Cancer cachexia syndrome developed in nude mice bearing melanoma cells producing leukemia-inhibitory factor

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ABSTRACT

Melanoma-derived lipoprotein lipase inhibitor (MLPLI) is a factor purified from the conditioned medium of a human melanoma cell line, SEKI, which induced severe cachexia in tumor-bearing nude mice. Amino acid sequencing revealed that the amino-terminal portion was identical to that of leukemia-inhibitory factor (LIF). To determine whether MLPLI is actually LIF, the expression of LIF mRNA was examined in the SEKI melanoma cell line. Northern blot analyses revealed that the cell line displayed an intense hybridizable band with a molecular size of 3.8 kilobases, suggesting that MLPLI is identical to LIF.

The relationship between the development of the cancer cachexia syndrome and the expression of LIF mRNA was examined in four melanoma xenografts, SEKI, G361, A375 and MEWO, in nude mice. SEKI- and G361-bearing nude mice developed cancer cachexia syndrome, and their body weights decreased by 73.6% and 73.8% of the control, respectively. A375- and MEWO-bearing nude mice, however, did not develop the syndrome. Northern blot analyses revealed that G361 as well as SEKI expressed a large amount of LIF mRNA, but A375 and MEWO did not, suggesting a close relationship between the expression of LIF mRNA and the development of the syndrome.

These data support the concept that MLPLI, or LIF, plays an important role in the development of the cancer cachexia syndrome observed in melanoma-bearing nude mice.

INTRODUCTION

Cancer cachexia is a morbidity that develops in animals or humans that bear cancers. Although the mechanisms responsible for this morbidity are multifactorial, humoral factors produced by cancer cells could partly explain this morbidity (1, 2). We reported previously (3) the purification of a candidate factor with the ability to inhibit LPL from the conditioned medium of a human melanoma cell line, SEKI, which induces severe cachexia in tumor-bearing nude mice (4); the suppression of LPL activity may lower the intake of fatty acids by adipocytes, resulting in lipid catabolism in adipose tissues (5). The factor was temporarly designated as MLPLI (3). Amino acid sequencing revealed that the amino-terminal portion was identical to that of LIF, suggesting that MLPLI is a protein closely related to LIF (3).

In the present study, we investigated whether MLPLI is actually LIF by examining the expression of LIF mRNA and found that the MLPLI-producing tumor expressed large amounts of LIF mRNA. We then examined the relationship between the development of the cancer cachexia syndrome and the expression of LIF mRNA in four melanoma xenografts in nude mice.

MATERIALS AND METHODS

Materials. RPMI 1640 was obtained from Nissui Seiyaku Co. (Tokyo, Japan); fetal calf serum was from Boehringer GmbH (Mannheim, Germany); penicillin and streptomycin were from GIBCO (Grand Island, NY); and sodium bicarbonate was from Otsuka Seiyaku Co. (Tokyo, Japan).

Cell Lines. Four melanoma cell lines were examined. SEKI cells were established at the National Cancer Center (Tokyo, Japan) (6) were a melanoma cell line which induced severe cachexia in tumor-bearing nude mice. MLPLI was purified from this cell line (3). G361 was purchased from the American Type Culture Collection (Rockville, MD); this melanoma cell line also induces cachexia in tumor-bearing nude mice. For comparison with the two melanoma cell lines associated with cachexia, we examined two other melanoma cell lines. A375 cells were purchased from the American Type Culture Collection (Rockville, MD), and the MEWO cell line was provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan); both cell lines were expected to have no activity to induce cachexia in tumor-bearing nude mice.

An adult T-cell leukemia cell line, MT-2, provided by Dr. I. Miyoshi (Kochi Medical College, Nangoku-city, Japan), was used as a cell line expressing TNF-α and IL-1α mRNAs in Northern blot hybridization. All cell lines were cultured in RPMI 164 medium supplemented with 5% heat-inactivated fetal calf serum, glutamine (0.3 mg/ml), penicillin (100 units/ml), streptomycin (200 mg/ml), and sodium bicarbonate (0.14%) in a humidified atmosphere of 5% CO₂/95% air at 37°C in 75-cm² plastic tissue culture flasks.

Tumor Xenografts. SEKI xenografts in nude mice were obtained as described previously (3). G361, A375, and MEWO xenografts were obtained by i.m. inoculation of each cultured cell line into nude mice. Each xenograft growing in muscle tissue was then transplanted s.c. into the flank region of the animals. Tumors were maintained by serial passage in vivo by s.c. transplantation of tumor blocks. After several passages, the tumors were used for the experiment. For the present study, specimens of about 5 x 5 mm² from the xenografts were transplanted s.c. into both sides of the flank region of nude mice.

Animals. Five-wk-old female BALB/c-nu/nu mice were purchased from Clea Japan, Inc. (Tokyo, Japan). They were housed five per plastic cage on hardwood chips with 12-h light-dark cycles in a temperature-controlled chamber at 23 ± 3°C and provided with autoclaved food and water ad libitum.

Experimental Protocol of Animals. Twenty-five animals were randomly allocated into five equal groups prior to the experiment. Tumor xenografts (Group 1, SEKI; Group 2, G361; Group 3, A375; Group 4, MEWO) were transplanted s.c. into the flank regions of the nude mice. In Group 5, no tumors were transplanted and used as control. All animals were weighed 3 times a week between 9 and 11 a.m. for 34 days.

Northern Blot Analyses. Four melanoma cell lines were examined for expression of LIF, TNF-α, IL-1α and IL-1β mRNA. Poly(A)⁺ RNA extraction, gel electrophoresis and Northern blot hybridization were performed by the previously described method (7). The blot hybridization was performed by the previously described method (7). For detecting LIF mRNA, synthetic oligodeoxynucleotides 5'-GGA GTG GCC AAG GTA CAC GAC TAT GCC GTA CAG CTC CAC CAG CTT GGC CTT CTC-3', containing the coding sequence for Glu⁷⁶-Ser⁷⁸ of human...
LIF (8), were used as the probe. The synthetic probes for other cytokines were oligodeoxyribonucleotides complementary to TNF-α (Gln17-Asn30), IL-1α (Phe16-Ile30), and IL-1β (Ala16-Met16). The MT-2 cell line was reported to produce TNF-α (9) and IL-1α (10); expression of TNF-α and IL-1α mRNAs in this cell line was examined by synthetic probes to ascertain the reliability of these probes. To assess the integrity of the tissue poly(A)+ RNA extracted, the expression of human β-actin mRNA was examined by the previously described method (7).

The expression of LIF mRNA in SEKI and G361 melanoma cells was compared with those in 15 transplantable tumors not associated with cachexia; these 15 tumors include five melanomas, three pancreatic cancers, two lung cancers, two breast cancers, an esophageal cancer, an epidermoid cancer, and a leukemia.

RESULTS

Northern Blot Analyses for Cytokines in Four Melanoma Cell Lines. We selected four human melanoma cell lines, SEKI, G361, A375, and MEWO. Autoradiographs for Northern blot analyses using the synthetic oligodeoxyribonucleotide probes for human LIF, IL-1β and β-actin are shown in Fig. 1. For LIF, one intense hybridizable band with a molecular size of 3.8 kilobases was detected in SEKI and G361 cell lines. In contrast, A375 and MEWO cell lines exhibited faint bands. It is worth noting that SEKI and G361 cell lines had stronger bands than 15 transplantable cancer cell lines not inducing cachexia in tumor-bearing mice. When the probe for β-actin mRNA was used, a 2.0-kilobase band was detected in each cell line. An autoradiograph for Northern blotting using the synthetic probe for human IL-1β showed that a 1.6-kilobase band was detected only in G361 cells. For TNF-α, no bands were detected by 5-day exposure in all the melanoma cell lines; at the same experimental condition, the probe could detect a 1.7-kilobase band in MT-2 cells (data not shown). In the case of IL-1α, there were no detectable bands corresponding to IL-1α mRNAs in all the melanoma cells; in contrast, a 2.2-kilobase band was detected in MT-2 cells (data not shown).

Weight Change after Transplantation of Four Melanoma Xenografts. Mice transplanted with SEKI and G361 tumor cells lost significant body weight 18 and 11 days after transplantation, respectively (Fig. 2). In addition, they developed a cachexia-like appearance, especially 2 to 3 wk after transplantation (Fig. 3). Nude mice bearing the other two melanoma cell lines displayed neither significant weight loss nor cachexia-like appearance compared with the controls. When G361 tumors were surgically resected, both body weight and the cachexia-like appearance of tumor-bearing nude mice completely regressed within 10 days (data not shown). This recovery was similar to that of SEKI tumors reported previously (4).

DISCUSSION

It is conceivable that MLPLI produced by SEKI cells is a protein identical or closely related to LIF based on the evidence described below. (a) As reported previously, 13 amino acids present at the amino-terminal portion of MLPLI are identical to those of human LIF (3). (b) The molecular weight of MLPLI is almost identical to that of LIF (3). (c) The present study demonstrates that SEKI cells expressed a large amount of LIF mRNA using a synthetic probe recognizing the portion of mRNA corresponding to the midportion of LIF protein. The size of the hybridizable band was almost identical to that of human LIF mRNA reported previously (11).

MLPLI purified from the SEKI cell line inhibits LPL activity in adipocytes (3); the suppression of LPL activity may lower the intake of fatty acids by adipocytes causing lipid catabolism (5). Furthermore, Metcalf et al. (12) recently reported that exogenously added recombinant LIF induced body weight loss in experimental animals. All these findings promote this protein as a strong candidate for the factor causing the cancer cachexia syndrome. However, these findings do not necessarily mean that MLPLI, or LIF, is responsible for the cancer cachexia syndrome in cancer patients; proof for this awaits the demon-

![Fig. 1. LIF, IL-1β, and β-actin mRNA expression in four human melanoma cell lines. Poly(A)+ RNA (5 μg) was prepared from four cultured cell lines. Two cell lines were associated with cachexia syndrome (a, SEKI; b, G361); and the other two were not (c, A375; d, MEWO).](image)
stration that human cancer cells which are associated with the cancer cachexia syndrome produce LIF. In this context, we analyzed four melanoma cell lines to examine the relationship between the expression of LIF mRNA and the development of the cancer cachexia syndrome in tumor-bearing nude mice. As might be expected, weight measurements (Fig. 2) and gross appearance (Fig. 3) in tumor-bearing nude mice revealed that SEKI and G361 induced a severe cancer cachexia syndrome. As described previously, the SEKI cell line was used for purification of MLPLI, since cachexia induced by the SEKI cell line is more severe than that of 264 other tumor cell lines transplantable into nude mice (3). Although G361 was not included in that series, the degree of the cancer cachexia syndrome developed by G361-bearing nude mice was almost equal to that of SEKI; the present data establish G361 as another cell line causing severe cachexia. Northern blot studies demonstrated that a large amount of LIF mRNA was expressed in cell lines of SEKI and G361, but not in the other two cell lines not inducing the cancer cachexia syndrome (Fig. 1). We further observed that the expression of LIF mRNA in SEKI and G361 melanoma cells was very high when compared with 15 transplantable tumors not associated with cachexia (data not shown). These results suggest a very close relationship between the expression of LIF mRNA and the development of the cancer cachexia syndrome, at least in melanoma.

TNF-α/cachectin possesses the ability to inhibit LPL activity (13) and therefore is claimed to be a factor responsible for the cancer cachexia syndrome (14, 15). We examined the expression of mRNAs for TNF-α in these four melanoma cell lines; TNF-α mRNA was not detected in melanoma cell lines associated with cancer cachexia. Moreover, Kawakami et al. (16) recently reported that TNF-α-like immunoreactivity was not detected in the conditioned medium of SEKI melanoma cells. These observations indicate that TNF-α was not the factor responsible for the cancer cachexia syndrome that developed in these two human melanoma xenografts. It has been reported (17) that IL-1 possesses the ability to inhibit the LPL activity expressed in adipocytes. We also examined the expression of mRNAs for IL-1α and IL-1β in these four melanoma cell lines and found only IL-1β mRNA in G361 cells (Fig. 1). When the potency of IL-1β to inhibit LPL activity was examined in the 3T3-L1 adipocyte, the relative activity of IL-1β was rather low, approximately 25 to 50% of highly purified MLPLI/LIF⁴; however, it is possible that IL-1β contributes to the induction of the cancer cachexia syndrome in G361-bearing nude mice.

The present study supports our view that MLPLI, or LIF, plays an important role in the cancer cachexia syndrome observed in melanoma-bearing nude mice. Further studies are required to investigate the mechanism by which MLPLI/LIF induces the cancer cachexia syndrome in vivo and its clinical importance in cancer patients.

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REFERENCES


Fig. 3. Photographs of mice inoculated s.c. with human melanoma cell lines. The photographs were taken 18 days following inoculation. All animals possessed tumors in both of the flank regions. A, SEKI; B, G361; C, MEWO; D, non-tumor-bearing control.


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