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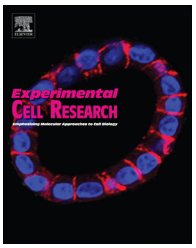

Research Article

Carcinoembryonic antigen promotes colorectal cancer progression by targeting adherens junction complexes

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ABSTRACT

Oncomarkers play important roles in the detection and management of human malignancies. Carcinoembryonic antigen (CEA, CEACAM5) and epithelial cadherin (E-cadherin) are considered as independent tumor markers in monitoring metastatic colorectal cancer. They are both expressed by cancer cells and can be detected in the blood serum. We investigated the effect of CEA production by MIP101 colorectal carcinoma cell lines on E-cadherin adherens junction (AJ) protein complexes. No direct interaction between E-cadherin and CEA was detected; however, the functional relationships between E-cadherin and its AJ partners: α-, β- and p120 catenins were impaired. We discovered a novel interaction between CEA and beta-catenin protein in the CEA producing cells. It is shown in the current study that CEA overexpression alters the splicing of *p*120 catenin and triggers the release of soluble E-cadherin. The influence of CEA production by colorectal cancer cells on the function of E-cadherin junction complexes may explain the link between the elevated levels of CEA and the increase in soluble E-cadherin during the progression of colorectal cancer.

Abbreviations: ACTN4, actinin-4 protein; AJs, adherens junctions; CEA, carcinoembryonic antigen; E-cadherin, epithelial cadherin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; hnRNPM, heterogeneous RNA-binding protein M; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse transcriptase-polymerase chain reaction

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Introduction

CEA (CEACAM5) is one of the most widely used tumor markers and has been associated with enhanced metastatic potential in colorectal cancers [1]. CEA is a large glycoprotein (~180 kD) and a member of a family of 29 related genes in the immunoglobulin-gene super family. One major function of CEA is to regulate intercellular adhesion. It is expressed on the luminal surface of the colonocytes and its expression pattern changes in the neoplastic cell so that it is also expressed on the basal and lateral membranes. CEA plays a critical role in establishing and maintaining tissue architecture and function in the colon [1]. CEA also appears to regulate a variety of cellular functions that include inhibition of cell differentiation, polarization, intercellular and matrix adhesions, signal transduction, cellular architecture, and anoikis (cell death due to the loss of cell-to-cell contacts) as well as cellular migration [1,2]. CEA has been identified as a selectin ligand in colon carcinoma cells that is important for cancer cell migration [3]. After separating from a primary tumor, metastasizing cells enter the circulation and interact with host cells before lodging in secondary organs. CEA and variant isoforms of surface glycoproteins CD44 cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow [3,4].

In preclinical models, it has been shown to have a causal effect of CEA production by cancer cells on liver metastasis. CEA injected into athymic nude mice prior to the intrasplenic injection of colorectal cancer cell lines with a low metastatic potential enhanced hepatic metastasis [5]. Anti-CEA antibodies against the NH2-terminal (MN-3) and A1B1 (MN-15) domains of CEA impeded metastasis; affected cell migration, invasion, and adhesion *in vitro*; and improved mouse survival *in vivo* [6]. In addition, weakly metastatic human colorectal carcinoma cells acquire a high metastatic potential when transfected with recombinant CEA [7,8]. To elucidate the role of CEA and CD44 in colorectal cancer metastasis the expression of these multifunctional molecules has been suppressed using siRNA in LS174T colon carcinoma cells. The ability of modified cells to metastasize was analyzed in 2 independent mouse models [9]. It has been shown that cell migration was decreased as a result of silencing CEA but was enhanced in CD44-knockdown cells. Collectively, the data indicate that CEA, but not CD44, is a viable target for therapeutics aimed at curbing colon carcinoma metastasis [9].

E-cadherin has been implicated in carcinogenesis as its expression is commonly down-regulated or lost in primary carcinomas [10] and E-cadherin is a tumor suppressor gene in colorectal carcinomas. We identified a novel CEA-binding protein – CEAR, also called heterogeneous RNA-binding protein M (hnRNPM) – in liver macrophages which is involved in the implantation and survival of tumor cells in the liver [11]. CEAR/hnRNPM belongs to a large family of heterogeneous nuclear RNA-binding proteins (hnRNPs A-U), also called "histones of RNA" [12]. The hnRNP family of proteins shares common structural domains and have central roles in regulating gene expression at both transcriptional and translational levels, alternative splicing, micro-RNA processing and stress response [13]. Individual hnRNPs are reported to act in tumor development and progression in a variety of cancers [14]. The exact role of hnRNPM in colorectal cancer progression is unknown. At present, a vast amount of data is available on molecular mechanisms of individual components of metastasis [15], but the data can be quite contradictory. In this context it is especially important to have an adequate biological model, which would make it possible to explore metastasis *in vitro* and *in vivo* as a complex physiological phenomenon. A very promising field is the comparison of genetically modified cell lines of the same origin with varying CEA production and metastatic potential. One such model is the colon cancer cell line collection based on the low-differentiated, poorly metastatic, non-CEA-producing MIP101 colorectal carcinoma cell line. Poorly and highly differentiated colorectal carcinoma cells differ in morphology, adhesion, CEA-production, motility and invasion. Poorly differentiated cells (MIP-101) are highly invasive, more adherent, scattered, elongated and motile than the moderate and well-differentiated colorectal carcinomas (CX-1, DLD-2) which produce high levels of CEA [16]. We used the poorly differentiated CEA negative human colorectal carcinoma line – MIP-101 and its CEA-producing clones (clones 6 and 8) developed by transfection with the full-length CEA cDNA [7]. In this study we discovered that CEA expression by colorectal cancer cells influences the function, and the interactions between the E-cadherin junction complex proteins may explain the link between the elevated levels of CEA and the increase in soluble E-cadherin during the progression of colorectal cancer.

Materials and methods

Cell lines

Cells were maintained at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium containing 10% fetal bovine serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 300 μg/ml glutamine. MIP 101 clone 6 and clone 8 cell lines produced by transfection of MIP-101with the full-length CEA cDNA [7,16] were selected on G 418. CX1, HT-29 and CaCO2 cells are all CEA-producing aggressive colorectal cancer cells.

Semi-quantitative RT-PCR of mRNA expression

The relative mRNA-expression levels of CEA, hnRNP M, and adherent junction proteins (E-cadherin, α-catenin, β-catenin) were determined by reverse transcriptase-polymerase chain reaction (RT-PCR). All primers were custom made by Integrated DNA Technology. Primers for CEA were forward (N-domain-F) 5′-caccactgccaagctcacta; reverse (CEA-A1R) 5′-ctgggttctgggtttcacat; β-actin, forward 5′-tgagcgcggctacagctt-3′ and reverse 5′-tccttaatgtcacggacgattt-3′; human hnRNPM forward, 5′-gagcggaagaccactgaaag-3′, and reverse 5′-agaatgtctgctcggaccac-3′. The human hnRNPM primers were designed to detect two isoforms that represent the wild type (full-length) and a splice variant with a 39 amino acid deletion with the expected PCR products of 321 and 204 base pairs. E-cadherin, α-catenin, and β-catenin primer sequences have been previously published [17]. RNA was extracted from colorectal carcinoma cells using RNAsol, according to the manufacturer's protocol (Ambion, Inc.). Superscript™ III First Strand Synthesis system for RT-PCR (Invitrogen) was used to generate the cDNAs. We diluted synthesized cDNAs in 50 μL of diethylpyrocarbonate-treated water and used 3 μL of each reaction in each 25-μL RT-PCR. DNA was amplified with the following parameters: 95 °C for 1 minute followed by 35 cycles of 95 °C for 30 s, 52–60 °C for 30 s, and 72 °C for 1 min. The gene expression was normalized with