Trends in Cancer

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Review

Targeting the Tumor Microenvironment in Colorectal Peritoneal Metastases

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Peritoneal metastasis (PM) occurs in approximately one in four colorectal cancer (CRC) patients. The pathophysiology of colorectal PM remains poorly characterized. Also, the efficacy of current treatment modalities, including surgery and intraperitoneal (IP) delivery of chemotherapy, is limited. Increasingly, therefore, efforts are being developed to unravel the PM cascade and at understanding the PM-associated tumor microenvironment (TME) and peritoneal ecosystem as potential therapeutic targets. Here, we review recent insights in the structure and components of the TME in colorectal PM, and discuss how these may translate into novel therapeutic approaches aimed at re-engineering the metastasis-promoting activity of the stroma.

Highlights

Up to one in four patients with CRC develops PM, which is notoriously difficult to treat. Despite the magnitude of this clinical challenge, little is known about the pathophysiology and molecular biology of PM.

Emerging data highlight the role of the peritoneal ecosystem and of the TME as key drivers of metastatic progression and treatment resistance.

Current treatment strategies, including surgery and IP chemotherapy, result in a shift of the ecosystem towards an overall immunosuppressive and metastasis enhancing environment.

Strategies targeting the TME may prevent or reverse peritoneal cancer progression. Advances in drug delivery platforms and tissue engineering hold considerable promise to allow prolonged stromal targeting.

PM in CRC: Setting the Stage

CRC represents a major cause of cancer-related mortality worldwide, and approximately 25% of patients presents with, or will develop **peritoneal metastasis** (PM; see Glossary) [1]. Despite this high incidence, and in sharp contrast with PM from ovarian cancer, surprisingly little is known about the biology of colorectal PM. Over the past decades, advances in surgical management and identification of novel therapeutic targets have led to significantly improved survival of patients with colorectal liver and lung metastases [2]. In contrast, however, modern chemotherapy is less effective in patients with colorectal PM [3]. At the same time, current treatments such as surgery and IP chemotherapy adversely affect the peritoneal host defense, leading to rapid recurrence in many patients.

The role of the **tumor microenvironment** (TME) in cancer progression is increasingly recognized. While the TME of colorectal PM is poorly understood, recent data suggest that the tumor stroma may harbor targets for re-engineering the adverse TME and for harnessing the patient's immune response. These developments are facilitated by the rapidly increasing interest in novel compounds and biomaterials for IP delivery such as hydrogels and nanomedicine formulations. Here, we review the pathophysiology (Box 1) and microenvironment of PM in CRC patients, discuss the impact of surgery on PM, and provide insights into novel experimental models (Box 2) and the potential of stromal targeting in these patients.

TME of colorectal PM

As in any solid cancer, the malignant cell population of PM is embedded in, and communicates with, a tissue consisting of a large array of cellular and acellular components. The cellular components include immune cells, vascular cells, and mesenchymal cells, while the acellular components include extracellular vesicles (EVs), nanosized fragments of cells containing lipids, proteins and nucleotides, structural extracellular matrix (ECM) proteins such as collagen, laminin, fibronectin, and proteoglycans, and the soluble elements including metabolites, growth factors, cytokines, chemokines, and proteases. For certain elements such as ECM composition, significant differences have been identified between normal colon, primary CRC, and liver metastases

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[4]. However, further deep mapping and biological understanding of these elements in PM of colorectal cancer needs more in depth research. An interesting study by Ubink and coworkers compared histopathological and molecular characteristics between paired primary colorectal cancers, and their associated PM. They found a >50% stromal content in 79% of primary cancers, compared to only 40% of PM. In addition, the majority of the primary as well as the metastatic tumors was classified as consensus molecular subtype (CMS) 4 [5].

The role of exosomes in the pathogenesis of PM was recently highlighted. Extracellular vesicles from CRC cells are enriched in the cell surface glycoprotein CD44, and vesicle transfer of CD44 to MC enhanced cancer cell invasion by inducing the mesothelial cells (MCs) to secrete matrix metalloproteinase (MMP), resulting in reduced mesothelial barrier activity [6]. EV-associated small RNAs from peritoneal lavage samples may have prognostic utility in CRC [7].

The most abundant mesenchymal cell types in the neoplastic stroma are cancer-associated fibroblasts (CAFs), a heterogeneous population that contributes to tumor initiation, (peritoneal) metastasis and immune escape [8,9]. The origins of CAFs are incompletely elucidated, but they do not stem from the cancer cell population. The largest source of CAFs are resident subperitoneal fibroblasts, which are driven to phenotypic diversity by multiple mediators including transforming growth factor (TGF)-β, tumor necrosis factor-β, and insulin-like growth factor I [10]. Indeed, *in vitro* and xenograft mouse models have demonstrated that subperitoneal fibroblasts create a permissive environment for invasion and metastasis in colorectal PM [11]. Other cell types that

Box 1. Pathophysiology of Colorectal PM

The pathophysiology of PM can be conceptualized as a stepwise process (Figure I). Isolated cancer cells or clusters of cells are shed from the surface of the primary cancer; a process that is facilitated by increased solid pressure and by changes in the activity of cell adhesion molecules such as E-cadherin [69,70]. In hypermethylated colorectal cancer, Zajac and coworkers observed that peritoneal spread and collective invasion is mediated by cancer spheroids displaying an outward apical pole, termed tumor spheres with inverted polarity (TSIPs) [71]. Malignant cells or clusters are transported throughout the peritoneal cavity with the physiological flow of peritoneal free fluid, resulting in a predictable tumor distribution (pelvis, right paracolic gutter, omentum, and subdiaphragmatic spaces).

The striking tropism of PM for the omentum is poorly understood. Recent data suggest that tumor-derived inflammatory factors stimulate omental neutrophils to release **neutrophil extracellular traps** (NETs), formed when stressed neutrophils expel their protein-studded chromatin to form local snares, and which act as a premetastatic niche [72]. It has also been suggested that cancer growth is stimulated by the proangiogenic environment of the omental 'milky spots', which consist of immune aggregates and a dense capillary network and that initial cancer cell binding is mediated by a network of type I collagen fibers overlaying the milky spots [73,74]. In ovarian cancer, the omentum was shown to harbor adipose-derived mesenchymal stem cells, which alter the metastatic microenvironment via paracrine mechanisms, and promote disease progression by stimulating proliferation, migration, and chemoresistance [75]. It is unknown whether a similar mechanism is relevant in CRC.

Loose cancer cells adhere to the MC layer and to the underlying ECM, an active process facilitated by a range of adhesion molecules. These belong largely to the integrins and their ligands, the proteoglycans such as CD44, the immunoglobulin superfamily, the blood group antigen proteins, the mucins, and EpCAM [76]. MCs express vascular cell adhesion molecule-1, intercellular adhesion molecule (ICAM)-1, and platelet endothelial cell adhesion molecule-1, but not ICAM-2 or E-selectin [77,78]. Senescence of peritoneal MCs is associated with increased expression of ICAM-1, and with increased CRC cell proliferation, migration, and invasion into the submesothelial tissue [79,80]. The expression of mesothelial adhesion molecules (and the resulting cancer cell adhesion) may be considerably enhanced by inflammatory stimuli induced by infection or surgical trauma [81]. NETs were recently shown to play an important role in colon cancer cell metastasis in the peritoneal cavity, and to regulate colon cancer cell migration and adhesion to ECM proteins [82]. Also, loose cancer cells gain access to submesothelial tissue at areas of peritoneal discontinuity or MC contraction. Alternatively, cancer cells can induce retraction and/or apoptosis of MCs. Heath *et al.* demonstrated FAS-dependent apoptosis of cultured human MCs induced by SW480 CRC cells [83]. Clusters of ovarian cancer cells induce retraction of MCs via myosin-mediated traction forces [84]. Once the mesothelial barrier is breached, cancer cells intermingle with fibroblasts and other stromal cell types in the underlying ECM. Eventually, invasion of the underlying abdominal wall or visceral organ structures will occur, along with the development of ascites.

Glossary

Chimeric antigen receptor T cells: expanded T cells from the patient (autologous) or from a donor (allogeneic), which are transduced with a gene encoding the engineered chimeric antigen receptor targeting a specific tumor antigen, and reinfused in the patient.

Epithelial-to-mesenchymal transition or epithelial plasticity: this process describes the plasticity that allows cancer cells to change from an epithelial to a mesenchymal-like phenotype, together with the acquisition of invasive behavior, immune evasion, and therapy resistance. EMT can be induced by a variety of growth factors, signaling pathways, and cellular stresses such as hypoxia, surgery, and chemotherapy treatment.

Hyperthermic intraperitoneal chemoperfusion: clinical treatment method for patients with peritoneal metastasis: during surgery and immediately following surgical removal of the tumor bulk, the peritoneal cavity is filled with a heated solution of chemotherapy (usually a platinum compound or mitomycin C).

Neutrophil extracellular traps: upon activating signals and conditions, neutrophils expel nuclear DNA which forms extracellular web-like structures decorated with nuclear histones and proteins, which serve primarily to trap microbial pathogens. However, recent data show that NET formation or NETosis is involved in a number of pathological conditions including intravascular thrombosis, respiratory disease, and cancer metastasis.

Peritoneal metastasis: this specific form of cancer metastasis typically originates from intra-abdominal cancers and is characterized by the formation of metastatic cancer nodules on the mesothelial surfaces, or on the underlying stroma.

Pressurized intraperitoneal aerosol chemotherapy: novel treatment approach in patients with widespread, unresectable peritoneal metastases, consisting of intraperitoneal delivery of chemotherapy as an aerosol, during a laparoscopic procedure.

Toll-like receptors: these are a family of receptors that recognize evolutionary highly conserved danger signals expressed by pathogens, and constitute an important part of the innate immune system. Depending on the receptor and

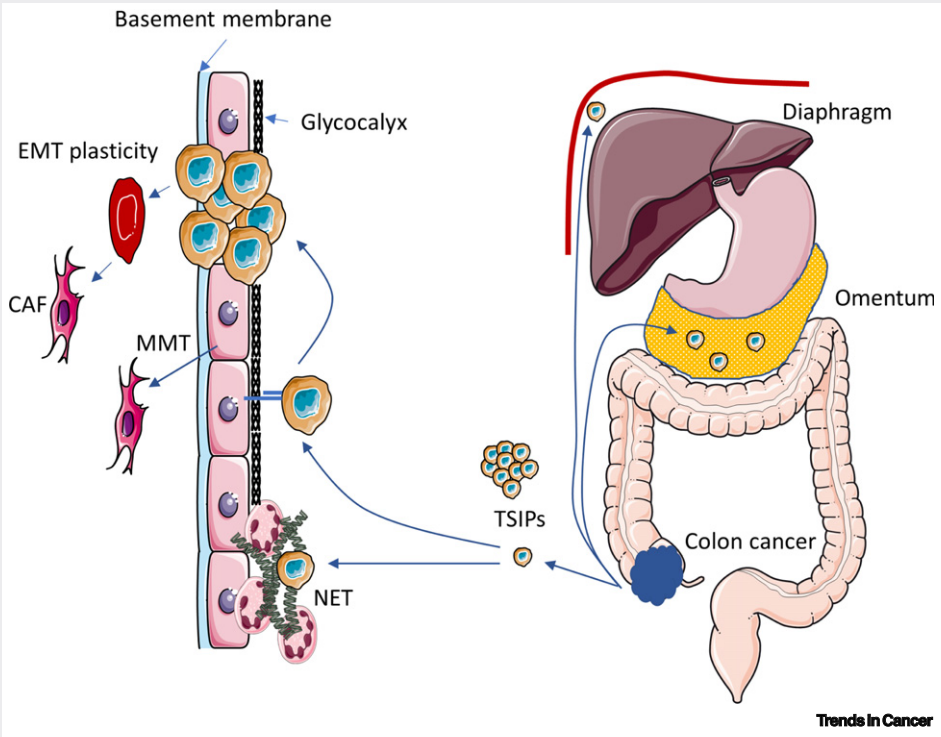

Figure I. The Peritoneal Metastasis (PM) Cascade. Cancer cells are shed from the primary tumor, either as isolated cells or as tumor spheres with inverted polarity (TSIPs), and are transported throughout the peritoneal cavity in a clockwise direction. Cancer cells adhere to the MCs, the glycocalyx, or the underlying stroma, or may be trapped in neutrophil extracellular traps (NETs). Once the mesothelial layer is breached, cancer cells undergo epithelial-to-mesenchymal (EMT) plasticity, resulting in a motile and invasive phenotype. Stromal cancer-associated fibroblasts (CAFs) play a central role in fueling the metastatic process. They may derive from multiple sources such as resident fibroblasts and MCs, the latter through a process of mesothelial-to-mesenchymal transition (MMT).

tumor type, agonists of TLR can have either tumor activating or tumor suppressing effects.

Tumor microenvironment: this refers to the cellular, biochemical, and mechanical constituents that surround and support the cancer cell population, with which they form a communicating ecosystem.

have been proposed as CAF precursors include endothelial cells, preadipocytes, and pericytes. Recent findings in PM from ovarian cancer suggest that MCs may undergo mesothelial-to-mesenchymal transition (MMT) to CAFs [12]. Preliminary experiments using patient-derived material do suggest that MMT occurs in colorectal PM (J. Demuytere *et al.*, unpublished). Recently, in pancreatic tumors, two CAF subtypes were identified: a population showing nuclear factor-κB signaling and expression of inflammatory cytokines/chemokines termed inflammatory CAF (iCAF), and a population that expresses α-smooth muscle actin and matrix proteins named myofibroblast CAF (myCAF)[13–15]. Similar heterogeneity in CAF populations has been observed in breast and lung cancer [16,17]. Whether iCAF, myCAF, or other CAF phenotypes participate in PM is not known but functional heterogeneity of CAFs may be an emerging feature of therapy resistance and immune escape of PM.

In the era of immunotherapy, increasing attention goes to the extent and composition of the stromal immune infiltrate. In primary CRC, extensive immune cell infiltration is observed in the subset (15%) of patients with deficient mismatch repair, resulting in a high tumor mutational burden and good response to immune checkpoint inhibitor (ICI) treatment [18]. In patients with colorectal liver metastasis, the location and extent of immune cells in the invasive margin was shown to predict response to chemotherapy [19]. The immune composition of colorectal PM remains, however, largely unexplored. A recent study compared the TME between primary CRC and PM clinical

samples, and found that the immune cell infiltrate in PM induces senescence and promotes extensive neovascularization [20]. Also, it appears that although there are multiple immune cells (including T cells) in the vicinity of colorectal PM cells, these appear to be excluded from the PM foci (Figure 1).

It is increasingly recognized that specific subpopulations of immune cells differ not only between cancer types but also between the primary and metastatic locations of the same cancer; this concept was recently termed immune contexture [21]. Tumor-associated macrophages (TAMs) are recruited to the TME by chemokines including CCL2, vascular endothelial growth factor (VEGF), CCL5, and TGF-β [22]. In general, TAMs stimulate tumor growth and metastasis by stimulating angiogenesis, priming the premetastatic niche, and exerting immunosuppressive effects [23]. Recent studies suggest that TAMs may elicit invasive behavior and epithelial-to-mesenchymal plasticity in colon cancer cells [24,25]. However, the prognostic significance of TAM infiltration differs according to tumor type: a high TAM density is correlated with a worse outcome in gastric cancer, urogenital cancer, and head and neck cancer, but with better survival in CRC [22]. In part, this may be explained by TAM-mediated enhanced response of CRC to chemotherapy [26]. In ovarian and gastric cancer, TAMs were shown to drive spheroid formation and to promote peritoneal dissemination [27,28].

The vascular component of the tumor stroma consists of blood vessels, lymphatic channels, and isolated endothelial cells (ECs). Angiogenic mediators secreted by cancer and stromal cells, including VEGFA, placental growth factor, basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF), drive the formation of microvessels that are morphologically and functionally abnormal. One of their key characteristics is hyperpermeability, explained by wide

Box 2. Novel Experimental Models of Colorectal PM

Syngeneic and xenografted models of colorectal PM can be created by IP injection or implantation of single cells or tissue fragments; the reader is referred to a recently published overview [85]. Although patient-derived xenografts may retrieve pharmacokinetic and pharmacodynamic information of drugs, these models are expensive, and do not allow high throughput molecular analyses or drug sensitivity screens. In addition, 2D cancer cell cultures do not recapitulate the phenotypic and genetic heterogeneity of the tumor cell population, and segregate cancer cells from interactions with the stromal microenvironment. Novel model systems that allow to faithfully reproduce the colorectal PM landscape include patient derived tumor organoids, bioengineered scaffolds, and organ-on-chip devices.

Patient-Derived Tumor Organoids

Organoids or tumoroids are self-organizing 3D structures derived from stem cells, cancer cells, and, dependent on the isolation and cultivation procedure, may include TME components such as immune cells and CAFs [86]. Patient-derived organoids hold considerable promise for molecular characterization and personalized therapy in CRC [87]. These self-organized bodies faithfully recapitulate the architecture and immunohistochemistry markers of the source tumor (Figure I). Several groups have succeeded in creating organoids from patients with colorectal PM, and used these to test drug sensitivity [88–91]. The recent introduction of repeated laparoscopic pressurized intraperitoneal aerosol chemotherapy (PIPAC) in patients with PM will allow us to predict individual treatment sensitivity, and study the process of emerging drug resistance over time [92].

Bioengineered Scaffolds

Continued developments in the fields of biomaterials, 3D modeling, and tissue engineering provide new opportunities to study tumor–stroma interactions in PM. The combination of scaffolds with hydrogels offers a highly innovative tool to construct a TME with the desired cellular and biomechanical properties. Hybrid type I collagen hydrogel-polylactic acid scaffolds coseeded with cancer cells, and CAFs showed *in vitro* colonization by heterocellular spheroid formation in the voids of the scaffold indicating mimicry of the PM architecture at the qualitative, quantitative, and spatial level (Figure I) [93]. Similarly, hybrid RGD-functionalized polyethylene-glycol-based hydrogel polycaprolactone scaffolds coseeded with cancer cells and MCs showed proliferative and communicative transcriptomic signatures that correlated well with overall and progression-free survival in high-grade serous ovarian cancer patients [94]. Orthotopic scaffold implantation into immunodeficient mice lead to ascites formation combined with omental and liver metastasis. In both scaffold models, patient-derived cell populations may be included and further development may allow to use bioengineered tumors as a platform technology to stratify the design of clinical trials.

Tumor-on-Chip Constructs

A further recent development of organoids as a model system is to connect multiple organoids using microfluidic channels. This allows to study the effect of nutrient distribution and mechanical stress, multiorgan metabolism, drug pharmacokinetics, and the integration of biosensors to monitor drug response [95]. Mazzocchi and coworkers used peritoneal mesothelioma samples to construct organoids within a tumor-on-a-chip microfluidic device, and demonstrated that the results of on-chip chemotherapy sensitivity screening correlated with those observed in the patients themselves [96].

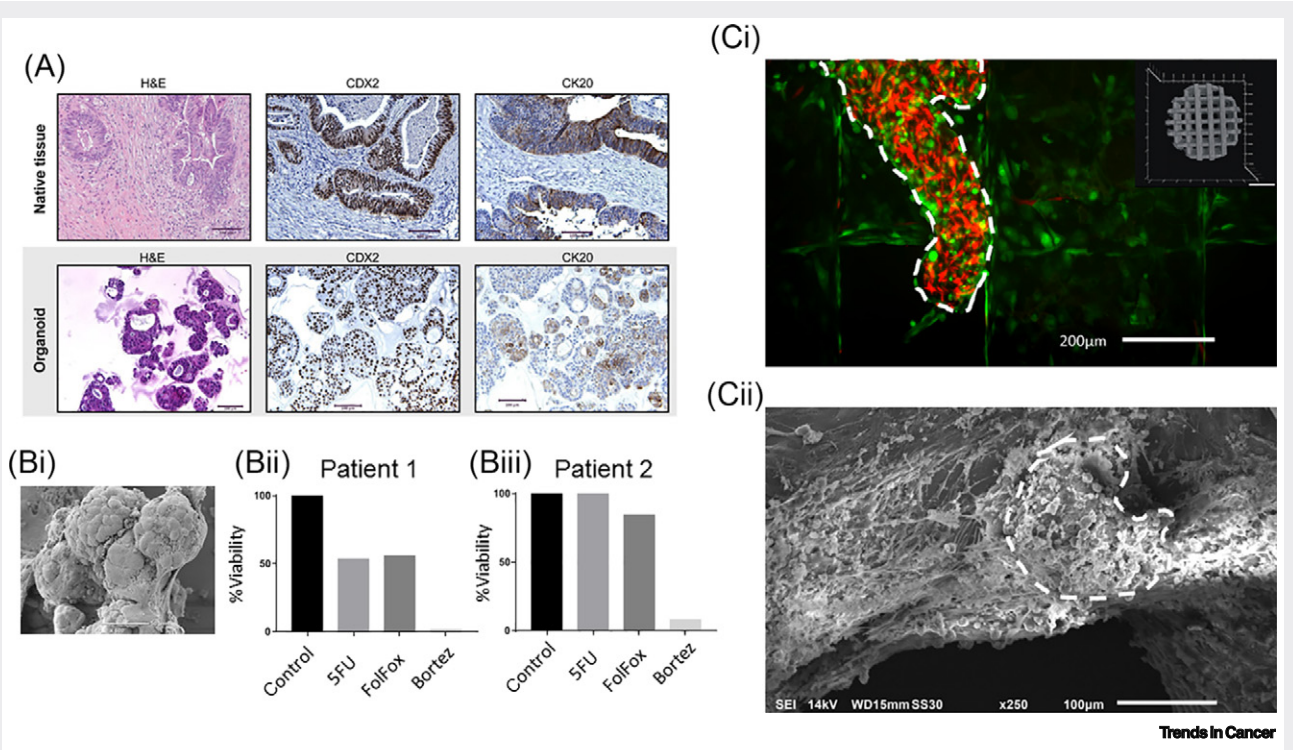

Figure I. Model Systems to Study Peritoneal Metastasis (PM). (A) PM can be used to generate 3D tumoroids that express the same diagnostic markers (homeobox protein, CDX2 and cytokeratin, CK20) as the source tissue. (B) PM tumoroids allow cytotoxic drug testing. (i) Scanning electron micrograph of a PM organoid (Bar = 50 mm). (ii and iii) Viability assays testing 5-fluorouracil (5FU) ± oxaliplatin (FolFox) and positive cytotoxic control bortezomib (Bortez). (Ci) Confocal image of a scaffold seeded with eGFP-labeled colorectal cancer cells (green) and tdTomato-labeled CAFs (red). Struts are mainly populated with colorectal cancer cells in contrast to spheroids that fill up the voids and contain a heterocellular population of cancer cells and CAFs. Inset shows top view of a μCT reconstruction of polylactic acid scaffold. (Cii) Scanning electron micrograph at an intersection of two struts. Both in Ci and Cii images were taken after 5 weeks of *in vitro* culture and dotted line indicates spheroid. Abbreviation: H&E, hematoxylin and eosin.

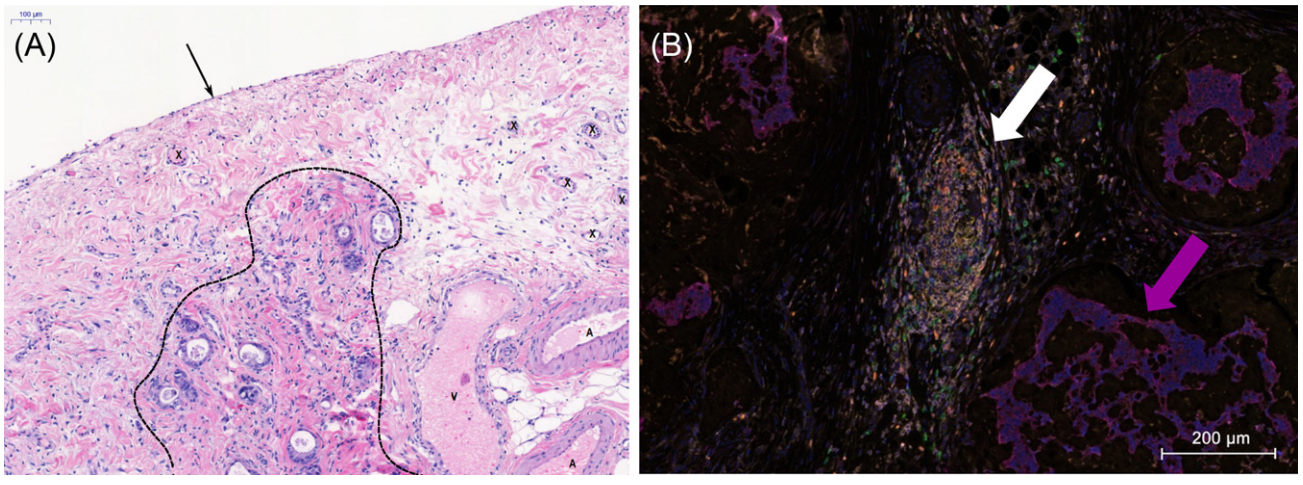

Figure 1. The Tumor Microenvironment of Two Clinical Colorectal Peritoneal Metastases. (A) Hematoxylin and eosin staining highlighting the mesothelial monolayer (black arrow), capillaries (x), arterioles (A), and vein (V). The interface between the submesothelial stroma and tumor stroma is indicated with the broken line. (B) Multiplex immunohistochemistry highlights immune cell exclusion (white arrow) away from tumor foci (purple arrow). Key: magenta, tumor cells; green, CD8; pink, CD4; orange, FoxP3; red, PD-1; yellow, PD-L1.

or absent intercellular junctions, the presence of lacunae and fenestrations, and incomplete coverage by support cells such as pericytes. As a consequence, large blood components leak into the interstitium and give rise to elevated oncotic and fluid pressures. The importance of the extent of angiogenesis in colorectal PM is poorly understood. In a retrospective histological analysis on PM resection samples, high levels of tissue versican and VEGF expression were associated with worse overall survival in a multivariable model [29]. However, microvessel density was not correlated with either VEGF expression or survival.

Effects of Surgery on the Peritoneal Ecosystem

It has been established that most of the growth factors, chemokines, and cytokines orchestrating surgical wound healing also promote tumor (re)growth, **epithelial-to-mesenchymal transition** (EMT), which is an important driver of cancer invasion, and angiogenesis [30]. Surgical trauma to the peritoneum is known to cause adhesions, which may lead to potentially serious complications such as bowel obstruction. Recent data in a mouse model suggest that the prime mover of adhesion formation is in fact traumatized MCs, which respond to hypoxia by nuclear activity of hypoxia-inducible factor (HIF)-1α, resulting in increased expression of podoplanin and mesothelin [31]. Antibodies neutralizing mesothelin diminish adhesion severity, suggesting potential clinical translation. Similarly, surgical stress to the peritoneal ecosystem may create a permissive environment for tumor growth. Clinical peritoneal samples from ovarian carcinoma patients taken at the incision site 1 h after manipulation showed higher abundance of cytokines involved in the inflammatory response [CD54, interleukin (IL)-6, IL-8, serpin E1, and Rantes], cell proliferation, and negative regulation of cell death [granulocyte colony-stimulating factor (CSF) and complement component C5/C5a] compared to the samples taken immediately after incision. Importantly, medium from the stressed samples induced cancer cell chemoresistance, a metastatic phenotype, and resistance to apoptosis. In agreement, the presence of chemokines and cytokines in peritoneal drain fluids of CRC patients was different in early versus late collections after incision [32]. FGF, granulocyte–macrophage (GM)-CSF, and IL-6 were the most prevalent postsurgical cytokines, while CCL2 (MCP-1), CXCL8 (IL-8), and CCL4 (MIP1β) were the most prevalent chemokines immediately after incision. At the end of the procedure, an 11-fold increase of IL-6 and fivefold increase of IL-1β, PDGF, and IL-1 receptor antagonist was observed compared to the starting point. Similarly, Berkovich *et al.* sampled drain fluid on consecutive days following colon surgery, and observed that, *in vitro*, drain fluid enhanced the migration capacity of colon cancer cells [33].

The perioperative period is characterized by a profound impairment of both innate and adaptive immunity [34,35]. Specifically, surgical stress results in an expansion of T regulatory (Treg) cells, myeloid-derived suppressor cells (MDSCs), and TAMs [36–38]. In addition, surgery results in upregulation of PD-1 expression, decreased T-cell proliferation, and impaired dendritic cell (DC) and natural killer (NK) cell cytotoxicity [39,40]. Angka and coworkers showed a profound reduction in NK-cell-secreted interferon (IFN)-γ; a cytokine with important roles in controlling infection and metastasis formation [41]. It has been suggested that locoregional hyperthermia may elicit an antitumor immune response, which may overcome the adverse effects of surgery [42]. There are, however, at present no clinical studies that support this hypothesis in the context of IP therapy. Anecdotal evidence from drain fluid analyses in a single patient showed that **hyperthermic chemoperfusion** (HIPEC) actually reverses tumor-induced NK cell suppression [43].

Laparoscopic surgery, although associated with less surgical trauma, is known to damage mesothelial structure and function by the insufflation of cold, dry CO_2 gas. The use of warm, humidified CO_2 gas mitigates expression of cyclo-oxygenase-2, VEGF, and HIF1α, prevents ultrastructural damage to the MCs and their apical network of microvilli, and impedes tumor cell implantation and subsequent growth [44,45].

Targeting the Peritoneal Microenvironment in Colorectal PM (Figure 2, Key Figure)

Intraperitoneal Immunotherapy and Immunomodulation

Advances in the field of implantable and injectable biomaterials open exciting new possibilities to engineer the peritoneal microenvironment. Several forms of IP immune therapy have met with success in preclinical models of colorectal PM. These include cell-based therapies, cancer vaccines, and IP antibodies. Katz and coworkers studied the effects of IP delivery of anti-carcionembryonic antigen (CEA) **chimeric antigen receptor T** (CAR-T) cells in a mouse model of colon adenocarcinoma [46]. They found that regional delivery resulted in superior antitumor efficacy compared to systemic administration. Also, the efficacy of anti-CEA CAR-T cells was improved when combined with targeting of MDSCs and Treg cells. Several IP cancer vaccines have shown promising results in preclinical colon cancer models. Liang *et al.* demonstrated the efficacy of a folate receptor alpha targeted lipoplex loading recombinant IL-15 plasmid in a CT26 colon cancer mouse model [47]. Other promising IP vaccines in preclinical CRC models include a virus-infected cell vaccine expressing IL-12, and a combination of immune checkpoint inhibitors with IL-18 [48,49]. The trifunctional antibody catumaxomab contains binding sites for epithelial cell adhesion molecule (EpCAM) and $CD3^+$ T cells, while the Fc domain binds to type I, IIa, and III Fcγ receptors on DCs, NKs, and macrophages. The molecule was approved by the European Medicines Agency for palliation of ascites in EpCAM-positive epithelial cancers. Analysis of clinical ascites samples showed that treatment with IP catumaxomab enhances the expression of the activation molecules CD69 and CD38 in T cells, NK cells, and macrophages, while accumulation of $CD8^+$ T cells into the peritoneal cavity was enhanced [50]. Also, IP catumaxomab was shown to promote recruitment of inflammatory TH1 cells (capable of degranulating and secreting IFN-γ) and to stimulate expression of TRAIL by NK cells, and co-stimulatory molecules by monocytes [51]. However, due to its high immunogenicity rate and narrow indications, commercial sale of catumaxomab was discontinued in 2017. A comparable approach was reported by Froysnes *et al.*, who successfully treated

Key Figure

Overview of Therapeutic Strategies Aimed at Reengineering the Tumor Microenvironment of Colorectal Peritoneal Metastases

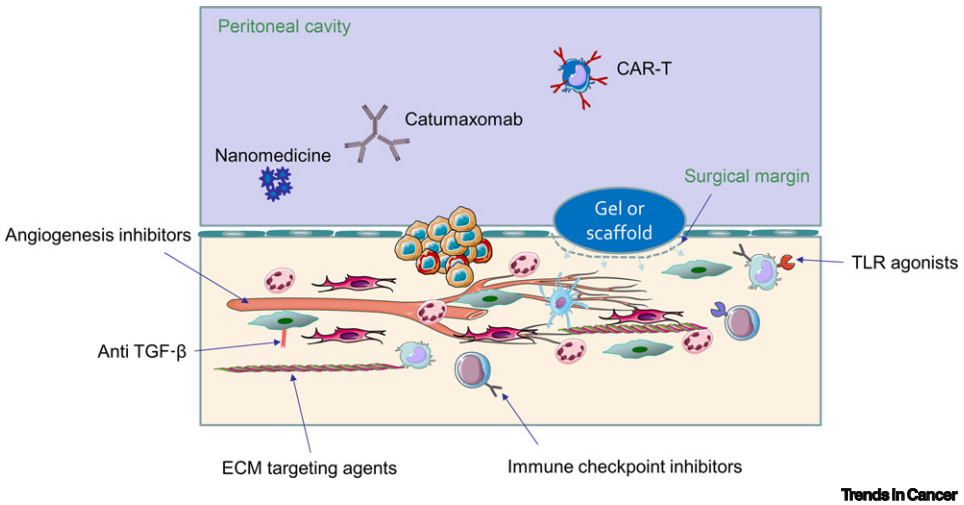

Figure 2. Abbreviations: CAR-T, chimeric antigen receptor T cell; ECM, extracellular matrix; TGF-β, transforming growth factor-β; TLR, Toll-like receptor.

colorectal PM patients with IP MOC31PE immunotoxin, consisting of an antibody recognizing EpCAM conjugated to the potent *Pseudomonas* exotoxin A [52].

An alternative, promising approach is IP delivery of natural or synthetic compounds that stimulate innate immunity. Examples include agonists of the **Toll-like receptors** (cytosine–guanine oligonucleotides, polyinosinic:polycytidylic acid, imidazoquinoline compounds) and activators of STING (stimulator of interferon genes) such as cyclic dinucleotides [53]. Locoregional delivery of these compounds in a suitable biomaterial such as a sprayable hydrogel or implantable scaffold in the postsurgical resection bed holds promise to reprogram the postoperative immunosuppressive peritoneal environment [54]. Lemdani and coworkers recently reported a combination of radiofrequency ablation with locoregional immunomodulation in a CT26 mouse model of CRC [55]. They injected a thermosensitive hydrogel loaded with recombinant GM-CSF and Bacille Calmette–Guerin (BCG) intralesionally, and when combined with RFA observed a strong immune response and complete cure of second, untreated tumors implanted in the opposite flank. This approach is now being tested in a Phase Ib/II study for unresectable colorectal liver metastases (LICoRN-01, NCT04062721). Similar strategies are currently being pursued in the treatment of colorectal PM [56]. A Phase I trial of IP ONCOS-102, an oncolytic adenovirus armed with human GM-CSF and an Ad5/3 chimeric capsid, in combination with an anti-PD-L1 antibody (durvalumab) is currently ongoing in patients with colorectal PM (NCT02963831).

Taken together, the available data suggest a potential role of IP immunomodulation in patients with colorectal PM. However, few clinical data are as yet available, and progress is hampered by the current lack of insight into the immune environment of the peritoneal cavity, and how it is affected by current treatment approaches.

Targeting the Biomechanical Environment of Colorectal PM

The increased mechanical stress governing the colorectal tumor stroma not only enhances metastatic behavior, but also constitutes a significant obstacle to systemic as well as IP drug delivery [57]. As a consequence, therapies aimed at structural remodeling of the stroma may result not only in biophysical normalization, but also in enhanced drug exposure and reduced metastatic behavior. Antifibrotic drugs such as pirfenidone inhibit TGF-β-mediated intestinal fibroblast proliferation, and may normalize fibrotic stroma [58]. Direct inhibition of stromal TGF-β signaling was shown to prevent metastasis formation in mice by patient-derived CRC organoids [59]. Also, recent data suggest a strong link between TGF-β signaling and immunosuppression. Tauriello and coworkers showed that TGF-β caused immune evasion in a mouse CRC model, and that pharmacological inhibition restored sensitivity to immune checkpoint inhibition, reduced the extent of peritoneal carcinomatosis, and blocked the appearance of liver metastases [60]. In addition, TGF-β-directed therapies inhibit the process of EMT, considered a key driver of the metastatic process. Multiple TGF-β-directed pharmacological approaches, including small molecule receptor kinase inhibitors, monoclonal antibodies, antisense oligonucleotides, chimeric proteins, and vaccines are in early phase clinical trials [61]. A Phase I clinical trial using galunisertib (LY2157299), a selective TGF-β receptor I inhibitor, is currently recruiting chemotherapy resistant advanced CRC patients (MoTriColor trial, NCT04031872).

Many solid tumors, including CRC, overexpress FGF and its receptor, and preclinical trials have shown the potential of blocking this pathway in CRC [62]. A clinical trial of dovitinib, an oral inhibitor of FGF and VEGF receptors, failed to show activity in advanced CRC [63]. Targeting hyaluronic acid, which is upregulated in fibrotic tumors, resulted in lowered solid pressure and improved drug delivery in experimental models. However, a recent clinical trial using recombinant human hyaluronidase (PEGPH20) in pancreatic cancer showed detrimental effects, raising the

question whether stromal elements act to restrain, rather than support, pancreatic cancer cells [64,65]. Similarly, early clinical trials targeting MMPs, which mediate ECM remodeling, have not met with success. Enzymes regulating post-translational modification of collagen including lysyl oxidase (LOX), LOX-like 2 (LOXL2), and lysyl hydroxylases have emerged as potential therapeutic targets [66]. However, the addition of simtuzumab, an antibody to LOXL2, to FOLFIRI did not improve outcome in metastatic KRAS mutant CRC [67].

Despite these initial clinical disappointments, several other novel strategies targeting the biomechanical tumor environment in PM are being investigated. The interested reader is referred to a recent review [68].

Concluding Remarks

The role of the peritoneal microenvironment is increasingly recognized in the pathogenesis and therapeutic resistance of colorectal PM. The presence of structural ECM components, a phenotypic diversity of MCs and CAFs, and impaired innate and adaptive immune cells result in an overall state of immunosuppression during PM development and treatment. The biochemical and biomechanical interactions among cancer cells and the peritoneal microenvironment may dynamically evolve in response to any given treatment including surgery and chemotherapy. A better understanding of this dynamic reciprocity will reveal the combinatorial signals that support and promote PM. Preclinical models with the desired cellular and biomechanical properties may generate novel insights in drug response and resistance. Future PM treatment methods should include interrogation of the peritoneal microenvironment during therapy, and should consider opportunities to prevent or redirect the tumor supportive microenvironment associated with PM (see Outstanding Questions). Inclusion of patient-derived cell populations in bioengineered tumors may enable personalized therapy testing.

Outstanding Questions

What are the structural and cellular components of the microenvironment of colorectal peritoneal metastases, and how do these affect therapy response?

How does surgery affect the immune contexture and peritoneal host defense in these patients?

What is the potential of organoids and bioengineered scaffolds as model systems to study PM?

Can we identify actionable targets in the biological and mechanical tumor microenvironment of PM?

What is the potential for intraperitoneal delivery of novel drug platforms such as nanomedicine and hydrogels as a tool to provide immune modulation and/or immunotherapy?

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