Biology of colorectal liver metastases

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Metastases formation is the principle cause of cancer treatment failure and is responsible for nearly all deaths from colorectal cancer. By the time primary colorectal cancers are discovered, subclinical or clinically relevant liver metastases have already occurred, and the presence of multiple metastases makes eradication by currently available therapeutic strategies extremely difficult. The formation of liver metastases represents a highly selective process in which a subpopulation of cells within a tumor express genes that allow them to progress through distinct steps and spread to distant organs. Alteration of gene expression in these cells leads to transformation, growth, angiogenesis, invasion, dissemination, survival in the circulation, and attachment in the organ of metastases. Once the tumor cell has attached in the liver, it must respond appropriately to the new microenvironment, which includes being able to use growth factors and blood vessels from the liver for the benefit of the tumor mass. This then enables the tumor cells to undergo further invasion, angiogenesis, and subsequent growth.

Current therapies directed at metastatic disease of the liver have had minimal impact on outcome. Modifications of current treatment regimens are unlikely to significantly alter the natural history of liver metastases. Therefore, understanding the molecular and biologic mechanisms of liver metastases allow for the development of rational therapeutic strategies.
aimed at preventing and treating liver metastases. This article focuses on the molecular and biologic mechanisms that occur during the development of liver metastases. In addition, therapeutic strategies aimed at liver metastases, angiogenesis, and apoptosis are reviewed.

The biology of liver metastases formation

The formation of metastases is an inefficient process. Primary tumor masses shed large numbers of cells into the vasculature; however, only 0.01% of these cells form tumor foci [1]. This inefficiency is because metastasis is a selective, nonrandom process consisting of a series of interrelated steps (Fig. 1). Each step is important, because failure of any one step will disrupt the metastatic cascade.

Fig. 1. The metastatic cascade in the pathogenesis of cancer metastasis. To produce metastases, tumor cells must detach from the primary tumor, invade the extracellular matrix and enter the circulation, survive in the circulation to arrest in the capillary bed, adhere to subendothelial basement membrane, gain entry into the organ parenchyma, respond to paracrine growth factors, proliferate and induce angiogenesis, and evade host defenses. The pathogenesis of metastasis is therefore complex and consists of multiple sequential, selective, and interdependent steps whose outcome depends on the interaction of tumor cells with homeostatic factors. (Modified from Stetler-Stevenson WG, Kleiner DE. Molecular biology of cancer: invasion and metastasis. In: De Vita JR, Hellman S, Rosenberg SA, editors. Cancer principles and practice of oncology. Philadelphia: Lippincott Williams & Wilkins; 2001. 123–36.)
Adhesion molecules play a vital role at various stages of tumor progression and metastases formation. First, after the initial transformation and growth, subpopulations of cells develop genetic alterations leading to varying degrees of a metastatic phenotype. These cells down-regulate cell-cell adhesion and cell-extracellular matrix (ECM) adhesion leading to increased cell motility and the ability for these cells to separate from the primary tumor. A variety of cell surface receptors that mediate these interactions have been characterized, including integrins, cadherins, selectins, and immunoglobulin-like proteins.

The integrin family of adhesion molecules acts as cell surface receptors to bind to the laminin, fibronectin, and collagen components of the ECM, and many integrins are expressed on the surface of human colon carcinoma cells [2,3]. For example, colon carcinoma expresses the α6β4 integrin, an adhesion molecule implicated in tumor cell motility and progression [2]. Another integrin, αvβ3, can directly bind matrix metalloproteinase 2 (MMP-2). Expression of αvβ3 helps invading cells to localize a proteolytically active form of MMP-2 on the surface of the cells, which in turn enables them to degrade and remodel the ECM during invasion [4]. Once tumor cells have adhered to the ECM and basement membrane (BM), and have produced degradable substances, the tumor cells must then detach to migrate into the stroma. In this setting, a decrease in integrin expression to promote detachment from the primary tumor correlates with tumor progression [3].

Similarly, the cadherins are a superfamily of single-pass transmembrane glycoproteins involved in cell-cell adhesions. The classic cadherin, E-cadherin, binds to intracellular actin cytoskeleton by cytoplasmic proteins termed catenins. Decreased expression of the E-cadherin/β-catenin/α-catenin cell-cell adhesion complex facilitates detachment of tumor cells from primary lesions and is associated with a higher metastatic potential, poor differentiation, and worse prognosis in colon cancer [5].

Cell-cell adhesive interactions can actually facilitate metastases formation during tumor cell arrest in the distant organ. Embolized tumor cells often circulate as clumps of cells adherent to other tumor cells or platelets. This ability to clump during tumor cell embolization may increase trapping of tumor cells in the microcirculation of distant organs, such as the liver. Molecules that mediate these interactions include the immunoglobulin superfamily and selectins. Molecules in the immunoglobulin superfamily, such as the vascular cell adhesion molecule and nerve cell adhesion molecule, mediate signal transduction, cellular immunity, and cell adhesion [6]. Carcinoembryonic antigen (CEA), another member of the immunoglobulin superfamily, may also facilitate adhesion of colon cancer cells to other tumor or host cells [7]. Jessup et al [8] have shown that CEA injected intravenously into athymic nude mice increases the ability of weakly
metastatic human colorectal carcinoma to form liver metastases. Once the metastatic cell has arrested, it must attach to hepatic or other distant organ endothelial cells or ECM components via expression of specific adhesion molecules. For colon cancer, these specific adhesion molecules include CD44 [9], the ganglioside GM2 [10], and the sialyl-LeX carbohydrate antigen [11].

**Invasion and metastases**

A tumor cell must be able to invade and traverse the BM and the ECM to metastasize. Numerous mechanisms of tumor cell invasion of the BM have been described [12]. These include mechanical pressure forcing tumor cells along paths of least resistance, increased cell motility [12], and degradation of the BM and ECM. This latter theory has been the focus of intense preclinical and clinical research in preventing the formation of liver metastases. Colon cancer cells produce a variety of proteases. The family of proteases that has received the most attention has been the matrix metalloproteinases (MMPs). MMPs play a vital role in normal physiology because the ECM and BM are a dynamic matrix of structural proteins, growth factors, and enzymes, which is constantly being remodeled. MMPs can directly cleave and activate growth factors, and regulate apoptosis, cell migration, and cell-cell communication [13]. There are at least 20 MMPs grouped into four subfamilies: the collagenases, the gelatinases, the stromelysins, and the metalloelastases (Table 1) [14]. There are also four naturally occurring MMP inhibitors, known as tissue inhibitors of metalloproteinases (TIMPs) [15]. Colon cancers produce increased levels of matrilysin and stromelysin-1, -2, and -3, compared with normal colon mucosa [14]. The expression of these proteases correlates with an invasive phenotype and tumor progression [14].

Other MMPs are important in colon cancer liver metastases formation. Masaki et al [16] have shown that increased expression of MMP-7 is associated with colon cancer liver metastases. Ogata et al [17] have also demonstrated that an imbalance of MMP-9 and TIMP-1 is associated with liver metastases from colon cancer. Increased expression of Mr 72,000 type IV collagenase has been observed in colon carcinoma cells when compared with normal cells [18].

More recently, scientists have focused on the urokinase-type plasminogen activator receptor (uPAR), which binds urokinase-type plasminogen activator (uPA) and facilitates a proteolytic cascade focused at the cell surface. uPAR has been recognized as a multifunctional protein that interacts with integrins and initiates signaling events that alter cell adhesion, migration, tumor proliferation, and angiogenesis. uPAR has also been implicated in formation of liver metastases from colon cancer [19]. The uPAR molecules are expressed at the invasive front of colon adenocarcinomas; high levels of uPAR protein expression correlate with reduced
survival (<5 years) and liver metastases formation in colon cancer patients [19]. Finally, transfection of colon cancer cells with antisense uPAR cDNA decreases their ability to form metastases [19]. MMP and uPAR family of proteases clearly are intimately involved with tumor cells’ ability to form metastases. Drugs targeting these systems are currently being investigated in clinical trials.

### Migration and motility

Cell migration is required for tumor cells to gain access to the microcirculation. Cell migration requires interaction of the tumor cells’ cytoskeleton, forming membrane ruffles, filopodia and pseudopodia, and the ECM. This complex interaction is stimulated by cytokines such as

| Table 1 | Effect of mammalian MMPs on nonmatrix substrates |
|-------------------|-------------------------------|-----------------|
| MMP               | Nonmatrix substrate           | Effect          |
| **Metalloelastases** |                              |                 |
| MMP-7             | β4 integrin                   | Release β4 integrin |
|                   | E-cadherin                    | Increases bioactivity |
|                   | Plasminogen                   | Angiostatic      |
|                   | TNF-α                         | Bioavailability  |
| MMP-26            | α1-proteinase inhibitor       | Bioavailability  |
|                   | MMP-9                         | Pro-MMP-9        |
| **Collagenases**  |                              |                 |
| Collagenase-1 (MMP-1) | Perlecan                  | Bioavailable FGF |
|                   | α1-antichymotrypsin           | Inactive serpin  |
| Collagenase-2 (MMP-8) | Pro-MMP-8                | MMP-8            |
| Collagenase-3 (MMP-13) | Pro-MMP-9, -13          | MMP-9, -13       |
| **Stromelysins**  |                              |                 |
| Stromelysin-1 (MMP-3) | Perlecan                  | Bioavailable FGF |
|                   | Decorin                       | Bioavailable TGF-β |
|                   | Pro-IL 1-B                    | IL 1-β           |
|                   | Plasminogen                   | Angiostatin      |
|                   | E-cadherin                    | Increases bioactivity |
| Stromelysin-2 (MMP-10) | Pro-MMP-1, -8 -10         | MMP-1, -8 -10    |
| Stromelysin-3 (MMP-11) | IGFBP-1                  | Bioavailable IGF |
| **Gelatinases**   |                              |                 |
| Gelatinase A (MMP-2) | Decorin                   | Bioavailable TGF-β |
|                   | Pro-TGF-β2                    | TGF-β2           |
|                   | Pro-IL1-B                     | IL-1β            |
|                   | Pro-TNF-α                     | TNF-α            |
| Gelatinase B (MMP-9) | Pro-TNF-α                     | TNF-α            |
|                   | Plasminogen                   | Angiostatin      |

**Abbreviations**: FGF, fibroblast growth factor; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; MMP, matrix metalloprotease; TGF, transforming growth factor; TNF, tumor necrosis factor.

hepatocyte growth factor (HGF, or scatter factor) and transforming growth factor β (TGF-β).

HGF is a naturally occurring peptide produced by mesodermal cells. The receptor for HGF, c-met, is located in the cellular membrane and plays an important role in the progression of human colorectal carcinoma. The addition of exogenous HGF to primary colon carcinoma cell lines leads to c-met activation and induces human colorectal carcinoma cell motility [20]. Levels of c-met mRNA have been shown to be higher in hepatic metastases than in primary colorectal tumors [21]. Only about 50% of primary colorectal carcinomas overexpress c-met [22]. Approximately 70% of liver metastases overexpress c-met compared with the primary colorectal cancer from the same patient. These studies suggest that overexpression of the c-met protein and HGF may be important in promoting the formation of colon cancer liver metastases.

TGF-β has also been implicated in the enhancement of colon cancer cell migration and motility. TGF-β is a potent negative regulator of epithelial cell growth and can cause complete inhibition of the growth of non-transformed colon cells. In contrast, colon cancer is commonly associated with the acquisition of resistance to TGF-β [23]. Markowitz et al [23] has shown that malignant progression of colon adenoma cell lines correlates with acquisition of TGF-β resistance and that acquisition of TGF-β receptor mutation is correlated with the adenoma to carcinoma transition [24].

Growth factors and growth factor receptors

Once a tumor cell has implanted in the liver, it must be able to interact appropriately with the new microenvironment. Proliferation, which is a necessary step in the development of clinically relevant metastases, may occur secondary to constitutively activated oncogenes or other environmental stimuli, such as growth factors. Growth factors act by binding to specific tyrosine kinase receptors. It is the activation of these receptors that leads to the transcription of genes that regulate metastatic tumor growth. Growth factor ligands implicated in the growth of colorectal liver metastases include epidermal growth factor (EGF), insulin-like growth factor (IGF), and HGF (previously discussed).

The EGF axis is composed of the EGF receptor (EGFR) and its ligands, EGF and transforming growth factor α. These ligands are involved with the growth, proliferation, and metastases of colon cancer cells [23]. Levels of expression of EGFR in primary cultures of established human colorectal carcinoma cell lines are significantly higher in cell lines established from patients with Duke’s stage D lesions than in cell lines established from patients with Duke’s stage B lesions. In vivo studies, increased levels of EGFR correlated directly with the ability to produce hepatic metastases [25].

Parker et al [26] have shown that increased EGFR gene expression and functional protein levels correlate with metastatic potential of human colon
carcinoma cell in nude mice. In this experiment, using monoclonal antibodies specific for the activated EGFR, increased immunoreactivity was observed in metastatic lesions growing in the liver when compared with primary tumors growing orthotopically in the cecum or ectopically in the subcutis of nude mice. These results suggest that colorectal liver metastases growth depend on response to organ-derived growth factors and activation of cell-surface tyrosine kinase receptors.

Another growth factor implicated in the progression and metastases of colorectal carcinoma is IGF. The IGF system is composed of three ligands (IGF-I, IGF-II, and insulin), three receptors (IGF-IR, IGF-IIR, and insulin receptor), and at least six structurally and functionally distinct IGF binding proteins [27]. IGFs circulate in the plasma complexed to a family of binding proteins (BP). The functions of IGF-BPs appear to be diverse but remain to be clarified. IGF-BP3, the most common circulating IGF-BP, modulates the activity of IGF-I by binding to it and transporting it from the circulatory system to the cell surface receptor. As long as IGF-I is bound to IGF-BP3, it is inactive, but after dissociation from IGF-BP3, IGF-I can bind to and activate IGF-IR.

Increased expression of IGF-I and its receptor have been demonstrated in colon cancers [28]. IGF-I functions as a survival factor for human colon carcinoma cells. Studies have also demonstrated IGF-I and insulin protect colon carcinoma cells from IFN-γ/TNF-α–induced apoptosis [29]. Therefore, blocking IGF-IR signaling may sensitize colon cancer cells to DNA-damaging agents. IGF-I and insulin have also been implicated in liver metastases formation [30]. Koenuma et al [30] have shown that IGF-I and insulin stimulated growth of highly metastatic colon cancer cell lines, compared with low-metastatic cell lines, suggesting a role for IGF-1 in liver metastases formation. These pathways are all inherently involved in metastases formation. No pharmacologic strategies are available to inhibit IGF-IR function; however, Reinmuth et al [31] have demonstrated that dominant-negative transfection of the IGF-IR led to decreased tumor growth, angiogenesis, increased apoptosis, and decreased tumorigenicity and growth in the liver.

Liver metastases and tumor angiogenesis

Once a tumor establishes an invasive phenotype in the organ of metastasis, it must establish its own neovascular blood supply to grow. Increased vascularity may allow not only an increase in tumor growth but also a greater chance of hematogenous tumor embolization. Early work in this field was based on a simple model in which a tumor cell would release a soluble factor that would bind to an endothelial cell and induce endothelial cell proliferation. Current models suggest that angiogenesis is dependent on the balance among stimulatory and inhibitory molecules (Table 2).
Numerous angiogenic growth factors contributing to metastases formation have been identified in colon cancer.

**Vascular endothelial growth factor**

Of all angiogenic factors identified, vascular endothelial growth factor (VEGF) is the best-characterized angiogenic effector molecule. VEGF is known to be upregulated in colorectal cancer, and the relationship of VEGF level with the metastatic potential of primary colon cancers and with overall patient survival has been extensively studied. Takahashi et al [32] used immunohistochemical techniques to analyze 52 human colon carcinoma specimens and 10 adenoma specimens for factor VIII (vessel counts), VEGF, and basic fibroblast growth factor levels. VEGF and VEGF receptors were present in greater amounts in metastatic versus nonmetastatic neoplasms and correlated directly with the extent of neovascularization and the degree of proliferation. Vessel counts were also higher in metastatic tumors than in nonmetastatic tumors (Fig. 2).

In a separate report, VEGF and vessel counts were assessed for their ability to serve as prognostic markers in patients with node-negative colon cancer [33]. Patients in this study were not given adjuvant chemotherapy and were observed for a minimum of 5 years. Relatively low vessel counts were associated with favorable prognosis and high vessel counts were associated with disease recurrence. Patients whose tumors had low VEGF expression had significantly better survival rates than did patients with high VEGF expression. These studies support the importance of vessel count and VEGF expression in colorectal metastases. Others studies also support these findings [34].

**Thrombospondin**

Thrombospondin (TSP) is a high molecular weight, multifunctional glycoprotein first described as a product of platelets released in response to thrombin activation. Of the five subtypes of TSP (TSP-1 to TSP-5), TSP-1

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Table 2

<table>
<thead>
<tr>
<th>Stimulatory</th>
<th>Inhibitory</th>
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<tbody>
<tr>
<td>Acidic and basic FGF</td>
<td>Angiostatin</td>
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<tr>
<td>Angiogenin</td>
<td>Endostatin</td>
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<tr>
<td>Hepatocyte growth factor</td>
<td>Vasculostatin</td>
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<tr>
<td>IL-8</td>
<td>Interferon α, β, γ</td>
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<tr>
<td>Placenta growth factor</td>
<td>IP-10</td>
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<tr>
<td>Platelet-derived endothelial growth factor</td>
<td>Maspin</td>
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<tr>
<td>TGF-α, TGF-β</td>
<td>Platelet factor 4</td>
</tr>
<tr>
<td>Tumor necrosis factor α</td>
<td>Prolactin fragment</td>
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<tr>
<td>Vascular endothelial growth factor</td>
<td>Thrombospondin</td>
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**Abbreviations**: FGF, fibroblast growth factor; IL, interleukin; IP, inducible protein; TGF, transforming growth factor.

and TSP-2 have been implicated in the inhibition of angiogenesis and liver metastases. Tokunaga et al [35] used reverse transcriptase polymerase chain reaction and immunohistochemical techniques to investigate the significance of TSP in colon cancer. Among 61 colon cancer specimens, 38 were positive for TSP-2 expression; the incidence of hepatic metastases in these patients was much lower than that in patients whose tumors did not express TSP-2. This suggests that primary tumors expressing TSP may have a better prognosis and that a balance between pro- and antiangiogenic factors may determine whether primary tumors are able to form liver metastases.

**Angiopoietins**

Angiopoietins constitute a novel family of angiogenic mediators that are ligands for the endothelium-specific tyrosine kinase receptor, Tie-2 [36]. Of the four currently known angiopoietins (Ang-1 to Ang-4), the best characterized are Ang-1 and Ang-2. Ang-1 helps to maintain and stabilize mature vessels by promoting interaction between endothelial cells and surrounding support cells [36]. Ang-2 is expressed at sites of vascular remodeling and is believed to antagonize the stabilizing action of Ang-1. In the presence of VEGF, vessel destabilization by Ang-2 has been hypothesized to induce an angiogenic response; however, in the absence of VEGF, Ang-2 leads to vessel regression.

Angiopoietins have been implicated in facilitating hepatic metastases formation. In contrast to the prevailing view that most tumors and metastases begin as avascular masses, Holash et al [37] have done studies to suggest that metastases initially grow by co-opting existing blood vessels. This co-opted host vasculature does not immediately undergo angiogenesis
to support the tumor but instead regresses, leading to a secondarily avascular tumor and massive tumor cell loss. Ultimately, the remaining tumor is rescued by robust angiogenesis at the tumor margin secondary to increased VEGF expression caused by hypoxia. The secretion patterns of Ang-1 and Ang-2 help mediate this balance between vascular regression and growth.

The authors’ lab has also demonstrated the expression of Ang-1 and Ang-2 by colorectal hepatic metastases [36]. Immunofluorescent staining of colorectal hepatic metastases revealed the presence of Ang-2 protein in cancer epithelium, but Ang-1 was not expressed in tumors. This suggests that the net gain of Ang-2 activity is possibly an initiating factor for tumor angiogenesis and may aid in hepatic metastases formation.

Liver metastases and apoptosis

Another mechanism by which colorectal liver metastases survive in their new microenvironment is by avoiding the process of apoptosis. Apoptosis was first described as a physiologic process of cellular suicide or “programmed cell death.” Apoptosis is critical for normal homeostasis and the apoptotic machinery is present in almost all eukaryotic cells. The activation of death-inducing factors or the withdrawal of survival factors can trigger the apoptotic process (Fig. 3). A complex balance of pro- and

Fig. 3. Common triggers of apoptosis. Numerous extrinsic and intrinsic triggers of apoptosis result in activation of common intracellular pathways that lead to a caspase cascade, which then leads to DNA fragmentation and cellular disassembly. (Modified from Berman RS, Portera CA, Ellis LM. Biology of liver metastases. In: Talamonti M, editor. Liver-directed therapy for primary and metastatic liver tumors. 2001. p. 1–29.)
antiapoptotic factors regulates this process. Colorectal liver metastases cells can exploit these factors to bypass the normal pathways that would trigger defective cells to undergo apoptosis. The up-regulation of antiapoptotic genes and the down-regulation of proapoptotic genes are mechanisms to avoid apoptosis. Alterations in the Bcl-2 family of proteins, a major apoptosis-regulatory protein family, often occur in cancers.

During the adenoma to carcinoma transition, decreased levels of apoptosis have been associated with expression of mutant p53, demonstrating a lack of function of this protein believed to be involved in the regulation of apoptosis and cell cycle arrest. Numerous groups have studied the expression of Bcl-2 family members during colon tumorigenesis. Bcl-XL overexpression has been observed in colon adenocarcinoma and in some adenomas, suggesting that up-regulation of Bcl-XL is a relatively early event in colon cancer progression. Bcl-2 expression, however, declines during progression [38]. Decreased expression of the proapoptotic gene Bak also may be associated with tumor progression.

Once colon cancer cells gain access to the circulation, they must be able to survive until they arrest in the liver or other distant organs. This requires the establishment of extracellular matrix-independent survival and resistance to anoikis. Anoikis, or detachment-induced apoptosis, is a normal physiologic mechanism discovered in epithelial cells that were experimentally dissociated from their ECM [39]. This physiologic process prevents normal cells from colonizing at ectopic sites. Resistance to anoikis is required for metastases formation. Berman et al [40] have evaluated rates of anoikis in paired colon cancer cell lines SW620 and SW480. The metastatic cell line SW620 had significantly increased anchorage-independent survival after 24, 48, and 72 hours when compared with the nonmetastatic cell line SW480. This resistance to anoikis in detachment conditions suggests a survival advantage for potentially metastatic cells.

Biologically based treatment of liver metastases

Anti–vascular endothelial growth factor therapy

Because VEGF expression is associated with colon tumor growth and metastases, strategies that affect the biologic activity of the VEGF receptor/ligand system have been investigated. Warren et al [41] first reported on the effects of VEGF monoclonal antibodies (mAB) on the growth of colorectal hepatic metastases. The administration of these antibodies significantly decreased the growth of several human colon cancer cell lines in the livers of nude mice when compared with mice administered with an isotopic control antibody.

Shaheen et al [42] investigated the use of SU5416 and SU6668, small-molecule inhibitors of the VEGF receptors, in a model of colon cancer liver metastases. In this study, Balb/c mice underwent splenic injection of
syngeneic CT-26 colon carcinoma cells and 4 days afterward were assigned to receive i. p. injections of a control solvent, SUGEN-5416 (SU5416), or SUGEN-6668 (SU6668). In comparison with control mice, liver weights (a gross measure of hepatic tumor burden) were lower and surface liver metastases were fewer in the SU5416 and SU6668 groups. Immunohistochemical staining of the hepatic metastases revealed decreased blood vessel counts, decreased tumor cell proliferation, and increased apoptosis in the treatment groups compared with controls. Moreover, median survival in mice treated with SU6668 was 40% longer than in mice receiving the control solvent. These studies show that antiangiogenesis therapy targeting the tyrosine kinase receptor for VEGF inhibits vascularity and proliferation and growth of colon cancer liver metastases, and may be a promising strategy for liver metastases.

Preliminary clinical trials using SU5416 have also shown promise [43]. In a phase 2 study of 28 patients, SU5416 was used in conjunction with 5-flurouracil (5-FU) and leucovorin (LV) chemotherapy. The overall response rate was 37%; however, the striking finding in this study was that 83% of patients responded to therapy or continued to have stable disease [43]. A randomized phase 3 trial using SU5416 in conjunction with 5-FU and LV has recently begun.

**Tissue inhibitors of metalloproteinases**

TIMPs serve to balance MMP activity in physiologic processes. The synthetic MMP inhibitor, BB-94, has been used to study the effects in colon cancer. Human colorectal cancer cell lines were injected systemically or into the peritoneal cavity of nude mice followed by treatment with batimastat. Batimastat significantly reduced the number of liver tumors compared with treatment with vehicle only [44]. Results of early clinical trials using MMP inhibitors have been disappointing; however, these trials were conducted in patients with advanced disease when metastases and invasion had already occurred [45].

**Inhibitors of epidermal growth factor**

Clinical phase 1/2 trials using anti-EGFR strategies have also shown promise. Saltz et al [46] have reported on the use of IMC-C225, a chimeric monoclonal antibody that binds selectively to EGFR. In this study, IMC-C225 was used in combination with CPT-11 (Irinotecan) chemotherapy in patients with refractory and advanced colorectal cancers, many of whom had liver metastases. Toxicities attributable to IMC-C225 were minor. Twenty-one patients (17%) achieved a partial response, and an additional 37 patients (31%) had stable disease or minor responses. Similar results have been reported with ZD1839 (Iressa), an orally administered selective EGFR tyrosine kinase inhibitor [47].
Inhibitors of cyclooxygenase

Another biologic strategy that has received considerable attention has been the inhibition of cyclooxygenase-2 (COX-2). Several previous studies indicate that nonsteroidal anti-inflammatory drugs (NSAIDs) can prevent the development of colorectal carcinoma in humans. One of the targets of NSAIDs is the enzyme COX-2, which catalyzes the conversion of arachidonic acid to prostaglandin H₂. Preclinical studies have demonstrated the role of COX-2 in colon cancer tumor growth, angiogenesis, and liver metastases formation. Chen et al [48] investigated COX-2 expression in primary colorectal cancer, metastatic hepatic lesions, and corresponding normal mucosa in 17 patients. Immunoblot-detectable COX-2 expression was higher in primary colon cancers when compared with adjacent normal mucosa. Furthermore, COX-2 expression was significantly higher in metastatic liver lesions when compared with the corresponding primary colon cancers. Other researchers have found similar results [49]. These results suggest that investigation of the effect of selective COX-2 inhibition on metastases growth may be warranted. Clinical trials using COX-2 inhibition for the treatment of liver metastases are currently being performed.

Summary

The elucidation of the interaction of the tumor and its microenvironment has increased surgeons’ understanding of the biologic mechanisms mediating metastases formation. The ability to develop effective biologic therapies will depend on understanding the complex interactions among endothelial cells and tumor cells and on the cytokine cross-talk among all cells within a particular tumor at a particular site. Biologic therapies must be targeted to both the site and the tumor. By the time metastases form, most steps in the metastatic cascade have been completed. Therapy to down-regulate or interrupt the last stages of metastases, proliferation, and angiogenesis and mechanisms to disrupt cell survival signals appear to be the most promising areas of investigation.

References


