An Evidence for Human Tumor Cell Lines Producing Different Carcinoembryonic and/or Its Related Antigens
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Carcinoembryonic antigen (CEA) is one of well established human tumor-associated antigens with a molecular weight of about 180 Kd-200 Kd consisting of 50-60% carbohydrate [14, 25]. In addition to CEA, several CEA cross-reacting antigens have been identified in normal tissues [6, 16, 19, 20, 23, 26]. It is still inconclusive at the present time whether these CEA and CEA-related antigens are cleavage products or different gene products. CEA itself isolated from human tumor tissues with perchloric acid [5] exhibits extensive heterogeneity in its physicochemical and immunological properties [7, 8, 24]. CEA has recently been shown to reveal uniform reactivities with monoclonal antibodies recognizing the peptide epitopes, whereas it showed heterogeneous reactivities with antibodies recognizing the carbohydrate epitopes [12, 18], suggesting that the antigenic heterogeneity of CEA may be mainly located at the carbohydrate moiety. Furthermore, 2 distinguishable CEA molecules have recently been isolated from the same human tumor tissues [9, 10]. Since CEA is usually isolated from human organs and/or tissues, it is not easy to study how many different CEA and/or CEA-related antigens are found in a single type of tumor cells. Therefore, attempts were made to study whether or not a human cell line composing of homogeneous cells produces heterogeneous CEA and/or CEA-related antigens.

Human tumor cell lines used in this study were MKN 28, MKN 45 and CCK-81. MKN 28 and MKN 45 are well and poorly differentiated adenocarcinoma cell lines derived from lymph node and liver metastases of stomach cancer, respectively [11,12]. CCK-81 is an adenocarcinoma cell line which is from the lymphnode metastasis of transverse colon cancer [13]. These cell lines were kindly provided by the Japanese Cancer Research Resources Bank.

Rabbit anti-CEA antibody was the product of Dakopatts, Denmark. Mouse monoclonal anti-CEA antibodies were purchased from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan and Immunobiology Laboratory (IBL), Takasaki, Japan. Monoclonal anti-CEA antibody prepared by IBL was an antibody against CEA produced by MKN 45.

Cell lines were cultured in RPMI 1640 medium supplemented with 9% heat-inactivated fetal calf serum (FCS, Gibco Laboratories, Grand Island, USA), 100 IU/ml, 100 µg/ml streptomycin and 4mM L-glutamine. Before labeling, cells (1×10⁶/ml) were incubated in the same medium for 6 hr at 37°C. Then, cells were transferred to leucine-free RPMI 1640 medium containing 20% dialyzed FCS and were incubated with 50 µCi/ml of ³H-leucine (Amersham International, Buckinghamshire, England) for further 16 hr at 37°C. After washing labeled cells twice with cold 0.15 M phosphate buffered saline, pH 7.2 (PBS), the labeled cells were solubilized on ice for 1 hr with 100 µl of 10 mM Tris-HCl buffer, pH 8.0, containing 0.14 M NaCl, 3 mM MgCl₂, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride, and with 0.5% Nonidet P-40 (NP-40) on ice for 10 min. After centrifugation at 4°C, the cell lysate was treated with normal rabbit or mouse serum-coupled Sepharose 4B by end-over-end rotation for 2 hr at 4°C. The treated cell lysate was similarly mixed with either polyclonal or monoclonal anti-CEA antibody. Then, an aliquot (150 µl) of 20% protein A-Sepharose 4B (Pharmacia, Sweden) was incubated with the mixture for 2hr at 4°C. The Sepharose beads were finally washed twice with 20 mM Tris-HCl buffer, pH 7.6, containing 0.1% bovine serum albumin (BSA), 0.15 M NaCl, 1 mM ethylenediaminetetraacetic acid, 0.5% NP-40, 1% Na-deoxycholate and 10% glycerol, and twice with the same buffer without BSA. The immunoprecipitates were analyzed by 7.5% slab polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) were run at
a constant voltage of 100 V according to the methods reported previously [17]. After electrophoresis, the gel was dried and exposed to an X-ray film for fluorography [3].

When CEA(s) in the supernatants of MKN 28, MKN 45 and CCK-81 was determined by enzyme-linked immunoassays using Dako polyclonal anti-CEA antibody, CEA's produced by MKN 28 and CCK-81 were found to be less than those produced by MKN 45 under the same culture conditions. After these three different cells were labeled with 3H-leucine and solubilized, immunoprecipitation was performed with three different anti-CEA antibodies as listed in Table 1. The results obtained were shown in Fig. 1 and summarized in Table 1. Immunologically specific bands detected in the lysates of MKN 28 and CCK-81 with anti-CEA antibodies were found to be weaker than those in the lysate of MKN 45 (Fig. 1), being consistent with less CEA production of MKN 28 and CCK-81 as mentioned above. As shown in Fig. 1, MKN 45 was found to mainly produce homogeneous antigen(s) (180 Kd) with different anti-CEA antibodies. On the other hand, MKN 28 and CCK-81 were found to produce different CEA and/or CEA-related antigens which were not easily all found in Fig. 1. At least three bands with 180, 100 and 50 Kd were immunologically detected in MKN 28, whereas four bands with 180, 160, 100 and 30 Kd in CCK-81. According to previous studies on molecular sizes of CEA and CEA-related antigens [1, 4, 6, 15, 22], antigens with 180, 160, 100

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a) No. 1, rabbit polyclonal anti-CEA antibody from Dako; No. 2, mouse monoclonal anti-CEA antibody from Mochida Co., No. 3, mouse monoclonal anti-CEA antibody from IBL.

b) Molecular weights were determined by SDS-PAGE and expressed in kilodaltons.

![Fig. 1. Polycrylamide slab gel (7.5%) electrophoresis of CEA and/or its related antigens produced by MKN 28, MKN 45 and CCK-81 in the presence of sodium dodecyl sulfate under reducing conditions. A, mouse monoclonal anti-CEA from IBL; B, mouse monoclonal anti-CEA from Mochida; C, rabbit polyclonal anti-CEA from Dako.](image-url)
and 50, and 30 Kd may correspond to CEA, nonspecific cross-reacting antigen (NCA)-2, NCA and normal fetal antigen (NFA), respectively. The reason why such multiple bands were immunologically detected in the lysates of MKN 28 and CCK-81 may not be well accounted for but may be accounted for by at least the following possibilities. One is that both highly and poorly glycosylated antigens might be detected in cell lysates since NP-40 allows the solubilization of a wide range of proteins and represents a much milder procedure for extraction as compared to perchloric acid [1]. The second one is that smaller-sized CEA antigens might be produced since monoclonal anti-CEA antibody prepared by IBL was unreactive with NCA-2, NCA and NFA according to the product information on the specificity. Although multiple CEA-related antigens have been detected in human normal lung and colonic carcinomas [2, 9, 10], it is the first report that a singly type of human tumor cells (MKN 28 and CCK-81) also produced different CEA and/or CEA-related antigens. At the present time, however, it is not known whether these antigens are merely degradation products or different gene products. In either case, gene cloning and analysis of genomic DNA of these cell lines will be helpful to determine the relationship among these antigens. 

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REFERENCES


要　約

ヒト株化細胞の産生する免疫学的に不均一な癌胚性抗原（CEA）については（短報）：杉井俊二（北里大学医学部部血清学教室）——ヒト株化細胞の産生する CEA 抗原（分子）を免疫学的に検討した結果、CEA 分子（180 Kd）のみを産生する細胞株と、複数の CEA あるいは CEA 関連抗原（180, 160, 100, 30 Kd）を同時に産生する細胞株とに分類できる可能性が示唆された。