MINI-REVIEW

Metastasis-associated Factors Facilitating the Progression of Colorectal Cancer

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Abstract

Tumor metastasis remains the principal cause of treatment failure and poor prognosis in patients with colorectal cancer. It is a multistage process which includes proteolysis, motility and migration of cells, proliferation in a new site, and neoangiogenesis. A crucial step in the process of intra- and extra-vasation is the activation of proteolytic enzymes capable of degrading the extracellular matrix (ECM). In this stage, urokinase plasminogen activator receptor (uPAR) and matrix metalloproteinases (MMPs) are necessary. Micrometastases need the presence of growth factor and vascular growth factor so that they can form macrometastasis. In addition, cell adhesion molecules (CAMs) and guanine nucleotide exchange factors (GEFs) play important roles in the progression of colorectal cancer and metastatic migration. Further elucidation of the mechanisms of how these molecules contribute will aid in the identification of diagnostic and prognostic markers as well as therapeutic targets for patients with colorectal metastasis.

Keywords: Adhesion molecules - proteolysis - angiogenesis - growth factor - metastasis - colorectal cancer

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Introduction

Colorectal cancer is one of the most common cancers and has a high mortality rate in the world. The prognosis of metastatic colorectal cancer (mCRC) remains poor regardless of the advances obtained in recent years with new therapeutic agents, surgical procedures, and diagnostic methods(McMillan & McArdle, 2007). Metastasis is an inefficient process governed by several rate-limiting steps, and that failure to negotiate these steps can lead to tumor dormancy. To produce a metastasis, tumor cells must complete a multistep progression through a series of sequential and selective events. In each step, causative molecules have been identified; these include matrix degradation enzymes, cell-adhesion molecules, growth factors and vascular growth factors, and many of these can be regarded as prognostic factors. A better knowledge of the molecular bases will lead to new paradigms and possible improvements in diagnostics and therapeutics with the ultimate goal of preventing metastasis and increasing patient survival. In this review, we will focus on the major molecular factors involved in the development of metastasis in colorectal cancer.

Adhesion molecules

CAMs play an important role in the progression of colorectal cancer and its metastatic migration (Wan et al., 2009; Buda & Pignatelli, 2011). Multiple cytokines, growth factors and cell populations, such as endothelial, epithelial, immune, and blood cells as well as platelets interact, express and secrete CAMs, which are essential for the integrity of epithelial tissues, cell polarity, and cellular differentiation (Bandres et al., 2009; Filiz et al., 2009). Several CAMs are implicated not only in malignant cell detachment from the primary site, but also in tumour cell attachment to distant tissue (Rudmik & Magliocco, 2005; Ogawa et al., 2008).

CD44 is a transmembrane glycoprotein which serves as a recyclable receptor for hyaluronan, an acidic glycosaminoglycan with roles in cell motility and proliferation. CD44 may also act as an anchor for epithelial cells in basement membranes thus maintaining their polar orientation (Amirghofran et al., 2008; Kunimura et al., 2009; Zlobec et al., 2009). CD44 variants, generated by alternative splicing, modulate cell-to-cell interaction, movement, and finally metastatic potential. In CRC, CD44 glycoprotein have been related to metastases and disease recurrence (Yamaguchi et al., 1996; Al-Maghrabi et al., 2012). There appears to be a relationship between the level of CD44 expression and metastasis when using in vivo studies and in vitro immunohistochemistry. Another immunohistochemical evaluation of CD44v6 in colorectal cancer highlighted a significant role of membranous CD44v6 expression in colorectal cancer progression and prognosis (Huh et al., 2009). However, there is quite a bit

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of controversy regarding the real value of CD44 in liver metastases formation because plasma levels have not been linked to advanced stages of the disease (Masson et al., 1999) and immunohistochemical studies have not shown significant difference of CD44v6 expression between CRCs with and without liver metastasis (Gotley et al., 1996; Choi et al., 2000; Bendardaf et al., 2005; Delektorskaia et al., 2005; Bendardaf et al., 2006; Amirghofran et al., 2008; Huh et al., 2009; Kopp et al., 2009).

Carcinoembryonic antigen (CEA) is a membrane glycoprotein normally present on fetal gastrointestinal and liver cells, however, it can become inappropriately expressed in a number of malignancies. CEA is secreted on the cell surface of the majority of metastatic colon cancer (Takeuchi et al., 2003; Hatate et al., 2008). CEA has long been recognized for its involvement in the development of liver metastasis in colorectal cancer (Katoh et al., 2008; Yuan et al., 2008; Mourtzikou et al., 2012). This event is mediated by the induction of altered cell adhesion properties leading to increased retention of metastasized cells in the liver. Several evidences suggested that carcinoembryonic antigen intervenes in colorectal metastatic process (Feng et al., 2009).Overexpression of the glycoprotein leads to inhibition of cell differentiation and disruption of cellular polarisation and tissue structure. It was observed that anoikis, an apoptotic process initiated when there is poor or no cell-ECM contact, is also downregulated by CEA overexpression (Lankiewicz et al., 2008; Emara et al., 2009).

CEA offers a selective advantage to form metastases in the liver, possibly by altering the hepatic micro-environment to favour the chance of tumor arrest and growth in the liver (Thomas, Forse, & Bajenova). Expression of CEA has been clearly correlated to generation of liver metastases in experiments transfecting CEA to CRC cell lines or administering CEA in animal models previous to CRC cell injection (Thomas et al., 1995). A recent study demonstrated that CEA was responsible for modifying the hepatic environment, making it more conducive for colon cancer cell survival (Gangopadhyay, Lazure, & Thomas, 1998). In the liver, kupffer cells express CEA receptor, which bind to CEA and activate a signaling cascade that ends up with releasing IL-1, 6 and TNF-α which, in turn, facilitates CRC cell metastasis and growth (Gangopadhyay, Lazure, & Thomas, 1997). Further investigations revealed that CEA facilitates CRC cell survival via the induction of IL-10 and subsequent decrease of nitric oxide (NO) concentration. IL-10 is probably released by stimulated Kupffer cells and the NO decrease is achieved due to inhibition of inducible nitric oxide synthetase (iNOS). Thus, it appears that CEA is not only a useful diagnostic marker, but also a molecule directly implicated in the molecular reactions of colorectal liver metastasis.

Proteolytic enzymes

The extracellular matrix of proteins forming the connective tissue around metastatic tumour cells is a physical barrier for cells to pass through. After the tumor cells become detached from neighboring cells, they release protease to break down this barrier in order to allow migration and invasion. With respect to normal migratory cells such as fibroblasts and macrophages, proteases are released in a coordinated manner in equilibrium with other factors that rebuild the matrix. The release of proteases for the degradation of extracellular matrix is not controlled in colorectal cancer metastasis. Among proteases, matrix metalloproteases (MMPs), plasminogen activators and Cathepsins are the most relevant.

MMPs are enzymes that degrade the collagen meshwork of the ECM and basement membrane components such as collagen type IV, proteoglycan, elastin, laminin, and fibronectin. In malignant diseases, MMPs are considered to be overexpressed leading to increased proteolytic activity (Kahlert et al., 2008; Yamamoto et al., 2008; de Lima et al., 2009). MMPs have been demonstrated to be expressed on the surface of invasive tumor cells and may be subjected to tumor-/fibroblast-cell interaction (Zuzga et al., 2008). In the majority of colorectal cancers, MMPs are overexpressed and its expression has been correlated with malignancy and hepatic metastasis (Adachi et al., 1999). In vivo studies demonstrate that secreted MMP-9 plays a crucial role in cell motility and metastatic ability of colorectal cancer cells (Koyama et al., 2008).

The urokinase plasminogen activator receptor (uPAR), also known as urokinase receptor, is multidomain glycoprotein tethered to the cell membrane with a glycosylphosphotidylinositol (GPI) anchor. uPAR was originally identified as a saturable binding site for urokinase on the cell surface. Urokinase plasminogen activator (uPA) is important due to its catalytic activity in converting plasminogen to plasmin, a broad-spectrum protease that in turn catalyses the degradation of diverse substrates in the extracellular matrix and activates certain metalloproteases (Kim et al., 2006; Klinge et al., 2007). uPAR is involved in metastasis development in several cancers. It has been shown that an increase of the activator u-PA, its receptor u-PA-R, and Plasminogen activator inhibitor-1(PAI-1) are associated with tumor progression, metastasis and shortened disease-free survival in patients afflicted with malignant tumors of multiple tissues (Zlobec et al., 2008; Van Buren et al., 2009; Lund et al., 2011). Specific inhibition of the u-PA receptor has been successful in reducing the establishment of primary tumors and metastasis after intravenous injection of tumor cells into immunodeficient mice (Ignar et al., 1998). In colorectal cancer, numerous studies have shown overexpression of u-PAR cell in contrast to the corresponding normal tissue and/or surrounding stromal cells (Cantero et al., 1997; Morita et al., 1998), and suggested u-PAR as a characteristic of the invasive or even the malignant phenotype (Sliutz et al., 1996). Colorectal cancer with a high uPAR expression has been correlated with a low 5-year survival (Ganesh et al., 1994). Inhibition of uPAR expression is associated with decreased motility and invasiveness in the human CRC cell line HCT116 (Ahmed et al., 2003a). Antisense uPAR mRNA could inhibited CRC liver metastasis development in a nude mice model (Ahmed et al., 2003b).

Cathepsins are a class of lysosomal peptidases which

Table 1. Genes Implicated in Metastatic Colorectal Cancers and Their Function

Gene	Function in metastasis	Reference
CD44	CD44 variants facilitates motility and metastatic ability	Kunimura et al.(2008)
Carcinoembryonic Antigen (CEA)	Facilitates CRC cell detachment, stasis and growth in distal organ	Pakdel et al. (2011)
Matrix metalloproteinases (MMP)	Degradation of ECM	Kahlert et al.(2008)
Urokinase Plasminogen Activator Receptor (uPAR)	Degradation of ECM and activates pro-MMPs	Illemann et al.(2009)
Epidermal Growth Factor Receptor(EGFR)	Promotion of cell proliferation and stimulate MMPs	Yarom et al.(2009)
Vascular endothelial growth factor (VEGF)	Induction of angiogenic activity	Bertin et al.(2011)
Platelet-derived endothelial cell growth factor(PDECGF)	Induction of angiogenic activity	Ionescu et al.(2011)
Guanine nucleotide exchange factors (GEFs)	Promotion of migration and angiogenesis	Buongiorno et al.(2008)
Phosphatase of regenerating liver-3(PRL3)	Promotion of migration and metastatic potential	Peng et al.(2009)

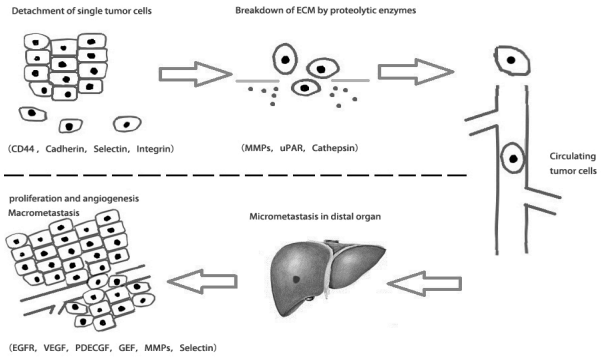

Figure 1. Multi-step Process Involved in Colorectal Cancer Metastasis Formation

belong to the cysteine, serine, or aspartic protease classes. Several investigations have confirmed a significantly higher mRNA content, antigen level, enzymatic activity, and immunohistochemical protein expression of cathepsins in colorectal carcinoma (CRC) tissues compared to matched controls of unchanged colorectal mucosa (Kuester et al., 2008; Szajda et al., 2008; Yu et al., 2005). These observation clearly indicated that these cathepsins might be involved in CRC development and growth. Staining intensity, as well as antigen and activity levels for cathepsins increased with the transition from adenoma to adenocarcinoma, suggesting that elevated cathepsin levels may be a sensitive marker of progression from premalignant colorectal adenoma to invasive CRC (Kaneko et al., 2007).

Growth factors and their receptors

Malignant tissue express a variety of growth factors and their receptors (Portera et al., 1998; Inaba et al., 2001; Yasui et al., 2001), including epidermal growth factor (EGF), transforming growth factor (TGF)-alpha and beta, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) and sex hormones. These factors induce not only cell growth but also extracellular matrix degradation for tumor invasion and proliferation (Miyazono et al., 2012). For CRC, the most common metastatic site is liver. Once established in the liver tissue microenvironment, micrometastases need growth factor stimuli for growth. Degradation of ECM results in an increased availability of growth factors. The resulting balance will then determine micrometastasic growth.

The epidermal growth factor receptor (EGFR) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracelluar protein ligands. EGF and TGF-alpha act as autocrine growth factors and then induce the expression of mRNAs for multi-growth factors and their receptors (EGF, TGF-alpha, EGFR, ERBB2, PDGF). Moreover, they stimulate the expression of metalloproteinase genes, suggesting that EGF and TGF-alpha successively evoke cascades which are convenient for tumor progression, invasion and metastasis (Tampellini et al., 2007). In addition, EGFR regulates cancer cell proliferation, apoptosis and tumor-induced neoangiogenesis, and has been implicated in several human cancers, including metastatic colorectal cancer (mCRC). EGFR has been reported to be highly expressed and amplificated in 72% to 82% of metastatic CRC tissue samples (Delektorskaia et al., 2003). Some studies have reported that expression of EGF receptors in CRC is correlated with aggressiveness and metastatic ability.

Monoclonal antibodies (MoAbs) designed to bind to the ectodomain of the EGFR have shown activity in chemorefractory mCRC and in the first-line setting. Cetuximab and panitumumab are MoAbs that bind to the EGFR and thereby inhibit cell proliferation, metastasis, and angiogenesis. Although EGFR is expressed in approximately 72% to 82% of patients with mCRC, the clinical efficacy of treatment with anti-EGFR antibodies is limited to a subset of patients (Delektorskaia et al., 2003). Recent clinical data confirm that the efficacy of cetuximab and panitumumab is confined to patients bearing tumors with wild-type K-ras (Weber & McCormack, 2008; Krupitskaya & Wakelee, 2009). Therefore, K-ras mutation analysis now is considered a new standard of care in the selection of patients for EGFR-targeted therapy.

Angiogenesis

A tumor can grow up to a mass of 10^6 cells and approximately 1-2 mm in diameter without blood supply. However, successful metastatic tumour growth depends upon appropriate tumour-host microenvironment interactions and, ultimately, the development of vascularised metastases extravasation in the target organ. Angiogenesis is a prerequisite for tumor growth and metastasis which depends on the production of angiogenic factors by host and tumor cells. Neovascularization enhances the growth of primary tumors and provides an avenue for hematogenous metastasis. Different angiogenic factors have been related to metastasis formation because they can promote primary tumor growth and increase tumor cell chances to contact blood and thus disseminate. However, it is likely that angiogenesis plays a major role in metastasis generation regulating micrometastases

outgrowth. Gastrointestinal cancer cells produce various angiogenic factors, including vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PDECGF). Because increasing vascularity correlates with metastasis and poor prognosis, they may be candidate prognostic factors of colorectal cancer.

VEGF is a direct-acting endothelial cell mitogen capable of inducing cell migration, proliferation, invasion, and increased vascular permeability. VEGF is secreted by the majority of solid malignant tumors and has been demonstrated to be a negative predictor for patients' prognoses. Five VEGF isoforms have been described, which are generated by different splicing of the VEGF gene. The N-terminal domain, which mediates binding to the VEGF receptor, is identical in all isoforms; therefore all isoforms are able to induce angiogenesis (Gisterek & Kornafel, 2006).

In colorectal cancer, many studies have identified VEGF to be important in the formation of liver metastasis (Kang et al., 1997). Increased blood supply and tumor vessel formation, as estimators of angiogenic activity, have been found to be higher in liver metastases of primary CRC. VEGF expression level has been defined using reverse transcription polymerase chain reaction (RT-PCR) analysis in two groups of primary CRC. Patients developing liver metastases showed aberrant expression of VEGF with a significantly higher incidence (12 of 16, 75%) than those without liver metastasis (20 of 45, 44%) (P = 0.036) (Tokunaga et al., 1998). VEGF expression in primary CRC seems clearly associated with increased chances of dissemination. Moreover, increased VEGF in the primary tumor has been associated with a poor prognosis as positive tumor expression has been correlated with a twofold increase risk of death and an increased risk of tumor recurrence (Ishigami et al., 1998; Vermeulen et al., 1999).

VEGF inhibitors have shown very promising results in animal studies and currently in clinical trials to evaluate their effectiveness against human cancers. A major problem with current cytotoxic chemotherapy is drug resistance, because malignant cells are genetically unstable and heterogeneous. By contrast, the target of anti-angiogenic drugs consists of a stable population of endothelial cells. In long-term preclinical studies, drug resistance to angio-suppressive molecules is not observed and affects tissue with active vascular growth only. While new blood vessels are constantly being formed during embryo genesis, normal tissue repair and wound healing, few vessels are being formed in adults. Thus a specific inhibitor of angiogenesis may have fewer side effects. Bevacizumab is the first humanized murine monoclonal antibody (mAb) against targeted VEGF. Bevacizumab recognizes and neutralizes all major isoforms of human VEGF. The vascular changes consisted of a reduction in the amount of endothelial cells and microcapillary counts within the tumor tissue, as well as reduced vascular permeability and associated decrease in interstitial pressure within the tumor (Assenat & Ychou, 2009; Iwasaki & Nihira, 2009). Treatment with bevacizumab is generally well tolerated, with hypertension and arterial thromboembolic events being the main side effects, which is directly related to receptors blockade (Tamiya et al., 2009).

PDECGF or thymidine phosphorylase (dThdPase) is another distinctive molecule related to angiogenesis and metastatic progression. It is an endothelial cell mitogen initially purified to homogeneity from human platelets. PDECGF has chemotactic activity for endothelial cells in vitro and has angiogenic activity in vivo. It has also been demonstrated to be identical to thymidine phosphorylase, an enzyme involved in pyrimidine nucleoside metabolism. PDECGF expression is elevated in several types of solid tumors, including colorectal cancer. PDECGF is involved in colorectal cancer angiogenesis (Beckner, 1999; Takahashi et al., 1996). Study demonstrated a high level of PDECGF in human colon cancer. The intensity for staining of PDECGF in infiltrating cells correlated with vessel counts, suggesting that infiltrating cells may contribute to angiogenesis in human colon cancer and may provide a redundant mechanism for tumor neovascularization.

Miscellaneous

Guanine nucleotide exchange factors (GEFs) are components of intracellular signaling network and function as activators of small GTPases. They activate G proteins by promoting the dissociation of GDP and binding of guanosine triphosphate (GTP). Tiam1 (T-cell lymphoma invasion and metastasis-inducing protein 1), one of GEFs that activates Rac, is a colorectal cancer metastasis-related gene which regulates numerous biologic properties including migration and invasion. Tiam1 was originally identified as the invasion- and metastasis-inducing gene by proviral tagging in combination with in vitro selection for invasiveness in T lymphoma cells. The role of Tiam1 in cellular migration, invasion and metastasis was implicated in a variety of cancers such as breast cancer, lung cancer and colorectal cancer (Adam et al., 2001; Liu et al., 2006; Liu et al., 2007; Buongiorno et al., 2008; Rudin et al., 2008). In colorectal carcinomas, overexpression of Tiam1 is associated with more malignant potential and metastatic ability. Malliri et al. (2006) identified that Tiam1 is a Wnt-responsive gene which is upregulated in human colon adenomas and implicated in intestinal tumorigenesis. Overexpression of Tiam1 in SW480 colon cancer cells induced a metastatic phenotype, accounting for more aggressive behaviour of these cells. Tiam1 contributes directly to the metastasis of colon cancer cells, and that ectopic overexpression of Tiam1 is sufficient to increase cellular migration and metastatic potential of these cells. Silencing of Tiam1 resulted in the effective inhibition of in vitro cell growth and of the invasive ability of colorectal cancer cells (Liu et al., 2006). The fact that targeting Tiam1 in vivo reduced the metastatic potential of colorectal cancer makes Tiam1 a promising target for therapeutic intervention. A recent study investigated the relationship between Tiam1 and lymphangiogenesis in human colorectal carcinoma (CRC) tissues after knockdown of the Tiam1 gene with RNA interference (RNAi) (Zhong et al., 2009). The results showed that the lymph microvessel density (LMVD) in the Tiam1 positive group was significantly higher than that in the Tiam1

negative group. This study suggests that expression of Tiam1 correlates with lymphangiogenesis in CRC and that Tiam1 gene may act as a crucial therapeutic target for lymphangiogenesis in CRC.

PRL-3 (phosphatase of regenerating liver-3/PTP4A3) is a 20 kDa prenylated protein tyrosine phosphatase which belongs to the PRL subfamily. Over expression of this gene in metastatic colon cancer was first demonstrated by comparing the global gene expression profile using a technique of serial analysis of gene expression (SAGE). By preparing libraries from microdissected liver metastases and comparing them to normal hepatic or inflammatory cell libraries, Saha et al.(2001) found that the PRL-3 gene was up-regulated in all of the metastases examined, with very little or no expression in normal colorectal epithelium and intermediate levels of expression in primary tumours. Bardelli et al. (2003) reported that PRL-3 was expressed in metastatic colorectal cancer but was not expressed in the metastases of other types of cancers to the same organs or in nonmetastatic colorectal cancers. PRL-3 was also expressed in the tumor vasculature, regardless of the tumor source. The elevated expression of PRL-3 in metastatic colorectal carcinoma indicated the important role of this subfamily of enzymes in neoplastic cell transformation. ICH studies revealed that expression of PRL-3 was undetectable in the normal colon and colonic adenoma tissues and weak in the primary colonic adenocarcinomas. In contrast, PRLs were expressed strongly in all lymph node metastases (Wang et al., 2007). A recent study suggested that PRL-3's roles in motility, invasion, and metastasis in colon cancer are critically controlled by the integrin β1-ERK1/2-MMP2 signaling (Marti et al., 2009). Another study indicated that PRL-3 may play an important role for the promotion of CRC cell migration and metastatic potential through direct KRT8 dephosphorylation (Kato et al., 2004). However, identification of the detailed mechanism in the PRL-3 signaling pathway awaits further study.

Conclusion

Whilst much work has been done in the exploration for biological basis in primary colorectal cancer, our understanding of the biology of metastasis remains far from complete. There is increasing evidence that liver metastasis from colorectal cancer, which represents disease progression, also represents disease evolution from the primary tumor. Genes codifying for proteins directly or indirectly involved in adhesion, invasion, angiogenesis, survival and cell growth have already been linked to mechanisms of metastases in colorectal cancer. Because metastasis is responsible for a large amount of cancer deaths, therapeutic strategies to prevent or arrest the development of metastases have the potential to significantly impact patient survival. Development of such strategies requires an understanding of the biology and molecular events responsible for this process. Conventionally, the prognosis of neoplastic disease and its treatment are based mainly on exact clinical and histopathological staging. This prognosis could, however, be improved by measuring the molecular markers which play key roles in tumor progression and metastasis. Because receptors and cytokine ligands that mediate metastasis formation are sparsely expressed in the adult healthy organism and are more readily reached by pharmaceuticals than intracellular drug targets they may represent a particularly suitable focus for therapeutic intervention. The inclusion of anti-metastatic agents in new generation of chemotherapy is expected to improve treatment efficacy.

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