Mechanisms regulating colorectal cancer cell metastasis into liver (Review)

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Abstract. The metastatic spread of tumor cells is one of the most common causes of mortality in cancer patients. The elucidation of the molecular mechanisms that underlie the formation of metastatic colonies has been one of the major objectives of cancer research. Organ-specific colonization of cancer cells is a significant and noteworthy feature of metastasis. Colorectal cancer (CRC) is one of the most common causes of cancer-related mortality. The liver is commonly the sole site of metastasis for CRC and represents a major cause of mortality in CRC patients. However, what regulates CRC cell metastasis into liver and the reasons for the liver-specific metastasis of CRC have yet to be adequately elucidated. Recent progress provides indications and a conceptual framework with which to investigate this issue. This review evaluated experimental and clinical evidence to support a mechanistic role for circulation patterns and microvessels in liver, metastasis-related genes, chemokines and their receptors, and cellular adhesion molecules in the process of CRC liver metastasis.

Contents

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1. Introduction

Despite the significant advances in diagnostic and therapeutic modalities in the treatment of cancer patients, metastasis, the spread of cancer from the primary site of tumor growth to other organs, remains the leading cause of cancer-related morbidity and mortality (1). Metastasis is a multistep process by which cancer cells disseminate from primary tumors and establish secondary lesions in distant organs. The metastatic process may be formalized into a series of discrete stages (2). These stages include escape from the primary tumor, intravasation into the lymphatic or vascular systems, survival in the circulation, avoidance of host defense mechanisms, arrest at a new site, extravasation into the tissue, and growth at the new site (3).

Cancer metastasis involves a series of complex interactions between tumor cells and their microenvironment that affect its biological effectiveness and facilitate tumor cell arrest in distant organs. Clinicians previously observed that certain types of primary tumor are more likely to metastasize to specific organs (4). For instance, colorectal cancer (CRC) metastasis predominantly affects the liver, but not bone or brain (5). Several factors are thought to be involved in the specific site of cancer metastasis. The cellular origin, intrinsic properties of the tumor, tissue affinities and circulation patterns determine the sites of tumor spread (6).

The liver is one of the most significant targets for organ-specific metastasis in various cancer types and is commonly the sole site of metastasis for CRC (7). Liver metastases are a major cause of mortality in patients with CRC. However, the reasons for the regulation of CRC cell metastasis into liver have yet to be elucidated. It is likely that the portal drainage of the gastrointestinal tract is partially responsible for the high rate of liver metastasis in CRC. However, additional molecular variables are undoubtedly critical in determining whether CRC is likely to metastasize into the liver. Recent progress provides indications and a conceptual framework with which to investigate this issue further. This review evaluated experimental and clinical evidence to support a mechanistic role in the process of CRC liver metastasis involving a number of factors, including circulation patterns and microvessels in liver, metastasis-related genes, chemokines and their receptors, and cellular adhesion molecules.

2. Regulation by circulation patterns and microvessels in liver

The liver specificity of the metastatic pattern of CRC is partially attributable to the circulation patterns. Previously, it was postulated that anatomic factors related to direct portal seeding from the colon and rectum to the liver, as well as the unique features of hepatic blood flow contribute to this metastatic pattern (8,9). In CRC, the mesenteric circulation from the bowels and the permissiveness of the liver capillary sinusoids are thought to favor liver metastasis (10,11). Colorectal tumors predominantly spread along the mesenteric circulation to the liver in 80% of patients with recurrent disease (12). Cancer cells dispersed from CRC, for example, draining via the portal vein into the liver likely give rise to liver metastases. Furthermore, Weiss *et al* revealed an association of metastatic incidence and organ blood flow consistent with the mechanical hypothesis of the metastatic pattern (13). However, the two hypotheses are not mutually exclusive. Tumor type-specific distribution of metastatic growth cannot solely be explained by anatomical considerations of blood supply in the majority of cases (14).

Findings of previous reports suggested that the stabilization of tumor cell adhesion to the microvessels of host organs is crucial for further stages of secondary tumor formation (15). The sinusoidal endothelial layer in liver is characterized by an incomplete covering of the microvessel structures (16). Fenestrated endothelial cells (ECs) are grouped in sieve plates and measure approximately 175 nm in diameter, occupying 6-8% of the sinusoidal surface connecting the sinusoidal system with the perisinusoidal space of 'Disse' (17). Due to an incomplete layer of hepatic ECs, these ECM components are directly accessible to circulating cells (16,18). Various studies have shown that interactions between these ECM components within the liver and tumor cells appear to be crucial to the formation of hepatic metastases (19-21). The liver is furthermore unique, since it lacks a basement membrane under the sinusoidal endothelium (22). This unique nature of the hepatic ECM is predicted by its special configuration and apparent continuity with the extraparenchymal areas of the connective tissue (23). In contrast, other epithelial organs have subendothelial basement membranes and a substantial ECM between the endothelial and epithelial cells.

3. Regulation by cellular adhesion molecules

A significant and early step during the formation of distant metastasis is the arrest of circulating tumor cells within the host organ (24). Interactions of both tumor and host tissue cells with the ECM play a pivotal role in tumor progression (25). Various types of cell adhesion molecules appear to be involved in the complex processes of metastatic tumor cell adhesion to the microvasculature, among which are integrins, members of the immunoglobulin superfamily, cadherins (26), and membrane-bound proteoglycans, such as syndecans and glypicans (27). These molecules mediate successful cell arrest, which appears to be dependent on the balance between adhesive and anti-adhesive forces, and the rate at which adhesive interactions are broken (28). Besides quantitative modulation of the expression of a number of cell adhesion molecules, qualitative alterations of their activity and affinity provides oncogenic mechanisms for the acquisition of metastatic phenotypes and prediction of the metastatic pattern (29). In addition to the effect of hematogenous dynamics, CRC cells preferentially adhere to the liver, suggesting the existence of specific molecular interactions that favor the retention of tumor cells in this organ (30).

Integrin-mediated interactions of tumor cells with ECM components appear to be among the most significant determinants for organ-specificity of the CRC metastatic process (31,32). The formation of liver metastasis is correlated with $\alpha_v\beta_3$- and $\alpha_v\beta_5$-integrin expression (33,34), binding to vitronectin and fibronectin, respectively. Kikkawa *et al* revealed that $\alpha_v\beta_3$-integrin-expressing CHO-K1 cells were present in the extravascular region of the liver 24 h after portal vein injection, whereas $\alpha_v\beta_3$-integrin-expressing CHO-K1 cells failed to adhere to sinusoidal endothelial cells, suggesting that the vitronectin receptor may solely promote the transendothelial migration step (33). Enns *et al* demonstrated $\alpha_v\beta_5$-integrin to mediate the adhesion of HT-29 cells to the endothelial lining, but not the subsequent transendothelial migration step into the liver parenchyma (34). By investigating adhesive and invasive interactions of circulating human colon carcinoma cells within the hepatic microvasculature by intravital fluorescence microscopy, investigators showed that their adhesion within the liver sinusoids appears to be mediated by the integrins $\alpha_6\beta_1$ and $\alpha_6\beta_4$ (35). Furthermore, $\alpha_v\beta_5$ and $\alpha_v\beta_6$ appear to be of paramount importance for this metastatic tumor cell adhesion (34). The study of HT-29 colon cancer clones with low or high metastatic potential revealed cell adhesion within the hepatic microcirculation and early tumor cell extravasation into the liver parenchyma to be directly mediated by α_6-, β_1-, and β_4-integrins (35). Additionally, the transendothelial migration of HT-29 cells is also mediated by α_2-integrins (35). Dynamic adhesion studies of colon carcinoma cells to ECM components under laminar flow further indicated the necessity of β1-integrins for firm adhesion (36).

In addition to integrins, sLe^a and sLe^x have also been associated with the organ-specific spreading of CRC. In their study, Okazaki *et al* showed that the metastatic activity of colon cancer is enhanced due to alterations in the expression levels of adhesion molecules (37). Comparison of the wild-type human colon cancer-derived cell line KM12SM with its highly metastatic counterpart, isolated by an *in vivo* selection procedure, revealed differences in the expression levels of various adhesion molecules. Wild-type KM12SM cells predominantly express sLe^a and, to a lesser extent, Lea, Le^x and sLe^x (37). In contrast, highly metastatic cells showed an upregulation of Le^x and sLe^x. Additionally, the percentage of Le^a- and sLe^a-positive cells was markedly higher in the metastatic cell line compared with KM12SM wild-type cells. As mentioned above, increased sLe^a and sLe^x levels have been associated with the progression and organ-specific spreading of CRC (38).

4. Regulation by chemokines and their receptors

Cancer metastasis develops from a non-random process, in which organ selectivity by the tumor cells is mostly delineated by factors that are expressed at the remote organs that eventually become the sites where metastasis occurs. These factors sustain the consecutive steps required for metastasis formation, including tumor cell adhesion to microvessel walls,

extravasation into target tissue and migration. Notably, chemokines and their receptors have been found to play a key role in the regulation of organ selectivity. There is mounting evidence that organ-specific metastasis is governed, in part, by interactions between chemokine receptors expressed in cancer cells and the corresponding chemokines secreted within the target organs (39). Chemokines expressed by specific organs promote tumor cell adhesion to microvessel walls, facilitate extravasation into target tissues and induce tumor cell migration (40).

Previous studies demonstrated a role for the chemokine receptor CXCR4 in the dissemination of CRC to the liver (41). In clinical studies, the overexpression of the chemokine receptor CXCR4 was associated with an impaired prognosis of patients undergoing surgery for CRC liver metastasis (41). In accordance with breast and prostate cancer, and malignant melanoma, CXCR4 is also involved in the organ-specific metastatic spreading of CRC (41-45). Thereby, CXCR4 is clearly associated with CRC-derived liver metastases (41) and an increased risk of tumor recurrence (43), thus being correlated with a poor prognosis (41,43). Low vs. high CXCR4 expression in CRC liver metastases correlated with a significant difference in overall survival (median $CXCR4^{low}$, 27 months vs. median $CXCR4^{high}$, 10 months) (41). The formation of CT-26 colon cancer cell-derived liver metastasis was clearly associated with marked CXCR4 upregulation in liver metastasis cells (45).

The chemokine receptor CCR6 is involved in colorectal liver metastasis. CCR6 is expressed in a subset of T cells and is associated with their migration to the liver; thus questions have arisen as to whether the metastatic spreading of CRC to liver may be regulated by this chemokine receptor (46). It is well known that the liver exhibits peak expression levels of the appropriate ligand CCL20, suggesting that an increased CCL20 expression may contribute to the selective recruitment of CCR6-expressing CRC cells (47). Immunohistology analysis clearly revealed CCR6 expression in primary CRC specimens, whereby CCR6 staining was stronger in tumor cells compared with adjacent colon epithelial cells, indicating that CCR6 expression was related to tumor tissue (46). Moreover, multiple logistic regression analysis, controlling for age, gender, tumor stage, nodal status, pathologic grade, and preoperative carcinoembryonic antigen levels, revealed that CCR6 expression in the primary tumor was independently associated with the presence of liver metastases (46).

Chemokine signaling is a key feature of site-directed metastasis where neoplastic cell-secreted chemokines signal to receptors expressed by a number of myeloid cell subtypes. This feature is crucial with regard to colon carcinoma metastasis to liver (48). Murine and human colon cancer cells are known to secrete the CC-chemokine ligands CCL9 and CCL15. Subsequently, $CD34^{+}Grl^{-}$ immature myeloid cells that express the CCL9/15 receptor CCR1, the activation of which directly induces MMP-2 and MMP-9 expression, are recruited. Lack of the *Ccr1*, *Mmp2* or *Mmp9* genes in myeloid cells suppresses disseminated tumor growth in the liver, resulting in a longer survival of tumor-bearing mice (48).

5. Regulation by metastasis-related genes

Several studies (49-51) have reported that tropism of circulating tumor cells to specific organ locales is regulated by the complexity of genetic alterations intrinsic to neoplastic cells, while also recognizing that an altered expression of significant genes also regulates tropism. With the advent of gene expression microarrays and the establishment of improved study models, several new factors have been added to the list of gene signatures responsible for liver metastases.

KAI1/CD82, a member of the transmembrane 4 superfamily (tetraspanin), has previously been shown to contribute to metastagenicity of malignant melanoma (49,50). A splice variant of KAI1 lacking exon 7 at the C-terminal region interacts with KITENIN, a tetraspanin family member found to be overexpressed in metastatic gastric tumors. Transfection of CT-26 CRC cells with this variant facilitated cytoskeletal reorganization and resulted in early liver metastases (51).

The tyrosine phosphatase PRL-3 is regarded as a significant marker for liver metastases (52). Increased PRL-3 expression was found in metastatic CRC and downregulation of PRL-3 in DLD-1 colon cancer cells prevented hepatic metastases without affecting cell proliferation, while its transfection increased metachronous liver metastases (53,54). SW480 CRC cells overexpressing PRL-1 and PRL-3 presented upregulation of RhoA and RhoC GTPases, and inhibition of Rho kinase activity abrogated their cell motility. In addition, farnesylation of PRL-3 and preservation of its phosphatase activity were essential for the invasion of tumor cells (55). Specific monoclonal antibodies against PRL-3 have been developed for predicting and diagnosing CRC hepatic metastasis (56).

Miyamoto *et al* investigated the role of insulin-like growth factors I and II (IGFI/II) in CRC. Blockade of IGFI/II using neutralising antibodies markedly diminished the formation of hepatic metastases from CRC cells injected intrasplenically (57). The inhibition of VEGF has offered new opportunities in the treatment of advanced/metastatic CRC to the liver. However, additional studies should be performed to optimize the therapeutic targeting of angiogenetic mechanisms for treating liver metastases (58-61). The inhibition of Src tyrosine kinase in combination with gemcitabine suppressed metastasis to the liver and locoregional lymph nodes (62).

6. Conclusions

Understanding the mechanisms by which CRC metastasis develops within the liver is clinically significant. Elucidation of the biology of metastatic phenotypes may provide useful insights into targeted drug development and further patient-specific therapies for CRC patients. Predicting the pattern of recurrence in specific CRC patients exhibiting a high risk of developing liver metastasis may allow for the identification of CRC, as well as of patients who may benefit from liver-directed therapy. Liver-directed adjuvant therapies and organ-specific screening may be considered in those patients identified to be at high risk of developing recurrence specifically within the liver.

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