CARCINOEMBRYONIC ANTIGEN (CEA):
DISCOVERY, FUNCTION, CHEMISTRY AND
DIAGNOSTIC SIGNIFICANCE

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ABSTRACT:
The CEA molecule is an onco-development human tumor marker, bearing the cluster differentiation designation of CD66e, a subtype of CD66 group of CEA family. It has a molecular weight of 180 kDa. This antigen was demonstrated in primary tumor of the pancreas and liver, followed by the study of its presence in 1st two Trimesters of gestation. Therefore, the component was designated as Carcinoembryonic Antigen of the human digestive system and subsequently abbreviated as CEA. It became one of the significant entities to diagnose and monitor solid tumor and the response to therapy, especially in GIT cancer. Due to an immense importance of CEA in present day diagnostic and health services, this article reviews the important discovery of CEA by Dr. Phil Gold, its biology, function, chemistry and the clinical role of CEA in categories of cancer screening, diagnosis, prognostic indicator and as a monitoring tool for treatment.

Key words:
CEA, antigen, colorectal carcinoma, malignant

Short Title:
Structure, function and Significance of CEA

INTRODUCTION:
The CEA molecule is an onco-development human tumor marker1-3. It bears the cluster differentiation designation of CD66e, a subtype of CD66 group of CEA family. It has a molecular weight of 180 kDa 2,4. In a series of studies in early 1960s, a tumor component in colon cancer tissue was found that was not present in the corresponding normal tissues5. In subsequent years5, same antigen was also noted in all GIT tumors derived from endoderm. This antigen was also demonstrated in primary tumor of the pancreas and liver2. Further investigations, performed using human embryo and fetus derived tissues, revealed that gut-derived tissue in the 1st two Trimesters of gestation expressed the same antigenic moiety1,2,5,6. In comparison, tissues from 3rd Trimester did not exhibit the antigenic component. Therefore, the component was designated as Carcinoembryonic Antigen of the human digestive system and subsequently abbreviated as CEA5.

After nearly 40 years since the discovery of CEA in 1965-664,5,6, until now, this tumor marker became one of the significant entities to diagnose and monitor solid tumor and the response to therapy7-10, especially in GIT cancer. Due to an immense importance of CEA in present day diagnostic and health services, this article reviews the discovery, biology, function, chemistry and the clinical role of CEA in categories of cancer screening, diagnosis, prognostic indicator and as a monitoring tool for treatment. This review is also a tribute to the inspirational work of the eminent Scientist Prof. Dr. Phil Gold, whose discovery of CEA and the subsequent research and scholarly efforts made the diagnosis of several malignant conditions a lot easier.

DISCOVERY OF CEA:
In early 1960s, Phil Gold, the discoverer of CEA, and colleagues initiated to demonstrate tumor components in colon cancer tissue that was not found in the corresponding normal tissues5. The idea came from previous attempts and failures to detect components or functions unique to cancer cells and the description of acquired immunological tolerance2,10,11. Experiments were carried out in adult male rabbits, immunized with colon cancer tissue extracts as well as in neonatal rabbits tolerant to normal tissue extracts5. Subsequent use of tissue extracts (possessing antigenic characteristics), anti-sera and the indicative immunological techniques, in vivo, in a series of studies, result in providing evidence that a tumor component do exist, that cannot be found in normal tissue2,5,6. Later studies also demonstrated
the presence of the same cancer antigen in all endodermally derived GIT tumors and the primary tumors of pancreas and liver. During the course of investigations, the scientists have also noted an exceptional "embryologic combined" of the tumor antigenic activity. The cancer component in question appeared to be related to cancer that arises from tissue lining between the embryonic stomatoderm, above, to the proctoderm. Further investigation with human embryo and fetus derived tissue, proved that the gut-derived tissue in the 1st two trimesters of gestation expressed the particular antigenic component, whereas the comparable tissue in the 3rd trimester did not. Therefore, Dr. Phil Gold and colleagues named that component as carcino-embryonic antigen of the human digestive system, and subsequently abbreviated as CEA.

STRUCTURE AND GENETIC RELATIONSHIP:
Early investigations concerning the biology of CEA involved the purification and characterization of its molecule. Later studies revolve around the cloning of CEA gene and functional biology of the CEA molecule. The CEA molecule is an Oncodevelopment human tumor marker. It bears the cluster differentiation designation of CD66e. CEA was shown to be a membrane constituent in adenocarcinoma of human digestive system. Earlier experiments suggest that it is a surface glycoprotein that interacts with the microskeleton of the cell. It was also indicated that CEA might be released from the cell surface to the interstitial space and into the circulation of the tumor-bearing patients. This has also been shown that CEA belong to a group of molecules that are closely related to each other due to molecular homology, therefore, indicating the presence of a "CEA molecular family". Beside CEA, other member of this family includes, non-specific cross-reacting antigen (NSA), biliary glycoprotein (BGP-I-III), the meconium antigen (MA), pregnancy-specific b-glycoprotein (PSG) and the tumor antigen derivative TEX. In late 1980s, scientists have achieved the isolation of cDNA clones for CEA and related family members.

FUNCTIONAL ACTIVITIES OF CEA:
Studies carried out in recent past have revealed that CEA mediate Ca++ and temperature dependent cellular aggregation. It was also shown to induce the homotypic sorting of cells in heterogeneous population of aggregating cells. Furthermore, CEA influx was also noted in development of epithelial cell layers. CEA molecule interferes with normal adult single palisade layer of epithelial cells, in consequence, replaced with a multilayered cellular array. It is also suggested that CEA may act as an inhibitor of intercellular contract. Furthermore, relationship between intercellular free CEA concentration and the metastatic potential of CEA producing tumors has also been established. This has also been emphasized that CEA may bind to a specific receptor on Kupffer cells, causing them to produce cytokines that stimulates the growth of metastatic cells.

CHEMISTRY:
It is a glycoprotein and has a molecular mass of 180 kDa. It consist of a protein core that makeup about 45-46% of the molecule. The protein portion consist of single polypeptide chain, containing of 107 amino acids NH-terminal domain followed by three highly homologous domains of 178 amino acids each. C-terminal domain consists of 26 amino acids. Carbohydrate side-chains, which comprise of around 54-55% of the CEA molecule, bound to protein core via 28 potential Asn (Asparagine)-linked glycosylation sites. When visualized by cellular microscopy after appropriate shadow casting, the CEA molecule appears as a screw or cruller shaped structure with dimensions of approximately 9 x 40 nm.

CLINICAL VALUATION AND DIAGNOSTIC LEVELS OF CEA:
It was stated that the role of CEA became a consideration in late 1960s, with the development of an RIA method for circulating CEA. Since then, enormous wealth of literature has accumulated describing the significance of CEA in patient care. Commonly considered cut-off point for CEA is 2.5-5.0 ng/ml for distinguishing normal from abnormal level of serum or plasma. Survey conducted with vast population of healthy persons showed that 85% to 87% had serum CEA level less than 2.5 ng/ml, 95% to 98% less than 5.0 ng/ml and none had level greater than 10.0 ng/ml. Moreover, CEA concentration is often found raised in smokers than in non-smokers. Elaborated efforts have also been made in 1970s and 1980s to review the clinical usefulness of CEA through a larger forum of international scientists and researchers. Keeping in view the facts and findings, it is suggested that the current clinical
application of CEA may be divided into five major and two minor categories. Major categories are, detection/screening of cancer, diagnosis, prognosis, monitoring of treatment and therapy. Minor categories are special pathological techniques such as Immunohistochemistry/cytochemistry of CEA and localization of tumor with imaging/radiolabeled CEA antibody techniques. All categories are briefly discussed below to clear the viewpoint.

SIGNIFICANCE OF CEA IN DETECTION, SCREENING AND DIAGNOSIS OF VARIOUS CANCERS:

As Phil Gold, the discoverer of CEA stated in one of his reviews, that the role of CEA in clinical medicine first become a consideration with the development of a RIA method of circulating CEA in 1969. Since then, a large body literature has accumulated describing the value of serum CEA measurements in patients care.

It is argued that currently available CEA assays cannot be used for general screening tests for colorectal cancer, because of the possibility of getting false-negative results. The argument is supported by the studies that CEA level is less likely to be elevated in early stage of colorectal cancer (such as Dukes stages A and B) than advanced stage of CRC of Dukes stages C and D. Screening ability of CEA is also not viable for other cancers, such as that of breast, pancreas and lungs and other non-adenocarcinoma because of the lack of sensitivity.

Use of tumor marker as diagnostic tool is used as "case-finding" in determining whether disease is present in individual at high risk for, or suspected of, having the disease. The diagnostic utility of CEA is established in adenocarcinoma of colon, where 80% or more of patients showed increased level of circulating CEA. However, suggestions were made that CEA assay alone should not be used as the sole diagnostic test. As stated earlier, CEA level, greater than the cut-off value of 5.00 ng/ml but less than 10.00 ng/ml, may also detected in non-malignant conditions, such as liver diseases.

This has also been reported that there is a rise in both frequency of positive CEA assay and the plasma CEA level, with increasing tumor burden. Example is the incidence of positive CEA assay in patients with Dukes stages A (20%), to Dukes stages D (90%) colon cancer. Significant CEA elevation ranging from 18% to 79%, was demonstrated in patients with colon cancer of Dukes stage A, B1, B2, C1 and C2.

In advanced breast cancer, the correlation between CEA and occurrence of metastasis from the breast has been found to be varying with the site of metastasis. It was reported that patients with bone or visceral involvement have more frequent elevation than the patients with soft tissue metastases. Early diagnosis of breast or GIT cancer has recently shown advancement through the use of RT-PCR assay, detecting CEA expressing cancer cells. In diagnosis of lung cancer, CEA can also be a useful tumor marker, because its sensitivity in two thirds of non-small cells lung cancer (NSCLC) patients and one third of small-cell lung cancer. Furthermore, the detection of CEA levels in cerebrospinal fluids seems to be useful in diagnosing meningal carcinomatosis, while analysis of gastric juices for CEA in identifying high-risk patients of gastric carcinoma.

It is argued that serum CEA assay alone, have not been a useful marker in the screening and diagnosis of colon cancer. However, it plays an important role in the clinical management of patients with colorectal adenocarcinoma. In this respect, the application of monoclonal antibody (mAB) technique targeting CEA have shown promising results due to high sensitivity for diagnosing colon adenocarcinoma.

CEA SIGNIFICANCE AS A PROGNOSTIC MARKER AND MONITORING THE COURSE OF TREATMENTS.

Prognostic utility of CEA in patients with CRC and some other cancers have been thoroughly investigated. Pre-operative serum levels are found to be elevated in 40% to 70% patients with CRC. Pre-operative CEA levels are also found to correlate inversely with tumor grade and directly with pathological grade. Therefore, it is documented that CEA is raised in 95% of patients with well-differentiated tumors, where as only 30% in poorly differentiated adenocarcinoma. Several studies have demonstrated that a significant inverse relationship exist between pre-operative plasma CEA levels and patients' survival, suggesting poor prognosis. CEA is also an important prognostic marker of breast cancer. In most case studies, the pre-operative CEA levels have been found to correlate with poor prognosis. Moreover, pre and post-operative or treatment levels of CEA may also serve as a good prognostic maker in breast, stomach and lung cancers.

Currently, treatment monitoring is the most significant area of CEA assay. A postoperative raise in CEA level in patients undergone a treatment regime strongly suggest...
recurrence of tumor\textsuperscript{1,81,82}. At present, serial CEA-monitoring is considered the best noninvasive technique for detecting recurrent CRC\textsuperscript{68,83,84}. Numerous studies substantiated that intensive follow-up CEA assays facilitate the identification of treatable recurrence at an early stage\textsuperscript{85-88}. It is also suggested that when CEA increases more rapidly than an average 12.6\% per month, recurrence should be strongly suspected \textsuperscript{87,88}.

In addition to the agreement of usefulness of CEA assay in CRC treatment monitoring, it is also agreed that such approach is also valuable in regression or progression of metastasis in patients receiving chemotherapy/radiotherapy in breast, lung or meningeval carcinoma \textsuperscript{89-93}. It is also concluded that the present available data strongly suggest that both pre and post-operative level of CEA assay is useful in the management of patients diagnosed with cancer.

**ADVANCED TECHNIQUES, FACILITATED BY INVASIVE METHODS, FOR THE DETECTION OF CEA:**

Immuno-histochemical detection of CEA is widely used and there are a number of monoclonal antibodies (mAB) to CEA, which exhibit variable cross reactivity\textsuperscript{1,94,95}. By this technique CEA have been identified in cancers of colo-rectum, breast, lung, cervix uterine, gall bladder, stomach, pancreas, liver, prostate, urinary bladder and neuroendocrine tumors \textsuperscript{1,95-99}. Numerous attempts have been made to compare the incidence of immuno-histochemical staining for CEA with plasma levels, tumor differentiation, disease stage, histological type, cellular localization and clinical prognosis \textsuperscript{1,95,97,98}. In conclusion, the positive tissue staining is similar to the frequency of elevated serum CEA levels, especially in CRC\textsuperscript{99}.

Radio-immunolocalization (RIL) is another method, used currently for research and advanced diagnostic purpose\textsuperscript{1}. This procedure uses tumor-specific radio labeled ABs to differentiate between malignant and benign tissue in vivo. It is reported that the affinity-purified, iodine labeled poly-colonel AB produce better results than the other related Abs. Considerable advances have been made in the clinical utility of RIL, particularly in detection of CRC. A trail using a 99mTc-labeled anti-CEA AB for immunoscintigraphic visualization of CEA expressing tumor showed an 80\% sensitivity rate in the detection of CRC and its abdomen-pelvic metastasis and a 90\% sensitivity rate in the detection of recurrent of disease \textsuperscript{84,100}. Presently researchers and scientists are in process of developing better techniques to improve tumor imaging through better comprehension of pharmacokinetics of radio labeled AB and clear understanding of the immuno-biology of CEA producing tumors.

**AKNOWLEDGEMENTS:**
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Fig. 1-A: Description of CEA variants and subgroups, including the gene specifications. (From CEA homepage by Prof. Dr. Zimmermann-Tumor Immunology Lab-Munich, Germany).

CEACAM subgroup (1/2)

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Domain Organization

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Splice Variants

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Fig. 1-B: Description of CEA variants and subgroups, including the gene specifications. (From CEA homepage by Prof. Dr. Zimmermann-Tumor Immunology Lab-Munich, Germany).

CEACAM subgroup (2/2)

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Gene Splice Variants

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Figure 2. Schematic representation of the human carcinoembryonic antigen (CEA) gene and protein: [Ref: Horig H, Medina FA, Conkright W A, Kaufman HL. Carcino embryonic antigen. Expert Reviews in Molecular Medicine. Cambridge University Press. 2000]. (a) The CEA gene is encoded by a segment of DNA that is 3100 base pairs in length and is derived from eight exons (N domain, Al-A3, Bl-B3 and M domain). (b) The CEA protein product contains a leader sequence and three highly conserved repeat domains (1-3), each comprising 178 amino acids. Each of these three repeat domains can be further divided into two sub-domains (A and B), which share significant sequence homology. Each domain contains four cysteine residues at similar positions, which pair up to form A and B 'loops' stabilised by disulphide bridges between the cysteines. (a) The domains and sub-domains in the CEA gene correspond to the labelled domains of the mature protein shown in (b). The CEA protein consists of 668 amino acids, and has a configuration that is similar to that of other members of the immunoglobulin gene superfamily. The protein extends out from the cell membrane into the extracellular space, and is anchored through a hydrophobic C-terminal region (the M domain). Most of the final molecular weight of CEA is provided by N-linked glycosylation, which occurs at the sites indicated in (b).
Fig 3: Represents the amino acid sequence of CEA with 702 units.

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