Subcellular Distribution, Synthesis, and Release of Carcinoembryonic Antigen in Cultured Human Colon Adenocarcinoma Cell Lines

Zuo R. Shi, Dean Tsao, and Young S. Kim

Gastrointestinal Research Laboratory, Veterans Administration Medical Center, San Francisco 94121, and the Department of Medicine, School of Medicine, University of California, San Francisco 94143, California

ABSTRACT

The content of carcinoembryonic antigen (CEA) and its subcellular distribution were studied in nine human colon carcinoma cell lines. A great variation in CEA content was found among different colon cancer cell lines. Well-differentiated colon cancer cell lines (LS174T and SKCO-1) contained the highest CEA activity which was 35 to 60 times greater than that of less well-differentiated cells (SW620, SW480, and HRT18). More than 80% of the CEA was associated with the cell membrane in all nine cell lines. With increasing cell density, the CEA content per cell was found to increase in SW1116, HCT8, and HCT48 cells, while no change was observed in SW620, HRT18, and HT29 cells. In SW480, LS174T, and SKCO-1 cells, CEA content actually decreased with increasing cell density. Investigation of the synthesis of CEA in cells and its release into the medium over an 8-day period showed that the rate of CEA synthesis at maximum cell density for LS174T and SKCO-1 cells decreased to 15 and 50% of that at low cell density, respectively. In contrast, the rate of CEA release into medium by these two cell lines was higher at maximum than at low cell density. For HCT48 cells, the increased rate of CEA synthesis with increasing cell density markedly elevated cellular CEA levels. These observations were confirmed by studying the rate of incorporation of N-acetyl[3H]glucosamine into cellular CEA in LS174T and HCT48 cell lines. The rate of incorporation of radioactivity into CEA was greater during the exponential phase of growth than during the stationary phase for LS174T, while the opposite was observed with HCT48 cells.

This study indicated that there is a great variation in CEA content among different human colon cancer cell lines and that it is associated predominantly with the membrane fraction. The rate of synthesis and release of CEA also varied among different cell lines. The growth phase had a varied effect on the CEA content, the rate of synthesis, and the release of CEA in these human colon cancer cell lines.

INTRODUCTION

CEA, a glycoprotein, is a tumor-associated oncofetal antigen which has been extensively investigated over the last 2 decades. Although originally thought to be specific for human colon adenocarcinomas, it is now well known that it shows immunological cross-reactivity with glycoproteins extracted from other carcinomas (3) and normal colon tissues (40). Elevated levels of serum CEA are found not only in patients with large-bowel cancer but also in association with other tumors and a variety of benign conditions (1, 5, 16, 24), including pregnancy and tobacco smoking (28). The large variations of the serum CEA level in colon cancer, both between different patients and at different stages of the disease in the same patient, have been attributed to the size of the tumor, its state of differentiation, the degree of invasiveness, and the extent of metastatic spread (30, 39). However, precise relationships between serum CEA levels and these factors have not been established. Leibovitz et al. (19) reported that the CEA content of the culture medium of 11 colon cancer cell lines varied considerably, but neither the CEA content of these cells nor its turnover rate was studied. Recently, Dreyfino and his coworkers (9, 10) measured the content and the turnover rate of CEA in one colon cancer cell line, LoVo, and reported that these parameters were affected by growth phase of these cells.

In this study, we investigated the cellular content, subcellular distribution, rate of synthesis, and release of CEA into culture medium at different growth phases of 9 human colon cancer cell lines.

MATERIALS AND METHODS

Materials. The Roche CEA assay kit was purchased from Hoffman-La Roche Inc. (Nutley, N. J.); affinity-purified fluorescein isothiocyanate-labeled goat anti-rabbit IgG (heavy and light) and Zysorbin (fixed and killed Staphylococcus aureus) were obtained from Zymed Laboratories (San Francisco, Calif.); N-acetyl[3H]glucosamine (specific activity, 3.0 Ci/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.); Autofluor was from Particle Data Laboratories (Elmhurst, Ill.); and anti-CEA antiserum was prepared in rabbit with CEA purified from a metastatic liver tumor of colon adenocarcinoma. The antiserum was absorbed with glutaraldehyde-insolubilized normal human plasma proteins, immobilized normal tissue extracts (including kidney, colon, liver, and lung), and human erythrocytes. Other chemicals were all of the highest quality available commercially.

Cell Culture. The human colon cancer cell lines HCT8, HCT48, and HRT18 were generously given to us by Dr. Richard M. Schultz of the National Cancer Institute (Bethesda, Md.) (31, 33). Human colon cancer cell lines HT29 and SKCO-1 were kindly provided by Dr. Jorgen Fogh of the Sloan-Kettering Institute for Cancer Research (Rye, N. Y.) (11). Human colon cancer cell lines SW480, SW620, and SW1116 were developed at the Sloan-Kettering Institute for Cancer Research (Rye, N. Y.) (11). Human colon cancer cell lines SW480, SW620, and SW1116 were developed at the Scott & White Clinic (Temple, Texas) and were generously provided by Col. Albert Leibovitz (19). LS174T colon cancer cell line was a gift from Dr. Barry D. Kahan of the University of Texas (Houston, Texas) (32). All of these cell lines were maintained as monolayers cultured in 75-cm2 tissue flasks or 60-mm Petri culture dishes in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). Media were changed at 4-day intervals after experimental samples had been taken. All cultures were free of Mycoplasma when these experiments were performed.

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Preparation of Cell Homogenate and Membrane and Cytoplasmic Fraction. For the determination of the subcellular distribution of CEA, cells cultured in 75-sq cm tissue culture flasks were harvested, and the growth media were collected for CEA assay. The cells were scraped after washing 4 times and suspended in isotonic PBS at pH 7.4. A cell homogenate was prepared by sonicating the cell suspension twice for 15 sec on ice. The homogenate was centrifuged at 100,000 × g for 1 hr to obtain a membrane pellet and a cytoplasmic fraction. Both the cell homogenate and cell membrane fraction were solubilized in 1% NP-40 with sonication and used for CEA assay.

CEA Assay. CEA levels were determined by a radioimmunoassay method as suggested by Hoffman-La Roche Inc. Determination of the CEA in culture media from cells which produced small amounts of CEA (HRT18, HT29, SW620, and SW480) was prevented by interference of the culture medium with the assay method when more than 50 μl medium had to be used. To overcome this difficulty, an indirect method with perchloric acid extraction, as suggested by Hoffman-La Roche Inc., for the measurement of low levels of serum CEA was used. However, there was some discrepancy between the CEA values obtained by the direct and indirect methods. Values were consistently lower when determined by the indirect method. The CEA values measured in this way were converted by a factor derived from measuring the CEA concentration in a medium sample containing a known amount of CEA processed under identical conditions. Preliminary studies showed that NP-40 did not interfere with the assay at concentrations of up to 0.1% (34). In all cases, duplicate dishes were harvested with trypsin for cell counting, and CEA content was expressed as ng/10^6 cells.

Incorporation of N-Acetyl[3H]glucosamine into CEA. HCT48 and LS174T cell lines were used for studying the rate of incorporation of a radioactive sugar precursor, N-acetyl[3H]glucosamine, into CEA. Each line was seeded in 60-mm Petri dishes containing 8 ml of medium. After each 24-hr period of incubation, a duplicate set of dishes was harvested, collecting the media and cells separately. CEA in both medium and cell homogenate was assayed by the direct radioimmunoassay method. Separate dishes under identical conditions were treated with trypsin to detach cells for counting. The number of cells was determined using a Coulter Counter. The net CEA synthesized or released by cells per day was calculated by subtracting from the current value the total cellular CEA content or total CEA in the medium of the previous day and dividing by the total number of cells counted that day.

Synthesis and Release of CEA. Human colon cancer cells chosen for this study were seeded as an aliquot of 3 × 10^6 cells into 60-mm Petri culture dishes containing 8 ml of medium. After each 24-hr period of incubation, a duplicate set of dishes was harvested, collecting the media and cells separately. CEA in both medium and cell homogenate was assayed by the direct radioimmunoassay method. Separate dishes under identical conditions were treated with trypsin to detach cells for counting. The number of cells was determined using a Coulter Counter. The net CEA synthesized or released by cells per day was calculated by subtracting from the current value the total cellular CEA content or total CEA in the medium of the previous day and dividing by the total number of cells counted that day.

Immunoprecipitation of Labeled Cellular CEA. Detergent-solubilized and dialyzed N-acetyl[3H]glucosamine-labeled cell homogenate containing 40 ng of CEA was incubated with 10 μl of rabbit anti-CEA antiserum overnight at 4°C. The mixture was then incubated with 50 μl of 10% protein A-S. aureus for 30 min at room temperature. The mixture was centrifuged at 2500 × g for 10 min with a Beckman table top centrifuge. The bacterial pellet was washed twice with PBS containing 0.1% NP-40 and then twice with PBS. The washed pellet was resuspended in 0.5 ml of 0.1 N NaOH and counted in 5 ml of scintillation fluid using a Beckman Model LS8000 scintillation counter. To correct for the nonspecific precipitation of 3H-labeled compounds other than CEA, normal rabbit serum was used in place of rabbit anti-CEA antiserum for immunoprecipitation. These values were subtracted from the test sample counts.

RESULTS

Subcellular Distribution of CEA. The CEA content varied widely among the human colon tumor cell lines examined (Table 1). LS174T and SKCO-1 had the highest cellular CEA contents while HRT18, SW480, and SW620 had very low contents. More than 80% of the CEA was membrane associated in all the cell lines studied (Table 2). The amount of CEA in the medium expressed as a percentage of the total cellular CEA showed that HCT48 had the highest (33%) and HT29 had the lowest (9.3%) amounts with the other cell lines falling between these values.

Specific Cellular CEA Activity at Different Cell Densities. Human colon cancer cells were harvested at different stages from sparse (30%) to maximum (100%) cell density, homogenized by sonication, and solubilized in NP-40 for CEA determination. The degree of cell density was estimated by measuring the number of cells per unit area of the culture dish using an inverted microscope. Separate dishes under identical conditions were used for counting the cells after detaching them with trypsin. SKCO-1 showed a 2-fold and LS174T showed a 1.5-fold decrease of cellular CEA content once cells reached maximum density (Chart 1). The CEA contents of HCT8 and SW1116 increased 1.7-fold as cell density increased from 30 to 70%, staying at this level with higher degrees of cell density. By far the largest change in CEA content with increasing cell density occurred in HCT48, which showed a 20-fold increase at 100%, compared to 30% of maximum density. In none of the cell lines was the content of CEA or the cell number influenced by the refeeding of the cells.

Synthesis and Release of CEA. The synthesis and release of CEA were studied in SKCO-1, LS174T, and HCT48 from the exponential phase (2 to 4 days) to the stationary phase (7 to 8 days) of cell growth (Chart 2). It is interesting that, in both LS174T and SKCO-1, CEA production fell consistently with increasing cell density. The fall was from 288.6 ng/10^6 cells/day at Day 2 to 451. ng/10^6 cells/day at Day 8 for LS174T and from 115.2 ng/10^6 cells/day at Day 2 to 45.4 ng/10^6 cells/day at Day 8 for SKCO-1. The opposite was found for HCT48. Production rose from 12.1 ng on Day 2 to 109.1 on Day 8. In all the cell lines, the CEA release rose with increasing cell density (Chart 2B). However, when the CEA content in the medium was expressed as a percentage of the total cell-bound and medium CEA, there was a 2-fold decrease in CEA release from Day 3 to Day 8 for HCT48.

### Table 1

<table>
<thead>
<tr>
<th>Cancer cell lines</th>
<th>CEA activity (ng/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA activity in colon cancer cell lines</td>
<td>Homogenate</td>
</tr>
<tr>
<td>HCT 8</td>
<td>120.0 ± 92.0^c</td>
</tr>
<tr>
<td>HCT 48</td>
<td>45.2 ± 6.5</td>
</tr>
<tr>
<td>HRT 18</td>
<td>13.3 ± 1.7</td>
</tr>
<tr>
<td>HT 29</td>
<td>56.0 ± 16.9</td>
</tr>
<tr>
<td>LS 174T</td>
<td>599.4 ± 104.4</td>
</tr>
<tr>
<td>SKCO-1</td>
<td>355.0 ± 64.7</td>
</tr>
<tr>
<td>SW 480</td>
<td>16.0 ± 1.1</td>
</tr>
<tr>
<td>SW 620</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>SW 1116</td>
<td>33.4 ± 7.7</td>
</tr>
</tbody>
</table>

* Accumulated CEA content in the growth medium.
* Mean ± S.D. of 4 experiments; cells were harvested at maximum density.
CEA in Human Colon Cancer Cell Lines

Table 2

Relative distribution of CEA in subcellular fractions of colon cancer cell lines

<table>
<thead>
<tr>
<th>Cancer cell lines</th>
<th>Total no. of cells (x 10^7)</th>
<th>Total CEA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogenate</td>
<td>Membrane pellet</td>
</tr>
<tr>
<td>HCT 8</td>
<td>3.2 ± 0.6a</td>
<td>3,780 ± 719.5</td>
</tr>
<tr>
<td>HCT 48</td>
<td>2.4 ± 0.3</td>
<td>1,074 ± 333.7</td>
</tr>
<tr>
<td>HRT 18</td>
<td>3.7 ± 0.7</td>
<td>492.2 ± 77.2</td>
</tr>
<tr>
<td>HT 29</td>
<td>2.6 ± 0.3</td>
<td>1,433 ± 245.1</td>
</tr>
<tr>
<td>LS174T</td>
<td>2.3 ± 0.4</td>
<td>13,800 ± 3,309.5</td>
</tr>
<tr>
<td>SKCO-1</td>
<td>1.1 ± 0.2</td>
<td>3,981 ± 985.7</td>
</tr>
<tr>
<td>SW480</td>
<td>3.1 ± 0.5</td>
<td>492.3 ± 22.7</td>
</tr>
<tr>
<td>SW620</td>
<td>4.7 ± 0.4</td>
<td>486.9 ± 65.1</td>
</tr>
<tr>
<td>SW1116</td>
<td>1.5 ± 0.3</td>
<td>503.5 ± 84.9</td>
</tr>
</tbody>
</table>

Mean ± S.D. of 4 experiments studied; cells were harvested at maximum density.

Numbers in parentheses, mean of percentage of total CEA of homogenate.

LS174T and SKCO-1, on the other hand, showed increased secretion of CEA at higher cell density (Chart 2C).

CEA synthesis during both exponential and stationary phases for LS174T and HCT48 was further studied by using CEA labeled with N-acetyl[3H]glucosamine to indicate the rate of CEA synthesis. LS174T cells incorporated the labeled sugar into CEA much more rapidly during the exponential than the stationary phase as shown in Table 3. In HCT48, the opposite applied, and CEA synthesis was more rapid during the stationary phase.

DISCUSSION

Over the last decade, many human colon carcinoma cell lines have been established in different research laboratories, and the CEA contents of some of these cell lines have been determined (3, 4, 7, 9, 15, 20, 22, 23, 25, 30, 33, 36–38). Although CEA content clearly varies between different cell lines, direct comparisons are not valid, because the conditions and the methods used to assay CEA also varied among different laboratories. We have assayed CEA levels in 9 different human colon carcinoma cell lines in culture using Hansen's Z-gel radioimmunoassay method. To provide the most favorable conditions for the assay of membrane-bound CEA, the cell membrane was solubilized in the nonionic detergent, NP-40, to release the integral membrane CEA. A great variation in CEA content was found among the 9 different human colon cancer cell lines, ranging from 10.3 to 599.4 ng/million cells (Table 1). Cells with high CEA levels (LS174T and SKCO-1) contain 35 to 60 times more CEA than do cells with low levels (SW620, SW480, and HRT18). The
degree of differentiation of colon adenocarcinoma cells is one possible explanation for these differences in CEA content. Other workers (2, 6, 13) have shown that the CEA content is higher in well-differentiated colon cancer cells than in the less well differentiated ones. Histological examination of tumors produced in nude mice by the cell lines under study showed that both LS174T and SKCO-1 lines consisted of well-differentiated adenocarcinomatous cells, while the other lines were poorly differentiated.

Evidence that CEA is an integral membrane glycoprotein of the cultured human colon cancer cell line SKCO-1 has been reported by our laboratory (34). The subcellular distribution of CEA in 8 other human colon cancer cell lines determined in the present study confirms this; more than 80% of cell-associated CEA was found with the cell membrane fraction in all 9 cell lines studied (Table 2). The remaining 10 to 20% existed in a soluble form in the cytosol. The CEA concentration was also measured in the culture media in which the colon cancer cells had been grown. In general, a greater amount of CEA was found in the culture media of cells containing higher cellular CEA. With the exception of HCT48 which had released about 33%, most cells had released about 15% of their cellular CEA into the surrounding medium at maximum cell density (Table 2).

Drewinko and Yang (10) reported that the CEA per cell of a human colon cancer cell line, LoVo, increased once the cell reached the stationary phase, but this was not a consistent finding in our study. The cellular CEA content did increase in the cell lines SW1116, HCT8, and HCT48 after the stationary phase of growth had been reached (Chart 1). HCT48 cells showed a 20-fold increase in CEA content over the level measured during the exponential phase of growth. SW620, HRT18, and HT29 showed no growth-dependent change, while SW480, LS174T, and SKCO-1 showed a decrease in the cellular CEA content at the stationary phase of growth (Chart 1). These results were not due to changes in cell size during growth, since the number of cells per mg of protein and the microscopic appearance of the cells did not vary with alterations in the cell density.

To study these findings in more detail, the net intracellular synthesis of CEA and its net release into the medium were studied over an 8-day period in LS174T, SKCO-1, and HCT48 (Chart 2). These 3 cell lines were chosen because of their high CEA content, which facilitated the determination of CEA levels in the media. By Day 8, CEA synthesis per day per million cells had decreased to 15 and 50% of the Day 2 values for LS174T and SKCO-1 cells, respectively. In contrast, both cells showed an increase in the release of CEA at the high cell density (stationary phase) by a factor of 5 for LS174T and 2 for SKCO-1 (Chart 2). The increased rate of CEA release into the medium at high cell density could in part explain the lower cellular CEA content in SKCO-1 and LS174T cells. In HCT48 cells, CEA synthesis accelerated right up to Day 8 (Chart 2). Coupled with a decrease in the proportion of CEA which was released into the medium, this resulted in very high cellular CEA levels when the cells were in the stationary phase. Alterations in the rate of CEA synthesis with changing cell density were confirmed by studies of the rate at which N-acetyl[3H]glucosamine was incorporated into cellular CEA. This was faster during the exponential than the stationary phase for LS174T. The opposite was found for HCT48 (Table 3).

It is noteworthy that the 2 cell lines (LS174T, SKCO-1) in which a decrease in cellular CEA content with increasing cell density is seen are also the cell lines which produce well-differentiated tumors in nude mice and which have a high CEA content. This shows that there is a strong correlation between the control of CEA metabolism and tumor morphology. The marked increase in cellular CEA content with increasing cell density which is found in HCT48 cells suggests that the control of CEA metabolism in human colon adenocarcinoma cells is subject to stimulatory as well as inhibitory influences.

The immunohistochemical localization of CEA in benign and malignant colorectal tissues has been well studied (2, 6, 13, 26, 27, 29). CEA staining is frequently positive in colon adenocarcinomatous tissue (2, 6). In general, the CEA content correlates with the degree of differentiation, and the most intense staining has been found in more differentiated cancer cells (2, 6, 13). CEA staining in well-differentiated adenocarcinomas was confined to the surface lining of the glands and the apical cytoplasm, but in poorly differentiated tumors, signet-ring carcinomas, and invasive cancers, both membrane and cytoplasmic staining have been demonstrated (2, 6, 13, 27). However, relatively few studies have been performed on cultured human colon cancer cell lines (10). CEA as a colon cancer marker has been studied widely in clinical investigations since Gold and Freedman (12) reported the presence of CEA in human colon adenocarcinomatous tissue 16
years ago. At present, the clinical usefulness of CEA is limited. This may be due in part to the varying cellular content of CEA or to its physicochemical and immunological heterogeneity (8, 14, 17, 28, 33, 35, 36). Our data demonstrated great differences in CEA content as well as CEA synthesis and release in different human colon cancer cell lines. These parameters are variedly affected by the growth phase of cells. Further elucidation of the factors and mechanisms controlling the synthesis, release, and immunological heterogeneity of this important oncotelant antigen using both in vitro and in vivo studies is necessary.

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