Carcinoembryonic antigen cell adhesion molecule1 (CEACAM1) in metastatic colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is the second most common cancer diagnosed in women and third most common cancer diagnosed in men. One in three deaths is observed among affected individuals. Colorectal liver metastasis (CRLM), is responsible for about two thirds of the deaths related to the disease. There is still no accurate or effective marker available for CRC. However, carcinoembryonic antigen (CEA) remains a standard marker used for monitoring metastatic disease responses to systemic therapy. Imaging modalities are still routinely used beside CEA, which requires further expenses and frequent follow-up visits. Thus, identifying a more accurate non-invasive marker for the diagnosis and the assessment of prognosis in CRC and CRCLM is of great significance.

Since cloning of CEA, a very large family of CEA related proteins has been uncovered. CEA has been grouped together with its paralogues under the CEA-related cell adhesion molecule (CEACAM) family, a subdivision of the immunoglobulin superfamily of cell adhesion molecules (IgCAMs). CEACAM1 was found to be one of the important members of the CEACAM family. CEACAM1 receptors are expressed on various mammalian cells, such as epithelial cells, endothelial cells, leukocytes and T cells. Recently, CEACAM1 was found to potentially modulate tumorigenesis and had variable expressions in different tumors. In CRC, CEACAM1 is thought to have a bimodal role. Loss of the expression of CEACAM1 was seen in early CRC and adenomas, which demonstrates a tumor suppression effect. Further studies showed that CEACAM1 is overexpressed in invasive CRC and is correlated with the clinical stage. More recently, CEACAM1 was found to be an independent risk factor for lymph node involvement, haematogenous metastasis and shorter patient survival. This signifies a promoting effect of CEACAM1 in advance disease. Nevertheless, studies investigating the expression of CEACAM1 in metastatic lesions are still lacking and the reason why CRC cells re-expresses CEACAM1 remains unclear.

Objectives: The hypothesis of this study states that the expression of CEACAM 1 is a prognostic factor for establishing the aggressiveness of CRC liver metastasis. Our objective is to identify the immunohistochemical expression of CEACAM1 in metastatic colorectal cancer by testing matched samples from primary and metastatic lesions. In addition, we aim to compare the expression of CEACAM1 to CEA in relation to relevant clinical factors.

Methods: The study included tumor tissue samples from 43 patients with colorectal liver metastasis following liver resection. Primary CRC tumor tissue was obtained from 18 patients out of 43. 92 hepatic lesions in total were analyzed from all 43 patients. Immunohistochemistry staining for CEACAM1 and CEA was performed using the Ventana BenchMark LT fully automated machine (Ventana Medical System Inc. Arizona, USA). Relevant clinical variables were analyzed in relation to CEACAM1 and CEA expression in the primary and metastatic tumors using Spearman's rank correlation or chi-squared statistical test.

Results: CEA expression in the primary tumor was found to be associated with lymph node involvement, disease staging and the number of metastatic liver lesions. High BMI was associated with lower hepatic expression of CEACAM1. Extensive lymph node involvement of the primary tumor was associated with loss of CEACAM1 expression in both the metastatic tumor and the background normal liver. Poorly differentiated metastatic liver tumors had high CEACAM1 expression. Finally, CEACAM1 apical staining was associated with progressive features of CRC.

Conclusion: Our study showed that CEACAM1 expression in CRC and CRCLM was associated with more relevant clinical variables compared to CEA, which may reflect an important modulatory role of CEACAM1 in CRC tumorigenesis. Therefore, CEACAM1 may potentially represent a more reliable marker for CRCLM compared to CEA.

Résumé

Introduction: Le cancer colorectal (CCR) est le deuxième cancer le plus souvent diagnostiqué chez les femmes et le troisième cancer le plus souvent diagnostiqué chez les hommes. Chez les personnes touchées, on observe un décès sur trois. Le cancer colorectal métastatique au foie (CCMF) représente environ deux tiers des décès. Il n'y a toujours pas de biomarqueur précis ou efficace pour diagnostiquer le CCR. Toutefois, l'antigène carcino-embryonique (CEA) reste un des meilleurs marqueurs utilisés, à nos jours, pour la surveillance de la maladie colorectale métastatique et l'évaluation d'une réponse ou non à la thérapie systémique. Les modalités d'imagerie, à part le niveau de l'antigène CEA dans le sang, sont encore couramment utilisés et nécessite des dépenses supplémentaires et des visites de suivis fréquents pour les patients. Ainsi, l'identification d'un biomarqueur non-invasif plus précis pour diagnostiquer et évaluer le pronostic du CCR et du CCMF demeure d'une grande importance.

Depuis le clonage du CEA, une très grande famille de protéines liées au CEA a été découverte. L'antigène CEA est regroupé, avec ses analogues, sous la famille de molécule d'adhésion cellulaire (CEACAM), une subdivision de la famille des immunoglobulines des molécules d'adhésion cellulaire (IgCAM). CEACAM1 représente un des éléments importants de la famille CEACAM. Les récepteurs CEACAM1 sont exprimés dans différentes cellules animales, tels que les cellules épithéliales, les cellules endothéliales, les leucocytes et les cellules T. Récemment, CEACAM1 a été trouvé à moduler la formation tumorale à des expressions variables dans différentes tumeurs. Dans le CCR, CEACAM1 a un rôle bimodal. La perte d'expression du CEACAM1 est remarquée au début de la formation du CCR et des adénomes, ce qui démontre un effet de suppression de la tumeur. D'autres études ont démontré que le niveau de CEACAM1 est surexprimé dans le CCR invasif et qu'il existe une corrélation entre le niveau de CEACAM1 et le stade clinique de la maladie. Plus récemment, on a démontré que l'expression de CEACAM1 peut représenter un facteur de risque indépendant de l'état des ganglions lymphatiques, des métastases hématogènes et de la durée de survie plus courte chez les patients. D'où une signification plus prometteuse du niveau de CEACAM1 dans les CCMF plus avancés. Néanmoins, les études sur l'expression de CEACAM1 dans les lésions métastatiques font encore défaut et la raison pour laquelle les cellules CRC qui ré-expriment CEACAM1 reste incertaine.

Objectifs: Nous émettons comme l'hypothèse que l'expression de CEACAM1 agit comme un facteur potentiel pronostique pour établir l'agressivité du CCMF. Notre objectif est d'identifier l'expression immuno-histochimique de CEACAM1 dans le cancer colorectal métastatique en testant des échantillons appariés de lésions primaires colorectals et hépatiques. En outre, nous cherchons à comparer l'expression de CEACAM1 à l'antigène CEA avec des variables cliniques.

Méthodes: L'étude s'est basée sur des échantillons de tissus tumoraux de 43 patients atteints d'un CCMF obtenus après une résection du foie. De plus, d'autres échantillons de tissu provenant de la tumeur primaire étaient disponibles pour 18 des 43 patients. Au total, 92 lésions hépatiques provenant des 43 patients ont été incluses dans nos analyses. Une coloration immuno-histochimique pour CEACAM1 et l'antigène CEA a été réalisée en utilisant l'indice de référence Ventana LT, machine entièrement automatisée (Ventana Medical System Inc. Arizona, Etats-Unis). Quelques variables cliniques pertinentes ont été analysées en fonction du CEACAM1 et et du CEA dans les tumeurs primaires et métastatiques en utilisant une corrélation de rang Spearman ou un test statistique « chi-square ».

Résultats: Une corrélation est présente entre le niveau d'expression du CEA dans la tumeur primaire, l'état des ganglions lymphatiques, le stade de la maladie et le nombre de lésions hépatiques. Un index de masse corporel (IMC) élevé a été associé avec un niveau d'expression bas du CEACAM1 dans les tissus hépatiques. De plus, on observe une perte d'expression de CEACAM1 à la fois dans la tumeur métastatique et la partie du foie normal du même échantillon. Une expression élevée du CEACAM1 a été remarquée dans les tumeurs métastatiques mal différenciées. Finalement, l'expression du CEACAM1 à la surface apicale a été associée à des caractéristiques de progression du cancer colorectal.

Conclusion: Notre étude démontre que l'expression de CEACAM1 dans le CCR et le CCCMF est associée à des variables cliniques plus pertinentes que le CEA, ce qui reflète un rôle modulateur potentiel et important du CEACAM1 dans la formation tumorale colorectale. Par conséquent, CEACAM1 peut potentiellement représenter un marqueur plus fiable pour CCMF que le CEA.

List of abbreviations

CEACAM1 = Carcinoembryonic antigen cell adhesion molecule 1

CRC = Colorectal cancer

CRCLM = Colorectal liver metastasis

CT = Computed tomography

MRI = Magnetic resonance imaging

CEA = Carcinoembryonic antigen
TTA = Tumor-associated antigens

MSI = Microsatellite instability

EGFR = Epidermal growth factor receptor

CEACAM = CEA-related cell adhesion molecule

GPI linkage = Glycosylphosphatidylinositol linkage

Bgp = Biliary glycoprotein

CEACAM1-L = Long isoform of CEACAM1

CEACAM1-S = Short isoform of CEACAM1

VEGF = Vascular endothelial growth factor

MDSC = Myeloid -derived mature immune cells

IHC = Immunohistochemistry

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Chapter 1: Introduction

1.1. Colorectal Cancer:

There are more than 1 million new cases of colorectal cancer (CRC) occurring globally each year [1]. It is the second most common cancer diagnosed in women and third most common cancer diagnosed in men, with 90 percent of cases occurring above the age of 50 [2]. The lifetime incidence of this disease in an average risk individual is about 5 percent [3]. CRC is considered as the second most common cause of cancer related deaths [4]. It results in approximately one in three deaths among affected individuals. The estimated number of deaths from CRC only in the United States is about 50000 patients every year [5]. Colorectal liver metastasis (CRLM), occurring in more than 30 percent of cases, is responsible for about two thirds of deaths related to the disease [6].

1.1.1. Clinical features:

The clinical features of cancers in general can be vague. Non-specific signs and symptoms such as, fatigue, anorexia, loss of weight and cachexia are seen in various types of cancers. Yet, the clinical presentation depends on the system involved. The presence of lower gastrointestinal symptoms may be seen in healthy individuals, CRC or any other lower gastrointestinal condition [7-10]. However, a number of signs and symptoms referred to as “alarm” features have been linked to identify patients with possible CRC. These features include having unexplained iron deficiency anemia, experiencing rectal bleeding, change in bowel habits, feeling a palpable right-sided abdominal mass or a rectal mass on physical examination [11]. Most of these features alone have poor sensitivity and specificity for the diagnosis of CRC. However, the presence of dark red rectal bleeding or an abdominal mass had superior specificity compared to the other features [12]. Nevertheless, awareness of these “alarm” features of CRC is important to prioritize patients requiring referral to a specialist for further assessment and evaluation within few weeks of the consultation [11].

1.1.2. Diagnosis:

Multiple investigation tests are typically required for an accurate diagnosis and staging of CRC. A complete colonoscopy to detect suspicious lesions and to obtain an immediate tissue biopsy for the histological diagnosis of CRC is usually recommended, unless clinical contraindications are present. Other modalities such as, flexible sigmoidoscopy, barium enema and computed tomographic (CT) colonography can be used in patients with coexisting comorbidities. Following the diagnosis of CRC, staging can be achieved by the use of contrast-enhanced CT of the chest, abdomen and pelvis. In addition, it is recommended to perform magnetic resonance imaging (MRI) in the staging process of rectal cancer to evaluate the tumor and lymph nodes status. In some cases of rectal cancer, endorectal ultrasound can be utilized adjunct to MRI for further assessment or when the use of MRI is contraindicated [13].

1.1.3. Treatment:

The management of CRC is a multidisciplinary task. Different treatment modalities are typically offered depending on the clinical stage and anatomical site of the primary tumor in addition to the associated risks and comorbidities. The general rule for local disease is to safely resect the whole tumor with an adequate safe margin, to lower the risk recurrence, and to excise a sufficient number of adjacent lymph nodes, for an accurate histopathological staging. Preoperative management of the primary tumor, by chemotherapy and/or radiotherapy, can be used in moderate and high risk cases of rectal cancer. Neoadjuvant chemotherapy can be offered, to allow tumor response and shrinkage, to patients with high risk locally advanced rectal cancer. Afterwards, adjuvant chemotherapy agents such as capecitabine, oxaliplatin and 5-fluorouracil, should be considered, mainly for Stage II and III of CRC, to reduce the risk of local and systemic disease, whereas the need of further treatment for Stage I CRC following complete resection depends on the clinical evaluation. The selection of chemotherapeutic agent(s) should be made following a detailed discussion between the clinicians and the patient to explain the contra-indications, method of administration and the possible side-effects of the treatment. Finally, patients presenting with stage IV CRC are evaluated to detect the resectability of both primary and metastatic tumors and to initiate systemic treatment followed by surgery when appropriate [13].

1.1.4. Screening:

The screening of CRC is usually recommended after the age of 50 years for asymptomatic average risk individuals. Individuals with clinical symptoms however, should undergo appropriate diagnostic work up. Average risk individuals, i.e. with negative family history of CRC, should undergo screening by one of the currently recommended strategies. Performing a full colonoscopy every ten years has the highest sensitivity and specificity among the available screening methods. Alternative methods includes, flexible sigmoidoscopy every five years, which can be combined with fecal occult blood testing, and Double contrast barium enema every five years. On the other hand, the screening of high risk individuals should start at the age of 40 years or ten years younger than the age of his/her relative at the time of CRC diagnosis, whichever comes first. These include patients who have first-degree relatives with the disease, a family history that suggests a definable genetic abnormality, familial adenomatous polyposis (FAP) or long-standing colonic inflammatory bowel disease [14].

1.1.5. Surveillance:

The overall five year survival of CRC is about 64.9%. However, survival rates vary according to the stage at the time of diagnosis. Localized disease has 90.3% five year survival. Whereas, regional and metastatic disease have 70.4% and 12.5% five year survival, respectively [3]. Hence, the availability of an effective screening tool is very important to facilitate the detection of CRC at an early stage. Furthermore, regular surveillance is recommended following successful treatment of CRC, in order to improve the chances of survival. Serum testing for carcinoembryonic antigen (CEA) should be done regularly every six months in the first three years for possible recurrence. In addition to CEA, a follow up CT scan of the chest, abdomen, and pelvis needs to be done at least twice during the first three years as well. It is also important to perform a colonoscopy one year following treatment, and to repeat it after five years if normal, and then as determined by the local cancer network thereafter [13].

1.2. Colorectal liver metastasis:

Liver metastasis occurs in more than 30 percent of patients with CRC. It is responsible for about two thirds of deaths related to the disease [6]. The median survival time for untreated CRCLM is only about 6.9 months [15]. Clinical suspicion of CRCLM is usually confirmed by radiological imaging to reach the diagnosis. Imaging modalities used in CRCLM include, but not limited to, ultrasound, CT scan, MRI and positron emission tomography (PET) [16]. Following the diagnosis and evaluation of the disease, CRCLM is usually categorized based on the treatment possibility into resectable disease, potentially resectable disease or unresectable disease [17]. Liver resection remains to be the most important modality in the treatment of CRCLM. It offers effective palliation, and a potential chance of cure [15]. In addition, the use of perioperative chemotherapy, such as the FOLFOX regimen (5-fluorouracil, leucovorin, and oxaliplatin), showed an added benefit to the survival [18]. Assessment of resectability is based on systemic and local factors. Extensive extrahepatic disease is a systemic contraindication for resection. Hepatic vascular involvement, adequate safe margin and the volume of future liver remnant are important local factors. For patients with normal liver function, 20% future liver remnant is required, whereas 30-40% is required in the presence of steatosis or cirrhosis [19-22]. For potentially resectable lesions, neoadjuvant chemotherapy to downstage the disease can be recommended in some cases to improve the survival [23]. Recent adjunct treatment modalities, including radiofrequency ablation (RFA), cryotherapy and radioembolization improved patient care, and prolonged survival in patients with unresectable disease [17]. Overall, CRCLM is potential cause of death in progressive CRC. Efforts should be directed towards the prevention and early detection of the disease to enhance the chances of cure.

1.3. Non-invasive Markers for CRC:

The early detection of potentially progressive CRC disease is extremely important in order to facilitate the use of early treatment options. However, there is still no such effective marker available. CEA has been used as an efficient marker for CRC clinical management for many years; elevated serum CEA levels indicate metastasis and poor prognosis. In fact, CEA is still the best marker for monitoring metastatic disease responses to systemic therapy [24]. However, the sensitivity and specificity of CEA in this context are about 68 percent and 83 percent, respectively [25]. Considering the average sensitivity level of CEA, imaging modalities, such as ultrasonography and computer tomography are still routinely used, which requires further expenses and frequent follow-up visits. Thus, identifying an effective non-invasive tumor marker for the diagnosis and the assessment of prognosis in CRC and CRCLM is of great significance.

Tumor markers in general are defined as substances that can be attributed to the development of normal cells or carcinogenesis at different cell development stages. These substances are typically proteins or, to a lesser extent, glycolipids. Tumor markers generally represent a highly diverse biological structure [26]. Overall, the largest identified category of tumor markers with clinical relevance is the group of Tumor-associated antigens (TAAs). TAAs are molecules that are mainly produced by neoplastic cells more than the normal cells [26]. The formerly mentioned CEA is one of the commonly used TAAs in clinical practice. TAAs group also include other markers such as Cancer antigen 19-9 (CA 19-9) and Cancer antigen 72-4 (CA 72-4). Most researchers found that these markers had poor sensitivity and specificity compared to CEA in CRC disease [27-29].

The second category of tumor markers, which is still being widely investigated for practical clinical application in CRC, is the molecular biomarkers group. This group includes markers such as *KRAS*, *BRAF*, *PIK3CA* mutations, microsatellite instability (MSI) and chromosome 18q loss of heterozygosity [26]. Recently, some of these molecular markers have been introduced into clinical practice in CRC, mainly in stage II and III, to help in selecting appropriate adjuvant chemotherapy regimens and avoiding potential drug resistance.

KRAS gene mutation is found in 40% of CRC patients. The most common mutation is located on the short arm of chromosome 12 at codon 12 or, less frequently, at codon 13 [26, 30]. Furthermore, *BRAF* mutations are found in about 10% of CRC patients [30, 31]. Both *KRAS* and *BRAF* gene mutations affects the epidermal growth factor receptor (EGFR) signaling pathways. Thus, CRC tumors in patients with one or both genes mutation are likely to be resistant to anti-EGFR therapies, such as cetuximab and panitumumab [31-34]. Similarly, recent reports have shown that *PIK3CA* mutations, found in about 15 percent of

CRC tumors, are also correlated with significant resistance to anti-EGFR therapies [30, 35, 36].

MSI is the condition of genetic hypermutability that results from impaired DNA Mismatch Repair (MMR), which leads to repeated errors in DNA sequences. CRC tumor cells can be classified according to the degree or presence of MSI into microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L) and microsatellite stable (MSS) [26, 37]. Most studies concluded that the degree of MSI may be a significant prognostic factor in CRC and that most tumors in patients with MSI-H are resistant to 5-FU-based chemotherapy [38-40].

Finally, the loss of chromosome 18q heterozygosity (18qLOH) has been linked to poor survival rates in patients with CRC [41, 42]. However, an exact correlation and effect on the response to chemotherapy has not been established. Thus, it cannot be used currently for clinical decisions regarding chemotherapy regimens [26].

Overall, despite the wide efforts being conducted to explore potential markers for CRC, none of the tumor markers mentioned above qualify to be an accurate and effective marker for clinical applications.

1.4. CEACAM Family:

Since cloning of CEA [43], a very large family of CEA-related proteins have been uncovered. These proteins have been grouped together and referred to as the CEA-related cell adhesion molecule (CEACAM) family, a subdivision of the immunoglobulin superfamily of cell adhesion molecules (IgCAMs) [44]. The CEACAM family mainly includes 12 proteins encoded by different genes that are located on the long arm of human chromosome 19 in the region 19q 13.1-13.2 [45-47]. These proteins share some common features in their overall structure, such as the extracellular variable and constant immunoglobulin (Ig) domains. These domains are essentially required for CEACAM functionality as intercellular adhesion molecules [48].

The molecular structure of the first identified member of the family, CEA, which was eventually renamed as CEACAM5, exhibits only one variable (V)-like domain. In addition, CEA (CEACAM5) has six constant C2-like Ig domains, preceded by a signaling peptide that is composed of 34 amino-acids [49, 50]. CEACAM5, as well as CEACAM6, are attached to the cellular membrane through a glycosylphosphatidylinositol (GPI) linkage, which is an important characteristic of these two CEACAM proteins that are only found in man and higher primates (Figure 1) [51, 52].

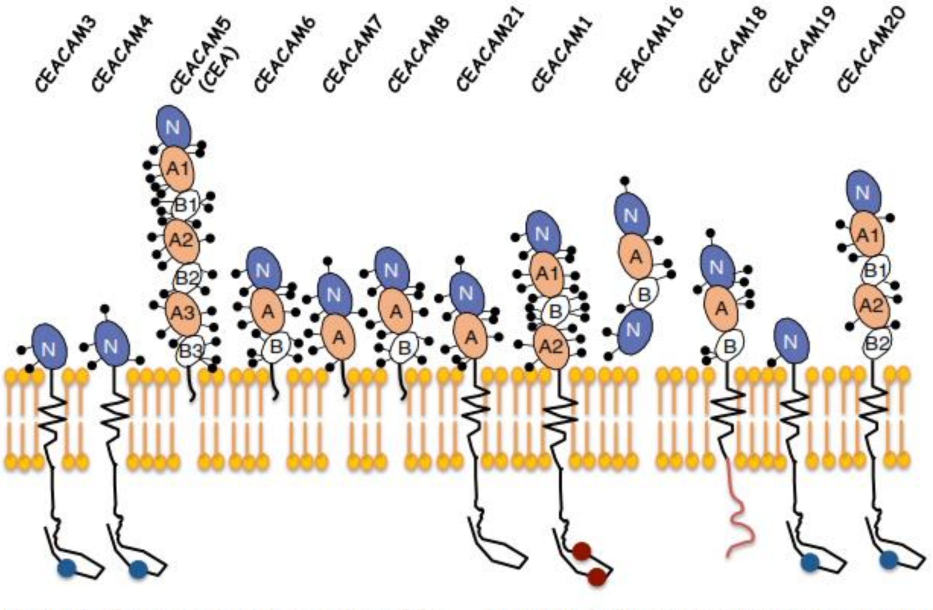

Fig. 1 Human CEACAM family members. Twelve members of the human CEA family, which belong to the CEACAM subgroup, are depicted here. CEACAM proteins generally have one variable (V)-like Ig domain, identified as the N domain (except CEACAM16 with two N domains) (*blue*), but they differ in the number of constant C2-like Ig domains, identified as A (*pink*) or B (*white*), as well as the membrane anchorage. CEACAM5, CEACAM6, CEACAM7, and CEACAM8 are associated with the membrane through a GPI linkage, whereas six CEACAM family members (CEACAM1, CEACAM3, CEACAM4, CEACAM19, CEACAM20, and CEACAM21) are anchored to the cellular membrane *via bona fide* transmembrane domains. CEACAM16 is a secreted version with no membrane anchorage. The CEACAM1 cytoplasmic domain has ITIM motifs (*red circles*), whereas CEACAM3, CEACAM4, CEACAM19, and CEACAM20 carry ITAM motifs (*blue circles*). All family members are highly glycosylated proteins highlighted by the *stick and balls* on the extracellular domains

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***Figure 1**: Human CEACAM1 family members.*

Beauchemin, N., et al. (2013). Cancer and Metastasis Reviews [53].

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1.5. CEACAM1:

CEACAM1, formerly referred to as biliary glycoprotein (Bgp) or CD66a, was found to be one of the most important CEACAMs. It is the most widely distributed protein within the family. CEACAM1 receptors are expressed on various mammalian tissues, such as epithelial cells, endothelial cells, leukocytes and T cells [54-57]. In addition to being an intercellular adhesion molecule, CEACAM1 was linked to various other important functions, such as the metabolism of insulin, the regulation of T-cells and neovascularization [58-61]. Furthermore, it is being investigated for being a potential modulator of tumorigenesis in different types of cancer, including colorectal cancer, which will be further discussed in greater detail below [62-66].

1.5.1. CEACAM1 Structure:

Similar to the structure of CEACAM5 and most other members of the family, CEACAM1 has only one variable (V)-like domain. This is followed by one to three constant C2-like Ig domains, as opposed to six found in CEACAM5. Moreover, compared to the GPI linkage described in CEACAM5, CEACAM1 is anchored to the cellular membrane through a transmembrane domain [48, 67]. The cytoplasmic part of CEACAM1 comes in either a long (L) or a short (S) domain. CEACAM1-L isoforms encompass 71-73 cytoplasmic amino acids and contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which are targets for phosphorylation and dephosphorylation modulating subsequent cellular functions [67-69]. In contrast, the CEACAM1-S isoforms only encode 10 cytoplasmic amino acids that are lacking any sites for phosphorylation [67-69]. Both isoforms are co-expressed with different ratios in most CEACAM1-expressing tissues, which is the reason implicated in altering the signaling outcome [70-72]. There are 12 different isoforms of CEACAM1 produced as a result of alternate splicing which vary in structure, depending on the extracellular C2-like Ig domain, membrane anchorage and the existing cytoplasmic tail i.e. CEACAM1-L or CEACAM1-S. Three of these 12 isoforms are secreted versions of CEACAM1, which are important markers for progression of different types of cancer, such as malignant melanoma, bladder and pancreatic cancers (Figure 2) [73-75].

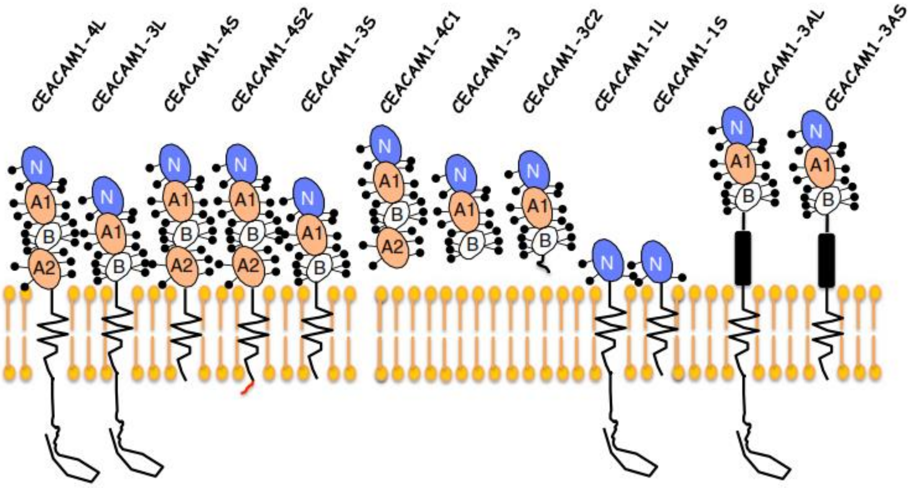

Fig. 2 Human CEACAM1 isoforms. Alternative splicing adds to the complexity of CEACAM proteins. CEACAM1 transcripts can be alternatively spliced in order to generate 12 different isoforms containing 1, 2, or 3 C2-like domains as well as 2 major cytoplasmic domains, termed as long and short tails. According to standardized nomenclature, the number after CEACAM1 is indicative of the number of extracellular Ig-like domains, while the letter following this number points to the presence of either a long (*L*) or short (*S*) cytoplasmic tail, a unique terminus (*C*), or an Alu family repeat sequence (*A*) (*black boxes*). Graphic domain features and glycosylation sites are the same as in Fig. 1

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Figure 2: *Human CEACAM1 isoforms.*
Beauchemin, N., et al. (2013). Cancer and Metastasis Reviews [53].
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1.5.2. CEACAM1 and Tumorigenesis:

The role of CEACAM1 in tumor cells has yet to be fully explained. However, significant correlations and findings were seen in different types of cancer. Since the process of angiogenesis regulates the growth of tumor cells [76, 77], the identified pro-angiogenic activity of CEACAM1 is thought to be a significant contributing factor to cancer growth and progression [78, 79]. This functional activity of CEACAM1 was explored by observing an up-regulated expression of CEACAM1 with vascular endothelial growth factor (VEGF) [78, 79]. Moreover, CEACAM1 was studied in different types of cancer, in order to further explain its role. The high expression of CEACAM1 has been associated with the progression of cutaneous malignant melanoma and lung adenocarcinoma [80-83]. On the other hand, a suppressing effect of CEACAM1 is implicated in early prostate, breast and endometrial cancers [84-86]. Thus, CEACAM1 is believed to have an important role in modulating the progression, invasion and metastatic potential of different types of cancer [87].

1.5.3. CEACAM1 and Colorectal Cancer:

Studying the expression of CEACAM1 in colorectal cancer revealed an interesting bimodal feature. It is thought to be linked to tumor suppression in hyperplasia and adenoma [88, 89] and to tumor progression later in the disease [90, 91]. Thus, CEACAM1 is implicated in having a significant modulatory effect in CRC.

In 1993, Neumaier, et al. [88] studied the mRNA expression of CEACAM1 in 21 CRC human tissue samples obtained from 20 patients following surgical resection. They found a low expression in the cancer tissue compared to the adjacent normal mucosa. In addition, the low mRNA expression of CEACAM1 was accompanied by a more extensive lymph node involvement. Therefore, the study concluded that losing the expression of CEACAM1 is a major event in CRC carcinogenesis [88].

Since it was not known at what stage of the disease low CEACAM1 levels will cause an effect, in 1997, Nollau P., et al. [89] studied the mRNA expression of CEACAM1 in relation to colorectal adenomas. The study included 22 tissue samples of human colorectal adenoma obtained from 16 patients during endoscopy. They have found a greater than 50% down-regulation of CEACAM1 mRNA expression in most samples. Considering the previous findings of studies done on CRC samples, Nollau P., et al. concluded that the early dysregulation seen in the expression of CEACAM1 suggests an important role for the development of malignant adenocarcinoma from colorectal adenomas [89].

Furthermore, experimental and animal studies in the literature suggested a tumor suppressor effect of CEACAM1 in the tumorigenesis of CRC [92, 93]. It was indicated that CEACAM1 acts as a regulator of apoptosis in the colonic epithelium and that failure of its expression contribute to the development of hyperplastic lesions, which eventually may transform into malignant neoplastic cells [94].

Despite that genetic alteration and loss of CEACAM1 expression may contribute to the development of early CRC and adenomas [88, 89, 95], recent reports however, have shown that CEACAM1 is overexpressed at the protein level in invasive CRC and is correlated with the clinical stage [90, 91]. In 2003, Jantscheff, P., et al. [96] conducted a human study on 243 patients, who underwent surgical resection for CRC. Immunohistochemical analysis was carried out on tissue microarrays of the cancer tissue. The median follow-up of patients was 8 years. The CEACAM1 expression was found to be high in 58% of patients. There was no significant difference neither in the overall survival nor in the disease-free survival between the two groups i.e. high vs low CEACAM1 expression. The authors concluded that CEACAM1 down-regulation has no prognostic impact in CRC. In addition, CEACAM1 can be highly expressed in many cases of CRC, which contradicts the end findings of previous studies [96]. However, there was no specification of the clinical stage in relation to CEACAM1 expression mentioned in this study.

In 2007, Kang, W.Y., et al. [97] studied the immunohistochemical CEACAM1 expression in Paraffin-embedded sections from 184 patients including 42 colorectal adenomas with low-grade dysplasia, 43 adenomas with high-grade dysplasia, and 99 adenocarcinomas. CEACAM1 was found to be expressed in the apical membrane and the lumen of neoplastic glands in both adenomas and adenocarcinomas. In addition, the results of this study showed that higher CEACAM1 expression significantly correlates with tumor invasion, stage, and pre-operation serum CEA level [97]

In 2011, Ieda, J., et al. [98] used antibodies to detect the isoform expression of CEACAM1-L and CEACAM1-S by immunohistochemistry. CRC tissue samples from 164 patients were sectioned from Paraffin-embedded blocks. The patients included 26 Stage I, 63 Stage II, 47 Stage III and 28 Stage IV colorectal carcinomas based on the TNM classification. The results of this study showed that CEACAM1-L overexpression at the invasion front of CRC adenocarcinomas is an independent risk factor for lymph node metastasis, haematogenous metastasis and shorter patient survival [98].

Recently in 2012, Arabzadeh, A., et al. [99] studied the effect of systemic CEACAM1 deletion on host metastatic development of CRC in detail. They used an experimental model of *Ceacam1*$^{-/-}$ mice that were injected intrasplenically by MC38 metastatic CRC cells. 14 days post-injection, CEACAM1 depletion resulted in a reduced metastatic burden in experimental metastasis, observed by a reduction in the size and number of liver metastatic lesions in *Ceacam1*$^{-/-}$ compared to wild-type mice. In addition, *Ceacam1*$^{-/-}$ livers were shown to be an unfavorable microenvironment for metastasis. This was assessed by intravital fluorescence microscopy to evaluate the survival of MC38 cells that were injected intrasplenically after labeling it by carboxyfluorescein succinimidyl ester (CSFE). Furthermore, KI-67 staining of samples showed that the absence of CEACAM1 in the liver significantly reduced MC38 tumor cell proliferative capacity. The results of this impressing study also included a reduction in vascular maturity and in myeloid -

derived mature immune cells (MDSCs) in *Ceacam1*$^{-/-}$ mice. These findings support the link of CEACAM1 to tumor angiogenesis and the involvement of immune cells in the process of metastasis, respectively. In addition, a regulatory effect on chemokine secretion was attributed to CEACAM1, which associates the process of inflammation to developing metastasis. Following these multiple experiments, the authors concluded that CEACAM1 may represent a novel metastatic CRC target for treatment [99].

After reviewing the literature, CEACAM1 is believed to have a significant modulating effect in colorectal cancer tumorigenesis. It is associated with tumor suppression early in CRC and tumor promotion in advance metastatic disease. Nevertheless, human studies investigating the expression of CEACAM1 in CRCLM are still lacking and the reason why CRC cells re-expresses CEACAM1 remains unclear. Thus, further exploring the role of CEACAM1 in metastatic CRC is a promising area of research, as we might be looking at a reliable biomarker for CRC that would potentially facilitate and improve the follow-up of patients in the future.

Chapter 2: Thesis Project

2.1. Objective:

The main aim of this research is to study the immunohistochemical expression of CEACAM1 in metastatic colorectal cancer in order to elucidate its role in colorectal cancer liver metastasis and to pave the way into developing a new reliable diagnostic or prognostic marker for this disease.

2.2. Hypothesis:

The expression of CEACAM1 is a prognostic factor for establishing the aggressiveness of CRC liver metastasis.

2.3. Specific Aims:

1. To identify the expression of CEACAM1 in CRC liver metastasis and their matched primaries.
2. To compare the expression of CEACAM1 to the expression of CEA in CRC liver metastasis.
3. To correlate the clinical findings and outcomes to the expression of CEACAM1 in CRC liver metastasis.

Chapter 3: Methodology

3.1. Selection of patients:

The study included 54 patients with colorectal liver metastasis who underwent liver resection at the Royal Victoria Hospital, Montreal, QC, Canada. The patients were consented through the liver disease biobank of the McGill University Health Center (MUHC) for the use of their samples and clinical data for clinical research purposes. 11 patients were excluded for having totally necrotic samples. We had access to CRCLM tissue samples from 18 patients out of 43 along with their matched primary CRC tissue. We additionally included CRCLM tissue samples of the remaining 25 patients. However, samples from these patients were not matched with the primary CRC, due to the fact that their initial surgery was done at different hospitals.

3.2. Clinical data:

The clinical data were collected by reviewing the MUHC electronic health record system (OACIS), in addition to the medical chart of each patient at the medical records department in the Royal Victoria Hospital. The information gathered included relevant clinical data such as patient’s demographics, comorbidities, prognosis, chemotherapy, tumor staging and pathology specifications. The data related to metastatic tumor response to chemotherapy were collected from the MUHC liver disease biobank database, which was originally evaluated by assessing tumor morphology changes on CT scans.

3.3. Tissue samples:

Specimens obtained through surgical resection or tissue biopsy procedures were fixed in neutral buffered formalin (10% v/v formalin in water, pH 7.4) and embedded in paraffin wax. Paraffin embedded blocks are routinely stored in the archives of the MUHC pathology department. Hospital pathology reports were reviewed to identify and locate the exact tissue blocks containing the desired tumor and adjacent normal liver tissue. Relevant blocks were then pulled from the pathology department archives in preparation for sectioning. The total tumor samples collected from 43 patients included 92 CRCLM lesions. The matched group accounted for 40 lesions, whereas the unmatched group accounted for the remaining 52 **(**Figure 3).

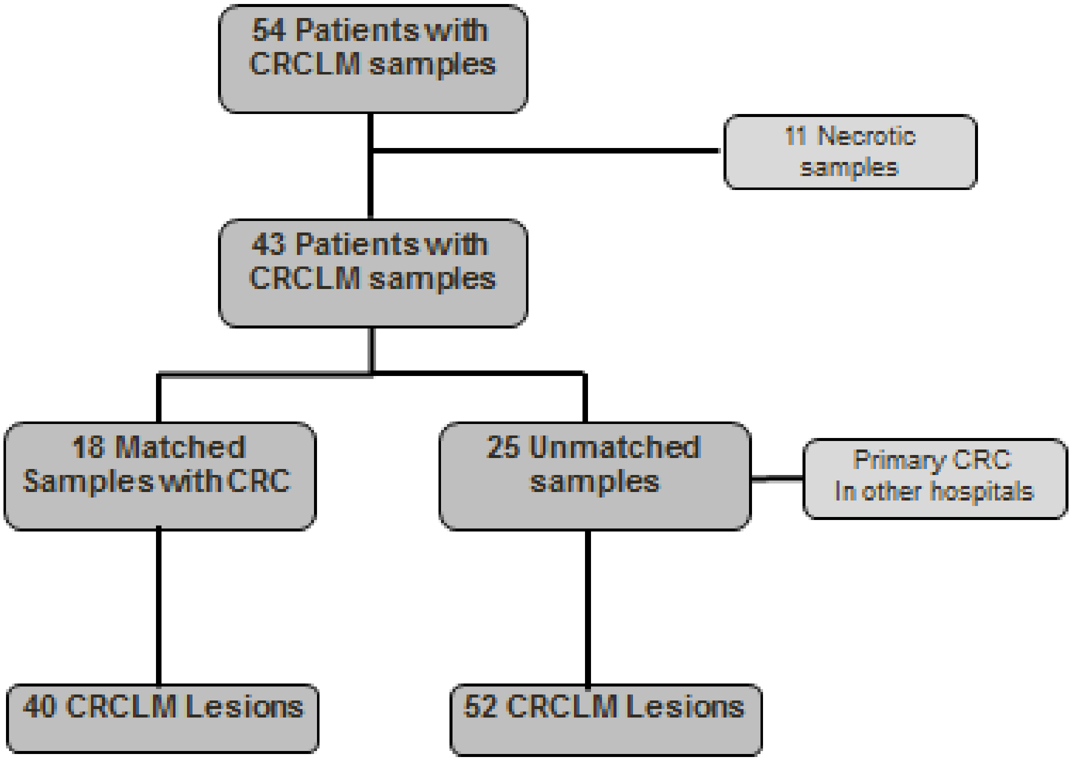


Figure 3: *Flow diagram of CRCLM patients included in the study.*

3.4. Sectioning of paraffin-embedded blocks:

Sectioning was performed at the histology core facility of the Goodman Cancer Research Center at McGill University for the majority of the samples. All CRC samples were cut through our Liver Disease Biobank infrastructure. Serial sections 4 μm thick were cut from each paraffin-embedded block. Sections were then mounted on charged glass slides (Superfrost Plus; Fisher Scientific, Waltham, MA) and kept in room temperature.

3.5. Antibodies:

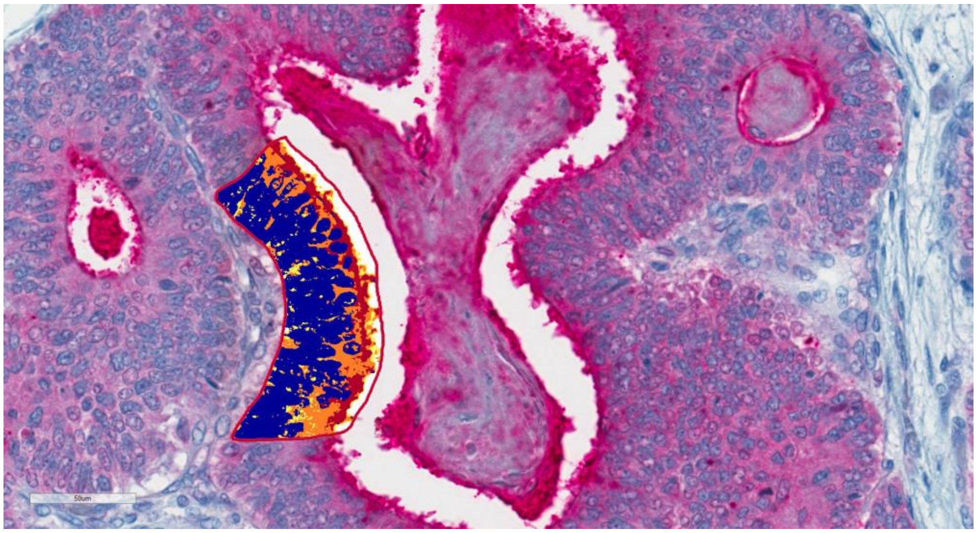
The antibodies for Immunohistochemistry included an IgG mouse monoclonal antibody 5F4 (3mg/ml) directed against human CEACAM1, provided by Dr. R. S. Blumberg (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) [100]. In addition, we have used the 5C8C4 antibody (2.1G/L) targeting CEA, from Dr. B.B. Singer (Anatomy, University Hospital Essen, Germany), which is an IgG mouse monoclonal antibody that is mono-specific anti-human CEACAM5.

3.6. Immunohistochemistry:

Immunohistochemistry was performed using the Ventana BenchMark LT fully automated machine (Ventana Medical System Inc. Arizona, USA) with the support of Dr. Bia Dias (Dr. M. Burnier Laboratory). The fully automated processing of bar code labeled slides included baking of the slides, solvent-free deparaffinization, and CEACAM1 (Citrate buffer) antigen retrieval. Slides were incubated with the primary antibody, either 5F4 (1:25) directed against human CEACAM1 or 5C8C4 (1:250) against human CEA, for 32 minutes at room temperature, followed by application of biotinylated secondary antibody (30 min, 37ºC). Finally the antibody was detected by Fast Red chromogenic substrate and counterstained with hematoxylin. For CEACAM1, human liver tissue was used as a positive control; negative controls used were taken from liver specimens of *Ceacam1*$^{-/-}$ knockout mice. For CEA, tissue from human colon cancer was used as a positive control and the negative control was human liver tissue. The primary antibody was also omitted as an additional negative control.

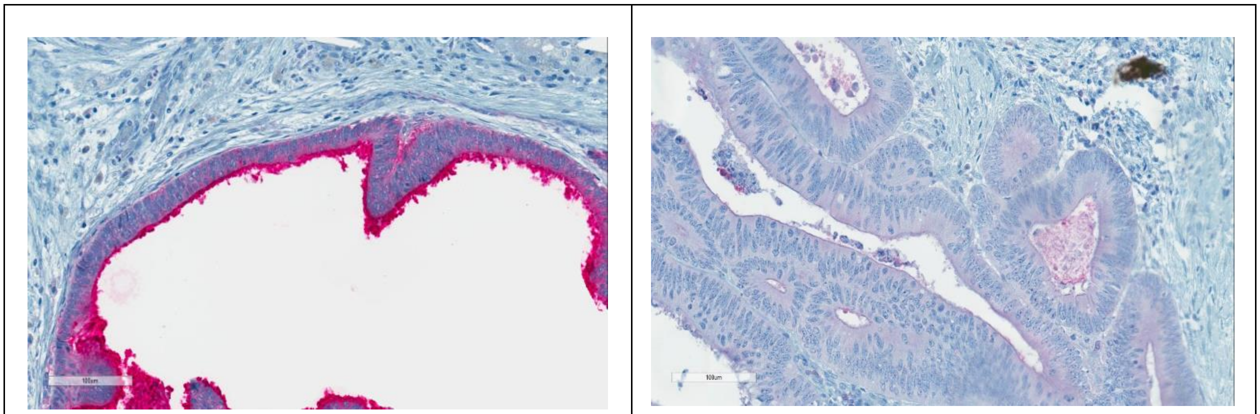
3.7. Microscopy and scoring:

All slides were scanned at 20X magnification using the Aperio AT Turbo system. Images were then viewed using the Aperio ImageScope ver.11.2.0.780 software program for scoring analysis and assessment of tumor differentiation. Slides were reviewed with a pathologist for accurate scoring (Dr. Zu-hua Gao). A positive reaction was manually scored into 4 grades, according to the intensity of the staining: 0, 1+, 2+, and 3+. Whereas the ratio of the strong positive IHC signals were automatically scored into 4 different categories: 0 (0%), 1 (1–33%), 2 (34–66%), and 3 (67–100%) **(**Figure 4**).** The final score, calculated as the product of the intensity score multiplied by the percentage score, was classified as follows: 0 for negative; 1–3 for weak; 4–6 for moderate; and 7–9 for strong. Samples with a final score ≤3 were grouped together as CEACAM1 expression negative while those with a score ≥4 were grouped together as CEACAM1 expression positive **(**Figure 5) [101]. Additionally, we noted the variation in the cellular staining location on our samples. Some tumors showed an isolated apical staining pattern, where the expression signal was seen only in the apical cells at the luminal side of the adenocarcinoma, whereas other tumors had a combined apical and cytoplasmic staining pattern (Figure 6). All slides were scored without knowledge of clinical data.



Automated IHC scoring to identify the ratio of the strong positive signal (Red stain) to the overall positive signal (Red, orange and yellow staining) using Aperio ImageScope ver.11.2.0.780 software

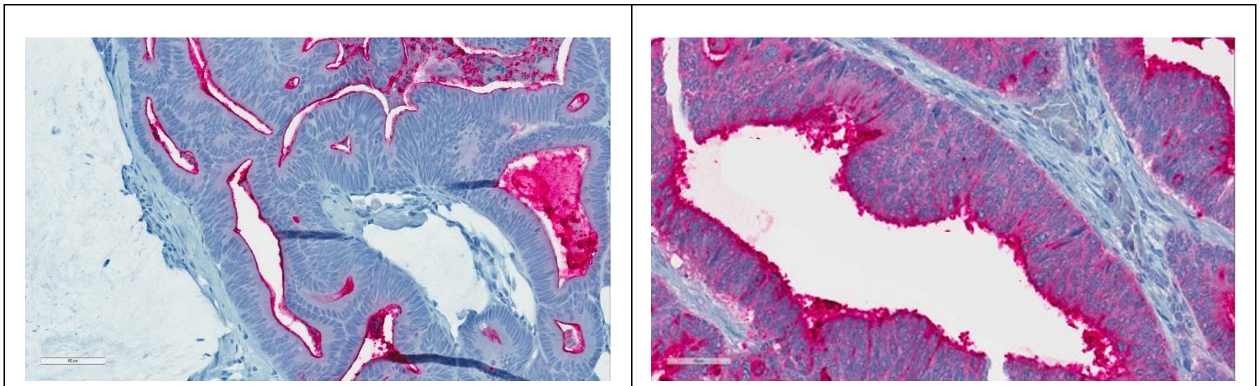
***Figure 4**: Aperio ImageScope ver.11.2.0.780 Automated scoring.*



a. Positive CEACAM1 expression

b. Negative CEACAM1 expression

***Figure 5:** CEACAM1 expression in different CRCLM lesions.*



a. Apical staining pattern

b. Mixed apical and cytoplasmic staining

***Figure 6:** CEACAM1 staining location in CRCLM lesions from different patients.*

3.8. Statistical Analysis:

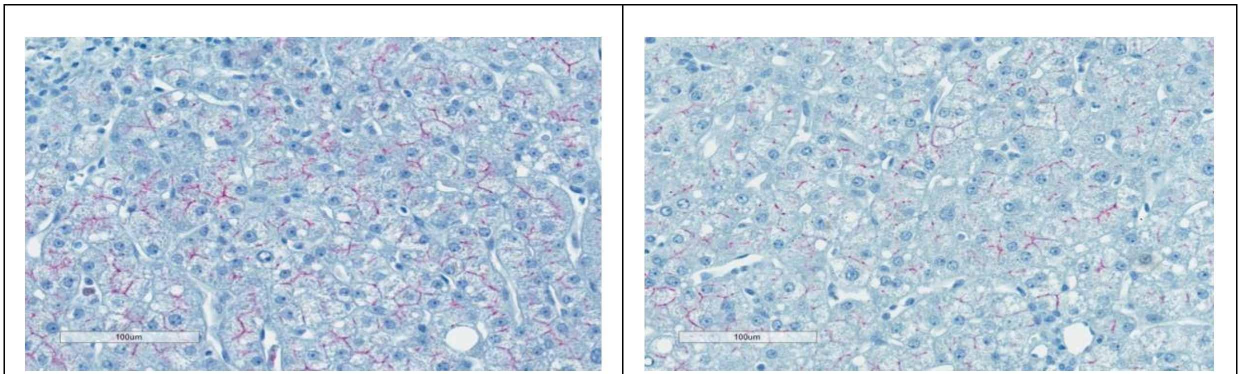
Data were expressed as means ± standard deviation or median and range if not normally distributed. The average immunohistochemistry expression score was calculated in patients with multiple tumor lesions. Each clinical variable was separately assessed in relation to CEACAM1 and CEA expression. Spearman's rank correlation coefficient was used for continuous and ordinal data. Categorical variables were analyzed using a chi-squared statistical test. A p-value of less than 0.05 was considered to be statistically significant. Statistical calculations were performed using the IBM SPSS ver.22 software program.

Chapter 4: Results

4.1. Antibody titration:

4.1.1. CEACAM1:

The initial CEACAM1 antibody titration included different dilutions tested on the positive control tissue (human liver) in order to identify the optimal concentration to be used. After reviewing the slides with our pathologist (Dr. Zu-hua Gao), the concentration found to be optimal in terms of signal to background, for the study samples was 1:25 (Figure 7).



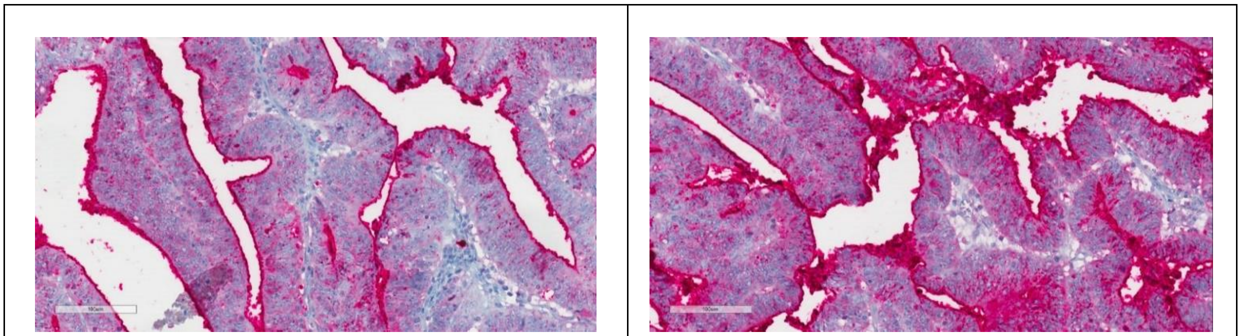
a. CEACAM1 (1:25) The optimal concentration

b. CEACAM1 (1:50) Low concentration showing faint staining.

Figure 7: *CEACAM1 antibody titration on human liver tissue.*

4.1.2. CEA:

Similarly, the CEA antibody was optimized using different dilutions tested on the positive control tissue (human colon cancer). The optimal concentration detected to proceed for testing our samples was 1:250 (Figure 8).



c. CEA (1:250) The optimal concentration

d. CEA (1:100) high concentration showing diffuse staining.

Figure 8: *CEA antibody titration on human colon cancer tissue.*

4.2. CRCLM matched pair's analysis:

4.2.1. Descriptive analysis:

The initial analysis was done on the matched samples from 18 patients (Figure 9). We first describe the overall CEACAM1 and CEA IHC expression scores in the primary tumor, liver metastasis and normal liver tissue of each patient (Table 1). CEACAM1 expression in the primary tumor was found to be positive in about 85% of the sample, whereas 95% of the liver metastasis samples were positive. On the other hand, CEA expression was positive in 95% and 90% of the primary and metastatic samples respectively. Since normal liver tissue does not express CEA, it was only tested for CEACAM1, which showed a positive expression in 67% of the matched samples.

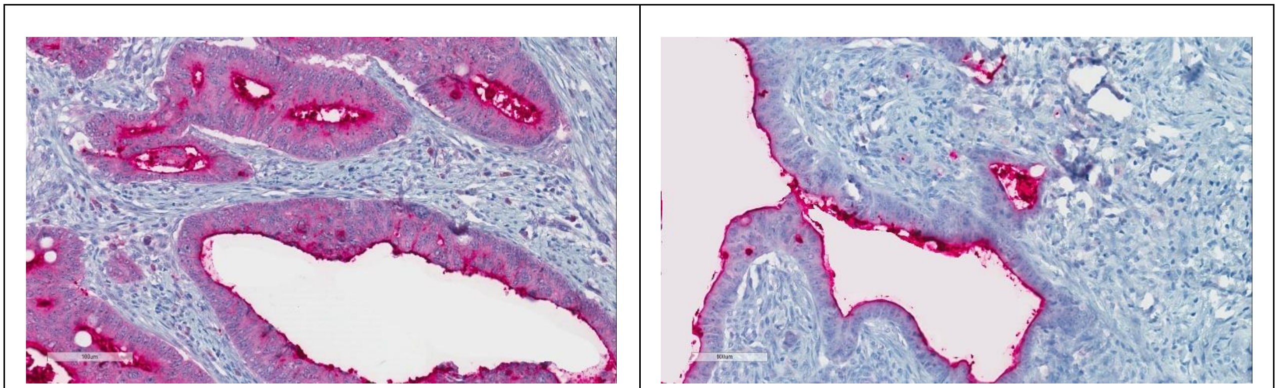
We then identified the staining location of the IHC markers in the matched samples (Table 1). In the primary tumor, CEACAM1 stained about 55% of the matched samples in both apical and cytoplasmic parts of the cancer cells and 39% only stained at the apical region. However, half of the samples from liver metastasis stained both apical and cytoplasmic regions, while the other half stained only the apical region. In contrast, most samples stained both the apical and cytoplasmic regions for CEA in both the primary and metastatic tumor samples.

Table 1: Descriptive analysis of immunohistochemistry in the matched group.

Variable	**IHC expression**			**IHC staining location**		
Patients with metastatic colorectal carcinoma **(n=18)**	Weak (0-3)	Moderate (4-6)	Strong (7-9)	None	Apical	Apical-Cytoplasmic
CEACAM1 in the primary tumor	3 16.67%	11 61.11%	4 22.22%	1 5.56%	7 38.89%	10 55.56%
CEA in the primary tumor	1 5.56%	9 50.00%	8 44.44%	0	1 5.56%	17 94.44%
CEACAM1 in liver metastasis	1 5.56%	9 50.00%	8 44.44%	0	9 50.00%	9 50.00%
CEA in liver metastasis	2 11.11%	7 38.89%	9 50.00%	0	0 0.00%	18 100.00%
CEACAM1 in normal liver tissue*	6 33.33%	10 55.56%	2 11.11%			

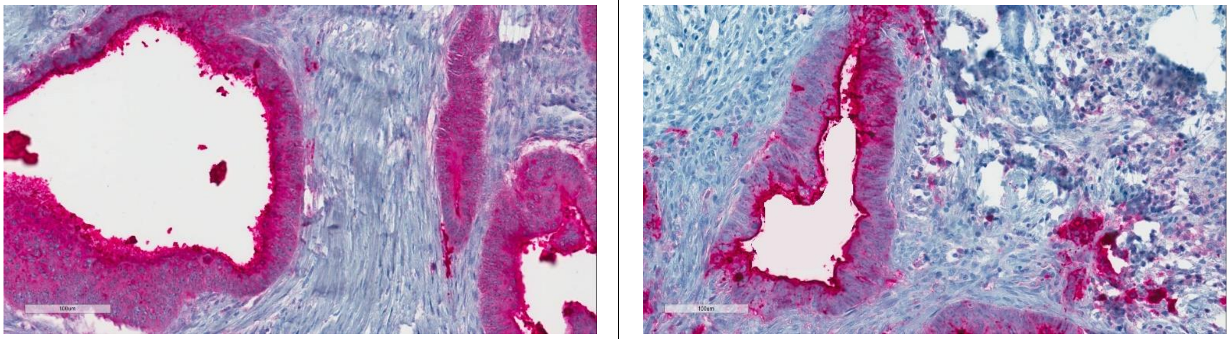
° IHC score ≥4 was considered as a positive expression

* CEACAM1 staining location in the normal liver tissue is typically membranous



a. Primary CRC CEACAM1

b. CRCLM CEACAM1



c. Primary CRC CEA

d. CRCLM CEA

***Figure 9:** CEACAM1 and CEA staining in matched samples from a single patient.*

4.2.2. Correlations with the demographic characteristics:

We performed a correlation analysis in the matched samples for the IHC expression of CEACAM1 and CEA in the primary tumor, liver metastasis and normal liver tissue with the demographic variables, i.e. sex, age and BMI (Table 2). There were no significant correlations identified with neither the primary nor the secondary tumor samples. However, a statistically significant inverse correlation of CEACAM1 expression in the background normal liver tissue with the body mass index was identified (p-value = 0.003).

Table 2: Correlations with the demographic characteristics of patients with matched samples.

	Number	**Primary tumor CEACAM1 expression**	**Primary tumor CEA expression**	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=18)**	***P* value[a]** **(*r* value)[b]**	***P* value[a]** **(*r* value)[b]**	***P* value[a]** **(*r* value)[b]**	***P* value[a]** **(*r* value)[b]**	***P* value[a]** **(*r* value)[b]**
Sex[d]		0.229	0.104	0.46	0.857	0.403
Male	12					
Female	6					
Age (y/o)[c]		0.107 (-0.392)	0.84 (-0.051)	0.791 (-0.067)	0.791 (0.067)	0.569 (-0.144)
Range	31–81					
Mean	63.56					
Median ± SD	66.56±12.4					
BMI (kg/m^2)[c]		0.517 (-0.198)	0.654 (0.138)	0.212 (0.371)	0.921 (0.031)	***0.003*** (-0.746)
Range	17–37					
Mean ± SD	24.17±6.04					
Median	21.26					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

4.2.3. Correlations with the clinical staging variables of the primary disease:

Correlating the primary disease staging with the IHC expression scores in the matched samples resulted in a significant correlation of CEA expression in the primary tumor with the overall stage (p-value = 0.012) and the N stage (p-value = 0.027), in addition to a near significant correlation with the M stage (p-value = 0.073). In contrast, there were no similar significant correlations seen with CEACAM1 (Table 3). However, the absolute number of positive lymph nodes in the initial primary disease was found to be inversely correlated with CEACAM1 score within the normal liver tissue (p-value = 0.029) (Table 4). In addition, a near significant negative correlation was found between the N stage and CEACAM1 expression in the background liver (p-value = 0.08) (Table 3). The correlation between lympho-vascular invasion in the primary tumor and CEACAM1 expression in the metastatic tumor was found to be negative and near significant (p-value = 0.61). Whereas, a positive near significant correlation between lympho-vascular invasion was found with CEA expression in the primary tumor (p-value = 0.65) (Table 4). Furthermore, the combined apical and cytoplasmic staining of CEACAM1 in the primary tumor was found to be significantly correlated with the larger sized primary tumors (p-value = 0.036) (Table 5).

Table 3: Correlations with the primary disease staging in the matched group.

Variable	**Number** **(n=18)**	**Primary tumor CEACAM1 expression** ***P value[a] (r value)[b]***	**Primary tumor CEA expression** ***P value[a] (r value)[b]***	**Metastatic tumor CEACAM1 expression** ***P value[a] (r value)[b]***	**Metastatic tumor CEA expression** ***P value[a] (r value)[b]***	**Normal Liver CEACAM1 expression** ***P value[a] (r value)[b]***
Primary AJCC Stage[c]		0.982 (0.006)	***0.012*** (0.61)	0.29 (-0.282)	0.336 (0.257)	0.433 (-0.211)
Stage I - II	3					
Stage III - IV	13					
Unknown	2					
Primary tumor T stage[c]		0.19 (0.346)	0.176 (-0.356)	0.596 (-0.144)	0.687 (-0.109)	0.838 (0.056)
T2-T3	13					
T4	3					
Unknown	2					
Primary tumor N stage[c]		0.634 (0.129)	***0.027*** (0.55)	0.114 (-0.411)	0.341 (0.255)	0.081 (-0.449)
N0	3					
N1	9					
N2	4					
Unknown	2					
Primary tumor M stage[c]		1 (0)	0.073 (0.476)	0.687 (-0.113)	0.326 (0.272)	0.949 (-0.018)
M0	10					
M1	5					
Unknown	3					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

Table 4: Correlations with the primary disease features in the matched group.

	Number	**Primary tumor CEACAM1 expression**	**Primary tumor CEA expression**	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=18)**	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]
Primary tumor source[d]		0.079	0.336	0.411	0.136	0.163
Colon	8					
Rectum	10					
Primary tumor size (cm)[c]		0.12 (0.42)	0.178 (-0.368)	0.146 (0.394)	0.582 (0.155)	0.355 (0.257)
Range	2–7					
Mean ± SD	3.57±1.34					
Median	3.5					
Primary tumor lympho-vascular invasion[c]		0.818 (0.063)	0.065 (0.471)	0.061 (-0.478)	0.649 (0.123)	0.817 (-0.063)
Yes	10					
No	6					
Unknown	2					
Number of positive lymph nodes[c]		0.164 (0.366)	0.120 (0.405)	0.616 (-0.136)	0.522 (0.173)	***0.029*** (-0.544)
Range	0–11					
Mean ± SD	3.06±3.57					
Median	2					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

Table 5: CEACAM1 localization vs. primary CRC size in the matched group.

	Number	**Primary tumor CEACAM1 location**			
Variable	**(n=18)**	***Apical***	***Apical-Cytoplasmic***	***P value***	***r value***
Primary tumor size (cm)[c]		5	10	***0.036***	0.545
Range	2–7				
Mean ± SD	3.57±1.3				
Median	3.5				

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

4.2.4. Correlations with the progression variables of the metastatic disease:

The correlation analysis with the metastatic disease progression variables in the matched samples showed a significant positive correlation of CEA expression in the primary tumor with the number of metastatic lesions in the liver (p-value = 0.01). A near significant correlation between lympho-vascular invasion in the metastatic tumor with CEA expression in the primary tumor was identified (p-value = 0.92) (Table 6). In addition, the isolated apical staining of CEACAM1 in the primary tumor was found to be significantly correlated with the number of metastatic liver lesions compared to the combined apical and cytoplasmic staining (p-value = 0.03) (Table 8) (Figure 10).

4.2.5. Correlations with chemotherapy:

There were no significant correlations with neither the primary nor the secondary tumor CEACAM1 and CEA IHC expression scores against chemotherapy cycles and response (Table 7). However, the combined apical and cytoplasmic staining of CEACAM1 in the primary tumor was found to be significantly correlated with the favorable response to chemotherapy compared to the isolated apical staining in the matched samples (p-value = 0.008) (Table 8).

Table 6: Correlations with the progression of metastatic disease in the matched group.

	Number	**Primary tumor CEACAM1 expression**	**Primary tumor CEA expression**	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=18)**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**
Synchronous vs metachronous[d]		0.102	0.6	0.136	0.472	0.438
Synchronous (within one Year)	12					
Metachronous	5					
Unknown	1					
Number of metastatic liver lesions[c]		0.2 (-0.32)	***0.01*** (0.58)	0.23 (-0.3)	0.65 (-0.11)	0.34 (-0.24)
Range	1–13					
Mean	3.5					
Median ± SD	2±3.49					
Size of metastatic liver tumors (cm)[c]		0.57 (-0.19)	0.6 (0.18)	0.94 (0.03)	0.66 (-0.15)	0.98 (-0.01)
Range	0.4–7					
Mean ± SD	3.7±1.66					
Median	3.6					
Liver tumor lympho-vascular invasion[c]		0.23 (-0.31)	0.092 (0.42)	0.63 (-0.13)	0.15 (0.37)	0.58 (-0.145)
Yes	2					
No	16					
Disease recurrence[c]		0.69 (1.44)	0.89 (-0.05)	0.78 (-0.10)	0.89 (0.05)	0.27 (-0.38)
Yes	10					
No	2					
Unknown	6					
Extra-hepatic metastasis[c]		031 (-0.36)	0.91 (-0.04)	0.54 (-0.22)	0.91 (0.04)	0.73 (-0.13)
Yes	7					
No	3					
Unknown	8					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

Table 7: Correlations with chemotherapy cycles and response in the matched group.

	Number	**Primary tumor CEACAM1 expression**	**Primary tumor CEA expression**	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=18)**	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]
Number of chemotherapy cycles to treat liver metastasis[c]		0.95 (0.02)	0.36 (-0.30)	0.17 (-0.44)	0.36 (-0.30)	0.47 (-0.24)
Range	4–11					
Mean ± SD	7.5±2.3					
Median	6					
Tumor response to chemotherapy[c] **‘** [d]		0.89 (0.04)	0.59 (0.16)	0.18 (-0.38)	0.87 (0.05)	0.15 (-0.4)
Complete	4					
Partial	8					
Progressive	2					
Unknown	4					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Tumor response to chemotherapy evaluated by the changes in CT morphology

Table 8: CEACAM1 localization vs. lesion count and chemotherapy response in the matched group.

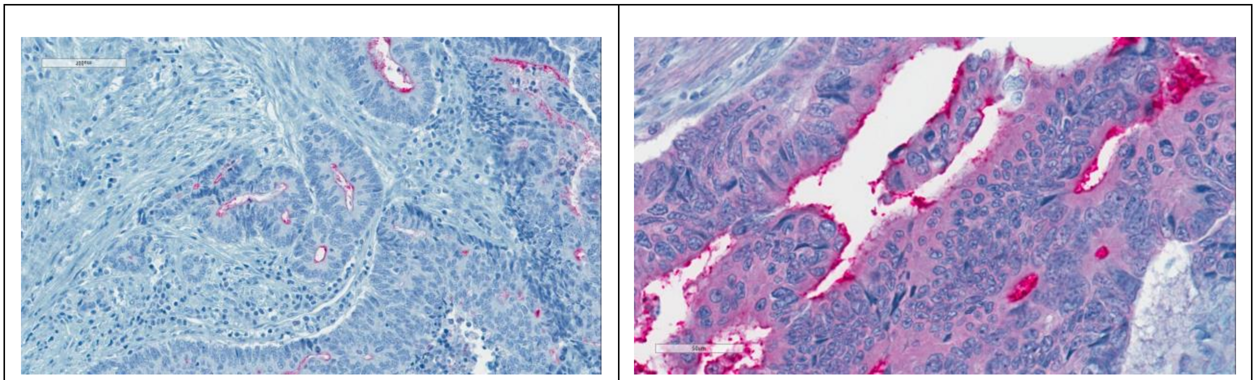
Variable	**Number (n=18)**	**Primary tumor CEACAM1 location**			
		Apical	***Apical-Cytoplasmic***	***P value***	***r value***
Number of metastatic liver lesions[c]		7	10	***0.03***	-0.51
Range	1–13				
Mean ± SD	3.5±3.49				
Median	2				
Tumor response to chemotherapy[c, d]		6	8	***0.008***	-.677
Complete	4				
Partial	8				
Progressive	2				
Unknown	4				

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Tumor response to chemotherapy evaluated by the changes in CT morphology



a. More metastatic lesions with apical staining.

b. Less metastatic lesions with mixed apical and cytoplasmic staining.

Figure 10: *CEACAM1 staining location in primary CRC in relation to the number of metastatic lesion in the matched group.*

4.3. CRCLM complete cohort analysis:

4.3.1. Descriptive analysis:

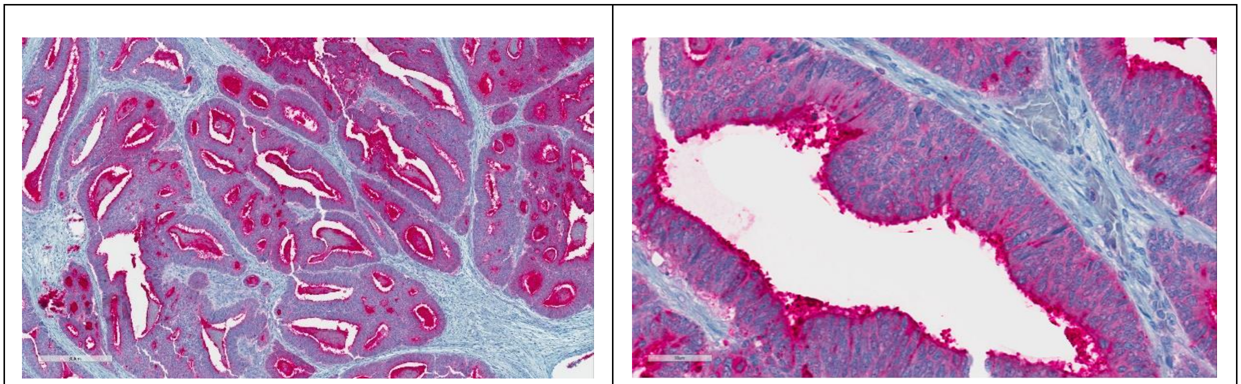
The second set of analyses were done on CRCLM samples from all 43 patients included in the study **(**Figure 11**)**. The overall descriptive analysis of CEACAM1 and CEA IHC expression scores in the liver metastasis and normal liver tissue of each patient is shown on Table 9. CEACAM1 expression in the liver metastasis was found to be positive in about 81% of the sample, whereas CEA expression was positive in about 91%. CEACAM1 expression had also been tested on normal liver tissue, which showed a positive expression in almost 56% of all samples.

Similar to our previous analysis on the matched group, we had identified the staining location of the IHC markers here as well for all CRCLM samples (Table 9). For CEACAM1, almost half of the samples from liver metastasis stained both apical and cytoplasmic regions, while the other half stained only the apical region of the tumor cells. In contrast, most samples stained the apical and cytoplasmic regions at the same time with CEA (97.7%).

Table 9: Descriptive analysis of immunohistochemistry in all samples.

Variable	**IHC expression**			**IHC staining location**		
Patients with metastatic colorectal carcinoma **(n=43)**	Weak (0-3)	Moderate (4-6)	Strong (7-9)	None	Apical	Apical-Cytoplasmic
CEACAM1 in liver metastasis	8	18	17	1	21	21
	18.6%	41.9%	39.5%	2.3	48.8%	48.8%
CEA in liver metastasis	4	18	21	0	1	42
	9.3%	41.9%	48.8%		2.3%	97.7%
CEACAM1 in normal liver tissue*	19	21	3			
	44.2%	48.8%	7%			

° IHC score ≥4 was considered as a positive expression
* CEACAM1 staining location in the normal liver tissue is typically membranous



a. 4X view

b. 20X view

Figure 11: CEACAM1 staining in CRCLM.

4.3.2. Correlations with the demographic characteristics:

The correlation of the demographic variables with the IHC expression of CEACAM1 and CEA in CRCLM and the normal liver tissue resulted in similar findings to the initial analysis on the matched subgroup. We continue to observe a statistically significant inverse correlation of CEACAM1 expression in the background normal liver tissue with the body mass index in all 43 patients (p-value = 0.003) (Table 10).

Table 10: Correlations with the demographic characteristics of all patients.

Variable	Number (n=43)	Metastatic tumor CEACAM1 expression	Metastatic tumor CEA expression	Normal Liver CEACAM1 expression
		P **value**[a] **(*r* value)**[b]	***P*** **value**[a] **(*r* value)**[b]	***P*** **value**[a] **(*r* value)**[b]
Sex[d]		0.52	0.86	0.97
Male	28			
Female	15			
Age (y/o)[c]		0.26 (0.176)	0.41 (0.13)	0.54 (0.1)
Range	31–81			
Mean ± SD	63.84±11.42			
Median	67			
BMI (kg/m^2)[c]		0.78 (-0.53)	0.91 (-0.02)	***0.003*** (-0.52)
Range	15–44			
Mean ± SD	26.16±6.59			
Median	26			

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

4.3.3. Correlations with the clinical staging variables of the primary disease:

Correlating the primary disease staging of all patients with the IHC expression scores in both the metastatic liver tumor and the normal liver tissue samples showed a significant inverse correlation of CEACAM1 with the N stage (p-value = 0.006 and 0.02, respectively). A near significant negative correlation was found between the overall primary tumor stage and CEACAM1 expression in the background liver (p-value = 0.09) (Table 11). In addition, the absolute number of positive lymph nodes in the initial primary disease was found to be inversely correlated with CEACAM1 score within the normal liver tissue as well (p-value = 0.004) (Table 12). Furthermore, the isolated apical staining of

CEACAM1 in the metastatic liver tumor was found to be significantly correlated with the advanced N stage compared to the combined apical and cytoplasmic staining (p-value = 0.004) (Table 13). On the other hand, there were no similar significant correlations seen with CEA except for a near significant correlation seen between the N stage and CEA expression in liver metastasis (p-value = 0.08) (Table 11)

Table 11: Correlations with the primary disease staging in all patients.

	Number	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=43)**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**
Primary AJCC Stage[c]		0.16 (-0.27)	0.63 (0.09)	0.09 (-0.32)
Stage I - II	5			
Stage III - IV	24			
Unknown	14			
Primary tumor T stage[c]		0.8 (-0.05)	0.95 (0.01)	0.31 (0.2)
T2-T3	21			
T4	8			
Unknown	14			
Primary tumor N stage[c]		***0.006*** (-0.5)	0.08 (0.33)	***0.02*** (-0.43)
N0	5			
N1	15			
N2	9			
Unknown	14			
Primary tumor M stage[c]		0.43 (-0.16)	0.98 (0.01)	0.33 (-0.2)
M0	19			
M1	7			
Unknown	17			

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

Table 12: Correlations with the primary disease features in all patients.

	Number	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**	**Normal Liver CEACAM1 expression**
Variable	**(n=43)**	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]	***P value***[a] ***(r value)***[b]
Primary tumor source[d]		0.76	0.47	0.18
Colon	18			
Rectum	17			
Unknown	8			
Primary tumor size (cm)[c]		0.32 (0.2)	0.67 (0.09)	067 (0.09)
Range	1–7			
Mean ± SD	3.54±1.24			
Median	4			
Primary tumor lympho-vascular invasion[c]		0.11 (-0.32)	0.29 (0.22)	0.74 (-0.07)
Yes	14			
No	12			
Unknown	17			
Number of positive lymph nodes[c]		0.33 (-0.19)	0.38 (0.18)	***0.004*** (-0.54)
Range	0–27			
Mean ± SD	3.96±6.34			
Median	2			

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

Table 13: CEACAM1 localization vs. lymph node status in all patients.

	Number	**Metastatic tumor CEACAM1 location**				
Variable	**(n=43)**	***No stain***	***Apical***	***Apical-Cytoplasmic***	***P value***	***r value***
Primary tumor N stage[c]					**0.004**	-0.52
N0	5		1	4		
N1	15		7	8		
N2	9	1	7	1		
Unknown	14					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

4.3.4. Correlations with the progression variables of the metastatic disease:

The metastatic disease progression variables included synchronicity, the number and size of lesions, lympho-vascular invasion, extra-hepatic metastasis and disease recurrence. The correlation analysis of these variables with the IHC expression scores of both metastatic liver tumor and the normal liver tissue samples from each patient did not result in any significant correlation (Table 14). However, considering that many patients had multiple liver lesions, correlating the IHC expression score from each liver lesion (92 lesions) with the tumor differentiation of the same lesion showed a significant correlation with CEACAM1. Specifically, poorly differentiated tumors had higher CEACAM1 expression compared to the moderately differentiated ones (p-value = 0.017) (Table 15).

Table 14: Correlations with the progression variables of metastatic disease in all patients.

Variable	Number (n=43)	Metastatic tumor CEACAM1 expression *P value*[a] *(r value)*[b]	Metastatic tumor CEA expression *P value*[a] *(r value)*[b]	Normal Liver CEACAM1 expression *P value*[a] *(r value)*[b]
Synchronous vs metachronous[d]		0.7	0.22	0.34
Synchronous (within one Year)	27			
Metachronous	11			
Unknown	5			
Number of metastatic liver lesions[c]		0.63 (0.08)	0.39 (0.15)	0.58 (-0.09)
Range	1–13			
Mean ± SD	3.02±2.66			
Median	2			
Size of metastatic liver tumors (cm)[c]		0.59 (0.11)	0.32 (-0.21)	0.74 (-0.07)
Range	0.4–10			
Mean ± SD	3.67±2.42			
Median	3.5			
Liver tumor lympho-vascular invasion[c]		0.46 (0.12)	0.12 (0.24)	0.43 (-0.13)
Yes	5			
No	38			
Disease recurrence[c]		0.3 (0.2)	0.82 (0.04)	1 (0)
Yes	19			
No	10			
Unknown	14			
Extra-hepatic metastasis[c]		0.58 (0.12)	0.65 (-0.09)	1 (0)
Yes	15			
No	13			
Unknown	15			

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Chi-square test

Table 15: Correlations with the level of differentiation in all metastatic liver lesions.

Variable	**Number** (n=92)	**Metastatic tumor CEACAM1 expression**	**Metastatic tumor CEA expression**
		***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**
Liver tumor differentiation[c]		***0.017*** (0.25)	0.37 (0.1)
Moderately differentiated	49		
Poorly differentiated	43		

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

4.3.5. Correlations with chemotherapy:

There were no significant correlations with neither CEACAM1 nor CEA IHC expression scores in the metastatic liver tumor against chemotherapy cycles and response (Table 16). However, the combined apical and cytoplasmic staining of CEACAM1 was found to be significantly correlated with the favorable response to chemotherapy compared to the isolated apical staining of the metastatic tumor cells in the liver (p-value = 0.016) (Table 17).

Table 16: Correlations with chemotherapy cycles and response in all patients.

Variable	Number (n=43)	Metastatic tumor CEACAM1 expression	Metastatic tumor CEA expression	Normal Liver CEACAM1 expression
		***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**	***P value*[a]** ***(r value)*[b]**
Number of chemotherapy cycles to treat liver metastasis[c]		0.36 (-0.18)	0.3 (-0.20)	0.57 (-0.11)
Range	0–13			
Mean ± SD	7.14±2.6			
Median	6			
Liver tumor response to chemotherapy[c] ‘ [d]		0.14 (-0.23)	0.72 (0.07)	0.32 (-0.19)
Complete	6			
Partial	13			
None	4			
Progressive	5			
Unknown	15			

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Tumor response to chemotherapy evaluated by the changes in CT morphology

Table 17: CEACAM1 localization vs. chemotherapy response in all patients.

Variable	Number (n=43)	Metastatic tumor CEACAM1 location				
		No stain	***Apical***	***Apical-Cytoplasmic***	***P value***	***r value***
Liver tumor response to chemotherapy[c] ‘ [d]		1	11	16	***0.016***	-.449
Complete	6					
Partial	13					
None	4					
Progressive	5					
Unknown	15					

[a] P values less than 0.05 indicate statistical significance

[b] r value represents Correlation coefficient

[c] Spearman's rank correlation

[d] Tumor response to chemotherapy evaluated by the changes in CT morphology

Chapter 5: Discussion

Colorectal cancer is a severe disease. Colorectal liver metastasis is responsible for about two thirds of its related deaths. There is still no accurate or effective marker available for CRC. However, CEA is currently still the standard marker used to indicate metastasis, poor prognosis and to monitor the response of metastatic disease to systemic therapy. Nevertheless, its sensitivity and specificity rates remain moderate. In an effort to identify an effective non-invasive marker for CRC, the CEACAM family has been explored for years. CEACAM1, one of the important identified members of the family, was found to potentially modulate tumorigenesis in addition to various other cellular activities. In colorectal cancer, CEACAM1 is thought to have a bimodal role. It is associated with tumor suppression early in CRC and tumor promotion in advance metastatic disease. Nevertheless, human studies investigating the expression of CEACAM1 in CRCLM are still lacking and the reason why CRC cells re-expresses CEACAM1 remains unclear.

In this study, we had identified the immunohistochemical expression of CEACAM1 in metastatic colorectal cancer for the first time by testing matched samples from primary and metastatic lesions. We also compared the expression of CEACAM1 to the expression of CEA in CRCLM. In addition, we correlated some of the clinical findings and outcomes to the immunohistochemical expression of CEACAM1. Our data showed that CEACAM1 expression in CRC and CRCLM was associated with more clinical variables compared to CEA.

5.1. CEACAM1 expression in CRC:

Our study showed that both CEACAM1 and CEA have high expression rates in metastatic colorectal cancer. In the primary tumor, CEACAM1 had a positive expression in 85% of our sample. In contrast, CEA expression was positive in 95% of our tested samples. When we tested the corresponding CRCLM lesions, CEACAM1 and CEA had positive expressions in 95% and 90% of the metastatic liver tumors respectively. Previous findings of Jantscheff, P., et al. obtained from studying primary CRC samples, showed a positive expression rate of CEA in CRC tumors that is similar to our finding. However, their study showed that CEACAM1 was positive in 58% primary tumors only [96]. Despite the different rate of CEACAM1 expression in the primary tumor identified in our study compared to the study by Jantscheff, P., et al., we certainly do agree with these authors that CEACAM1 can be highly expressed in many cases of CRC. Since CEACAM1 is associated with tumor suppression early in CRC and tumor promotion in advance metastatic disease, the observed differences can be attributed mainly to the different stages of disease in both studies. Our study included only patients with metastatic colorectal cancer, whereas, the study done by Jantscheff, P., et al. included patients with resected CRC with no specification of the disease staging that were not necessarily metastatic. Another variable in these respective studies is the specificity and affinity of the anti-CEACAM1 mAbs used. The Jantscheff study used the 4D1/C2 mAb recognizing an epitope in the last CEACAM1 constant domain [102], whereas the 5F4 mAb used in this work recognizes a distinct epitope in the highly N domain [100]. Therefore, the observed differences may be part of the potential bimodal role of CEACAM1 in CRC tumorigenesis.

By including all CRCLM samples in our study, i.e. matched and unmatched lesions, CEACAM1 and CEA had positive expressions in 81% and 91% of the metastatic liver tumors, respectively. In addition, normal background liver tissue showed a positive expression in 56% of our sample. These variations in CEACAM1 expression may represent a significant process in CRC tumorigenesis.

We also identified some variation in the cellular staining location of CEACAM1 in CRC and CRCLM. In the primary tumor, CEACAM1 stained about 55% of the matched samples in both apical and cytoplasmic parts of the cancer cells and 39% only stained at the apical region. However, almost half of the samples from liver metastasis stained both apical and cytoplasmic regions, while the other half stained only the apical region of the tumor cells. In contrast, most samples stained the apical and cytoplasmic regions at the same time with CEA in both the primary and metastatic tumor samples. We will be discussing the correlation of these findings and variations in CEACAM1 expression with some of the clinical findings and outcomes to further elucidate its role in colorectal cancer liver metastasis.

5.2. Hepatic CEACAM1 and obesity:

We started our analysis by studying the demographic variables of our patient population. Our data showed an inverse correlation between CEACAM1 expression in the background normal liver tissue and the body mass index. Patients with higher BMI tend to have lower hepatic expression of CEACAM1. These findings confirm previous data shown in the literature. The study by Lee W. identified lower immunohistochemical expression of CEACAM1 with high grade fatty liver and severe obesity [103]. In addition, previous data by Xu E, et al. indicated that CEACAM1 is a key regulator of hepatic lipogenesis and that Ceacam1-/- mice with chronic dietary fat exposure are predisposed to liver steatosis, leading to hepatic insulin resistance and liver damage [104]. Severe obesity is known to be associated with insulin resistance [105], which may explain the observed correlation with CEACAM1. The role of CEACAM1 in regulating insulin clearance and degradation by the liver was studied by Najjar SM group in 2002. The paper demonstrated that CEACAM1 increases insulin clearance to maintain insulin sensitivity by studying a transgenic mouse model. They concluded that CEACAM1 might modify insulin action by increasing its degradation and by altering the signaling pathway of receptor-mediated insulin endocytosis [106]. Thus, the observed loss in hepatic CEACAM1 expression in obese patients may reflect its possible association with the development of insulin resistance.

5.3. Primary disease stage:

We analyzed the overall primary disease stage in addition to each individual element of the staging in relation to CEACAM1 and CEA. In the matched samples, CEA expression in the primary tumor was significantly correlated with both the overall TNM stage, as well as the isolated N stage. In contrast, there were no similar significant correlations seen with CEACAM1 expression score in the tumor samples. However, the combined apical and cytoplasmic staining pattern of CEACAM1 in the primary tumor was found to be significantly correlated with the larger size primary tumors. The previous study of Kang, W.Y., et al. did in fact show a significant correlation of CEACAM1 in the primary tumor with the overall stage [91]. However, our study included more CRCLM samples than primary tumors. Thus, the small sample size of primary CRC lesions in our study could be the reason behind the different findings compared to Kang’s study.

When we included the complete cohort of CRCLM samples, CEACAM1 expression showed significant correlations with the lymph node status. Extensive lymph node involvement of the primary tumor were associated with loss of CEACAM1 expression in both the metastatic tumor and the background normal liver as well. In addition, the isolated apical staining pattern of CEACAM1 in the metastatic liver tumor was associated with advanced lymph node involvement. The Ieda, J., et al., paper from Japan, showed a significant correlation of CEACAM1-L dominance in the primary CRC with the advanced lymph node status and the presence of distant metastasis. However, CEACAM1 in human CRCLM samples was not previously studied. Therefore, combining previous data with our findings may further clarify the picture. The apparent trend in extensive primary lymph node involvement is directed towards seeing high expression of CEACAM1 in the primary CRC and low expression in CRCLM and the background normal liver.

5.4. Liver metastasis:

We attempted to identify the relevant variables of liver metastasis in relation to CEACAM1 and CEA expression. Our analysis included synchronicity, the number and size of lesions, lympho-vascular invasion, extra-hepatic metastasis and disease recurrence. In the matched group, high expression of CEA in the primary tumor was associated with more metastatic liver lesions. Similar correlation was not seen with CEACAM1 expression score, but, the isolated apical staining pattern of CEACAM1 in the primary tumor was also associated with more metastatic liver lesions.

Considering that many patients had multiple liver lesions and each tumor may have a different degree of differentiation, we analyzed CEACAM1 expression in each lesion. Our data showed that poorly differentiated metastatic tumors had higher CEACAM1 expression compared to the moderately differentiated ones. Kang's study showed similar findings, but in primary CRC tumors. Their study included adenomas with low or high grade dysplasia and adenocarcinoma lesions as well. Higher expression trend of CEACAM1 was seen towards adenocarcinoma [91].

The response of liver metastasis to chemotherapy can be used as an indicator of disease regression, stability or progression. Our data showed that progressive lesions following chemotherapy were associated with an isolated apical staining pattern of CEACAM1 in the primary tumor of the matched group and the metastatic tumors of our complete cohort. These findings may represent a role of CEACAM1 in the disease progression process.

5.5. CEACAM1 Staining location:

Our study identified some variation in the cellular staining location of CEACAM1 in both the primary and metastatic lesions. Part of our samples stained both apical and cytoplasmic parts of the cancer cells and the remaining stained at the apical region only. Our data showed five significant findings related CEACAM1 staining location described above (Figure 14). Four of which were suggestive of disease progression with the isolated apical staining pattern of tumors compared to the combined apical and cytoplasmic staining. Apical staining in the primary tumor was associated with the number of metastatic lesion and liver tumor progression in response to chemotherapy. Whereas CEACAM1 apical staining in metastatic tumors was associated with advanced primary lymph node involvement in addition to tumor progression in response to chemotherapy. CEACAM1 staining location was previously studied in gastric adenocarcinoma. Zhou, C.J. et al., showed that cytoplasmic CEACAM1 was seen with diffuse carcinoma. However, they found that membranous CEACAM1, including apical and uniform staining patterns, promoted angiogenesis in the gastric carcinoma areas, and cytoplasmic CEACAM1 inhibited angiogenesis. They concluded that the transformation of CEACAM1 expression from a membranous to a cytoplasmic distribution is an important event to reverse tumor angiogenesis effects [107]. Our study mainly showed an association of CEACAM1 apical staining with progressive features of CRC, which may be due to a modulatory effect on tumor angiogenesis in addition to other unknown factors.

5.6. Limitations:

Even though this study was carefully planned, there were several limitations that should be taken into account. First, the small sample size of the primary CRC tumors due to the limited access to tissue samples, as many patients had their primary tumor resection surgery done at different hospitals. This affected the matched sample group of CRCLM and CRC. However, we performed our analysis on the complete cohort of CRCLM samples and the matched subset group separately. Second, our study only included patients known to progress to stage IV disease. Including patients with early stage disease would have been beneficial. However, previous CEACAM1 studies were mostly done on primary CRC samples of earlier disease stages with no previous studies done on human CRCLM. Finally, the retrospective nature of collecting the required clinical information had led to some missing data, despite our careful chart review and search of clinical health records.

5.7. Conclusion:

Colorectal cancer is a severe disease. Colorectal liver metastasis is responsible for about two thirds of its related deaths. CEA is currently still the standard marker used to indicate metastasis, prognosis and response to treatment. However, CEACAM1 is believed to potentially be a more reliable and effective marker for CRC. In this study, we identified the immunohistochemical expression of CEACAM1 in metastatic colorectal cancer for the first time by testing matched samples from primary and metastatic lesions. We then compared the expression of CEACAM1 to CEA in relation to the clinical data. Our study showed that CEA and CEACAM1 have high expression rates in advanced colorectal cancer both in the primary and metastatic tumors. CEA expression in the primary tumor was found to be associated with lymph node involvement, disease staging and the number of metastatic liver lesions. On the other hand, CEACAM1 expression showed different significant correlations with the clinical variables. We first identified loss of hepatic CEACAM1 expression in obese patients with CRCLM, which could be related to the potential role of CEACAM1 in insulin resistance. In addition, our data showed that extensive lymph node involvement of the primary tumor is associated with loss of CEACAM1 expression in both the metastatic tumor and the background normal liver. Previous studies of CEACAM1 showed that higher expression in the primary CRC is associated with advanced lymph node status. Therefore, the apparent trend in extensive primary lymph node involvement is directed towards seeing high expression of CEACAM1 in the primary CRC and low expression in CRCLM and the background normal liver. We additionally found that poorly differentiated metastatic liver tumors had higher CEACAM1 expression compared to the moderately differentiated ones. Finally, our study showed an association of CEACAM1 apical staining with progressive features of CRC. Apical staining in the primary tumor was associated with the number of metastatic lesions and tumor progression in response to chemotherapy. Whereas in metastatic tumors, apical staining of CEACAM1 was associated with advanced primary lymph node involvement in addition to tumor progression following chemotherapy. Overall, our experience showed that CEACAM1 expression in CRC and CRCLM was associated with more relevant clinical variables compared to CEA, which may represent an important modulatory effect of CEACAM1 in CRC tumorigenesis.

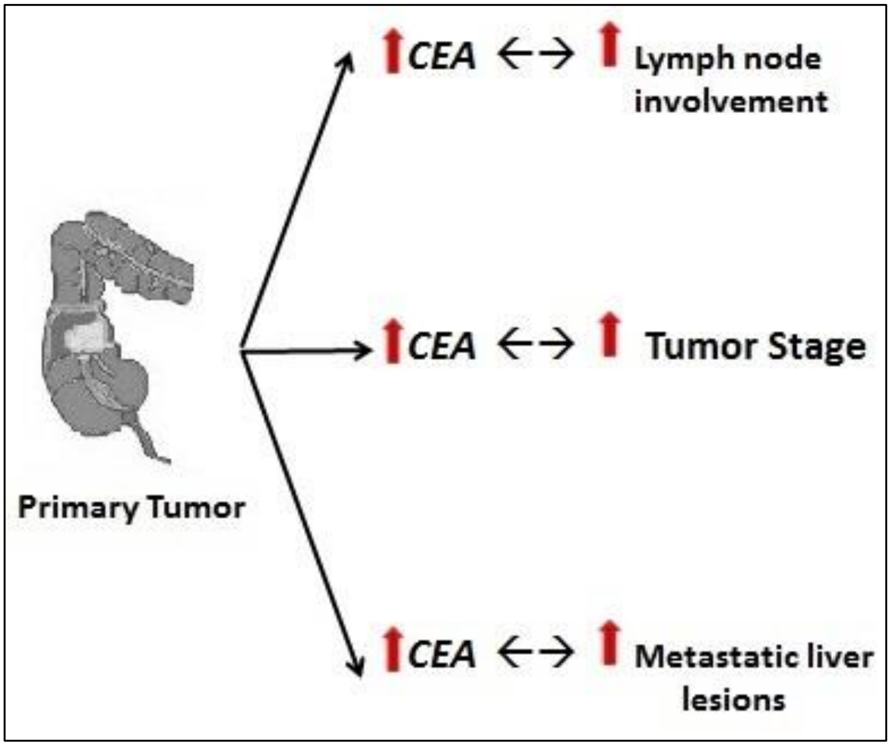


Figure 12: *Summary of the significant findings in the primary CRC.*

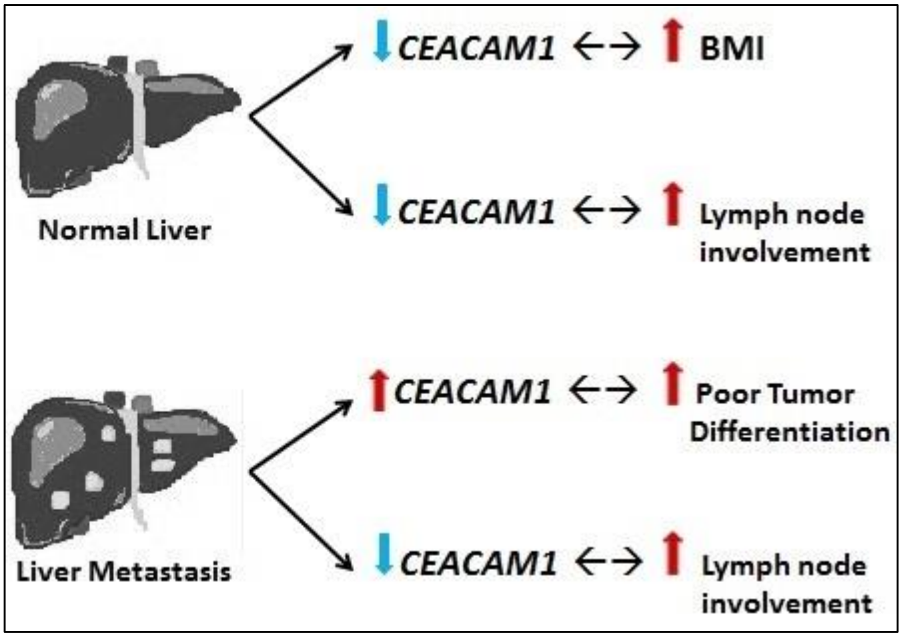


Figure 13: *Summary of the significant findings in liver metastasis and the background normal liver.* ***(*** Novel findings***)***

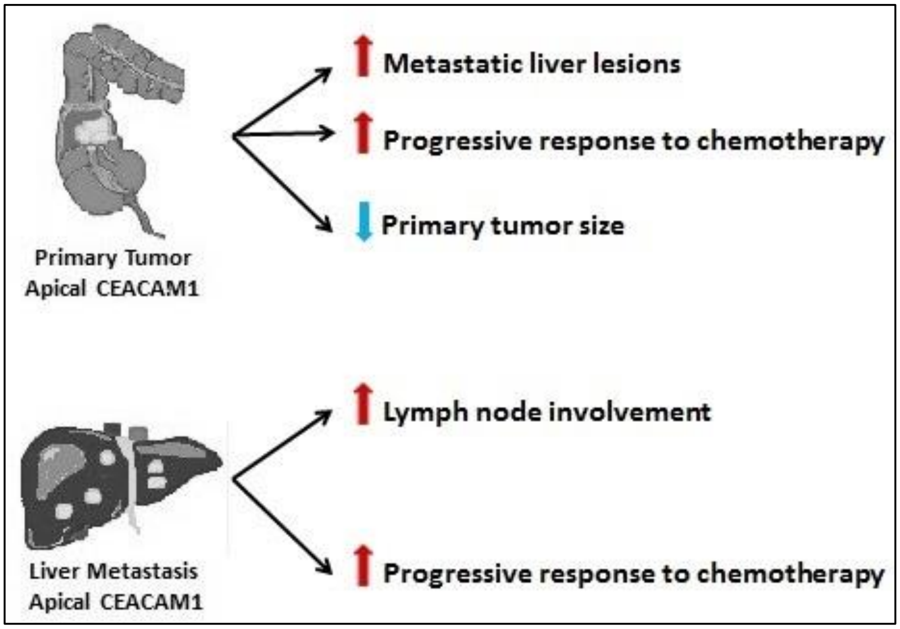


Figure 14: *Summary of the significant findings related to the apical staining location of CEACAM1.* ***(*** Novel findings***)***

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