

The Molecular Biology of Peritoneal Carcinomatosis from Gastrointestinal Cancer

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Abstract

Introduction: Peritoneal carcinomatosis is a frequent form of disease progression in gastrointestinal cancer, and all too often is a preterminal event with a median survival of only 6 months. Despite the introduction of aggressive surgical and chemotherapeutic approaches, any significant improvement in survival is unlikely until we better understand the molecular biology of peritoneal metastasis. **Methods:** A Medline search and review of references was undertaken to identify all manuscripts in the English language concerned with peritoneal metastasis from gastrointestinal cancer. **Results:** Peritoneal carcinomatosis involves a complex sequence of interdependent steps. The injured peritoneum is a rich source of cytokines and growth factors that facilitate tumour proliferation and invasion in the postoperative abdomen. Peritoneal tumour adhesion is dependent on adhesion molecules, such as CD44, and the β -1 integrins. Invasion of the mesothelium involves, at least in part, a process of tumour-induced mesothelial apoptosis. Matrix metalloproteinases, such as MMP-7, facilitate stromal invasion, but the role of other proteases in invasion remains to be elucidated. To date, the significance of angiogenesis in the peritoneal metastatic cascade is unknown. **Conclusion:** The molecular biology of peritoneal carcinomatosis is only just beginning to be understood. Further research into the mediators of the peritoneal metastatic cascade is needed if more effective therapeutic strategies are to be developed for this invariably fatal, yet unfortunately common, condition.

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Key words: Gastrointestinal neoplasms, Neoplasm metastasis, Peritoneum

Introduction

The term “peritoneal carcinomatosis” was first used by Sampson in 1931 to describe the intra-abdominal spread of ovarian carcinoma.¹ Although ovarian cancers account for the majority of cases, peritoneal carcinomatosis is also frequently associated with gastrointestinal malignancies. With the exception of pseudomyxoma peritonei, peritoneal carcinomatosis of gastrointestinal origin is generally regarded as a terminal stage of disease progression, only suitable for palliative treatment.^{2,3} This view has been challenged with the recognition that peritoneal dissemination may occur in isolation of other systemic disease and, as such, may represent a form of regional recurrence amenable to surgical cure. This has inspired a number of aggressive therapeutic approaches with the promise of improved long-term survival.⁴⁻⁸ Despite these advances, it is unlikely that any further impact will be made until the molecular biology underlying the disease is better understood.

This article aims to review the current knowledge on the molecular biology of peritoneal carcinomatosis and to

suggest possible avenues for the development of novel therapeutic strategies. For convenience, the peritoneal metastatic cascade has been subdivided into its component parts, each of which, although mutually inclusive, must be successfully completed for the formation of a metastatic tumour. First, tumour cells must separate from the primary tumour, disseminate through the abdominal cavity and adhere to the innermost layer of the peritoneum, the mesothelium. Secondly, they must invade the mesothelium and its basement membrane to gain access to the underlying stromal tissue. Thirdly, the tumour-stromal interaction must be compatible with further tumour proliferation. Finally, the developing metastasis must induce angiogenesis to ensure its continuing survival. In addition, the role of the peritoneum as a favourable “host” tissue for metastasis formation will be reviewed with an emphasis on the role of surgical injury in the promotion of tumour metastases.

Methods

A Medline search and review of references was performed to identify all manuscripts in the English language concerned with original research into the molecular biology of

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peritoneal metastasis from gastrointestinal cancer. Mesh headings used in the search included peritoneum, neoplasm metastasis and gastrointestinal neoplasms. Manuscripts were assigned to one or more sections of the peritoneal metastatic cascade based on the primary focus of the research. Review of the manuscripts was combined with data from our own original research into this field to build up as complete a picture of the molecular biology of peritoneal carcinomatosis as possible.

Results

The Peritoneal Host Environment

The importance of the host environment in the formation of secondary metastases was first recognised by Paget⁹ in 1889 in his “seed and soil” hypothesis. Our understanding of the metastatic process has subsequently exploded thanks largely to an increased knowledge of the influence of cytokines and growth factors on tumour growth and development. Consideration of the cytokine/growth factor environment of the peritoneum is, therefore, central to understanding its ability to act as a suitable host tissue for secondary metastases.

Abdominal surgery, by its very nature, has a profound influence on the peritoneal cytokine environment, stimulating the influx of inflammatory cells into the peritoneal cavity and inducing the production of a multitude of inflammatory cytokines and growth factors. Unwittingly, this peritoneal inflammatory response can have a stimulatory effect on the growth and invasion of any residual tumour cells, and may account for the enhanced tumour growth often witnessed following palliative surgery. Although this tumour enhancement may be attributed partly to the immunosuppressive effects of surgery,¹⁰⁻¹² surgical trauma itself appears to have an important local effect.

Eggermont et al¹³ demonstrated the influences of operative stress and immunomodulation on peritoneal tumour growth in a murine model. Intra-peritoneal administration of interleukin-2 (IL-2) and lymphokine-activated killer (LAK) cells were highly effective in preventing the growth of inoculated tumour cells. However, when laparotomy was performed prior to tumour inoculation, the suppressive effects of IL-2 and LAK cells were completely abrogated. Tumour growth was greatest in the vicinity of the laparotomy wound, whilst a wound outside the abdomen had no effect. van den Tol et al¹⁴ investigated the preference of tumour cells for areas of peritoneal trauma. In their rat model, surgical gauze of differing texture was used to inflict trauma by standardised peritoneal rubbing. Rat colon carcinoma cells preferentially adhered to and grew at the sites of peritoneal trauma, with the amount of tumour growth being proportional to the degree of peritoneal injury. Several authors have used laparoscopic models to

determine the influence of surgical wounds on peritoneal tumour growth.¹⁵⁻¹⁸ Surgical injury, whether inflicted by laparotomy or laparoscopy, increases intra-peritoneal growth compared to unoperated controls. However, the growth-promoting effect is always greater after laparotomy than laparoscopy, and is related to the increased size of the abdominal wound.

The injured peritoneum is rich in cytokines and growth factors and provides fertile soil for tumour growth. There is a rapid induction of many pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), tumour necrosis factor (TNF- α) and IL-6, which can be detected in peritoneal fluid within hours of laparotomy.^{19,20} At the cellular level, a residential population of peritoneal macrophages is believed to be the prime instigator of peritoneal inflammation, secreting the early inflammatory cytokines of IL-1 β and TNF- α . These activate the local mesothelium to produce more cytokines (TNF- α , IL-1 β , IL-6 and IL-8),²¹⁻²³ chemokines [RANTES and macrophage chemotactic protein-1 (MCP-1)]²⁴ and prostaglandins (PGI₂ and PGE₂),²⁵ which amplify the inflammatory response. Peritoneal fibroblasts may also contribute to the cytokine network.²⁶⁻²⁸

The relevance of these inflammatory cytokines to metastasis formation lies in their ability to up-regulate the peritoneal growth factors that bring about tissue repair and, secondarily, stimulate tumour growth. Little is known about the growth factors produced by the human peritoneum. In rabbits, peritoneal “tissue repair cells” respond to treatment with fibroblast growth factor (FGF), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), but are inhibited by transforming growth factor-beta (TGF- β).^{29,30} In rats, TGF- β has been implicated in peritoneal repair following uterine horn injury.³¹ *In vitro* studies, using human mesothelial cell cultures, have shown that both EGF and PDGF induce mesothelial proliferation.^{32,33} In three-dimensional culture systems, PDGF and FGF, but not TGF- β , induce peritoneal fibroblast proliferation, as does incubation with peritoneal macrophage or mesothelial cell conditioned media.²⁸

Recently, we have explored the role of the heparin-binding growth factors (HBGFs) as potential mediators of peritoneal metastasis.³⁴ This heterogeneous group of growth factors includes, amongst others, heparin-binding epidermal growth factor (HB-EGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and interleukin-8 (IL-8). They have all been implicated in various stages of the metastatic process, including cell adhesion,³⁵⁻³⁷ proliferation³⁸⁻⁴⁰ and angiogenesis.^{41,42}

Using primary mesothelial cultures, we have shown that human mesothelial cells constitutively expressed bFGF,

HB-EGF and two VEGF spliced variants, VEGF₁₂₁ and VEGF₁₆₅. Activation with IL-1 β or TNF- α up-regulated HB-EGF and VEGF expression, whilst IL-6 had no such effect and IL-2 produced a marked suppression in HB-EGF and bFGF. It is possible that IL-2-mediated suppression of mesothelial growth factors may explain some of the anti-tumour effects observed by Eggermont et al.¹³

High levels of HBGFs can be measured in postoperative drain fluid from patients who have undergone laparotomy (Jayne and Guillou, unpublished observations), and may contribute to the favourable conditions for metastasis. Exogenous heparin can inhibit the action of these growth factors.⁴³⁻⁴⁵ In animal models, intraperitoneal heparin reduces tumour cell growth⁴⁶⁻⁴⁸ while, *in vitro*, modified heparin oligosaccharides inhibit HBGF-induced colonic tumour cell division and migration.⁴⁹ The anti-metastatic properties of heparin are independent of its anti-coagulative effects, thus enabling modified heparin-like molecules to be synthesised that possess solely anti-metastatic⁵⁰ and anti-angiogenic⁵¹ properties. Their efficacy in clinical practice has yet to be tested.

Peritoneal Tumour Dissemination

Dissemination of tumour cells from the primary cancer may result from spontaneous exfoliation, aided by the down-regulation of intercellular adhesion molecules,⁵² or as a direct consequence of surgical intervention. Serosal involvement by the primary tumour has been shown to be predictive of peritoneal recurrence.⁵³⁻⁵⁶ Similarly, tumour perforation, whether spontaneous or iatrogenic, increases the rate of local recurrence and reduces survival.⁵⁷⁻⁵⁹ In addition, tumour cells may be inadvertently liberated from transected lymphatics and blood vessels during the course of surgical resection. Whatever the mechanism of spillage, malignant cells can be detected in 3.7% to 13% of peritoneal washings from patients with colorectal cancer, increasing to 13% to 26% if the washings are collected post-resection.^{60,61} These tumour cells are viable and can be cultured *in vitro*.⁶⁰ Similar rates of detection have been described in preoperative washings from patients with pancreatic cancer^{62,63} and gastric cancer,⁶⁴ and have been shown to be independent markers of prognosis.^{65,66}

Peritoneal Tumour Adhesion

The mesothelium is the first surface encountered by disseminated tumour cells and successful adhesion is, therefore, of paramount importance in metastasis formation. Although the precise mechanisms orchestrating this interaction remain to be fully elucidated, several candidate adhesion molecules including the lymphocyte-homing molecule, CD44, and the integrin superfamily have been implicated.

Kotanagi et al⁶⁷ established two cancer cell lines from a

liver metastasis (AKT-CC-K-LM) and a peritoneal metastasis (AKT-CC-K-PC) of a patient with colon cancer. The two cell lines differed in their expression of cell adhesion molecules, with AKT-CC-K-LM cells overexpressing carcinoembryonic antigen, E-cadherin and sialyl-Lewis antigens, and AKT-CC-K-PC cells overexpressing CD44v6. Injection of AKT-CC-K-LM cells into the spleen or peritoneal cavity of mice resulted in liver metastases only, whilst injection of AKT-CC-K-PC cells yielded peritoneal metastases only. It was postulated that CD44 might be instrumental in tumour-peritoneal interaction, binding to its ligand, hyaluronic acid, which is expressed by mesothelium.⁶⁸ Schlaeppi et al⁶⁹ studied the adhesion of four colorectal cell lines to mesothelial monolayers and various extracellular matrix proteins. All cell lines adhered rapidly to the mesothelium, but both anti-integrin and anti-CD44 antibodies failed to inhibit this interaction. However, adhesion to extracellular matrix components was completely integrin-dependent. Yonemura et al⁷⁰ used a gastric cell line, MKN-45, to establish a highly metastatic variant, MKN-45-P, by serial peritoneal passages in a mouse model. The differential expression of various metastasis-related genes (integrin subunits, motility factors, proteases, growth factors, etc.) between MKN-45 and MKN-45-P were examined by reverse transcription polymerase chain reaction. Integrin α 2 and α 3 subunits were significantly elevated in MKN-45-P compared to MKN-45. These α -integrins dimerise with β 1 subunits to form adhesion molecules for various basement membrane proteins, including fibronectin, laminin and collagen IV, which are secreted by human mesothelium.⁷¹ Treatment with anti- β 1-integrin antibodies significantly inhibited the adherence of MKN-45-P in an *ex vivo* peritoneal model, thereby suggesting a role for β 1-mediated integrin adhesion to the submesothelial basement membrane.

Peritoneal Invasion

Before invading tumour cells can gain firm adherence to the submesothelial connective tissue, they must penetrate the mesothelial monolayer. Buck et al⁷² illustrated the protective effect of the mesothelium in a rat model. When Walker 256 tumour cells were injected into the peritoneal cavity of rats, in which the parietal peritoneum had been stripped of its mesothelial lining, leaving the basement membrane intact, rapid implantation occurred in denuded areas. However, in unoperated rats, with an intact mesothelium, peritoneal implantation was a rare event.

Akedo et al⁷³ observed three patterns of tumour growth when rat ascites hepatoma cells were co-cultured with mesothelial monolayers. Tumour cells either formed “piled-up” nests upon the mesothelial monolayer, exhibited invasive growth between adjacent mesothelial cells, or failed to attach and grew in suspension. Using a three-

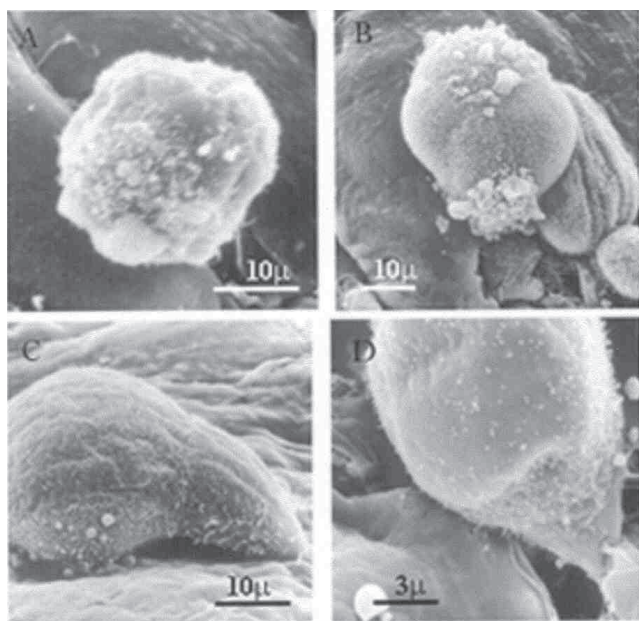


Fig. 1. Scanning electron photomicrographs of colonic tumour cells (SW480) seeded onto a peritoneal model. Rounded cells adhere to the mesothelial surface (12 hrs, A), exhibit vesicle formation and spreading (24 hrs, B and C), and invade between gaps in the mesothelial monolayer (48 hrs, D). Reproduced with kind permission from Kluwer Academic Publishers: *Clin Exp Metastases* 1999; 17(6):515-23 (Fig. 7).

dimensional *in vitro* model, we have observed similar patterns of peritoneal tumour growth.⁷⁴ Adherent tumour cells spread quickly upon the mesothelial surface and, within 24 hours, began to invade the underlying connective tissue (Fig. 1). However, not all tumour cells exhibited invasive growth. While some cells invaded and proliferated in the connective tissue to form distinct metastatic deposits, a subpopulation of cells showed proliferative growth on the peritoneal surface without invasion. The degree of invasive growth varied with the cell line studied.

Several authors have commented on a change in mesothelial morphology that occurs in areas of tumour cell invasion.⁷²⁻⁷⁵ Mesothelial cells take up a characteristic “rounded” morphology with separation of cell-cell contacts to expose the submesothelial basement membrane. Yonemura et al⁷⁶ explored this observation further using a mouse model and the gastric cell line, MKN-45-P. Intraperitoneal inoculation of MKN-45-P resulted in mesothelial contraction and eventual exfoliation. Similar effects could be induced *in vivo* by intra-peritoneal injection of IL-6, TNF- α and IL-8, and *in vitro* by cytokine stimulation of mesothelial monolayers. It was postulated that tumour-derived cytokines were responsible for the disruption of the mesothelial barrier, thereby exposing the submesothelial basement membrane, and facilitating tumour adhesion.

We have observed similar changes in mesothelial morphology when monolayers were co-cultured with

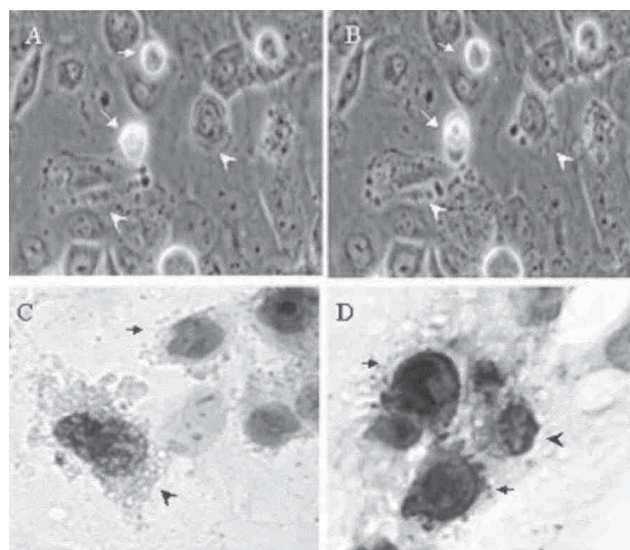


Fig. 2. Mesothelial-SW480 co-cultures. A and B: Phase contrast photomicrographs illustrating the cellular changes of apoptosis (membrane blebbing, cell shrinkage and nuclear fragmentation) observed in mesothelial cells (arrows) adjacent to adherent SW480 tumour cells (original magnification $\times 200$). C and D: Immunocytochemistry of mesothelial-SW480 co-cultures. Mesothelial cells (arrows) adjacent to SW480 tumour cells (small arrows) show apoptotic changes with characteristic nuclear fragmentation (original magnification $\times 400$).

colorectal cell lines. However, mesothelial retraction is only the beginning of an elaborate process that occurs over a period of 20 to 30 minutes and culminates in mesothelial exfoliation and death. Under phase-contrast microscopy, mesothelial cells in close proximity to adherent tumour cells are seen to undergo cytoplasmic shrinkage, nuclear fragmentation and membrane-blebbing characteristic of apoptosis (Fig. 2). This tumour-induced mesothelial apoptosis has been confirmed by DNA fragmentation assays and immunocytochemistry. We are currently investigating the potential mediators involved in this apoptotic killing, and believe that the death-ligand/receptor system, Fas/FasL, may play an integral role.⁷⁷ This novel mechanism of peritoneal invasion opens up further exciting avenues for investigation.

Peritoneal Tumour Proliferation and Angiogenesis

Having attached themselves to the peritoneum and penetrated the mesothelial barrier, tumour cells must invade the submesothelial connective tissue and proliferate to form metastatic deposits. The connective tissue degrading proteases, matrix metalloproteinases (MMPs), play a central role in stromal invasion. Yonemura et al⁷⁸ studied the role of MMP-7 in a mouse model of peritoneal carcinomatosis. Specific antisense oligonucleotides inhibited the expression of MMP-7 by the highly metastatic gastric cell line, MKN-45-P, and suppressed invasion without altering cell proliferation. In addition, survival of MKN-45-P-bearing

mice, which had been pre-treated with antisense oligonucleotides, was significantly better than that of control mice. Aparicio et al⁷⁹ found that the MMP inhibitor, batimastat, significantly reduced metastasis formation and prolonged survival in a rat model of peritoneal carcinomatosis. However, batimastat treatment was associated with marked peritoneal inflammation and ascites, thereby raising concerns about its potential as an anti-metastatic agent in humans.

The proliferation of invading tumour cells is a function of the genetic profile of the cells and their response to external growth signals. We have alluded to some of the growth factors produced by the injured peritoneum, but their effect on tumour proliferation has been relatively unexplored. EGF enhances the invasive potential of mammary carcinoma cells when injected into the peritoneal cavities of rats.⁸⁰ Davies et al⁸¹ showed that, in mice, this growth-promoting effect was due to the production of EGF by the peritoneal host tissue. Injection of a murine mammary carcinoma cell line, which was negative for EGF production, resulted in the production of multiple small peritoneal deposits, which could be abolished by simultaneous injection of anti-EGF antibodies. No such effect was seen with subcutaneous tumour growth, suggesting a site-specific requirement for EGF in peritoneal metastases.

Using an *in vitro* transwell system, van der Wal et al⁸² found that mesothelial cells inhibited the growth of CC531 colon carcinoma cells, while CC531 cells stimulated mesothelial cell growth. Both cell types produced insulin growth factor-1 (IGF-1) and possessed IGF receptors. In co-culture, IGF-1 potentiated the inhibitory effect of mesothelial cells on CC531 proliferation, while enhancing mesothelial proliferation. It is postulated that the inhibitory effects of IGF-1-like molecules might explain why tumour cells grow poorly in a surgically uncompromised abdomen.

Once tumour growth has been established, continual survival is dependent on the formation of a new blood supply. The deeper layers of the peritoneum contain a rich capillary network and are ideally suited to this function. However, the majority of peritoneal metastases consist of small, discrete nodules, with more substantial deposits usually only seen at sites of previous injury. The significance of angiogenesis to peritoneal metastasis is, therefore, uncertain, and awaits further histological and molecular investigation.

Summary

Peritoneal metastasis involves a complex sequence of interdependent steps. The molecular mechanisms involved in peritoneal tumour dissemination, adhesion, invasion, proliferation and angiogenesis are only just beginning to be unravelled. The mediators of initial tumour-mesothelial

adhesion remain largely unknown, but may involve CD44, while firm adherence to the submesothelial basement membrane appears to be a function of β 1-integrin binding. Adherence of tumour cells is followed shortly by invasion of the mesothelium. This appears, at least in part, to be accomplished via tumour-induced mesothelial apoptosis. Although MMPs have been implicated in peritoneal invasion, the role of other proteases has not been explored. The injured peritoneum is a rich source of cytokines and growth factors, such as the HBGFs, that stimulate tumour proliferation and metastatic growth. Modulation of this growth factor milieu may have a role in the prevention of tumour recurrence following cancer surgery. To date, the significance of angiogenesis in the peritoneal metastatic cascade is unknown. Further research is required to solve the many aspects of the metastatic cascade that remain elusive. Only then will more effective treatments be developed that will hopefully improve the poor prognosis associated with peritoneal carcinomatosis from gastrointestinal cancer.

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