

PDF issue: 2025-06-06

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(Citation) Bulletin of allied medical sciences Kobe : BAMS (Kobe),13:163-169

(Issue Date) 1997-12-26

(Resource Type) departmental bulletin paper

(Version) Version of Record

(URL) https://hdl.handle.net/20.500.14094/00188184



# Mutual Relations of Serum Carcinoembryonic Antigen (CEA) Levels to Tumor Volume and Proliferative Activity of Tumor Cells: An Experimental Study

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Carcinoembryonic antigen (CEA) producing tumor, MKN45, cells were transplanted into 15 nude mice, and the mutual relations of serum CEA levels to tumor volume and proliferative activity of tumor cells were analyzed. The proliferative activity was evaluated by proliferating cell nuclear antigen (PCNA) labeling index (LI, %). Respective 2 or 3 mice were sacrificed 1, 2, 3, 4, 5, 6 and 7 weeks after transplantation under etherization. Transplanted tumors grew semi-logarithmically during the experimental period. Correlations represented by the formulas, Y (CEA, ng/ml) = 0.002X (tumor volume, mm<sup>3</sup>) + 1.515 (r = 0.745, P < 0.01) and Y (log CEA, ng/ml) = 0.026X (PCNA LI) - 0.26 (r = 0.543, P < 0.08), were found among serum CEA levels, tumor volume and PCNA LI. A formula, Y (log tumor volume, mm<sup>3</sup>) = 0.031X (PCNA LI) + 2.052 (r = 0.582, P < 0.06), was found between tumor volume and PCNA LI. The results suggest that serum CEA levels, tumor volume and proliferative activity of tumor cells are mutually correlated in the genetically identical tumors and that the biological malignancy of tumor increases in proportion as tumor grows.

#### Key Words

Nude mouse, Carcinoembryonic antigen (CEA), Proliferating cell nuclear antigen (PCNA), Tumor volume.

## INTRODUCTION

Carcinoembryonic antigen (CEA) is widely used as one of the indispensable tools for the management of various cancer patients, because the antigen has been found to be useful as a monitor for detection, staging, identifying recurrence, determining the response to therapy and estimating the

survival or prognosis. The elevation mechanism in the peripheral venous blood has been gradually clarified by the precise clinical and experimental studies.<sup>1-14)</sup> Recently, we have reported that the positive relation of serum CEA levels to the proliferative activity of cancer cells or lesions are found in the human gastrointestinal cancer patients.<sup>14-18)</sup> However, the gastrointestinal cancer patients and/or lesions consisting of CEA producing ability, tumor volume and proliferating activity are different in each of the clinical cases. Mutual relations of CEA producing ability to tumor volume and proliferative activity of tumor cells have not yet been examined in the animal models. Therefore, it is not proved whether

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the positive relation of serum CEA levels to proliferative activity in cancer lesions are true, even though the relation is theoretically thought to be true.

In the present study, in order to analyze and prove the aforementioned phenomena, the mutual relations of serum CEA levels to tumor progression and proliferative activity of the tumor cells were examined using an animal model transplanted a CEA producing tumor cell line with a genetically same biological property.

# MATERIALS AND METHODS

1. Gastric cancer cell line

Human gastric cancer cell line with CEA producing ability, MKN<sup>45,19</sup>) supplied by the Japanese Cancer Re-Resources Bank (Tokvo. search Japan) was used in this study. Cells in **RPMI1640** were propagated medium (Nihonseiyaku. Inc. Tokvo. Japan) supplemented with 10% heatinactivated fetal bovine serum and maintained at 37 °C in a humidified atmosphere consisted of 5% CO2 and Single-cell suspension was 95% air. obtained by trypsinization.

2. Xenotransplantation of MKN45 cells

Five-weeks-old female athymic nude mice (BALB/cAJcl, nu/nu) weighing approximately 20g were purchased from Clea Japan, Inc. (Osaka, Japan), and employed for the experiments. They were maintained under the pathogen-free conditions on a standard diet and water throughout the experiments. Xenotransplantation was initiated by the subcutaneous injection of 106 cancer cells into the right flank of the nude mouse. Five weeks later, the subcutaneous tumor was resected from the flank under etherization, and then 3 mm pieces of the transplanted tumor were subcutaneously by 14G trocar into the right flanks of 15 nude mice respectively. Respective 2 mice were sacrificed at intervals of 1 week up to 7 weeks after transplantation but 3 mice were sacrificed only six weeks after transplantation under etheriza-The whole blood was collected tion. from the heart by a syringe (1 ml) with 25 G needle. Immediately after the measurement of length and width of the resected tumors, they were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Tumor volume (V) was calculated by the formula according to Tibbetts and co-worker,<sup>20)</sup>  $V = (a \times b^2) / 2$ , where "a" and "b" are the measurements (mm) of "length" and "width" respectively.

3. Determination of serum CEA

Serum CEA levels were determined by two-site immunoradiometric assay, CEA-Kit "Daiichi II" (Daiichi Radioisotope Institute, Co., Ltd., Tokyo). CEA values under 0.5 ng/ml could not be correctly determined, because of the limit in this kit.

4. Immunohistochemical analysis of PCNA

The proliferative activity of the tumor cells was immunohistochemically evaluated by proliferating cell nuclear antigen (PCNA) labeling index (LI), as already reported by us.<sup>15-16)</sup> In brief, 4  $\mu$ m sections were made from paraffin embedded specimens. The paraffin embedded sections were dewaxed and dehydrated, and then en-



Figure 1. Growth of tumors. Transplanted tumors grow semi-logarithmically.

blocked dogenous peroxidase was with 3% hydrogen peroxide. Nonspecific bindings were blocked by preincubation with normal bovine serum. PCNA was stained with monoclonal antibody PC10 (Nichirei, Tokyo) overnight at 4°C using modified avidin biotin method. The specimens were counter-stained with hematoxy-A minimum of 1000 cancer cells lin. were microscopically examined in each of the invasive parts into the muscle layers of the lesions. PCNA LI was expressed as the percentage (%) of PCNA positive tumor cell nuclei to all of the examined nuclei.

5. Statistical analysis of regression scattergrams

The regression scattergrams among CEA levels, tumor volume and PCNA LI were analyzed using a personal computer software ("Stat View SE + Graphics", Abacus Concepts Inc., Berkeley, CA). The relationships between tumor volume and growth after transplantation, between CEA levels and PCNA LI and between tumor

volume and PCNA LI were analyzed after the logarithmical exchange of the tumor volume and CEA values, because their relationships showed concave or convex curves and the statistical analyses were very difficult even aforementioned bv the software. Growth curve of the transplanted tumors was also examined using the same computer software. P values less than 0.05 were taken to be significant, and the values less than 0.1were estimated to have a tendency to be significant.

#### RESULTS

#### 1. Growth of transplanted tumors

All of the transplanted tumors grew almost semi-logarithmically after transplantation. A formula represented by Y (log tumor volume, mm<sup>3</sup>) = 0.252X (days after transplantation) + 1.804 (r = 0.986, P < 0.01) was found, as shown in Figure 1 (Fig. 1). Tumor doubling time of the transplanted tumors was 8.362 days.

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Figure 2. Relationship between serum CEA levels and tumor volume.

2. Relationship between CEA levels and tumor volume

The whole blood could not be collected in 2 mice sacrificed 1 and 3 weeks after transplantation. Serum CEA level in 1 mouse sacrificed 1 week after transplantation was not determined, because of the level under the determinative limit of the diagnostic kit. Thus, these mice were excluded following analyses of the data.

A significant correlation represented by the formula, Y (CEA, ng/ ml) = 0.002X (tumor volume, mm<sup>3</sup>) + 1.515 (r = 0.754, P < 0.01), was found between them (Fig. 2).

3. Relations of CEA levels to PCNA LI and of tumor volume to PCNA LI

As shown in Figure 3, formulas represented by Y (log CEA, ng/ml) = 0.026X (PCNA LI) - 0.26 (r = 0.543, P < 0.08) and Y (log tumor volume, mm<sup>3</sup>) = 0.031X (PCNA LI) + 2.052 (r = 0.582, P < 0.06) were found in the relationships between tumor volume and PCNA LI and between tumor volume and PCNA LI (Fig. 3).

# DISCUSSION

Close correlations between serum CEA levels and tumor volume or depth of cancer invasion have shown by many investigators.<sup>1-14)</sup> Significant correlations between serum CEA levels and proliferation activity of tumor cells represented by PCNA LI and/or between the proliferative activity and tumor progression have been reported also by some investigators.<sup>14-18)</sup> However, relationships among the CEA levels, proliferative activity and stage of tumor growth have not yet been simultaneously examined in human malignant tumors and also in animal models transplanted CEA producing tumor cell lines. Thus, it remains unclear how serum CEA levels and proliferation activity of tumor cells change according to the stage of tumor growth. In the present study, in order to analyze and prove the



Figure 3. Relationships between serum CEA levels and PCNA LI (left) and between tumor volume and PCNA LI (right).

aforementioned unclear phenomena, the mutual relations of serum CEA levels to tumor progression and proliferative activity of the tumor cells were examined using an animal model transplanted a CEA producing tumor cell line with a genetically same biological property.

The result obtained in this study clearly demonstrates that serum CEA levels increase in parallel with tumor volume, as already suggested in human gastrointestinal cancer by many investigators.<sup>1-14)</sup> Furthermore. serum CEA levels and tumor volume had a tendency to increase semilogarithmically in parallel with PCNA LI of the invasive parts of tumors. This result suggests that proliferative activity in the tumor lesions may change due to the stage of tumor Since it has also been congrowth. firmed that PCNA LI is one of the indicators of the malignancy of gastrointestinal cancer,<sup>15,16,21-22)</sup> these results and reported facts strongly suggest that the biological malignancy of tumors increases in proportion as tumors grow.

In conclusion, analysis of the mutual relations of serum CEA levels to tumor volume and proliferative activity of tumor cells in an animal model reveals that serum CEA levels increases in parallel with tumor volume and that serum CEA levels and tumor volume increase semilogarithmically in parallel with proliferative activity. From the results, it may be concluded to be true that the biological malignancy of tumor increases in proportion as tumor grows. Therefore, the determination of serum CEA and/or PCNA LI of tumor tissues seems to be clinically useful for the evaluation of tumor progression and extension.

#### REFERENCES

- 1. Shuster J, Silverman M, Gold P. Metabolism of human carcinoembryonic antigen in xenogenic animals. Cancer Res 33:65-68, 1973
- 2. Bivins BA, Meeker WR, Griffin Wo, et al. Carcinoembryonic antigen (CEA) levels and his-

topathology in colon cancer. J Surg Res 18:257-261, 1975

- 3. Sugarbaker PH. Carcinoembryonic antigen (CEA) in obstructive colorectal cancer. Am Surg 184:752-757, 1976
- 4. Phil E, McNaughtan J, Ma J, et al. Immunohistological patterns of carcinoembryonic antigen (CEA) in colorectal carcinoma: correlation with staging and blood levels. Pathology 12:7-13, 1980
- 5. Wagener C, Wallraf RM, Nisson C, et al. Localization and concentration of carcinoembryonic antigen (CEA) in gastrointestinal tumor: correlation with CEA levels in plasma. JNCA 67:539-547, 1981
- 6. Sugarbaker PH, Dunnick NR, Sugarbaker EW. Diagnosis and staging. In Cancer, Principles and Practice of Oncology. Edited by DeVita VT Jr, Hellman S, Rosenberg SA. JB Lippincot, Philadelphia, pp 226-263, 1982
- 7. Schmitz R, Nikolaizik W, Izbicki JR, et al. Tumor volume correlated serum CEA levels in nude mice after primary xenotransplantation of human colorectal carcinoma. Cancer Det Prev 5:325-329, 1982
- 8. Nagura H, Tsutsumi Y, Shiodo Y, et al. Immunohistochemistry of gastric carcinoma and associated diseases: nobel distribution of carcinoembryonic and secretory component on the surface of gastric cancer cells. J Histochem Cytochem 31:193-198, 1983
- 9. Staab HJ, Anderer FA. Circulating carcinoembryonic antigen (CEA), a growth parameter in malignant disease. Cancer Det Prev 6:33-39, 1983
- 10. Martin KW, Halpern SE. Carcinoembryonic antigen production, secretion and kinetics in BALB/c mice and a nude mouse-human tumor model. Cancer Res 44:5475-5481, 1984
- Hamada Y, Yamamura M, Hioki K, et al. Immunohistochemical study of carcinoembryonic antigen in patients with colorectal cancer. gen levels. Cancer 55:136-141, 1985
- 12. Tabuchi Y, Deguchi H, Imanishi K, et al. Comparison of carcinoembryonic antigen levels between portal and peripheral blood in patients with colorectal cancer. Correlation with histopathologic variables. Cancer 59:1283-1288, 1987
- 13. Tabuchi Y, Deguchi H, Imanishi K, et al. Carcinoembryonic antigen and carbohydrate antigen 19-9 levels of peripheral and draining venous blood in colorectal cancer patients. Correlation with histopathologic and immunohistopathologic variables. Cancer 62:1605-1613, 1988.
- Tabuchi Y, Nakamura T, Nakae S, et al. Elevation mechanism of serum carcinoembryonic antigen (CEA) in colorectal cancer patients. In Recent Advances in Gastroenterological carcinogenesis I. Tahara E, Sugimachi K and Oohara T (Ed.) Monduzzi Editore, Bologna, pp 1003-1006, 1996
- 15. Nakamura T, Tabuchi Y, Nakae S, et al. Serum carcinoembryonic antigen levels and proliferating cell nuclear antigen labeling index for patients with colorectal cancer. Cancer 77:1741-1746, 1996
- 16. Tabuchi Y, Nakamura T, Nakae S. Proliferative activity of colorectal cancer cells represented by proliferating nuclear cell antigen (PCNA): a clinical role. Bull Allied Med Sci (Kobe) 12:21-26, 1996
- 17. Tabuchi Y, Nakamura T, Nakae S. Argyrophilic nucleolar organizer legions in colorectal cancer: correlation with tumor progression and nuclear DAN ploidy. Bull Allied Med Sci (Kobe) 12:151-156, 1996
- Tabuchi Y, Nakamura T, Nakae S. Relations of serum CEA levels to clinicopathologic variables and proliferating cell nuclear antigen (PCNA) labeling indices in colorectal cancer. Bull Allied Med Sci (Kobe) 12:163-169, 1996
- 19. Hojo H. Establishment of cultured cell lines of human stomach cancer origin and their morphological characteristics. Niigata Igaku Zatsusi 91:737-767, 1977 (in Japanese with English abstract)
- 20. Tibbetts L, Chu MY, Hager JC, et al. Chemotherapy of cell-line-derived human colon carcinomas in mice immunosuppressed with antithymocyte serum. Cancer 40:2651-2659, 1977
- 21. Al-Sheneber IF, Shibata HR, Sampalis J, et al. Prognostic significance of proliferating cell nnuclear antigen expression in colorectal cancer. Cancer 71:1954-1959, 1993
- 22. Mayer A, Takimoto M, Fritz E, et al. The prognostic significance of proliferating cell nuc-

lear antigen, epidermal growth factor receptor, and mdr gene expression in colorectal cancer. Cancer  $71{:}2457{\cdot}2460,\,1993$