Vol. 98, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1981 Pages 535-540

IDENTIFICATION OF THE mRNA CODING FOR THE ACTH-β-LIPOTROPIN PRECURSOR IN A HUMAN ECTOPIC ACTH-PRODUCING TUMOR

Toshihiko Tsukada*, Yoshikatsu Nakai*, Hisato Jingami*, Hiroo Imura*,
Shunzo Taii**, Shigetada Nakanishi**, and Shosaku Numa**
*Second Division, Department of Internal Medicine
and **Department of Medical Chemistry,
Kyoto University Faculty of Medicine, Kyoto 606, Japan.

Received December 11,1980

SUMMARY

The mRNA coding for the ACTH-β-lipotropin precursor from a human ectopic ACTH-producing thymic carcinoid was identified by blot hybridization analysis with the bovine cDNA as a probe. The mRNA from the tumor had the same length (approximately. 1,100 nucleotides) as that from the human pituitary. An additional hybridization-positive RNA species of a larger size was found in the tumor. Cell-free translation of the mRNA from the tumor as well as from the pituitary yielded a product with an apparent molecular weight of 38,000 that was reactive both with antibody to ACTH and with antibody to β-endorphin.

INTRODUCTION

The production of peptide hormones by tumors derived from tissues that do not normally elaborate the hormones is not an uncommon event. This phenomenon, designated as ectopic hormone production, can be explained by an abnormality in gene expression in neoplasms, although its exact mechanism is unknown (1). Ectopic ACTH-producing tumors elaborate not only ACTH but also β-LPH, γ-LPH, β-endorphin and γ-MSH, as does the pituitary (2, 3). This suggests that all these peptides are derived from a common precursor in the tumors as is the case in the pituitary and that the structural gene coding for the precursor protein may be identical in these tissues. However, there has been little information concerning

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Abbreviations: β-LPH, β-lipotropin; γ-LPH, γ-lipotropin; γ-MSH, γ-melanotropin; IgG, immunoglobulin G; ACTH(1-24), synthetic peptide with residues 1-24 of the amino acid sequence of corticotropin; SDS, sodium dodecylsulfate; DBM, diazobenzyloxymethyl

0006-291X/81/020535-06$01.00/0

the mRNA coding for ACTH and related peptides in such tumors. The present study deals with the identification of the mRNA coding for the ACTH-β-LPH precursor in a human ectopic ACTH-producing thymic carcinoid.

METHODS

Preparation of RNA. An ectopic ACTH-producing tumor, histologically proven to be a thymic carcinoid, was excised from a patient with Cushing's syndrome. A human pituitary gland was obtained at the time of autopsy. The tissues were immediately frozen and stored at -70°C until they were used. The contents of immunoreactive ACTH, immunoreactive β-endorphin and immunoreactive γ-MSH in the tumor extract, measured by the respective radioimmunoassays (2, 3), were 4,400, 5,500 and 820 ng/g tissue, respectively. RNA was isolated from the tissues according to the procedure used previously for the isolation of rat liver RNA (4), except that the oligo(dT)-cellulose chromatography step was omitted. Approximately 1.7 mg of RNA was isolated from 1.4 g of the tumor tissue, and approximately 0.3 mg of RNA from 0.24 g of the pituitary gland. RNA isolated from a thymoma and from a medullary thyroid carcinoma obtained at surgery served as control RNA preparations; these tumors contained no measurable amount of immunoreactive ACTH.

Hybridization analysis. Blot hybridization analysis was carried out with 10 μg of RNA according to the procedure of Alwine et al. (5); RNA was denatured with 1M glyoxal (6). The hybridization probe used was the PstI-excised insert of plasmid pSNAC20 carrying cDNA for the bovine ACTH-β-LPH precursor (7); it was labeled by nick translation (8) with [α-^{32}P] dCTP (3000 Ci/mmol, The Radiochemical Centre, Amersham).

Cell-free translation. The cell-free protein-synthesizing system used was derived from rabbit reticulocytes as described previously (9). The reticulocyte lysate was treated with micrococcal nuclease (EC 3.1.31.1, Worthington Biochemical Corporation) to destroy the endogenous mRNA (10). The reaction mixture (total volume, 750 μl) contained 375 μl of the nuclease-treated reticulocyte lysate, 600 μCi of L-[^{35}S]-methionine (1000 Ci/mmol, New England Nuclear), either 180 μg of tumor RNA or 75 μg of pituitary RNA and the remaining components at the concentrations described previously (9). Incubation was carried out at 25°C for 90 min. Indirect immunoprecipitation of the cell-free translation product was performed as described previously (9). The post-ribosomal fraction derived from the reaction mixture was incubated with the IgG fraction prepared from antiserum to ACTH(1-24) or from antiserum to β-endorphin (RB100-11/76 provided by Dr. R. Guillemin) under the specified conditions. The immunoprecipitated translation product was analyzed by electrophoresis on discontinuous SDS-polyacrylamide slab gel containing 10 % acrylamide, followed by fluorography, as described previously (11).

RESULTS

Identification of the ACTH-β-LPH precursor mRNA by blot hybridization analysis

RNA isolated from a human ectopic ACTH-producing thymic carcinoid as well as

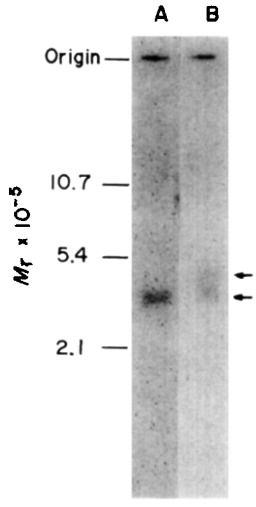

Fig. 1 Hybridization analysis of RNA from the human pituitary (A) and from the ectopic ACTH-producing tumor (B) with the ^{32}P-labeled cDNA. The RNA was electrophoresed on 1.5 % agarose gel. The positions of hybridization-positive bands are indicated by arrows. The RNA markers used were Escherichia coli 23 S rRNA (M_r 10.7 x 10^5), 16 S rRNA (M_r 5.4 x 10^5) and rabbit α- and β-globin mRNA (M_r 2.1 x 10^5).

from a human pituitary was electrophoresed on agarose gel, transferred onto DBM-paper and hybridized with the ^{32}P-labeled DNA complementary to the bovine ACTH-β-LPH precursor mRNA. The autoradiogram of the blot hybridization analysis is presented in Fig. 1. The RNA from the tumor showed two hybridization-positive bands (lane B), whereas a single band was observed for the RNA from the pituitary (lane A). The major RNA species derived from the tumor co-migrated with the RNA species derived from the pituitary, exhibiting an apparent molecular weight of approximately 3.7 x 10^5 (1,100 nucleotides). The second, minor RNA species derived from the tumor moved more slowly. Control RNA preparations obtained from a thymoma and a medullary thyroid carcinoma, which did not produce ACTH, yielded no hybridization-positive bands (data not shown).

Cell-free translation of the ACTH-β-LPH precursor mRNA

RNA from the tumor as well as from the pituitary was translated in the reticulocyte lysate cell-free protein-synthesizing system, and the products formed were

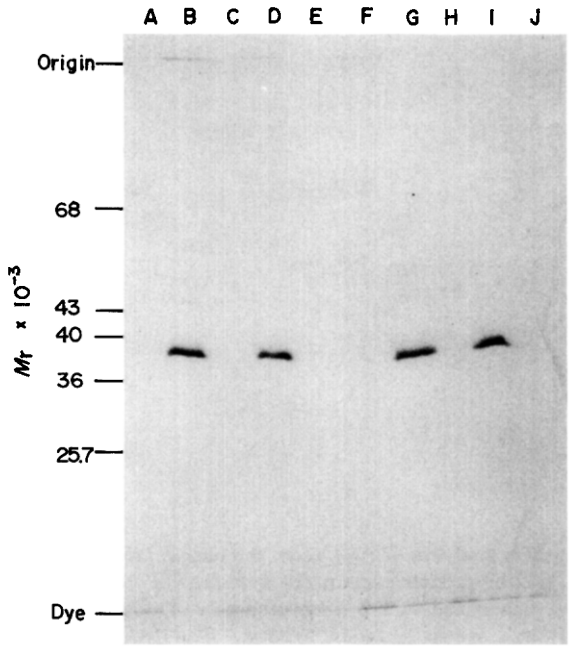

Fig. 2 SDS-polyacrylamide gel electrophoresis of the immunoprecipitates derived from the translation products formed with RNA from the human pituitary (A-E) and from the ectopic ACTH-producing tumor (F-J). Aliquots of the translation product corresponding to 150 μl of the reaction mixture were subjected to indirect immunoprecipitation with the additions indicated: A and F, control IgG; B and G, anti-ACTH; C and H, anti-ACTH and 5 μg ACTH(1-24); D and I, anti-β-endorphin; E and J, anti-β-endorphin and 5 μg porcine β-endorphin. The polypeptide markers used were bovine serum albumin (M_r 68,000), chicken ovalbumin (M_r 43,000), rabbit muscle aldolase (M_r 40,000), rabbit muscle lactate dehydrogenase (M_r 36,000) and bovine pancreas chymotrypsinogen (M_r 25,700). Bromphenol blue served as the tracking dye.

analyzed by indirect immunoprecipitation with antibody to ACTH and antibody to β-endorphin, followed by SDS-polyacrylamide gel electrophoresis. The autoradiogram of this electrophoretic analysis is shown in Fig. 2. The RNA from the two tissues yielded a single radioactive product with an identical mobility corresponding to an apparent molecular weight of 38,000 (lanes B, D, G and I). This product was not precipitated with control IgG from a nonimmunized rabbit (lanes A and F). An excess of ACTH(1-24) (lanes C and H) or β-endorphin (lanes E and J) competed with the cell-free translation product for binding to the respective antibodies. It has previously

been demonstrated that the anti-ACTH and anti-β-endorphin used do not cross-react with β-endorphin and ACTH, respectively (9). Thus, our results indicate that both antibodies specifically reacted with the translation product we identified, supporting the view that this product contains both ACTH and β-endorphin.

DISCUSSION

By means of blot hybridization analysis with the bovine ACTH-β-LPH precursor cDNA as a probe, we have shown that the human ectopic ACTH-producing tumor contains two hybridization-positive RNA species. The major RNA species has the same size as the hybridization-positive RNA species from the human pituitary and thus probably represents the mRNA coding for the ACTH-β-LPH precursor. The nature of the minor RNA species, which has a larger size, remains to be elucidated. It may be an RNA species that is distinct from the ACTH-β-LPH precursor mRNA but that contains sequences homologous to the latter. It is also possible that the minor RNA species may be an immature RNA species that codes for the ACTH-β-LPH precursor but that has not been spliced properly. Another possibility is that it may be the mRNA coding for the ACTH-β-LPH precursor with additional or different untranslated sequences.

The RNA from the ectopic ACTH-producing tumor directs the synthesis of a protein with an apparent molecular weight of 38,000 in the cell-free translation system, as does the RNA from the human pituitary. The product synthesized with the mRNA from the tumor is indistinguishable in molecular size as well as in immunoreactivity with anti-ACTH and anti-β-endorphin from that synthesized with the mRNA from the human pituitary.

Thus, the mRNA coding for the ACTH-β-LPH precursor in the ectopic ACTH-producing tumor and that in the pituitary most likely have the same size and encode a translation product with the same molecular weight and immunoreactivity. This

is consistent with our previous finding that ectopic ACTH-producing tumors elaborate various component peptides of the ACTH-β-LPH precursor, such as ACTH, β-LPH, β-endorphin and γ-MSH (2, 3). The observed differences in the size distribution of immunoreactive ACTH and related peptides between ectopic ACTH-producing tumors and the pituitary (2, 3) may be due to variations in the post-translational cleavage or glycosylation of the same ACTH-β-LPH precursor. Recently, Miller et al. (12) have reported that the ACTH-β-LPH precursor encoded by the mRNA from an ectopic ACTH-producing pancreatic carcinoma is indistinguishable from that synthesized in a pituitary adenoma in organ culture.

ACKNOWLEDGEMENTS

We are indebted to Dr. K. Noma, Kure National Hospital for a specimen of the ectopic ACTH-producing thymic carcinoid, to Dr. T. Kameya, National Cancer Center, for the thymoma, and to Dr. S. Takai, Osaka University, for the medullary thyroid carcinoma. Our thanks are also due to Dr. R. Guillemin and Dr. N. Ling, the Salk Institute, for the generous gift of antiserum to β-endorphin and of porcine β-endorphin.

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