and cytokines that guide the composition and behavior of the immune microenvironment in GI malignancies. Many of these are likely to be viable therapeutic targets. Over the coming decade, it is probable that we will see the maturation of these strategies into clinically relevant modalities.

These results in total highlight that it is likely not the actions of individual chemokines and cytokines, or even the actions of individual immune cell subsets that are important in gastrointestinal malignancies. Instead, it is the overall product of these factors acting in concert to either promote or combat tumor progression. As more high-throughput technical assays, like cytometry by time of flight (CyTOF), single-cell RNA sequencing, and highly multiplexed chemokine and cytokine profiling become more widely prevalent, it is likely that we will gain a better understanding of how exactly these factors influence one another, and which factors are key orchestrators of pro-tumoral and anti-tumoral responses. Furthermore, it is possible that novel bioinformatic techniques like machine learning and artificial intelligence algorithms will usher in a new age of personalized medicine where therapeutic approaches are guided by pre-treatment assessment of circulating and tumor intrinsic chemokine, cytokine, and immune cell profiles. As it stands currently, however, we do have at least a basic understanding of the tumor microenvironment and how it dictates response to currently utilized immunotherapies. Finally, we should emphasize that this opening chapter is only meant to provide a basic framework for the reader to understand concepts presented in the remainder of the

book. This is by no means a comprehensive review and there are other cellular subsets and soluble factors that are undoubtedly important components of the tumor microenvironment in GI malignancies.

10 The Tumor Microenvironment and Response to Immunotherapy in GI Malignancies

Major advances in the field of clinical oncology have been made over the past decade through the introduction and clinical approval of immunotherapeutic approaches for cancer therapy. The first reports of strategies that invoke the immune system to attack tumors span to 1891 when William B. Coley applied the idea to inject streptococcal bacteria into a patient with inoperable cancer [175]. He observed notable tumor regression and refined this strategy over the years treating nearly 1000 patients with his "Coley Toxins." This approach demonstrated success in managing the clinical progression of bone and soft-tissue sarcomas. Thankfully, the immunotherapeutic approaches used today are substantially more refined and mainly involve the direct targeting of T cell suppressive immune checkpoint molecules with antibody-based therapies. These targeted therapies have concentrated a great deal on disrupting inhibitory immune checkpoint pathways, including PD-1/PD-L1 and CTLA-4, and have illustrated variable impacts across gastrointestinal diseases. While some tumor types respond well, others illustrate negligible response. Furthermore, it has become apparent that in some cases, subsets of individual tumor types, for example, microsatellite instable CRC, are quite responsive while others are not [176]. The precise biology underpinning this variable response appears to depend on tumor cell intrinsic factors as well as factors surrounding the tumor microenvironment. For example, esophageal cancer remains largely refractory to immunotherapy [177].

Esophageal squamous cell carcinoma is slightly more responsive than adenocarcinoma, but overall response remains low. This limited response has been attributed to low expression of PD-L1 in esophageal cancer, where the cell subsets expressing this marker are of myeloid lineage, and those with >1% of these cells in the tumor display a response rate of ~25%, versus ~15% in those with <1% of these cells [178].

Similarly, ***gastric cancer*** remains minimally responsive to checkpoint blockade with the CheckMate649 trial illustrating an extension of median survival of 2.2 months to just over 1 year in patients that received nivolumab plus chemotherapy versus chemotherapy alone [179]. Overall, checkpoint blockade for gastric cancer and gastro-esophageal junction adenocarcinoma remains second- or third-line therapy following progression on standard chemotherapeutic regimens [180].

PDAC displays similarly limited response to immunotherapy. In this disease context, combination of immunotherapy with chemotherapy has been demonstrated

as safe, but no notable extensions in median survival have yet been demonstrated [181, 182]. Studies of murine models of PDAC have indicated that combination of "traditional" immunotherapeutic approaches like PD-1 and CTLA-4 blockade, or cytotoxic chemotherapy, may be effective when combined with agents that target myeloid components such as CD40 agonism [183]. In fact, a recent clinical trial demonstrated the tolerability and clinical activity of combined CD40 agonism and chemotherapy in metastatic PDAC [184]. Considering the notable immunosuppressive microenvironment in PDAC, it is unsurprising that single-agent immunotherapy has proven unsuccessful. Combination therapies that simultaneously stimulate T cells while additionally remodeling the microenvironment are in preclinical development and may hold promise [185, 186]. Further, groups are investigating combining radiation with immunotherapy, various cell-therapy approaches, and oncolytic viruses in PDAC although these investigations remain at relatively early stages [187–189].

In contrast to PDAC, results in **HCC and CCA** have demonstrated more encouraging results. Single agent PD-1/PD-L1 blockade provides an objective response rate of around 5–20% in CCA [190]. Although still relatively low overall, for CCA, this disease control rate is clinically meaningful. Single agent CTLA-4 and PD-1 blockade have both only shown modest efficacy in HCC [191–194]. It should be noted that in some of these trials although a significant extension in median progression-free survival and overall survival was not observed, a favorable risk-benefit ratio was, supporting the implementation of these approaches in certain settings [194]. Moreover, combination of the anti-PD-L1 therapy atezolizumab with the vascular endothelial growth factor (VEGF) inhibitor bevacizumab has been shown to extend overall and progression-free survival relative to the standard of care sorafenib in a recent phase III clinical trial [195]. This result emphasizes that the most efficacious treatments for GI malignancies are likely to combine immunotherapy with other chemotherapeutic or biologic agents. Certainly, the rise of clinical trials testing these combinatorial approaches within the past decade illustrates the excitement the field has for these modalities.

Patients with some subsets of gastrointestinal malignancies can derive clinical benefit from immune checkpoint blockade alone. For example, patients with **CRC** largely do not respond to immunotherapy [196–198]. However, in early trials assessing PD-1 blockade in solid tumors, including CRC, it was found that a very small subset of patients derived notable clinical benefit from this approach [199]. In particular, patients with microsatellite instable/DNA mismatch repair deficient tumors (MSI-H/dMMR) display durable responses when compared to their "wildtype" counterparts [200]. These patients display tumors with higher mutational burden, thereby offering more antigens for the adaptive immune system to recognize and attack [201]. This concept has further led to the development of an immunoscore that can predict patients with CRC that will respond well to immunotherapy [202]. Moreover, MSI-H/dMMR has proven to be a positive prognostic factor for response to immunotherapy across a wide range of solid tumors [203].

11 Concluding Remarks

In total, it has become apparent over the past decade that immunotherapy offers a fundamental shift in how we treat a variety of cancers. Further, we have learned more about how tumor cell intrinsic properties (e.g. mutational burden) and tumor microenvironmental factors (e.g., extent of PD-L1-positive cells) influence response to this class of therapy. Future work will hone our understanding of these factors, in particular, interactions between microenvironmental stromal and immune cells with tumor cells, and uncover further therapeutic targets that can be leveraged to enhance the efficacy of immunotherapy in the setting of gastrointestinal tumors. Our burgeoning understanding of immunology and the receptors and soluble factors that govern the activation and polarization of immune cell subsets will also direct these efforts and provide novel modalities to advance immunotherapy for gastrointestinal cancer.

The tumor microenvironment clearly plays a role in regulating all stages of tumor development. From the earliest stages, cancer cells, the surrounding stromal cells, and tissue-resident as well as infiltrating immune cells converse through a variety of mechanisms, driving diverse microenvironmental compositions and behaviors. An easy way to conceptualize this interaction comes from considering cancer as "a wound that never heals" where the overall response to the tissue insult (cancer) is one that is characterized by tissue remodeling, scar formation, and suppression of cell-killing immune responses. Through our increased understanding of these interactions, we are constantly developing tools that allow for modulation of the composition and behavior of the tumor microenvironment to promote anti-tumor immunity. As this understanding matures, we will develop context-specific pharmacological approaches that combine tumor microenvironment-targeted therapies with immunotherapy to drive potential curative responses. This chapter sets the foundation for what will be discussed in detail throughout the remainder of the book. In particular, specifics regarding the tumor microenvironment, microenvironment-targeted therapies, and immunotherapies for all GI malignancies will be discussed in detail.

Acknowledgements The authors would like to thank the patients and clinicians who continue to advance the field of cancer immunotherapy through their commitment to clinical research. The figures presented here were generated with BioRender.com.

Compliance with Ethical Standards

Funding This work was supported by NIH grants R01CA208253, R01CA228406 and P30CA138292. *The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.*

Conflict of Interest Dr. Lesinski has consulted for ProDa Biotech, LLC and received compensation. Dr. Lesinski has received research funding through a sponsored research agreement between Emory University and Merck and Co., Bristol-Myers Squibb, Boerhinger-Ingelheim, and Vaccinex. Dr. Herting has no conflicts to disclose.

References

1. Blander JM (2016) Death in the intestinal epithelium-basic biology and implications for inflammatory bowel disease. FEBS J 283(14):2720–2730
2. Ullman TA, Itzkowitz SH (2011) Intestinal inflammation and cancer. Gastroenterology 140(6):1807–1816
3. Kulkarni MS, Yielding KL (1985) DNA damage and repair in epithelial (mucous) cells and crypt cells from isolated colon. Chem Biol Interact 52(3):311–318
4. Napier KJ, Scheerer M, Misra S (2014) Esophageal cancer: a review of epidemiology, pathogenesis, staging workup and treatment modalities. World J Gastrointest Oncol 6(5):112–120
5. Carcas LP (2014) Gastric cancer review. J Carcinog 13:14
6. Villanueva A (2019) Hepatocellular carcinoma. N Engl J Med 380(15):1450–1462
7. Benedict M, Zhang X (2017) Non-alcoholic fatty liver disease: an expanded review. World J Hepatol 9(16):715–732
8. El-Serag HB (2012) Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 142(6):1264–73.e1
9. Banales JM, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR et al (2020) Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 17(9):557–588
10. Adamska A, Domenichini A, Falasca M (2017) Pancreatic ductal adenocarcinoma: current and evolving therapies. Int J Mol Sci 18(7):1338
11. Machado NO, Al Qadhi H, Al Wahibi K (2015) Intraductal papillary mucinous neoplasm of pancreas. N Am J Med Sci 7(5):160–175
12. Sun J (2017) Pancreatic neuroendocrine tumors. Intractable Rare Dis Res 6(1):21–28
13. Williamson JM, Williamson RC (2014) Small bowel tumors: pathology and management. J Med Assoc Thail 97(1):126–137
14. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB (2019) Colorectal cancer. Lancet 394(10207):1467–1480
15. Tiwari AK, Laird-Fick HS, Wali RK, Roy HK (2012) Surveillance for gastrointestinal malignancies. World J Gastroenterol 18(33):4507–4516
16. Orth M, Metzger P, Gerum S, Mayerle J, Schneider G, Belka C et al (2019) Pancreatic ductal adenocarcinoma: biological hallmarks, current status, and future perspectives of combined modality treatment approaches. Radiat Oncol 14(1):141
17. Tariq NU, McNamara MG, Valle JW (2019) Biliary tract cancers: current knowledge, clinical candidates and future challenges. Cancer Manag Res 11:2623–2642
18. Ferrone CR, Marchegiani G, Hong TS, Ryan DP, Deshpande V, McDonnell EI et al (2015) Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. Ann Surg 261(1):12–17
19. Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 315(26):1650–1659
20. Pandolfi F, Altamura S, Frosali S, Conti P (2016) Key role of DAMP in inflammation, cancer, and tissue repair. Clin Ther 38(5):1017–1028
21. Baker KJ, Houston A, Brint E (2019) IL-1 family members in cancer; two sides to every story. Front Immunol 10:1197
22. O'Toole A, Michielsen AJ, Nolan B, Tosetto M, Sheahan K, Mulcahy HE et al (2014) Tumour microenvironment of both early- and late-stage colorectal cancer is equally immunosuppressive. Br J Cancer 111(5):927–932
23. Karamitopoulou E (2019) Tumour microenvironment of pancreatic cancer: immune landscape is dictated by molecular and histopathological features. Br J Cancer 121(1):5–14
24. Papaccio F, Paino F, Regad T, Papaccio G, Desiderio V, Tirino V (2017) Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development. Stem Cells Transl Med 6(12):2115–2125

25. Pon JR, Marra MA (2015) Driver and passenger mutations in cancer. Annu Rev Pathol 10:25–50
26. Cannon A, Thompson C, Hall BR, Jain M, Kumar S, Batra SK (2018) Desmoplasia in pancreatic ductal adenocarcinoma: insight into pathological function and therapeutic potential. Genes Cancer 9(3–4):78–86
27. Apte MV, Wilson JS, Lugea A, Pandol SJ (2013) A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology 144(6):1210–1219
28. Lohr M, Schmidt C, Ringel J, Kluth M, Muller P, Nizze H et al (2001) Transforming growth factor-beta1 induces desmoplasia in an experimental model of human pancreatic carcinoma. Cancer Res 61(2):550–555
29. Bachem MG, Schunemann M, Ramadani M, Siech M, Beger H, Buck A et al (2005) Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology 128(4):907–921
30. Murakami T, Hiroshima Y, Matsuyama R, Homma Y, Hoffman RM, Endo I (2019) Role of the tumor microenvironment in pancreatic cancer. Ann Gastroenterol Surg 3(2): 130–137
31. Shan T, Chen S, Chen X, Lin WR, Li W, Ma J et al (2017) Cancer-associated fibroblasts enhance pancreatic cancer cell invasion by remodeling the metabolic conversion mechanism. Oncol Rep 37(4):1971–1979
32. Mace TA, Ameen Z, Collins A, Wojcik S, Mair M, Young GS et al (2013) Pancreatic cancer-associated stellate cells promote differentiation of myeloid-derived suppressor cells in a STAT3-dependent manner. Cancer Res 73(10):3007–3018
33. Halbrook CJ, Lyssiotis CA (2017) Employing metabolism to improve the diagnosis and treatment of pancreatic cancer. Cancer Cell 31(1):5–19
34. Lyssiotis CA, Kimmelman AC (2017) Metabolic interactions in the tumor microenvironment. Trends Cell Biol 27(11):863–875
35. Brabletz T, Kalluri R, Nieto MA, Weinberg RA (2018) EMT in cancer. Nat Rev Cancer 18(2):128–134
36. Jolly MK, Boareto M, Huang B, Jia D, Lu M, Ben-Jacob E et al (2015) Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. Front Oncol 5:155
37. Roche J (2018) The epithelial-to-mesenchymal transition in cancer. Cancers (Basel) 10(2):52
38. Gaianigo N, Melisi D, Carbone C (2017) EMT and treatment resistance in pancreatic cancer. Cancers (Basel) 9(9):122
39. Vu T, Datta PK (2017) Regulation of EMT in colorectal cancer: a culprit in metastasis. Cancers (Basel) 9(12):171
40. Ramesh V, Brabletz T, Ceppi P (2020) Targeting EMT in cancer with repurposed metabolic inhibitors. Trends Cancer 6(11):942–950
41. Huels DJ, Sansom OJ (2015) Stem vs non-stem cell origin of colorectal cancer. Br J Cancer 113(1):1–5
42. Xu Y, Liu J, Nipper M, Wang P (2019) Ductal vs. acinar? Recent insights into identifying cell lineage of pancreatic ductal adenocarcinoma. Ann Pancreat Cancer 2:11
43. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M et al (2009) Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457(7229): 608–611
44. Powell AE, Vlacich G, Zhao ZY, McKinley ET, Washington MK, Manning HC et al (2014) Inducible loss of one Apc allele in Lrig1-expressing progenitor cells results in multiple distal colonic tumors with features of familial adenomatous polyposis. Am J Physiol Gastrointest Liver Physiol 307(1):G16–G23
45. Zhu L, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT et al (2009) Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 457(7229):603–607
46. Davis H, Irshad S, Bansal M, Rafferty H, Boitsova T, Bardella C et al (2015) Aberrant epithelial GREM1 expression initiates colonic tumorigenesis from cells outside the stem cell niche. Nat Med 21(1):62–70

47. Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Goktuna SI, Ziegler PK et al (2013) Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 152(1–2):25–38
48. Busnardo AC, DiDio LJ, Tidrick RT, Thomford NR (1983) History of the pancreas. Am J Surg 146(5):539–550
49. Brembeck FH, Schreiber FS, Deramaudt TB, Craig L, Rhoades B, Swain G et al (2003) The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. Cancer Res 63(9):2005–2009
50. Lee AYL, Dubois CL, Sarai K, Zarei S, Schaeffer DF, Sander M et al (2019) Cell of origin affects tumour development and phenotype in pancreatic ductal adenocarcinoma. Gut 68(3):487–498
51. Lynch MD, Watt FM (2018) Fibroblast heterogeneity: implications for human disease. J Clin Invest 128(1):26–35
52. Kalluri R (2016) The biology and function of fibroblasts in cancer. Nat Rev Cancer 16(9):582–598
53. Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M et al (2017) Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med 214(3):579–596
54. Koshida Y, Kuranami M, Watanabe M (2006) Interaction between stromal fibroblasts and colorectal cancer cells in the expression of vascular endothelial growth factor. J Surg Res 134(2):270–277
55. Zhu HF, Zhang XH, Gu CS, Zhong Y, Long T, Ma YD et al (2019) Cancer-associated fibroblasts promote colorectal cancer progression by secreting CLEC3B. Cancer Biol Ther 20(7):967–978
56. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA et al (2014) Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 25(6):735–747
57. Ozdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR et al (2014) Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell 25(6):719–734
58. Lee JJ, Rothenberg ME, Seeley ES, Zimdahl B, Kawano S, Lu WJ et al (2016) Control of inflammation by stromal Hedgehog pathway activation restrains colitis. Proc Natl Acad Sci U S A 113(47):E7545–E7553
59. Ohlund D, Elyada E, Tuveson D (2014) Fibroblast heterogeneity in the cancer wound. J Exp Med 211(8):1503–1523
60. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA et al (2019) Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov 9(8):1102–1123
61. Dominguez CX, Muller S, Keerthivasan S, Koeppen H, Hung J, Gierke S et al (2020) Single-cell RNA sequencing reveals stromal evolution into LRRC15(+) myofibroblasts as a determinant of patient response to cancer immunotherapy. Cancer Discov 10(2):232–253
62. Mace TA, Shakya R, Pitarresi JR, Swanson B, McQuinn CW, Loftus S et al (2018) IL-6 and PD-L1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer. Gut 67(2):320–332
63. Zhang R, Qi F, Zhao F, Li G, Shao S, Zhang X et al (2019) Cancer-associated fibroblasts enhance tumor-associated macrophages enrichment and suppress NK cells function in colorectal cancer. Cell Death Dis 10(4):273
64. Ahmadzadeh M, Rosenberg SA (2005) TGF-beta 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. J Immunol 174(9):5215–5223
65. Thomas DA, Massague J (2005) TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 8(5):369–380
66. Krenkel O, Tacke F (2017) Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol 17(5):306–321