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Changes in Serum and Tissue Carcinoembryonic Antigen with Growth of a Human Gastric Cancer Xenograft in Nude Mice

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We established a human gastric cancer xenograft which, when inoculated into nude mice, showed a positive correlation between tumor growth and the serum level of carcinoembryonic antigen (CEA). Serum CEA levels in the mice rose continuously with increasing tumor weight after inoculation, showing a correlation coefficient of 0.96. A positive correlation was also observed between the tissue CEA level and tumor weight, the former increasing along with the latter. Furthermore, the level of serum CEA closely paralleled that of tissue CEA. The serum CEA level fell after tumor extirpation, with a half-life of approximately 86 h. These results suggest that the elevation of serum CEA is attributable to the gain in tumor weight as well as the increase of CEA production in the tumor tissue. Thus, human gastric cancer xenografts in nude mice are a good model for examining the biological role of CEA.

Key words: Carcinoembryonic antigen — Gastric cancer — Nude mouse

Serum carcinoembryonic antigen (CEA) is one of the most important tumor markers for monitoring cancer patients during the post-therapeutic follow-up period. Effective surgical therapy for tumors results in normalization of raised serum CEA levels. Postoperative sequential measurements of serum CEA provide an earlier indication of recurrence than can be obtained clinically.[1] Many tumors express CEA to a variable degree, including those of the gastrointestinal tract, breast and bronchus.[2] The preoperative serum CEA level in patients with gastrointestinal cancer has been shown to be correlated with prognosis.[3–6] It is accepted that patients with a high serum CEA level have a poorer prognosis than those with a low level.

The growth potential of human colorectal carcinoma in nude mice is associated with the preoperative serum CEA concentration in the patients from which the carcinoma was derived.[7] In the present experiment, which was designed to examine the changes in the serum CEA level with the growth of human gastric cancer xenografts in nude mice, we found for the first time that the amount of tissue CEA is directly related to tumor weight.

MATERIALS AND METHODS

Animals Four-week-old male athymic nude mice were obtained from Shizuoka Experimental Animals Corp. (Hamamatsu). They were maintained under specific-pathogen-free conditions, which involved the use of a laminar air-flow rack and sterilized food, water, bedding and cages. Experiments were begun after observing the mice for two weeks.

Human gastric cancer xenograft A human gastric cancer xenograft, originally obtained from a 61-year-old woman with gastric cancer, was established in our laboratory. The implantation techniques used for serial transfer have been described previously.[8] Fresh tissues removed from back tumors of nude mice were washed with saline and cut into pieces 2 mm in diameter. Each piece was implanted with a trocar into the flank of a nude mouse. The implantation sites were carefully inspected and measured twice a week. Relative tumor volume (mm^3) was calculated as $1/2ab^2$ (a, long diameter; b, short diameter).[9]

CEA clearance study Three groups of 15 nude mice were observed 6 weeks after tumor inoculation. Five mice in a control group were examined for serum CEA levels and tumor weight. Ten other mice were anesthetized with ether, and the tumors were extirpated and weighed. The feeding artery was ligated, and the skin sutured with 3–0 silk thread. Five mice were examined for serum CEA 1 week after extirpation, and the other 5 mice 2 weeks after.

Assay of CEA Serum CEA levels were measured by direct radioimmunoassay using a glazyme CEA-EIA test kit (Wako Pure Chemicals Co. Ltd., Osaka). Serum CEA levels in healthy nude mice averaged less than 2.5 ng/ml, which was considered below the positive level.

Cancerous tissue was homogenized with phosphate buffer, and then centrifuged at 15,000 rpm for 140 min. The supernatant was obtained and CEA was measured

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using the glazyme CEA-EIA test kit. Protein concentration was measured by the method of Lowry *et al.*[10] Tissue CEA levels per mg protein were measured using the same method as that for serum samples.

Histological examination Immunohistochemical study of CEA was carried out by the method of Hsu *et al.*[11] using avidin-biotin-peroxidase complex (ABC). The CEA antiserum was purchased from Dakopatts (Copenhagen). Avidin and biotin reagents were obtained from BioGenex Laboratories (Biotin-Streptavidin Immunostaining Kit, Dublin, CA). As a control, normal rabbit serum was used instead of the primary or secondary antibody.

Statistics Statistical analysis was performed by use of Student's t test. The criterion of significance was $P<0.05$.

RESULTS

Tumor growth The tumor grew slowly during the latent period (first 7 days), and then began to show logarithmic growth, up to 2.0×10^3 (mm^3) relative tumor volume. Doubling time was 6.4 days (Fig. 1).

Histological findings The histological features of the tumor were those of poorly differentiated adenocarcinoma. Immunohistochemically, CEA was demonstrated in the cytoplasm and on the cell surface of the tumor cells (Fig. 2). The proportion of CEA-positive cells was higher in tumors weighing over 3 g than in those weighing less than 1 g.

Correlation between serum CEA level and tumor weight The correlation between serum CEA level and tumor weight in mice was studied (Fig. 3). The serum CEA

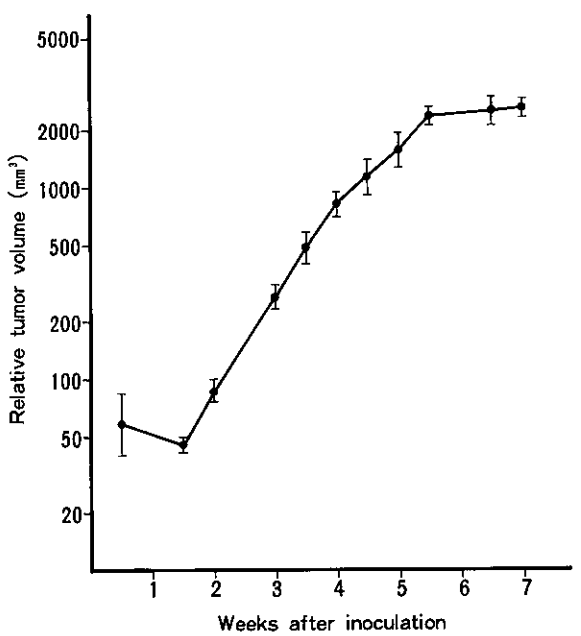

Fig. 1. Growth curve of the tumor. The xenograft tumor proliferated exponentially after inoculation up to a relative tumor volume of 2.0×10^3 (mm^3). Mean±SD.

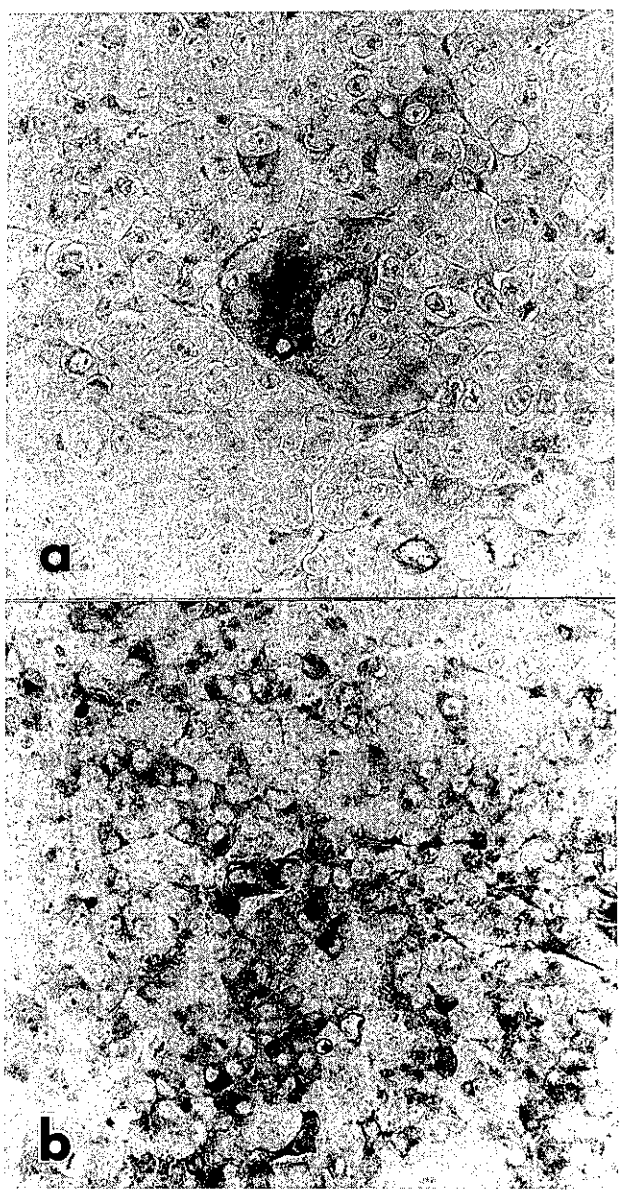

Fig. 2. Histochemical features of the tumor. The appearance is one of poorly differentiated adenocarcinoma. CEA is seen in the cytoplasm and on the cell surface of the tumor cells. (a) shows minor CEA positivity in cells of a tumor weighing 0.27 g (less than 1 g) and (b) shows major staining in a tumor weighing 3.6 g (more than 3 g). (×200).

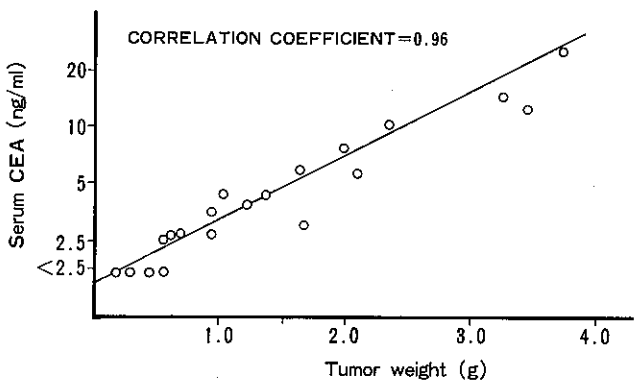

Fig. 3. Correlation of serum CEA level with tumor weight in each animal shows a direct relationship within the size limits studied.

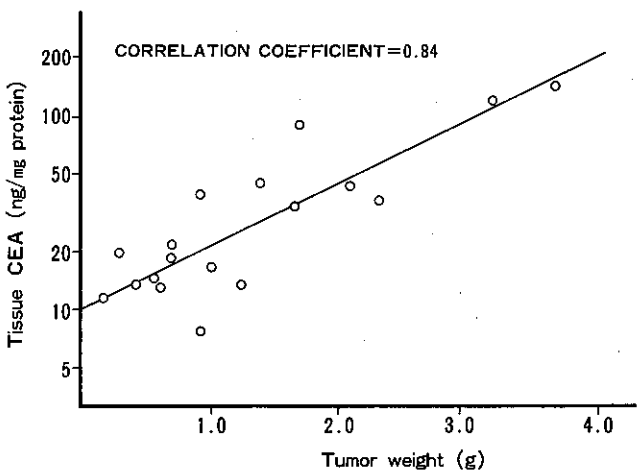

Fig. 4. Correlation of tissue CEA level with tumor weight in each animal. Tissue CEA increases proportionally to tumor weight.

level rose continuously with increasing tumor weight, with a correlation coefficient of 0.96.

Correlation between tissue CEA level and tumor weight The correlation between tissue CEA level and tumor weight was also studied (Fig. 4). The tissue CEA level rose along with increasing tumor weight, with a correlation coefficient of 0.84.

Correlation between CEA levels in serum and tissue The correlation between serum and tissue CEA levels was studied (Fig. 5). Serum CEA levels rose along with increasing tissue CEA levels, with a correlation coefficient of 0.76.

CEA clearance study The changes in serum CEA levels after tumor extirpation were then studied (Fig. 6). Serum CEA levels had fallen significantly at 1 and 2

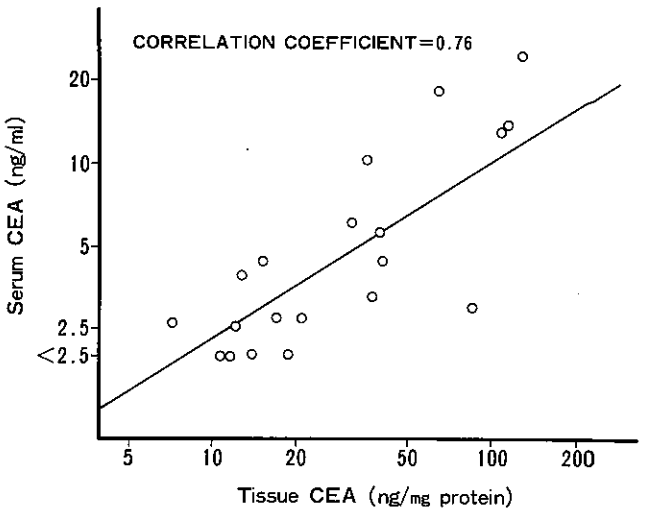

Fig. 5. Correlation of serum CEA level with tissue CEA level. Serum CEA level increases with the tissue CEA level.

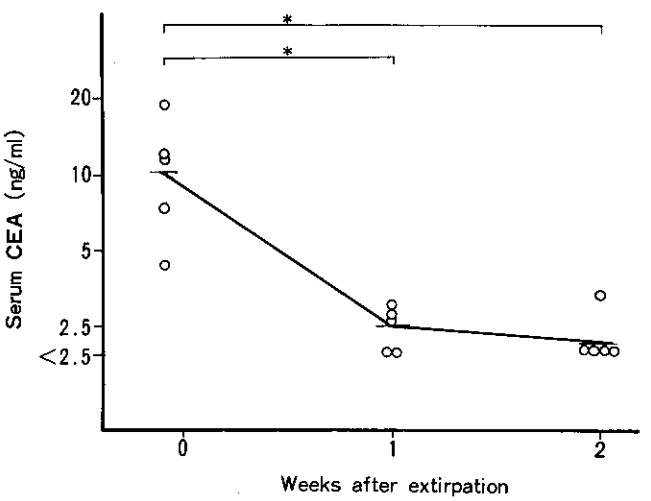

Fig. 6. Changes in serum CEA level after tumor extirpation. Serum CEA level fell significantly after tumor extirpation with a half-life of about 86 h. $*P<0.01$.

weeks after tumor extirpation in comparison with animals without the treatment ($P<0.01$). The half-life of CEA in serum was approximately 86 h.

DISCUSSION

The present study showed that the serum CEA level is directly related to tumor weight. It was clear that the serum CEA level rose with the gain in tumor weight. Similar findings were also obtained in a study of human

colon cancer xenografts in nude mice by Martin and Halpern.[12] On the other hand, Stragand *et al.*[13] and Lewis and Keep[14] reported different phenomena in studies of human colon cancer xenografts. It is possible that their findings differed because they were unable to measure serum CEA levels as low as those measurable in our study.

A correlation between the tissue CEA level and tumor weight was also observed. It has been reported that the production of CEA in gastrointestinal cancer cells *in vitro* increases with the period of culture.[15] CEA production has also been shown to be associated with the proliferative activity of gastric cancer cells.[16] An association between CEA secretion and tumor growth has been observed, but no correlation between CEA in the tumor and tumor size was apparent.[12] Our present result, however, indicates that CEA productivity changes with tumor growth.

Furthermore, a correlation between tissue CEA and serum CEA was observed. The CEA positivity of cells tended to increase with the tumor growth, and indeed a correlation between CEA localization in cancer cells and serum CEA has already been reported.[17, 18] Thus, the observed elevation of serum CEA is attributable not only to a gain in tumor weight but also to an increase of CEA production in the tumor tissue.

The serum CEA level in mice fell to within the normal range after tumor extirpation, and thus it was confirmed that the tumor was the source of elevated serum CEA. However, 80% of labeled CEA was previously shown to be removed within 1.5 h after injection in experimental animals.[19] In the present study, xenograft tumor-derived CEA showed a slow decrease in sera of nude mice, with a half-life of about 86 h. Serum CEA levels in nude mice were decreased at 1 or 2 weeks after tumor extirpation, and a mouse with local recurrence continued to show a raised serum CEA level, much in line with previous clinical observations.

The present results therefore suggest that human gastric cancer xenografts in nude mice are a good model for investigating the biological role of CEA, and that serum CEA can be a marker of tumor growth.

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