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Review

Malignant cell interactions with cells of the hepatic sinusoids mediate primarily the development of colorectal cancer liver metastasis

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SUMMARY

Metastases are the main cause of death for patients with colorectal cancer and the liver is the primary host organ. However, macrometastases constitute the final step of a complicated and poorly-defined multistage process, named invasion- metastasis cascade. Before they metastasise, malignant cells undergo partial or complete transformation and acquire new properties. They present intensive growth, provoke neoangiogenesis, invade the surrounding extracellular matrix, detach from their primary site and intravasate. Some succeed in surviving in the systemic circulation, adhere to hepatic sinusoids and extravasate. Eventually, by evading the hepatic immune system, few cancer cells colonise the liver and form metastases. While a vast number of cells leave the primary tumour and intravasate, only a small minority reaches the liver blood network. Thus, the possibility of metastases formation is very low. The entrapment of colorectal cancer cells in the sinusoids and their interactions with the resident cells are considered very important initial steps in the liver invasion. Sinusoidal endothelial cells, pit cells, stellate cells and Kupffer cells all mediate the metastatic process in complex ways, through a variety of biological compounds and intercellular actions. Current research aims to elucidate the role of these cells in colorectal cancer liver metastasis.

Key words: colorectal cancer, liver metastasis, hepatic sinusoids, sinusoidal endothelial cells, pit cells, stellate cells, Kupffer cells

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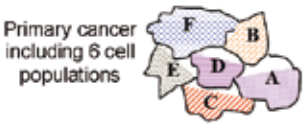
INTRODUCTION

Metastasis is considered a different entity from the primary tumour, because metastatic cells show phenotypical and genetic differences from their ancestors[1-4]. Studies in multiple tumour types, by numerous laboratories confirmed that various subpopulations in the primary sites differ from each other[5-7]. The biological heterogeneity of cancer cells in their primary as well as in their metastatic site is probably the main obstacle for effective treatment[7].

Genetic and phenotypic alterations in cancer cells are obviously necessary, in order to accomplish all successive stages of the metastatic process. Progressive growth, vascula-risation, invasion, detachment from the primary tumour and intravasation initially occur; then, evasion of the immune system, survival in the hostile environment of the systemic circulation, arrest, adhesion to foreign vessels and extravasation follow. The final stages of the cascade include evasion of host defence, establishment of an adequate blood supply network and colonization. All these stages demand numerous cell properties and failure or inadequacy in any of them cancels the entire metastatic process (Figure 1)[7].

Due to the resemblance of tumour behaviour with embryogenesis and healing, a theory named epithelial mesenchymal transition (EMT) has prevailed[8-10]. According



	A	B	C	D	E	F
Progressive growth	+	+	+	+	+	+
Neoangiogenesis	+	+	+	+	+	+
Invasion	+		+		+	+
Detachment from the primary tumour	+	+	+		+	+
Intravasation-Embolisation	+	+	+			+
Survival in the systemic circulation	+	+	+			+
Arrest and adhesion to blood vessel endothelium		+				+
Extravasation		+	+			+
Evasion of host defence		+	+			+
COLONIZATION						+

Figure 1. Sequential steps in the process of metastasis for 6 cell populations A-F. Most cancer cells can not fulfil all the necessary alterations in order to achieve metastasis. They may present invasion deficiency (B), deficiency in adhesion (C), multiple incomplete steps (A, D and E). Very few cells finally succeed colonization and metastasis (F)[7,13]

to this, before they metastasise, primary carcinoma cells lose many of their epithelial phenotypes, such as epithelial polarity, cytokeratin expression and ability of E-cadherin composition (a key protein in epithelial adherens junctions). Instead, they acquire fibroblast morphology, become motile and invasive. They express N-cadherin (a mesenchymal protein in adherens junctions) and αvβ6 integrin, secrete proteases such as matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) and present platelet derived growth factor (PDGF) receptors[11]. The responsible signals which induce EMT may originate in the stroma of the primary carcinoma. EMT was considered to be reversible for most of the cancer cells, which regain many of their original characteristics, when they have completed metastasis[12].

It is not known if cancer cells acquire these properties gradually or if they already accumulate most of them, when they begin the metastatic sequence. Only the colonizing ability is strongly believed to be acquired later in the tumourogenicity[13]. While micrometastases may be achieved by several cancer cells, macrometastases rarely occur[10]. Dormant micro-metastases are usually the final stage of the metastatic process for the vast majority of malignant cells, which never succeed to survive or adopt in the inhospitable environment of the foreign tissue. In accordance with that, cancer patients usually present myriad of micrometastases in their body without any clinical evidence [14,15]. In a clinical study by Tarin et al, patients with ovarian cancer and malignant ascites were treated with peritoneovenous shunts, which drained ascites in the systemic circulation. Interestingly, despite constant entry of innumerable cancer cells in the venous blood, metastases were rare [16].

THE METASTATIC CASCADE

Colorectal cancer is the commonest among other primary tumours, which colonizes the liver. 50-60% of patients with colorectal cancer will present hepatic metastases and their life expectancy is mainly determined by the progression of secondary liver disease[17]. The progression from a local tumour to a systemic metastatic disease is called the invasion-metastasis cascade (Figure 2).

Following the cascade process, malignant cells of the primary colorectal tumour, after intense local proliferation, induce a breach in the basement membrane, succeed in invading the extracellular matrix and reach a blood vessel,

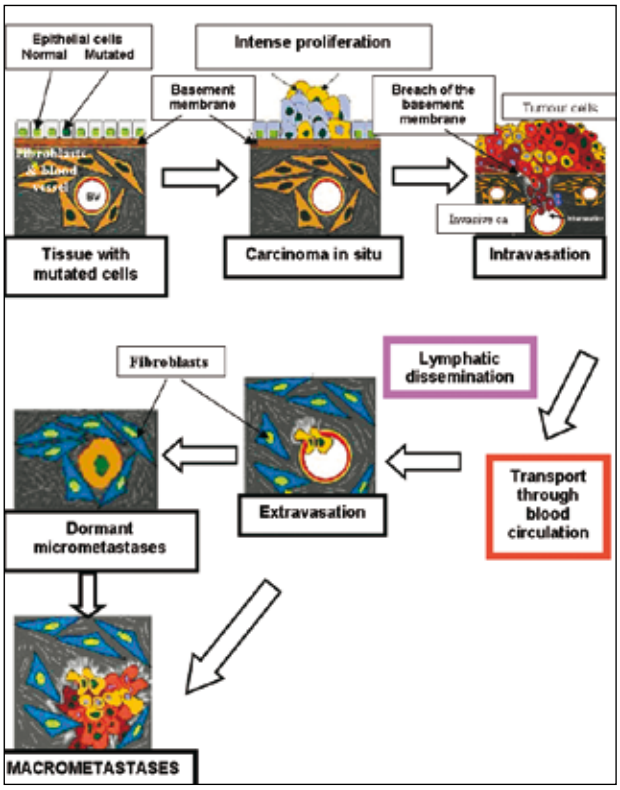


Figure 2. The invasion-metastasis cascade. Successive stages until the formation of macrometastases[13,18]

which offers access to the systemic circulation. They intravasate and some reach very small portal branches or hepatic sinusoids and may be trapped there, as they are of a larger diameter than the capillaries. Their volume increases even further, as they tend to adhere to platelets in the circulation, thus partly evading the immune defence[18]. Sinusoidal entrapment exposes malignant colorectal cells to the highly competent hepatic sinusoidal immune system. Endothelial cells, pit cells and Kupffer cells eliminate about 90% of the arriving tumour cell population[19,20].

While local immune cells act in a tumouricidal way, colorectal cancer cells adhere to the sinusoidal endothelium through specific adhesion molecules. Active pit cells and endothelial cells release interferon gamma (IFNγ) and NO in the sinusoids and cause the expression of functional Fas by 5% of colorectal cells[18]. As endothelial cells express the Fas ligand (FasL), the apoptosis pathway is followed by Fas expressing cancer cells, which finally die. Eventually, a small remnant of metastatic colorectal cells, less than 5%, succeed to survive and having evaded all defensive mechanisms, pass through the hepatic endothelial cells and reach the space of Disse, in close proximity to the hepatocytes[21]. From the moment of extravasation, cytotoxic T cells, monocytes and macrophages, which occupy extrasinusoidal hepatic tissue, are activated against the metastatic cells, though not always successfully[18]. Ultimately, few cancer cells cause micrometastases in the hepatic parenchyma. They remain in a dormant state, the duration of which is unknown. It is highly probable, that sooner or later these micrometastases will be reactivated and create macrometastases[13]. The last stage of the cascade is then accomplished.

Generally, invasion and metastasis are responsible for 90% of cancer associated deaths. Interestingly, the majority of cancer cells, when the patient succumbs to the disease, are usually traced in metastatic sites, rather than the primary tumour itself[13].

TUMOUR CELL- HOST CELL INTERACTIONS

In order to succeed in metastasising, malignant cells tend to invade more frequently the tissue, which imposes the fewer adaptive alterations; the tissue, which presents the most "fertile soil" for growth. This observation was first described by Paget in the "seed and soil" hypothesis in 1889. According to this, colonisation is achieved by cells, which find the suitable environment- "soil" in a distant organ. The interaction between cancer cells and host cells defines the fate and the direction of a metastasis. Though, other parameters like mechanical and anatomical features, venous circulation or lymphatic drainage also influence the metastatic process[7]. It is obvious that Paget's theory can not explain why contralateral organs are so unusual metastatic targets of the primary tumour, in spite of their apparent provision of the perfect "soil". Contralateral breast metastases are infrequent and constitute less than 6% of the total breast metastatic incidents[22,23]. Similarly, primary renal cancer also fails to create contralateral metastases[24,25].

In the case of colorectal liver metastases, it is not certain, if the hepatic parenchyma, protected by a highly competitive and multifactorious immune system constitutes the suitable "soil" for colorectal cells. The most probable cause for the high incidence of colorectal liver metastases is the hepatic blood supply. The portal vein connects the gut with the liver and when cancer cells detach from their original site, they inevitably arrive in the sinusoidal network and most of them are trapped there. Even if it is very difficult for tumour cells to colonize the liver, due to a variety of reasons, the vast number of them, which are immobilised in the sinusoids, guarantee that after a considerable time period, some will eventually acquire the appropriate genetic and phenotypic changes to provoke macrometastases. However, there are also factors, which favour liver colonization; the affluent sinusoidal blood flow is apparently one of them, as it eliminates the necessity of production of endothelial factors and neoangiogenesis[26].

HEPATIC SINUSOIDS & CANCER CELL ARREST IN THE LIVER

Sinusoids are the hepatic blood capillaries, where the circulating tumour cells may arrest or be trapped. Then, they interact with various cells, which are present in the sinusoids and may be destroyed or adhered to the endothelium and continue the metastatic process, which may lead to colonization[26]. Liver sinusoidal cells consist of endothelial cells, Kupffer cells, stellate cells and pit cells- the hepatic natural killer cells (Figure 3)[20]. Each of these cell types plays a different and important role in the hepatic homeostasis and tumour progression.

Sinusoidal endothelial cells (SECs)

SECs were first described by Wisse at the beginning of 1970s. They differ from other endothelial cells, as their structure includes characteristic transcytoplasmic canals arranged in sieve plates named fenestrae. Fenestration distinguishes SECs from all other liver and endothelial cells and constitutes a reliable marker for these cells[27,28]. They

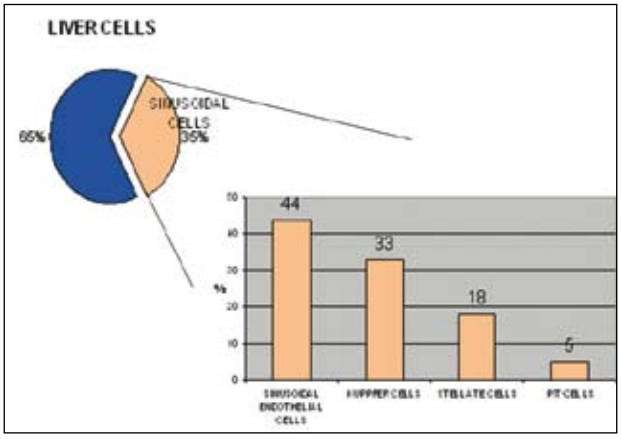


Figure 3. Types of sinusoidal cells. Liver sinusoidal cells consist of endothelial cells, Kupffer cells, stellate cells and pit cells[20]

do not form a continuous wall in the sinusoids, because they are not always attached to each other; additionally, there is no basement membrane proximally to the space of Disse, so various molecules may find easy access, from the sinusoids to the hepatic parenchyma[29].

Sinusoidal endothelial cells present a multifunctional character, which has recently been elucidated to a certain extent. They form a major scavenger cell system in the liver and accomplish receptor- mediated endocytosis and pinocytosis. They remove molecules from the circulation, such as modified albumins, hyaluran, ceruloplasmin, iron-transferrin and acetylated low-density lipoproteins. They may also phagocytose ECM substances and regulate collagen balance[29].

In vivo experiments on rodents with latex particles demonstrated that under normal circumstances SECs appeared very active in endocytosing soluble waste macromolecules and colloidal materials from the circulation. They were unable to uptake particles greater than 0.23μm, which were eliminated by Kupffer cells. However, when Kupffer cell activity was considerably impaired with the use of alcohol, SECs phagocytosed particles greater than 1μm. These results may show that liver sinusoidal endothelial cells constitute a second line of defence after Kupffer cells and pit cells, against foreign materials and organisms[27,30]. Furthermore, studies on duck liver cell populations infected with duck hepatitis B virus indicated that SECs mediated the elimination of viruses and inhibited the infection of hepatocytes[31].

SECs play an important role in the innate immune system, act as antigen presenting cells to lymphocytes and also regulate immune tolerance. Several factors relevant to antigen presentation were found to be present on SECs: CD40, CD54, CD80, CD 86 and MHC class I and II[20,32]. However, other studies, reported that SECs could not activate naove T cells by themselves and that they lacked the expression of MHC class II. These important differences were possibly attributed to different ways of cell isolation and cultivation. While many methods have been developed, the isolation of pure SEC population remains a very difficult task. On the other hand there is still no agreement if these cells could keep their natural biological characteristics after cultivation of more than 1or 2 days or if serum affects their well being[27].

Experiments on rats have indicated that SECs undergo apoptosis, when exposed to hypoxia-reoxygenation, due to liver surgery or severe systemic shock[29,33]. Furthermore, experiments on mice have revealed, that SECs are able to produce NO, which may lead lymphoma or colorectal cancer cells to apoptosis[34,35]. This cytotoxic action through NO secretion was observed in experiments with melanoma cells, as well[36]. Although, they appear to play a protective role against cancer, SECs may also aid tumour cells to arrest and metastasise into the liver. Under cytokine activation, they express adhesion molecules, such as E-selectin, which attach cancer cells to the endothelium and facilitate their extravasation in the hepatic parenchyma[37].

Pit cells

Discovered by Wisse et al. in 1976, they are hepatic natural killer (NK) cells, which always remain in contact with sinusoidal endothelial cells and Kupffer cells. Apart from the name pit cells, which is related to their cytoplasmic granules, they are also called hepatic NK cells and hepatic large granular lymphocytes. Their shape varies, due to the presence of pseudopodia, but their structural characteristic is the presence of rod vesicles in the cytoplasm. They also contain granules with lysosomal enzymes, perforin and phosphatase[20,38,39].

Rat experiments on the morphology of pit cells, revealed that their population could be separated into low and high density. The former contained more rod vesicles and more but smaller granules than NK cells in the blood; the latter presented intermediate numbers, between low density and blood NK cells. Moreover, functional differences also occurred. Low density NKs showed the highest cytotoxicity, while high density cells had intermediate cytotoxic activity in comparison with low density and blood NKs[39,40].

The hepatic NKs were believed to be descendents of blood NKs. The latter migrate into the hepatic sinusoids and differentiate into high and then low density pit cells[41].

Multiple factors of the sinusoidal environment were considered to induce and influence this transformation, with Kupffer cells to play a substantial role[42]. In general, after differentiation pit cells remain in direct contact with the blood. However, using their pseudopodia, they may penetrate the endothelial cell fenestrae and enter the space of Disse, which is an uncommon feature; in that way they are able to contact hepatocyte microvilli[39].

Pit cells substantially contribute to hepatic immunity and present antitumour action. Experimental data on rats demonstrated that pit cells were highly cytotoxic against multiple malignant cell lines, such as mastocytoma tumour cells P815, Lewis lung carcinoma cells 3LL, murine fibrosarcoma L929, rat colorectal carcinoma DHD-K12 and colon carcinoma cells CC531s[39].

In order to exert cytotoxicity, pit cells require binding with the target cells, named conjugation. Various adhesion molecules on NK cells mediated this process, like CD2, a member of the immunoglobulin superfamily, CD28 and lymphocyte function-associated antigen 1 (LFA-1), while CD58 and CD54 may be present on the target cells[43,44]. Additionally, interactions between β_2 integrins and intercellular cell adhesion molecules (ICAMs) were considered important in these cell- cell conjugations[39,43,45].

After conjugation, stimulation of various receptors may trigger or inhibit NK cytotoxicity. Three superfamilies of natural killer cell receptors were presented primarily on human NKs, while others, named co-receptors still remain under investigation: the killer immuno-globulin receptor (KIR) that recognised MHC class I molecules, the c-type lectin, which recognised non classical MHC class I or class I- like molecules and the natural cytotoxicity receptor (NRC) superfamily, which is not well studied, yet[46].

Pit cells in collaboration with Kupffer cells represented the first line of liver defence against metastasising cancer cells. They were able to destroy tumour cells as well as virus and transformed cells, by various mechanisms[38,47]:

i. Perforin/ granzyme pathway: This was a Ca^{2+} dependent molecular pathway, where pit cells adhered to tumour cells and release perforin and proteases into the intercellular space. Perforin induced pores in the tumour cytoplasmic membrane and proteases provoked DNA segmentation.

ii. Apoptosis pathway: Pit cells expressed Fas ligand (FasL) and tumour necrosis factor- related apoptosis-inducing ligand (TRAIL). When they adhered to the tumour cells, these ligands bound to their receptors and led cancer cells to apoptosis.

iii. Cytokine pathway: By secreting cytokines, like interferon-γ, they activated lymphocytes and macrophages against invading cancer cells.

Hepatic stellate cells (HSCs)

They were called Ito cells in the past, by the Japanese anatomist Toshio Ito, who described them as fat-storing cells in 1952[48]. Other names also used were peri- or parasinusoidal cells and fat storing cells. Their present name was agreed in 1996 referring to their resting shape in normal liver[49]. They are located in the space of Disse, comprising about 15% of the resident cells in normal liver. Stellate cells present a unique morphology, due to their long cytoplasmic processes that form a spindle-shaped cellular body[50]. These projections serve as sensory organs for chemotactic signals, generating contractile actions and cell motility[51].

In pathological conditions, such as liver cirrhosis or hepatic injury their fine structure substantially differentiated. Rough endoplasmic reticulum was enlarged, Golgi apparatus were better developed and protein production was induced. HSCs were transformed to a myofibroblast-like appearance, by losing their processes and lipid droplets and forming collagen fibres[20,49,50].

HSCs demonstrate multiple similarities with SECs. They both share a mesenchymal phenotype, *in situ* close proximity and express several angiogenic effectors, such as vascular endothelial factor (VEGF)[52].

HSCs constitute the major vitamin A reservoir in the body, because they contain over 80% of the total vitamin A in lipid droplets, though, cells storing vitamin A exist in various tissues, including kidneys, lungs and intestine[49]. Additionally, significant amounts of a variety of other compounds were present in stellate cells, like phospholipids, cholesterol, triglycerides and free fatty acids[53].

HSCs play a significant role in producing ECM and matrix metalloproteinases in the hepatic tissue that is regulated by fibrogenic cytokines, including transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF)[50]. Moreover, they contribute to hepatocyte proliferation after liver injury, through the secretion of mesenchymal morphogenic proteins epimorphin and pleiotrophin[54,55], hepatocyte growth factor (HGF) and epidermal growth factor (EGF)[49,56].

They also exert immuno-regulatory activity. By producing chemokines, they promote mono- and polymorphonuclear leukocyte infiltration, activate neutrophils and

regulate lymphocyte populations[49,57]. They also act as professional antigen presenting cells that may activate T lymphocytes[48,58]. Furthermore, they express toll-like receptors (TLRs), which lead to HSC activation when interacting with bacteria[58,59].

HSCs secrete and respond to a wide variety of cytokines (Table 1). They modify the activity of various growth factors, express adhesion molecules such as intercellular cell adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), neural cell adhesion molecule (NCAM) and mediate detoxification of ethanol and xenobiotics[49,60].

In general, stellate cells display proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation activity and retinoid loss, when activated[49]. They were implicated in inflammation[61], cell survival and apoptosis[62], fibrinogenesis, MMP expression, liver regeneration[49] and monitoring of cellular pH[63].

HSCs participate in tumour growth and metastatic process. Experimental studies on rats revealed that conditioned medium from cultures of hepatocellular carcinoma hepatocytes could induce HSC activation[64]. Moreover, *in vitro* experiments with melanoma cells which caused liver metastases, concluded that tumour cells activated HSCs, which in turn promoted angiogenesis through VEGF expression[65].

Injection of colon carcinoma cells in nude mice provoked the formation of hepatic metastatic foci and the activation of HSCs. The latter produced HGF and TGF-β1, which induced tumour cell migration and proliferation. Similarly, tumour cells secreted PDGF-AB and enhanced stellate cell locomotion and proliferation[66].

Experiments on rats reported that co-cultures of SECs and HSCs presented spontaneous differentiation, with HSCs forming the core of the cell population and SECs the surface. *In vitro* activated stellate cells, cultured with SECs, expressed functional smooth muscle cell phenotype and formed capillary-like structures in angiogenesis assays. As tumours may activate HSCs, their mediation in neoangiogenesis through interactions with SECs was implicated in these studies[67].

Kupffer cells (KCs)

They were named after Carl von Kupffer, the German histologist and embryologist, who discovered them. They constitute the biggest, more than 80%, tissue macrophage population in the human body and approximately 15% of all hepatic cells. Their progenitors are monocytes from the bone marrow, but they also present ability for self-renewal[68]. Their shape varies due to cytoplasmic extensions. They present microvilli and lamellipodia in their surface and contain abundant lysosomes and phagosomes[69].

Table 1. Cytokines associated with HCS activity. The expression and interaction with a large variety of biological molecules allow HSCs to mediate multiple activities and functions [49]

CYTOKINES	CYTOKINE ACTION
• Transforming Growth Factors: TGFβ1, TGFα • Platelet derived growth factors (PDGF-B) • Hepatocyte growth factor (HGF) • Stem cell factor • Fibroblast growth factors (a-, bFGF) • Vascular endothelial growth factor (VEGF) • Insulin like growth factors: IGF-I, II • Endothelin-1: ET-1, ECE • Leptin • Plasminogen: UPA, PAI-1 • Fibrillar collagens: Collagens I, II • Renin, angiotensin II	Proliferative-Fibrogenic
• Macrophage colony stimulating factor (M-CSF) • Platelet activating factor (PAF) • CD40 • Tumour necrosis factor α (TNF-α) • Opioids • Toll like receptor ligands (TLR4, CD14)	Chemotactic-Inflammatory
• Interleukine-6 (IL-6) • Neurotrophins: NGF, BDNF, NT-4	Regenerative
• Interleukin-10 (IL-10) • Adiponectin • Folistatin	Antifibrogenic
• Fas signalling	Apoptotic

KCs support and maintain liver homeostasis and participate in reactions against toxic agents. They mainly act as scavengers around the sinusoids and remove foreign particles from the blood and the hepatic tissue[20]. When activated, they produce a variety of inflammatory agents, growth control mediators and oxygen radicals. These products, modulate acute and chronic liver responses to injury, drugs, chemicals and cancer[70].

The protective role of KCs against damage is assisted by their ability to migrate, from the sinusoids to the hepatic parenchyma and in reverse, without facing any barrier. Additionally, they can act as antigen presenting cells, regulating hepatic immune reactions [70]. Furthermore, their population differs in size and functional characteristics in the liver tissue. KCs in periportal regions are larger and

double in number comparing with their homologue cells in centrilobular regions, have more active lysosomes and greater phagocytic activity; though they secrete less superoxide anions[69].

While they act as main liver protectors, among other hepatic cells, when activated they may contribute to liver damage. KCs exercise their actions through the production of numerous molecules, including cytokines, oxygen radicals, proteolytic enzymes, nitrogen species and lipid metabolites such as prostaglandins (Table 2). These molecules may interact directly or through neutrophil activation, with hepatocytes and cause their death[70,71]. Sinusoidal endothelial cells are also activated by these mediators and induce increased coagulation in the liver. Consequently, fibrin is deposited and the increased hypoxia may harm the hepatocytes[70].

It seems that there is a threshold above which KCs become harmful. High doses of inflammatory agents may activate these macrophages to secrete injurious amounts of cytokines in the hepatic parenchyma. The same result may occur due to persistent inflammatory stimuli, which cause long term cytokine production[70]. Experiments in rats, with bacterial lipopolysaccharide (LPS) showed that low doses of LPS activate KCs, without any associated damage, whereas large doses induced harmful results[73].

The hepatic macrophages also mediate growth and regeneration in the liver, by producing numerous mitogens and co-mitogens, such as TNF-α. In rat experiments this cytokine induced proliferation and decreased apoptosis in hepatocyte cultures[74-76].

Table 2. Molecules secreted by Kupffer cells. KCs exercise their actions through the production of cytokines, oxygen radicals, nitrogen species, proteolytic enzymes and lipid metabolites such as prostanoids [70,72]

GROUP	MEMBERS
Peptide mediators	• Tumour necrosis factor α (TNF-α) • Interleukin 1α (IL-1α) • Interleukin 6 (IL-6) • Transforming growth factor β (TGF-β)
Oxygen Species (superoxide)	
Nitrogen Species (nitric oxide)	
Proteases	
Lipid metabolites (Prostanoids)	• Prostaglandin D2 • Prostaglandin E2 • Thromboxane

KCs can be activated rapidly in the whole organ. Rat liver treated with peroxisome proliferator WY-14643, produced nuclear factor kB (NF-kB), a major regulator of macrophage cytokines, in about 2 hours[76]. Other rat experiments, where peroxisome proliferators were used to activate Kupffer cells, revealed a production of oxygen species, also within 2 hours[77].

The characteristics already mentioned above are also implicated in hepatic macrophage role within the "host tumoural surveillance system". As they constantly reside around the sinusoids, they discriminate and remove bacteria, foreign particles and tumour cells, which reach the sinusoids. The latter become vulnerable to macrophage tumourocidal activity, especially during endothelial adhesion and extravasation[78,79]. Destruction of metastasising tumour cells occurs after binding with macrophages[80], by several mechanisms: release of tumour necrosis factor, secretion of proteases, production of oxygen metabolites and phagocytosis[80-82].

However, the interaction between KCs and arriving tumour cells is not always in favour of liver homeostasis. Tumour cell binding with the resident hepatic macrophages leads necessarily to a cancer cell arrest in the liver. If the killing process is not immediately accomplished or is partially completed, then the binding process becomes the first step of tumour colonization[79]. Experimental data show that Kupffer cells exert a limited capacity of tumour surveillance and arrest; when cancer cells reach the liver in high numbers, they are eventually saturated and metastasis occurs[79]. Furthermore, if liver metastasis progresses, then KCs produce growth factors, such as hepatocyte growth factor (HGF) and proteases, which facilitate tumour cell proliferation and invasion [68]. In vitro experiments have also indicated, that highly malignant cells are able to reduce in their favour the phagocytic capacity of KCs and promote colonization[26].

In conjunction with cytotoxic ability, KCs also exert a cytostatic and immune regulatory function. In the early stages of metastasis, they control tumour growth and keep metastatic cell proliferation rate low[79]. In addition, KCs actively proliferate, possibly in an attempt to deal with large populations of tumour cells and secrete chemotactic agents to attract monocytes and other immune cells from the systemic circulation. Nevertheless, these properties are limited and may be overcome by a very large number of invading metastatic cells or their antigenic diversity[79].

KCs present CEA receptors (CEA-R), which are responsible for binding and subsequent degradation of CEA. When the carcinoembryonic antigen is connected, KCs are

activated and they secrete high amounts of cytokines, including IL-1β, IL-6, IL-10 and TNF-α. These molecules cause alterations in sinusoidal endothelium and activation of SECs, which in turn may express adhesion molecules aiding arrest and extravasation of tumour cells[26,83]. Thus, experimental studies on mice have observed adhesion between Kupffer cells or SECs and colorectal cancer cells, without CEA adhesion; these observations suggest a non adhesive mechanism, which may facilitate hepatic colonisation [84]. Furthermore, Minami et al showed that CEA mediated metastasis indirectly, through cytokine production; the use of cytokine inhibitors prevented tumour cells from adhering to SECs in this study[85].

In general, *in vitro* and *in vivo* studies in animal models observed that KCs protected the liver against a variety of tumour cells, which reached the organ. They also exerted cytotoxic action against colon adenocarcinoma metastasising cells[72,86]. KC activation was mediated by immunomodulators, such as lipopolysaccharides, muramyl peptides, lymphokines and interferon γ[72,87]

CONCLUSIONS

Metastasis formation is a multistage process. In the case of colorectal cancer, malignant cells undergo epithelial mesenchymal transition while they still belong to the primary tumour, develop multiple properties and commence the invasion- metastasis cascade. The initial target is the liver, which constitutes the commonest host organ for colorectal cancer cells.

The entrapment of metastatic cells in the sinusoids and their interactions with local cells are considered particularly important among metastatic stages. All four types of hepatic sinusoidal cells present immune activities, secrete and express numerous biological active molecules and influence substantially tumour cells. Phagocytosis, antigen presentation, foreign immune cell activation, apoptosis, cell adhesion, matrix degradation, fibrinogenesis and angiogenesis are some of the intercellular procedures which are accomplished by sinusoidal cells during colorectal liver metastasis.

However, many aspects of these interrelated molecular pathways need to be better elucidated. Current research has added significant knowledge and ongoing studies attempt to explain them further. Future therapeutic applications may succeed in inhibiting malignant cell arrest and their interactions with hepatic sinusoidal cells, thus cancelling liver invasion in an initial, premature stage.

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