Carcinoembryonic Antigen: a Multifunctional Molecule in Colorectal Cancer Progression and Metastasis

Authors
Peter Thomas[1]
Murali Kuracha[1]
Brian Loggie[1]
Venkatesh Govindarajan[2]

Affiliations
Depts. Of Surgery[1] and Biomedical Science[2],
Creighton University,
Omaha, Nebraska 68178
USA

Corresponding author:
Peter Thomas, Ph.D.
Professor of Surgery and Biomedical Sciences
Creighton University
2500 California Plaza, Criss III Rm 254
Omaha, NE 68178

Tel: 402-280-3921
Fax:402-280-3461
E-mail:
peterthomas@creighton.edu

Abstract

Colorectal cancer is a major public health problem and the majority of deaths associated with the disease are the result of metastatic spread to the liver. The biology of colorectal cancer metastasis is slowly being unraveled and involves multiple mechanisms that provide the cancer cell with an advantage for invasion, survival in the circulation and for implantation and growth in the liver. A glycoprotein tumor product that seems to offer an advantage in all these areas is carcinoembryonic antigen (CEA, CEACAM5, CD66e). While the function of CEA in normal tissues is still a subject of speculation, CEA seems to play a critical role in cancer and is involved in multiple mechanisms that are designed to provide the cancer cell with an advantage to invade and metastasize. These mechanisms include induction of changes in the tumor microenvironment at both the primary and distant metastatic site, protection against both apoptosis and cytotoxicity and implementation of angiogenisis. Thus, this review is focused on the role of CEA in promoting colorectal cancer progression and metastasis especially to the liver.

Keywords: carcinoembryonic antigen; liver metastasis, colorectal cancer, anoikis, angiogenesis, cytokines

1. Introduction/Background:

Carcinoembryonic antigen (CEA) is a well-known glycoprotein marker for colorectal and other epithelial cancers. The discovery of CEA in 1965 [Gold and Freedman 1965], marked a turning point in the study of cancer and led to what might be called the age of tumor markers. CEA was the first commercially available tumor marker and may be considered the prototype [Zamcheck, 1981]. While the early studies on CEA showed great promise, the hope that CEA levels could be used as a screening test for colorectal cancer was not satisfied. CEA was found not to be specific to any particular cancer and the high false positive and false negative results made its use for early detection of colon cancer unfeasible. Nevertheless, CEA is the most used serum marker for colorectal cancer and give an indication of prognosis [Dhar et al 1972, Duffy et al, 2001, Moertel et al, 1986] It has also been used successfully for the detection of recurrences following surgery in colorectal cancer patients and can often detect new tumor growth before the onset of clinical symptoms [Arnaud et al, 1980, Goldstein et al, 2005]. CEA is also used for the detection of occult tumor using radiolabeled CEA specific antibodies (radioimmuno-detection) though lack of sensitivity has precluded its routine use [Goldenberg, et al, 1980]. CEA is also used as a target antigen for treatment using radioimmuno-therapy [Behr et al, 1996]. There are also ongoing investigations into CEA as a target antigen for immunotherapy [Kaufman et al, 1991, Morse et al, 2003, Ueda et al, 2004, Bacac, 2016]. Though CEA has not lived up to its initial promise it is useful in clinical medicine and studies of CEA are still ongoing particularly in immunotherapy. Until the cloning of its gene in 1989 [see Thompson et al 1991] very little was known about the molecular structure of CEA and its function in both normal and malignant tissues was unknown. Structural information was limited to CEA being an 180kD glycoprotein with about 60% N-linked sugar chains and 6 disulfide bridges [Thomas et al 1990]. Serum CEA was elevated in a number of different cancers including breast, stomach lung and thyroid but its major use was as a marker for colorectal cancers [Jessup and Thomas, 1998]. Initially thought to be tumor specific later studies showed moderate serum elevations of CEA in benign conditions including inflammatory bowel disease, heavy smokers and a variety of liver diseases including hepatitis and cirrhosis [Thomas and Zamcheck 1983]. Once the gene was cloned further studies showed that CEA was one member of a family of 28 genes and this family was related to the much larger immunoglobulin super family [Hammerstrom 1999]. A nomenclature for the complete CEA family of proteins can be found in Beauchemin, et al 1989.

While some earlier studies had been done on the metabolism of CEA including its interaction with macrophages [Toth et al 1985] and its potential role in promoting liver metastasis [Thomas et al 2011] it was only after 1989 that information on its possible function(s) became forthcoming. One of the first clues to function came when it was shown that CEA and other members of its gene family could participate in homotypic binding [Benchimol et al 1989; Beauchemin, and Arabzadehl 2013]. These interactions though weak were sufficient to cause adhesion between cells expressing CEA on the cell surface [Benchimol et al 1989, Thomas et al 1995]. Further the greatest elevations in serum CEA is associated with liver metastasis in colorectal cancers and is an indicator of poor prognosis [Zamcheck, 1981; Jessup and Thomas, 1998]. Thus a role for CEA in the establishment of colorectal cancer liver metastasis was proposed [Jessup and

Thomas, 1989] In this review we will examine the biology of CEA in reference to its function in the formation of distant metastasis particularly in the liver and also its potential function at the site of the primary tumor. The scope of this review is restricted to colorectal cancers though many of the functions ascribed to CEA may also impact other cancers.

2. Carcinoembryonic Antigen (CEA, CEACAM5, CD66e). Structure and Function.

CEA is a relatively large glycoprotein with a molecular weight in the region of 180kD. About 60% of this mass is composed of N-linked complex carbohydrate chains [Chandrasekeran et al. 1983]. These carbohydrate chains confer great solubility to the molecule. The protein backbone comprises of 651 amino acids that are arranged in 7 immunoglobulin-like domains. These domains comprise of an N-terminal V like immunoglobulin domain followed by six IgC domains each of which are stabilized by a disulfide bridge. In the mature protein there is a GPI tail that allows anchorage to the plasma membrane. This tail can be cleaved by phospholipases to release CEA from the cell [Sack et al, 1988].

CEA is produced in the normal colon and is located on the apical membrane of mature colonocytes. CEA released from the membrane is secreted into the lumen of the colon. In cancer CEA is distributed throughout the plasma membrane. The normal function of CEA is unclear. However, it can act as a receptor for bacteria and this likely confers protection to the normal colonic mucosa [Leusch et al, 1990, 1991]. Whether CEA performs functions in cancer cells that are distinct from the normal colonocyte, is not known.

2.1 The CEA Receptor (CEAR, hnRNP M)

The CEA receptor was first identified in Kupffer cells (hepatic fixed macrophages) as an 80kD surface protein that bound with CEA [Toth et al 1982]. Subsequently the gene for this protein was cloned and sequenced (Bajenova et al 2001). The CEA receptor thus cloned was found to be identical to the heterogeneous RNA binding protein (hnRNP) M4 and showed approximately 97% sequence homology between human and rat genes. [Bajenova et al 2001]. Four isoforms of hnRNP M have been described [Datar et al 1993] and two of these are known to bind CEA [Bajenova et al 2001]. One isoform (hnRNP M4) which was originally identified as the CEA receptor is 38 amino acid shorter than the longer form due to a deletion between the first and second RNA binding domains. The longer form also binds CEA; however the two other isoforms have not been investigated for interaction with CEA so their function in this respect is not known [Bajenova et al 2001]. CEAR is a highly expressed protein that is involved in binding both RNA and DNA. It also functions in the transport of mRNA to the cytoplasm [Marko et al 2010]. Though CEAR is most commonly expressed in the nucleus it can also be found both on the cell surface and in the cytoplasm of Kupffer cells [Bajenova et al, 2003]. In macrophage cell lines such as THP-1 CEAR is not expressed on the cell surface until the cells are activated by phorbol ester when CEAR translocates from the nucleus to the cell surface [Aarons et al 2007]. CEAR was also shown to bind to CEA in HT-29 colon cancer cells although the functional significance of this is not known [Laguinge et al 2005]. It has been suggested that both CEA and CEAR may be involved with the resistance of cancer cells to anoikis (induction of apoptosis when cells are unattached to extracellular matrix) [Soeth et al 2001,

Samara et al 2007]. Binding of CEA to CEAR occurs *via* a penta-peptide motif (PELPK) located at the hinge region between the CEA's N-terminal and first immunoglobulin loop domain. More recently it has been shown that PELPK binding occurs at the C-terminal end of CEAR and overlaps the third RNA binding domain [Palermo et al 2012]. This interaction produces cytokines by activating a signaling cascade and these cytokines alter the liver microenvironment such that it becomes more hospitable to the implantation and growth of the cancer cells [Gangopadhyay et al 1996, Gangopadhyay et al, 1998; Aarons et al 2007]. Production of both IL-6, IL-1, TNF-α and IL-10 by CEA stimulated Kupffer cells increases the survival and subsequent growth of weakly metastatic human colorectal cancer cells by upregulating endothelial cell adhesion molecules (CCAM-1, V-CAM-1 and E-selectin) and protecting against cytotoxicity in experimental models for liver metastasis [Thomas et al 2011]. Mutations in the PELPK motif results in extremely elevated serum CEA levels. These patients also tend to not develop liver metastasis [Zimmer and Thomas, 2000]. A similar situation is seen in the Cotton-top Tamarin that shows alterations in the PELPK sequence. Though they have a high incidence of colorectal cancers they also have a high CEA serum level and few if any liver metastasis [Tobi et al, 2011, Tobi et al 2016]. In general hnRNP proteins have been shown to be actively involved in cancer metastasis having a role in apoptosis, angiogenesis, cell invasion and involvement in the epithelial mesenchymal transition [Han, et al 2013). Using quantitative proteomics Chen et al (2013) suggested a role for hnRNP M specifically, as a biomarker for colorectal cancer.

3. CEA: a Promotor of Metastasis in colorectal cancer

Elevated serum CEA levels are associated with liver disease [Thomas and Zamcheck 1981]. Further the liver is the major organ for clearance of CEA from the circulation. CEA accumulates in Kupffer cells (liver fixed macrophages) is modified and passed to hepatocytes via the asialo-glycoprotein receptor for final degradation [Toth et al 1982, Toth et al 1985]. A study by Jessup et al, (1988) showed that the growth potential of tumors from patients with CEA >5ng/ml was greater than that for patients with normal levels (<2.5ng/ml). In a follow up study with 82 patients he showed that metastatic potential (i.e. ability to grow in the liver) was also correlated to serum CEA levels. (Jessup et al 1989). Taken together, these observations suggested an association between CEA and liver metastasis in colorectal cancer.

3.1. *Colorectal Cancer*

In the Western world, colorectal cancer is a major public health problem {Torre et al 2015]. In the United States, colorectal cancer is the second leading cause of cancer-related deaths and the third most common cancer that affect both men and women in equal proportions [Siegel, 2015]. The majority of these deaths (70%) are related to distant metastases. It is estimated that at the time of surgery for their primary colorectal cancer, approximately 10-25% of patients will have liver metastasis [Fortner et al 1984. Ruers et al 2002]. Another 20-30% will develop liver metastasis following resection of the primary colorectal cancer [Kemeny et al 1989]. Treatment options for patients with liver metastasis are limited. Few patients benefit from surgical removal of the liver tumors as most are not operable. With the extent of liver involvement being such a strong prognostic factor of median and 5-year survival rates

[Milikan 1997], it is imperative to investigate the mechanisms underlying the metastatic processes in colorectal cancer with a view to improving therapeutic options.

3.2 *Colorectal Cancer Metastasis*

The 5-year survival rate for patients with localized colorectal cancer is between 80-90%. However, those patients with distant metastases, most commonly to the liver and lung, have a 5-year survival rate of only 10-20% [Fortner et al 1984]. The unique circulatory system of the liver makes it a common site for metastatic invasion from gastrointestinal malignancies. Although only a subset of patients are suitable candidates, complete surgical resection of all liver metastasis does increase the 5-year survival rate [Bengtsson et al 1981]. However, it is estimated that relapse after resection occurs in approximately 75% of patients [Rees et al 2008].

Of those patients in whom metastases to the liver develop, approximately 25% of these will be candidates for surgical resection [Nordlinger et al 1996]. The introduction of fluorouracil combined with levamisole as an adjuvant therapy was a major advance in colorectal cancer treatment. Notably, Moertel et. al (1995) demonstrated that in patients with stage III disease, the drug combination showed a 33% reduction in mortality rate following surgical resection as compared to the surveillance group [Adson et al 1987]. However, in the context of hepatic metastasis, chemotherapeutic agents have proved much less efficacious. Systemic chemotherapy for metastatic colorectal cancer to the liver offers potential palliative management, but less than one-third of patients will respond and long-term survival is rare [Moertel et al]. Regional chemotherapy through hepatic arterial infusion (HIA) shows a better response rate compared to systemic chemotherapy, but no survival benefit between local and systemic chemotherapy has been demonstrated [Fong et al 1996; Hughes et al 1996. Vernook et al 1996]. Although the incorporation of oxaliplatin-based chemotherapy has improved overall survival in metastatic colorectal cancer recurrence rates over a five year period range from 20% to 75% [Andre et al 2004].

The high recurrence of liver metastaseis following liver resection suggests that undetected disease remains, either at the primary or metastatic site, or elsewhere. Patients with node-negative disease, recur at a very low rate for Stage 1 but up to 30% for stage II advances. The treatment of hepatic metastasis is therefore, still a major clinical problem. Thus more information on the mechanisms of metastasis development is needed if more effective therapies are to be designed. Because of the relationship with liver disease and colorectal cancer a study of CEA as a mediator for metastasis and its possibility as a target molecule is likely to throw insight into possible therapeutic advances

3.3 CEA as a Mediator of Colorectal Cancer Liver Metastasis

In colorectal cancers CEA is associated with a more differentiated phenotype. The CEA producing cells were more metastatic to the liver in a mouse model of hepatic metastases [Wagner et al 1992, Tibbetts et al 1993]. Colorectal cancer cells that produce low or no CEA are less well differentiated with none or few glandular structures, and are poorly metastatic to the liver [Wagner et al, 1989, Thomas et al 1995]. Intravenous injection of CEA in nude mice prior to intra-splenic injection of weakly metastatic human colorectal cancer cells significantly increased the number of mice with tumor deposits in the liver [Hostetter et al. 1990], suggesting a role for circulating CEA in liver metastasis development. Transfection of tumor cells with low CEA production, with the CEA gene

also results in increased metastatic potential [Hashino et al 1994, Thomas et al 1995]. These cells have a more mesenchymal like morphology and an increased capacity to invade through Matrigel [Danaker et al 1989]. CEA has been associated with a mechanism that increases the metastatic potential of the cancer cells to the liver and the lungs [Toth et al.1989] by interacting with the CEAR expressed on Kupffer cells and lung alveolar macrophages. [Bajenova et al, 2001]. CEA binding to CEAR induces the expression of pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) that allow adhesion of tumor cells to the liver sinusoidal epithelium by increasing adhesion molecules such as ICAM-1 [Gangopadhyay, 1998, Jessup et al 2004]. Release of IL-8 may also affect metastatic potential of the tumor cells [Wang et al 2017]. Toxic levels of nitric oxide and other reactive oxygen species can be released during liver metastasis formation by blockage of the sinusoids by cancer cells. This hypoxia initiates the synthesis of ROS. Release of IL-10 by CEA activated macrophages protects the cancer cells from cytotoxicity due to hypoxia by inhibiting inducible nitric oxide synthetase (iNOS) and inhibiting production of ROS particularly from endothelial cells [Jessup et al, 2005,]. This reduces the chances of cancer cell death and is another mechanism by which CEA gives the metastatic tumor cell a survival advantage.

The effects of alcohol on the development of cancer including liver breast and colon are well known and is a public health problem [Boffetta et al 2006, Rehm et al, 2014, Nelson et al. 2013]. However, very little is known about the potential effects of alcohol ingestion on the development of liver metastases from other primary sites including colorectal cancer [Stangle et al, 1994; Maeda et al, 1998]. Recent studies have suggested a role for CEA in this process. Early studies in rhesus monkeys fed an alcohol containing diet showed impaired clearance of CEA from the circulation [Thomas et al, 1982]. When CEA metabolism in the liver is impaired by alcohol, circulating levels of CEA are raised and Kupffer cells become more sensitive to CEA, increasing cytokine production and inflammatory changes conducive to cancer cell implantation and growth. [McVicker et al 2013, Mohr et al, 2017]. This may represent a mechanism for increased liver metastasis in the alcoholic with colon cancer.

CEA has also been implicated in other pro-metastatic mechanisms including the promotion of angiogenesis [Bramswig, et al 2013] and the inhibition of anoikis [Ordonez, et al 2000]. TGF-β signaling is important for tumor progression [Massague, 2008] and CEA has been shown to interact with the TGF-β receptor and interferes with signaling in colorectal cancer [Li et al 2010]. CEA and its family member CEACAM-6 are also targets for Smad3 related TGF-β signaling. Active TGF-β signaling increases CEA synthesis by increasing the activity of the CEA promotor. [Han et al, 2008]. Chen et al 2016, have also shown increased CEA in adenomas associated with reduced TGF-β signaling. Li et al 2016 showed that both TNF-α and TGF-β1 produced by macrophages can increase the rate of migration of breast cancer and melanoma cell lines using a matrix metalloproteinase dependent mechanism. As both TNF-α and TGF-β1 production by macrophages can be influenced by soluble CEA, at least in colorectal cancer, this suggests a further mechanism for CEA involving migration at the primary site and could represent an additional role for CEA in the development of a metastatic phenotype. Therefore a number of systems that can be effected by CEA, all seem to give the tumor cell a selective advantage to invade metastasize and survive.

. A summary of CEAs role in the metastatic cascade can be seen in Figure 1. The next

sections of this review will provide additional insights into these areas.

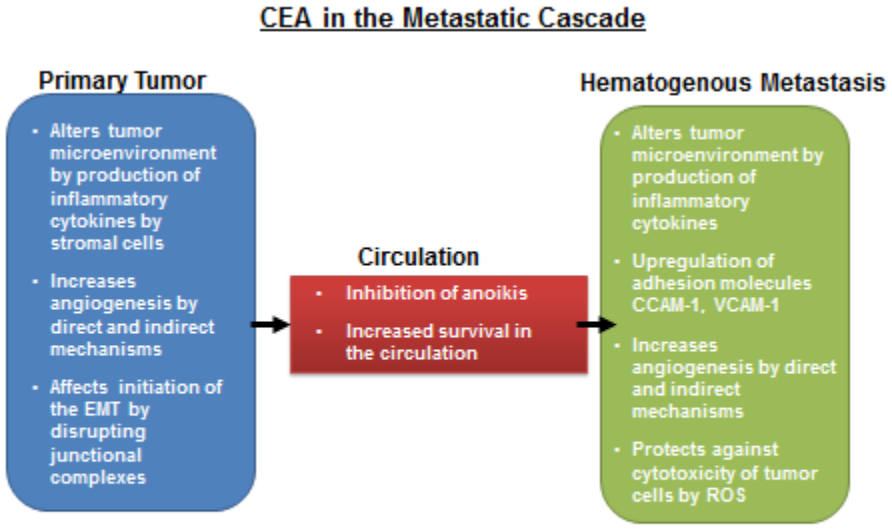

Figure 1: representation of the involvement of CEA in the metastatic cascade

4. CEA an Adhesion Molecule.

Previous work has shown a direct role for CEA in promoting cell/cell adhesion. CEA and other members of its family can form homotypic complexes [Benchimol et al, 1989, Zhou, et al 1993] that can result in increased cell aggregation. These homotypic adhesions occur between the N and A3B3 immunoglobulin-like domains of CEA through protein/protein interactions. In spite of the high degree of glycosylation (over 50% of MW) the highly branched N-linked sugar chains do not seem to be involved in this binding. Binding between CEA producing cells can be inhibited by anti CEA antibodies (Benchimol et al 1989) Up-regulation of CEA in normally non-CEA producing colorectal cancer cell lines results in clumping of the cells presumably by the formation of these homotypic interactions between CEA on the surface of adjacent cells. A more recent study has also shown interactions between CEA and proteins in cell junctional complexes [Bajenova, et al. 2014] and these may also contribute to changes in intra-cellular adhesion. These studies showed that CEA overexpression disrupted interactions between adherens junction proteins. These proteins (cadherins and catenins) are needed to maintain the function of adherens junction complexes, and influence both cell signaling and epithelial tissue architecture. These changes may also effect the epithelial mesenchymal transition (EMT) a process that is important for both invasion and metastases [Baum et al 2008, Hugo et al 2007]

5. Anti-Apoptotic Effects of CEA

Apoptosis is an important biological process that regulates cell numbers. In cancer this process can go awry and reduced apoptosis can contribute to increased cell growth in cancer (Shanmugatherson and Jothy 2000). .A

number of studies have shown that CEA is an inhibitor of anoikis a form of apoptosis that occurs when cells are unable to attach to a matrix (Ordonez 2000. Soeth 2001). The mechanism of the anti-apoptotic effect of CEA relates to inhibition of the death receptor DR5 (Trail R2). (Samara et al 2007, Camacho-Leal et al 2008). Site directed mutagenesis studies showed that there was a requirement for the CEA/CEAR five amino acid binding sequence PELPK for interaction and inhibition of DR5 [Samara et al 2007]. This suggests that CEAR is also involved in this inhibition and it may be the CEA/CEAR complex that binds with DR5 though this remains to be proven. Camacho-Leal 2008, have suggested that CEA can also inhibit anoikis by inactivating the intrinsic caspase death pathway through CEAs GPI linkage. This inhibition of anoikis either through the PELPK sequence or the GPI link gives CEA-producing cancer cells a survival advantage particularly when they become detached and travel through the circulation to a distant metastatic site. Wirth et al, (2002) also showed that inhibition of CEA production in colon cancer cells increased their rate of apoptosis and inhibited lung metastases in a nude mouse model. Recently Yan et al (2016) have also reported that the protection of cells from anoikis by secreted CEA may enhance metastasis and could be a target for therapy. This data shows that CEA plays an important part in protecting cancer cells from programmed cell death and thus provides another mechanism for tumor cells to survive and proliferate.

6. CEA as a Pro-Angiogenesis molecule

It has been known for some time that the CEA related transmembrane receptor CEACAM-1 has a role in angiogenesis. (Kuesport 2006, Ergun 2000). The first indication that CEA itself may have a direct effect on angiogenisis was reported by Bramswig et al, 2013. Because CEA is a secreted molecule it can react directly with CEAR on the surface of endothelial cells Bramswig et al(2013), showed that CEA was pro-angiogenic via a VEGFR independent mechanism and secreted (soluble) CEA can directly activate endothelial cells *via* integrin β3 signaling [24,47]. In vivo they showed angiogenesis was reduced when CEA production was inhibited. The same group (Praeger et al 2014) also reported that the response of patients to the anti VEGF antibody bevacizumab inversely correlated with plasma CEA levels. In addition to the mechanism proposed by Bramswig et al (2013) we propose that CEA can also influence angiogenisis in a less direct way. CEA can interact with Kupffer cells and other macrophages to secrete certain cytokines including the pro-angiogenic cytokines IL-6 and IL-8. In Pseudomyxoma Peritonei (PMP) a peritoneal surface cancer, large amounts of IL-6 and IL-8 are secreted into the ascites associated with this disease [Lohani et al, 2014) and these cytokines appear to originate from stromal cells rather than the tumor itself [Kuracha et al 2016]. PMP ascites also contain large amounts of CEA. [Canbay et al 2013], [Thomas et al 2015]. Toth 1992 showed that elicited peritoneal macrophages bind CEA through an 80kD protein that is probably CEAR, though at that time the effects of CEA on cytokine production was not known. More recently we have shown that macrophages isolated from PMP ascites respond to CEA and produce IL-6, IL-8 and MCP-1 (CCL-2) a macrophage attractant [Thomas et al, 2015). The CEA mediated regulation of angiogenisis at both the primary and metastatic sites is depicted diagrammatically in Fig. 2.

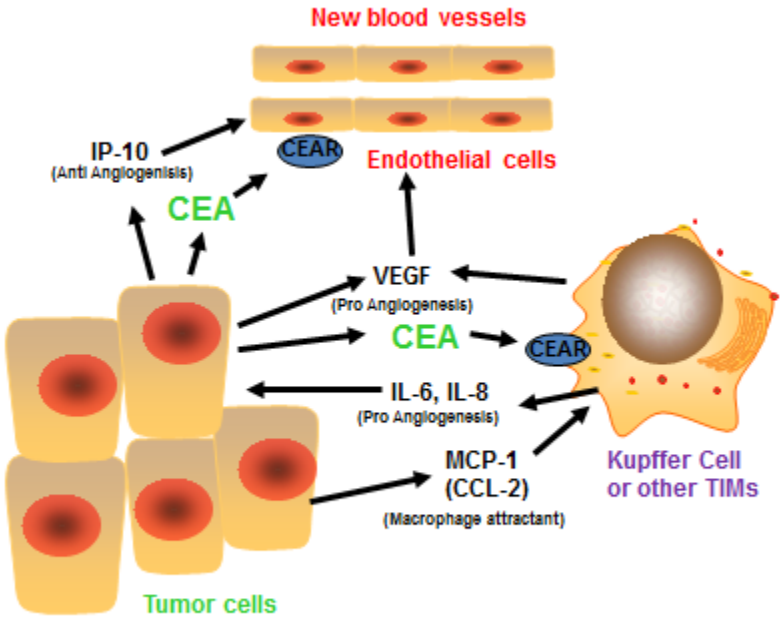

Figure 2: Angiogenesis in colorectal cancer. Mechanisms involving CEA

7. CEA may be involved in the Epithelial Mesenchymal Transition.

The physical events of cancer metastases have been well established – (i) tumor detachment and migration from the primary site, (ii) penetration through the basement membrane, (iii) entrance into blood or lymphatic circulation, (iv) extravasation into distant tissue sites, (v) formation of micro-metastases and, (vi) growth [Steeg, 2006]. While various theories have been proposed to explain the cellular changes that allow for such metastatic behavior, the idea of cancer cells transitioning from an epithelial to mesenchymal histology to enhance their invasive capacity has garnered much attention in the last few decades.

First described in 1982 by Greenburg and Hay, the process now known as Epithelial Mesenchymal Transition (EMT) refers to cellular changes in cancers that occur in response to extra-cellular stimuli. These changes cause cells to revert from a non-motile, polarized epithelium to a motile, non-polarized mesenchymal phenotype – a transition that may or may not be reversible [Hugo et al, 2007]. When reversal does occur this is known as the Mesenchymal Epithelial Transition (MET). Once in the mesenchymal state, there is increased potential for cell migration and dissemination into the circulation.

EMT is a normal developmental process and occurs during certain phases of embryological development to allow for migration of mesenchymal cells to sites of future organ development. During development, classical examples of EMT (e.g., neural crest cell migration) give rise to motile cell populations. These cells later differentiate into various epithelial and other cohesive cell structures, including muscular and neural cells that express specialized cell–cell adhesions. As ongoing research further elucidates the mechanisms underlying EMT, more parallels are being drawn between the events of EMT and tumor progression.

As is true for most cancers, acquisition of

mesenchymal characteristics correlates to more advanced tumor progression and poorer prognosis [Polyak et al, 2009]. Using the TNM staging system of colorectal cancer, more severe disease correlates with increased mesenchymal characteristics [Edge, 2010]. In the context of cancer this mesenchymal phenotype allows the cells to separate, invade, and intravasate at the primary site; later at the distant organ site, it allows these cells to extravasate and invade the parenchyma. Once the tumor cells enter the parenchyma of the target organ, they receive a new set of signals that results in a reversion of the EMT. This change to a mesothelial epithelial transition (MET), a reversion of the EMT, reestablishes the histological features of the primary tumor. We have shown that down regulation of the CEA receptor can cause changes in expression of EMT related proteins including E-cadherin and Snail (Bajenova and Thomas, unpublished results). Recent studies have also shown that a closely related member of the CEA gene family CEACAM-6 (NCA) can promote invasion in gastric cancer by inducing EMT through the PI3K/AKT signaling pathway [Zhang, et al 2014]. Inhibitors of PI3K were also shown to reverse the EMT in these cancers. This pathway may also involve the CEAR as like CEA, CEACAM-6 expresses the peptide binding motif (PELPK) for CEAR [Thomas et al, 2011]. Zang et al 2014 have suggested that CEACAM-6 may be a target for therapy in gastric cancer. Since both CEA and CEACAM-6 are overexpressed in colorectal cancers [Beauchemin 2013] they may also be targets for therapy. IL-6 is also known to affect EMT and production of IL-6 by stromal cells in response to CEA may also be another area in which CEA influences this process [Rockavec et al 2014]. Further evidence for the possible influence of CEA in the EMT is that there is crosstalk between CEA and TGF-β both CEA and CEACAM-5 are targets for the Smad3 signaling pathway [Han et al 2008]. Using Smad3 null mice they also suggested that TGF-β is involved in the induction of CEA. As TGF-β is a key regulator of EMT this could be another pathway in which CEA could influence tumor progression by interfering with the EMT [Jenson-Jerome et al, 2015]. A recent study by Bajenova et al 2016 showed using genome wide analysis that in a comparison of CEA producing with CEA negative colorectal cancer cells, the CEA producing cells showed changes in 100 genes including those involved in the TGF-β signaling pathway. They suggested that CEA can trigger colorectal cancer cell invasion and metastases by stimulating the EMT and reducing stress and apoptotic signaling.

8. Potential for CEA as a Target for Therapy

Because CEA is involved in multiple processes that give tumor cells a selective advantage to metastasize. It seems logical to investigate inhibition of the CEA/CEAR interaction as a potential therapy. Lee et al (2012) have described an RNA aptamer directed against the amino acid sequence PELPK which is the binding site on CEA for CEAR. This RNA aptamer (80μg/Kg) has been shown to be effective in inhibiting liver metastasis from cell line derived xenografts in animal models of colon cancer presumably by interfering with CEA binding to CEAR on Kupffer cells. . Recently DNA aptamers have also been used against the homotypic adhesion sites of CEA to block tumor cell/cell interactions (Oreva et al 2013) further emphasizing the feasibility of this approach to inhibit the effects of CEA on colorectal cancer metastasis. CEA also appears to be a target for immunotherapy. For example Bacac et al 2016 have used an IgG based T-cell specific antibody to CEA to prevent metastasis in a xenograft model of colon

cancer. Methods to eliminate CEA induced metastasis would be especially important in cases where there are increased circulation cancer cells due to other therapies [Martin et al. 2017]

9. Conclusions

Liver metastasis is a very complex process that involves many cell types and many phenotypic changes expressed by tumor cells and the surrounding stroma [Weidle et al 2015, Pashos et al 2014.] One of the many factors that affect colorectal cancer metastasis is the production of CEA.

There is no doubt that CEA affects many functions in the colorectal cancer cell. These functions generally promotes metastasis and ensures cancer cell survival, altering the microenvironment at the distant site by producing an inflammatory response resulting in up-regulation of endothelial cell adhesion molecules and thus increasing the chances of tumor cell implantation.

CEA also provides protection against endothelial cell mediated cytotoxicity due to Reactive oxygen species (ROS). At the primary site CEA/CEAR can influence the EMT leading to breakdown of cell/cell interactions and promoting invasion. In the circulation it can inhibit anoikis and therefore promote tumor cell survival. At both the primary and metastatic sites it can increase angiogenesis through a variety of mechanisms. For a long time CEA was regarded as just a useful biomarker for colon and other cancers. Now that we are beginning to understand its biological role it opens up new lines of enquiry into the mechanisms that effect metastasis and tumor progression. This new knowledge is likely to have a strong translational impact.

10. References

1. Aarons, C.B., Bajenova, O., Andrews, C., Heydrick, S., Bushell, K.N., Reed, K.L., Thomas, P., Becker, J.M. and Stucchi, A.F., 2007. Carcinoembryonic antigen-stimulated THP-1 macrophages activate endothelial cells and increase cell–cell adhesion of colorectal cancer cells. *Clinical & experimental metastasis*, *24*(3), pp.201-209.

2. Adson, M.A., 1987. Resection of liver metastases—when is it worthwhile? *World journal of surgery*, *11*(4), pp.511-520.

3. André, T., Boni, C., Mounedji-Boudiaf, L., Navarro, M., Tabernero, J., Hickish, T., Topham, C., Zaninelli, M., Clingan, P., Bridgewater, J. and Tabah-Fisch, I., 2004. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *New England Journal of Medicine*, *350*(23), pp.2343-2351.

4. Arnaud, J.P., Koehl, C. and Adloff, M., 1980. Carcinoembryonic antigen (CEA) in diagnosis and prognosis of colorectal carcinoma. *Diseases of the Colon & Rectum*, *23*(3), pp.141-144.

5. Bacac, M., Fauti, T., Sam, J., Colombetti, S., Weinzierl, T., Ouaret, D., Bodmer, W., Lehmann, S., Hofer, T., Hosse, R.J. and Moessner, E., 2016. A novel carcinoembryonic antigen T-cell bispecific antibody (CEA TCB) for the treatment of solid tumors. *Clinical Cancer Research*, *22*(13), pp.3286-3297.

6. Baum, B., Settleman, J. and Quinlan, M.P., 2008, June. Transitions between epithelial and mesenchymal states in development and disease. In *Seminars in cell & developmental biology* (Vol. 19, No. 3, pp. 294-308). Academic Press.

7. Bajenova, O., Chaika, N., Tolkunova, E., Davydov-Sinitsyn, A., Gapon, S., Thomas, P. and O'Brien, S., 2014. Carcinoembryonic antigen promotes colorectal cancer progression by targeting adherens junction complexes. *Experimental cell research*, 324 (2),pp.115-123.

8. Bajenova, O., Gorbunova, A., Evsyukov, I., Rayko, M., Gapon, S., Bozhokina, E., Shishkin, A. and O'Brien, S.J., 2016. The Genome-Wide Analysis of Carcinoembryonic Antigen Signaling by Colorectal Cancer Cells Using RNA Sequencing. *PloS one*, *11*(9), p.e0161256.

9. Bajenova, O., Stolper, E., Gapon, S., Sundina, N., Zimmer, R. and Thomas, P., 2003. Surface expression of heterogeneous nuclear RNA binding protein M4 on Kupffer cell relates to its function as a carcinoembryonic antigen receptor. *Experimental cell research*, 291(1), pp.228-241\

10. Bajenova, O.V., Zimmer, R., Stolper, E., Salisbury-Rowswell, J., Nanji, A. and Thomas, P., 2001. Heterogeneous RNA-binding protein M4 is a receptor for carcinoembryonic antigen in Kupffer cells. *Journal of Biological Chemistry*, *276*(33), pp.31067-31073.

11. Beauchemin, N. and Arabzadeh, A., 2013. Carcinoembryonic antigen-related cell adhesion molecules

(CEACAMs) in cancer progression and metastasis. *Cancer and Metastasis Reviews*, *32*(3-4), pp.643-671.

12. Beauchemin N, Draber P, Dveksler G, Gold P, Gray-Owen S, Grunert F, Hammarström S, Holmes KV, Karlsson A, Kuroki M, Lin SH et al.. Redefined nomenclature for members of the carcinoembryonic antigen family. Experimental cell research. 1999 Nov 1; 252(2):243.

13. Behr, T.M., Sharkey, R.M., Juweid, M.I., Dunn, R.M., Ying, Z., Zhang, C.H., Siegel, J.A., Gold, D.V. and Goldenberg, D.M., 1996. Factors influencing the pharmacokinetics, dosimetry, and diagnostic accuracy of radioimmunodetection and radioimmunotherapy of carcinoembryonic antigen-expressing tumors. *Cancer research*, *56*(8), pp.1805-1816.

14. Benchimol, S., Fuks, A., Jothy, S., Beauchemin, N., Shirota, K. and Stanners, C.P., 1989. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. *Cell*, *57*(2), pp.327-334.

15. Bramswig, K.H., Poettler, M., Unseld, M., Wrba, F., Uhrin, P., Zimmermann, W., Zielinski, C.C. and Prager, G.W., 2013. Soluble carcinoembryonic antigen activates endothelial cells and tumor angiogenesis. *Cancer research*, *73*(22), pp.6584-6596.

16. Bengtsson, G., Carlsson, G., Hafström, L. and Jönsson, P.E., 1981. Natural history of patients with untreated liver metastases from colorectal cancer. *The American Journal of Surgery*, *141*(5), pp.586-589.

17. Boffetta, P., Hashibe, M., La Vecchia, C., Zatonski, W. and Rehm, J., 2006. The burden of cancer attributable to alcohol drinking. *international Journal of Cancer*, *119*(4), pp.884-887.

18. Camacho-Leal, P. and Stanners, C.P., 2008. The human carcinoembryonic antigen (CEA) GPI anchor mediates anoikis inhibition by inactivation of the intrinsic death pathway. *Oncogene*, *27*(11), pp.1545-1553.

19. Canbay, E., Ishibashi, H., Sako, S., Mizumoto, A., Hirano, M., Ichinose, M., Takao, N. and Yonemura, Y., 2013. Preoperative carcinoembryonic antigen level predicts prognosis in patients with Pseudomyxoma Peritonei treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *World journal of surgery*, *37*(6), pp.1271-1276.

20. Chandrasekaran, E.V., Davila, M., Nixon, D.W., Goldfarb, M. and Mendicino, J., 1983. Isolation and structures of the oligosaccharide units of carcinoembryonic antigen. *Journal of Biological Chemistry*, *258*(11), pp.7213-7222.

21. Chen, J., Raju, G.S., Jogunoori, W., Menon, V., Majumdar, A., Chen, J.S., Gi, Y.J., Jeong, Y.S., Phan, L., Belkin, M. and Gu, S., 2016. Mutational Profiles Reveal an Aberrant TGF-β-

CEA Regulated Pathway in Colon Adenomas. *PloS one*, *11*(4), p.e0153933.

22. Chen, S., Zhang, J., Duan, L., Zhang, Y., Li, C., Liu, D., Ouyang, C., Lu, F. and Liu, X., 2013. Identification of HnRNP M as a Novel Biomarker for Colorectal Carcinoma by Quantitative Proteomics. *American Journal of Physiology-Gastrointestinal and Liver Physiology*.

23. Dhar, P., Moore, T., Zamcheck, N. and Kupchik, H.Z., 1972. Carcinoembryonic antigen (CEA) in colonic cancer: Use in preoperative and postoperative diagnosis and prognosis. *Jama*, *221*(1), pp.31-35.

24. Daneker, G.W., Piazza, A.J., Steele, G.D. and Mercurio, A.M., 1989. Relationship between extracellular matrix interactions and degree of differentiation in human colon carcinoma cell lines. *Cancer research*, *49*(3), pp.681-686.

25. Datar, K.V., Dreyfuss, G. and Swanson, M.S., 1993. The human hnRNP M proteins: identification of a methionine/arginine-rich repeat motif in ribonucleoproteins. *Nucleic acids research*, *21*(3), pp.439-446.

26. Duffy, M.J., 2001. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful?. *Clinical chemistry*, *47*(4), pp.624-630.

27. Edge, S.B., 2010. American Cancer Society. AJCC cancer staging handbook: from the AJCC cancer staging manual.

28. Fong, Y., Kemeny, N., Paty, P., Blumgart, L.H. and Cohen, A.M., 1996, July. Treatment of colorectal cancer: hepatic metastasis. In *Seminars in surgical oncology* (Vol. 12, No. 4, pp. 219-252). John Wiley & Sons, Inc..

29. Fortner, J.G., Silva, J.S., Golbey, R.B., Cox, E.B. and Maclean, B.J., 1984. Multivariate analysis of a personal series of 247 consecutive patients with liver metastases from colorectal cancer. I. Treatment by hepatic resection. *Annals of surgery*, *199*(3), p.306.

30. Gangopadhyay, A., Bajenova, O., Kelly, T.M. and Thomas, P., 1996. Carcinoembryonic antigen induces cytokine expression in Kupffer cells: implications for hepatic metastasis from colorectal cancer. *Cancer research*, *56*(20), pp.4805-4810.

31. Gangopadhyay, A., Lazure, D.A. and Thomas, P., 1998. Adhesion of colorectal carcinoma cells to the endothelium is mediated by cytokines from CEA stimulated Kupffer cells. *Clinical & experimental metastasis*, *16*(8), pp.703-712.

32. Gold, P. and Freedman, S.O., 1965. Specific carcinoembryonic antigens of the human digestive system. *Journal of Experimental Medicine*, *122*(3), pp.467-481.

33. Goldstein, M.J. and Mitchell, E.P., 2005. Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. *Cancer investigation*, *23*(4), pp.338-351.

34. Goldenberg, D.M., Kim, E.E., DeLand, F.H., Bennett, S. and Primus, F.J., 1980. Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. *Cancer Research*, *40*(8 Part 2), pp.2984-2992.

35. Greenburg, G. and Hay, E.D., 1982. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *The Journal of cell biology*, *95*(1), pp.333-339.

36. Hammarström, S., 1999, April. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. In *Seminars in cancer biology* (Vol. 9, No. 2, pp. 67-81). Academic Press.

37. Han, S.U., Kwak, T.H., Her, K.H., Cho, Y.H., Choi, C., Lee, H.J., Hong, S., Park, Y.S., Kim, Y.S., Kim, T.A. and Kim, S.J., 2008. CEACAM5 and CEACAM6 are major target genes for Smad3-mediated TGF-β signaling. *Oncogene*, *27*(5), pp.675-683.

38. Hashino, J., Fukuda, Y., Oikawa, S., Nakazato, H. and Nakanishi, T., 1994. Metastatic potential of human colorectal carcinoma SW1222 cells transfected with cDNA encoding carcinoembryonic antigen. *Clinical & experimental metastasis*, 12(4), pp.324-328.

39. Hostetter, R.B., Augustus, L.B., Mankarious, R., Chi, K., Fan, D., Toth, C., Thomas, P. and Jessup, J.M., 1990. Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis. *Journal of the National Cancer Institute*, *82*(5), pp.380-385.

40. Hughes, K. S., et al. "Resection of the liver for colorectal carcinoma metastases: a multi-institutional study of patterns of recurrence." *Surgery* 100.2 (1986): 278-284.

41. Hugo, H., Ackland, M.L., Blick, T., Lawrence, M.G., Clements, J.A., Williams, E.D. and Thompson, E.W., 2007. Epithelial—mesenchymal and mesenchymal—epithelial transitions in carcinoma progression. *Journal of cellular physiology*, *213*(2), pp.374-383.

42. Iddings, D. and Bilchik, A., 2007. The biologic significance of micrometastatic disease and sentinel lymph node technology on colorectal cancer. *Journal of surgical oncology*, *96*(8), pp.671-677.

43. Jensen-Jarolim, E., Fazekas, J., Singer, J., Hofstetter, G., Oida, K., Matsuda, H. and Tanaka, A., 2015. Crosstalk of carcinoembryonic antigen and transforming growth factor-β via their receptors: comparing human and canine cancer. *Cancer Immunology, Immunotherapy*, *64*(5), pp.531-537.

44. Jessup, J.M., Giavazzi, R., Campbell, D., Cleary, K., Morikawa, K. and Fidler, I.J., 1988. Growth potential of human colorectal carcinomas in nude mice: association with the preoperative serum concentration of carcinoembryonic antigen in patients. *Cancer research*, *48*(6), pp.1689-1692.

45. Jessup, J.M., Giavazzi, R., Campbell, D., Cleary, K.R., Morikawa, K., Hostetter, R., Atkinson, E.N. and Fidler, I.J., 1989. Metastatic potential of human colorectal carcinomas implanted into nude mice: prediction of clinical outcome in patients operated upon for cure. *Cancer research*, *49*(24 Part 1), pp.6906-6910.

46. Jessup, J.M., Laguinge, L., Lin, S., Samara, R., Aufman, K., Battle, P., Frantz, M., Edmiston, K.H. and Thomas, P., 2004. Carcinoembryonic antigen induction of IL-10 and IL-6 inhibits hepatic ischemic/reperfusion injury to colorectal carcinoma cells. *Intern ational journal of cancer*, *111*(3), pp.332-337.

47. Jessup, J.M., Samara, R., Battle, P. and Laguinge, L.M., 2005. Carcinoembryonic antigen promotes tumor cell survival in liver through an IL-10-dependent pathway. *Clinical & experimental metastasis*, 21(8), pp.709-717.

48. Jessup, J.M. and Thomas, P., 1998. CEA and metastasis: a facilitator of site-specific metastasis. *Cell Adhesion and Commu nications Mediated by the CEA Family*, 5, pp.195-222.

49. Jessup, M. and Thomas, P., 1989. Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. *Cancer and Metastasis Reviews*, 8(3), pp.263-280.

50. Kaufman, H., Schlom, J. and Kantor, J., 1991. A recombinant vaccinia virus expressing human carcinoembryonic antigen (CEA). *International journal of cancer*, *48*(6), pp.900-907.

51. Kemeny, M.M., Goldberg, D., Beatty, J.D., Blayney, D., Browning, S., Doroshow, J., Ganteaume, L., Hill, R.L., Kokal, W.A., Riihimaki, D.U. and Terz, J.J., 1986. Results of a prospective randomized trial of continuous regional chemotherapy and hepatic resection as treatment of hepatic metastases from colorectal primaries. *Cancer*, *57*(3), pp.492-498.

52. Kemeny, N.E. and Schneider, A.M., 1989. Regional treatment of hepatic metastases and hepatocellular carcinoma. *Current problems in cancer*, *13*(4), pp.203-283.

53. Kuracha, M.R., Thomas, P., Loggie, B.W. and Govindarajan, V., 2016. Patient-derived xenograft mouse models of pseudomyxoma peritonei recapitulate the human inflammatory tumor microenvironment. Cancer medicine. 5(4) 711-719

54. Kurkjian, C., Murgo, A.J. and Kummar, S., 2008. Treatment of recurrent metastatic colon cancer in the age of modern adjuvant therapy. *Clinical colorectal cancer*, *7*(5), pp.321-324.

55. Laguinge, L., Bajenova, O., Bowden, E., Sayyah, J., Thomas, P. and Juhl, H., 2005. Surface expression and CEA binding of hnRNP M4 protein in HT29 colon cancer cells. *Anticancer research*, *25*(1A), pp.23-31.

56. Lee, Y.J., Han, S.R., Kim, N.Y., Lee, S.H., Jeong, J.S. and Lee, S.W., 2012. An RNA aptamer that binds

carcinoembryonic antigen inhibits hepatic metastasis of colon cancer cells in mice. *Gastroenterology*, *143*(1), pp.155-165.

57. Leusch, H.G., Drzeniek, Z., Hefta, S.A., Markos-Pusztai, Z. and Wagener, C., 1991. The putative role of members of the CEA-gene family (CEA, NCA and BGP) as ligands for the bacterial colonization of different human epithelial tissues. *Zentralblatt für Bakteriologie*, *275*(1), pp.118-122.

58. Leusch, H.G., Hefta, S.A., Drzeniek, Z., Hummel, K., Markos-Pusztai, Z. and Wagener, C., 1990. Escherichia coli of human origin binds to carcinoembryonic antigen (CEA) and non-specific crossreacting antigen (NCA). *FEBS letters*, *261*(2), pp.405-409.

59. Li, Y., Cao, H., Jiao, Z., Pakala, S.B., Sirigiri, D.N.R., Li, W., Kumar, R. and Mishra, L., 2010. Carcinoembryonic antigen interacts with TGF-β receptor and inhibits TGF-β signaling in colorectal cancers. *Cancer research*, *70*(20), pp.8159-8168.

60. Li, R., Hebert, J.D., Lee, T.A., Xing, H., Boussommier-Calleja, A., Hynes, R.O., Lauffenburger, D.A. and Kamm, R.D., 2016. Macrophage-secreted TNFα and TGFβ1 Influence Migration Speed and Persistence of Cancer Cells in 3D Tissue Culture via Independent Pathways. *Cancer Research*, pp.canres-0442.

61. Lohani, K., Shetty, S., Sharma, P., Govindarajan, V., Thomas, P. and Loggie, B., 2014. Pseudomyxoma Peritonei: inflammatory responses in the peritoneal microenvironment. *Annals of surgical oncology*, *21*(5), pp.1441-1447.

62. Maeda, M., Nagawa, H., Maeda, T., Koike, H. and Kasai, H., 1998. Alcohol consumption enhances liver metastasis in colorectal carcinoma patients. *Cancer*, *83*(8), pp.1483-1488.

63. Marko, M., Leichter, M., Patrinou-Georgoula, M. and Guialis, A., 2010. hnRNP M interacts with PSF and p54 nrb and co-localizes within defined nuclear structures. *Experimental cell research*, *316*(3), pp.390-400

64. Martin, O.A., Anderson, R.L., Narayan, K. and MacManus, M.P., 2017. Does the mobilization of circulating tumor cells during cancer therapy cause metastasis?. *Nature Reviews Clinical Oncology*, *14*(1), pp.32-44.

65. Massagué, J., 2008. TGFβ in cancer. *Cell*, *134*(2), pp.215-230.

66. McVicker, B., Tuma, D.J., Lazure, K.E., Thomas, P. and Casey, C.A., 2015. Alcohol, carcinoembryonic antigen processing and colorectal liver metastases. In *Biological Basis of Alcohol-Induced Cancer* (pp. 295-311). Springer International Publishing.

67. Millikan, K.W., Staren, E.D. and Doolas, A., 1997. Invasive therapy of metastatic colorectal cancer to the liver. *Surgical Clinics of North America*, *77*(1), pp.27-48.

68. Moertel, C.G., Fleming, T.R., Macdonald, J.S., Haller, D.G., Laurie, J.A., Tangen, C.M., Ungerleider, J.S., Emerson, W.A., Tormey, D.C., Glick, J.H. and Veeder, M.H., 1995. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Annals of internal medicine*, *122*(5), pp.321-326.

69. Moertel, C.G., O'Fallon, J.R., Go, V.L., O'Connell, M.J. and Thynne, G.S., 1986. The preoperative carcinoembryonic antigen test in the diagnosis, staging, and prognosis of colorectal cancer. *Cancer*, *58*(3), pp.603-610.

70. Mohr, AM. Gould, JJ. Kubik, JL. Talmon, GA. Casey, CA, Thomas, P. Tuma, D.J. McVicker, BL. Enhanced colorectal cancer metastases in the alcohol-injured liver, *Clin Exp. Metastasis 2017, (in press)*

71. Morse, M.A., Nair, S.K., Mosca, P.J., Hobeika, A.C., Clay, T.M., Deng, Y., Boczkowski, D., Proia, A., Neidzwiecki, D., Clavien#, P.A. and Hurwitz, H.I., 2003. Immunotherapy with Autologous, Human Dendritic Cells Transfected with Carcinoembryonic Antigen mRNA: . *Cancer investigation*, *21*(3), pp.341-349.

72. Nelson, D.E., Jarman, D.W., Rehm, J., Greenfield, T.K., Rey, G., Kerr, W.C., Miller, P., Shield, K.D., Ye, Y. and Naimi, T.S., 2013. Alcohol-attributable cancer deaths and years of potential life lost in the United States. *American Journal of Public Health*, *103*(4), pp.641-648.

73. Nordlinger, B., Guiguet, M., Vaillant, J.C., Balladur, P., Boudjema, K., Bachellier, P. and Jaeck, D., 1996. Surgical resection of colorectal carcinoma metastases to the liver: a prognostic scoring system to improve case selection, based on 1568 patients. *Cancer*, *77*(7), pp.1254-1262.

74. Orava, E.W., Abdul-Wahid, A., Huang, E.H.B., Mallick, A.I. and Gariépy, J., 2013. Blocking the attachment of cancer cells in vivo with DNA aptamers displaying anti-adhesive properties against the carcinoembryonic antigen. *Molecular oncology*, *7*(4), pp.799-811.

75. Ordoñez, C., Screaton, R.A., Ilantzis, C. and Stanners, C.P., 2000. Human carcinoembryonic antigen functions as a general inhibitor of anoikis. *Cancer Research*, *60*(13), pp.3419-3424.

76. Palermo, N.Y., Thomas, P., Murphy, R.F. and Lovas, S., 2012. Hexapeptide fragment of carcinoembryonic antigen which acts as an agonist of heterogeneous ribonucleoprotein M. *Journal of Peptide Science*, *18*(4), pp.252-260

77. Paschos, K.A., Majeed, A.W. and Bird, N.C., 2014. Natural history of hepatic metastases from colorectal cancer-pathobiological pathways with clinical significance. *World Journal of Gastroenterology: WJG*, *20*(14), p.3719

78. Polyak, K. and Weinberg, R.A., 2009. Transitions between epithelial and mesenchymal states: acquisition of

malignant and stem cell traits. *Nature Reviews Cancer*, *9*(4), pp.265-273.

79. Prager, G.W., Braemswig, K.H., Martel, A., Unseld, M., Heinze, G., Brodowicz, T., Scheithauer, W., Kornek, G. and Zielinski, C.C., 2014. Baseline carcinoembryonic antigen (CEA) serum levels predict bevacizumab-based treatment response in metastatic colorectal cancer. *Cancer science*, *105*(8), pp.996-1001.

80. Rees, M., Tekkis, P.P., Welsh, F.K., O'rourke, T. and John, T.G., 2008. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Annals of surgery*, *247*(1), pp.125-135.

81. Rehm, J. and Shield, K.D., 2014. Alcohol and mortality: global alcohol-attributable deaths from cancer, liver cirrhosis, and injury in 2010. *Alcohol research: current reviews*, *35*(2), p.174.

82. Rokavec, M., Öner, M.G., Li, H., Jackstadt, R., Jiang, L., Lodygin, D., Kaller, M., Horst, D., Ziegler, P.K., Schwitalla, S. and Slotta-Huspenina, J., 2014. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *The Journal of clinical investigation*, *124*(4), pp.1853-1867.

83. Rougier, P., Laplanche, A., Huguier, M., Hay, J.M., Ollivier, J.M., Escat, J., Salmon, R., Julien, M., Roullet Audy, J.C. and Gallot, D., 1992. Hepatic arterial infusion of floxuridine in patients with liver metastases from colorectal carcinoma: long-term results of a prospective randomized trial. *Journal of Clinical Oncology*, *10*(7), pp.1112-1118.

84. Ruers, T. and Bleichrodt, R.P., 2002. Treatment of liver metastases, an update on the possibilities and results. *European Journal of Cancer*, *38*(7), pp.1023-1033.

85. Sack, T.L., Gum, J.R., Low, M.G. and Kim, Y.S., 1988. Release of carcinoembryonic antigen from human colon cancer cells by phosphatidylinositol-specific phospholipase C. *Journal of Clinical Investigation*, *82*(2), p.586.

86. Samara, R.N., Laguinge, L.M. and Jessup, J.M., 2007. Carcinoembryonic antigen inhibits anoikis in colorectal carcinoma cells by interfering with TRAIL-R2 (DR5) signaling. *Cancer research*, *67*(10), pp.4774-4782.

87. Shanmugathasan, M. and Jothy, S., 2000. Apoptosis, anoikis and their relevance to the pathobiology of colon cancer. *Pathology international*, *50*(4), pp.273-279.

88. Siegel, R.L., Miller, K.D. and Jemal, A., 2015. Cancer statistics, 2015. *CA: a cancer journal for clinicians*, *65*(1), pp.5-29.

89. Soeth, E., Wirth, T., List, H.J., Kumbhani, S., Petersen, A., Neumaier, M., Czubayko, F. and Juhl, H., 2001. Controlled ribozyme targeting demonstrates an anti-apoptotic effect of carcinoembryonic antigen in HT29 colon cancer cells. *Clinical cancer research*, *7*(7), pp.2022-2030.

90. Stangl, R., Altendorf-Hofmann, A., Charnley, R.M. and Scheele, J., 1994. Factors influencing the natural history of colorectal liver metastases. *The Lancet*, *343*(8910), pp.1405-1410.

91. Steeg, P.S., 2006. Tumor metastasis: mechanistic insights and clinical challenges. *Nature medicine*, *12*(8), p.895.

92. Tibbetts, L.M., Doremus, C.M., Tzanakakis, G.N. and Vezeridis, M.P., 1993. Liver metastases with 10 human colon carcinoma cell lines in nude mice and association with carcinoembryonic antigen production. *Cancer*, *71*(2), pp.315-321.

93. Thomas, P., Forse, R.A. and Bajenova, O., 2011. Carcinoembryonic antigen (CEA) and its receptor hnRNP M are mediators of metastasis and the inflammatory response in the liver. *Clinical & experimental metastasis*, *28*(8), pp.923-932.

94. Thomas, P., Gangopadhyay, A., Steele, G., Andrews, C., Nakazato, H., Oikawa, S. and Jessup, J.M., 1995. The effect of transfection of the CEA gene on the metastatic behavior of the human colorectal cancer cell line MIP-101. *Cancer letters*, *92*(1), pp.59-66.

95. Thomas P, Kuracha M, Loggie, B and Govindarajan V. Carcinoembryonic Antigen (CEA): A Potential Mediator of Inflammation in Pseudomyxoma Peritonei**.** 25th Annual International CEA Symposium, Washington DC, August 17th 2015.

96. Thomas, P., Toth, C.A., Saini, K.S., Jessup, J.M. and Steele, G., 1990. The structure, metabolism and function of the carcinoembryonic antigen gene family. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1032*(2-3), pp.177-189.

97. Thomas, P. and Zamcheck, N., 1983. Role of the liver in clearance and excretion of circulating carcinoembryonic antigen (CEA). *Digestive diseases and sciences*, *28*(3), pp.216-224.

98. Thomas, P., Zamcheck, N., Rogers, A.E. and Fox, J.G., 1982. Plasma clearance of carcinoembryonic antigen and asialo carcinoembryonic antigen by the liver of the nutritionally deficient rhesus monkey. *Clinics in laboratory medicine*, *2*(3), p.459.

99. Thompson, J.A., Grunert, F. and Zimmermann, W., 1991. Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. *Journal of clinical laboratory analysis*, *5*(5), pp.344-366.

100. Tobi, M., Kim, M., Zimmer, R., Hatfield, J., Kam, M., Khoury, N., Carville, A., Lawson, M.J., Schiemann, W.P. and Thomas, P., 2011. Colorectal cancer in the cotton top tamarin (Saguinus oedipus): how do they evade liver metastasis?. *Digestive diseases and sciences*, *56*(2), pp.397-405.

101. Tobi, M., Thomas, P. and Ezekwudo, D., 2016. Avoiding hepatic metastasis naturally: Lessons from the cotton top tamarin (Saguinus

oedipus). *World Journal of Gastroenterology*, *22*(24), p.5479.

102. Toth, C.A., 1992. Carcinoembryonic antigen binding proteins on elicited peritoneal macrophages. *Journal of leukocyte biology*, *51*(5), pp.466-471.

103. Toth, C.A., Rapoza, A., Zamcheck, N., Steele, G. and Thomas, P., 1989. Receptor-mediated endocytosis of carcinoembryonic antigen by rat alveolar macrophages in vitro. *Journal of leukocyte biology*, *45*(4), pp.370-376.

104. Toth, C.A., Thomas, P., Broitman, S.A. and Zamcheck, N., 1982. A new Kupffer cell receptor mediating plasma clearance of carcinoembryonic antigen by the rat. *Biochemical Journal*, *204*(2), pp.377-381.

105. Toth, C.A., Thomas, P., Broitman, S.A. and Zamcheck, N., 1985. Receptor-mediated endocytosis of carcinoembryonic antigen by rat liver Kupffer cells. *Cancer research*, *45*(1), pp.392-397.

106. Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. and Jemal, A., 2015. Global cancer statistics, 2012. *CA: a cancer journal for clinicians*, *65*(2), pp.87-108.

107. Vauthey, J.N., Marsh, R.D.W., Cendan, J.C., Chu, N.M. and Copeland, E.M., 1996. Arterial therapy of hepatic colorectal metastases. *British journal of surgery*, *83*(4), pp.447-455.

108. Ueda, Y., Itoh, T., Nukaya, I., Kawashima, I., Okugawa, K., Yano, Y., Yamamoto, Y., Naitoh, K., Shimizu, K., Imura, K. and Fuji, N., 2004. Dendritic cell-based immunotherapy of cancer with carcinoembryonic antigen-derived. HLA-A24-restricted CTL epitope: Clinical outcomes of 18 patients with metastatic gastrointestinal or lung adenocarcinomas. *International journal of oncology*, *24*(4), pp.909-918.

109. Venook, A.P. and Warren, R.S., 1996. Regional chemotherapy approaches for primary and metastatic liver tumors. *Surgical oncology clinics of North America*, *5*(2), pp.411-427.

110. Wagner, H.E., Thomas, P., Wolf, B.C., Zamcheck, N., Jessup, J.M. and Steele Jr, G.D., 1989. Characterization of the tumorigenic and metastatic potential of a poorly differentiated human colon cancer cell line. *Invasion & metastasis*, *10*(5), pp.253-266.

111. Wagner, H.E., Toth, C.A., Steele, G.D. and Thomas, P., 1992. Metastatic potential of human colon cancer cell lines: relationship to cellular differentiation and carcinoembryonic antigen production. *Clinical & experimental metastasis*, *10*(1), pp.25-31.

112. Wang, T., Notta, F., Navab, R., Joseph, J., Ibrahimov, E., Xu, J., Zhu, C.Q., Borgida, A., Gallinger, S. and Tsao, M.S., 2017. Senescent Carcinoma-Associated Fibroblasts Upregulate IL8 to Enhance

Prometastatic Phenotypes. *Molecular Cancer Research,* 15(1), pp.3-14.

113. Weidle, U.H., Birzele, F. and Krüger, A., 2015. Molecular targets and pathways involved in liver metastasis of colorectal cancer. *Clinical & experimental metastasis*, *32*(6), pp.623-635.

114. Wirth, T., Soeth, E., Czubayko, F. and Juhl, H., 2002. Inhibition of endogenous carcinoembryonic antigen (CEA) increases the apoptotic rate of colon cancer cells and inhibits metastatic tumor growth. *Clinical & experimental metastasis*, *19*(2), pp.155-160.

115. Yan,, C. Hu, Y. Zhang, B. Mu, L. Huang, K. Zhao, H. Ma, C. Li, X. Tao, D. Gong, J. Qin, J. 2016. The CEA-/I0 colorectal cancer cell population harbors cancer stem cells and metastatic cells. Oncotarget, 7 (49) pp.80700-80715

116. Zamcheck, N., 1981. The expanding field of colorectal cancer markers: CEA, the prototype. *Cancer Bull*, *33*, pp.141-151.

117. Zang, M., Zhang, B., Zhang, Y., Li, J., Su, L., Zhu, Z., Gu, Q., Liu, B. and Yan, M., 2014. CEACAM6 promotes gastric cancer invasion and metastasis by inducing epithelial-mesenchymal transition via PI3K/AKT signaling pathway. *PloS one*, *9*(11), p.e112908.

118. Zhang, Y., Zang, M., Li, J., Ji, J., Zhang, J., Liu, X., Qu, Y., Su, L., Li, C., Yu, Y. and Zhu, Z., 2014. CEACAM6 promotes tumor migration, invasion, and metastasis in gastric cancer. *Acta biochimica et biophysica Sinica*, *46*(4), pp.283-290.

119. Zimmer, R. and Thomas, P., 2001. Mutations in the Carcinoembryonic Antigen Gene in Colorectal Cancer Patients. *Cancer research*, *61*(7), pp.2822-2826.

120. Zhou, H., Fuks, A., Alcaraz, G., Bolling, T.J. and Stanners, C.P., 1993. Homophilic adhesion between Ig superfamily carcinoembryonic antigen molecules involves double reciprocal bonds. *The Journal of cell biology*, *122*(4), pp.951-960.