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TOPIC HIGHLIGHT

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Natural history of hepatic metastases from colorectal cancer - pathobiological pathways with clinical significance

Konstantinos A Paschos, Ali W Majeed, Nigel C Bird

Konstantinos A Paschos, Ali W Majeed, Nigel C Bird, Liver Research Group, Department of Oncology, School of Medicine, Royal Hallamshire Hospital, The University of Sheffield, Sheffield S10 2JF, United Kingdom
Konstantinos A Paschos, Department of Surgery, General Hospital of Drama, GR66100 Drama, Greece
Author contributions: Paschos KA analysed the data and wrote the manuscript; Majeed AW analysed the data and reviewed the manuscript; Bird NC analysed the data and reviewed the manuscript
Correspondence to: Konstantinos A Paschos, MD, MSc, PhD, Department of Surgery, General Hospital of Drama, Terma Hippokratous Str., GR66100 Drama, Greece. kostaspaschos@yahoo.gr
Telephone: +30-252-1350413 Fax: +30-252-1023881
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Abstract

Colorectal cancer hepatic metastases represent the final stage of a multi-step biological process. This process starts with a series of mutations in colonic epithelial cells, continues with their detachment from the large intestine, dissemination through the blood and/or lymphatic circulation, attachment to the hepatic sinusoids and interactions with the sinusoidal cells, such as sinusoidal endothelial cells, Kupffer cells, stellate cells and pit cells. The metastatic sequence terminates with colorectal cancer cell invasion, adaptation and colonisation of the hepatic parenchyma. All these events, termed the colorectal cancer invasion-metastasis cascade, include multiple molecular pathways, intercellular interactions and expression of a plethora of chemokines and growth factors, and adhesion molecules, such as the selectins, the integrins or the cadherins, as well as enzymes including matrix metalloproteinases. This review aims to present recent advances that provide insights into these cell-biological events and emphasizes those that may be amenable to therapeutic targeting.

Key words: Colorectal cancer; Metastatic cascade; Liver metastasis; Liver sinusoids; Neovascularization

Core tip: The multi-step process of colorectal hepatic metastases includes numerous molecular pathways and cellular interactions with potential clinical interest. Mutations at the initial site of colorectal carcinogenesis, such as *p53* and *APC*, neoplastic cell interrelationship with the stromal macrophages, neoangiogenesis through growth factors, such as the vascular endothelial growth factor and platelet-derived growth factor, the role of hepatic sinusoidal cells, such as Kupffer cells, the expression of adhesion molecules, including the selectins and the integrins, are all crucial stages/events within the metastatic process. The exploration and analysis of recent research data may contribute to a better understanding of their clinical significance and may lead to new therapeutic strategies.

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INTRODUCTION

Carcinogenesis [carcino (καρκίνος) = cancer, and genesis (γέννεσις) = birth] and metastasis [meta (μετά) = after, next and stasis (στάση) = arrest] are both words of Greek origin, expressing the onset and the advanced progression (spread to another location) of cancer, respectively. Cancer development is a complicated biological process

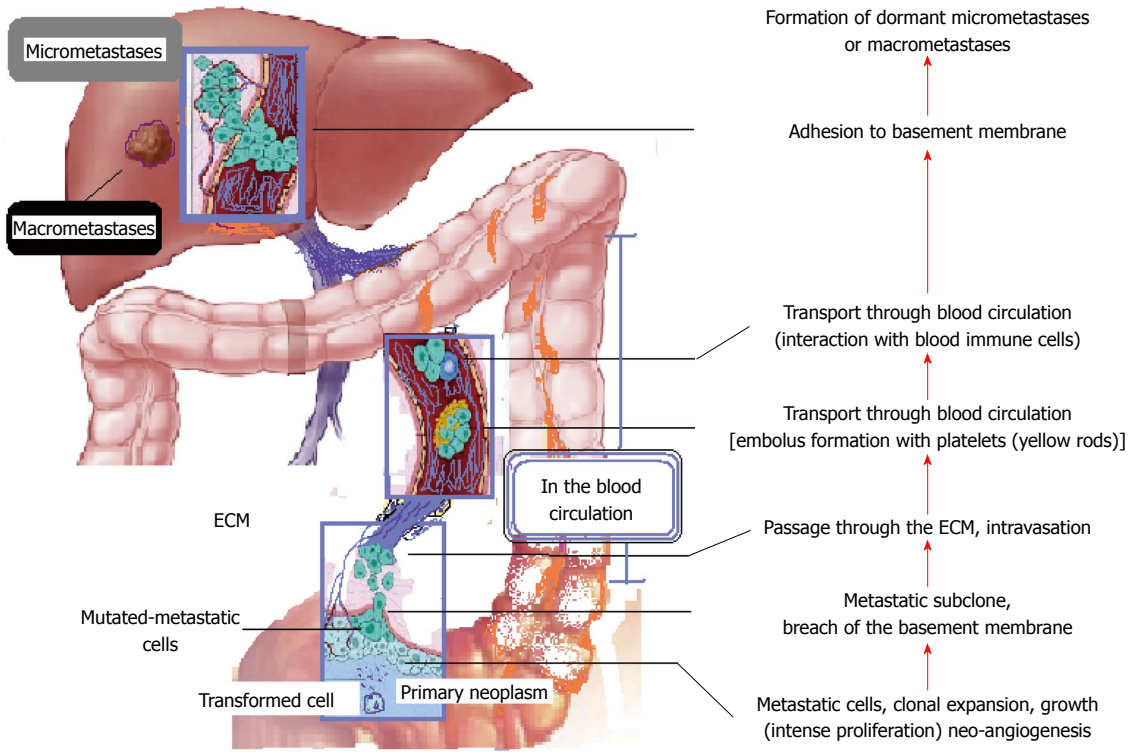

Figure 1 The invasion-metastasis cascade[1,178]. Successive interrelated stages/steps until foreign tissue colonisation and the formation of macrometastases. The paradigm of colorectal liver metastasis, from the large intestine through the superior and inferior mesenteric veins and the portal vein to the liver. ECM: Extra cellular matrix.

that includes numerous poorly understood aspects and attracts the greatest interest of biomedical sciences. It is a succession of interrelated, well-defined stages, usually termed the invasion-metastasis cascade[1,2].

The primary stage in the natural history of carcinogenesis starts with the transformation of normal cells into malignant derivatives at their initial site. Most interestingly, this transformation is also multistep and includes several genetic and phenotypic cellular alterations. Thus, there are two processes that progress in parallel, the metastatic cascade and the cancer cell transformation[3,4].

Primary mutations that signal the initiation of carcinogenesis lead to a progressive cellular proliferation and tumour formation. Neovascularisation (neoangiogenesis) and invasion of normal tissue follow, until several malignant cells are detached from the primary tumour (disaggregation), migrate and intravasate; this is the onset of metastasis. The next steps may be evasion of the immune system, cell survival in the hostile environment of the systemic circulation, arrest of the circulating tumour cells and adhesion to the endothelium lining the capillary bed of the target organ, and extravasation. The final stages of the metastatic cascade are evasion of host defences, establishment of a new blood network to supply the development of a new secondary tumour, and colonisation (Figure 1)[3,5,6].

All these successive stages demand multiple properties from malignant cells, and failure or inadequacy in any of them cancels the entire metastatic cascade. These properties are acquired through multiple mutations. It is uncertain whether cancer cells already possess most of their new potential when they begin the metastatic sequence or if this is gradually acquired. However, the colonising ability is strongly believed to appear later in the tumourigenicity[4,7]. Importantly, various laboratories have confirmed different cell subpopulations in the primary tumour site. Concurrently, the metastatic tumour created in a distant location by cancer cells is considered a different entity from the primary one, because metastatic cells show phenotypical and genetic differences from their ancestors[8-10].

While micrometastases are achieved by several cancer cells, macrometastases rarely occur. It is believed that micrometastases are the final metastatic stage for the vast majority of malignant cells, which never succeed in surviving or adapting in the inhospitable environment of the foreign tissue. In accordance with that belief, cancer patients may present myriad of micrometastases in multiple organs and tissues, although without any clinical evidence[11,12].

This general pattern of carcinogenesis and metastasis applies to most cancer types with certain modifications and adaptations according to implicated cells, tissues and organs[1]. Colorectal hepatic metastasis attracts a particular scientific interest due to the high frequency of colorectal cancer (CRC) (3rd most common cancer in the developed countries and 2nd leading cause of cancer-related mortality) and the unique properties, functions and role of the liver in the human body[13,14]. This review will describe the metastatic journey of CRC cells from the large intestine

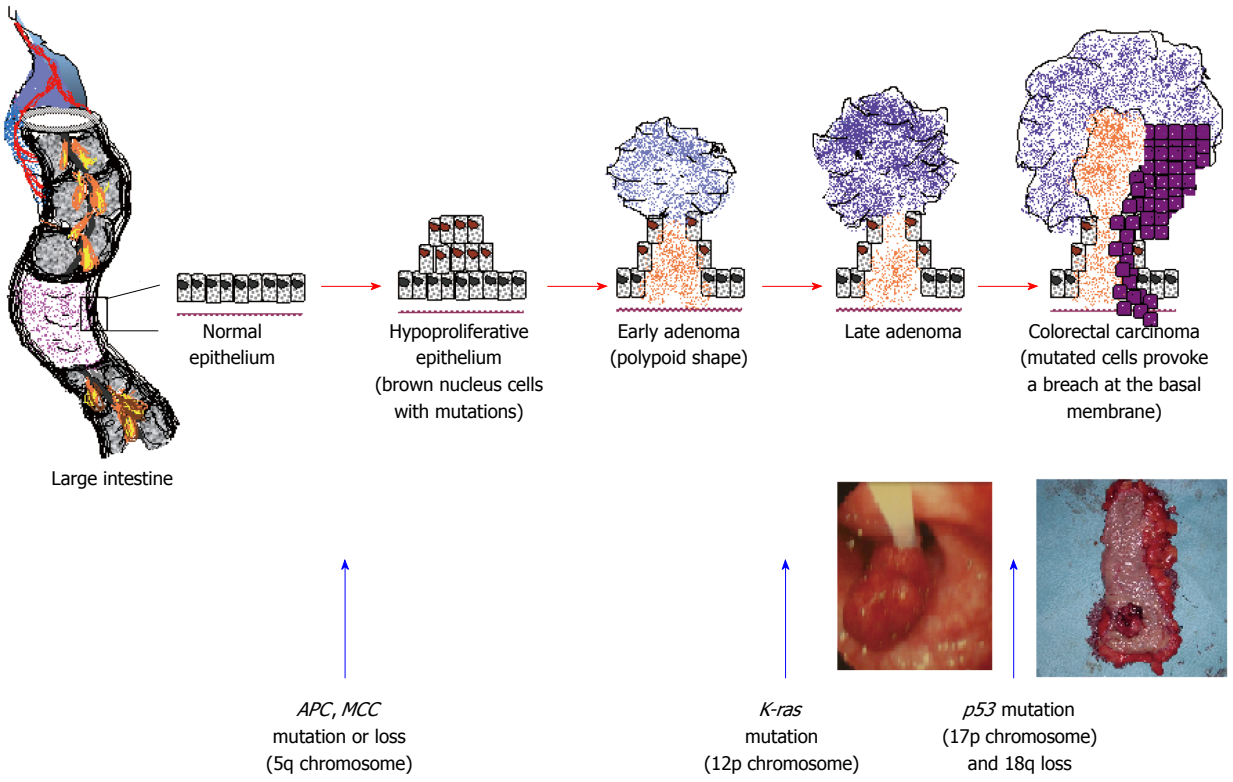

Figure 2 A sequence of mutations for colorectal epithelial cells generates the formation of polypoids, adenomas and finally carcinoma[15,16,19]. Polypoid and carcinoma clinical photos (authors' archives) underneath the figures.

to the liver and will investigate the normal-malignant cell interactions, the role of the tissue microenvironment and the genetic and molecular pathways that mediate this carcinogenic-metastatic process.

INITIAL GENETIC ALTERATIONS OF EPITHELIAL CELLS IN COLORECTAL CARCINOGENESIS

Mutations in CRC include alterations in tumour suppressor genes such as *p53* and *APC*, DNA repair genes such as *hMLH1* and *hMSH2*, and/or oncogenes such as *K-ras* and *c-Myc*. In this way, normal colorectal epithelial cells gain, among other abilities, the capacity of uncontrolled growth and evasion of apoptosis (programmed cellular death). Rapid cell proliferation usually generates the formation of a polypoid structure in the epithelium that may evolve to adenoma, which is a precancerous lesion. An adenoma may progress to CRC, as the mutations accumulate and CRC cells acquire all the necessary properties; single cancer cells or small clusters of them may finally detach from the initial intestinal tumour and migrate (Figure 2)[15,16].

There are at least two well-described genetic pathways that may generate CRC. The most common, which mediates up to 60% of carcinomas, is the chromosomal instability pathway. This is the result of mutations of the *p53*, *APC* and protooncogene *K-ras*, allelic loss of 18q and aneuploidy. The role of the APC gene is crucial in tumourigenesis, as 100% of patients with the familial adenomatous polyposis (FAP) syndrome who carry this mutation develop CRC if they receive no treatment. Another genetic pathway is the microsatellite instability (MIN) one, also termed replication error pathway, which is responsible for approximately 20% of carcinomas. These neoplasms have aberrant DNA mismatch, a near-diploid karyotype, sparse *p53*, *K-ras* and *SMAD4* alterations, but also frequent *BAX*, *BRAF* and *TGF-BIIR* mutations. Hereditary nonpolyposis colon cancer (HNPCC) is attributed to the MIN pathway, accounts for 5%-6% of CRC, and 80% of these patients develop cancer in their lifetime. In HNPCC, MIN is a consequence of mutations in DNA mismatch repair genes (*hMSH2*, *hPMS1*, *hPMS2* and *hMLH1*). Notably, other genetic pathways also exist, such as the cytosine phosphodiester guanosine island methylator phenotype pathway. While in the absence of methylation genes are normally expressed, in the presence of promoter methylation, genes are transcriptionally downregulated. When tumour suppressor genes are involved, carcinogenic mutations occur. A whole subclass of CRC tumours include high proportions of hypermethylated genes[17-19].

INTERACTIONS OF NEOPLASTIC CELLS WITH THE STROMA CELLS AND THE EXTRACELLULAR MATRIX AT THEIR INITIAL SITE - LARGE INTESTINE

The promotion of the initial stages of colorectal carcino-

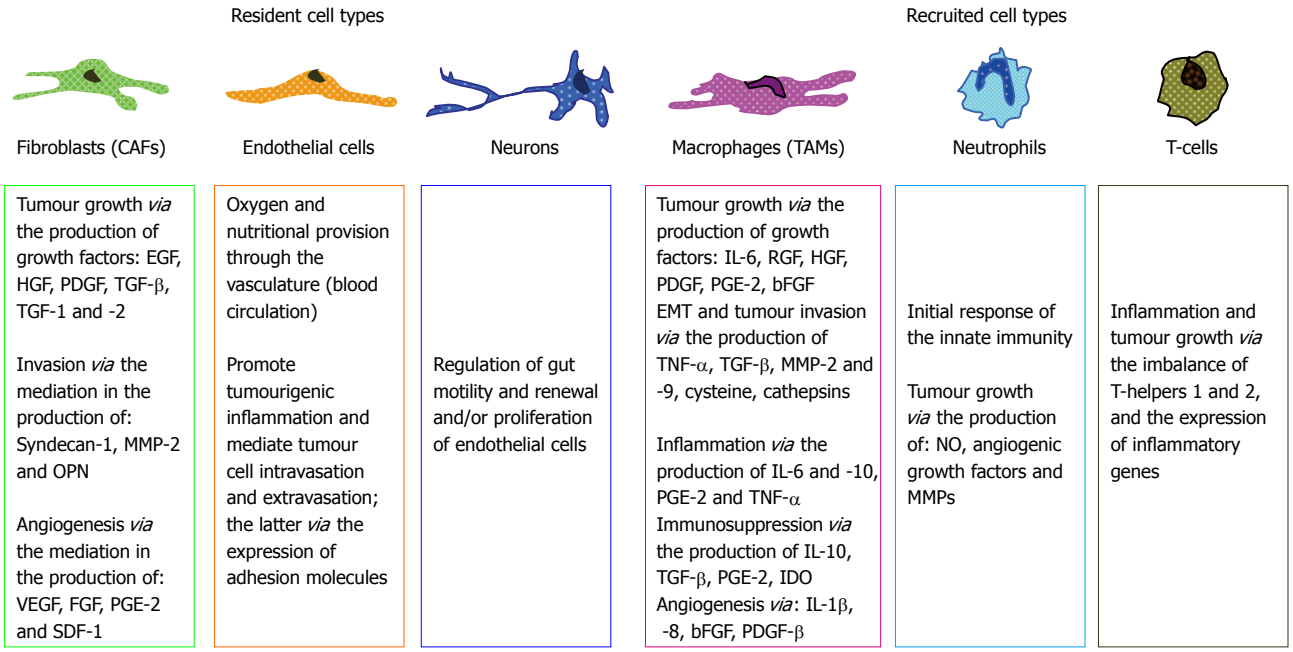

Figure 3 Various cellular types[22,23,112]. Various cellular types (resident: fibroblasts, endothelial cells and neurons, or recruited: macrophages, neutrophils and lymphocytes) which mediate cancer progression and growth in the colorectal microenvironment. bFGF: Basic fibroblast growth factor; CAF: Cancer associated fibroblasts; ECM: Extracellular matrix; EGF: Epidermal growth factor; EMT: Epithelial-to-mesenchymal transition; HGF: Hepatocyte growth factor; IDO: Indoleamine 2,3-dioxygenase; IGF: Insulin growth factor; IL-10: Interleukin 10; MMP: Matrix metalloprotease; NO: Nitric oxide; OPN: Osteopontin; PDGF-β: Platelet-derived growth factor-beta; PGE2: Prostaglandin E2; SDF-1: Stromal cell-derived factor-1; TAM: Tumor-associated macrophages; TGF-β: Transforming growth factor-beta; TNF-α: Tumour necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

genesis depends on the communication and interrelationship of neoplastic cells with the stroma. The term stroma, also referred to as reactive stroma in carcinogenesis, includes the endothelial cells, fibroblasts, immune cells (such as macrophages, lymphocytes and neutrophils), as well as non-cellular elements, such as the extracellular matrix (ECM). It appears that numerous special characteristics of the reactive tumour stroma, concerning cellular, architectural or chemical elements, support a reciprocal tumourigenic communication with neoplastic cells *via* the basement membrane[20]. These characteristics may be a high number of fibroblasts, altered molecular expression on the cellular surface and the cytoplasm of endothelial cells, macrophage recruitment, increased capillary density, ECM rich in fibrin and collagen-1. Furthermore, the production and secretion of a plethora of chemical compounds, including cytokines and growth factors in the colorectal stroma, mediate the promotion of carcinogenesis (Figure 3)[21-23].

Fibroblasts

Fibroblasts within a tumour appear to harbour mutations that transform them into myofibroblasts that are termed cancer-associated fibroblasts (CAFs). Apart from normal fibroblasts, CAFs may also originate from endothelial cells, epithelial cells, preadipocytes and bone marrow-derived progenitors[24,25]. Interestingly, *CAF* mutations may refer to a variety of genes encoding multiple growth factors, cytokines, enzymes and ECM-related proteins. Various studies have shown that CAFs have the potential to produce transforming growth factor beta (TGF-β) in an autocrine or paracrine way, triggering CRC cell detachment from their initial site[26,27]. Moreover, a recent study from Zhu *et al*[28] has demonstrated that TGF-1 may induce plasminogen activator 1 (PAI-1) transcription in CAFs. PAI-1 mediates the fibrinolytic activity in the vasculature, is widely expressed throughout tumours and is associated with malignant invasion and neoangiogenesis[29,30]. Taking together these experimental data, CAFs appear to play an important role in various aspects of carcinogenesis and metastasis, including migration, matrix degradation, invasion and angiogenesis[26,31].

Macrophages

The development of a tumour causes an inflammatory reaction where immune cells may be implicated. Macrophages are potentially the most important tumour-associated immune cells. They may constitute a considerable amount of the initial tumour mass and they correlate with tumour poor prognosis. Although macrophages act as tissue scavengers in general, eliminating any potential harmful element (invading cells or chemicals), cancer cells may use macrophage products in their favour, masking their surface antigens and thus avoiding the tumouricidal action of immune cells. In the invasion-metastasis cascade, macrophages play a significant role in the promotion of inflammation, stroma and ECM remodeling, angiogenesis, neoplastic cell invasion, intravasation and seeding at foreign sites[32-34].

Neoangiogenesis at the initial site of CRC is crucial for tumour development since oxygen diffusion alone from the normal capillary network is unable to supply a

tumour larger than 1-2 mm. Macrophages regulate the critical process of neovascularisation through vascular endothelial growth factor (VEGF) production[35]. VEGF acts directly on endothelial cells promoting their proliferation, migration, invasion and high vascular permeability[36,37]. Another paradigm of the macrophage supporting role for malignant colorectal cells is through the macrophagic removal of apoptotic CRC cells that express sulfoglycolipids SM4. While such a process initially appears to be tumouricidal, the increased secretion of interleukins and TGF-β1 may contribute to tumour development and angiogenesis activation[38].

Lymphocytes

Lymphocytes constitute another immune cell category implicated in tumourigenesis with a favourable prognosis. In advanced CRC, the presence of T lymphocytes favours a better clinical outcome for patients suffering from the disease[39-41]. A recent study by de Miranda *et al*[42] showed that high tumour infiltration by activated $CD8^+$ T cells in patients with Lynch CRC correlated with early staging of the primary tumour and absence of lymphatic metastases. While immune cells mainly support the defending system of normal tissue against neoplastic cells, the latter use genetic and molecular pathways that promote the evasion of immunosurveillance. CRC cells may express the Fas ligand on their surface and bind Fas-expressing immune cells, thus triggering apoptotic mechanisms for the latter[43,44]. An alternative mechanism of escaping immunosurveillance for tumour cells is the high expression of carbohydrates on their cellular membrane. This alters the CRC cell surface antigen profile, impeding their recognition and destruction by immune cells[45]. Furthermore, cells of normal colorectal tissue may also support neoplastic cells in evading immunosurveillance. Tumour-associated macrophages are able to produce arginine metabolites, which cause T-lymphocyte death. Similarly, in the lymph nodes of advanced neoplasms, dendritic cells play an immunosuppressive role rather than an immunoreactive one. Thus, instead of activating T-lymphocytes, they induce tumour tolerance[46,47].

ECM

ECM supports the cells mechanically and assists their physicochemical functions through the provision of pathways for migration and the maintenance of growth factors. Adhesion molecules play a critical role in the ECM-cell connection, with the integrins being particularly important. The ECM consists of 5 classes of molecules (collagens, fibronectins, glycans, hyaluronans, laminins and proteoglycans). Every class includes multiple isoforms, which are cell-dependent. ECM composition and structure vary with developmental stage, tissue and disease[48,49].

The basement membrane (BM) or basal lamina is a specific ECM-type meshwork produced by epithelial and stromal cells, and it plays a crucial role in carcinogenesis and metastasis[50,51]. BM chemical structure includes Type Ⅳ and Ⅶ collagen, laminins and heparan sulphate proteoglycans. Type Ⅳ collagen in particular is of great importance and provides the basic scaffold that supports other BM components, such as laminin networks and perlecan oligomers[52-54].

Any alteration in ECM structural profile or degradation of any of its molecules may cause cellular and tissue dysfunction and therefore disease. CRC induces numerous ECM changes, the BM included. Type Ⅶ collagen is altered or disappears during the development of multiple cancers, such as melanoma, breast and prostate. Moreover, laminin-5 is usually absent from the ECM structure in advanced CRC. Interestingly, new synthesis or accumulation of BM molecules (*e.g.*, laminin-1 or 5) may also be imposed by tumours, and this phenomenon suggests the important role of BM in tumour invasion[53,54]. Similarly, modifications in ECM adhesion molecules also occur, while neoplastic cells express the appropriate adhesive receptors on their cellular membrane. Integrin α6β4, a molecule that normally supports cellular adhesion to the BM, is such a paradigm as, following the tumour-imposed ECM modifications, it changes its role and induces actin-mediated cell motility. In this way, integrin new distribution appears to correlate with considerable CRC aggressiveness[55]. Most importantly, neoplastic cells may also interfere in ECM metabolism through proteinase expression, of the matrix metalloproteinases (MMPs) in particular. These multi-functional degrading enzymes play a critical role in carcinogenesis but also in other stages of the invasion-metastasis cascade. CRC cells may induce the expression of MMP-2 and -9 by stromal cells, either directly or *via* a paracrine mechanism, thus modifying ECM and promoting tumour development[56-58].

EPITHELIAL-MESENCHYMAL TRANSITION OF EPITHELIAL CELLS IN THE LARGE INTESTINE

Epithelial-mesenchymal transition (EMT) is a reversible morphogenetic biological process that involves the transition from stationary polarized epithelial cells to motile, multipolar or spindle-shaped mesenchymal cells. Epithelial cells are characterised by an atypical-basal polarity, the formation of tight junctions and the expression of intercellular adhesion molecules, such as E-cadherin. On the other hand, mesenchymal cells appear to be unable to build mature intercellular contacts, invade through the ECM and express molecules including fibronectin, vimentin, N-cadherin, Twist and Snail. While EMT occurs in embryogenesis and normal early development, accumulating evidence indicates that it also plays a crucial role in tumour development and metastasis; neoplastic cells usurp EMT to obtain properties that promote their detachment from their initial site and favour their migration to distant tissues[59,60].

The process of EMT is induced, promoted and regulated by multiple effectors that also regulate EMT dur-

ing embryogenesis, including the cytokines interleukin 8 (IL-8), growth factors [epidermal growth factor (EGF), platelet-derived growth factor (PDGF), TGFβ] and ECM components. Interestingly, it has been shown that CRC cells are able to produce such molecules, including IL-1α, IL-1β and tumour necrosis factor alpha (TNF-α)[60-62]. Specifically, TGFβ plays a critical role through autocrine and/or paracrine mechanisms. This growth factor may be triggered by TNF-α, which is produced by tumour-infiltrating macrophages. The binding of TGFβ with TGFβR1 and/or TGFβR2 receptors triggers the phosphorylation of Smad2 and Smad3 dimers, which dissociate from the receptors to interact with Smad4; subsequently these dimers enter the nucleus to regulate EMT[63]. Additionally, the TGFβ-Smad pathway induces the high motility group A2 gene (*HMGA2*), which mediates EMT; HMGA2 is a nuclear factor that links TGFβ with the EMT-inducing transcription factors Twist and Snail1 and 2. TGFβ/Smad activities may also be associated with PDGF and PDGF receptor signalling, such as the phosphorylation of p68 RNA helicase to trigger nuclear translocation of β-catenin through a wingless Int (Wnt)-independent pathway[64-66].

In CRC, tumour cell EMT predominantly concerns cell adhesion mediated by E-cadherin. CRC cells present either mutations of the E-cadherin gene, proteolytic degradation of E-cadherin, insulin growth factor 1-promoted internalization of E-cadherin or disarrangement of E-cadherin and β-catenin connections[67,68]. Notably, intact cadherin-catenin complexes support the normal function of intercellular adhesion and guarantee stable "adherens junctions" and an unimpaired Wnt signalling pathway (the latter being a complex protein network controlling signal transduction)[69]. Neoplastic cells along with the E-cadherin deregulation present an accumulation of Src kinase and phospho-myosin associated with EMT[70,71], as well as high expression of guanine nucleotide exchange factor Tiam 1 that promotes metastatic potential through cellular adhesion reduction and resistance to anoikis[72]. Furthermore, CRC cells present a variable level of Ras homolog gene family, member C (RhoC), a protein that functions as a switch in signal transduction, promoting reorganisation of actin and regulating cell shape. It is noteworthy that increased expression of RhoC is associated with poor prognosis, as well as with an aberrant localization and expression of E-cadherin. The disruption of E-cadherin-mediated cell adhesion contributes to CRC cell detachment and motility. Moreover, its replacement with N-cadherin, a phenomenon named cadherin switch, further promotes cancer cell translocation and invasion of the surrounding stroma. In this way, EMT favours carcinogenesis and cellular transformation towards a metastatic phenotype[73].

ANGIOGENESIS IN THE LARGE INTESTINE

Angiogenesis is defined as the formation of new vessels from preexisting ones[74]. Hypoxia or low oxygen tension is the primary triggering factor for the onset of this process. Early in carcinogenesis, tumour growth requires angiogenesis for the provision of oxygen and nutritional factors, while later new vessels provide CRC cells with a way to the systemic circulation (intravasation) and metastasis to distant sites. In normal conditions, the angiogenic process is accurately regulated by pro- and anti-angiogenic agents. In CRC, hypoxia induces the activation of hypoxia inducible factor-1 and subsequently the expression of angiogenic factors including VEGF, basic fibroblast growth factor and PDGF by CRC cells. Notably, prostaglandin E_2 directly triggers CRC cells to express VEGF, appearing as a promising therapeutic target[75,76].

Moreover, stromal cells, such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages, mast cells, neutrophils and others, also produce proangiogenic factors inducing angiogenesis. The aforementioned factors interact with their respective receptors at the endothelial cell surface initiating signalling cascades involving p38, eNOS, as well as PI3 and ERK MAP kinases; the latter induce vasodilation, endothelial cell proliferation/migration and vessel assembly. Concurrently, angiogenesis suppressor proteins are downregulated, such as thrombospondin. The resulting vascular networks of tumours are chaotic and poorly functional due to abnormal endothelial cell structure, proliferation and apoptosis; additionally, abnormal pericytes appear to be loosely attached and do not fully cover the vessels. Consequently, neoplastic vasculature is leaky, haemorrhagic, with excessive branching, which results in oxygen depletion and extracellular acidosis[77,78].

It has been demonstrated that the microvascular density has a considerable prognostic value for tumours and may predict survival in patients with CRC[79]. To study this, a colour Doppler vascularity index (DVI) (ratio of coloured pixels/total pixels per tumour section) has been introduced, in an effort to predict distant metastasis and survival in CRC patients. It was concluded that patients who had a DVI > 15% at the primary site had worse overall survival compared to patients with DVI < 15%[80]. A similar parameter is the Doppler hepatic perfusion index (DHPI) (ratio of hepatic arterial flow/total liver flow), which appeared to be increased even in patients with occult micrometastases and is predictive of metastatic liver tumour recurrence[81]. The association between angiogenesis at the primary CRC site and in the hepatic metastases is further supported by imaging techniques, such as dynamic contrast-enhanced magnetic resonance imaging and contrast-enhanced computed tomography[82].

LYMPHANGIOGENESIS IN THE LARGE INTESTINE

The lymphatic network is a major metastatic route and lymphatic metastases constitute an important prognostic indicator in solid tumours and CRC[83]. Excessive lymphangiogenesis is associated with metastasis in CRC.

Lymphatic vessel density (LVD) has been introduced as a quantitative parameter for lymphangiogenesis. *In vitro* models demonstrated that VEGF-A and VEGF-C are the major regulators of this process and their levels correlate with LVD. In addition, PDGF-BB, FGF-2, hepatocyte growth factor (HGF), angiopoietin by binding to its receptor Tie2 and sphingosine-1-phosphate (S1P) cause lymphangiogenic effects. Neoplastic cells with high levels of sphingosine kinase 1 release S1P in the ECM, which in turn may lead to paracrine-induced angio- and lymphangiogenesis[84-86]. *In vivo* models suggested new pathways for lymphangiogenesis in CRC. The high levels of VEGF-C and VEGF-D appear to direct tumour lymphangiogenesis *via* the VEGF-C and -D pathway and their receptor VEGFR-3 (present on lymphatic endothelial cells), while VEGF-A may play a regulatory role during this process[85,87]. The activation of VEGFR-3 along with the β1 integrin subunit triggers multiple signalling pathways in the lymphatic endothelial cells, including Pyk2, NF-κB, ERK and JNK MAP kinases, which mediate proliferation and survival[88]. A recent study by Du *et al*[89] on human CRC samples indicated that metastasis associated protein 1 (MTA-1), which is expressed in various epithelial cancers and plays a critical role in metastasis, correlated with VEGF-C and mediated its expression. Hence, it was suggested that MTA-1 promotes lymphangiogenesis and therefore CRC metastasis.

Lymphangiogenesis may be initiated with the formation of lymphatic vessels by circulating endothelial progenitors (CEPs); the latter constitute a subpopulation of circulating endothelial cells derived from the bone marrow. It has been shown that CEPs are increased in the circulation in multiple pathologies, cancer included[27,90,91]. However, alternative pathways for the initiation and progress of lymphangiogenesis may also exist. The differentiation of other cells into lymphatic endothelial cells, such as macrophages and stromal cells, may be such pathways[92,93].

Interestingly, apart from invasion to lymph nodes, CRC LVD is also associated with liver metastasis. Although this association is rather unclear, a possible explanation may be that CRC cells express chemokine receptor CXCR5; its ligand BCA-1/CXCL13 is mutually expressed on lymphatic endothelial cells and the liver[94]. CRC cells may be directed through the lymphatic network to both lymph nodes and the liver. Moreover, lymphatic metastases are traced at the liver lymphatic drainage network, including portal, mediastinal and coeliac lymph nodes. These liver-associated lymphatic metastases may be generated from previous CRC liver metastases. All these findings show clearly the importance of angio- and lymphangiogenesis for carcinogenesis and metastasis and explain why they attract great research interest and constitute promising targets for anticancer therapy[27,95].

INTRAVASATION AND CIRCULATION - SURVIVAL IN THE LYMPHATIC AND/OR THE BLOOD VESSELS

Malignant cell detachment from their primary tumour is the necessary step for their intravasation into existing or newly formed lymphatic or blood vessels. Certain molecular changes concerning the expression of degrading enzymes (such as the MMPs) and adhesion molecules (such as selectins, integrins and others) appear to contribute to CRC cell intravasation. Notably, the tortuous leaky neoplastic blood network, with its loose junctions among endothelial cells and the defective pericyte coverage, may substantially promote tumour cells entering into the lumina of vessels[78].

Sonoshita *et al*[96] studied murine and human tissue and showed that the transcriptional modulator enhancer of split (Aes) prevented CRC cells from intravasation through the impairment of trans-endothelial invasion that followed Notch-dependent mechanisms. Concurrently, urokinase plasminogen activator (uPA) expression is increased in patients with FAP during the transformation of normal to dysplastic epithelia[97,98]. The uPA system consists of uPA, the uPA receptor (uPAR), the tissue-type plasminogen activator (tPA), the plasminogen (Plg) and plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2). This biological system is involved in multiple molecular pathways, including adhesion, chemotaxis, cytokine release, protease expression, neutrophil proliferation and activation for oxidant production. It modulates inflammation, growth, invasion, angiogenesis and metastasis of multiple tumour types[99,100]. When uPA connects with uPAR, it activates Plg along with other proteases such as MMP-2 and -9. These events promote ECM fragmentation and CRC cell detachment and intravasation.

Interestingly, when normal cells lose their contact with the ECM and their neighbouring cells, they undergo apoptosis that is triggered by broken integrin bonds. The ability of CRC cells to survive in the presence of fragmented ECM constitutes a crucial property of metastatic cells. This apoptosis resistance might be attributed to the overexpression of focal adhesion kinase by CRC cells, which contributes to conferring survival by activating certain molecular pathways, including ERK and AKT. Hence, tumour cells can by-pass integrin signalling and survive without contact with the ECM[101,102].

Following their detachment, access to the blood and lymphatic vessels is the next step for metastatic CRC cells. The microvessel diameter and their leaky structure are important factors that promote passive CRC cell entry into the circulation. Although lymphangiogenesis is less studied than angiogenesis, it appears that LECs, stimulated by VEGF-C, secrete mitogenic and/or chemotactic factors, which may attract tumour cells to adhere to, pass through and intravasate into the newly-built lymph vessels[90,103].

CRC cells are transported through the hepatic-portal circulatory system to reach the liver. The inferior and superior mesenteric veins and the portal vein, along with their neighbouring lymphatic vessels, constitute the dominant metastatic routes for hepatic metastases. In cancer patients, a large number of circulating tumour cells may be detected, although the intravasated cellular populations do not correlate with the density and the extension of metastases. Accumulating experimental evidence has

suggested that entrance into the systemic circulation is lethal for the vast majority of tumour cells; only 0.01% of metastatic cancer cells may trigger the formation of metastasis when injected into the systemic circulation[27]. *In vitro* studies using videomicroscopy to monitor intravasated cancer cells have indicated that most of the cells undergo apoptosis during their passage through the vascular wall or soon after their entrance into the circulation. It is generally accepted that two major mechanisms contribute to tumour cell death following their intravasation: mechanical stress and the immune system[5,104].

Haematogenous dissemination exposes tumour cells to the strong mechanical forces generated by blood flow. In particular, when they circulate within a narrow capillary network or the microvasculature of contractile organs, such as the heart and the skeletal muscles, they are forced to transform their shape from spherical to cylindrical, which is lethal to the majority of tumour cells[5]. However, as tumour cells get progressively more invasive and metastatic through a series of mutations, they display larger cell deformations and shape alterations. *In vivo* studies have demonstrated that metastatic cells are quite deformable and both the cell nucleus and cytoplasm may undergo strong compression and shape deformation in small capillaries. Specifically, the length of the cell nucleus may increase 1.6-fold and the cytoplasmic major axis up to 3.9-fold in comparison with the same cells in larger microvessels[105-107]. Also, tumour cells reaching capillary bifurcation points can stretch and extend their cytoplasms in both directions[105,106]. Mechanical forces, such as endothelial contraction, shear or pulsatile stresses, cause the production of oxygen radicals including NO and ROS, which generate apoptosis of CRC cells.

Metastatic tumour cells have developed multiple molecular pathways in order to survive the mechanical forces in the circulation. CRC cells interact with platelets and/or themselves to form large emboli that protect them from shear stress[108]. Cancer cells may activate adhesion pathways involving integrins, leading to their attachment to endothelial cells and thus evasion of anoikis. Programmed cell death may also implicate metabolic pathways including pentose phosphate and control of glucose uptake[109]. Moreover, the tyrosine kinase TrkB was demonstrated to suppress anoikis and appeared to be crucial for metastatic progression in CRC cells[110].

The second major mechanism responsible for metastatic cell elimination in the circulation is immunosurveillance. Numerous cytokines released by cancer cells, endothelial cells or immune cells, such as IL-2, -12 and -18, may activate various subsets of immune cells including T-lymphocytes and natural killer (NK) cells. The latter eliminate cancer cells through Trail and/or NKG2/perforin pathways. Similar to mechanical forces adaptations, metastatic cells have developed mechanisms to evade immunosurveillance. It has been shown that cancer patients present high levels of acute phase glycoproteins in their blood, correlated with the disease. These proteins may protect malignant cells against anoikis and immune cell attacks[111]. Furthermore, tumour cells interact with platelets forming large emboli, a process mediated by the expression of tissue factor and L- and P-selectins. In this way, they shield themselves against both immune detection and shear forces[112]. Undoubtedly, immune system and cancer cell interactions attract great research interest and the manipulation of immunesurveillance is a major strategy in anticancer treatment.

EXTRAVASATION - CRC CELL ARREST IN THE HEPATIC SINUSOIDS

The sinusoids constitute the specific hepatic capillary network where four different cell populations reside within the sinusoidal lumen or in proximity to the sinusoidal wall: sinusoidal endothelial cells (SECs), Kupffer cells (KCs), hepatic stellate cells (HSCs) and pit cells. Each of these cell types plays a crucial role in hepatic homeostasis and CRC metastasis[113].

The progression of CRC hepatic metastasis is divided into four interrelated phases: (1) microvascular phase of liver-infiltrating malignant cells; (2) interlobular micrometastasis phase; (3) angiogenic micrometastasis phase; and (4) established hepatic metastasis phase. The first phase mainly occurs within the sinusoids, whereas the following ones describe further metastatic steps that affect the inner hepatic parenchyma. Although sinusoidal cells contribute to all four, they predominantly mediate the first phase[114].

During the microvascular phase, resident cells generate multiple tumouricidal and tumourigenic effects, which may either promote the invading cell elimination or liver colonisation. A plethora of molecular pathways are involved, including NO and reactive oxygen release by SECs, expression of adhesion molecules, such as selectins, integrins and others by the same cells, phagocytosis, cytokine and growth factor release by KCs and HSCs, release of cytotoxic agents from pit cells. The aforementioned examples demonstrate the complexity and the importance of the intercellular reactions that occur within the sinusoids in colorectal liver metastasis[114-116].

Sinusoidal endothelial cells

SECs were first described by Wisse at the beginning of 1970s. Their cytoplasm includes characteristic canals, arranged in sieve plates named fenestrae. Fenestration constitutes a unique marker and distinguishes SECs from all other endothelial and liver cells (Figure 4)[117,118]. They form a major scavenger cell system and accomplish receptor-mediated endocytosis and pinocytosis through three major types of endocytic receptors: the liver sinusoidal cell mannose receptor, the liver sinusoidal cell scavenger receptor and the liver sinusoidal cell receptor IIb2, which is an Fc-γ receptor[114]. The scavenging properties of SECs are critical in CRC metastasis. *In vivo* murine experiments indicated that autotaxin, a phosphodiesterase that promotes metastasis and angiogenesis, was rapidly removed from the blood circulation and degraded by SECs[119].

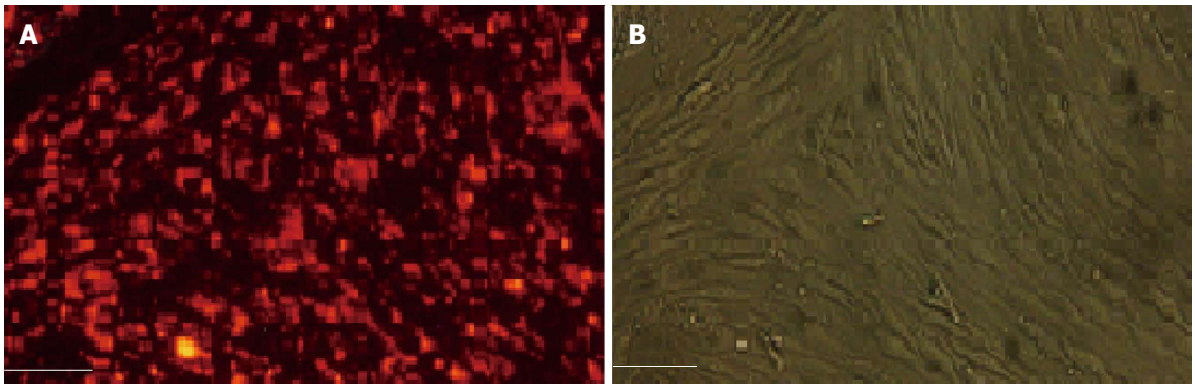

Figure 4 Sinusoidal endothelial cells. Fluorescence labeled (AcLDL) (A) and light microscopy (B). The cells have a cobblestone architecture on the sixth day in culture (authors' archive). Magnification × 200. Scale bars = 100 μm. AcLDL: Acetylated low density lipoprotein.

Table 1 Molecules produced by Kupffer cells that may mediate their interactions with metastasising colorectal cancer cells within the hepatic sinusoids[126,127]

Group	Molecules
Cytokines and chemokines	Tumour necrosis factor alpha
	Transforming growth factor beta
	Interleukins (IL-1α, -1β, -6, -8, -10, -12 and -18)
	Interferon gamma
	Platelet-activating factor
	Monocyte chemotactic protein-1
	Macrophage inflammatory protein (MIP-1)
Hydrolytic and proteolytic enzymes	Urokinase-type plasminogen activator
	Metalloproteinases (MMP-1, -7 and -13)
Lipid metabolites (prostanoids)	Prostaglandin E2
	Thromboxane
Oxygen species (superoxide) and hydrogen peroxide	
Nitrogen species (nitric oxide)	

This study further supported previous data which demonstrated that hepatectomy or chemically-induced injury resulted in an increase of serum autotoxin[120].

Although SECs appear to play a tumouricidal role, they may also aid tumour cells to arrest and metastasise within the liver. Under cytokine activation, SECs may express adhesion molecules, such as E-selectin, which mediate cancer cell attachment to the endothelium, thus facilitating their extravasation into the hepatic parenchyma[121,122].

Experimental studies have attempted to investigate and exploit SEC properties in anticancer therapy. Gene therapy uses certain vectors, such as adenoviruses, to reach target cells and is considered a new and promising treatment of acquired diseases, including liver neoplasms and metastases. SECs, in conjunction with KCs, may endocytose and destroy these vectors, cancelling any therapy. On the other hand, as hepatocytes constitute the primary target cells for anti-cancer therapy, gene vectors should reach the space of Disse, in order to interrelate with hepatocytes. The space of Disse may be accessible only through SEC fenestration, and the diameter of the latter ranges according to species and liver condition; ageing or liver diseases substantially alter fenestrae, impairing the vector access to the space of Disse[123].

Kupffer cells

These constitute the biggest (more than 80%) tissue macrophage population in the body of vertebrates and approximately 15% of all liver cells[124,125]. KCs mainly act as scavengers around the sinusoids, but also as antigen-presenting cells and liver regeneration mediators. When activated, KCs produce a wide variety of molecules, such as growth control mediators, inflammatory agents, proteolytic and hydrolytic enzymes, oxygen and nitrogen species and lipid metabolites. All these products modulate acute and chronic liver responses to pathogens, chemicals, drugs, injury, as well as cancer and metastasis (Table 1)[126-128].

KCs play a crucial role in the "host tumoural surveillance system". As they constantly reside around the sinusoids, they discriminate and remove neoplastic cells that reach the liver. Interestingly, CRC cells become vulnerable to macrophage tumouricidal activity during endothelial adhesion and extravasation[129,130]. Specifically, KCs may recruit inflammatory cells, they may arrest CRC cells and inhibit their growth acting in a cytostatic way, and they may bind and eliminate them in a cytotoxic way[130]; the latter occurs by several mechanisms: phagocytic release of TNF, secretion of proteases and production of oxygen metabolites[131-133].

Rat experiments demonstrated that in the early stages of CRC liver metastasis, KCs exerted tumouricidal activity in conjunction with NK cells. One hour after CRC cells had reached the liver, more than 70% were already in contact with KCs; the KC population was considerably increased from the first day and phagocytosed more than 90% of malignant cells. However, NK depletion left 35% of the cancer free from KC interactions. The responsible mechanism may be that activated NK cells secrete pro-inflammatory cytokines, such as granulocyte macrophage colony stimulating factor (GM-CSF) and interferon gamma (IFN-γ), which in turn activate KCs or sensitise tumour cells to cytotoxic effects. Alternatively, NKs may induce CRC cell apoptosis, causing exposure of phosphatidylserine and enhancing phagocytosis by KCs[115]. The opposite interaction was also reported: activated KCs may produce IL-12 and/or IL-18, which enhance IFN-γ release by pit cells that exhibit high tumouricidal activity,

thus inhibiting CRC haematogenous metastasis in murine livers[134,135]. It appears that in the metastatic process, KCs and pit cells act in close cooperation against the invading cancer cells. Both produce cytokines and interact stimulating one another, eliminating cancer cells directly or mediating cancer cell death by their counterparts.

High mobility group box 1 (HMGB1) is a protein produced by normal and cancer cells which triggers apoptosis in macrophages and monocytes. Experimental data from Dukes' C and D surgical specimens showed that HGMB1 levels in the portal blood were higher for Dukes' D and correlated with the levels in the primary tumours. When HMGB1 concentration was raised, KC numbers were substantially decreased. Moreover, *in vitro* murine experiments performed in the same study showed that the administration of the protein decreased KC populations and promoted liver metastases[136]. These findings support the tumouricidal role of KCs in the metastatic process.

The interaction between KCs and invading tumour cells is not always in favour of liver homeostasis. Binding to KCs facilitates CRC cell arrest in the liver; if the killing process is not accomplished promptly or is partially completed, then the binding process substantially contributes to liver colonisation. Experimental data in rats advocated that KCs exert a limited capacity for tumour surveillance; when malignant cells reach the liver in very high numbers or present great antigenic diversity, KCs are eventually saturated and metastasis progresses[130].

Additionally, KCs produce growth factors, such as HGF, which facilitate tumour cell proliferation[137]. Furthermore, the ability of KCs to secrete MMPs, especially MMP-9 and MMP-14, as well as their inhibitors, may enhance angiogenesis and tumour invasion, *via* alterations of the ECM[138]. Notably, MMP-9-deficient mice presented considerably fewer liver metastatic lesions when CRC cells were injected in their spleen. MMP-9 was primarily derived from KCs, independently of its expression by tumour cells[139]. Moreover, *in vitro* experiments have indicated that highly malignant cells reduce, in their favour, the phagocytic capacity of KCs and promote colonisation[140]. The preceding experimental evidence suggests that KCs prevent tumour outgrowth and liver metastasis when the burden and the rate of invading cells are relatively low. However, KCs may contribute to liver colonisation if their tumouricidal ability is saturated due to excessive numbers of invading cells or when metastases are already established.

KCs present an 80 kDa carcinoembryonic antigen (CEA) receptor, which mediates binding and subsequent degradation of CEA[141,142]. When CEA is complexed, KCs are activated and secrete through β-2 adrenergic pathways large amounts of cytokines, including TNF-α, IL-1β, IL-6 and IL-10[141,143]. These pro- and anti-inflammatory agents generate alterations in the sinusoidal endothelium and SECs express adhesion molecules of the selectin family, which promote the arrest and extravasation of tumour cells[144]. Murine studies indicated successful adhesion of CRC cells to KCs and SECs, without the immediate mediation of CEA, excluding CEA's function as an adhesion molecule in hepatic colonisation[145]. The last observation was further investigated and it was revealed that CEA supports CRC cell survival *via* the induction of IL-10 and subsequent decrease of NO concentration. IL-10 is probably released by stimulated KCs and NO levels decrease due to inhibition of inducible nitric oxide synthetase[146].

Accumulating data have demonstrated the important role of immunoglobulin superfamily adhesion molecules in colorectal liver metastases, referring to KCs. Murine experiments demonstrated that CRC cells trigger KCs to produce pro-inflammatory cytokines, which in turn stimulate SECs to express high levels of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)[147] or VCAM-1 and platelet endothelial cell adhesion molecule 1[148]. These molecules mediate cancer cell adhesion, follow E-selectin expression and promote subsequent extravasation. Similarly, *in vitro* experiments using CEA-activated murine KCs indicated that cytokines were released, which activated endothelial cells to express ICAM-1 and VCAM-1[122,149].

Consequently, several lines of evidence support the hypothesis that when CRC cells reach the hepatic sinusoids, they secrete CEA that may activate KCs to produce cytokines; the latter stimulate SECs to express cell adhesion molecules that bind colorectal metastasising cells. This mechanism obviously contributes to malignant cell arrest, proliferation and invasion (Figure 5).

The cytostatic and cytotoxic actions of KCs were taken into consideration in liver metastasis research and adjuvant strategies were created in order to maintain and increase their potential antitumour role. As transient peri- and post-operative immunosuppression has been observed in patients undergoing major surgical interventions, further enhancement of KC activities is particularly useful during and after hepatectomy[150]. GM-CSF was tested for this purpose and experimental data from human liver wedge biopsies showed that it could stimulate KCs to exert increased cytotoxicity against a SW948 colon carcinoma cell line[151]. In syngenic rat models, the administration of this factor increased KC numbers and significantly enhanced their cytotoxicity *ex vivo*, cancelling the development of small metastatic foci[152]. IFN-γ was also tested targeting KC activity; in syngenic murine models, the preoperative administration of IFN-γ caused high anti-metastatic function of KCs and NKs in early liver metastasis[153]. These promising results remain to be further analysed and repeated in bigger studies using human hepatic tissue.

Hepatic stellate cells

HSCs are located in the space of Disse, comprising about 15% of the nonparenchymal hepatic cells. They have a unique morphology, due to long cytoplasmic processes that form a spindle-shaped cellular body (Figure 6)[154]. KCs produce chemokines that induce mono- and poly-

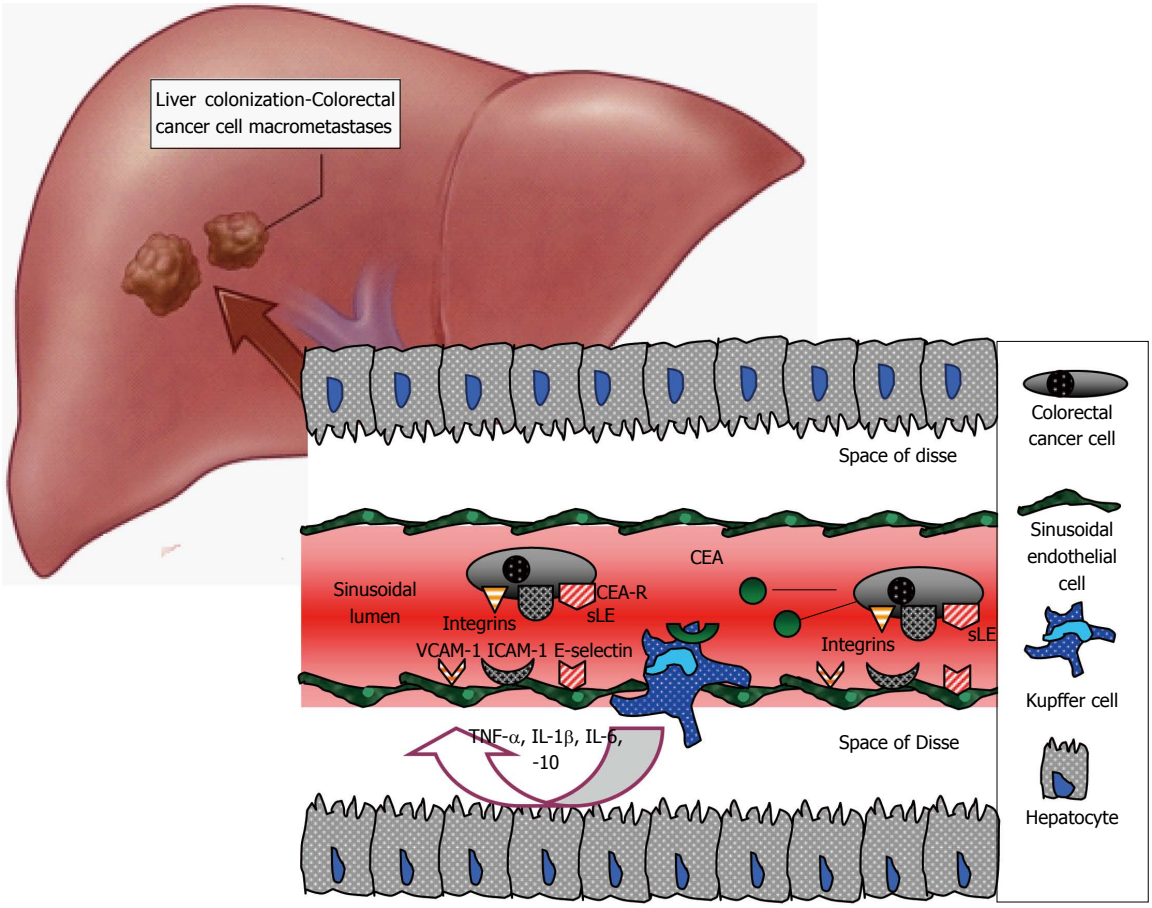

Figure 5 The role of Kupffer cells in colorectal cell adhesion-arrest within the sinusoids during haematogenous liver metastases. Kupffer cells are activated through carcinoembryonic antigen (CEA), release multiple chemokines and stimulate sinusoidal endothelial cells to express adhesion molecules, inducing colorectal cancer cell arrest. CEA-R: CEA receptor; ICAM-1: Intercellular adhesion molecule 1; IL: Interleukin; sLE: Sialyl Lewis antigen; TNF-α: Tumour necrosis factor alpha; VCAM-1: Vascular cell adhesion molecule 1.

morphonuclear leukocyte infiltration, activate neutrophils and control lymphocyte populations exerting immunoregulatory activity[155,156]. Furthermore, HSCs act as antigen-presenting cells that may activate T lymphocytes[157,158] and release pro-inflammatory cytokines when exposed to bacterial endotoxins through toll-like receptors[158,159].

HSCs secrete and respond to a wide variety of cytokines. They modify the activity of various growth factors, express adhesion molecules, such as ICAM-1 and VCAM-1, and regulate the detoxification of ethanol and xenobiotics[156,160]. Activated HSCs display contractility, chemotaxis, fibrogenesis, matrix degradation activity, proliferation, and retinoid loss. They mediate inflammation, cell survival and apoptosis, liver regeneration and monitoring of cellular pH[161-163].

HSCs are also key cells in tumour growth and CRC metastatic processes. Experimental studies in rats revealed that conditioned media from cultures of hepatocellular carcinoma hepatocytes could activate HSCs[164]. Moreover, *in vitro* experiments with metastatic melanoma cells concluded that tumour cells triggered HSC activation; the latter promoted angiogenesis through VEGF expression[165]. Injection of colon carcinoma cells in nude mice favoured the formation of hepatic metastatic foci through HGF and TGF-β1 produced by HSCs. Similarly, tumour cells secreted PDGF-AB and enhanced HSC proliferation and locomotion[166].

Co-cultures of SECs and HSCs caused spontaneous cellular differentiation, with HSCs forming the core and SECs the surface of the cell population. Concurrently, activated HSCs cultured with SECs expressed functional smooth muscle cell phenotype and formed capillary-like structures in angiogenesis assays. Taking into consideration that tumours activate HSCs, their contribution to neoangiogenesis through interactions with SECs was implicated in these studies[114,167].

Pit cells

The name pit cell is related to their cytoplasmic granules. The cells contain granules with lysosomal enzymes, perforin and various phosphatases, but their structural characteristic is the presence of cytoplasmic rod vesicles. Their shape varies, due to the presence of pseudopodia[113,168,169].

Pit cells substantially contribute to hepatic immunity and exert strong antitumour action. In collaboration with KCs, they represent the first line of liver defence against cancer cells. Experimental studies in rodents demonstrated that pit cells are highly cytotoxic against multiple

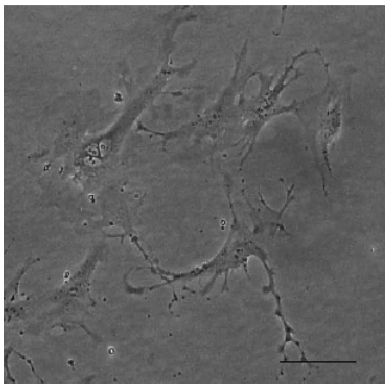

Figure 6 Hepatic stellate cells with their cytoplasmic processes, after 4 d in culture (authors' archive). Light microscopy (magnification × 200). Scale bar = 100 μm.

malignant cell lines, including murine fibrosarcoma L929, colon carcinoma CC531 and colorectal carcinoma DHD-K12 rat cells[169].

Pit cells exert their cytotoxicity through binding to target cells, a process named conjugation. Various adhesion molecules on pit cells mediate this process, such as CD2, a member of the immunoglobulin superfamily, CD28 and lymphocyte function-associated antigen 1, while CD54 and CD58 may be present on target cells[170,171]. In addition, interactions between β_2 integrins and ICAMs are crucial, supporting these cell-cell conjugations[169,170,172].

Following conjugation, various receptors may be stimulated, triggering or inhibiting pit cell cytotoxicity. Three superfamilies of NK cell receptors are presented primarily on human pit cells, while others, named co-receptors, still remain under investigation: the killer immunoglobulin receptor that recognizes major histocompatibility complex (MHC) class I molecules, the c-type lectin, recognising non classical MHC class I or class I-like molecules, and the natural cytotoxicity receptor superfamily, which remains to be further studied[173].

Pit cell tumouricidal actions are exerted mainly through three mechanisms[168,174], as follows:

Perforin-granzyme pathway: Through calcium-dependent molecular reactions pit cells adhere to tumour cells and release perforin and proteases into the intercellular space. Subsequently, perforin induces pores in the tumour cytoplasmic membrane and then proteases may generate DNA segmentation.

Apoptosis pathway: Pit cells express both the FasL and the tumour necrosis factor-related apoptosis-inducing ligand. When pit cells interact with tumour cells, these ligands bind to respective receptors and cancer cells undergo apoptosis.

Cytokine pathway: Pit cells secrete cytokines, such as IFN-γ, and thus activate lymphocytes and macrophages against invading cancer cells.

LIVER COLONISATION

The adherence of metastasising CRC cells to SECs is a primary step of great importance toward liver invasion and colonisation[140]. Malignant cells bind to SECs initially through selectins. However, these bonds do not appear to be strong enough to guarantee stable cell adhesion. Integrins are necessary to stabilise tumour cell-SEC adhesion. If integrins do not conform, then the bonds are broken and cancer cells are released into the blood or undergo mechanical damage[175]. The development of strong intercellular bonds allows CRC cells to resist the attractive forces of plasma flow and circulating blood cells, when they adhere to the hepatic sinusoids[176]. Multiple signalling molecules, including focal adhesion kinase, paxillin, and cytoskeletal proteins, are probably required for tumour cell adhesion and stabilisation under the hydrodynamic conditions of blood flow[175].

Shimizu *et al*[177], studying CRC liver metastases in murine models, reported that shortly after endothelial adhesion occurred within the sinusoids, metastasising cells extended cytoplasmic projections towards the space of Disse, through the pores of SECs. Forty-eight hours after their injection in mice, CRC cells may reach the hepatocytes and enter their cytoplasm, and 72 h later, they developed metastatic foci.

From the moment of extravasation, cytotoxic T cells, monocytes and macrophages which occupy extra-sinusoidal hepatic tissue are activated against the metastatic cells, though not always successfully[178]. Ultimately, few CRC cells cause micrometastases in the hepatic parenchyma. They remain in a dormant state, the duration of which is unknown. It is probable that these micrometastases will be reactivated after an unspecified time period and will create macrometastases. The last stage of the invasion-metastasis cascade is then accomplished[179,180].

It has been proposed that carcinoma cells address the problem of an incompatible microenvironment at the distant metastatic site through the establishment of a "premetastatic niche"[181]. In that state, CRC cells may release soluble CD44 that mediates resistance to apoptosis[182]. The crosstalk among cancer cells, immune cells, endothelial and stromal cells causes the production of various chemokines and growth factors, such as the EGF and TGF-α, which promote metastatic cell growth in the liver. Interestingly, in order to survive and develop a secondary neoplasm in the liver, CRC cells must undergo the reverse transition from EMT, which is termed mesenchymal-epithelial transition (MET). Consequently, CRC cells express epithelial cell markers such as E-cadherin[183,184]. Additionally, Belluco *et al*[185] showed that CRC cell kinase profile at the hepatic metastatic sites differs considerably from the initial intestinal site. These data indicate that CRC cells adjust their signalling network at the hepatic microenvironment in order to survive and generate metastatic foci. The activation of MET is probably triggered by the liver ECM. Hepatic CAFs appear to promote this process through overexpression of COX2 and TGF-β2.

Table 2 Adhesion molecules with potential prognostic value in colorectal cancer progression and hepatic metastases[176,194]

Adhesion molecule family	Adhesion molecule	Expression
Cadherins	E-cadherin	Colon epithelial cells
	P-cadherin	Colon epithelial cells
Lectins	sLEx	Colon epithelial cells
	sLEa (CA 19-9)	
	Galectin-3	
Selectins	E-Selectin	ECs
	L-Selectin	Leukocytes
	P-Selectin	ECs and platelets
Integrins	LFA-1 ($\alpha_L\beta_2$)	Colon epithelial cells, ECs, fibroblasts, leukocytes and platelets
	VLA-4 ($\alpha_4\beta_1$)	
Immunoglobulin superfamily CAMs	ICAMs	ECs, fibroblasts and leukocytes
	PECAM-1	ECs, platelets and leukocytes
	VCAM-1	ECs and epithelial cells
	MadCAM	Colon epithelial cells
	CEA	Colon epithelial cells
Proteoglycan receptors	CD44	Colon cells and ECs

CA 19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; EC: Endothelial cell; ICAM: Intercellular adhesion molecule; LFA-1: Leukocyte function-associated antigen 1; MadCAM: Mucosal addressin cell adhesion molecule; PECAM: Platelet endothelial cell adhesion molecule; sLEx: Sialyl Lewis x antigen; VCAM: Vascular cell adhesion molecule; VLA-4: Very late activation antigen 4.

The transition of metastatic microcolonies to macroscopic metastases may occur after weeks or months or they may persist as microcolonies in a state of long-term dormancy. However, similarly to the initial CRC tumours, this transition also needs additional vascular supply through neoangiogenesis. VEGF-A is the major growth factor that regulates neovascularisation and VEGFR1 is the main receptor.

CONCLUSION

The invasion-metastatic process from the initial site at the large intestine to the liver is a long process, where multiple molecular pathways and numerous cell types are involved. Recent research has elucidated various aspects of this process, such as the role of EMT and stromal microenvironment cellular crosstalk, the importance of adhesion molecules (Table 2), the significance of proteases, as well as the role of VEGF members in angio- and lymphangiogenesis.

Furthermore, technological advances have revolutionised the study of metastasis, since imaging techniques have provided real-time visualisation of metastatic cells in the ECM and the circulation[186]. Also, new genetic, molecular and biochemical techniques may permit the investigation of tumour cell heterogeneity at the initial and their metastatic sites and may explain its functional significance[187].

The progress in basic research concerning CRC hepatic metastasis over the last decade has been accompanied by the rapid translation of experimental data to oncological treatment. An anti-VEGF monoclonal antibody, named bevacizumab, has been introduced in CRC therapy and has contributed to prolonged patient survival[188]. Concurrently, clinical trials are in progress for antiCEA antibodies[189], MMP inhibitors[190], antibodies against integrins[191] or molecules that increase immunosurveillance[192,193]. Although comprehension of CRC hepatic metastasis has substantially evolved, the invasion-metastasis cascade remains partially understood and future basic and clinical research still has multiple issues to clarify.

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