Tumour Microenvironment: Overview with an Emphasis on the Colorectal Liver Metastasis Pathway

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Abstract The tumour microenvironment (TME) represents a dynamic network that plays an important role in tumour initiation, proliferation, growth, and metastasis. Cell behaviour may be regulated by interplay of molecular interactions involving positive and negative reinforcement as well as a high level of cross-talk, which determines this system. Additionally, cancer involves cell proliferation, its malignancy defined by the tumour's ability to break down normal tissue architecture and by a dynamic process of invasion and metastasis. The metastatic cascade is regulated by a chain of molecular steps which triggers the progression of the developing cancer cell in the primary tumour into a number of transformations, leading to invasion and proceeding to metastases. Tumour-associated macrophages (TAMs) play a key-role in the progression from inflammatory conditions to cancer; TAMs are also capable of infiltrating the tumour microenvironment. Furthermore, myeloid-derived suppressor cells (MDSCs), a population of inhibitory immune cells, have been reported to increase in various cancer types, although characterising human MDSCs remains difficult, as their phenotype is quite variable. The future of cancer treatment is likely to involve creating more drugs that target these elements

as well as others. An overview of the tumour's microenvironment is, therefore, presented in this paper, focusing on the metastatic pathways of primary colorectal cancer to the liver.

Keywords Tumour microenvironment · Colorectal liver metastasis · TAMs · Myeloid-derived suppressor cells

Introduction

The tumour microenvironment (TME) represents a dynamic network that plays an important role in tumour initiation, proliferation, growth, and metastasis. This network is a highly dynamic system, the regulation of which depends on the interaction between tumour cells, stromal matrix and extracellular matrix [1]. When studying the tumour microenvironment, emphasis should not be restricted to cancer cells, but, also –and, potentially, more importantly– on fibroblasts, cytokines, and vascular tissue, as well as the extracellular matrix surrounding all these cells [2].

A cell's behaviour can be regulated by interplay of molecular interactions involving positive and negative reinforcement, as well as a high level of cross-talk determinative of this system [3, 4]. This dynamic regulatory network involves a switch between different states or phenotypes and would require a number of activities by signalling molecules in several pathways [5, 6].

On the other hand, cancer involves cell proliferation, its malignancy further defined by the tumour's ability to break down normal tissue architecture and by a dynamic process of invasion and metastasis. A loss of natural control occurs, which, under normal circumstances, would have directed cells to assemble into tissues, and then would keep these tissues within a specific organ [7, 8]. A tumour's metastatic pathway is also determined by cancer stem cells, epithelial–mesenchymal transitions, and, also, angiogenesis [9–11]. The metastatic

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cascade is regulated by a chain of molecular steps which triggers the progression of the developing cancer cell in the primary tumour into a number of transformations leading to invasion and proceeding to metastases. Epithelial–mesenchymal transition (EMT) starts with proteolysis of the basement membrane (BM) and of the extra-cellular matrix (ECM) and is followed by the dissociation of cancer cells from the tumour itself due to a change of their adhesive properties. Local invasion and cell migration ensues, along with angiogenesis, preceding viable vascular dissemination with immune evasion, extravasation with distant transportation and survival of cancer cells at another organ, resulting in metastases. Wanebo et al. have recently presented a very detailed description of these phenomena [12].

Apart from cancer cells, the TME also contains immune cells, tumour vasculature, lymphocytes, fibroblasts and other cells. The stromal compartment is composed of an abundant accumulation of a special group of innate immune cells, namely myeloid cells, which have a significant impact on the biological behaviour of malignant disease [13].

Tumour Associated Macrophages

Tumour associated macrophages (TAMs) are leukocytes that have been reported to play a key-role in the progression from inflammatory conditions to cancer; TAMs are also capable of infiltrating the tumour microenvironment [14]. There is pre-clinical and clinical evidence supporting the theory that an abundance of TAMs in the TME is associated with poor prognosis [15].

TAMs can also lead to the production of growth factors that can result in angiogenesis and suppression of the host immune system [16]. TAMs seem to play a leading role in the malignant process or its initiation, via the activation of macrophages and the infiltration of inflammatory cells, which often take place before the actual malignancy is formed [17]. Transcriptional profiling of TAMs has demonstrated them to be highly enriched in transcripts encoding angiogenic molecules, while comparison of TAM transcriptomes with available clinical databases has further supported these transcriptional signatures and has indicated a survival prediction [18, 19].

TAMs have a key-role in angiogenesis because they secrete vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF), as well as nitric oxide synthase (NOS). NOS has an effect on both endothelial cell migration and proliferation as well as vasodilatation and increased flow to the tumour's surrounding environment [17, 20]. VEGF, which is also secreted by cancer associated fibroblasts (CAF), can affect angiogenesis via several functions, including stimulation and proliferation of endothelial cells, increase of vascular permeability and changes to the ECM [17]. Once

again, tumour hypoxia seems to be a very important factor, as many TAMs accumulate in hypoxic or necrotic areas of tumours. Hypoxia and re-oxygenation, with the production of reactive oxygen species that occurs in newly formed vascular architecture, enhance tumour cell proliferation and increase metastatic rates [20]. Additionally, capillaries formed during such angiogenesis also contribute to the metastatic pathway due to their increased permeability. Hypoxic areas attract TAMs by releasing hypoxia-induced chemo-attractants, including vascular endothelial growth factor (VEGF), endothelins and endothelial monocyte-activating polypeptide II [21]. A distinct, hypoxia-induced, pro-angiogenic human macrophage phenotype has been identified [22, 23].

TAMs have been reported to be highly enriched in transcripts that encode angiogenic molecules [24]. It has also been shown that these transcriptional signatures are predictive of survival [24, 25]. Most TAMs have been shown to have an IL-10high, IL-12low phenotype, with expression of the mannose receptor and the scavenger receptor class A [26, 27].

Myeloid-Derived Suppressor Cells and Dendritic Cells

Myeloid-derived suppressor cells (MDSCs) comprise a population of inhibitory immune cells that have been reported to rise in numbers in various cancer types, although the characterisation of human MDSCs remains difficult, as their phenotype is quite variable [20]. There are two main MDSCs, namely monocytic MDSCs (M-MDSC) and polymorphonuclear MDSCs (PMN-MDSC). PMN-MDSCs and PMNs appear to be morphologically similar, but functionally and phenotypically different. PMN-MDSCs are immunosuppressive whereas PMNs are not [28]. As for M-MDSCs and inflammatory monocytes, they demonstrate common phenotype and morphology; however, these cell populations are functionally distinct. M-MDSCs are highly immunosuppressive, expressing high levels of both iNOS and ARG1, apart from other factors. MDSCs seem to use a plethora of mechanisms to influence both innate and adaptive immune responses: being capable of differentiating into TAMs, they can potentially inhibit CD8+ T cell activation via expression of nitric oxide synthase 2 (NOS2) and arginase (ARG1), but they can also initiate the expression of Tregs [27, 28].

Dendritic cells (DCs) are terminally differentiated myeloid cells specialised in antigen processing and presentation; when found in the TME they are considered to be defective, in the sense that they cannot cause proper stimulation of an immune response against tumour-associated antigens [28]. There are two major sub-sets of DCs, the conventional (cDCs) and the plasmacytoid (pDCs), which share certain common progenitors, but differentiate along distinct genetic programmes and have different morphologies, markers, and functions. Hypoxic conditions, such as those of the inflammatory microenvironment of

the TME, also seem to have an effect on DC function. DCs have also been reported to suppress T cell responses at the tumour site [29]. pDCs form a small population of DCs with a morphology reminiscent of plasma cells. They also express TLR7 and TLR9, but only in human cells, and also large amounts of IFN α , as a reaction to activation of TLRs caused by viruses or self-DNA. It has been reported that abnormal myelopoiesis is the dominant mechanism responsible for DC defects in cancer. This abnormal differentiation leads to decreased expression of mature, functionally competent DCs, increased accumulation of immature DCs at the tumour site and high production of immature myeloid cells [28]. Such immunosuppressive activities are directly associated with DCs localised in tumour sites and with abnormal DC differentiation, when defective DC function is a systemic phenomenon with an effect on myeloid cell lineage during cancer. ZBTB46 has recently been highlighted as a new transcription factor, specifically expressed in all conventional human and murine DCs [30, 31]. These recent reports indicate DCs to be a unique immune cell lineage and help further understanding of the role of DCs in the TME.

Pathway of Colorectal Liver Metastasis

Colon cancer is the cause of more than 600,000 annual deaths, and is diagnosed in 5 % of the global population [32]. Colon cancer is characterised by high mortality rates and often linked with metastatic dissemination, in particular to the liver. Colorectal cancer liver metastases (CRLM) account for 20 % of cases with Stage II colon cancer, and 50 % of cases categorised as stage III colon cancer [33]. Colorectal cancer, which mostly originates from dysplastic adenomatous polyps, entails a multistep pathway, including the inactivation of a variety of tumour suppressing genes, which can also repair DNA. Additionally, the pathway includes concurrent activation of proto-oncogenes (e.g.: K-ras, NRas and c-Myc), giving colonic epithelial cells a selective growth advantage by triggering the transformation of normal epithelium into pre-cancerous adenomatous polyps and, thus, leading to local invasion and metastasis [34].

On the other hand, the liver is the body's largest organ with a unique architecture that enables its diverse functions, the main ones being protein biosynthesis and portal blood detoxification. Liver metastasis is defined by four phases: (a) the microvascular phase, involving tumour cell arrest in the sinusoidal vessels, which can lead to the tumour cells' death; (b) the extravascular, pre-angiogenic phase, during which avascular micrometastatic formation appears via recruitment of host stromal cells; (c) the angiogenic phase, during which recruitment of endothelial cells is initiated and tumours become vascularised via a number of possible interactions with the microenvironment, and (d) the growth phase, which

results in the establishment of what is manifested as clinical metastases (Fig. 1) [35].

After detaching from the primary tumour, cancer cells enter the existing or newly formed blood or lymph vessel system and disseminate. This pathway of intravasation, however, has not yet been fully clarified in colon cancer. To date a number of molecular steps have been described; they involve matrix metalloproteinases and interaction between cancer cells and endothelial adhesion molecules [34]. It has been reported that the urokinase plasminogen activator (u-PA) plays a decisive role in colorectal cancer intravasation [36], as it binds to its receptor (u-PAR) and then activates plasminogen and other proteases, such as, for example, matrix metalloproteinases-2 (MMP-2) and MMP-9. All this adds to the breakdown of the ECM, which, in turn, contributes to intravasation.

Colon cancer cells seem to be able to disseminate through lymph vessels, as liver metastasis and lymphangiogenesis are closely related. However, the mechanisms by which lymphangiogenesis supports colon cancer metastasis still remain unclear. One possibility is that VEGF-C can stimulate lymphatic endothelial cells by secreting chemotactic or mitogenic factors able to attract cancer cells to the newly growing lymph vessels, thus enabling cell adhesion onto them and intravasation through the lymphatic endothelium [37]. It has been shown that colorectal cancer cells can express the CXCR5 chemokine receptor and both lymph endothelial cells and the liver express its ligand BCA-1/CXCL13 [38], which might be indicative of the possibility that lymphangiogenesis may direct cancer cells to both lymph nodes and the liver.

Circulation imposes a major stress on cancer cells, because they need to find ways to survive in order to form metastases; mechanical destruction of circulating cancer cells is the first line of defence against haematogenous cancer dissemination in the host microenvironment [34]. Blood flow appears to exercise strong mechanical forces on cancer cells circulating in the blood, and, in addition to this, such mechanical forces can lead to increased NO and ROS production, which then trigger cancer cell apoptosis [34, 39, 40]. It may well be assumed that the balanced response of tumour cells to such mechanical destruction and initiation of their adhesion following circulating stress are necessary so that cancer cells may spread haematogenously.

Endothelial Adhesion Receptors

Interaction between invading cancer cells and the tumour microenvironment is critical in the metastatic pathway [41], while adhesive interactions between cancer cells and endothelial cells of targeted organs can outline parts of the metastatic process, such as circulatory cell arrest in the capillaries, and trigger a cascade of events that would result in extravasation of cancer cells within the organs targeted. This process,

Fig. 1 The four phases of colorectal liver metastasis pathway. *VCAM*: Vascular cell adhesion molecule *TAMs*: Tumour associated macrophages *MDSCs*: myeloid-derived suppressor cells

however, would require successive adhesive interactions between endothelial cells and their ligand [34]. Interactions between colon cancer cells and endothelial cells in the hepatic environment include selectin-mediated initial attachment as well as chemokine-activated transport of circulating cancer cells on to the endothelium. Integrins from cancer cells are activated and allow their firmer adhesion to members of the Ig-CAM family, such as ICAM and VCAM; this how trans-endothelial migration and extravasation processes take place [42]. The expression of endothelial adhesion receptors can be initiated via a number of pathways. Cancer cells can release cytokines, such as $\text{TNF-}\alpha$, $\text{IL-}\beta$ or $\text{INF-}\gamma$, which, in turn, could activate endothelial cells to express E-selectin, as well as P-selectin, ICAM-2 or VCAM [34, 43].

When colorectal cancer cells enter the liver microcirculation, a rapid host inflammatory response is initiated via induction of $\text{TNF-}\alpha$ expression in resident Kupffer cells. This leads to the expression of E-selectin and VCAM by endothelial cells and results in cancer cell binding and extravasation [44]. The kinetics of the host inflammatory response seem to be tumour-type specific and the process of E-selectin, as well as other endothelial adhesion receptor-mediated metastases, appear to have a local character. A macromolecular scaffolding complex known as selectin counter-receptor seems to be essential for cell binding [45]. Colon cancer cells have been shown to release soluble CD44, which attenuates the interaction of colon cancer cells with its ligand within the ECM, resulting in apoptosis resistance [46]. Additionally, ECM seems to modulate the proliferation of such hepatic cancer cells. The expression of the EGF receptor, as well as that of growth factors, is among the molecular parameters playing an important role in colorectal liver metastasis [47]. In the hepatic microenvironment among cases of colorectal cancer metastasis, a sub-population of CAFs may well be involved in changing the microenvironment, thus contributing to activating the MET process. The role of CAFs in colorectal cancer metastasis may be modulated via the expression of IL-8, a chemokine related to invasion and angiogenesis [48].

TAMs in the CLRM Pathway

Tumor-associated macrophages (TAMs) play an important role in the progression from inflammation to cancer as well as in tumour invasion and metastasis [49, 50]. TAMs are located within, but also surrounding, most tumours and, once activated, they can potentially release a number of factors which could influence the tumour cell pathway. TAMs infiltrate the tumour microenvironment and accumulate in tumour hypoxic areas; furthermore, TAMs lead to the production of growth factors resulting in angiogenesis, enzyme induced ECM remodelling as well as host immune system suppression, which allows tumour cells to evade normal regulatory pathways. However, the exact mechanisms involved are not fully understood. The presence and frequency of TAMs detected in a tumour are linked to the cancer prognosis: higher prevalence of TAMs is associated with worse prognosis [49], indicating TAMs as an attractive target for anti-cancer treatment.

Macrophages can adapt to their environment, and two major, polarised macrophage phenotypes have been identified [51, 52]. Activated M1 macrophages are characterised by the expression of nitric oxide synthase 2 (NOS2) and several pro-inflammatory cytokines with a reported tumoricidal capacity. However, the main functions of the alternatively activated M2 macrophages include their capacity to scavenge debris and promote tissue repair; they also have immune regulatory functions with many of the factors they produce acting in favour of tumour progression and growth. In theory, the different roles played by macrophages in various cancers could be regulated by variations in the equilibrium between M1 and M2 phenotypes, with specific cancers and their microenvironment influencing them.

Edin et al., in a study of 485 consecutive CRC specimens stained for nitric oxide synthase 2 (NOS2), as a marker for the M1 macrophage phenotype, and the scavenger receptor CD163, as a marker for the M2 macrophage phenotype, demonstrated that a high infiltration rate of M1 macrophages

is correlated to better CRC prognosis, yet in a stage-dependent manner [49]. On the other hand, it was reported that increased infiltration of M1 macrophages into the tumour front seems to be accompanied by parallel increase in the number of M2 macrophages, leading to the hypothesis that the production of M1 macrophages is positive for survival among CRC patients, although there may also be concomitant expression of M2 macrophages [49].

Clinical and pathological indicators for the prognosis of colorectal cancer include tumour and vascular invasion [53–55]. It has been reported that CD14⁺ macrophages are mainly distributed within the tumour invasive front, and that presence of a large number of CD14⁺ macrophages in the tumour invasive front is an indicator for good prognosis [56]. TAMs have been found to promote colorectal cancer growth and metastasis and this is more closely linked to the M2 functional subtype; besides, when influenced by cytokines, TAMs have been found to induce colon cancer cell migration [57]. Cui et al. reported that the effects of TAMs on liver metastases from colorectal cancer do not depend on the total number of TAMs, but on the number and ratio of M1 and M2 subtypes, pointing to the M2 number and the M2/M1 ratio as more significant predictors for hepatic metastases [56]. M1 was reported to be negatively correlated with the liver metastatic process and lymphatic metastasis, indicating an anti-tumour property of this sub-type. On the other hand, M2 have been reported to be correlated with all parameters linked to tumour invasiveness, including pre-operative CEA level tumour differentiation and liver and lymphatic metastatic potential [58].

The Importance of MDSCs in the CRLM Pathway

Myeloid-derived suppressor cells (MDSCs) comprise a population of inhibitory immune cells that have been reported to rise in numbers in various cancer types, although characterisation of human MDSCs remains difficult, as their phenotype is quite variable [27]. MDSCs represent a heterogeneous population, either of mono-cytic or granulocytic origin, expressed by the bone marrow as a reaction to a wide array of stimuli [59, 60]; MDSCs have the capacity to potentially compromise adaptive and innate immunity, since they play a central role in cancer progression. MDSCs can exhibit pro-tumourigenic activity both via direct mechanisms and by employing a myriad of immunosuppressive mechanisms at the tumour site. MDSCs are characterised by surface expression of CD11b and Gr-1 markers, their common functional feature being attenuation of T lymphocytes' and NK cells' effector functions [61]. MDSCs can differentiate into TAMs, potentially inhibiting CD8⁺ T cell activation through the expression of nitric oxide synthase 2 (NOS2) and arginase (ARG1), but they also initiate the expression of Tregs [27]. STAT signalling has been shown to be involved in the

immunosuppressive activity of MDSCs in cancer cases indicating the protein's key-role [62, 63]. Human MDSC phenotype is quite variable, which makes their characterisation very difficult. MDSCs have been reported to differentiate into TAMs [64, 65], while it has also been shown that murine and human MDSCs can inhibit CD8⁺ T cell activation via an expression of nitric oxide synthase 2 (NOS2) and arginase (ARG1) [66]. Furthermore, it has also been reported that MDSCs can initiate the development of Tregs [67] and the polarisation of macrophages to a phenotype similar to that of TAMs [68].

One of the main properties of MDSCs is the contrasting immune-surveillance mechanisms of CD8⁺ and CD4⁺ T lymphocytes. This fact indicates the importance of T cells and MDSCs equilibrium within the tumour microenvironment and it may play a leading role in the progression of the disease.

It has been reported that, following an injection of 4 T1 cells in mice and subsequent treatment with cyclophosphamide, tumour progression and metastatic spread were observed and the tumour microenvironment shown great infiltration by MDSCs; this effect also has been observed in cells infiltrated by CD4⁺ and CD8⁺ T cells via IFN- γ release [69]. On the basis of various reports, it has been suggested that there is close correlation between the MDSC spread into the tumour microenvironment and the actual tumour progression and metastatic pathway. This entails NK cells potentially rendering T cells resistant to the suppressive activity of MDSC and MDSC accumulation triggered by the Macrophage Migration Inhibitory Factor, an inflammatory cytokine produced by the tumour [70, 71].

MDSCs have been reported to mediate T cell suppression via a cell–cell effect or, through the release of soluble mediators, such as reactive oxygen species or cytokines [28, 72]. Another one of their features is MDSCs' ability to assist tumour growth by expressing pro-angiogenic factors, such as VEGF-A [73]. Although the role of myeloid cells in CRC metastatic dissemination to the liver has been less extensively studied, the presence of tumour-infiltrating myeloid cell population (CD11b⁺/CD34⁺/Gr1[–] cells) has been demonstrated to enhance hepatic CRC cell spread. The role of myeloid cells in the metastatic dissemination of CRC to the liver is being studied and also intricate interactions are found to be influencing the development of metastases [74]. Lim et al. have reported three myeloid cell population characterisations (CD11b/Gr1^{high}, CD11b/Gr1^{mid} and CD11b/Gr1^{low}) in the liver tumour microenvironment in murine models, with each of these exhibiting a distinct morphology, as well as surface marker expression and cascade of secreted inflammatory mediators. Specifically, CD11b/Gr1^{mid} cells were shown to increase dramatically in number in parallel to metastatic progress [75].

It has been shown that tumour-infiltrating myeloid cells support the metastatic cascade both at its early stages -

including invasion, extravasation and colonisation [76–78] - and also at its later ones. Lim et al. were able to show the functional importance of CD11b/Gr1mid and CD11b/Gr1low myeloid cells, in the development of liver metastases through their findings that depletion of these cells in animals with formed liver metastases caused an impressive decrease in the hepatic tumour burden. They also showed that said cells were important for the sustained growth of metastatic tumour cells at the late stages of the metastatic pathway [75].

The CCL2/CCR2 ratio disturbance observed in cancer has been attributed a role in the process of several primary neoplasms [79]. It has also been shown that the release of CCL2 by CRC cells leads to CD11b/Gr1mid cells being recruited to the hepatic tumour microenvironment. Consequently, the inhibition of CCL2 signalling, as well as the absence of its cognate receptor in Ccr2 knockout mice, resulted in attenuating the recruitment of CD11b/Gr1mid cells and in a marked reduction of the tumour burden. Inhibiting CCL2 leads to temporary delay in metastatic growth, indicating that the efficacy of targeting a single component may well be limited [75].

Zhang et al. investigated the clinical significance of circulating and tumour-infiltrating Lin2/lowHLA-DR2CD11b + CD33+ MDSCs in colorectal cancer, in a study involving patients with varying stages of colorectal cancer, and demonstrated that peripheral blood from said patients showed increased percentages and absolute number of Lin2/lowHLA-DR2CD11b + CD33+ MDSCs when compared with healthy individuals [80]. It was also concluded that this increase seems to correlate with the cancer stage and tumour metastatic process, but not with the actual size of the primary tumour, with a similar increase in the number of MDSCs detected in metastatic tumour tissues as well. Only MDSCs from cancer patients exhibited immunosuppressive activity and the capacity to inhibit autologous T-cell proliferation *in vitro*, indicating, once again, the importance of a possible pharmacologic blockade of MDSCs and the need to further explore this field [80].

CXCR4/CXCL12 Axis and the CRLM Pathway

Chemokines are chemotactic cytokines that can cause an accumulation of leukocytes directed towards a chemical gradient; this leads to leukocyte adaptation to specific organs [81] and can be stimulated by - among others - inflammatory cytokines and growth factors. Chemokines and their receptors, can be used by cancer cells in order to modulate the host microenvironment and also ease cancer progression [82, 83]. Chemokines and their receptors have been shown to regulate various metastatic and advanced cancers, with interleukin-8 (IL-8) and its receptor CXCR2 being two of the most significantly upregulated chemokines in colon cancer [84]. More specifically, when IL-8 is expressed and secreted by tumour

cells, cell proliferation and survival are enhanced and angiogenesis and neutrophil infiltration into the tumour are promoted.

CXCR4, an established inducer of colorectal cancer progression, has been found to be significantly higher in liver metastases than in primary colorectal cancer [82, 85]. The CXCR4 ligand, CXCL12/stromal-derived factor-1 (SDF-1), is also linked to tumour progression and accumulates in CD133-positive tumour cells [86–88]; the hypothesis is that there is interaction between CXCR4 and CXCL12 expression, highlighting this as causing tumours to proliferate, migrate, and invade other organs. Sakai et al. reported that increased levels of nuclear CXCR4 and hepatocyte CXCL12 expression are associated with overall and liver-disease free survival, when compared with other clinical factors [82]. Additionally, CXCR4 and CXCL12 expression profiles could be a predictor of remnant liver cancer recurrence and of poor prognosis. Sakai et al. concluded that CXCR4/CXCL12 ratio could have an important role in the biologically aggressive behaviour of colorectal liver metastasis, indicating that CXCR4 expression in colorectal liver metastases together with CXCL12 upregulation in hepatocytes have the potential of helping predict clinical outcomes following hepatectomy [82].

The Role of Infiltrating T Lymphocytes in the CRLM Pathway

Current management of diffuse CRLM is defined by multi-drug chemotherapy regimens making the development of novel anticancer therapies necessary [88]. The presence of immune cells within primary colorectal tumours has been linked with improved clinical outcomes. Patients with CRC have been found to have improved survival rates when CD8+ TILs had been detected in the colon cancer [89]. It has been reported that, in CRLM cases, there is an association between increased CD4 and CD8 T-cell infiltrates, as well as decreased Foxp3+ T regulatory cell percentages and improved overall survival [90]. Galon's group demonstrated how the presence of T-cell infiltrates in primary colon cancer could be more prognostic than the classic TNM staging currently used worldwide (A system of tumour evaluation based on three variables: primary tumour (T), regional nodes (N), and metastasis (M)) [91]. Additionally, it has been shown, in preliminary gene array studies, that T-cell proliferation seems to be a key-factor in the survival of patients with CRLM [92], indicating that attenuation of the T-cell response could prove to be a promising treatment pathway for such patients. It has been suggested that tumour infiltrating lymphocytes (TIL) from resected human CRLM responded to tumour antigens in a high percentage of patients studied, while the percentage of activated effector T-cells were identified using TIL flow cytometry [93]. All these findings corroborate the hypothesis

that T-cell infiltration into primary and metastatic colon cancer tumours is linked with improved survival and could also represent an immune response with anti-tumour and antigen-specific properties.

Qin et al. used an immune-competent syngeneic animal model of CRLM and demonstrated Th1 cell-mediated immunity, thus supporting a CD8⁺ cytotoxic T-lymphocyte-based tumour-specific immune response [32]. Furthermore, the presence of CD3⁺ CD4⁺ TIL was limited, while there was increased expression of CD3⁺ CD8⁺ TIL, and the majority of CD8⁺ cells were peritumoural with LIGHT (TNFSF14) being only slightly detectable on most CD8⁺ TIL. These findings supported the hypothesis of an immunosuppressive activity on TIL that migrate and then penetrate metastatic tumours. LIGHT (TNFSF14) is an immune-stimulatory cytokine which has been reported to attenuate the anti-tumour immune response and to be linked with improved overall and recurrence-free survival among CRLM patients [93].

The hepatic cytokine network seems to be regulating the immune response to liver malignancy [94], with CD4⁺ CD25⁺ regulatory T cells (Tregs) possibly having a key-role in ineffective anti-tumour immune responses [95]. Additionally, the Transforming Growth Factor beta (TGF- β), which is a secreted ligand, has been reported to have influence on both tumour initiation and metastasis [96]. TGF- β comprises three ligands: TGF- β 1, TGF- β 2, and TGF- β 3. In cancer cases, TGF- β 1 has been shown to be more abundant than TGF- β 2 or TGF- β 3, and the most commonly upregulated cytokine in tumour cells; therefore, it has been the focal point of attention in many of the mechanistic studies currently in progress both in vitro and in vivo [97]. Expression of the TGF- β ligand also increases when a tumour progresses and metastasizes [98]. On the other hand, IL-10 is a cytokine that could potentially play an important role in tumour development and metastatic pathway, due to its anti-inflammatory and immunosuppressive properties [99].

In an experimental model of liver metastasis, Huang et al. showed that there was a decrease in the number of CD4⁺ and CD8⁺ T cells and an increase in the number of circulating tumour cells, with the percentage of CD4⁺ CD25⁺ Treg cells increasing [100]. As CD4⁺ and CD8⁺ T cells were decreasing, enhanced expression of both CD4⁺ CD25⁺ Treg cells and immunosuppressive cytokines IL-10 and TGF- β 1 could be considered responsible for the suppression of local anti-tumour responses, leading to tumour cells evading host surveillance and progressing to liver metastasis [100].

Chemokines and their receptors are attractive therapeutic targets; however, clinical trial results so far have not provided substantial evidence to this effect. Myeloid cell recruitment to the tumour microenvironment is a key-process in the metastatic pathway, influencing the host immune system. In order to achieve some clinical benefit, it would be essential to obtain a strategy for targeting several chemokines and their receptors;

this also highlights the importance of more clearly outlining the role of myeloid cells in the metastatic pathway through future research.

Conclusive Remarks

Summarising the description of tumour microenvironment and delineating the metastatic pathway of colorectal cancer into the liver is not straightforward. Pathways cross-talk and several factors play key-roles in the process. Specifically, myeloid cell recruitment into the tumour's microenvironment seems to be a key-process in the metastatic pathway, influencing the host immune system, with TAMs also playing a very important role. In addition, the challenge is to determine the optimal immunotherapeutic strategy for this disease, since the full characterisation of the tumour's microenvironment would be necessary. Furthermore, the heterogeneity of colorectal cancer liver metastasis cases and the difficulty of reproducing accurate animal models need to be overcome. A multitude of targeted cancer treatments is the wave of the future and targeting cellular receptors and molecules in pathways causing cancer initiation, proliferation and metastasis may lead to successful cancer chemotherapy results. Considering that the tumour microenvironment is also a very complex and dynamic network, a combination of drugs targeting different tumours appears to be the most appropriate approach.

Conflict of Interest The authors declare no conflict of interest.

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