

Towards understanding the mechanisms of actions of carcinoembryonic antigen-related cell adhesion molecule 6 in cancer progression

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Human carcinoembryonic antigen (CEA) is the prototypic member of a family of highly related cell surface glycoproteins that includes carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) and others. CEACAM6 (formerly NCA), which belongs to the immunoglobulin superfamily, is a cell adhesion protein of the CEA family. It is normally expressed on the epithelial surfaces and on the surface of myeloid cells (CD66c). CEACAM6 is a multi-functional glycoprotein that mediates homotypic binding with other CEA family members and heterotypic binding with integrin receptors. It functions by organizing tissue architecture and regulating different signal transduction, while aberrant expression leads to the development of human malignancies. It was first discovered in proliferating cells of adenomas and hyperplastic polyps in comparison to benign colonic tissue when overexpressed on the surface of various cell types in model systems. CEACAM6 functions as a pan-inhibitor of cell differentiation and cell polarization, and it also causes distortion of tissue architecture. Moreover, overexpression of CEACAM6 modulates cancer progression through aberrant cell differentiation, anti-apoptosis, cell growth and resistance to therapeutic agents. In addition, CEACAM6 overexpression in multiple malignancies promotes cell invasion and metastasis, thereby representing an acquired advantage of tumor cells directly responsible for an invasive phenotype. This review focuses on the findings supporting the mechanisms of actions linking the oncogenic potential of CEACAM6 to the onset of cancer progression and pathogenesis, especially in breast cancer, and to validating CEACAM6 as a target to pave the way towards the design of efficient therapeutic strategies against breast cancer.

KEYWORDS

adhesion, cancer, CEACAM6, invasion and metastasis, review

1 | INTRODUCTION

Human carcinoembryonic antigen (CEA) is part of a larger family of related glycoproteins made up of 2 separate branches: the CEA cellular adhesion molecules (CEACAM) and the pregnancy-specific glycoprotein (PSG) subgroup.¹ In addition, cell adhesion molecules

(CAM) are predominantly transmembrane glycoproteins, involved in intercellular binding of adjacent cells to each other or to the extracellular matrix (ECM). They play a crucial role in a number of biological processes, including homeostasis, embryogenesis and development of neural tissue, inflammation, immune cell transmigration and immune response.² Four major groups of CAM have been

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described: the immunoglobulin (Ig) superfamily, integrins, selectins and cadherins.³ The CEACAM are highly glycosylated transmembrane proteins of the Ig superfamily, located on chromosome 19q13.1-13.2, and were described in 1972 (Figure 1).^{4,5} They are involved in modulate cellular processes, such as neovascularization, shaping of tissue architecture, angiogenesis, T-cell proliferation and cell-cell recognition, and regulation of insulin action, and have also been described as bacterial and viral pathogen receptors, such as *Neisseria* species, *Escherichia coli* and *Salmonella*.⁶ Moreover, CEACAM play an important role in tumor-associated mechanisms.⁷ The CEACAM family consists of membrane-linked glycoproteins anchored to the cell surface either by glycoposphatidyl-inositol (GPI) anchor or a transmembrane domain, as well as secretory glycoproteins.⁸ The GPI-anchored members include CEACAM7 (formerly CGM2), CEACAM8 (formerly CGM6), CEACAM5 and CEACAM6 (formerly NCA).⁹ CEACAM1 (formerly BGP), CEACAM3 (formerly CGM1), CEACAM4 (formerly CGM7), CEACAM19, CEACAM20 and CEACAM21 are all attached to the cell membrane through transmembrane domains.² CEACAMs manifest their function as an intercellular adhesion molecule through GPI anchorage to the cell surface or transmembrane domains.⁸

Most of the research has been directed at CEACAM1 due to the homology identified in a number of mammals, making it more

accessible as an in vivo model,¹⁰ as well as towards CEACAM5, the gastrointestinal oncofetal antigen first described in 1965.⁸ It is found in most carcinomas of the respiratory and genitourinary systems, in breast cancer, and in cancers of the gastrointestinal tract.¹¹ In contrast, CEACAM6, also referred to as CD66c, is a multi-functional glycoprotein that mediates homotypic binding with other CEA family members and heterotypic binding with integrin receptors.¹² It functions by organizing tissue architecture and regulation of signal transduction, while aberrant expression leads to the development of human malignancies.¹² It was first discovered in proliferating cells of adenomas and hyperplastic polyps in comparison to benign colonic tissue.¹³ Overexpression of CEACAM6 modulates cancer progression through aberrant cell differentiation, anti-apoptosis, cell growth and resistance to therapeutic agents.¹⁴ CEACAM6 overexpression in multiple malignancies promotes cell invasion and metastasis, thereby representing an acquired advantage of tumor cells directly responsible for an invasive phenotype.¹⁵ *In vitro* studies have shown that antibodies directed against CEACAM6 on overexpressing cells inhibited cell migration, invasion and adhesion.¹⁵

This article will serve as a comprehensive review highlighting the role of CEACAM6 in various malignancies, identifying common and unique pathways suspected of playing a central role in the malignant process. Furthermore, targeting CEACAM6 with novel therapeutic approaches provides an opportunity to treat several human malignancies.

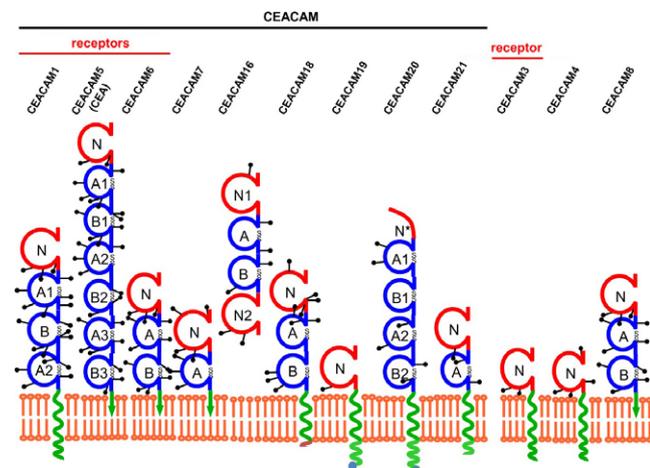


FIGURE 1 The carcinoembryonic antigen-related cell adhesion molecule (CEACAM) gene superfamily.⁷⁹ The CEACAM immunoglobulin-like domain superfamily is a large family of proteins encoded by 12 independent genes and located on chromosome 19. The Ig domain consists generally of one IgV-like N-domain (red), except CEACAM 16, which has 2 N-domains and IgC-like domains identified as (A and B) shown in (blue). The membrane-linked glycoproteins are anchored to the cell surface either by transmembrane domain (green zigzag lines), such as CEACAM5-8, or anchored to the membrane by glycoposphatidyl-inositol anchor (green arrowhead), including CEACAM1, 3, 4, 19, 20 and 21. CEACAM16 is a secreted version with no membrane anchorage. The CEACAM 19 and 20 cytoplasmic domain has immune-receptor tyrosine-based activator (ITAM) motifs (blue circles). Heavily N-Glycosylation sites in all family members are shown as black lippops on the extracellular domains

2 | CARCINOEMBRYONIC ANTIGEN/ CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE FAMILY: CHROMOSOMAL LOCATION, EXPRESSION AND REGULATION

2.1 | Chromosomal location of carcinoembryonic antigen/carcinoembryonic antigen-related cell adhesion molecules

The human CEACAM gene family is composed of 29 genes/pseudogenes and gene-like sequences that are clustered in human chromosome 19 (q13.2).¹⁶ This large gene family can be divided into the CEA subgroup (n = 12, where 5 of them are pseudogenes), the PSG-subgroup (n = 11) and the incomplete non-expressed CGM (CEA gene family member) subgroup (n = 6).^{17,18} These genes were arranged during evolution into 850-kb distal clusters and 250-kb proximal clusters in relation to the centromere, separated by 700 kb of genomic DNA containing a few unrelated genes.¹⁹ CEACAM4, CEACAM7, CEACAM5, CEACAM6 and CEACAM3 are closely clustered in the proximal cluster; CEACAM1, CEACAM8 and PSG/CGM genes are clustered in the distal cluster.¹⁶

Gene amplification is an essential mechanism of insertional mutagenesis, in addition to loss of control mechanisms, structural alterations, chromosome translocations and oncogene activation. The comparative genome hybridization analysis identified DNA copy number changes in all cancers.²⁰ The region 19q13.2-13.32, spanning

3.25 MB, is amplified in hepatocellular carcinoma as well as being amplified in other cancers such as follicular lymphoma (19q13), mantle cell lymphoma (19q13), respiratory tract small cell lung cancer (19q13.1), non-small cell lung cancer (19qcen-q13.3), hepatocellular carcinoma (19q13.1), breast carcinoma (19q13.1-qter), and chondrosarcoma (19q13.2),²¹ whereas deletion of this locus occurs infrequently and was only observed in colon tumors.²² It is clear that the precise characterization of chromosomal amplicon areas will be of prognostic and therapeutic value to revolutionize clinical molecular genetics in oncology.

2.2 | Transcriptional regulation of carcinoembryonic antigen/carcinoembryonic antigen-related cell adhesion molecule 6

Studies on transcriptional regulation of the CEACAM family genes have previously been performed with human CEACAM1 and CEACAM6 genes.²³ Research on the regulation of other CEACAM family genes in humans and other species is still scarce. The upstream promoter sequences of CEACAM1 and CEACAM6 genes lack the classical TATA and CCAAT boxes. TATNCCAAT-less genes can generally be grouped into: (i) constitutively active house-keeping genes with relatively G/C-rich promoter regions, SPI sites and often multiple transcriptional start sites; or (ii) genes lacking G/C-rich regions that have tightly clustered transcriptional start sites that are differentially or developmentally regulated.²⁴ However, in contrast to other TATNCCAAT-less genes, CEACAM family genes possess features from both groups.²³ They have G/C-rich promoter regions and SPI sites, but they also have clusters of transcriptional start sites and are differentially expressed. CEACAM6 promoters show a sequence homology of 80% within the first 230 nucleotides upstream of the translational start site, where they could share the same transcriptional binding factors. The sequences farther upstream diverge significantly from each other.²⁵

In TGF- β signaling, CEACAM6 was defined as a major SMAD3-mediated target gene. Moreover, HER2 expression was associated significantly with SMAD3 phosphorylation in only CEACAM6-positive cancers.²⁶ TGF- β elaborated in the malignant tumor microenvironment (TME) binds to the type II receptor (T_{RII}), promoting hetero-tetramerization with the type I receptor (T_{RI}) and increasing the phosphorylation of SMAD3 by activated T_{RI}. Phosphorylated SMAD3 forms a complex with Co-Smad (SD4), which promotes translocation into the nucleus and activate expression of target genes, specifically CEACAM6.²⁷ Hence, HER2 signaling through the TGF- β pathway may regulate CEACAM6 expression.

Although genetics is concerned with gene expression as a function of DNA sequence, epigenetic alteration of gene expression can be stably modulated by different mechanisms. It has been reported that HDAC inhibitor (NaB) and DNA methyltransferase inhibitor (5-AZA) upregulate CEA expression in different cancer cells.²⁸ In colon carcinoma cells, NaB and 5-AZA appear to have major effects in increasing the rate of transcription through epigenetic modifications

of chromatin and DNA methylation, respectively.²⁸ Furthermore, epigenetic analysis of CEACAM6 promoter in different primary breast cancers, identified downregulation of expression level by hypermethylation of CEACAM6 promoter among triple-negative breast cancers specifically.²⁹ Strongly suggesting that aberrant DNA hypermethylation of CEACAM6 promoter by overexpression of DNMT3b, DNA methyltransferase enzymes, and concurrent methylation-dependent silencing might trigger breast cancer progression.

2.3 | Expression of carcinoembryonic antigen/carcinoembryonic antigen-related cell adhesion molecule 6

Each CEACAM family member has a distinct tissue-specific expression pattern that varies during different stages of development. In healthy human adults, the expression of CEA is mostly found in columnar epithelial cells and mucus-secreting cells of the colon.³⁰ CEA can also be found at a lower level in secretory epithelial cells present in the stomach and small intestine, in epithelial cells of the prostate, in secretory epithelial and duct cells of the sweat glands, in transitional epithelial cells of the urinary bladder, as well as in squamous epithelial cells of the esophagus, tongue and cervix.¹³ Expression of CEA in lung, breast and pancreas is still controversial. The expression pattern of CEACAM6 is somewhat broader but generally similar to that of CEA with a few exceptions.³⁰ CEACAM6 is not expressed in small intestine, but in duct cells of the breast and pancreas, in myeloid cells of the bone marrow and spleen, and in pneumocytes and bronchiole epithelial cells of the lung, as well as in many other tissues.¹³ Over the past 10 years, a tremendous amount of information regarding the expression patterns and potential of CEACAM6 to serve as a prognostic indicator as well as a therapeutic target has emerged.

3 | STRUCTURE OF CARCINOEMBRYONIC ANTIGEN/CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 6

The ECM domains of CEACAM6 consist of an Ig variable-like N-terminal domain followed by 6 (A1B1, A2B2, A3B3), 3 (A1B1, A2) or 2 (A1B1) C2-type Ig domains, respectively, as shown in Figure 2.³¹ CEACAM6 and CEACAM1 share striking homology at both the nucleotide and amino acid levels. Relative to the CEA family, CEACAM1 and CEACAM6 are 80/90% homologous, respectively, at the nucleotide level and 70/85% homologous, respectively, at the amino acid level.³¹ While CEACAM6 molecules are bound to the plasma membrane by GPI anchors, CEACAM1 contains a transmembrane domain followed by a cytoplasmic tail.³² This distinctive structural feature may explain the opposite roles in human cancer of CEACAM1 vs CEACAM6.

As mentioned above, CEACAM6 is bound to the plasma membrane through a GPI anchor. Such anchors are synthesized in the endoplasmic reticulum.³³ The eukaryotic GPI anchor consists of a

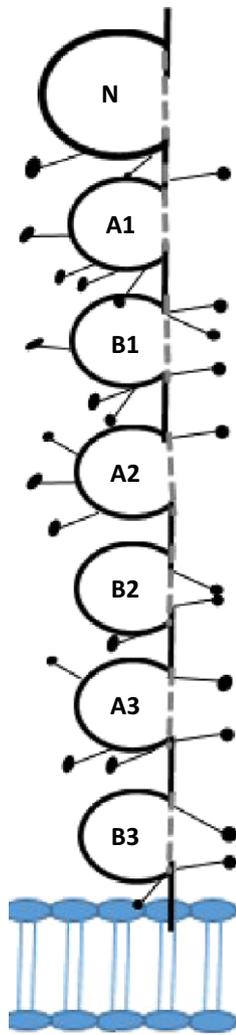


FIGURE 2 Model of a CEA molecule. CEA has been named in the CD system as CD66e. It consists of one IgV-like N-domain and 6 IgC-like domains (A and B). Glycosylation sites are shown as lollipop. The glycosylphosphatidylinositol linkage to the cell membrane is shown by an arrowhead

backbone of N-acetyl-glucosamine, 3 mannose residues, and ethanolamine bound to a phosphoinositol molecule covalently linked to a glycerolipid moiety located within the outer leaflet of the plasma membrane. CEACAM6 are likely attached to the GPI anchor through an amide linkage between their C-terminus and the core phosphoethanolamine moiety of the anchor. There are variations of the core structure of the eukaryotic GPI anchor, such as palmitoylation of the inositol ring, number and length of lipid chains attached to glycerol, and modifications of mannose residues.³⁴ GPI-anchored proteins, such as CEA and CEACAM6, are excluded from the polarized epithelial cells and are only specifically targeted to apical membranes.³⁵ Moreover, GPI-anchored proteins are found in specific rafts or micro-domains of the plasma membrane known as detergent insoluble glycolipid domains (DIG).³⁶ Several members of the Src-family of GTP-binding proteins and protein tyrosine kinases involved in signal transduction pathways are often associated with the cytoplasmic side of DIG.³⁶

4 | FUNCTIONS OF CARCINOEMBRYONIC ANTIGEN/CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 6

Carcinoembryonic antigen is expressed on the apical surface of columnar epithelial cells and mucus-secreting goblet cells of the human gastrointestinal tract, mainly in the upper one-third of the colonic crypts. In addition, CEA is expressed in squamous epithelial cells of the cervix and tongue,¹³ in sweat glands³⁷ and in epithelial cells of the prostate.³⁸ CEACAM6 is expressed in epithelial cells of the colon,³⁹ lung and stomach, and in granulocytes and monocytes as well.⁴⁰

4.1 | Physiological functions of carcinoembryonic antigen/carcinoembryonic antigen-related cell adhesion molecule 6 in normal tissues

Cell adhesion molecules play a crucial role in a number of biological functions, including homeostasis, embryogenesis, development of neural tissue, inflammation, immune cell transmigration and immune response.³ Moreover, expression of CEA and CEACAM6, but not CEACAM1, inhibit colonocyte differentiation.⁴¹ CEA was also shown to inhibit neurogenic and adipogenic differentiation.⁴² Altogether, these results suggested that the differentiation block by CEA is possibly a result of clustering of multiple CEA molecules on the cell surface and activation of downstream signaling cascades.³⁵ In addition, findings showed that opposite effects can be mediated by the 2 groups of CEACAM, as in cell differentiation. Hence, it is advantageous for the tumor cells to overexpress CEACAM6 and to downregulate CEACAM1, so that they can escape apoptosis as well.⁴³

4.2 | Physiological functions of carcinoembryonic antigen/carcinoembryonic antigen-related cell adhesion molecule 6 in cancer

The human CEACAM family genes are deregulated in many human cancers. CEA is overexpressed in many gastrointestinal cancers, including colorectal, esophageal, gastric, pancreatic, hepatocellular and gallbladder carcinomas, breast cancer, lung cancer, the female reproductive tract cancer (ovarian, cervical and endometrial carcinomas), medullary thyroid carcinomas, urinary bladder cancer and prostate cancer.⁴⁴ The question of the potential mechanism of CEA/CEACAM6 effects arises. There are at least 3 key physiological processes that function to maintain the homeostasis of a normal human epithelium: cell proliferation, cell differentiation and apoptosis.⁴⁵ The disturbance of these processes, such as through an increase in cell proliferation or inhibition of cell differentiation and apoptosis, or a combination of these events, could contribute to malignant transformation of the epithelial cells. The forced overexpression of CEA/CEACAM6 on the cell surface of several cell lines inhibited cell differentiation without increasing the rate of cell proliferation.⁴⁴ CEA/CEACAM6 are mostly expressed on the apical surface of the human colonial epithelium but are also expressed in other cell types.³⁵ This

normal pattern of expression is dramatically disrupted upon malignant transformation when CEA and CEACAM6 are overexpressed over the entire surface of the malignant cells whereas CEACAM1 is largely absent.⁴⁶ CEACAM6 are overexpressed in more than 50% of all human adenocarcinomas. Based on these observations, the prevailing hypothesis is that CEACAM6 overexpression plays an oncogenic role.¹⁵ In contrast, transmembrane CEACAM1 was proposed to be a tumor suppressor protein because it is downregulated in many cancers and its forced expression in various cancer cell lines, including prostate, colon and breast, decreases proliferation and increases apoptosis.⁴³ However, this generalization becomes blurred based on the following observations: (i) detection of its overexpression in various cancers;⁴⁷ (ii) its contribution to tumor aggressiveness;⁴⁸ (iii) the nature of its immune suppressive effects;⁴⁹ and (iv) its pro-angiogenic properties.⁵⁰

The GPI-anchored CEA/CEACAM6 have a variety of tumorigenic effects on cells cultured *in vitro* and in xenograft mouse models. Overexpression of CEA and CEACAM6 impedes myogenic, adipogenic, neurogenic and colonic differentiation programs,⁴¹ inhibits anoikis/apoptosis in colon and pancreatic cancer cells,^{51,52} disrupts cell polarization and tissue architecture,⁴¹ increases chemoresistance⁵³ and enhances liver metastasis.⁵²

Although earlier studies examined the role of CEACAM6 in gastrointestinal malignancies such as colon and pancreas, numerous other carcinomas (e.g. breast, gastric, thyroid, B-ALL and multiple myeloma) have been shown to overexpress CEACAM6, resulting in increased metastatic potential.

The fundamental role of CEACAM 6 in breast cancer progression will be discussed in detail below.

4.2.1 | Physiological functions of carcinoembryonic antigen-related cell adhesion molecule 6 in breast cancer

Carcinoembryonic antigen-related cell adhesion molecule 6 expression may represent an early event in the development of human epithelial malignancies. Breast cancer cells illustrate CEACAM6 better than normal breast tissue.⁵⁴ As we have seen in precursor lesions for both colorectal cancer and pancreatic ductal adenocarcinoma (PDA), overexpression of CEACAM6 is predictive of progression to breast cancer.⁵⁵ A retrospective analysis of 243 patient samples revealed that CEACAM6 overexpression correlated with tamoxifen resistance.⁵⁶ In a multivariate analysis, CEACAM6 overexpression was an independent predictor of disease recurrence.⁵⁷ Furthermore, siRNA-mediated gene silencing of CEACAM6 in tamoxifen-resistant MCF7 cell line derivatives reversed anchorage independence and re-established endocrine sensitivity.⁵⁷ Previous work revealed that the development of resistance to tamoxifen in breast tumor cells is related to augmented invasive potential⁵⁸ as well as CEACAM6 overexpression.⁵⁴ The relationship between CEACAM6 overexpression and acquired tamoxifen resistance leads to invasion and migration in MCF-7.2A and MCF-7.5C breast cancer cells.⁵⁹ CEACAM6 is markedly overexpressed in estrogen-diminished

breast cancer cells. These *in vitro* data were further validated in human breast cancer as CEACAM6 is recurrently overexpressed in the setting of post-adjuvant endocrine therapy (e.g. tamoxifen). In a cohort study of 840 invasive breast cancers and a separate validation cohort of 300 invasive breast cancers, CEACAM6 expression was found to be present in 37.1% (312/840 patients) cases.²⁶ Interestingly, CEACAM6 expression was highest in HER2+ tumors (67%) and lowest in triple negative breast cancer (25%).⁶⁰ The patients in this study were followed for a mean of 56.4 months, and 105 patients developed distant metastatic disease.²⁶ Expression rates for CEACAM6 were highest for patients with bone metastasis (42%). The study reported that CEACAM6 adverse prognostic significance for OS was independent of traditional prognostic factors (age, tumor grade, size and Ki-67 expression level). One unique feature of the signal transduction in HER2+ breast cancer cell lines and CEACAM6 expression is the interaction of the HER2 pathway and TGF- β signaling.¹⁷ Overexpression of CEACAM6 results in alteration and reorganization of the ECM and activation of the TME playing a key role in tumor invasion.⁵⁴ CEACAM6 signaling increases SRC activity, leading to increased IGF-I secretion, with autocrine and paracrine stimulation of the IGF-1R activating the PI3K/AKT pathway. Increased IGF-I expression results in MMP-2 elaboration, subsequently altering the ECM and promoting a malignant TME. Aggregation of CEACAM6 at the cell surface enhances anoikis resistance through activation of SRC in a caveolin-1-dependent manner, allowing for phosphorylation of its substrate focal adhesion kinase.¹⁷

In addition, TGF- β signals through the TGF- β receptors activate alternate pathways, such as AKT/PI3K.¹⁷ CEACAM6 expression also serves as an indicator of response to therapy in Her2+ breast cancer. CEACAM6 underexpression indicated trastuzumab responsive disease, whereas overexpression reflected trastuzumab-resistant breast cancer.⁶¹ Further investigation is warranted to identify whether CEACAM6 overexpression in metastatic breast cancer patients also serves as a biomarker of response to gemcitabine, an approved third line therapy.⁶¹ CEACAM6 expression would then serve as an avenue to identify high-risk cohorts of breast cancer patients whose therapy could be specifically tailored based on their underlying tumor biology, highlighting CEACAM6's role as both prognostic and underscoring its potential as a therapeutic target in epithelial carcinomas.

Based on the observations described above and the research work ongoing in our research lab, we generated the following dual hypothesis depicted in (Figure 3) below: (i) CEACAM6, beyond its normal functions, including intercellular adhesion, cell death regulation, and also its function as a general inhibitor of cell differentiation, might play a novel role in malignant transformation and tumor cell invasion/metastasis in breast cancer; and (ii) in addition to a better understanding of the mechanisms that underpin CEACAM6-mediated tumor cell invasion, our recent research findings have the potential to validate CEACAM6 as a target to pave the way towards the design of efficient therapeutic strategies against breast cancer.

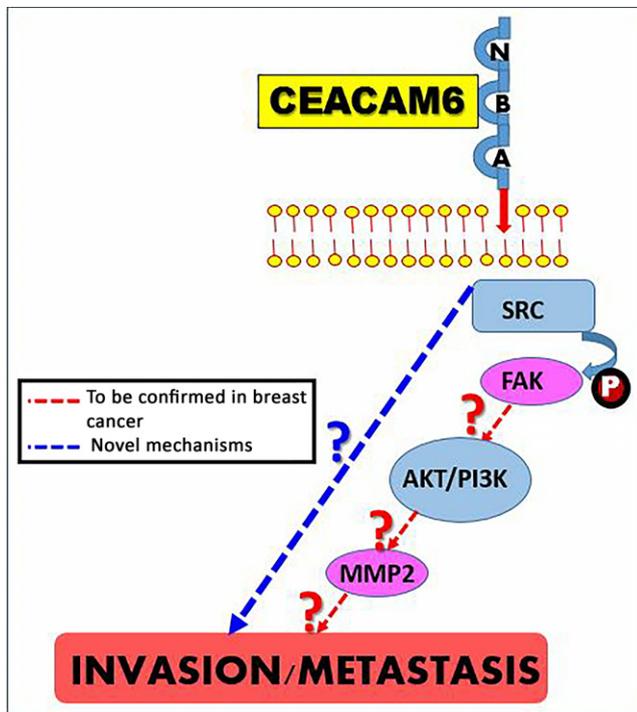


FIGURE 3 The novel predicted molecular mechanisms underlying carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) promoting breast cancer progression

4.2.2 | Physiological functions of carcinoembryonic antigen-related cell adhesion molecule 6 in other cancers

Over the past 2 decades, physiological and pathological roles of this large gene family, mainly in cancer, infectious diseases, immunology and diabetes, have been gradually elucidated in different model systems.⁴⁹ Although the growing knowledge on the functions of CEACAM has already been beneficial to clinical practice and therapy, further understanding of their mechanism of action in associated diseases and discovery of their participation in other diseases may have greater impact on both clinical and biological sciences in the future.

Carcinoembryonic antigen-related cell adhesion molecule 6 is overexpressed primarily by colorectal, gastric and pancreatic cancers, with the vast majority of these tumors (>90%) expressing CEA.⁶² In addition, it is expressed by the majority of medullary thyroid cancers⁶³ and more than 70% of non-small cell lung cancers.⁶⁴ Reports of expression on many other cancers exist. Overexpression of CEA colorectal cancer was described by inhibition of terminal differentiation, loss of cellular polarization, distortion of tissue architecture, and anoikis resistance,⁸ while in lung adenocarcinoma, it induces cell proliferation and anchorage-independent growth.^{65,66} In gastric cancer, CEACAM6 promotes lymph node metastasis, migration and invasion through increasing SRC phosphorylation.⁵⁰ In contrast, in B-cell acute lymphoblastic lymphoma, presumed enhancement of anoikis through increased caspase

activation as well as upregulation of the AKT cell survival pathway.⁵⁰ In addition, in multiple myeloma, CEACAM6 inactivation of CTL (cytotoxic lymphocytes), through binding and cross-linking of CEACAM6 on plasma cells, resulted in T-cell unresponsiveness immune evasion.^{51,67} In addition, in pancreatic ductal adenocarcinoma, increased metastatic potential through increased IGF-I resulted in expression of proteolytic MMP-2 and MMP-9, altering the ECM through SRC-AKT signaling pathway.⁶⁸ These findings correlate CEACAM6 overexpression with an increased metastatic phenotype through elevated SRC activity and matrix metalloproteinase 2 (MMP2) expression. Hence, CEACAM6 overexpression through activation of the SRC-AKT signaling provides chemoresistance to gemcitabine in PDA cell lines.

5 | CONTRIBUTION OF CARCINOEMBRYONIC ANTIGEN/ CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 6 IN CELL SIGNALING

The CEA family plays an active role in multiple signal transduction processes.⁶⁹ Using transfected basophile leukemia cells as a model, it has been demonstrated that GPI-bound CEA participates in a stimulatory signal transduction pathway.⁸

Many cell surface molecules, including receptors and adhesion molecules, can convey information from the milieu to the interior of the cells by means of signal transduction, so that the cells can respond to the changes in the environment. The CEACAM gene family of cell surface adhesion molecules is no exception. Nevertheless, Sreaton et al³⁵ suggested that the presence of CEA-specific GPI anchors, which reside in specific membrane microdomains (lipid rafts), is essential for their cellular functions, at least as inhibitors of cell differentiation. Sreaton et al further suggested that perturbation of integrin functions and integrin-ECM interactions may be the underlying mechanism responsible for their inhibitory effect on anoikis.³⁵ When CEACAM6 is cross-linked in BxPC3 cells, anoikis resistance develops through activation of SRC in a caveolin-1-dependant manner.⁵³ SRC was identified as a key player in tumor progression, providing oncogenic signals for mitogenesis, cell survival, angiogenesis, invasion and metastasis.⁷⁰ Caveolin-1 is an integral membrane protein found to co-precipitate with SRC and is required for SRC kinases to activate integrin signaling.⁷¹ The co-clustering of cell surface lipid raft-associated CEACAM6 and integrin $\alpha_5\beta_1$ leads to increased fibronectin binding to the cells and recruitment of downstream signaling elements, including ILK, Src family kinases, PBK and Akt/PKB.⁷² and, hence, it has been proposed that clustering could activate the integrin signaling pathway.¹¹ A similar observation was made by another group involving CEACAM6 and integrin $\alpha_7\beta_3$ in pancreatic adenocarcinoma cell lines.⁵³ Altogether, the GPI-anchored CEACAM can mediate cell signaling through modulation of integrin functions (Figure 4).

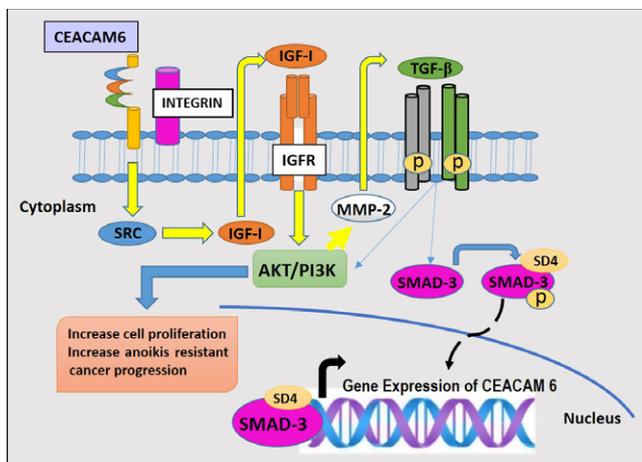


FIGURE 4 Carcinoembryonic antigen-related cell adhesion molecule (CEACAM6) signaling pathways

6 | CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 6 TARGETED CANCER THERAPIES

Combinations of surgery, chemotherapy and radiation therapy represent the conventional treatment for cancer. However, with the growing knowledge of cancer molecular biology, novel cancer therapies are focused on personalized tumor targets to minimize systemic toxicity and increase efficacy.⁷³

In the present review, the role of CEACAM6 in various malignancies has been discussed, as well as the identification of common and unique pathways suspected to play a central role in the malignant process. Furthermore, targeting CEACAM6 provides an opportunity to treat several human malignancies, including the following potential approaches.

6.1 | Anti-carcinoembryonic antigen-related cell adhesion molecule 6 single chain variable fragment therapeutic humanized

Mouse monoclonal antibody (Mab) 13-1, targeting a unique epitope on human CEACAM6, leads to apoptosis of PDA cells expressing CEACAM6 with an IC₅₀ independent of antibody cytotoxicity.⁷⁴ In silico CDR grafting and antibody engineering generated single chain variable fragment (scFv) humanized anti-CEACAM6 with a linker that could be PEGylated to enhanced plasma half-life. The unique features of humanized anti-CEACAM6 scFv include the ability to produce targeted tumor cell apoptosis and also represent an effective combination with standard chemotherapy at very low doses.⁷⁴

6.2 | Monoclonal antibodies

Utilizing GW-39 human colonic cancer cells, the effects of 3 Mabs that target specific epitopes A1B1 domains (MN-15) and the NH₂-terminal (MN-3) present in CEACAM6, CEACAM5 and the A3B3

domain (MN-14) were investigated.¹⁵ Although treatment with MN-15 and MN-3 resulted in an increase in survival, decreased adhesion to the ECM and abrogated metastatic potential, these Mabs were not efficacious with regard to tumor growth inhibition or regression.¹⁵ The availability of therapeutic monoclonal antibodies (unconjugated and conjugated) targeting CEACAM6 provides potential therapeutic opportunities likely to lead to a plethora of pre-clinical and clinical activity in the next decade.

6.3 | Antibody-drug conjugates

A study of an antibody-drug conjugate targeting CEACAM6 investigated the efficacy of the anti-CEACAM6-maytansinoid (DM1) immune-conjugate in a murine xenograft model of PDA.⁷⁵ The non-conjugated Mab did not have anti-tumor activity, and showed reversible and modest neutropenia. Further development of an antibody-drug conjugate-based therapeutic approach in PDA has yet to be pursued in early phase clinical trials.⁷⁵

6.4 | Immunotoxin-based therapy

Targeting CEACAM6 in PDA using immunotherapeutic strategies has been classified as direct or indirect.⁷⁶ Indirectly using the Mab (114) specific for CEACAM6 with no anti-tumor activity mediated cross-linking of CEACAM6, which resulted in cytoplasmic accumulation that increased efficacy of immunotherapy with saporin with caspase-mediated apoptosis.⁷⁶

6.5 | Knockdown mediated gene silencing

Knockdown models in CEACAM6-positive tumors identify key mechanistic pathways and potential targets involved in the development of the malignant phenotype.⁷⁷ Mice CEACAM6-specific siRNA studies diminished tumor proliferation significantly, with a decreased Ki-67 level impairing angiogenesis and increased apoptosis. Furthermore, CEACAM6-specific siRNA prevented metastasis and improved survival without toxicity. Knockdown models inform us of the critical role CEACAM6 plays in cancer development, further solidifying a focus on this molecule as a therapeutic target.⁷⁷

7 | SUMMARY AND PERSPECTIVE

The migration of cancerous cells from their primary location to distant sites is the cause of 90% of human cancer mortality.⁷⁸ Dysregulated overexpression of CEACAM6 plays a role in several of the hallmarks of cancer, including uncontrolled proliferation, anoikis resistance, neoangiogenesis, immune evasion, invasion and metastasis. We believe that safely targeting CEACAM6 is most likely the key to changing the natural history of the tumor and tumor microenvironment to allow novel combination therapies to be developed for treating both solid and hematologic malignancies. We believe that safely targeting CEACAM6 is most likely to change the natural

history of the tumor and TME and allow novel combination therapies to be developed for treating both solid and hematologic malignancies.

In conclusion, our review highlights the pathologic roles of CEACAM6 in the development of multiple human malignancies, warranting an ongoing focus to successfully transition this cell adhesion protein as a primary or adjunctive therapeutic target for anti-cancer therapy in the clinic.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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