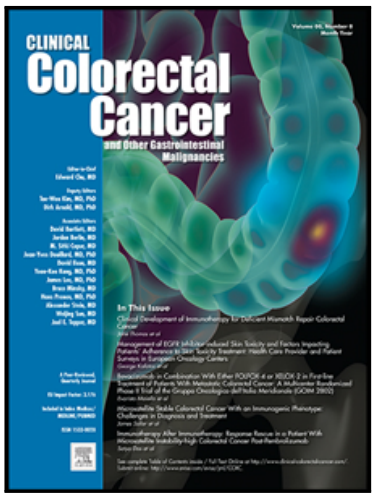

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Targeting Hyaluronic Acid and Peritoneal Dissemination in Colorectal Cancer

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Abstract

Peritoneal metastasis (PM) from colorectal cancer (CRC) carries a significant mortality rate for patients and treatment is challenging. The development of PM is a multistep process involving detachment, adhesion, invasion and colonisation of the peritoneal cavity.

Cytoreductive surgery and HIPEC (hyperthermic intraperitoneal chemotherapy) for PM from CRC has some benefit but overall survival is poor and recurrence rates are high. Treatments to prevent the development of peritoneal metastasis could have the potential to improve CRC survival and disease-free outcomes.

The ability of cancer cells to invade the peritoneum and become established as metastatic tumours is influenced by a multifactorial process. Hyaluronic acid (HA) has been shown to coat the mesothelial cells of the peritoneum and has been demonstrated to be utilised in various malignancies as part of the metastatic process in peritoneal dissemination. CD44, RHAMM (CD168) and ICAM-1 have all been shown to be binding partners for HA. Targeting HA-mediated binding may prevent adhesion to distant sites within the peritoneum through suppression of interaction of these molecules. Here we review the current literature and discuss key molecules involved with PM dissemination, with the potential to target these mechanisms in the delivery of future treatments.

Key Words: Hyaluronan, CD44, RHAMM, ICAM-1, Colorectal Cancer, Peritoneum

Introduction

Colorectal cancer (CRC) is the third most common cancer across the world [1]. However, metastatic disease remains a challenge with regard to both survival rates and quality of life. It is reported that patients diagnosed with synchronous or metachronous metastatic adenocarcinoma of the colon have an overall 5-year survival rate of 24% and 34% respectively [2]. Peritoneal metastatic disease in CRC often leads to palliative outcomes for these patients. More recently the development of the technique of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) is aimed at treating selected groups of PM patients [3, 4]. Patients with advanced CRC who had peritoneal metastases and had been treated with an aggressive surgical R0 resection [5] have been found to experience a recurrence rate of 79.1% and a 5-year overall survival of 36.2% [6].

The exact incidence of peritoneal metastasis (PM) in patients with CRC is not known due to limitations of current imaging modalities which rarely detect metastatic deposits less than

one centimetre in size. One prospective cohort group of 3019 CRC patients, who were surgically treated, reported a 13% rate of PM, where 61% were synchronous and 39% metachronous metastases demonstrated on imaging after initial treatment [7]. However, the overall frequency of involvement of the peritoneum in CRC dissemination is likely to be higher amongst the total CRC population.

Biology of the Peritoneum

Within the abdomen, peritoneum is split into two components, the parietal peritoneum and the visceral peritoneum. The parietal peritoneum lines the inner surface of the abdominal cavity, and the visceral peritoneum covers the visceral organs by integrating with the organs' outer serosal layers. The role of the peritoneum is of significance in maintenance of homeostasis within the abdominal cavity. Where pathology ensues, the equilibrium is disrupted leading to abnormal function. An understanding of both the anatomy and histophysiology of the peritoneum is crucial in developing targeted treatments for PM.

Peritoneal development begins in the fifth week of gestation. [8]. The peritoneum is derived from the mesodermal cells. The mesoderm splits in to three components; the lateral plate mesoderm, the intermediate mesoderm and paraxial mesoderm. The peritoneum develops from the lateral plate and separates, which separates again to form the visceral and parietal peritoneum (Figure 1) [9]. There are double layers of peritoneum, which form mesenteries to provide a pathway for both vessels and lymphatics to supply organs. The omentum is also formed from a double layer of peritoneum.

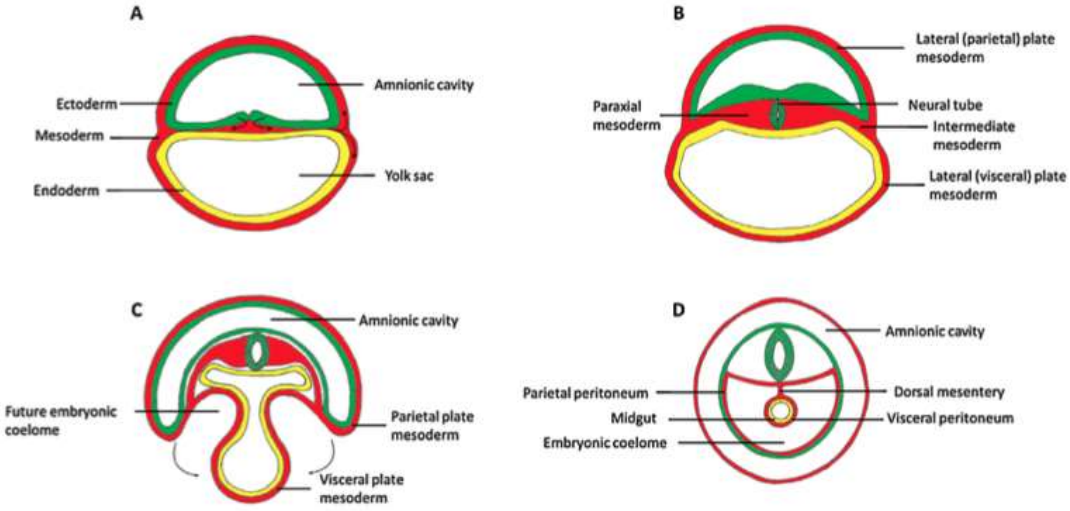

Figure 1. Cross section images of peritoneal development. Images taken from Van Baal et al (2016) [9]. A. Trilaminar embryo at day 17. Inward migrating cells sequentially form the endoderm, mesoderm and ectoderm. B. Day 19 embryo where the mesoderm differentiates into the lateral plate, intermediate and paraxial mesoderms. C. At day 22, the parietal plate mesoderm elongates to eventually enclose the endoderm. D. At week 4, the embryonic coelome is created, where the parietal peritoneum surrounds the embryonic cavity, and the visceral peritoneum surrounds the organs. Both are lined with mesothelial cells which are derived from the mesoderm.

The visceral and parietal peritoneum are both structurally similar and are composed of three fundamental layers, together with a fluid film which is a key component towards cellular functions.

- *Fluid film*

Sitting on top of the mesothelial cells is a glycoprotein called a glycocalyx, which serves to trap fluid and create a stagnant fluid layer. This layer is made up of proteoglycans and glycosaminoglycans (GAG). The Hyaluronan family is the predominant GAG, which plays a significant role in cell signalling and diffusion at the peritoneal surface [10, 11]. The fluid film can influence both cell-cell adhesion and de-adhesion. It can serve to regulate tissue function including proliferation and locomotion of cells [10]. Hyaluronic acid (HA) is synthesised by single transmembrane HA synthase (HAS) along the inner aspect of the plasma membrane. HA is extruded through the plasma membrane to the extracellular

matrix (ECM) to form long large molecules. For a matrix to form on the cell surface it needs to be anchored to the cell, often mediated by CD44 or chains of HA that are still attached to the HAS molecules. Retaining the fluid film at the cell surface allows capture and incorporation of HA binding molecules into the immediate cell environment[12]. There are three classes of HAS gene (HAS 1-3) [13, 14] - HAS1 & HAS2 produce high molecular weight HA (>300Kda) and HAS3 synthesises low molecular weight HA.

HA is able to influence hydration, biomechanical properties and homeostasis of different tissues. The full extent HA plays in physiological functions is still not completely understood. However, it has been described to have multifunctional roles including matrix/scaffold role, water balance and osmotic buffering, lubrication between tissues, scavenger of free radicals. HA has been shown to also play a role in cell regulation, including cell proliferation, locomotion, protection and wound healing [15, 16].

Degradation of HA is thought not to be completely possible through extracellular mechanisms alone. However, it has been demonstrated that intracellular mediated uptake of HA facilitates complete break-down via delivery to intracellular lysosomes by binding to molecules with HA binding receptors[17]. HA has a high, but variable, turnover rate, ranging from a few hours to a few days in most tissues. Intestine modelling has predicted a half-life in the peritoneum of 0.1-1.2 days [18].

- *Mesothelial Cells*

This is a monolayer of mesothelial cells which have both epithelial and mesenchymal characteristics. These cells are located covering the entire area of the three serosal surfaces of the body cavities in humans - the pericardium, the pleura and the peritoneum. In males, it also surrounds the testis. The cells have a central round or oval nucleus and are approximately 25μm in diameter. These cells are able to adapt their functional and structural characteristics under different conditions. The majority of mesothelial cells are structured in a squamous flattened arrangement. However, under certain conditions or regions within the peritoneum, such as the 'milky spots' of the omentum, the peritoneal side of the diaphragm and the parenchymal organs, the arrangement of cells is predominantly cuboidal [19, 20]. The cells have been shown to play roles in fluid and cell

transport, inflammation, tissue repair, lysis of fibrin deposition, a protective barrier and a frictionless interface for organs and tissues [21, 22].

- *Basal lamina*

The role of the basal lamina is thought to support the mesothelial cells at the basal surface. It is made up of an extracellular matrix composed of a mixture of collagens, with type IV collagen and laminin predominately. The binding of mesothelial cells to the basal lamina is weak and detachment regularly ensues following minor trauma [9, 23].

- *Interstitium*

The interstitium is also called the sub-mesothelial stroma. It provides further support to the peritoneal structure particularly with type I collagen fibres. Fibronectin, proteoglycans, GAGs, fibroblasts, adipocytes, lymphatics and blood vessels are present within this structure. The interstitium is also a readily available source of immune cells which are activated in various pathologies [24].

Physiology

Peritoneal fluid is in constant contact with the peritoneum, which circulates within the abdominal cavity. The fluid is composed of water, electrolytes, cells and proteins. Molecules in the fluid are able to enter or leave via transudation, exudation or through the lymphatics. In response to injury not only do mesothelial cells heal from the wound edges, but they have also been shown to demonstrate the ability to detach from distant sites, migrate and settle on a site of mesothelial injury [21]. The concept of free floating mesothelial cells in the peritoneal fluid is thought to increase the speed of repair of injured sites [25]. The peritoneum contains surface microvilli [26] and as such covers a large surface area approximately $140m^2$ (+/-$80m^2$) [27, 28].

<u>Peritoneal metastases in colorectal carcinogenesis</u>

The development of PM in CRC is thought to be caused by four principal mechanisms:

- Direct invasion
- Intraperitoneal seeding
- Lymphatic spread
- Haematogenous embolic dissemination.

Peritoneal seeding involves individual or collections of malignant cells detaching from the primary tumour and gaining access to the peritoneal space. This is a multistep process. Once detached from the primary site, these cells are able to be transported along the predicable physiological routes, which are responsible for clearance of fluid from the peritoneal circulation. It has been reported that the distribution of peritoneal metastases is dependent on the primary location of the tumour, its subsequent circulation of spread and the nature of the metastatic deposits, with malignant mucinous ascites having different peritoneal surface metastatic patterns [29].

Following initial adhesion to the peritoneum, invasion through the peritoneum is required to enable the final process of colonisation of the cancer cells to form metastatic tumours. Adhesion is a multifactorial process, where the ability to attach to the peritoneum is dependent on both the biological properties of the cancerous cell and the biological conditions of the tissues. This is termed the so called 'soil and seed' mechanism.

Tumours are able to exploit the natural mechanisms of cell-cell interaction and physiology within the peritoneal cavity to promote distant spread at various stages of the soil and seed mechanism of spread, stromal interactions within the tumour microenvironment can enhance the chance of tumour cells adhering and proliferating at a distant site.

Two mechanisms of attachment to the peritoneum have been described in the process of intraperitoneal seeding. The first is via trans-lymphatic metastasis (TLM) and the second being trans-mesothelial metastasis (TMM) [30, 31]. In TLM, free tumour cells find access to the sub-mesothelial lymphatics through openings at the junctions of mesothelial cells, called lymphatic stomata (LS). LS serve as drainage channels for ascitic absorption of fluid and cells from the peritoneal cavities. They can be found on the greater omentum, falciform ligament, mesentery and throughout the peritoneal lining of the abdominal cavity. In TMM,

free tumour cells are believed to exploit the mesothelium expressing a distinct pattern of adhesion receptors, which are thought to be part of leukocyte migration in peritoneal inflammation. Cytokines released in this reaction to peritoneal inflammation include tumour necrosis factor-α (TNF-α), interleukin (IL)- 1β, IL-6 and interferon-γ which have been seen to create a beneficial environment for tumour cells interacting with mesothelial cells. This cytokine release increases the expression of some cell adhesion molecules including vascular-cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), allowing for more readily available mesothelial cell - tumour cell interaction [32, 33].

Mesothelial cell-tumour interaction is theoretically split into the following steps (figure 2):

1. *Detachment, local invasion and entry into the peritoneal cavity*

Mechanisms of shedding of cancer cells from a primary colon cancer mass can either be spontaneous or iatrogenic. Spontaneous causes can include downregulation of cell-cell adhesion molecules within the tumour. Incomplete resection or breech of tumour integrity are potential causes of iatrogenic detachment. Cells, once free, are then able to be transported around the peritoneum utilising the peritoneal circulation. Cells are normally unable to survive following detachment since they require anchorage to the ECM and surrounding cells in order to function optimally. Anoikis is a type of programmed cell death to prevent tissue proliferation when anchorage dependent cells become detached and are unable to continue normal cell-cell signalling. One mechanism described in cancer cell survival is the aggregation of cells into clusters, which when detached are able to continue to survive and grow thus reducing apoptotic anoikis mechanisms [34].

2. *Adherence to peritoneal surface*

These detached malignant cells adhere to mesothelial cells of the peritoneum through interaction with adhesion molecules. Inflammatory mediators have been reported to promote expression of adhesion molecules and increase the propensity for peritoneal carcinomatosis [35, 36]. HA coating the mesothelial cells has also been described to facilitate cell-cell adhesive properties.

3. Invasion of the peritoneum

Access to the sub-mesothelial layers of the peritoneum is thought to occur where disruptions of mesothelial continuity occurs: Surgical trauma could be an iatrogenic cause of such disruption. Alternatively, cancer cells themselves may cause disruption to the mesothelial layer, through inducing apoptotic mechanisms of mesothelial cells [37]. Another theory is that the changing shape and rounding of mesothelial cells in response to inflammation and cytokine expression is thought to expose the basement membrane making it susceptible to invasion by malignant cells [38].

4. Colonisation and angiogenesis

Metastatic tumour deposits, once they have breached the mesothelial layer, are able to proliferate through the production and utilisation of growth factors [39, 40]. In order to enrich tumour cells with nutrients and oxygen delivery, tumour cells are able to produce angiogenic promoting molecules that induce angiogenesis in the patient and bring a blood supply to these tumour cells [41, 42].

All of these steps are regulated by multi-molecular receptor interaction and signalling mechanisms which facilitate the process of peritoneal spread.

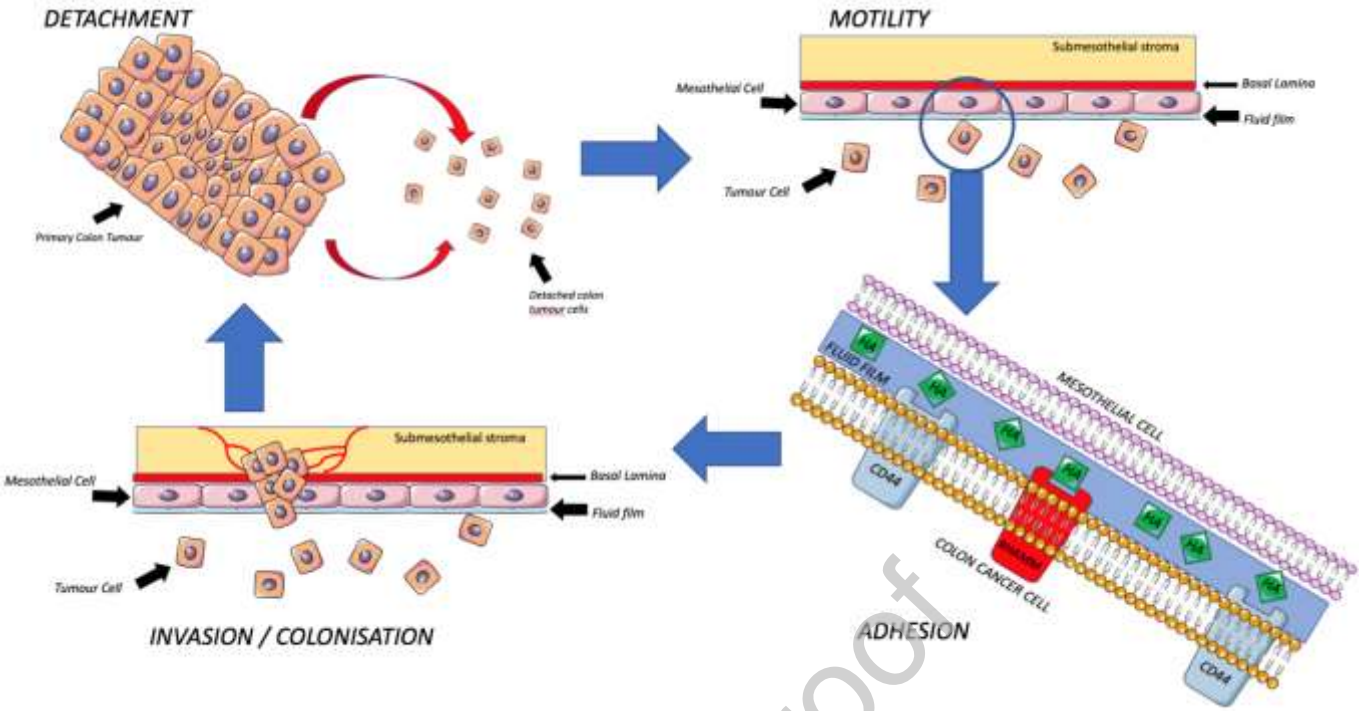

Figure 2. Cycle of peritoneal metastatic dissemination. Image created using SMART Sevier Medical Art Package.

Adhesion in CRC

The process of cellular adhesion in PM is achieved utilising cellular adhesion molecules which are able to interact with distant sites. Adhesion molecules can either be independent adhesion molecules or important in a cascade of activation of adhesion molecules. Broadly speaking in the context of PM, adhesion can be split into HA-dependent adhesion and HA-independent adhesion.

HA has been shown to interact with three cell surface receptors, namely CD44, RHAMM and ICAM-1 [43-45]. In the context of CD44-HA-dependent signalling, two CD44 molecules are required to be cross-linked to facilitate interaction with other signalling proteins. Studies looking into comparing the effects of expression of molecules following the addition of HA have seen upregulation of CD44 [46, 47]. Other studies have reported that the introduction of HA causes upregulation of downstream signalling cascades, and therefore it may be a key effector of particular signalling mechanisms [48, 49].

CD44

CD44 is a transmembrane glycoprotein that was first found to have cellular adhesion and homing functions on lymphocytes [50]. It has since been found to have multifunctional responsibilities within most human tissues both due to multiple binding sites on the protein molecule and isomer variants [51]. It has also been shown to be involved in cell migration, extravasation, proliferation, haematopoiesis and immune cell modulation [52, 53]. CD44 is encoded on the short arm of chromosome 11 [54] and can produce variations of the protein due to the combination of constant and variable exons within the sequence. There are 10 constant exons and 9 variable exons. The most common isoform of CD44 is CD44 standard (CD44s), in which there are no variable exons. In humans, there are nine exons (CD44v2-10) involved in the different splicing during synthesis of CD44 mRNA strands. These isomers can be generated by an alteration in splicing involving one or more of the nine variant exons, thus allowing for a large number of possible variant isomers to be produced (figure 3).

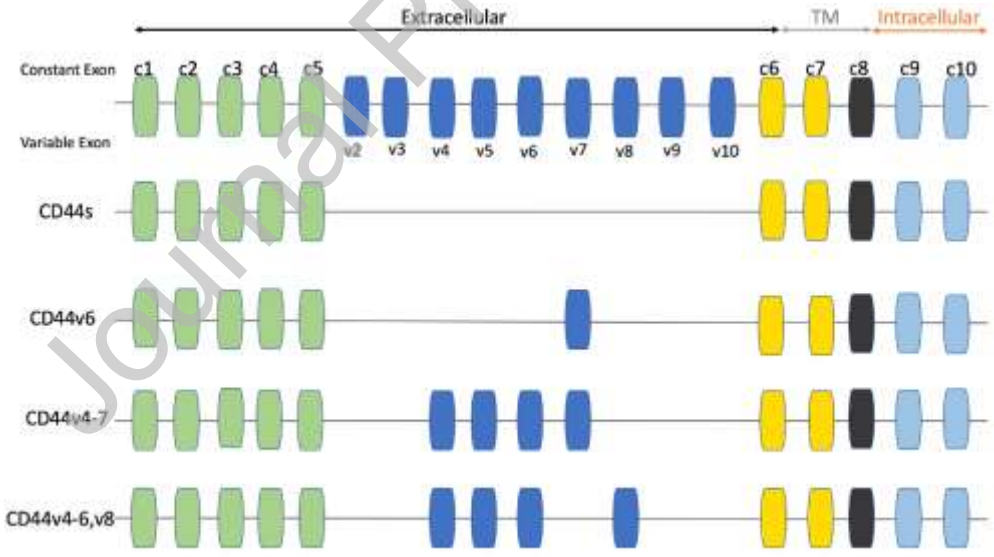

Figure 3. Example variant isoform combinations of CD44. Image adapted from Sneath and Mangham (1998) [50]

In normal colonic tissue, CD44s has the strongest expression whilst CD44v6, CD44v7, CD44v8, CD44v7&8, CD44v7&9, CD44v7, CD44v8&v9 all have moderate expression [55].

In the early 1990s, it was demonstrated that metastatic potential could be transferred to a non-metastatic pancreatic cell through transfection of a CD44 variant [56]. The work involved initially identifying membrane proteins on metastatic rat adenocarcinoma with monoclonal antibodies. The antibodies were screened against a bacterial cDNA library, where a CD44v was found to be encoded. The cDNA was transfected to normal pancreatic rat cells which did not express this particular CD44v. The normal cell line, now transfected, was found to have gained metastatic properties when injected into rats. Expression of CD44s or certain isoform variants of CD44 in CRC may be a potential biomarker in predicting risk of peritoneal metastases in patients, and could alter treatment strategies of such patients in terms of personalised therapies.

Receptor for Hyaluronic Acid Mediated Motility (RHAMM)

RHAMM, also called CD168, was originally identified on murine fibroblasts and fibrosarcoma cells. It is located on chromosome 5q33.2, where alternative splicing of its mRNA produces four known isoforms of the protein [57]. Initially it was described as a protein involved in cell locomotion [58], however today it is known to be implicated in many other functional roles at cellular level. It is found on the cell surface, the cytoplasm and the nuclei of various types of cell. In normal tissue, RHAMM has been found to be responsible for cell signalling cascades, cell cycle progression and expression of genes regulating the extracellular matrix. Interaction with HA has been shown to induce tyrosine phosphorylation of cellular proteins including focal adhesion kinase ($p125^{FAK}$) in fibroblasts [59-61]. In CRC, it is implicated in unfavourable prognostic outcomes and also thought to contribute to metastatic dissemination.

Intracellular adhesion molecule 1 (ICAM-1)

There are five ICAMs which are a subfamily of the immunoglobulin superfamily of cell adhesion molecules. Increased expression of ICAM-1 has demonstrated increased adhesion of malignant cells in CRC cell lines [62, 63]. ICAM-1 is a single chain protein 80-114kDa with a core polypeptide structure of 55kDa. ICAM-1 has been reported as a binding receptor for

HA. The molecule has been found to share some basic amino acid clusters similar to both CD44 and RHAMM [43]. Interestingly, the literature on ICAM-1 is mixed Although downregulating ICAM-1 expression has been shown to reduce CRC cell adhesion [32], it has also been demonstrated that ICAM-1 negative tumours have a higher rate of metastasis to liver and lymph node than ICAM-1 positive CRC [64]. Involvement of other adhesion molecules in the enhanced dissemination of ICAM-1 negative CRC tumour cells is yet to be explored.

CD44 and Peritoneal Metastases in Colorectal Cancer

HA binding sites in the extracellular domain of CD44 have been identified through truncation mutagenesis techniques[65]. CD44 and HA binding has been reported to be stabilised by disulphide bonding, where cysteine residues have been found to be important components of binding [66]. One study has demonstrated residues Cys77 and Cys97 have been identified as one such bond. Destabilising or preventing such bonding from occurring through redox reactions have been shown to destabilise CD44/HA interaction [67].

Expression studies have demonstrated CD44 and its isoforms to be present in CRC, with overexpression being associated with aggressive metastatic CRC phenotypes [68]. Tumour suppressors, such as NDRG1 (N-Myc downstream-regulated gene 1), which regulate CD44 expression, have been shown to affect the degree of dissemination of PM in certain CRC cell lines [69]. However, it has been observed that CD44 expression appears to be independent of P53 mutations in CRC [70]. Certain isoforms of CD44, such as CD44V6, seen in CRC, have been shown to have increased preponderance for metastatic spread [71-75].

CD44 crosslinking and fragmented HA has been shown to upregulate adhesive molecules and expression of integrins to enhance adhesive properties to endothelial cells. Fujisaki, Tanaka [76] demonstrated c-MET expression was also increased and was able to enhance integrin-facilitated adhesion of CRC cell lines. They suggested that the expression of CD44 created an adhesion cascade for CRC cells to facilitate metastasis.

There is potential that inhibitors which can disrupt CD44/HA interaction have the potential to affect various disease processes, in which they have been demonstrated to play a role [77, 78].

RHAMM and Peritoneal Metastases in Colorectal Cancer

The HA binding domain on RHAMM was first discovered by Turley and their interaction researched by her research group [79-83]. The two binding domains found on RHAMM have been shown to contribute equally to the HA binding ability of this protein. A common HA binding protein motif has been demonstrated in both CD44 and HA, shown through site-directed mutagenesis[84].

Disease progression in many cancers has been linked with overexpression of RHAMM. RHAMM expression has even been ranked as a highly significant prognostic factor in terms of predicting overall outcomes. In CRC, higher expression of RHAMM is correlated strongly with an overall poorer prognosis [85]. Literature speculation describes RHAMM as being responsible for increased motility and invasion of metastatic cells and a significant factor driving metastatic spread [86].

Increased surface expression of RHAMM has been shown to be amplified through the addition of HA. RHAMM overexpression has demonstrated increased ability of metastatic cells to migrate and colonise distant secondary sites. Tumour buds, which are clusters of cells found at the invasive fronts of tumours, are seen as a more aggressive subpopulation of cells within a tumour. In CRC, RHAMM positivity within tumour budding cells has been associated with more aggressive tumour histopathological features [87]. Conversely silencing of RHAMM expression has been shown to decrease tumorigenicity within CRC cells both in-*vitro* and in-*vivo* [86].

Colorectal Cancer and Anoikis

If CRC cells are impeded long enough to prevent attachment to host cells, such as through the use of a competitive inhibitor, anoikis evasion may be disrupted and CRC cells will fail to

survive in the peritoneal cavity environment. The KLKs (Kallikrein-related peptidases) and V-Src (V-Sarcoma viral oncogene homolog) family molecules have also been implicated in cell-cell dependent clustering of CRC cells and resistance to anoikis [88]. It may be possible that inhibition of CD44-HA interaction may not only affect adherence to distant tissues, but also affect the cell-cell adhesive properties of the CRC cells themselves. While the cells may not induce anoikis spontaneously, delayed adhesion in the peritoneal microenvironment could increase free-floating cell vulnerability to targeted treatment with multimodal therapy.

Targeting HA-mediated interaction in malignancy

Although not specifically targeting HA-dependent adhesion molecular interaction, there are two ongoing trials which describe utilising HA to enable increased delivery of chemotherapeutic agents in metastatic CRC [89-91]. Both trials are from the same group, in Australia, examining the delivery of Irinotecan which is used as a chemotherapeutic agent in metastatic colorectal cancer. Irinotecan is combined with HA in the treatment of metastatic CRC. The drug delivery platform is based on the use of HA as a novel excipient in which formulation of Irinotecan with HA results in optimisation of cytotoxic drug uptake and retention within solid tumours. Early studies have shown enhanced efficacy in both non-clinical and early clinical studies. The first is a phase II single arm trial of FOLF(HA)iri plus cetuximab in irinotecan-naïve second line patients with KRAS wild type metastatic CRC [90]. The second is a randomised double blind phase III trial comparing FolF(HA)iri versus standard FOLFIRI chemotherapeutic regimens for second- or third-line therapy in Irinotecan-naïve patients [91].

Cisplatin based chemotherapeutic regimens have formed the basis of treatment of a number of cancers including colonic, gastric and ovarian cancer. PIPAC (pressurised intraperitoneal aerosolised chemotherapy) has been a fairly new novel treatment modality option for palliative patients with metastatic intraperitoneal disease. Modifying the chemotherapeutic aerosol to exploit the mechanism of CD44 over-expression on many tumour types in combination with CD44-hyaluronic acid interaction has led to the use of HA-

linked cisplatin in some PIPAC regimens, in an attempt to more effectively enhance the chemotherapeutic effect on tumour cells [92].

In Vivo Xenograft models studying ovarian cancer have demonstrated that targeting CD44-HA interaction, using a monoclonal antibody targeting CD44, can affect tumour binding [93]. To date there has been one in-human phase I clinical trial published, using a monoclonal antibody (mAb) specifically targeting CD44-HA in advanced solid tumours. Whilst this study was not targeting peritoneal spread of GI tumours, it is relevant to mention simply due to being the only human trial targeting the specific CD44-HA mechanism of interaction. The antibody selectively binds near the HA-binding region of all CD44 isoforms. Solid tumours including colorectal, thymus and skin primary malignancies demonstrated some modest tumour shrinkage in primary and distant disease, but no overall significant response. Patient response was evaluated through FDG-PET (flurodeoxyglucose-Positron emission tomography) imaging. However, the study was terminated due to no evidence of clinical or pharmacodynamic dose-response relationship. There were no safety concerns identified with the treatment [94]. The effects could possibly have been negligible in this particular study due to assessing the effects on established solid tumours. It may be more appropriate to attempt to exploit disruption of distant site binding by targeting this CD44-HA interaction, in the adhesion phase of the metastatic model. Many clinical trials, examining drug response to novel agents, assess effect on established advanced solid tumours, in what are in effect palliative patients. The timing of the cellular interaction and treatment initiation, which affects a different phase of the metastatic model, is crucial to take into account. For example, adjusting the study following complete cytoreductive resection of peritoneal tumours and administering mAb targeting CD44-HA against a placebo. Alternatively assessing the effect of resection of a perforated or T4 tumour to the initiation of mAb treatment targeting CD44-HA interaction and assessing for local recurrence could be a complementary study approach to such novel therapy.

Conclusions

It could be argued that current radical surgical and chemotherapeutic regimens, such as cytoreductive surgery, HIPEC or even PIPAC (Pressurised intraperitoneal aerosolised chemotherapy) regimens may potentially affect cell-cell interaction, and also affect the

peritoneal tumour microenvironment. It could be hypothesised that these changes may also affect cell-cell interaction of the natïve host tissue and molecules interacting with the peritoneum. HA is a molecule not only involved with the peritoneum ,but found throughout the body playing a role in many cellular interactions. It has been demonstrated that the extent of parietal peritonectomy in cytoreductive surgery does not influence or change the pharmacokinetics of chemotherapy drug concentration and is not directly related to plasma absorption of chemotherapeutic drugs [95]. Non-peritoneal cells, which could become exposed within the intrabdominal cavity, following such treatments, are likely to interact with both chemotherapeutic drugs, as well as free-floating cancer cells in a similar fashion to that of the peritoneum due to cross over of cell surface receptor molecules. Cell plasticity and ability to adapt to changing environments through upregulation or down regulation of cell surface receptors may also contribute to this.

Targeting HA dependent adhesion may have the potential to prevent peritoneal metastatic disease in CRC. Targeted immediate disruption of CRC-HA interaction at the time of cancer surgery, which could be utilised where macroscopic disease has been resected but where there is a risk of micro-metastases. Locally advanced or perforated colorectal tumours are highly likely to allow malignant cells to enter the peritoneal cavity. Prevention of adhesion and CRC-HA interaction could lead to reduced ability of cells to attach to distant tissues (such as the peritoneum). Disruption of adhesion dependent CRC cells could promote tumour cell anoikis and is a potential mechanism to explore for new treatment modalities. Facilitating conditions for tumour cells to be more vulnerable in a free-floating state, could allow optimisation of conditions toward multimodal therapy. A more complete understanding of how HA influences cellular interaction with CRC tumour cells may be a key influence in preventing or reducing the burden of peritoneal metastatic disease.

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Authors' Contributions

FS designed, drafted and edited article. LY, WG and RH designed, edited and were senior authors.

Competing interests

None declared

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