Structure, Function and Expression of the CEA Gene Family: Diagnostic and Therapeutic Implications

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Introduction

During the search for biochemical differences between tumorous and corresponding normal tissues a quarter of a century ago, the carcinoembryonic antigen (CEA) was found in colonic tumors, but not in normal colonic mucosa (Gold and Freedman 1965; von Kleist and Burtin 1966). With the development of more sensitive analytical methods, CEA and most other "tumor-specific" markers were also detected in normal tissues or in sera of individuals without tumors (for review see Shively and Beatty 1985). Despite this fact, CEA is widely used for the monitoring of tumor patients for recurrence of malignant disease after surgery (Fantini and DeCosse 1990). A continuous rise in the CEA concentration in serum, detected by serial determinations, is an indicator of tumor regrowth or metastasis in patients with adenocarcinomas of the colon, rectum, breast, lung and pancreas. Since an increase of the CEA concentration is often observed before other clinical symptoms are obvious, early therapeutic measures can be taken (e.g., "second-look" surgery in patients with colorectal tumors (Fantini and DeCosse 1990)). However, the diagnostic value of CEA, e.g., for early detection of primary tumors by routine screening, is limited due to the low sensitivity and specificity of CEA measurements. Therefore, in general, only patients with advanced malignant disease show increased preoperative CEA serum concentrations. On the other hand, elevated CEA concentrations can also be detected in patients with benign disease (e.g., colitis) and in smokers (Shively and Beatty 1985). Clinical trials indicate that radioactively labeled antibodies directed against CEA can be used to localize primary tumors and metastases (Bischoff-Delaloye et al. 1989). Due to the availability of other extremely potent imaging methods, such as computed tomography and magnetic resonance imaging, immunoscintigraphy will probably not be used routinely in the future. However, another immunolocalization technique might soon become invaluable.

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Currently, the use of fluorescent dye-labeled, anti-CEA antibodies for the intraoperative detection of small tumors (e.g., tumor cells in regional lymph nodes) is being evaluated in athymic mice carrying human colonic carcinoma xenografts (Pelgrin et al. 1991). Furthermore, it is hoped that by the application of toxin- or radioactive isotope-coupled anti-CEA antibodies, the specificity of tumor therapy will be increased. Tumor regression has been observed in animal models by several groups using this approach (Buchegger et al. 1988; Sharkey et al. 1991). In the athymic mouse model, enhanced tumor localization and radioimmunotherapy by anti-CEA antibodies was achieved after administration of cytokines, which selectively stimulate CEA expression (Kuhn et al. 1991).

Besides CEA, a large family of closely related cross-reacting antigens have been described which differ in size and tissue distribution (reviewed in Thompson and Zimmermann 1988). For example, nonspecific cross-reacting antigen (NCA) is found in many tumors of epithelial origin, normal lung and spleen and polymorphonuclear cells (Bordes et al. 1975). Biliary glycoprotein, on the other hand, is expressed in hepatocellular carcinomas and normal epithelial cells of bile canaliculi (Svenberg 1976; Hinoda et al. 1990). The presence of CEA-cross-reacting antigens in normal tissues can interfere with measurement in sera and targeting on tumor cells of CEA by antibodies. Due to the high degree of glycosylation of this protein family, biochemical characterization proved to be very difficult. It was hoped, therefore, that cloning of the CEA gene and possibly of related genes would help to clarify the relationship of this complex protein family. This approach was also expected to yield information that would allow the production of more specific probes for diagnosis and therapy. Furthermore, using these probes for determination of the expression pattern of the various CEA family members, new tumor markers might be identified.

Structure, Function and Expression of the CEA Gene Family

Genomic and cDNA cloning have revealed that CEA and related antigens are encoded by a family of genes which belong to the immunoglobulin superfamily. To date 22 genes have been identified (Table 1). Based on sequence similarity they can be subdivided into two main subgroups: the CEA subgroup, which contains the CEA gene and the genes for the classical cross-reacting antigens, and the pregnancy-specific glycoprotein (PSG) gene subgroup. The latter group of genes code for highly similar proteins formerly not known to be related to CEA. PSGs are produced in large amounts in the fetal part of the placenta and secreted into the maternal blood. At term, PSGs comprise the most abundant placental proteins in sera of pregnant women.

The deduced primary structure reveals that the CEA-related antigens are composed of a leader peptide, which is removed after transport into the

Table 1. The presently known members of the CEA gene family

CEA subgroup	PSG subgroup
CEAa,b	PSG1a,b,c,d,e,f
NCA	PSG2n
BGPa,b,c,d,e,f,g,h,i	PSG3m
CGM1a,b,c	PSG4a
CGM2	PSG5n,m
CGM6	PSG6r,s
CGM7	PSG7
CGM8[a]	PSG8a
CGM9	PSG11s,w
CGM10[a,b]	PSG12
CGM11[a,b]	PSG13
	PSG14
	PSG15

The lower case letters indicate the various splice or polyadenylation mRNA variants. CGM, CEA gene family member; PSG, pregnancy-specific glycoprotein.
[a] Probably a pseudogene.
[b] W. Khan and S. Hammarström, personal communication.

endoplasmic reticulum, one immunoglobulin variable (IgV)-like domain and a varying number (none, two, three or six) of Ig constant (C) region-like domains (review: Thompson et al. 1991). Most members of the CEA subgroup seem to be membrane-bound either via a glycosyl-phosphatidylinositol anchor or a transmembrane domain. PSGs lack hydrophobic domains, which is in agreement with their accumulation in the maternal blood. Differential splicing increases the complexity of the CEA family. Up to seven proteins can be predicted for a single gene (BGP) which differ in the number of Ig C region-like domains or the size of their cytoplasmic tails.

All members of the CEA gene family are located on the long arm of chromosome 19 (19q13.2–3; review: Thompson et al. 1991). Mapping aided by pulsed field gel electrophoresis and "contig" analyses (Branscomb et al. 1990) demonstrated arrangement of the CEA-related genes in clusters on a 1.2-Mb chromosome segment. The members of the CEA subgroup are located in two smaller clusters followed by the tightly clustered PSG genes toward the telomere (Thompson et al. 1992). The close vicinity of the members of the CEA family could allow coordinate expression of pairs or groups of genes by the use of common regulatory elements.

All members of the CEA subgroup so far analyzed are able to convey in vitro cell adhesion properties to transfectants expressing individual CEA-related cDNAs (Table 2). This property has also been reported for a number of other members of the immunoglobulin superfamily, such as neural cell adhesion molecule (N-CAM) and myelin-associated glycoprotein (MAG; Williams 1987). CEA, NCA and BGP allow both homophilic and

heterophilic cell adhesion, whereas the CGM6 gene product interacts only heterophilically with NCA and not homophilically with itself (Table 2). Detailed histological studies on the localization of rat CEA-related antigens imply that some members are involved in intercellular adhesion while others might aid in the organization of microvilli (Öbrink 1991). Disturbances in the expression of CEA or related antigens in tumor cells might contribute to the malignant phenotype (Benchimol et al. 1989), as is assumed to be the case for the recently discovered recessive oncogene product DCC ("deleted in colonic cancer"), a presumed cell adhesion molecule (Fearon et al. 1990). As an additional function of CEA subgroup members, binding of enterobacteria such as *Escherichia coli* and *Salmonella typhi* via lectin molecules on type 1 fimbriae has been reported (Table 2). This interaction, which involves D-mannosyl residues, might be important for the colonization by bacteria of the colonic mucosa as well as for the recognition of bacteria by granulocytes, which express, with the exception of CEA, all so far characterized members of the CEA subgroup (see below). Recently, the presumed mouse BGP homologue, mmCGM2, was reported to represent the receptor of the mouse coronavirus, which causes hepatitis (Williams et al. 1991). It might, therefore, be possible that human coronaviruses, which also cause common respiratory illnesses, use members of the human CEA family

Table 2. Function(s) of CEA family members

Member	Function(s)	Reference
CEA	Homo- and heterophilic cell adhesion (Ca^{2+}- and temperature-independent)	Benchimol et al. 1989 Oikawa et al. 1989
	Binding of bacteria	Leusch et al. 1990
	Accessory molecule for collagen type I binding	Pignatelli et al. 1990
NCA-50/90	Homo- and heterophilic cell adhesion (Ca^{2+}- and temperature-independent)	Oikawa et al. 1989 Zhou et al. 1990
	Binding of bacteria	Leusch et al. 1990
BGP	Homophilic cell adhesion (Ca^{2+}- and temperature-dependent)	Rojas et al. 1990
	Binding of bacteria	Leusch et al. 1991
NCA-95 (CGM6)	Heterophilic cell adhesion with NCA-50/90	Oikawa et al. 1991
Ecto-ATPase/ Cell-CAM 105 (rat)	Homophilic cell adhesion (Ca^{2+}-independent)	Lin and Giodotti 1989 Aurivillius et al. 1990
mmCGM1/2 (mouse)	Homophilic cell adhesion (Ca^{2+}- and temperature-dependent)	Turbide et al. 1991
	Mouse hepatitis virus receptor	Williams et al. 1991

as cell entry vehicles. The function of PSGs is unknown. Based on the inhibitory influence of PSGs on certain in vitro immunological reactions, it is speculated that they might be involved in the protection of the allotypic fetus from the maternal immune system by specific immunosuppression.

As a basis for improvement of the specificity and sensitivity of CEA detection, we have started to characterize the recognition pattern of a large panel of monoclonal antibodies. Among them are a number of anti-CEA monoclonal antibodies, the epitopes of which have been compared recently (Hammarström et al. 1989). To this end, we have tested a set of transfectants which express individual members of the CEA gene family (CEA, NCA, BGP, CGM1, CGM6) with each monoclonal antibody. After tagging with fluorescein-labeled second antibody, the antibody binding to the transfectants was determined by FACScan analyses. The results obtained by this approach also allow the assignment of biochemically characterized members of the CEA family to their respective genes by comparing reactivity patterns (Berling et al. 1990). After analysis of more than 110 monoclonal antibodies, three have been identified that react with only one transfectant each (CEA, NCA, CGM6). Two antibodies were found which react with the CEA transfectant and with the CGM1 or the BGP transfectant respectively. Since CEA is not found on granulocytes these antigens can be detected specifically on these cells and studied individually. The above-mentioned approach has also been used to characterize antibodies which define the clusters of differentiation (CD) 66 and 67 and have been shown to cross-react with CEA-related antigens. Whereas the CD67 antibody seems to be specific for the CGM6 product, the CD66 antibodies exhibit a broader recognition pattern, reacting with CEA, NCA, BGP and CGM1 (Watt et al. 1991 and unpublished results). Since CD66 and CD67 antibodies have been shown to react within the hematopoietic system exclusively with mature granulocytes and some precursors, CEA-related antigens therefore represent surface markers for the myeloid lineage.

As long as not all members of the CEA family can be discriminated by monoclonal antibodies, in parallel we have applied gene-specific hybridzation probes and primers to screen normal and tumorous tissues for the expression of individual CEA-related genes. These and other studies have shown that, in general, NCA mRNA levels are significantly higher in colon adenomas and adenocarcinomas than in normal colonic mucosa, whereas CEA mRNA levels do not change dramatically upon malignant transformation (Boucher et al. 1989; Sato et al. 1988; Cournoyer et al. 1988; Higashide et al. 1990; Hinoda et al. 1991). In order to be able to study large numbers of tissue samples we have developed an assay system where we can specifically identify CEA, NCA, BGP, CGM1 and CGM6 mRNAs using the polymerase chain reaction (PCR). The feasibility of this approach is currently being tested with a larger number of gynecological tumors. RNAs, the integrity of which has been proven by amplification of a β-actin mRNA fragment, are reacted with a pair of primers recognizing all known

members of the CEA gene family. The positive samples are then analyzed for the presence of the mRNA of each of the above-mentioned members of the CEA gene family. Preliminary results indicate that, in general, CEA mRNA is coexpressed with NCA mRNA in tumors. Therefore, the CEA and NCA genes, which are next to each other in the CEA gene locus, might share common regulatory elements. Interestingly, most mucinous ovarian carcinomas contained both CEA and NCA mRNAs, whereas ovarian adenocarcinomas of the serous subtype did not express any of the CEA-related mRNAs tested. The expression pattern of the CEA gene family at the mRNA level is summarized in Table 3.

Clinical Implications

What is the relevance of these findings for the diagnosis and therapy of benign and malignant disease? The sensitivity of detection and targeting of CEA could possibly be increased by using cocktails of CEA-specific antibodies. Since we have demonstrated that most of the so far characterized members of the CEA gene subgroup (with the exception of the CEA gene) are expressed on granulocytes, the commonly used approach to test anti-

Table 3. Expression pattern of the CEA gene subgroup

Gene	mRNA size (kb)	Encoded protein	Tissue or cells
CEA	3.5, 3.0	CEA	Normal colon mucosa Colonic polyps Colonic adenocarcinomas (~100%) Less in other carcinomas of epithelial origin (mucinous ovarian carcinomas, lung, pancreas)
BGP	3,9, 3.7, 2.2, 1.8	BGP I (NCA-160)	Normal hepatocyte Hepatocellular carcinoma
NCA	2.5	NCA-50/90	Seems to be always coexpressed with CEA, though to a lesser degree in normal colonic mucosa and polyps CML leukocytes Bone marrow
CGM1	1.3	?	CML leukocytes
CGM2	?	?	?
CGM6	2.2	NCA-95	CML leukocytes Bone marrow

mRNA levels were assessed by northern blot or PCR analyses.

CEA antibodies for cross-reaction against granulocytes is still appropriate, if cross-reactivity with PSGs can be excluded. The monitoring of patients with colorectal tumors might be improved by measuring NCA serum concentrations. Preliminary findings from analysis of a large number of sera of tumor patients, patients with benign disease and healthy individuals with certain antibody combinations which recognize single family members or sets of CEA-related antigens indicate that the sensitivity of tumor detection can be increased without unacceptable loss of specificity. Furthermore, NCA could turn out to be useful as a marker for tumor progression, as the expression of the NCA gene seems to increase with progressing malignancy. The PCR technology would allow determination of NCA mRNA levels in small amounts of biopsy material. Radiolabeled antibodies specific for the CGM6 product might improve the detection of occult inflammatory lesions. Presently, for this purpose, CEA cross-reactive antibodies are employed (D'Amico et al. 1991). CEA and NCA mRNAs (and possibly the corresponding proteins) represent biological markers for certain tumor subtypes as shown for ovarian carcinomas. Therefore, identification of these mRNAs or proteins, respectively, might aid diagnosis in tumor cases of ambiguous histology. These promising results, however, have to be confirmed by analyses of a larger number of tumors and sera, as well as corresponding normal tissues.

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