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Review

The intervention of immunoglobulin superfamily cell adhesion molecules in the progression of colorectal cancer and liver metastasis

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SUMMARY

Colorectal cancer (CRC) is a common malignancy which presents a high metastatic potential toward the liver. The hepatic colonisation determines the prognosis of the disease and cell adhesion molecules play a critical role in this process. The immunoglobulin superfamily includes numerous proteins that mediate adhesion among malignant and normal cells in the primary site of CRC, but also in the hepatic metastases. These molecules intervene in cell detachment, cancer progression, intra- and extravasation, as well as attachment within the liver sinusoids. Carcinoembryonic antigen, probably the most popular member of the immunoglobulin superfamily, is a well-established prognostic clinical factor of particular value. Current research attempts to exploit these adhesion molecules in new diagnostic and therapeutic applications.

Keywords: carcinoembryonic antigen, cell adhesion molecule, colorectal cancer, immunoglobulin superfamily, liver metastasis.

INTRODUCTION

Colorectal cancer (CRC) is the third most common form of malignant tumours and affects about 650000 people worldwide. The patients are mainly of advanced age, as half of them are older than 70 years; cases before the age of 50 are sparse, unless hereditary pattern occurs. CRC is the second leading cause of cancer-related death in the "developed world", killing around 205000 individuals in Europe every year[1-3]. Interestingly, the prognosis and the overall life expectancy of the disease are predominantly determined by the progression of metastatic lesions and not by the primary carcinoma. The liver constitutes the main host organ for colorectal metastasis and despite the progress in diagnostic modalities, over 25% of patients present metastatic hepatic lesions at the time of initial diagnosis. Surgical resection still remains the best therapeutic approach, although only one third are potentially resectable metastases. Curative resections may achieve five year survival exceeding 50%. However, if colorectal liver metastases remain untreated, life expectancy is less than a year.[4-6]

The progression of CRC and the development of hepatic lesions is a long not completely understood process. Malignant cells initially provoke a breach in the basement membrane, reach blood vessels and intravasate. In the systemic circulation, if they survive the mechanical pressure, collisions with other cells and the attacks by immune cells, they may reach the portal vein, which is the gateway to the hepatic sinusoids. The latter are specific capillaries which form a dense network, where CRC cells interact with multiple resident cells, in an attempt to colonise the liver.[7,8]

The maintenance, promotion or disruption of cell adhesion is crucial for CRC outgrowth and liver infiltration. Numerous cell adhesion molecules are implicated not only in the proliferation and detachment of malignant cells from the primary carcinoma, but also in their attachment in distant tissue.[9] Immunoglobulin superfamily cell adhesion molecules (IgSFCAM)s mediate multiple aspects of CRC initial progression and are suggested as important diagnostic factors. Furthermore, they are present in

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important cellular interactions within the sinusoids, regulating CRC cell arrest and extravasation in the liver. IgSFCAMs or their receptors are expressed either on malignant cells or on non-parenchymal hepatic cells, such as Kupffer cells (KC)s, sinusoidal endothelial cells (SEC)s and stellate cells and thus stand in a molecular crossroad, substantially mediating the metastatic process.[10]

THE ROLE OF IMMUNOGLOBULIN SUPERFAMILY CELL ADHESION MOLECULES

The immunoglobulin superfamily includes multiple cell surface and soluble proteins, which mediate intercellular recognition, adhesion and binding. It consists of numerous subgroups, such as antigen receptors (e.g. immunoglobulins), growth factor and cytokine receptors (e.g. PDGFR, IL-1 and IL-6 receptor), tumour cell antigens (e.g. CEA), T-lymphocyte and natural killer (NK) cell receptors (e.g. CD4, CD8), as well as cellular adhesion molecules[11]. Interestingly, IgSFCAMs represent one of the most ancient and diverse groups of cell adhesion proteins, display an immunoglobulin-like structure in their extracellular part and either regulate homophilic and heterophilic cellular adhesion or interact with other cell surface proteins, such as the integrins (Table I).[12-14].

Intercellular adhesion molecules (ICAMs) comprise a subfamily of 5 members with a sequence homology of 30 to 50%. They are transmembrane glycoproteins, composed by 2 to 9 domains of the immunoglobulin superfamily, a hydrophobic transmembrane region and a short intracellular part.[16,22] ICAM-1 (CD54) is primarily expressed on venular endothelium and leukocytes, although under cytokine stimulation it may be traced on every human cell type.[15] ICAM-2 (CD102) is mainly located on endothelial cells, leukocytes and platelets and unlike ICAM-1, its expression is highly resistant to inflammatory agents.[23] ICAM-3 (CD50) presents morphological similarities with ICAM-1, is predominantly expressed on leukocytes and constitutes the only ICAM that significantly appears on neutrophils. ICAM-4 (CD242), is restricted to erythrocytes,[24] while ICAM-5, also termed telencephalin, is found on neurons of the central nervous system.[25]

Vascular cell adhesion molecule 1 (VCAM-1), also named CD106, includes 6 or 7 extracellular immunoglobulin domains, one transmembrane and a short intracellular domain. It is widely expressed on multiple cells, such as endothelial, thymic epithelial, spleen and bone marrow stromal cells, and peripheral lymph nodes. VCAM-1 is upregulated by several chemokines and cytokines, including interleukin 1 beta (IL-1β), IL-4, interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α), and serves as a ligand for $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins.[13,26] Notably, the co-expression of VCAM-1 with $\alpha_4\beta_1$ integrin on T cells in neoplasms may cause T-cell migration away from the latter, resulting in limited accumulation of immune cells in the tumour microenvironment. Under these circumstances, VCAM-1 overexpressing neoplasms may present a higher ability to escape immune surveillance and attack.[17]
Platelet endothelial cell adhesion molecule (PECAM-1) or CD31 belongs to another IgSFCAM subfamily. While primarily expressed on the endothelium, it is also evident on every cell of the vascular circulation, including leukocytes, mast cells and platelets. PECAM-1 plays a critical role as an adhesion and molecular signalling mediator in angiogenesis, thrombosis, endothelial cell response to fluid shear stress and leukocyte migration.[27,28]

Immunohistochemical studies reported a low or non-expression of ICAM-1 on normal colon epithelial cells, in contrast with vascular endothelial cells and the extracellular matrix (ECM). Increased expression was revealed on colon cancer cells and well-differentiated tumours showed the highest levels. It was also discovered that endothelial cells of small CRC blood vessels expressed significant levels of ICAM-1 and VCAM-1.[29,30] These two CAMs are also produced by hepatic stellate cells and this production is increased under the influence of cytokines like IL-6.[31] This last observation is considered quite important, how-

Table I. Immunoglobulin superfamily cell adhesion molecules (IgSFCAM)s with potential prognostic value in colorectal cancer

IgSFCAMs	Members mediating colorectal cancer progression	Expression
ICAMs15,16	ICAM-1 (CD54), ICAM-2 (CD102), ICAM-3 (CD50), ICAM-4 (CD242), ICAM-5 (telencephalin)	Endothelial cells, fibroblasts and leukocytes
VCAMs17	VCAM-1 (CD106)	Endothelial and epithelial cells
PECAMs18,19	PECAM-1 (CD31)	Endothelial cells, leukocytes and platelets
CEACAMs20,21	CEACAM5 (CEA), CEACAM1 (BGP)	Colon epithelial cells

BGP: Biliary GlycoProtein, CEA: CarcinoEmbryonic Antigen, CEACAM: CarcinoEmbryonic Antigen Cell Adhesion Molecule, ICAM: InterCellular Adhesion Molecule, PECAM: Platelet Endothelial Cell Adhesion Molecule, VCAM: Vascular Cell Adhesion Molecule

ever there are no experimental results referring to colorectal metastasis.

Holubec *et al* investigated the diagnostic and therapeutic value of IgSFCAMs in patients with Dukes' stages B-D colorectal cancer. They concluded that ICAM-1 and VCAM-1 were significantly increased in primary tumours, in cases of distant metastases irrespective of localisation.[32] Similar results were reported in a Greek study, which suggested that these molecules had a significant predictive value in the chemotherapeutic outcome of advanced disease.[33] Furthermore, a clinical trial on the relationship between serum concentration of soluble ICAM-1 and CRC stage, advocated that this adhesion molecule is an independent prognostic factor for stage II of the disease.[34]

On the contrary, clinical studies assessing various tumour markers in the prognosis of CRC concluded that both ICAM-1 and VCAM-1 present no significant difference between early and advanced-metastatic stage of the disease[35] and did not contribute to Dukes' classification, referring to node and liver invasion.[36] On the other hand, a recent meta-analysis of published research concerning microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in colorectal cancer prognosis, revealed that among 10 studies from 1995 to 2002, survival was inversely related to MVD, when the latter was assessed with PECAM-1; therefore this adhesion molecule was recommended as a reliable marker of angiogenesis which occurs in the onset of colon carcinogenesis and metastasis.[37] In conclusion, while accumulating evidence suggests the clinical value of ICAM-1, VCAM-1 and PECAM-1 in colorectal cancer progression, further investigation through larger, better designed and preferably multicentric studies, appears necessary in order to reach sound conclusions.

The molecular role of IgSFCAMs also attracts great interest concerning colorectal liver metastases. Experimental data suggested that CRC cells trigger murine KCs, the macrophages that predominantly reside the liver, which produce inflammatory cytokines like TNF-α within the hepatic sinusoids; subsequently, these molecules stimulate SECs to express high levels of ICAM-1 and VCAM-1[38] or VCAM-1 and PECAM-1.[39] These adhesion molecules mediate CRC cell adhesion, follow the expression of E-selectin (an adhesion molecule of the selectin family mainly expressed on endothelia) and support subsequent extravasation. Moreover, *in vitro* experiments on mice with KCs indicated that these hepatic macrophages may secrete cy-

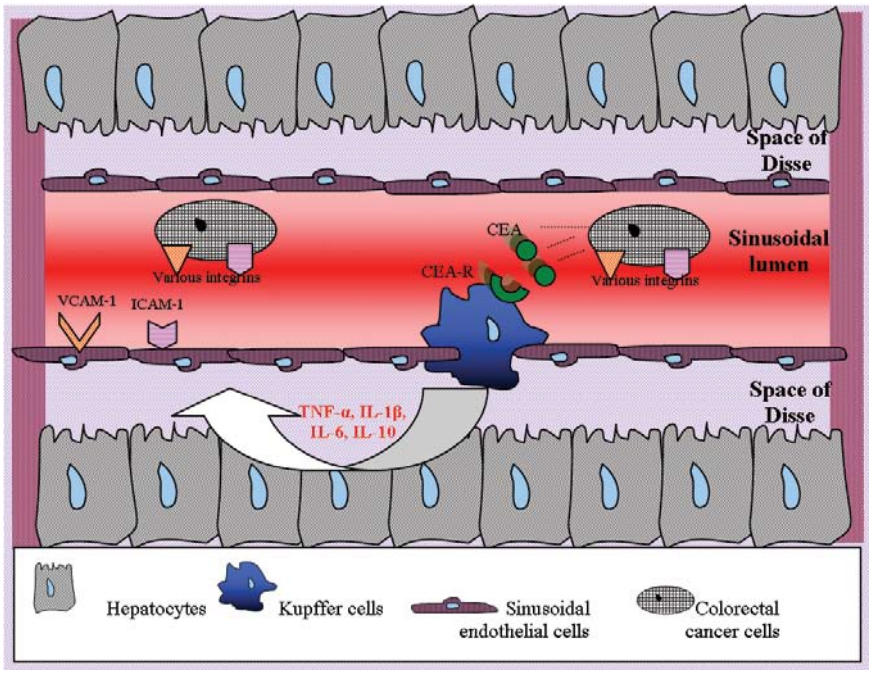

Figure 1. Kupffer cells are activated by CEA, produce numerous cytokines and stimulate sinusoidal endothelial cells to secrete adhesion molecules of the immunoglobulin superfamily, thus mediating colorectal cancer cell arrest.

CEA: Carcinoembryonic Antigen, CEA-R: CEA Receptor, ICAM-1: Intercellular Adhesion Molecule 1, IL-1β, -6, -10: Interleukin 1 beta, -6, -10, TNF-α: Tumour Necrosis Factor alpha, VCAM-1: Vascular Cell Adhesion Molecule 1.

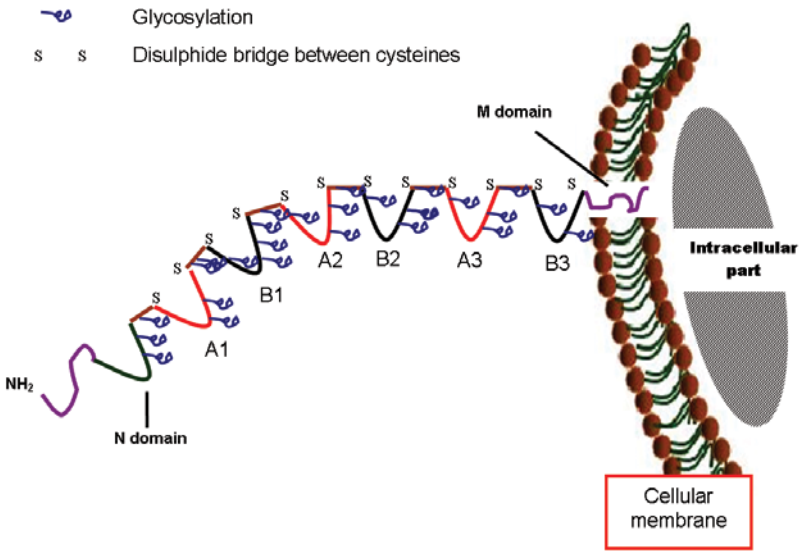

Figure 2: The molecular structure of CEA protein. It consists of the N domain and three repeated domains (1-3), divided into two subdomains (A and B). Each domain includes four cysteine residues, which in pairs form A and B "loops". The "loops" are stabilised through disulphide bridges between cysteines. CEA is anchored to the cellular membrane by a hydrophobic C-terminal region (M domain).[21,46]

tokines under carcinoembryonic antigen (CEA) activation, which stimulate endothelial cells to express adhesion molecules, including ICAM-1 and VCAM-1.[40,41] Another study on human liver tissue, received through partial hepatectomy from patients with gastrointestinal cancer liver metastases, also declared that gastric and colorectal cancer promoted an increased sinusoidal expression of ICAM-1 and VCAM-1.[42] Consequently, several lines of evidence support the hypothesis that CEA may trigger KC activation and lead to the production of cytokines, which in turn stimulate SECs to express IgSFCAMs members that bind to colorectal metastasising cells and cause their arrest within the sinusoids (Figure 1). Then, malignant cells may extravasate and invade the hepatic parenchyma, succeeding liver colonisation. However, as these experiments were performed mainly on rodents and human umbilical vein endothelial cells (HuVEC)s, further research on human liver tissue is necessary, in order the above theory to be confirmed. Notably, the preceded experimental work has already guided therapeutically oriented studies and cyclooxygenase-2 inhibitors, such as celecoxib, exerted down-regulatory effects on ICAM-1 and VCAM-1, affecting endothelial adhesion of CRC cells.[43,44]

The Carcinoembryonic Antigen

CEA is an oncofoetal antigen expressed in human colon cancer, was first described by Gold and Freedman and belongs to a family of CEA-related proteins.[45] Contemporary techniques discovered 29 different genes in humans, forming three subgroups: the CEA with 12 members, the pregnancy specific glycoprotein (PSG) with 11 and a third one with 6 members. The first subgroup, includes among others carcinoembryonic antigen cell adhesion molecule 5 (CEACAM5) gene, which controls the synthesis of the clinically-used CEA protein on the apical surface of gastrointestinal epithelia.[20,21]

CEA is a 180 kDa cell surface glycoprotein, belongs to the immunoglobulin superfamily (Figure 2) and is expressed in squamous epithelia of the tongue, oesophagus and cervix, in columnar colon epithelium, in mucous cells of the stomach, in ducts of sweat glands and in prostate.[46,47] It is localised on the apical luminal surface of mature colonocytes in normal human colon and is released in large amounts, approximately 50-70mg per day, through the faeces. More differentiated normal or malignant cells express higher CEA levels.[48-50] CEA can also be expressed throughout the cellular surface of the colon adenocarcinoma cells. During cancer progression, proliferating CRC cells invade the basal lamina and cellular membrane particles enter the systemic circulation through adjacent lymph or blood vessels. As CEA is highly expressed on malignant cell membrane, its serum concentration substantially increases. While the tumour is developing, higher CEA values may be detected in the blood[21] (Figure 3).

When this glycoprotein was initially used in CRC di-

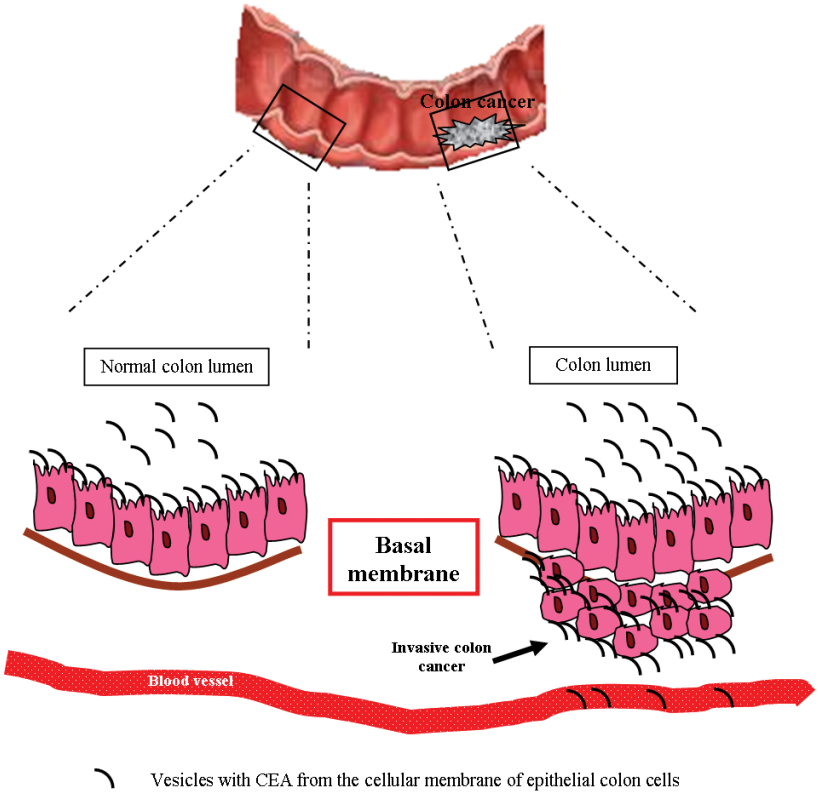

Figure 3. Excretion of CEA in normal and infiltrated colon. In normal tissue, polarised epithelial cells express CEA on the apical surface, which is released only in the lumen. However, epithelial cells in the deep layers of colon cancer are unpolarised and express CEA around their surface. When the basal membrane is invaded, exfoliated CEA reaches blood vessels.

agnosis, almost 100% accuracy was enthusiastically reported. However, subsequent data limited CEA use as a diagnostic tool, due to low reliability. Experimental data revealed that its serum concentration is influenced by several co-factors, such as liver function, tumour burden and differentiation and liver function[10]. In addition, CEA levels are considerably increased in neoplasms of the gastrointestinal system,[51] respiratory tract,[52] breast[53] and pancreas,[54] but also in non-malignant pathologies, including cirrhosis[55], hepatitis, renal failure,[56] bronchitis and in smokers.[57,58] In current clinical practice, CEA is useful for the surveillance of stage II-III colorectal cancer patients, before and after curative liver resection, and for monitoring advanced disease.[59,60] A recent clinical study reported that tumour-expressed CEA was a significant prognostic marker of equal value with serum CEA in CRC patients.[61]

In normal tissue, carcinoembryonic antigen is unlikely to contribute to intercellular adhesion, as it is located toward the colon lumen on polarised cells. Moreover, it probably mediates the innate immune defence, protecting the colon and possibly the upper alimentary tract and the skin from bacterial colonisation. This notion is based on numerous observations: the CEA position facing the colonic lumen where the microbial burden is quite high, its abundant glycosylation which permits interactions with fimbriated bacteria and its regulation by inflammatory cytokines.[62,63]

Mounting evidence suggests that CEA intervenes in colorectal metastatic process, as its over-expression causes inhibition of cell differentiation,[64,65] disruption of cellular polarisation and distortion of tissue structure.[66] Furthermore, anoikis, an apoptotic process triggered when cell-ECM contact is poor or absent, is down-regulated by CEA over-expression. Recent data indicated that this phenomenon involves on the one hand TRAIL-R2 binding and signalling,[67] and on the other the glycoprotein clustering in conjunction with activation of $\alpha_5\beta_1$ integrin.[68]

Kupffer cells present an 80 kDa CEA receptor (CEA-R), classified as β-2 adrenergic, that mediates the degradation of the glycoprotein.[69,70] When CEA binds to this

receptor, KCs are activated and secrete large amounts of cytokines, such as IL-1β, IL-6, IL-10 and TNF-α.[69,71] These products interact with the sinusoidal endothelium, activate SECs which in turn express adhesion molecules of the immunoglobulin and other families, assisting tumour cell arrest and extravasation. Current research, including our group's, attempts to support this model, which may explain important aspects of CRC liver metastasis.[72] Nevertheless, studies on mice reported adhesion between either KCs or SECs with CRC cells, without immediate CEA intervention[73]. The same group revealed that CEA facilitates malignant cell survival via the induction of IL-10 and showed a subsequent decrease of nitric oxide (NO) concentration. IL-10 is probably produced by activated KCs and the NO decrease is caused by the inhibition of inducible nitric oxide synthetase (iNOS).[74]

Current research has been targeting toward therapeutic application of CEA vaccines. This immunotherapy was designed mainly to eradicate CRC cells and was tested on animal models with no adverse effects.[75,76] Unfortunately, a recent clinical study on the treatment of CRC liver metastases demonstrated no therapeutic value of these vaccines, as the recurrence-free survival was similar with hepatic resection's alone.[77]

CONCLUSIONS

IgSFCAMs are present in multiple molecular pathways during CRC progression and liver metastasis. They determine malignant cell proliferation, tumour burden and migration within the primary site and appear to be potentially valuable prognostic tools. On the other hand, they are expressed by multiple non-parenchymal hepatic cells, regulating their interactions with invading CRC cells.

Carcinoembryonic antigen, a well-established diagnostic marker, is also involved in molecular actions within the sinusoids, controlling the metastatic process. Current research investigates its influence on Kupffer cells and a popular theory, based on this interrelationship, attempts to explain the very early stages of colorectal liver metastasis.

Recently published data demonstrate that therapeutically oriented trials already test IgSFCAMs in patients with unresectable hepatic lesions. The initial results are still ambiguous and additional studies are necessary. However, it appears that these adhesion molecules play a pivotal role in primary and advanced CRC and therefore could be further exploited in the diagnosis and treatment of this lethal disease.

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