Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/15/20240464

The extracellular matrix in peritoneal metastatic carcinoma

Yangsong Qiu

School of Biological Sciences, Faculty of Medical and Human Sciences, The University of Manchester, Oxford Road Manchester, M139PL, UK

yangsong.qiu921@gmail.com

Abstract. The peritoneum is a relatively common site for the metastasis of cancers that develop near the peritoneal cavity, such as gastric cancer, colorectal cancer (CRC), ovarian cancer, and low-grade appendiceal mucinous adenocarcinoma (LAMN). Peritoneal metastasis (PM) results from direct implantation and growth or microvascular metastasis of cancer cells to the peritoneum and is often associated with a poor prognosis. The biological features of peritoneal metastatic tumours are significantly altered, and the tumour microenvironment (TME) is profoundly abnormal. The extracellular matrix (ECM), a highly dynamic part of the TME, exhibits unique biological properties and influences tumour cells (TCs) behaviour and invasion. In this review, I focus on the hallmarks of cancer; the biology of CRC, PM from CRC, LAMN and pseudomyxoma peritonei (PMP, resulting from the intraperitoneal spread of LAMN); and the structural features of the cancer ECM and mucus and their roles in tumour growth, TCs invasion and drug resistance. The study of the ECM has led to a deeper understanding of peritoneal metastatic tumours and provides new insights for developing new biomarkers and targeted drugs.

Keywords: Extracellular matrix, Peritoneal metastasis, Colorectal cancer, Low-grade appendiceal mucinous neoplasm, Mucus.

1. Introduction

Cancer is the second leading cause of death worldwide and imposes a heavy economic burden on public health systems. Due to an increasingly ageing population, the cancer burden is expected to increase by 50% in 2040 compared to 2020, when the number of new cancer diagnoses is likely to reach almost 30 million [1, 2]. According to GLOBOCAN 2020 estimates, 19,292,789 new cancer cases (including nonmelanoma skin cancers) were diagnosed worldwide in 2020 [3]. A total of 9.96 million cancer deaths were recorded worldwide in 2020, with 3 million cancer deaths in China, 610,000 in the US and 180,000 in the UK [3].

Metastasis is a complex process with multiple regulatory mechanisms, among which extracellular matrix (ECM) remodelling makes a significant contribution [4]. The remodelled ECM composition, physical properties, and spatial structure are altered and play essential roles in tumour cells (TCs) invasion and metastasis [4]. Therefore, the characteristics and molecules associated with ECM remodelling can be used as critical indicators for clinical staging, the early diagnosis and treatment of tumours, and prognostics [5]. This review focuses on peritoneal metastasis (PM) from CRC and pseudomyxoma peritonei (PMP) from low-grade appendiceal mucinous neoplasms (LAMNs), as well

DOI: 10.54254/2753-8818/15/20240464

as the ECM and its relationship with cancer. Gaining insight into the mechanisms and determinants of peritoneal metastasis in tumours can aid healthcare professionals and researchers in the identification of efficacious treatments and suitable combinations of chemotherapeutic agents, thereby enhancing patient survival rates and overall quality of life.

2. Hallmarks of cancer

Genetic alterations usually trigger cancer in a single cell, which undergoes extensive changes to transform an ordinary into a cancerous cell. As our understanding of the mechanisms of cancer deepens, a few cancer-related principles will be distilled from the vast and complex plethora of cancer phenotypes and genotypes. Cancer is characterised mainly by fourteen basic features, such as self-sufficiency via growth signalling, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, and senescent cells and so on [6-8].

In addition to oncogenic mutations, abnormalities in the tumour microenvironment (TME) can lead to extensive changes in the epigenome of cancer cells, resulting in their clonal growth and enhanced adaptability. For example, hypoxia leads to a decrease in ten-eleven translocation (TET) demethylase activity, leading to hypermethylation [9]. Alterations in the ECM impact the stiffness of solid tumours and the migration of TCs. Cells can release soluble factors that bind to the ECM and participate in migration-associated cell signalling pathways. For example, growth factors and cytokines bind to G protein-coupled receptors (GPCRs) or cytokine receptors, which can interact with integrins or calmodulin to influence intracellular signalling pathways and alter whole-cell functions, such as cell–cell adhesion and cytoskeletal dynamics, and the ECM structure to promote the migration, survival, and proliferation of TCs [10].

2.1. Epigenetic plasticity

Epigenetic alterations can be caused by genetic, environmental, or metabolic damage. Plastic chromatin may activate proto-oncogenes and regulate the cellular state, enabling TCs to undergo dynamic and reversible transitions between multiple phenotypes [11]. Epigenetic modifications promote heterogeneity and plasticity in TCs by altering the transcriptional programme through chromatin remodelling, histone modification and DNA methylation [11]. The H3K4 demethylase *KDM5B* is overexpressed in various TCs and correlates with TCs proliferation and the expression of cancer stem cell marker pairs [12]. The resistance of estrogen receptor-positive (ER^+) breast cancer cells to endocrine drugs is not due to ERα expression or mutation but to enhanced phenotype plasticity due to changes in enhancers, suggesting ideas for new therapeutic targets for ER^+ breast cancer [13].

2.2. Cell-in-cell mechanism

TCs can acquire macromolecules from the microenvironment through the integrin acquisition of ECM proteins, receptor-mediated uptake, catabolism of albumin, and endocytosis of multiple components of the TME, which are then hydrolysed by lysosomes [14]. TCs exhibit cell-in-cell mechanism, including cannibalism and entosis, to adapt to nutrient-deprived environment and promote their evolution [15].

The behaviour of TCs engulfing living and dead cells is called cannibalism. The living cells consumed by TCs include T cells, neutrophils, natural killer (NK) cells and mesenchymal stem cells (MSCs) [16]. The mechanism of cannibalism is somewhat similar to phagocytosis in that both cells engaged in exocytosis and cannibalism express *CD68*, and both involve motor protein-dependent phagocytic activity [15]. For example, TM9SF4 promotes autophagy and cannibalism by inhibiting mTORC1 activity to facilitate cell survival under nutrient starvation conditions [17]. However, while phagocytosis usually involves dying or dead cells, cannibalistic cells can engulf living cells, suggesting that cannibalism is triggered by unique mechanisms [15]. Cannibalism is also associated with cortical cellular proteins and vesicles of the caveolar network [15]. In addition, under chemotherapeutic conditions, TCs feed on each other to obtain the energy and materials to survive and trigger tumour recurrence when the chemotherapy has been completed. When breast cancer cells were exposed to the

DOI: 10.54254/2753-8818/15/20240464

chemotherapeutic drugs doxorubicin or paclitaxel, senescent TCs frequently cannibalised neighbouring TCs, allowing them to survive longer in the culture medium [18].

Entosis mainly involves the same types of cells that engage in cannibalism and requires mediation through E-calmodulin and P-calmodulin [15, 17]. When adhering to a substrate, TCs actively induce neighbouring cells to engulf them, but the mechanisms remain unclear. Recent studies have shown that entosis can promote competition between TCs while possibly facilitating the evolution of TCs populations [17]. Starvation regulates TCs invagination through AMP-activated protein kinase (AMPK), and cells with lower energy levels and higher AMPK activity are sacrificed to 'feed' those with lower AMPK activity [17].

2.3. Intratumour heterogeneity (TH) and evolution

Cancer progresses through clonal evolution, and genetic variation is the primary condition. TCs variations arise through different mechanisms underlying genome instability, including endogenous and exogenous processes that generate point mutations and cause chromosomal instability [19]. Cancer is an evolutionary and genetic disease, as determined based on the study of ITH.

Bulk sequencing helps to have a comprehensive understanding of the oncogenes, revealing that mutations are acquired gradually. Miles *et al*. analysed mutations in 31 commonly mutated genes in 740529 cells at the single-cell level to study the clonal characteristics of myeloid malignancies [20]. They found that epigenetically related genes were expressed early in carcinogenesis and tended to undergo simultaneous mutation. In contrast, genes associated with cell signalling pathways were mutated later and appeared in other clones, resulting in a diversity of acute myeloid leukaemia (AML) clones [20]. Erikson *et al*. [21] used spatial transcriptomic techniques to study the spatial evolution of tumour clones in prostate cancer at the whole-organ level. Somatic mutations in benign tissues may be important mechanisms for initiating the formation of tumours, which gradually break through tissue boundaries and form focal areas. Gast *et al*. [22] found that the fusion of TCs and macrophages facilitated the formation of tumour heterogeneity. In addition, TCs grow under the selective pressure of the immune system, which directly determines the genetic heterogeneity within a tumour [23].

As the cell heterogeneity of primary tumours provides insights into their evolutionary history, analysing inter- and intrametastatic tumour heterogeneity can shape our understanding of metastatic malignancies. ITH exerts a significant adaptive impact on TCs and is considered a proxy for functional subclonal diversity, which has been associated with poor prognosis [19].

2.4. The tumour microbiome

The tumour microbiome or intratumoural microbiome is an essential member of the TME and is ubiquitous in different types of tumours [24]. Because tumour tissues are hypoxic, enriched with nutrients, and free from interference by the host immune system, tumours create an ideal environment for bacterial invasion, survival, and growth. Multiple contact-dependent and contact-independent immune mechanisms drive intricate host-microbe interactions to promote tumour formation and progression [25]. Microorganisms influence tumorigenesis through pleiotropic interactions that may be facilitated by the expression and secretion of virulence factors, physical binding-induced signalling, and recruitment of immune cells that promote oncogenic effects [25].

In vitro and preclinical animal models have shown that bacteria in the tumour-associated microbiota play roles in cancer development, metastasis, immune surveillance, and chemoresistance. A variety of unique intracellular bacteria in breast cancer tissues modulate the actin cytoskeleton of their host cells to help TCs resist fluid shear stress in the circulatory system during metastasis and promote their survival [26]. The distribution of microbiota-derived species within tumours is not random. Within the CD45 immune cell-enriched regions of oral squamous cell carcinoma and CRC tissues, bacteria were found in highly immunosuppressive microecological sites, as neutrophils were recruited and $CD3^+$ T cells were excluded [27]. Moreover, the mucus layer on the surface intestine plays a vital role in interacting with commensal flora. The absence of the mucus layer leads to the invasion of commensal flora, increasing the risk of CRC and promoting the development and progression of cancer [28, 29].

DOI: 10.54254/2753-8818/15/20240464

2.5. Tumour microenvironment (TME)

The TME is a complex environment. It contains TCs, stromal cells, fibroblasts, infiltrating immune cells, and ECM [30]. Ionic homeostatic imbalance, acidity, hypoxia, an increased lactate level, a reduced glucose concentration, nutrient competition and changes in the secretome of the TME can lead to metabolic reprogramming of immune cells and thus alter their proper function, causing a diminished inflammatory response or enhanced suppressive function and assisting in the immune escape of TCs [31].

The metabolic reprogramming of TCs due to genetic mutation is a crucial factor in the formation of the TME, which is characterised by the Warburg effect. TCs mainly adopt aerobic glycolysis to supply energy [32]. The changes of TME are dominated mainly by TCs, while the various non-TCs are also complex and dynamic. For example, immune cells within the TME differentiate into subpopulations with different phenotypes, different metabolic profiles and different functions, acting as antitumour or tumour-promoting agents and further altering the TME through their own metabolism, forming a sophisticated, interactive network [33]. Under inflammatory conditions, NK cell glycolytic pathways are upregulated, supporting their nonspecific recognition of target cells and the secretion of TCs killing mediators such as perforin, NK cytotoxic factor and TNF. In the TME, TCs and other cells secrete NK cell inhibitory factors, including IL-6, IL-10, transforming growth factor-β (TGF-β), and prostaglandin E2 to suppress their TC-killing activity [34]. Excessive accumulation of TC metabolites in the TME and a hypoxic environment inhibit the activity of NK cells. Moreover, lipids accumulate in the TME, causing immune suppression. Cholesterol in the TME induces $CD8^+$ T cells to express immune checkpoint-programmed cell death receptor-1 (PD-1), leading to T-cell failure in tumours [35]. In addition to metabolites derived from TCs, metabolites or suppressive factors secreted by immunosuppressive cells in the TME are also crucial to T cells. For example, ammonia oxidase in myeloid-derived suppressor cells (MDSCs) catalyses the production and accumulation of dicarbonyl methylglyoxal in these cells, leading to a reduction in the glycolysis rate [36]. Dicarbonyl methylglyoxal enters T cells when MDSCs contact T cells. It then reacts with L-arginine, resulting in the selective depletion of the L-arginine required for T-cell activation and thus suppresses T-cell immune function [36]. The ECM is also an important component of the TME, which can promote TCs development and metastasis and increase drug resistance [5]. It will be discussed in more detail below.

3. Colorectal Cancer (CRC)

3.1. Overview of CRC

CRC involves complex pathogenesis involving multiple genes and pathways and is a malignancy with a high incidence and mortality rate. A total of 1.93 million new cases of CRC are diagnosed worldwide each year, and the number of new cases of CRC is expected to increase to 2.5 million by 2035, surpassing common cancers such as liver cancer and gastric cancer [3]. CRC is primarily a tumour derived from benign adenomatous polyps that develop into adenocarcinoma through multistep mutations, many of which have not yet been elucidated and refined. The genetic basis of CRC is complex and heterogeneous, including cell conversion and epigenetic modifications that lead to chromosome instability, weakening of apoptotic mechanisms, overexpression of telomerase, and disruption of signal transduction regulation. More than one-half of patients are diagnosed with CRC associated with environmental factors such as smoking, unhealthy diet, heavy alcohol consumption, physical inactivity, and excessive weight; hence, CRC may be preventable [37]. The incidence and mortality of CRC can be reduced through early screening and surveillance [38].

3.2. Genomics of CRC

CRC involves the accumulation of mutations in a range of genes. The proto-oncogenes (POGs) encode proteins that accelerate the cell cycle, promote cell proliferation, inhibit differentiation, inhibit apoptosis, and participate in various intracellular signalling pathways. The classical POGs closely associated with CRC include *K-RAS*, *PIK3CA*, *C-MYC*, *BRAF*, *C-FOS*, *C-JUN*, *C-RAF*, and FPS [39]. The *RAS* family,

DOI: 10.54254/2753-8818/15/20240464

the most frequently mutated CRC-associated gene, is found in more than 50% of disseminated cases of CRC. *KRAS* is the most frequently mutated *RAS* family member and is located on chromosome 12p12.1, with codons 12, 13, 15, 18, 20, 30, 31, 61 and 63 being mutation hotspots [40]. P21, the protein encoded by *KRAS*, is involved in the EGFR/PI3K signalling pathway and EGFR/MAPK signalling pathway to maintain normal cell growth, proliferation, and differentiation [40]. Mutations in *KRAS* result in the loss of GTPase activity of p21, which maintains the signalling pathway in a continuously active state that is unaffected by upstream EGFR signalling [40]. *PIK3CA* is localised on chromosome 3q26.3, and the hotspots for *PIK3CA* mutations are exon nine and exon 20 [41]. Mutations in *PIK3CA* enhance the phosphatidylinositol kinase activity of the PI3K encoded by *PIK3CA*, and the PI3K/Akt/mTOR pathway continues to be abnormally activated, leading to the development of CRC [41]. Tumour suppressor genes (TSGs) inhibit excessive cell growth, and mutations in TSGs promote the progression of carcinogenesis into tumorigenesis. The main TSGs associated with CRC are *TP53*, *APC*, *DCC*, *PTEN*, *SLC5A8*, *RB*, *NDRG2*, *P16*, *DPC4*, and KLF6 [42]. *APC* is located on chromosome 5q21-q22 and is commonly mutated at codons 1309-1356, with codon 1356 being a hotspot for mutation. *APC* controls cell proliferation and differentiation of gastrointestinal cells by negatively regulating the Wnt signalling pathway; regulates cell adhesion by controlling the distribution of TGF-β and E-calmodulin between the cytoplasm and cell membrane; interacts with microtubules; and inhibits TC migration, DNA repair and regulation of the cell cycle [39]. *TP53* is located on chromosome 17p13.1, and the mutation hotspots are in four highly conserved regions from exons 5 to 8. *TP53* mutations are involved in advanced adenoma carcinogenesis [39, 42].

Microsatellite instability is another cause of CRC. Microsatellite sequences are duplicated or missing due to DNA mismatch repair defects. The primary human genes with mismatch repair are *hMSH2*, *hMSH6*, *hMSH3*, *hMLH1*, *hPSM2*, *hPSM1*, *hMLH3* and others [43]. The CpG island methylation phenotype (CIMP) leads to the formation of CRC by promoting epigenetic instability. It is typically characterised by CpG island site promoter hypermethylation, which results in the activation of many oncogenes. Many genes are associated with CIMP, including *MLH1*, *P16*, *MINT1* and *MINT31* [44].

3.3. The relationship between gut microbiota and CRC

The gut microbiota consists of anaerobic, partly anaerobic, and aerobic bacteria, with a total of 100 trillion bacteria comprising more than 1,000 species and known as the ‘second genome’ of the human body [45]. The gut microbiota plays roles in protecting the structure of the intestinal mucosa, promoting metabolism, providing nutrients, participating in signalling networks, regulating epithelial cell development and modulating the immune system [46]. The composition and distribution of microflora species in the intestine of patients with CRC differ significantly from those of the healthy population, with increased abundance of the thick-walled bacteria in the phylum Bacteroides and *Clostridium spp*. in the intestines of CRC patients and increased abundance of Lactococcus and Clostridium in cancerous tissue compared to paracancerous tissue [47].

Dysbiosis is a risk factor for the development of gastrointestinal tumours. Toxic substances produced by bacterial metabolism in dysbiosis include hydrogen sulfide, reactive oxygen and nitrogen species, and bile acid metabolites, which damage intestinal epithelial cells, promote chronic inflammation and the production of carcinogenic metabolites, and induce tumour formation [48]. Bile acids are standard regulators of the microbiota in gut and contain lithotrophic and deoxycholic acids that are potentially carcinogenic [49]. The metabolism of bile acids produces secondary bile acids to promote DNA damage in epithelial cells, induce oxidative stress, activate NF-KB signalling pathways and induce inflammatory responses [49, 50].

The gut microbiota is involved in the regulation of homeostasis of an organism’s internal environment. Once an organism's homeostasis is disrupted, the gut microbiota will be affected, leading to enteritis and intestinal cancer [47]. *Bifidobacterium bifidum* promotes the expression of the apoptotic gene *Bax* in CRC, decreases *Bcl-2* expression, and promotes apoptosis in TCs [51]. *Enterococcus faecalis* can cause DNA damage to intestinal epithelial cells and chromosome instability, inducing the development of CRC [48]. *Bacteroides fragilis* in the intestinal flora of CRC patients induces DNA

DOI: 10.54254/2753-8818/15/20240464

damage and increases the incidence of CRC by producing various toxic metabolites, such as reactive oxygen species, reactive nitrogen species, nitroso compounds and β-glucuronidase [50].

3.4. The immune microenvironment of CRC

The stroma and TCs exchange substances and develop together to form the TME. The TME contains cancer-associated fibroblasts (CAFs), mesenchymal fibroblasts, MSCs, dendritic cells (DCs), endothelial cells, inflammatory response immune cells and histiocytes. Various components of the TME, such as cytokines, transcription factors, extracellular vesicles, and free radicals, respond to and show functional crossover with each other [52]. The CRC TME can reprogram super enhancers to activate *PDZK1IP1*, which acts as an oncogenic driver of tumour growth and collaborates with the genetic drivers of disease in colorectal epithelial cells [53].

CAFs and tumour-associated macrophages (TAMs) are essential components of the TME, causing immunosuppression and promoting tumour progression. Vascular cell adhesion molecule-1 (VCAM-1) is widely expressed on the surface of macrophages and fibroblasts, and its expression level is associated with the enrichment and infiltration of TAMs into CRC tissues [54]. CAFs can upregulate the expression of VCAM-1 in the immune microenvironment of CRC, which promotes monocyte adhesion and secretion of interleukin-8 (IL-8), polarising M2 TAMs, which synergistically inhibit the function of NK cells with CAFs, ultimately leading to the development of CRC [54].

In addition, the microenvironment regulates tumour-infiltrating regulatory T cells (Tregs) via the MondoA-TXNIP axis, causing these cells to undergo metabolic reprogramming, exhibit high glucose consumption, compete for glucose in the microenvironment and induce dysfunction of effector T cells; thus, Treg migration is promoted via glucose metabolism, leading to their impaired immunosuppressive function [55].

4. CRC-associated peritoneal metastasis (CRC-PM)

Metastasis from CRC has always been a complex problem, with approximately 20% of patients exhibiting metastasis at initial diagnosis. The PM is the third most common site of CRC, following the liver and lung [56]. The metastasis rate is higher in patients with colon cancer (10.2%) than in those with rectal cancer (4.2%) [56]. Patients with PM from CRC indicate a poor prognosis with severe complications such as bowel obstruction, peritoneal effusion, hypoproteinaemia and anaemia, and if not treated aggressively, the median overall survival (MOS) is only 6 months, which is worse than that for patients exhibiting metastasis to other sites [57]. A genome analysis of 1,779 CRC-PM samples identified *KRAS*, *APC*, *SMAD4*, *BRAF* and *PIK3CA* as common mutated genes, and mutations in *KRAS* and *BRAF* are associated with overall survival and recurrence [58].

4.1. Mechanisms of PM

PM is caused largely by implantation metastasis. First, individual cancer cells are shed, and tumour masses or tumour spheroids with inverted polarity (TSIPs) are generated after the downregulation of adhesion molecules (especially E-cadherin) on the surface of TCs [56]. With its expression products calponin 3 binding to actin, calmodulin, troponin C and myosin, *CNN3* regulates smooth muscle contraction. In addition, *CNN3* may promote the initial spread of TCs by downregulating E-cadherin in the primary tumour [59]. Although CAFs in tumour-derived microvesicles (TMVs) are subjected to lipidome reprogramming and accumulate large amounts of fatty acids and phospholipids, CRC cells show an increased ability to migrate after taking up lipid metabolites secreted by CAFs [60].

Decidualised TCs undergo epithelial-mesenchymal transition and acquire mesenchymal features such as upregulated N-cadherin expression and loss of cell polarity, which enhance their invasiveness and tolerance to apoptosis [7]. Subsequently, free TCs enter the peritoneum via mesothelial or lymphatic pathways and accumulate in milky spots. Immunoglobulin superfamily adhesion molecules, cytokines and CD44 are essential factors in the TCs adhesion process [7, 61]. Milky spots are peritoneal surface structures comprising aggregates of macrophage-rich cells. Milky spots in the mouse omentum appear to exhibit a distinct vascular microenvironment and may promote early survival of cancer cells through

DOI: 10.54254/2753-8818/15/20240464

the expression of VEGF and CD105 [56]. Following adhesion, cancer cells continue to invade the subperitoneal region.

Once invading the subperitoneal region, CRC cells and cancer cell-derived TMEs remodel the ECM by secreting matrix metalloproteinases (MMPs), leading to TC invasion and migration. At the same time, TCs secrete hypoxia-inducible factor-1 (HIF-1) and insulin-like growth factor-1 (IGF-1) to promote their own growth and multiplication, eventually forming metastatic cancer foci [61].

4.2. TMEs of PM

After CRC cells metastasise to the peritoneum and colonise it, TMEs promote TCs invasion and proliferation in the peritoneum. Macrophages are the most abundant immune cells in ascites, accounting for 45-60% of the cellular component of ascites [33]. Macrophages in the ascitic fluid interact with TCs and gradually acquire a tumour-associated macrophage (TAM) phenotype. TAMs promote cancer stem cells (CSCs) to form multicellular spheres (MSCs) around them and maintain the stemness of CSCs by secreting IL-6 and activating the WNT signalling pathway, which improves MCS resistance and accelerates peritoneal invasion [62]. In addition to acting on TCs, TAMs inhibit the function of T cells in ascites, suppress T-cell infiltration into the TME, promote the establishment of Tregs and suppress the clearance of TCs by the immune system [33]. Moreover, TCs in ascites inhibit T-cell mitochondrial respiratory function by inducing T-cell endoplasmic reticulum stress, thereby suppressing T-cell toxicity [63]. In the presence of antigenic stimuli in the peritoneum, neutrophils release cytokines, chemokines and granulins, creating a microenvironment suitable for tumour growth. In the presence of appropriate stimuli, neutrophils release granulins and chromatin to form neutrophil extracellular traps (NETs) [64]. When NET formation is inhibited, the peritoneal metastasis rate is reduced, indicating that neutrophils can induce cancer cells to colonise the peritoneum [64].

In addition to immune cells in TMEs, CAFs are essential to the immune evasion of CRC cells by promoting the recruitment of immunosuppressive cells through the secretion of the chemokines CCL2 and CXCL12 and suppressing effector T cells through the secretion of TGF-β [60]. In cocultures, CAFs from CRC tissues significantly inhibited the expression of the NK cell surface receptors perforin and telomerase. They suppressed the secretion of the cytokines TNF-α and IFN-γ by NK cells [63]. In addition, CAFs surrounding CRC-PM cells abundantly expressed VEGF at a much higher level than TCs to promote angiogenesis in the peritoneal microenvironment [63].

The peritoneum is enriched with fat, and adipocytes are vital components and provide energy for the metabolism of TCs. The TCs in ovarian cancer PM are enriched with lipid droplets that they absorb from adipocytes. When left untreated, this lipid droplet uptake can transform the fat-enriched omentum into a solid tumour with virtually no adipocytes [65]. Fatty acid-binding protein 4 (FABP4) is a lipid chaperone protein that plays an essential role in the uptake, transport and metabolic regulation of long-chain fatty acids and is abundantly expressed in the ovarian cancer PM. Inhibition of FABP4 results in reduced lipid accumulation in TCs, decreased proliferation capacity, lower peritoneal invasion capacity and less tumour microvessel density [65].

5. LAMNs and PMP

Appendix tumours in the digestive system are rare and consist mainly of adenocarcinomas and carcinoid tumours. An adenocarcinoma composed of more than 50% extracellular mucus is called a mucinous adenocarcinoma [66]. In the WHO classification, mucinous gland tumours of the appendix are divided into LAMNs and adenocarcinomas of the appendix. LAMN is a low-grade cancer resembling adenoma but can spread distally, and mucinous neoplasms with undetermined malignant potential are included in this category [66]. LAMN is characterised by a broad frontal infiltrate in which mucus penetrates the appendiceal wall and spreads into the peritoneal cavity as a gelatinous deposit, leading to PMP [67].

LAMNs do not exhibit typical infiltration-based invasion; the cells grow slowly with a villous, jagged, and wavy structure, TCs can be sparse or even appear as acellular mucous in these lesions, and mucus deposition in the periappendiceal tissue or in the peritoneum containing TCs is associated with a poor outcome [68]. LAMNs usually show positive staining for CK20 (90-100%), MATH1 (100%), MUC2

DOI: 10.54254/2753-8818/15/20240464

(92%), PGP (83%), MUC5AC (72%) and CDX2 (92-100%), with patchy positive staining for CK7 (14-36%); markers such as P53, PAX8, MUC1, ER, C-Kit and SMAD4 are generally negative for staining. However, overlapping expression of markers makes the accurate diagnosis of these tumours difficult [69]. CDX2 is measured to help determine the origin of intestinal tumours. Nevertheless, CDX2 is expressed in primary mucinous carcinoma of the ovary; therefore, CDX2 should be assessed with CK7 expression [69]. In recent years, SATB2 expression has been reported in almost all patients with LAMNs (96-100%), and SATB2 staining was found to be negative in patients with primary ovarian carcinoma [66, 69]. Therefore, SATB2 expression can be used as a marker for the origin of appendix tumours.

According to available reports, the high frequency of mutations in *KRAS* and *GNAS* in LAMN samples indicates the activation of both the Ras-PI3K-Akt and cAMP-PKA signalling pathways to promote cell proliferation and is an independent predictor of poor progression-free survival [70]. *GNAS* mutations are common in LAMNs, and mutated *GNAS* may promote mucin production [71]. There are some genetic similarities between LAMN and CRC, but there are also significant differences. Both LAMN and CRC carry *KRAS* mutations, but the mutation rate is approximately 90% in LAMN and less than 50% in CRC, and both types of lesions exhibit frequent amplification of chromosome 1q and loss of the 1q36 locus. Although both show Wnt signalling pathway activation, *APC* mutations are uncommon in LAMNs [58]. In addition, disruption to the TGF-β signalling pathway has been detected in some cases of LAMN but not in CRC. The common mutation *TP53* in CRC is rarely seen in LAMN [58].

The TCs of PMP mainly originate from the appendix, and onset is insidious, with early symptoms often like appendicitis. In the middle to late stages of PMP, a large amount of mucus and many TCs infiltrate the abdominal cavity, and because they are difficult to remove entirely, PMP is prone to recurrence [66]. The accumulation of excessive mucus can place pressure on normal organs of the abdominal cavity, leading to intestinal obstruction. The main components of PMP mucus are MUC2 and MUC5AC. The hypoxic environment in the TCs in the region induces the expression of HIF-1α, which binds to the MUC2 promoter and promotes mucin expression. Both cell and animal studies have shown that BAY87-2243, a specific inhibitor of HIF-1α, inhibits the expression of MUC2 and tumour growth in PMP cells [72]. The development of drugs to dissolve mucus and liquefy it to increase drainage is another perspective; for example, the combination of H_2O_2 and ascorbic acid and the combination of bromelain and azathioprine have shown robust mucolytic effects [73, 74]. However, they have not been sufficiently studied for clinical use. The mutated genes in PMP are similar to those in LAMN, with high-frequency mutations in *KRAS* and *GENAS* and low-frequency mutations in *TP53* and in TGF-β signalling pathway- and PI3K-AKT signalling pathway-related genes [75, 76].

6. The extracellular matrix (ECM)

6.1. Composition and function of the ECM

The ECM, a significant part of TME, is a three-dimensional macromolecular network that provides essential fluid infiltration and spatial support [5]. Located in the basement membrane (BM) and mesenchyme of tissues, the ECM (Fig. 1) is a dynamic compartment undergoing constant remodelling that is tightly regulated and tissue-specific *in vivo*. The mammalian ECM consists of approximately 300 combinations of proteins, mainly fibronectin, proteoglycans, glycoproteins, and other cell-secreted proteins. Fibrous proteins, including collagen, elastin, fibronectin, and laminin, can wrap around and link surrounding nonfibrous components, forming the reticular skeleton of the ECM [77]. Proteoglycans freely suspended in the ECM include chondroitin sulfate, heparin, acetyl heparin sulfate, keratan sulfate, hyaluronic acid (HA), and short proteoglycans in the ECM of the adult brain, which play vital roles in cell communication, angiogenesis, and cell migration [2, 77]. Glycoproteins mainly include laminin, cell adhesin and fibronectin, all of which bind to cell surface receptors and are associated with cell adhesion [2, 5]. Other cellular secretagogues include transforming growth factors and protein hydrolases, which are bioactive molecules produced and secreted by cells [5]. Together, these components maintain the stability of the ECM. When one or more of these components are overexpressed or behave

DOI: 10.54254/2753-8818/15/20240464

abnormally, ECM homeostasis can be disrupted and may lead to tumour aggressiveness [5] (Fig. 2). More interestingly, degradation of the ECM is accompanied by the deposition of different tumour specific ECM, leading to an increase in density and stiffness.

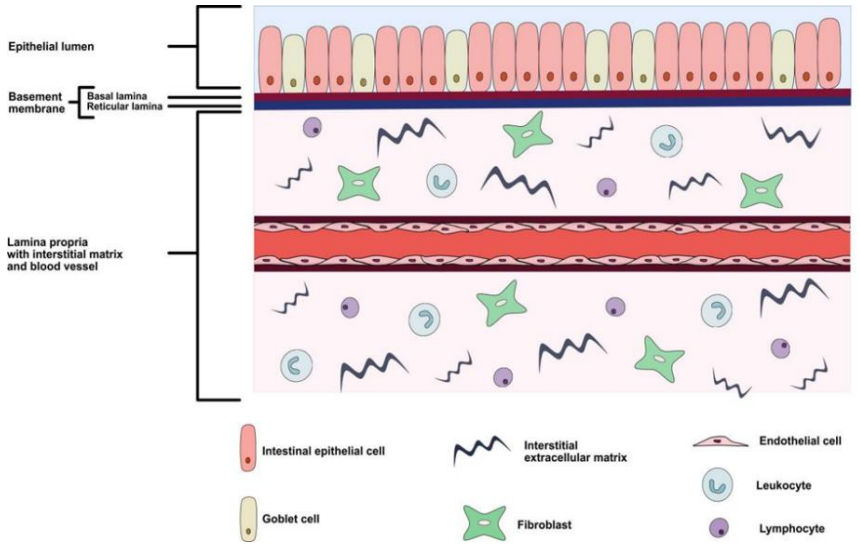

Figure 1. The extracellular matrix (ECM) of Normal colorectum. ECM distribution and localization in the colorectum, with basement membrane encompassing basal and reticular lamina. Interstitial ECM, fibroblasts, leukocytes, and lymphocytes are found in the lamina propria's connective tissue. Taken from Karlsson et al. [2].

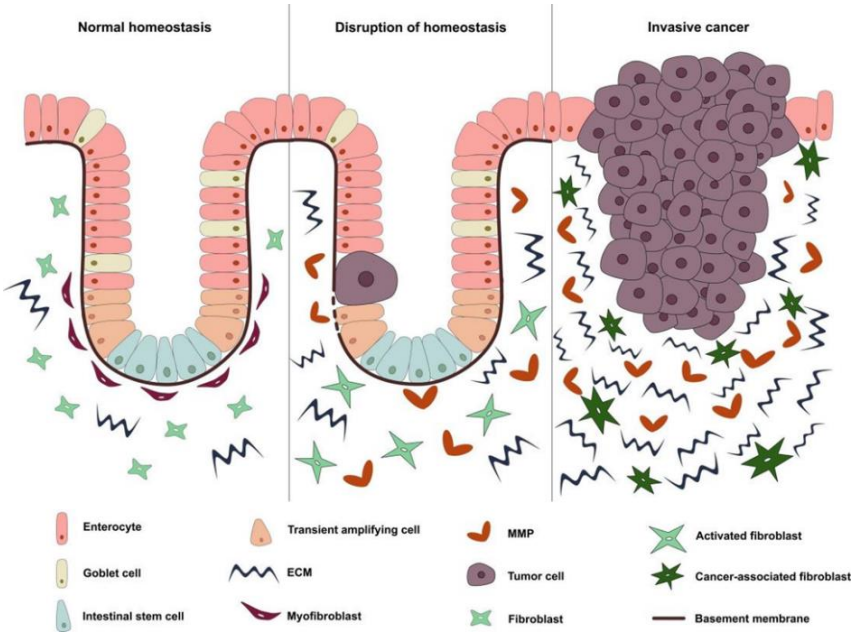

Figure 2. The microenvironment in the normal colorectum and colorectal cancer. The homeostasis of the tumour microenvironment is disrupted as colorectal cancer progresses, and the extracellular matrix (ECM) is constantly remodelled. Matrix metalloproteinase (MMP) can break down the ECM, creating a pathway for tumour cells and accelerating the migration of tumour cells to the surrounding area. Changes in the physical properties and chemical composition of the extracellular matrix alter tumour cell turgidity and drug resistance, promoting tumour invasion and metastasis. Taken from Karlsson et al. [2].

DOI: 10.54254/2753-8818/15/20240464

6.2. Role of the ECM in cancer

The remodelled ECM modulates TCs behaviour. Extracellular matrix protein 1 (ECM1) binds to various structural proteins and promotes tumorigenesis. It promotes drug resistance and the PKM2-mediated Warburg effect by activating epidermal growth factor. It controls cancer stem cell-like properties and the epithelial-mesenchymal transition by stabilising β-catenin expression and regulates gastric cancer cell metastasis and glucose metabolism through ITB4/FAK/SOX2/HIF-1α signalling [5, 78]. In addition, the ECM may control actin cytoskeletal structure, leading to metastasis of invasive breast cancer cells. One protein subtype, ECM1a, activates AKT/FAK/Paxillin/Rac/cytoskeletal signalling via the Gly-Pro-Arg (GPR)-mediated interaction with the integrin αXβ2 and upregulates CD326 expression to control ovarian carcinogenesis and resistance to cisplatin [78].

ECM remodelling is a pathological marker that promotes the migration of TCs, a process that requires additional energy, which is acquired from enhanced glycolysis. HA and hyaluronidase have both been implicated in tumour formation [5]. Hyaluronidase promotes TCs invasion by promoting an increase in the receptor complex kinase-mediated mRNA decay factor ZFP26, promoting the degradation of TXNIP transcripts, reducing the internalisation of glucose transporter protein 1 (GLUT1), enriching GLUT1 in the cell membrane and increasing glucose uptake and glycolysis to promote ATP production [79].

When the ECM is remodelled, the cross-links between proteins in the ECM are disrupted, and a certain degree of matrix degradation occurs, affecting the invasiveness of the tumour. TCs and CAFs act together and secrete multiple ECM–degrading enzymes, including MMPs, urokinase fibrinogen activator, and disintegrin-metalloproteinase [5]. These activated enzymes degrade the BM and destroy the tumour compartment, making it easier for TCs to spread and invade into the surrounding area [80]. Moreover, TCs form cytoskeletal protrusions known as invasive pedicles, and MMPs are released from these structures around TCs. MMPs degrade collagen I, laminin, and fibronectin, facilitating CRC cell passage through the BM of the blood–brain barrier and enhancing TCs migration. The use of MMP inhibitors significantly reduces the incidence of experimental brain metastasis [81].

The ECM is critical for shaping tumour inflammation and the immune environment by regulating the TME. The tumour ECM is approximately 1.5–fold stiffer than normal tissue, and the intracellular mechanosensitive protein complex EPHA2/LYN in TCs regulates EMT and tumour metastasis via TWIST1 in response to the changed ECM [80, 82]. Phosphatidylinositol 3-kinase (PI3K) activity is enhanced due to the induction of collagen cross-linking, thereby increasing cancer cell viability [80]. Thickened ECM around TCs restricts the movement of immune cells, thereby inhibiting their cytotoxicity [80]. The ECM may induce the acquisition of a low immunogenic phenotype by DCs, whereas antigens presented by immature DCs can lead to immune tolerance and failure to generate a T-cell response [77]. In the collagen-rich tumour ECM, monocytes are biased towards differentiation into M2 TAMs, which promote TCs growth, angiogenesis, invasion and metastasis by secreting cytokines such as interleukin-6, interleukin-18, tumour necrosis factor-α, tumour necrosis factor-β and interferon-γ and enzymes such as arginase 1 and ECM-modifying enzymes into the ECM [83]. A stiff ECM may act as a physical barrier, affecting the distribution of T cells, hindering their proximity to TCs, and even leading to proliferation failure, downregulation of cytotoxic activity markers (CD101) and upregulation of Treg markers (CIP2A), inhibiting immunity against tumours [77].

The tumour ECM enhances drug resistance. The rich and dense fibrous network of the ECM blocks drugs around tumour vessels, significantly reducing the efficiency of drug diffusion and acting as a barrier to drug transport into tumour tissue [77]. ECM stiffness increases multidrug resistance protein 1 (MRP1) in the TCs membrane. The stiff ECM enhances the functional activity of MRP1 in breast cancer cells, increasing the efflux of vincristine (VCR) [84]. BRAF leads to high expression of membrane-type I matrix metalloproteinase (MT1-MMP) in melanoma, CRC, and glioblastoma, which activates MAPK and PI3K/Akt signalling pathways in TCs via MET receptors to resist BRAF inhibitors [85].

The tumour matrix index (TMi), obtained by calculating the expression levels of 29 matrix genes, has predictive value for prognosis and response to adjuvant chemotherapy [86]. Pearce *et al*. found that TMi was positively correlated with disease score and immunosuppression and negatively correlated

DOI: 10.54254/2753-8818/15/20240464

with overall survival in 36 high-grade serous ovarian carcinoma (HGSOC) patients. It is a measure of tissue and ECM remodelling in HGSOC [87].

7. Mucus

Mucus is protective gel-like structure located on the surface of the mammalian intestinal epithelium and consist of mucin, secretory immunoglobulin (sIgA), antimicrobial peptides, inorganic salts, and water [28]. Mucus forms the chemical barrier of the intestine, protecting the intestinal mucosa from microbial attack and acid and alkaline erosion. Intestinal mucus is closely related to infectious intestinal diseases, inflammatory bowel disease and intestinal tumours and is involved in the development and progression of acute pancreatitis, nonalcoholic fatty liver disease and neurodegenerative diseases and may become a target for the treatment of various related diseases [88]. Few relevant studies concerning mucus in the appendix have been published, and more follow-up investigation is needed.

There are differences between the mucosal layers of the small intestine and colon (Fig. 3). There is only one layer of mucus in the small intestine, and the pore size allows bacterial-sized substances to penetrate [89]. Colonic mucus has two layers. The outer layer is similar in structure to that in the small intestine. It is the site of bacterial colonisation. The inner layer exhibits a smaller pore size and prevents the entry of microorganisms. The thickness of the mucus layer varies between different sections of the intestine. In the small intestine, it is relatively thin, at approximately 100-500 μm, while in the colon, it can reach a thickness of approximately 830 μm [89]. Mucins are highly glycosylated proteins secreted by goblet cells (GCs) and are the key compounds in mucous. Twenty mucins have been identified, named MUC1 to MUC20 in ascending order of their discovery [89]. MUC2 is the core component of mucous, with its oligosaccharide chain structure providing an adhesion binding site for normal intestinal flora, assisting probiotic colonisation of the intestine, and reducing the adhesion of pathogenic bacteria. This structure also provides binding sites for SIgA and antimicrobial peptides [88].

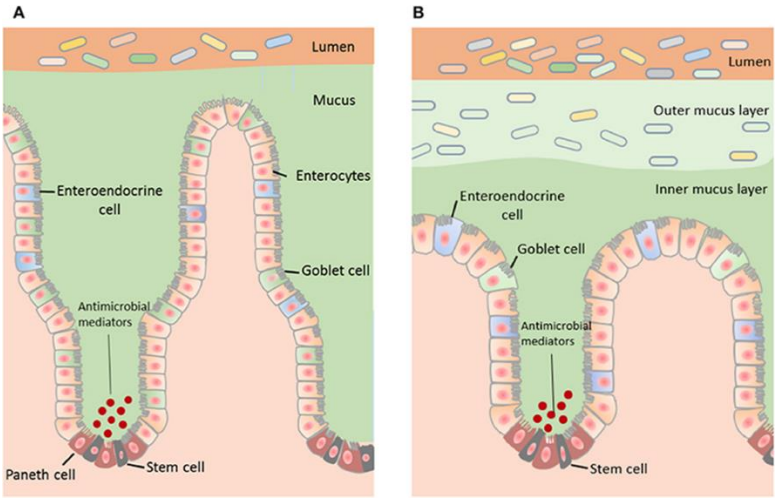

Figure 3. Differences in mucus in the small intestine (A) and colon (B). (A) The small intestine consists of a single layer of mucus that is loosely adherent to the epithelium and easily passable. The bacteria in the small intestine are repelled from the epithelium mainly by antimicrobial regulators. (B) The colon consists of two layers of mucus: the tight inner layer and the loose outer layer. The inner mucus of the colon is almost sterile, but microorganisms live on the outer mucus. Adopted form Herath et al. [88]

Mucus also shows an immune function. The immune molecules, such as sIgA, antimicrobial peptides, can exert antimicrobial effects on the mucus barrier. The intestinal trefoil factor (ITF) secreted by GCs interacts with mucin to increase the viscosity of the mucus layer [90]. Resistin-like molecule-β (RELM-β) directly kills commensal bacteria and pathogens that penetrate the mucosal layer [90]. α-Defensin,

DOI: 10.54254/2753-8818/15/20240464

the main antimicrobial peptide secreted by Paneth cells, binds to bacterial cell membranes to form ion channels that cause the leakage of bacterial cell contents, thereby killing bacteria [91].

Changes in the mucus composition have been correlated with CRC and PMP. Abnormal intestinal mucin O-glycosylation and an impaired mucus barrier trigger bacterial invasion and activation of caspase-1 inflammatory vesicles, promoting the development of enteritis and colon cancer [89]. MUC2-knockout mice develop spontaneous CRC and colitis [92]. In CRC patients, mucus is abnormal due to altered mucin expression and abnormal glycosylation. Mucus plays a dual role in colon cancer; on the one hand, it prevents the development of CRC, and on the other hand, in advanced stages of CRC, it promotes tumour growth and invasion [93]. Mucinous colorectal adenocarcinoma is associated with a poorer prognosis than nonmucinous adenocarcinoma, and high expression of MUC2 promotes CRC liver metastasis [94]. The mucus surrounding CRC cells blocks immune surveillance, promotes cancer cell migration, and increases resistance to anticancer drugs [89]. High expression of MUC2, abnormal glycosylation and ectopic expression of MUC5AC are accompanying characteristics of mucinous carcinoma [89]. In PMP, mucin secretion is significantly increased, and mucin is progressively deposited in the peritoneal cavity, playing a key role in the progressive sclerosis of the mucosal layer [95]. TCs are encapsulated in large amounts of mucus, which allows metastasis, dissemination, and redistribution of TCs within the peritoneal cavity. It is a barrier against immune cells and chemotherapy and produces a suitable microenvironment for tumour growth [28].

8. Conclusion

This review focuses on the hallmarks of tumours, the characteristics of CRC, CRC-PM, LAMN and PMP, and the impact of the tumour ECM and mucus. Many studies have focused on cells in tumour tissue and the TME, and more research needs to be done on the ECM in PM. A survey of the ECM involves many challenges, such as the acquisition of study materials and the choice of analytic methods. Specifically, LAMN and PMP are rare diseases, and therefore, samples are lacking. Commercial Matrigel, which is somewhat different from human ECM, are generally used instead of *in vitro* organoid cultures and may not be valid systems for studying cancer cell proliferation, metastasis, and other behaviours [4]. In CRC liver metastasis, the premetastatic niche (PMN) stimulates the liver microenvironment before circulating factors are released [2]. The difference between the ECM in PM and the ECM in the primary tumour and the factors in the ECM of the primary tumour that promote PM need to be addressed. The impact of TCs on the peritoneum and changes in the peritoneal ECM during metastasis are not well understood. An in-depth study of ECM in metastatic peritoneal cancer will help determine the mechanisms of peritoneal metastasis and provide important clues for developing novel therapeutic approaches.

References

[1] Xia, C., et al., *Cancer statistics in China and United States, 2022: profiles, trends, and determinants.* Chinese medical journal, 2022. 135(5): p. 584-590.

[2] Karlsson, S. and H. Nyström, *The extracellular matrix in colorectal cancer and its metastatic settling – Alterations and biological implications.* Critical reviews in oncology/hematology, 2022. 175: p. 103712-103712.

[3] Sung, H., 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.

[4] *Decellularized Normal and Tumor Extracellular Matrix as Scaffold for Cancer Organoid Cultures of Colorectal Peritoneal Metastases.* Obesity, fitness, & wellness week, 2022: p. 901.

[5] Cox, T.R., *The matrix in cancer.* Nature reviews. Cancer, 2021. 21(4): p. 217-238.

[6] Hanahan, D. and R.A. Weinberg, *The Hallmarks of Cancer.* Cell, 2000. 100(1): p. 57-70.

[7] Hanahan, D. and Robert A. Weinberg, *Hallmarks of Cancer: The Next Generation.* Cell, 2011. 144(5): p. 646-674.

[8] Hanahan, D., *Hallmarks of Cancer: New Dimensions.* Cancer discovery, 2022. 12(1): p. 31-46.

[9] Thienpont, B., L. Van Dyck, and D. Lambrechts, *Tumors smother their epigenome.* Molecular & cellular oncology, 2016. 3(6): p. e1240549-e1240549.
[10] Mohammadi, H. and E. Sahai, *Mechanisms and impact of altered tumour mechanics.* Nature cell biology, 2018. 20(7): p. 766-774.
[11] Flavahan, W.A., E. Gaskell, and B.E. Bernstein, *Epigenetic plasticity and the hallmarks of cancer.* Science (American Association for the Advancement of Science), 2017. 357(6348).
[12] He, R., et al., *H3K4 demethylase KDM5B regulates cancer cell identity and epigenetic plasticity.* Oncogene, 2022. 41(21): p. 2958-2972.
[13] Bi, M., et al., *Enhancer reprogramming driven by high-order assemblies of transcription factors promotes phenotypic plasticity and breast cancer endocrine resistance.* Nature cell biology, 2020. 22(6): p. 701-715.
[14] Finicle, B.T., V. Jayashankar, and A.L. Edinger, *Nutrient scavenging in cancer.* Nature reviews. Cancer, 2018. 18(10): p. 619-633.
[15] Fais, S. and M. Overholtzer, *Cell-in-cell phenomena in cancer.* Nature reviews. Cancer, 2018. 18(12): p. 758-766.
[16] Bartosh, T.J., et al., *Cancer cells enter dormancy after cannibalizing mesenchymal stem/stromal cells (MSCs).* Proceedings of the National Academy of Sciences - PNAS, 2016. 113(42): p. E6447-E6456.
[17] Fais, S. and M. Overholtzer, *Cell-in-cell phenomena, cannibalism, and autophagy: is there a relationship?* Cell death & disease, 2018. 9(2): p. 95-3.
[18] Tonnessen-Murray, C.A., et al., *Chemotherapy-induced senescent cancer cells engulf other cells to enhance their survival.* The Journal of cell biology, 2019. 218(11): p. 3827-3844.
[19] Black, J.R.M. and N. McGranahan, *Genetic and non-genetic clonal diversity in cancer evolution.* Nature reviews. Cancer, 2021. 21(6): p. 379-392.
[20] Miles, L.A., et al., *Single-cell mutation analysis of clonal evolution in myeloid malignancies.* Nature (London), 2020. 587(7834): p. 477-482.
[21] Erickson, A., et al., *Spatially resolved clonal copy number alterations in benign and malignant tissue.* Nature (London), 2022. 608(7922): p. 360-3.
[22] Gast, C.E., et al., *Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival.* Science advances, 2018. 4(9): p. eaat7828-eaat7828.
[23] Milo, I., et al., *The immune system profoundly restricts intratumor genetic heterogeneity.* Science immunology, 2018. 3(29): p. eaat1435.
[24] Nejman, D., et al., *The human tumor microbiome is composed of tumor type-specific intracellular bacteria.* Science (American Association for the Advancement of Science), 2020. 368(6494): p. 973-980.
[25] Cullin, N., et al., *Microbiome and cancer.* Cancer cell, 2021. 39(10): p. 1317-1341.
[26] Fu, A., et al., *Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer.* Cell, 2022. 185(8): p. 1356-1372.e26.
[27] Niño, J.L.G., et al., *Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer.* Nature (London), 2022. 611(7937): p. 810-4.
[28] Paone, P. and P.D. Cani, *Mucus barrier, mucins and gut microbiota: the expected slimy partners?* Gut, 2020. 69(12): p. 2232-2243.
[29] Tajima, Y., et al., *Differential analysis of microbiomes in mucus and tissues obtained from colorectal cancer patients.* Scientific reports, 2022. 12(1): p. 18193-18193.
[30] Xia, L., et al., *The cancer metabolic reprogramming and immune response.* Molecular cancer, 2021. 20(1): p. 28-28.
[31] Virtuoso, A., et al., *Tumor Microenvironment and Immune Escape in the Time Course of Glioblastoma.* Molecular neurobiology, 2022. 59(11): p. 6857-6873.
[32] Vaupel, P. and G. Multhoff, *Revisiting the Warburg effect: historical dogma versus current understanding.* The Journal of physiology, 2021. 599(6): p. 1745-1757.

DOI: 10.54254/2753-8818/15/20240464

[33] Hinshaw, D.C. and L.A. Shevde, *The tumor microenvironment innately modulates cancer progression.* Cancer research (Chicago, Ill.), 2019. 79(18): p. 4557-4566.
[34] Chambers, A.M., et al., *Adenosinergic Signaling Alters Natural Killer Cell Functional Responses.* Frontiers in immunology, 2018. 9: p. 2533-2533.
[35] Ma, X., et al., *Cholesterol Induces CD8+ T Cell Exhaustion in the Tumor Microenvironment.* Cell metabolism, 2019. 30(1): p. 143-156.e5.
[36] Baumann, T., et al., *Regulatory myeloid cells paralyze T cells through cell–cell transfer of the metabolite methylglyoxal.* Nature immunology, 2020. 21(5): p. 555-566.
[37] Myer, P.A., et al., *The Genomics of Colorectal Cancer in Populations with African and European Ancestry.* Cancer discovery, 2022. 12(5): p. 1282-1293.
[38] Siegel, R.L., et al., *Colorectal cancer statistics, 2020.* CA: a cancer journal for clinicians, 2020. 70(3): p. 145-164.
[39] Al-Shamsi, H.O., et al., *Molecular spectrum of KRAS , NRAS , BRAF , PIK3CA , TP53 , and APC somatic gene mutations in Arab patients with colorectal cancer: determination of frequency and distribution pattern.* Journal of gastrointestinal oncology, 2016. 7(6): p. 882-902.
[40] Zhu, G., et al., *Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer.* Molecular cancer, 2021. 20(1): p. 143-143.
[41] Chen, Z., et al., *Aspirin has a better effect on PIK3CA mutant colorectal cancer cells by PI3K/Akt/Raptor pathway.* Molecular medicine (Cambridge, Mass.), 2020. 26(1): p. 14-14.
[42] Dienstmann, R. and J. Tabernero, *Spectrum of Gene Mutations in Colorectal Cancer: Implications for Treatment.* The cancer journal (Sudbury, Mass.), 2016. 22(3): p. 149-155.
[43] Saller, J., et al., *Microsatellite Stable Colorectal Cancer With an Immunogenic Phenotype: Challenges in Diagnosis and Treatment.* Clinical colorectal cancer, 2020. 19(2): p. 123-131.
[44] Kawasaki, T., et al., *CpG island methylator phenotype-low (CIMP-low) colorectal cancer shows not only few methylated CIMP-high-specific CpG islands, but also low-level methylation at individual loci.* Modern pathology, 2008. 21(3): p. 245-255.
[45] Qin, J., et al., *A human gut microbial gene catalogue established by metagenomic sequencing.* Nature (London), 2010. 464(7285): p. 59-65.
[46] Fang, Y., et al., *The roles of microbial products in the development of colorectal cancer: a review.* Bioengineered, 2021. 12(1): p. 720-735.
[47] Perillo, F., et al., *Gut Microbiota Manipulation as a Tool for Colorectal Cancer Management: Recent Advances in Its Use for Therapeutic Purposes.* International journal of molecular sciences, 2020. 21(15): p. 5389.
[48] Zhao, L., W.C. Cho, and M.R. Nicolls, *Colorectal Cancer-Associated Microbiome Patterns and Signatures.* Frontiers in genetics, 2021. 12: p. 787176-787176.
[49] Cao, H., et al., *Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis: Bile acid, dysbiosis and CRC.* International journal of cancer, 2017. 140(11): p. 2545-2556.
[50] Hwang, S., et al., *Zerumbone Suppresses Enterotoxigenic Bacteroides fragilis Infection-Induced Colonic Inflammation through Inhibition of NF-κB.* International journal of molecular sciences, 2019. 20(18): p. 4560.
[51] Guo, P., et al., *FadA promotes DNA damage and progression of Fusobacterium nucleatum-induced colorectal cancer through up-regulation of chk2.* Journal of experimental & clinical cancer research, 2020. 39(1): p. 1-202.
[52] Colangelo, T., et al., *Friend or foe?: The tumour microenvironment dilemma in colorectal cancer.* Biochimica et biophysica acta. Reviews on cancer, 2017. 1867(1): p. 1-18.
[53] Zhou, R.W., et al., *A local tumor microenvironment acquired super-enhancer induces an oncogenic driver in colorectal carcinoma.* Nature communications, 2022. 13(1): p. 6041-6041.
[54] Zhang, R., et al., *Cancer-associated fibroblasts enhance tumor-associated macrophages enrichment and suppress NK cells function in colorectal cancer.* Cell death & disease, 2019. 10(4): p. 273-273.

[55] Lu, Y., et al., *MondoA–Thioredoxin-Interacting Protein Axis Maintains Regulatory T-Cell Identity and Function in Colorectal Cancer Microenvironment.* Gastroenterology (New York, N.Y. 1943), 2021. 161(2): p. 575-591.e16.
[56] Roth, L., et al., *Peritoneal metastasis: Current status and treatment options.* Cancers, 2022. 14(1): p. 60.
[57] Klevansky, M., et al., *The impact of primary tumour resection and sidedness in patients with synchronous metastatic colorectal cancer (mCRC): Findings from the South Australian Metastatic Colorectal Cancer Registry (SAMCRC).* Journal of clinical oncology, 2018. 36(4_suppl): p. 739-739.
[58] Lund-Andersen, C., et al., *Omics analyses in peritoneal metastasis-utility in the management of peritoneal metastases from colorectal cancer and pseudomyxoma peritonei: A narrative review.* Journal of gastrointestinal oncology, 2021. 12(Suppl 1): p. S191-S203.
[59] Kim, S.-C., et al., *Establishment and Characterization of Paired Primary and Peritoneal Seeding Human Colorectal Cancer Cell Lines: Identification of Genes That Mediate Metastatic Potential.* Translational oncology, 2018. 11(5): p. 1232-1243.
[60] Gong, J., et al., *Reprogramming of lipid metabolism in cancer-associated fibroblasts potentiates migration of colorectal cancer cells.* Cell death & disease, 2020. 11(4): p. 267-267.
[61] Lemoine, L., P. Sugarbaker, and K. Van Der Speeten, *Pathophysiology of colorectal peritoneal carcinomatosis: Role of the peritoneum.* World journal of gastroenterology : WJG, 2016. 22(34): p. 7692-7707.
[62] Raghavan, S., et al., *Ovarian cancer stem cells and macrophages reciprocally interact through the WNT pathway to promote pro-tumoral and malignant phenotypes in 3D engineered microenvironments.* Journal for immunotherapy of cancer, 2019. 7(1): p. 190-190.
[63] Demuytere, J., et al., *The role of the peritoneal microenvironment in the pathogenesis of colorectal peritoneal carcinomatosis.* Experimental and molecular pathology, 2020. 115: p. 104442-104442.
[64] Li, P., et al., *PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps.* The Journal of experimental medicine, 2010. 207(9): p. 1853-1862.
[65] Nieman, K.M., et al., *Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth.* Nature medicine, 2011. 17(11): p. 1498-1503.
[66] Hissong, E. and R.K. Yantiss, *The Frontiers of Appendiceal Controversies: Mucinous Neoplasms and Pseudomyxoma Peritonei.* The American journal of surgical pathology, 2022. 46(1): p. E27-E42.
[67] Hegg, K.S., et al., *Macroscopic and microscopic characteristics of low grade appendiceal mucinous neoplasms (LAMN) on appendectomy specimens and correlations with pseudomyxoma peritonei development risk.* Annals of diagnostic pathology, 2020. 48: p. 151606-151606.
[68] Yanai, Y., et al., *Molecular and clinicopathological features of appendiceal mucinous neoplasms.* Virchows Archiv : an international journal of pathology, 2021. 478(3): p. 413-426.
[69] Yajima, N., et al., *Immunohistochemical expressions of cytokeratins, mucin core proteins, p53, and neuroendocrine cell markers in epithelial neoplasm of appendix.* Human pathology, 2005. 36(11): p. 1217-1225.
[70] Alakus, H., et al., *Genome-wide mutational landscape of mucinous carcinomatosis peritonei of appendiceal origin.* Genome medicine, 2014. 6(5): p. 43-43.
[71] Nishikawa, G., et al., *Frequent GNAS mutations in low-grade appendiceal mucinous neoplasms.* British journal of cancer, 2013. 108(4): p. 951-958.
[72] Dilly, A.K., et al., *Targeting hypoxia-mediated mucin 2 production as a therapeutic strategy for mucinous tumors.* Translational research : the journal of laboratory and clinical medicine, 2016. 169: p. 19-30.e1.
[73] Pillai, K., et al., *A formulation for in situ lysis of mucin secreted in pseudomyxoma peritonei.* International journal of cancer, 2014. 134(2): p. 478-486.

DOI: 10.54254/2753-8818/15/20240464

[74] Pillai, K.M.S., et al., *Mucolysis by Ascorbic Acid and Hydrogen Peroxide on Compact Mucin Secreted in Pseudomyxoma Peritonei.* The Journal of surgical research, 2012. 174(2): p. e69-e73.

[75] Liu, X., et al., *Molecular profiling of appendiceal epithelial tumors using massively parallel sequencing to identify somatic mutations.* Clinical chemistry (Baltimore, Md.), 2014. 60(7): p. 1004-1011.

[76] Saarinen, L., et al., *Multiple components of PKA and TGF-β pathways are mutated in pseudomyxoma peritonei.* PloS one, 2017. 12(4): p. e0174898-e0174898.

[77] He, Y., et al., *Tumor-Associated Extracellular Matrix: How to Be a Potential Aide to Anti-tumor Immunotherapy?* Frontiers in cell and developmental biology, 2021. 9: p. 739161-739161.

[78] Yin, H., et al., *Extracellular matrix protein-1 secretory isoform promotes ovarian cancer through increasing alternative mRNA splicing and stemness.* Nature communications, 2021. 12(1): p. 4230-4230.

[79] Sullivan, W.J., et al., *Extracellular Matrix Remodeling Regulates Glucose Metabolism through TXNIP Destabilization.* Cell, 2018. 175(1): p. 117-132.e21.

[80] Henke, E., R. Nandigama, and S. Ergün, *Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy.* Frontiers in molecular biosciences, 2020. 6: p. 160-160.

[81] Kumara, H.M.C.S., et al., *Plasma MMP-2 and MMP-7 levels are elevated first month after surgery and may promote growth of residual metastases.* World journal of gastrointestinal oncology, 2021. 13(8): p. 880-892.

[82] Patwardhan, S., et al., *ECM stiffness-tuned exosomes drive breast cancer motility through thrombospondin-1.* Biomaterials, 2021. 279: p. 121185-121185.

[83] Deligne, C. and K.S. Midwood, *Macrophages and Extracellular Matrix in Breast Cancer: Partners in Crime or Protective Allies?* Frontiers in oncology, 2021. 11: p. 620773-620773.

[84] Kuermanbayi, S., et al., *In situ monitoring of functional activity of extracellular matrix stiffness-dependent multidrug resistance protein 1 using scanning electrochemical microscopy.* Chemical science (Cambridge), 2022. 13(35): p. 10349-10360.

[85] Lau, Eunice Yuen T., et al., *Cancer-Associated Fibroblasts Regulate Tumor-Initiating Cell Plasticity in Hepatocellular Carcinoma through c-Met/FRA1/HEY1 Signaling.* Cell reports (Cambridge), 2016. 15(6): p. 1175-1189.

[86] Lim, S.B., et al., *Addressing cellular heterogeneity in tumor and circulation for refined prognostication.* Proceedings of the National Academy of Sciences - PNAS, 2019. 116(36): p. 17957-17962.

[87] Pearce, O.M.T., et al., *Deconstruction of a metastatic tumor microenvironment reveals a common matrix response in human cancers.* Cancer discovery, 2018. 8(3): p. 304-319.

[88] Herath, M., et al., *The Role of the Gastrointestinal Mucus System in Intestinal Homeostasis: Implications for Neurological Disorders.* Frontiers in cellular and infection microbiology, 2020. 10: p. 248-248.

[89] Coleman, O.I. and D. Haller, *Microbe–mucus interface in the pathogenesis of colorectal cancer.* Cancers, 2021. 13(4): p. 1-18.

[90] Belle, N.M., et al., *TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths.* Nature communications, 2019. 10(1): p. 4408-13.

[91] Morampudi, V., et al., *The goblet cell-derived mediator RELM-β drives spontaneous colitis in Muc2-deficient mice by promoting commensal microbial dysbiosis.* Mucosal immunology, 2016. 9(5): p. 1218-1233.

[92] Zeng, Y., et al., *MUC1 predicts colorectal cancer metastasis: A systematic review and meta-analysis of case controlled studies.* PloS one, 2015. 10(9): p. e0138049-e0138049.

[93] De Arcangelis, A., et al., *Hemidesmosome integrity protects the colon against colitis and colorectal cancer.* Gut, 2017. 66(10): p. 1748-1760.

DOI: 10.54254/2753-8818/15/20240464

[94] Ribeirinho-Soares, S., et al., *Prognostic significance of MUC2, CDX2 and SOX2 in stage II colorectal cancer patients.* BMC cancer, 2021. 21(1): p. 359-359.
[95] Lin, Y.-L. and Y. Li, *The biological synthesis and the function of mucin 2 in pseudomyxoma peritonei.* Cancer management and research, 2021. 13: p. 7909-7917.