Recent advances in the tumour biology of the GPI-anchored carcinoembryonic antigen family members *CEACAM5* and *CEACAM6*

C.H.F. Chan PhD and C.P. Stanners PhD

KEY WORDS

Carcinoembryonic antigen, carcinoembryonic antigen–related cell adhesion molecule 6, cancer, colon cancer, tumour biology

INTRODUCTION

On its discovery in 1965 by Gold and Freedman [1] in the blood of patients with colorectal cancer, human carcinoembryonic antigen [*CEA* (since re-designated *CEACAM5*)] was initially thought to be a tumour-specific antigen. Although *CEACAM5* was subsequently found in normal tissues, its consistent overexpression in many cancers has made it a tumour marker widely used for patient management and a popular molecular target for novel cancer therapies.

After the cloning of *CEACAM5* cDNA in 1986 [2], other *CEACAM5*-related cell adhesion molecules were also identified in humans and other mammalian species [3–5]. The *CEACAM* family members are highly glycosylated proteins that belong to the immunoglobulin gene superfamily [6]. In humans, the *CEACAM* family consists of membrane-linked and secretory glycoproteins. The former are anchored to the cell surface either by a glycophosphatidyl–inositol (GPI) anchor or a transmembrane domain. The GPI-anchored members include *CEACAM5* (the original *CEA*), *CEACAM6*, *CEACAM7*, and *CEACAM8* [3]. Thus far, the GPI-anchored *CEACAM*s have been detected only in primates, and not in lower mammals [3–5,7].

The enormous volume of literature describing the aberrant expression of *CEACAM5* and *CEACAM6* in various types of cancers, the prognostic values of such expression, and *CEACAM5*-targeted therapies has tended to dilute studies revealing the significant biologic functions of these antigens and their potential clinical implications. This editorial overview highlights current knowledge of the biologic functions of *CEACAM5* and *CEACAM6* in relation to tumorigenesis.

Richard J. Ablin, PhD, Research Professor of Immunobiology, University of Arizona College of Medicine and the Arizona Cancer Center, Tucson, Arizona, U.S.A., and Phil Gold, PhD MD, Professor of Medicine, Physiology, and Oncology, McGill University, Montreal, Quebec, Canada, Section Editors.

CEACAM5 AND *CEACAM6* IN HUMAN CANCERS

CEACAM5 is overexpressed in cancers of the gastrointestinal tract, pancreas, liver, gallbladder, lung, breast, female reproductive system, medullary thyroid, urinary bladder, and prostate [3,8–11]. Similarly, *CEACAM6* is overexpressed in cancers of the colon, stomach, pancreas, lung, breast, and female reproductive system, and in leukemia [3,8]. Overall, *CEACAM5* or *CEACAM6*, or both, are overexpressed in as many as 70% of all human tumours [12]. In addition, that overexpression is often associated with poor prognosis—specifically, poor clinical outcome and reduced survival [13–16].

This overwhelming correlation suggests an instrumental role for these molecules in tumorigenesis. In fact, *CEACAM5* and *CEACAM6* have a variety of tumorigenic effects on cells cultured *in vitro* and in *in vivo* model systems. Overexpression of *CEACAM5* and *CEACAM6* impedes myogenic, adipogenic, neurogenic, and colonic differentiation programs [17–19], inhibits anoikis and apoptosis in colon and pancreatic cancer cells [20–22], disrupts cell polarization and tissue architecture [19], enhances liver metastasis [22,23], increases chemoresistance [24], and increases colon-tumour [25] and lung-tumour (Chan *et al.* Higher incidence of spontaneous lung tumours in the CEABAC mice. In preparation) susceptibility in a transgenic mouse model.

This broad spectrum of tumorigenic effects arises from functions at the molecular level. *CEACAM5* and *CEACAM6* have been shown to activate integrin signalling pathways [26,27]. Proteins that are GPI-anchored, including *CEACAM5* and *CEACAM6*, are often localized in the membrane microdomains called "lipid rafts" [28]. These rafts carry specific subsets of

signalling molecules and are freely mobile on the cell membrane. Growing evidence suggests the presence of specific types of lipid rafts [28,29]. *CEACAM5* and *CEACAM6* have been shown to be co-localized with integrin $\alpha5\beta1$ in the same specific lipid rafts [30].

CEACAM5 and *CEACAM6* function as intercellular adhesion molecules because of parallel and antiparallel self-binding of their extracellular domains [31], and therefore small *CEACAM5*- and *CEACAM6*-containing lipid rafts can cluster together to form bigger rafts [29], thus co-clustering their associated signalling elements. This co-clustering could underlie the observed activation of downstream signalling cascades, such as the integrin signalling pathway, including elements *ILK*, *PI3K*, and *AKT* [26]. This mode of signal activation would critically depend on the cell-surface level of *CEACAM5* and *CEACAM6*. That is, the downstream signal and consequent cellular behaviour would depend in a nonlinear threshold fashion on the concentration of *CEACAM5*, *CEACAM6*, or both.

CEACAM5 AND *CEACAM6* IN COLORECTAL CANCER

Colorectal cancers are the end result of multiple transformational events in normal epithelia. A set of neoplastic events, termed the adenoma–carcinoma sequence, was originally proposed by Vogelstein and colleagues for traditional adenomas [32]. The loss of functional *APC* causes a transition from normal epithelium to aberrant crypt foci (ACF), the earliest detectable tumorigenic change, followed by Kras activation (adenoma formation), loss of *SMAD2* and *SMAD4*, and *TP53* inactivation (carcinoma formation). With growing knowledge of the genetics of colorectal cancers, more gene mutations are being placed into this basic paradigm, although all the events are not necessarily present and their sequence can vary [33,34].

In contrast to the traditional adenomatous polyps, hyperplastic polyps are commonly believed not to progress to malignant lesions [35]. However, in recent years, sessile serrated adenoma, serrated adenoma, and mixed polyps (a subgroup of hyperplastic lesions showing a serrated feature) have been shown to have malignant potential [36]. These serrated lesions show frequent *BRAF* (a member of the *RAF* family of serine and threonine kinases) mutations and widespread DNA methylation, and they have recently been considered premalignant lesions that follow the serrated pathway of neoplastic transformation as proposed by Jass and colleagues [36–38]. A general inhibition of anoikis caused by mutation in a specific gene can lead to serrated polyp formation [37]. Mutations in or downregulation of *hMLH1* or *MGMT* (methylguanine methyltransferase) can then lead to progression to MSI-H (high level of microsatellite instability) and MSI-L (low level of microsatellite instability) colorectal cancers respectively [38].

Although *CEACAM5*—and to a lesser extent *CEACAM6*—are consistently overexpressed in most colorectal cancers and have a broad range of tumorigenic effects, they have not yet been assigned to any proposed pathway. On the one hand, the overexpression of *CEACAM5* in 30%–90% of ACFs suggests that this overexpression can be an early event in the adenoma–carcinoma sequence [39,40]. On the other hand, *CEACAM5* overexpression in serrated polyps and its anti-apoptotic ability may suggest its involvement in the serrated pathway [41]. Similarly, the overexpression of *CEACAM6* in hyperplastic polyps and traditional adenomas alike suggests that *CEACAM6* may also be involved in these neoplastic pathways [42].

A transgenic mouse containing both the *CEACAM5* and *CEACAM6* genes in a large (187 kb) piece of human genomic DNA (the CEABAC mouse) has recently been constructed [43]. At low-to-moderate expression levels of *CEACAM5* or *CEACAM6* (or both), a partial block in cell differentiation, a mild-to-moderate colonocyte hyperproliferation, and an inhibition of anoikis or apoptosis are evident in the transgenic colon. These mice are found to be significantly more prone to develop carcinogen-induced colon tumours, specifically the traditional adenomatous type [25]. At higher (tumour-like) expression levels, a complete block in cell differentiation and extreme colonocyte hyperproliferation can be observed. These mice show massively enlarged colons comprising continuous non-focal cytologic and architectural abnormalities, including dysplastic features and serrated morphology. These results suggest that, although moderate expression levels of *CEACAM5* and *CEACAM6* can cause an imbalance of tissue homeostasis leading to increased tumour susceptibility following the classical pathway of colonic neoplasia, tumour-like expression levels alone produce a severe imbalance leading directly to tumour formation, specifically the serrated subtype. Hence, we propose that *CEACAM5* and *CEACAM6* can play a significant role in both neoplastic pathways (Chan *et al.* Colorectal hyperplasia and dysplasia due to human *CEA* and *CEACAM6* expression in transgenic mice. Submitted manuscript).

CONCLUSION

CEACAM5 and *CEACAM6* are commonly considered inert tumour markers, despite the discovery and documentation of their tumorigenic functions over the past two decades. Nevertheless, because of their ectopic or deregulated overexpression in up to 70% of all tumours, *CEACAM5* and *CEACAM6* represent popular targets for novel cancer therapies, including cancer vaccines, cellular immunotherapy, radioimmunotherapy, and antibody therapy. With growing knowledge of the effects of *CEACAM5* and *CEACAM6* on tumour biology, novel therapeutic strategies that focus

more on perturbing the tumorigenic functions of these antigens may now be indicated.

REFERENCES

1. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* 1965;121:439–62.
2. Beauchemin N, Benchimol S, Cournoyer D, Fuks A, Stanners CP. Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen. *Mol Cell Biol* 1987;7:3221–30.
3. Hammarstrom S, Olsen A, Teglund S, Baranov V. The nature and expression of the human *CEA* family. In: Stanners CP, ed. *Cell Adhesion and Communication Mediated by the* CEA *Family: Basic and Clinical Perspectives.* Amsterdam: Harwood Academic Publishers; 1998: 1–30.
4. Zimmermann W. The nature and expression of the rodent *CEA* families: evolutionary considerations. In: Stanners CP, ed. *Cell Adhesion and Communication Mediated by the* CEA *Family: Basic and Clinical Perspectives.* Amsterdam: Harwood Academic Publishers; 1998: 31–55.
5. Zhou GQ, Zhang Y, Hammarstrom S. The carcinoembryonic antigen (*CEA*) gene family in non-human primates. *Gene* 2001; 264:105–12.
6. Paxton RJ, Mooser G, Pande H, Lee TD, Shively JE. Sequence analysis of carcinoembryonic antigen: identification of glycosylation sites and homology with the immunoglobulin supergene family. *Proc Natl Acad Sci U S A* 1987;84:920–4.
7. Naghibalhossaini F, Yoder AD, Tobi M, Stanners CP. Evolution of a tumorigenic property conferred by glycophosphatidyl–inositol membrane anchors of *CEA* gene family members during the primate radiation. *Mol Biol Cell* 2007;[in press]. [Epub February 7, 2007]
8. Logsdon CD, Simeone DM, Binkley C, *et al.* Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res* 2003;63:2649–57.
9. Chu PG, Ishizawa S, Wu E, Weiss, LM. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and α-fetoprotein. *Am J Surg Pathol* 2002;26:978–88.
10. Takano T, Miyauchi A, Matsuzuka F, *et al.* Preoperative diagnosis of medullary thyroid carcinoma by RT-PCR using RNA extracted from leftover cells within a needle used for fine needle aspiration biopsy. *J Clin Endocrinol Metab* 1999;84:951–5.
11. Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. *Mod Pathol* 2000;13:1186–91.
12. Chevinsky AH. *CEA* in tumors of other than colorectal origin. *Semin Surg Oncol* 1991;7:162–6.
13. Gaglia P, Caldarola B, Bussone R, *et al.* Prognostic value of *CEA* and ferritin assay in breast cancer: a multivariate analysis. *Eur J Cancer Clin Oncol* 1988;24:1151–5.
14. Ballesta AM, Molina R, Filella X, Jo J, Gimenez N. Carcinoembryonic antigen in staging and follow-up of patients with solid tumors. *Tumour Biol* 1995;16:32–41.
15. Jantscheff P, Terracciano L, Lowy A, *et al.* Expression of *CEACAM6* in resectable colorectal cancer: a factor of independent prognostic significance. *J Clin Oncol* 2003;21: 3638–46.
16. Sakao Y, Nakazono T, Sakuragi T, Natsuaki M, Itoh T. Predictive factors for survival in surgically resected clinical IA peripheral adenocarcinoma of the lung. *Ann Thorac Surg* 2004; 77:1157–62.
17. Eidelman FJ, Fuks A, DeMarte L, Taheri M, Stanners CP. Human carcinoembryonic antigen, an intercellular adhesion molecule, blocks fusion and differentiation of rat myoblasts. *J Cell Biol* 1993;123:467–75.
18. Stanners CP. Contributions of the human *CEA* family to malignant transformation. In: Stanners CP, ed. *Cell Adhesion and Communication Mediated by the* CEA *Family: Basic and Clinical Perspectives.* Amsterdam: Harwood Academic Publishers; 1998: 141–54.
19. Ilantzis C, DeMarte L, Screaton RA, Stanners CP. Deregulated expression of the human tumor marker *CEA* and *CEA* family member *CEACAM6* disrupts tissue architecture and blocks colonocyte differentiation. *Neoplasia* 2002;4:151–63.
20. Ordonez C, Screaton RA, Ilantzis C, Stanners CP. Human carcinoembryonic antigen functions as a general inhibitor of anoikis. *Cancer Res* 2000;60:3419–24.
21. Soeth E, Wirth T, List HJ, *et al.* Controlled ribozyme targeting demonstrates an antiapoptotic effect of carcinoembryonic antigen in HT29 colon cancer cells. *Clin Cancer Res* 2001;7: 2022–30.
22. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. *CEACAM6* gene silencing impairs anoikis resistance and *in vivo* metastatic ability of pancreatic adenocarcinoma cells. *Oncogene* 2004;23:465–73.
23. Jessup JM, Thomas P. *CEA* and metastasis: a facilitator of site-specific metastasis. In: Stanners CP, ed. *Cell Adhesion and Communication Mediated by the* CEA *Family: Basic and Clinical Perspectives.* Amsterdam: Harwood Academic Publishers; 1998: 195–222.
24. Duxbury MS, Ito H, Benoit E, Waseem T, Ashley SW, Whang EE. A novel role for carcinoembryonic antigen–related cell adhesion molecule 6 as a determinant of gemcitabine chemoresistance in pancreatic adenocarcinoma cells. *Cancer Res* 2004; 64:3987–93.
25. Chan CH, Cook D, Stanners CP. Increased colon tumor susceptibility in azoxymethane treated *CEABAC* transgenic mice. *Carcinogenesis* 2006;27:1909–16.
26. Camacho–Leal P, Zhai AB, Stanners CP. A co-clustering model involving α5β1 integrin for the biological effects of GPI-anchored human carcinoembryonic antigen (*CEA*). *J Cell Physiol* 2007;[in press]. [Epub February 7, 2007]
27. Duxbury MS, Ito H, Ashley SW, Whang EE. c-Src-dependent cross-talk between *CEACAM6* and αvβ3 integrin enhances pancreatic adenocarcinoma cell adhesion to extracellular matrix components. *Biochem Biophys Res Commun* 2004;317: 133–41.
28. Screaton RA, DeMarte L, Draber P, Stanners CP. The specificity for the differentiation blocking activity of carcinoembryonic antigen resides in its glycophosphatidyl–inositol anchor. *J Cell Biol* 2000;150:613–26.
29. Nicholson TB, Stanners CP. Specific inhibition of GPI-anchored protein function by homing and self-association of specific GPI anchors. *J Cell Biol* 2006;175:647–59.

30. Ordonez C, Zhai AB, Camacho–Leal P, DeMarte L, Fan MM, Stanners CP. GPI-anchored *CEA* family glycoproteins *CEA* and *CEACAM6* mediate their biological effects through enhanced integrin α5β1–fibronectin interaction. *J Cell Physiol* 2007; 210:757–65.
31. Taheri M, Saragovi U, Fuks A, Makkerh J, Mort J, Stanners CP. Self recognition in the Ig superfamily. Identification of precise subdomains in carcinoembryonic antigen required for intercellular adhesion. *J Biol Chem* 2000;275:26935–43.
32. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159–70.
33. Smith G, Carey FA, Beattie J, *et al.* Mutations in *APC*, Kirsten-*ras*, and *p53*—alternative genetic pathways to colorectal cancer. *Proc Natl Acad Sci U S A* 2002;99:9433–8.
34. Takayama T, Ohi M, Hayashi T, *et al.* Analysis of K-*ras*, *APC*, and β-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. *Gastroenterology* 2001;121:599–611.
35. Crawford JM. The gastrointestinal tract. In: Cotran RS, Robbins SL, Kumar V, eds. *Robbins Pathologic Basis of Disease.* 5th ed. Philadelphia: WB Saunders; 1994; 755–829.
36. Jass JR, Baker K, Zlobec I, *et al.* Advanced colorectal polyps with the molecular and morphological features of serrated polyps and adenomas: concept of a "fusion" pathway to colorectal cancer. *Histopathology* 2006;49:121–31.
37. Jass JR, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. *Gastroenterology* 2002;123: 862–76.
38. Jass JR. Serrated adenoma of the colorectum and the DNA–methylator phenotype. *Nat Clin Pract Oncol* 2005;2:398–405.
39. Pretlow TP, Roukhadze EV, O'Riordan MA, Chan JC, Amini SB, Stellato TA. Carcinoembryonic antigen in human colonic aberrant crypt foci. *Gastroenterology* 1994;107:1719–25.
40. Ilantzis C, Jothy S, Alpert LC, Draber P, Stanners CP. Cell-surface levels of human carcinoembryonic antigen are inversely correlated with colonocyte differentiation in colon carcinogenesis. *Lab Invest* 1997;76:703–16.
41. Jass JR, Filipe MI, Abbas S, Falcon CA, Wilson Y, Lovell D. A morphologic and histochemical study of metaplastic polyps of the colorectum. *Cancer* 1984;53:510–15.
42. Scholzel S, Zimmermann W, Schwarzkopf G, Grunert F, Rogaczewski B, Thompson J. Carcinoembryonic antigen family members *CEACAM6* and *CEACAM7* are differentially expressed in normal tissues and oppositely deregulated in hyperplastic colorectal polyps and early adenomas. *Am J Pathol* 2000;156:595–605.
43. Chan CH, Stanners CP. Novel mouse model for carcinoembryonic antigen–based therapy. *Mol Ther* 2004;9:775–85.

Correspondence to: Clifford P. Stanners, McGill University, Department of Biochemistry, 3655 Promenade Sir William Osler, Montreal, Quebec H3G 1Y6.
E-mail: cliff.stanners@mcgill.ca