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Biology of Human Colon Cancer Metastasis

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Abstract. The process of metastasis is highly selective and favors the survival and growth of a few subpopulations of cells that preexist within a heterogeneous primary neoplasm. To produce metastases, tumor cells must succeed in invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication in organ parenchyma. The outcome of this process depends on the interaction of metastatic cells with multiple host factors. To assess metastatic potential accurately, it is necessary to orthotopically implant human tumor cells recovered from surgical specimens into nude mice. This orthotopic implantation of tumor cells is invariably associated with trauma to the specific organ of implantation, which is followed by the processes of inflammation and repair. Tissue-specific growth factors may be responsible for stimulation of tumor cells that possess specific surface receptors. Understanding the factors that regulate cancer metastasis should allow for the design of rational therapy.

Carcinoma of the colon is the third most common cancer in the United States [1]. Despite significant improvements in early diagnosis, surgical techniques, and adjuvant chemotherapy, at least one-third of patients operated on "for cure" die of recurrent local disease and metastases that are resistant to conventional therapies [2–7]. For this reason, once the colon cancer is diagnosed, the urgent question facing the surgeon is whether the cancer is localized or has already spread to the regional lymph nodes and distant organs [8]. The major obstacle to the effective treatment of colon carcinoma metastasis is the biologic heterogeneity of neoplasms. By the time of initial diagnosis, a malignant tumor contains multiple cell populations with different properties: growth rate, karyotype, cell surface properties, antigenicity, immunogenicity, marker enzymes, sensitivity to various cytokines and cytotoxic drugs, and the ability to invade and produce metastases [4, 5, 7, 9–15]. Another challenge to therapy is the finding that different organ environments can modify a metastatic tumor cell's response to systemic therapy [7]. Understanding the mechanisms responsible for the development of biologic heterogeneity of colon cancer and the processes by which tumor cells can invade local stroma and spread to grow in distant organs is therefore a primary goal of cancer research. In this article, we review some recent data on the biology of human colon cancer metastasis and host factors that influence this process.

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Pathogenesis of Colon Cancer Metastasis

The process of cancer metastasis consists of a series of sequential, interrelated steps, shown in Figure 1. The outcome of metastasis depends on the continuous interactions between the tumor cells and various host factors. To produce a clinically relevant lesion, metastatic cells must complete *all* the steps of the process [3, 11, 13–17]. After the initial transformation of cells in the intestinal mucosa and initial growth of these cells, vascularization must occur if a tumor mass is to exceed 2 mm in diameter [18]. The synthesis and secretion of several angiogenic factors by tumor and host cells therefore play a key role in establishing a capillary network from the surrounding host tissues [19, 20]. Local invasion of the host stroma occurs next [21–24]. Because thin-walled lymphatic channels and venules offer little resistance to penetration by tumor cells they provide the tumor cell with easy entry into the circulation. Detachment and embolization of small tumor cell aggregates occur next, and most of these circulating tumor emboli are rapidly destroyed. Tumor cells that survive the circulation must then arrest in the capillary beds of organs where extravasation into the organ parenchyma occurs, probably by the same mechanisms that influence intravasation. Proliferation within the organ parenchyma completes the metastatic process. Again, the lesion must develop a vascular network and evade the host immune system [25, 26].

Mechanisms of Tumor Cell Invasion

After initial growth in the mucosa, the tumor protrudes into the lumen of the gut. Subsequent growth is mainly in the transverse axis, leading to circumferential tumors [27–29]. A tumor that traverses the muscularis mucosa and infiltrates the submucosa is termed invasive. As the tumor penetrates the bowel wall, it can invade neighboring structures; adjacent organ involvement occurs in 10% of patients [30–32]. An additional pattern of local spread is perineural invasion, or spread along the perineural spaces, which may reach as far as 10 cm from the primary tumor [33].

Several independent mechanisms can be involved in tumor cell invasion of host tissues. First, mechanical pressure produced by a rapidly proliferating neoplasm may force cords of tumor cells along tissue planes of least resistance [21]. Second, increased cell motility can contribute to tumor cell invasion. Third, invasive

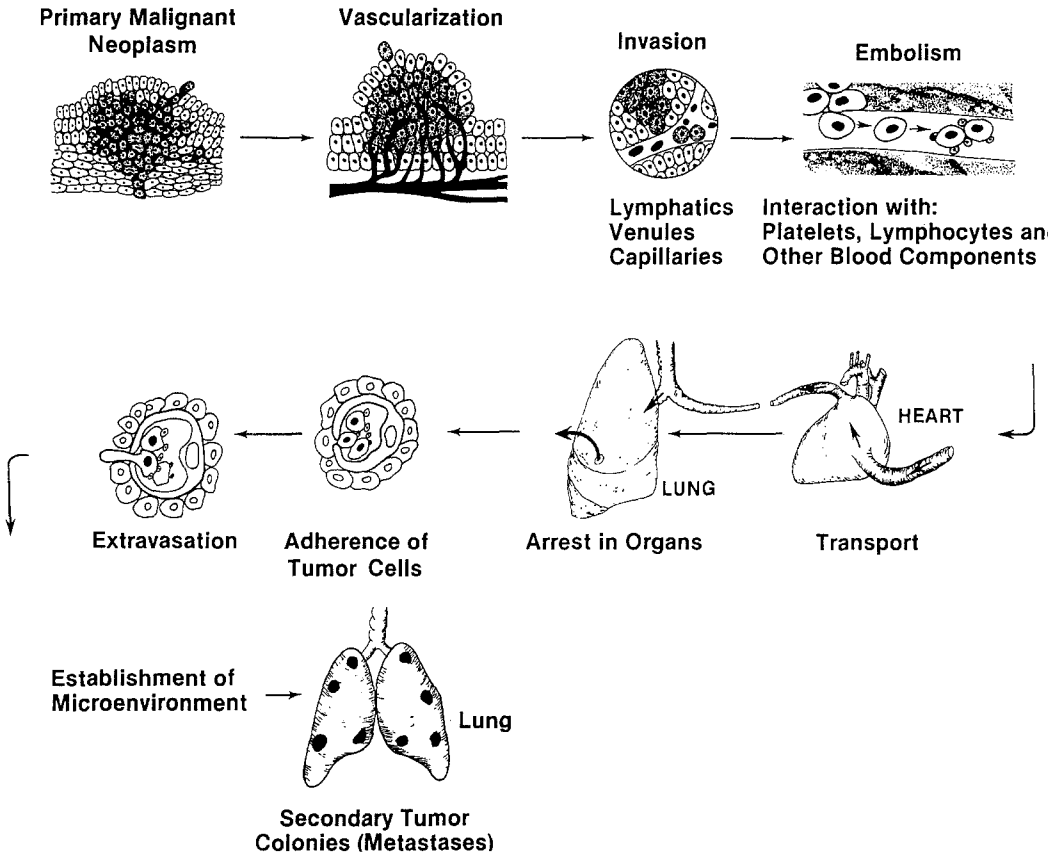

Fig. 1. Pathogenesis of cancer metastasis. The process of cancer metastasis consists of sequential, interlinked, and selective steps. The outcome of each step is influenced by the interaction of metastatic cells with homeostatic mechanisms. If a cell fails to complete any of the steps, it is eliminated; hence the formation of clinically relevant metastases represents the survival and growth of unique subpopulations of cells that preexist in primary neoplasms.

tumor cells may secrete enzymes capable of degrading basement membranes, which constitute a barrier between epithelial cells and the stroma. Epithelial cells and stromal cells produce a complex mixture of laminin, collagens, proteoglycans, and other molecules, which contains ligands for adhesion receptors and is permeable to molecules but not to cells [22, 34–38].

Colon cancer cells also produce and secrete basement membrane components such as laminin [39]. Well differentiated human colon carcinomas produce abundant amounts of laminin, whereas poorly differentiated colon carcinomas produce discontinuous basement membranes that are low in laminin [40]. A decrease in laminin content has also been demonstrated in basement membranes of dysplastic adenomatous polyps, but discontinuity of basement membranes was noted only in colon carcinomas [41–43]. The co-cultivation of human colon cancer cells with fibroblasts decreases the amount of collagen, laminin, and proteoglycans present in the interface between tumor cells and stromal cells [44].

Proteoglycans are another major constituent of the extracellular matrix. They include chondroitin sulfate, heparan sulfate, hyaluronate, and heparin. The function of the proteoglycans is not clear. The speculation is that these molecules serve as a reservoir for growth factors, such as transforming growth factor β (TGF-β) [45]. As enzymes degrade the extracellular matrix, TGF-β is released in an active form [46]. Thus proteoglycans may have an important role in regulating the growth and differentiation of tumor cells.

To invade the basement membrane, a tumor cell must first attach to the extracellular matrix by a receptor–ligand interaction. One group of such cell surface receptors are the integrins. Some members of the integrin family specifically bind cells to laminin, collagen, or fibronectin [47, 48]. Indeed, many integrins are expressed on the surface of human colon carcinoma cells [48–50], with each binding to different components of the extracellular matrix. A gradual decrease of integrin expression is associated with tumor progression, suggesting that the more aggressive cancers lose integrins to facilitate detachment from a primary neoplasm [49].

Another group of proteins that bind to extracellular matrix components are the lectins. These proteins bind sugars or liposaccharides with high specificity. Normal intestinal epithelial cells contain two highly conserved lectins of 31.0 and 14.5 kDa [51, 52]. The expression of the 31 kDa lectin is increased in carcinomas, absent in adenomas, and weak in normal epithelium. Its intramural content is significantly associated with carcinoembryonic antigen (CEA) [53]. CD44, a receptor responsible for lymphocyte homing [54, 55], binds to extracellular matrix components and to CEA [55, 56]. It is expressed as a 90 kDa protein in lymphocytes and as a 150 to 180 kDa protein in epithelial cells [57]. The production of CD44 gene transcript is markedly increased in human colon cancer cells compared to that in normal adjacent mucosa [57]. CD44 may regulate migration through the extracellular matrix. An abnormal pattern of activity of the CD44 gene has been reported in malignant colon carcinomas but not in normal tissues [58].

Subsequent to binding, tumor cells must degrade connective tissue extracellular matrix and basement membrane components [59]. Metastatic tumor cells produce various proteases and gluco-

sidases capable of degrading extracellular matrix components. The production of enzymes such as type IV collagenase (gelatinase, matrix metalloproteinase) and heparinase in metastatic tumor cells correlates with the invasive capacity of human colon cancer cells. Type IV collagenolytic metalloproteinases with apparent molecular masses of 98, 92, 80, 68, and 64 kDa have been detected in highly metastatic cells. Poorly metastatic cells, on the other hand, appear to secrete low amounts of only the 92 kDa metalloproteinase [60].

Under experimental conditions, collagenase activity can be stimulated by purified mucin products, which are associated with a poor prognosis [61]. Human colon cancer cells also secrete a plasminogen activator, urokinase, which activates the serine protease plasmin from plasminogen. Plasmin induces basement membrane (laminin) degradation and invasion. It further degrades the basement membrane by activation of collagenase type IV, which in turn depletes the membrane of both laminin and collagen type IV [22, 62]. Furthermore, plasmin acts as a chemoattractant for tumor cells [63].

Carcinoembryonic Antigen

Carcinoembryonic antigen is a member of the immunoglobulin supergene family, which participates in intercellular recognition and attachment [64]. CEA is the most commonly used marker for detecting human colon cancer [64]. However, because CEA is elevated in the serum of 6% to 12% of hepatocellular carcinoma (HCC) patients, as well as in some patients with benign disease, it is not useful for screening for colon cancer [64]. CEA can be used as a prognostic marker because in most cases an elevated preoperative serum CEA level is associated with a poor prognosis. Other factors, however, such as venous drainage of the tumor (portal versus systemic) or the degree of differentiation might decrease the sensitivity of CEA serum levels as a prognostic marker [64]. For patient follow-up, CEA is a useful tool because CEA serum levels usually increase before the appearance of clinical metastases. Second-look surgery based on elevation of CEA alone has been associated with the successful resection of local recurrences and a 5-year disease-free survival rate of 31% [65]. The recent introduction of radioimmuno-guided surgery using monoclonal antibodies has increased the postresection 5-year survival rate to 45% [66].

Cells expressing CEA have been shown to aggregate under in vitro conditions [67], which suggests that CEA may promote the adhesion of tumor cells to each other or to host cells. Tumor cells in aggregates have an increased capacity to arrest in a capillary bed and hence have increased potential for metastasis. Kupffer cells in the liver and alveolar macrophages in the lung are known to bind CEA through specialized receptors [68]. Systemic administration of CEA to nude mice was shown to increase the number of experimental liver metastases by increasing the arrest of tumor cells in the liver [69].

Lymphatic Metastasis

The lymphatic flow in the normal colon is through the lymphatic channels along the major arteries, with three echelons of lymph nodes: pericolic, intermediate, and principal lymph nodes [70]. In the neoplasms located between two major vascular pedicles, the lymphatics may drain to the left or the right or in both directions. If the central lymph nodes are replaced by actively growing tumor cells, the lymphatic flow can become retrograde along the marginal arcades both proximally and distally [71].

Tumor cells can enter the lymphatic or vascular circulations, and numerous connections allow the disseminating tumor cells to pass rapidly from one system to another [5, 71]. During lymphatic invasion, tumor cells are passively transported in the lymph to be trapped in the first draining lymph node, or they may bypass the regional lymph nodes to form distant nodal metastases, the "skip metastases" [4, 5, 72]. Whether regional lymph nodes can retain tumor cells and serve as a temporary barrier for cell dissemination has been a controversial issue. This question has significant clinical implications regarding the extent of surgical resection and lymph node dissection needed for colon cancer treatment. Answering this question experimentally is not simple because in experimental models normal lymph nodes are usually subjected to a sudden challenge with a large number of tumor cells, a situation that may not be analogous to small numbers of cancer cells reaching the lymph nodes continuously [73].

Hematogenous Metastasis

The most common site of hematogenous metastases is the liver, followed by the lung. In 40% of autopsies, the liver is the only site involved. Involvement of other sites in the absence of metastases in the liver or lung is rare [74]. During hematogenous metastasis, the malignant cell must survive transport in the circulation, adhere to small blood vessels or capillaries, and invade the vessel wall; but the mere presence of tumor cells in the circulation does not constitute a metastasis because most circulating cells die rapidly [72, 75, 76]. Using radiolabeled mouse B16 melanoma cells, we found that by 24 hours after entry into the circulation fewer than 1% of the cells were still viable and fewer than 0.1% of these tumor cells eventually produced metastases [75]. Therefore the greater the number of cells released by a primary tumor, the greater is the probability that some cells will survive to form metastases, as most cells are obviously destroyed in the bloodstream. The development of necrosis and hemorrhage in large tumors facilitates tumor cell entry into the circulation [72]. The rapid death of most circulating tumor cells is probably due to simple mechanical factors, such as blood turbulence or host defense mechanisms that include immune cells and nonimmune cells (e.g., endothelial cells) and the production of nitric oxide [75, 76]. Tumor cell survival can be increased if tumor cells aggregate with each other [14, 77, 78] or with host cells such as platelets [79] or lymphocytes [80]. Once metastatic cells reach the microcirculation, they interact with cells of the vascular endothelium. These interactions include nonspecific mechanical lodgment of tumor cell emboli and formation of adhesions to the endothelial cells. These properties of tumor cells in part determine their organ distribution [14, 81, 82].

The formation of fibrin clots at sites of tumor cell arrest in the microcirculation can damage blood vessels [83]. The increased coagulability often observed in the blood of patients with cancer may be related to the high levels of thromboplastin found in certain tumors, the production of high levels of procoagulant A activity [84], or the presence of phosphatidylserine in the outer leaflet of tumor cell membranes [3]. Arrested tumor cells probably extravasate by mechanisms similar to those responsible for local invasion. Growth of malignant cells at particular secondary sites

also involves the cells' responses to tissue or organ factors. Tumor cells can recognize tissue-specific motility factors, which direct their movement and invasion properties. After invasion, the cells respond to organ-specific factors that influence their further growth [14, 82, 85].

Metastasis by Direct Extension

Intraluminal spread occurs by the release of viable cells from the mucosal surface of the primary tumor and their distal implantation, usually at the site of a raw surface, such as a fistula, ulcer, or hemorrhoid [86–88]. Serosal cell shedding accounts for intraperitoneal seeding and carcinomatosis seen even in the absence of lymphatic or hematogenous spread. Other forms of implantation are related to surgical manipulations. The incidence of suture line recurrence is about 10%. About half of these cases are believed to be the result of either cell shedding or inadequate excision during surgery [8, 89]. Tumors can also develop in an abdominal scar [90] or at the mucocutaneous margin of a colostomy [91].

Metastatic Heterogeneity

Three general approaches have been used to isolate cells with different metastatic capacities. With the first, metastatic cells are selected in vivo: Tumor cells are implanted into syngeneic mice, and metastatic lesions are harvested; these cells are then expanded in culture or injected immediately into mice. The cycle is repeated several times, and the behavior of the recovered-isolated cells is compared with the cells in the parent tumor. With the second approach, cells are selected in vitro for the enhanced expression or lack thereof, a property believed to be important in one step of the metastatic process. The third approach involves cloning of primary tumors and then testing the metastatic potential of the clones in syngeneic mice. This procedure was used in the first experimental proof of metastatic heterogeneity in neoplasms provided by Fidler and Kripke, who showed that different tumor cell clones, each derived from individual cells isolated from the heterogeneous wild-type B16 melanoma, varied in their ability to form lung metastases following intravenous injection into mice [92]. Subsequent experiments with different cell lines produced similar results and demonstrated significant variability in size and pigmentation of metastases growing in different organs [93]. The biologic heterogeneity of metastases is not restricted to rodent tumors [94, 95].

Many human neoplasms, including colon carcinoma, have a clonal origin [94, 95]; nevertheless, by the time of diagnosis, the neoplasms have become heterogeneous and consist of multiple subpopulations of cells with different metastatic potential [85, 93, 96]. Data from our laboratory and many others clearly demonstrate that the process of metastasis for rodent and human neoplasms is also selective and favors the survival of unique metastatic cells that preexist within the parental neoplasm [92].

More than 100 years ago Stephen Paget studied the pattern of metastatic spread in breast cancer patients [97]. He concluded that the process was not due to chance. Paget proposed that certain tumor cells (the "seed") had a specific affinity for the milieu of certain organs (the "soil"); metastasis resulted only when the right seed interacted with the right soil. By this model, certain tumors produce metastases to specific organs independent of anatomic considerations. In 1928 Ewing challenged Paget's "seed and soil" theory and hypothesized that metastatic dissemination occurred by purely mechanical factors that are a result of the anatomic structure of the vascular system [98]. Sugarbaker, by comparison, concluded that regional metastases could be attributed to anatomic or mechanical factors, but that distant organ colonization was a result of specific tumor–host interactions [99]. Organ distribution and "fate" studies of radiolabeled cancer cells injected into the circulation of syngeneic mice demonstrated that tumor cells can reach the vascular bed of many organs, but metastasis formation occurs in only a few. Therefore the initial arrival of viable tumor cells to a particular organ does not always predict that the cells will proliferate to produce metastases [76].

Experimental support for the "seed and soil" hypothesis was derived from studies on the preferential metastasis of the B16 melanoma to specific organs. In these studies B16 melanoma cells injected intravenously into syngeneic mice produced metastases in the lungs and ovarian tissues but not in kidneys even when implanted intramuscularly [100]. The introduction of peritoneovenous shunts for palliation of malignant ascites provided similar data in humans. The autopsy findings in 15 of these patients substantiated the clinical observations that the shunts did not increase the incidence of visceral organ metastasis. In fact, despite continuous entry of billions of tumor cells into the circulation, metastases in the lung (the first capillary bed encountered) were rare [101].

Models for Human Colon Cancer Metastases

Appropriate animal models are mandatory for advancing our understanding of the biology of colon cancer metastasis. We have developed a model of regional lymph node metastases by injecting tumor cells into the apical lymphoid follicle of the cecum in mice [73]. Dye distribution studies have shown rapid distribution of the injected material in the lymphatic channels, and the procedure has yielded a high incidence of mesenteric lymphatic metastases [73].

To develop a reproducible model of hepatic metastasis, we implanted tumor cells into the spleens of nude mice [102]. Splenic injections are easier to perform than portal or mesenteric vein injections; access to the portal bloodstream is gained from the spleen, and tumor cells can then reach the liver to proliferate into experimental liver metastases [103]. Merely implanting tumor cells in the spleen did not guarantee liver metastasis. Metastatic human renal cell carcinoma, for example, injected into the spleen produces only spleen tumors, but these cells produce extensive lung metastases when they are injected into the kidney [104]. Also, orthotopic implantation of human colon cancer cells in nude mice produces liver metastases, depending on the nature of the tumor cells [105]. In our study we were able to distinguish between human colon cancer cells with low or high malignant potential. Specifically, intrasplenically injected cells isolated from liver metastases in colon cancer patients produced rapidly growing liver lesions, and the mice became moribund within 30 days, whereas cells from Dukes' stage B2 primary tumors produced only a few visible tumor foci by 90 days [106].

We next undertook a series of orthotopic implantation experiments to select and isolate cells with increased liver-metastasizing potential from heterogeneous primary human colon cancers. Cells derived from a surgical specimen of a primary human colon cancer, classified as Dukes' stage B2, were immediately estab-

lished in culture or were injected directly into the subcutis, spleen (for liver metastasis), or cecal wall of nude mice. Progressively growing tumors were excised, enzymatically dissociated, and then established in culture. Subsequent implantation into the cecal wall or spleen of nude mice produced only a few hepatic metastases. Human colon cancer cells from these rare liver metastases were recovered, expanded in culture, and then injected into the spleens of nude mice, thereby allowing additional cycles of selection. With each successive in vivo selection cycle, the metastatic ability of the isolated-propagated cells increased. After four cycles of selection, we obtained cell lines with high liver-colonizing efficiency in nude mice [61, 106].

Consequence of Tissue Damage Repair on Cancer Growth and Metastasis

The outcome of metastasis is influenced by host factors probably related to homeostatic processes, such as organ repair or regeneration, that are known to be organ-specific. For example, after a partial hepatectomy the liver undergoes rapid cell division termed *regeneration*. In a hepatectomized mouse, however, no such cell division can be found in the kidneys. Similarly, the mouse kidney compensates for unilateral nephrectomy by hypertrophy and hyperplasia, but there is no change in liver growth [107].

We have recently completed transplantation experiments on human colon cancers and human renal cell carcinomas in nude mice that have been subjected to either hepatectomy, nephrectomy, or abdominal surgery (used as a trauma control) [107; Gutman et al., submitted]. The results were interesting. Human colon cancer cells implanted subcutaneously demonstrated accelerated growth in partially hepatectomized mice but not in nephrectomized mice. Human renal cell carcinoma cells established as micrometastases in the lungs of nude mice underwent significant growth acceleration subsequent to unilateral nephrectomy but not hepatectomy. These results indicate that metastatic cells can respond to physiologic signals produced when homeostasis is disturbed. Tumor cells that either originate from or have an affinity for growth in this particular organ can also respond to these signals.

One possible mechanism to explain the accelerated growth of human colon cancer in hepatectomized mice is the production of organ-specific growth factors. Evidence supporting organ-specific growth factors for metastatic cells has been obtained, in part, from experiments on the effects of organ-conditioned medium on the growth of particular neoplastic cells. The presence of stimulatory or inhibitory tissue factors correlates with the site-specific pattern of metastasis [108]. Liver regeneration that follows major hepatectomy involves quantitative changes in hepatocyte gene expression [109, 110]. Recently, transforming growth factor α (TGF-α) mRNA was shown to increase approximately twofold in rat hepatocytes during the first 8 to 24 hours after partial hepatectomy, coinciding with an increase in epidermal growth factor receptor (EGF-R) mRNA and down-regulation of these receptor proteins, as well as a loss of EGF-R protein kinase activity [111, 112]. These results suggest that TGF-α is a physiologic regulator of liver regeneration by means of an autocrine mechanism [112]. Moreover, TGF-α production by hepatocytes might also have a paracrine role, stimulating proliferation of adjacent nonparenchymal cells or tumor cells [113].

Hepatocyte growth factor (HGF), another liver mitogen, is synthesized and secreted from nonparenchymal liver cells (endothelial and Kupffer cells). Subsequent to liver damage, a rapid increase is observed in HGF mRNA in Kupffer cells [114], paralleling the down-regulation of its receptor, the c-*met* proto-oncogene, in hepatocytes. Similar to EGF-R, the receptor for HGF (c-*met*) belongs to the tyrosine kinase family of receptors [115]. HGF is a potent mitogen for hepatocytes, melanocytes, and prostate cells; and it enhances the invasive capacity of carcinomas [116]. Levels of TGF-β mRNAs increase in normal nonparenchymal liver cells coinciding with hepatocyte DNA replication and mitosis, and TGF-β inhibits EGF-stimulated DNA synthesis, implying that it may be a component of a paracrine regulatory loop controlling hepatocyte replication at the late stages of liver regeneration [117]. Therefore when the liver is damaged, growth factors are likely to be released and stimulate the proliferation of receptive malignant tumor cells (i.e., those cells that possess the appropriate receptors).

The most widely investigated growth factor receptor in human colon cancer is EGF-R. We assessed the genes encoding for growth factor receptors of low- and high-metastatic human colon cancer variants [118]. Analyses of human colon cancer cells from nonselected surgical specimens that differed in malignant potential showed no amplification or rearrangements in the genes encoding EGF-R. In contrast, highly metastatic human colon cancer variants (either Dukes' stage D or variant cells selected in nude mice from a Dukes' stage B2 tumor) expressed significantly increased EGF-R mRNA transcripts when compared with low-metastatic human colon cancer cell types [118]. The in vitro growth stimulation of cells with high- or low-metastatic potential to TGF-α demonstrated the functional significance of increased EGF-R numbers on specific cell types. Overexpression or altered expression of EGF-R was reported for a variety of human carcinomas including breast, liver, pancreas, melanoma, glioblastoma, and metastatic human colon cancer [118–120]. In general, overexpression of EGF-Rs is associated with increased malignancy [120].

Related to, but distinct from, the EGF-R is the c-*met* proto-oncogene, whose encoded protein is the receptor for HGF [121]. In human tissues the highest levels of c-*met* mRNA expression are found in the liver, kidney, stomach, and thyroid. Studies with anti-c-*met* antibodies have revealed that receptor protein levels are high in hepatocytes and in gastric and intestinal epithelium (including colon and rectum), indicating a role for HGF and c-*met* in the growth and turnover of epithelial tissues [122]. Preliminary studies from our laboratory indicate high levels of c-*met* expression in colon cancer cell lines that have been adapted to grow in culture from either Dukes' stage B2 or D, or from liver metastases [118]. Analyses of mRNA isolated directly from human colon cancer specimens and normal colon mucosa also suggest increased c-*met* transcripts in the tumor tissues [118].

Effects of the Organ Environment on Human Colon Cancer Sensitivity to Cytotoxic Drugs

Clinical observations have shown that subsequent to systemic chemotherapy metastases in one organ may regress whereas those in others progress; and recent experimental data support these observations. For example, a murine fibrosarcoma growing subcutaneously in syngeneic mice is more sensitive to doxorubicin (DXR) than the same tumor growing as a lung metastasis [123].

DXR is a cytotoxic agent affected by the multidrug resistance (MDR) phenotype. We also found differences in the sensitivity to DXR of murine and human colon cancer tumors growing in the subcutis, spleen, liver, and lung. The sensitivity of the cells to DXR was highest in the subcutaneous environment, intermediate in the spleen and cecum, and lowest at metastatic sites, such as the liver and lungs. Different patterns of organ-specific chemosensitivity were found for 5-fluorouracil (5-FU) (a compound unaffected by MDR). Tumor cells growing in the lung were most sensitive to systemic administration of 5-FU; those growing in the subcutis, spleen, and cecum demonstrated intermediate sensitivity; those in the liver were resistant. Organ site-associated differences in drug sensitivity to either DXR or 5-FU were not associated with drug distribution patterns in the tumor [123, 124].

Multiple factors in the environment can influence tumor cell response to therapy, for example, the nutritional status of cells, the presence of organ-specific growth factors and other signal-transducing agents, the degree of oxygenation [125], the pH [126], the extent of the vascular network and its functionality [127], local immunity, extracellular matrix components, and drug metabolism and encapsulation [128]. Current studies are under way to determine if any of these mechanisms influence the sensitivity of colon cancer cells to chemotherapy.

Conclusions

Human colon cancer is heterogeneous for a variety of biologic properties that affect invasion and metastasis. The presence of subpopulations of cells with high metastatic capacity has important implications for the diagnosis and therapy of human colon cancer. Current staging systems (e.g., Dukes' TNM) are based on the anatomic site of the tumor and are not capable of recognizing the few highly metastatic cells that may have already metastasized at the time of diagnosis. Identification of these populations of cells requires a better understanding of the biology of human colon cancer metastasis, which explains the importance of developing relevant animal models. Our data indicate that the appropriate in vivo model for studying the biology and therapy of human colon cancer is based on the orthotopic implantation of tumor cells into nude mice, supplemented by methods of intralymphatic and intrasplenic injections to form metastases and by in vivo isolation techniques of various tumor cell populations. The growth and spread of human colon cancers depend on the interaction of specific tumor cells with homeostatic mechanisms. Cells populating metastases respond to organ-specific growth factors, which can also modify the response of the tumor cell to therapy.

Suggesting that human colon cancer metastasis is a selective process is an optimistic view of cancer biology. The belief that certain rules govern the spread of neoplastic disease implies that the elucidation and understanding of these rules will lead to better therapy.

Résumé

Les métastases relèvent d'un phénomène hautement sélectif responsable de la survie et de la croissance d'une quantité réduite de sous-populations cellulaires préexistantes à l'intérieur de la tumeur primitive. Pour pouvoir métastaser, les cellules tumorales doivent franchir la première barrière tissulaire, emboliser et survivre dans la circulation, s'arrêter dans un lit capillaire à distance, s'extravaser et se multiplier dans le parenchyme de l'organe cible métastasé. Le résultat de ce processus dépend de l'interaction des cellules métastatiques avec des facteurs multiples dépendant de l'hôte. Pour évaluer le potentiel métastatique, il faut implanter des cellules humaines tumorales chez la sourie nude orthotopiquement. Cette implantation orthotopique est associée invariablement avec le traumatisme de l'organe spécifique, suivie d'inflammation et ensuite de réparation. Les facteurs de croissance spécifiques pourraient être responsables de la stimulation des cellules tumorales qui lisent des récepteurs spécifques de surface. La compréhension des facteurs qui régissent le phénomène de métastases devraient aider à établir une meilleure thérapeutique.

Resumen

El proceso del desarrollo de metástasis neoplásicas es altamente selectivo y favorece la supervivencia y el crecimiento de las pocas subpoblaciones de las células preexistentes dentro de un neoplasma primario heterogéneo. Para producir metástasis, las células tumorales deben lograr con éxito la invasión, embolización, supervivencia en la circulación permanencia en una red capilar distante y extra-vasación para multiplicarse en el parénquima de un órgano. El resultado final de este proceso depende de la interacción de las células metastáticas con múltiples factores presentes en el huésped. Para determinar con precisión el potencial metastático es necesario implantar ortotópicamente células tumorales humanas recuperadas de especímenes quirúrgicos en ratones atímicos. Esta implantación ortotópica de células tumorales invariablemente se ve asociada con trauma al órgano específico de implantación trauma que es seguido por el proceso de inflamación y reparación. Los factores de crecimiento tisular pueden ser responsables del estímulo de las células tumorales que posean los correspondientes receptores específicos de superficie. La mejor comprensión de los factores que regulan las metástasis cancerosas habrá de permitir el diseño de terapias racionales.

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