Synthesis of somatomedin C/insulin-like growth factor I by human placenta

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Abstract

We have reported the presence of insulin-related poly A+ RNA sequences in human placenta by RNA to DNA hybridization. In this study we have used a monoclonal antibody to somatomedin C/insulin-like growth factor I (Sm-C/IGF-I) to identify somatomedin-like proteins whose synthesis is directed by placental mRNA. Poly A+ RNA from first trimester and term placenta was translated in a cell-free system using micrococcal nuclease-treated reticulocyte-lysate and [35S]methionine as a label. From 2.0 × 10⁶ cpm of specifically incorporated [35S]methionine labeled protein, an immunoprecipitate with an apparent molecular weight of 14000 represented about 0.1% of total radioactivity in the translational products of poly A+ RNA of first trimester placenta. A less prominent band (0.006%) of the same apparent molecular weight was also evident from translational products of term placental mRNAs. This protein could be competed with either acromegalic serum or synthetic Sm-C/IGF-I when added prior to immunoprecipitation. Translational products synthesized from mRNA of term placenta showed a second labeled band of 24000 daltons. This band was less effectively competed by acromegalic serum and not competed with either Sm-C/IGF-I or IGF-II and therefore its identity is uncertain. A protein similar to Sm-C/IGF-I is, therefore synthesized in first trimester placenta and to a lesser extent at term, suggesting developmental changes in Sm-C/IGF-I synthesis. Because Sm-C/IGF-I may act in a paracrine fashion, our findings suggest a role for Sm-C/IGF-I in growth of the placenta during early gestation.

Introduction

Somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I), which is believed to be an important stimulator of post-natal growth (1), may also be involved in fetal growth (2). Receptors for this peptide are widespread in fetal tissues (3, 4), concentrations in fetal and cord blood correlate with fetal size (5), and a variety of fetal tissues respond to (2, 6, 7) and produce Sm-C/IGF-I or closely related peptides (8, 9). Maternal serum concentrations of Sm-C/IGF-I rise during pregnancy in women with growth hormone deficiency, so that by the third trimester, they equal those of normal non-pregnant adults (10, 11). After parturition, concentrations drop precipitously to pregestational levels (10, 11). In rats that are hypophysectomized during pregnancy, Sm-C/IGF-I concentrations do not decline until the feto-placental unit is delivered (12). These observations suggest that the placenta might produce Sm-C/IGF-I, or that placental lactogen is exerting a somatotrophic action on somatomedin producing tissues (13).

In recent studies on the synthesis and secretion of placental proteins during normal and diabetic pregnancy (14–16) we used cDNA probes for insulin and insulin-related sequences to observe the expression of insulin-related mRNA in human placenta (15). With high stringency criteria for hybridization, we detected the expression of insulin-related species of mRNA, but not insulin. To further examine the insulin-related peptides syn-
thesized by placenta, we have performed immuno-
precipitations from \textit{in vitro} translation products
directed by placental mRNA. Using a monoclonal
antibody raised against the somatomedins, we re-
port evidence for the synthesis of protein with
immunodeterminants similar to Sm-C/IGF-I.

\textbf{Material and methods}

\textit{Preparation of mRNA from placenta}

Placentas were obtained within 20 minutes of
delivery at the MacDonald Hospital for Women,
University Hospitals of Cleveland, from normal
pregnant women at term, diabetic pregnant women
near term, and women having preterm therapeutic
abortions. Procurement of tissues was approved by
the committee for the protection of human subjects
of Case Western Reserve University and University
Hospitals of Cleveland. Most term and near term
placentas were obtained from cesarean sections.
Placentas were frozen in liquid nitrogen, and either
stored for up to 3 months, or processed immediate-
ly. First trimester placentas were pooled.

After placentas were rinsed, pieces of tissue
(0.2 – 1.0 cm) were excised and washed twice in 2 – 4
volumes of phosphate-buffered saline (PBS). Total
RNA was isolated using the guanidine thiocyanate
(Fluka, purum) procedure of Chirgwin \textit{et al.} (17),
with the exception that the tissue to guanidium
thiocyanate solution ratio was 1 : 4 and 100 g of
tissue was usually processed. RNA (100
$A_{260}$ nm/0.5 g oligo(dT) cellulose) was then eluted
twice from oligo(dT) cellulose (type 3, Collabora-
tive Research) (18), and the mRNA samples were
stored at –80°C in a concentration of 1 mg/ml.

\textit{In vitro translation with reticulocyte lysate}

Placental mRNA (0.1 – 1.0 $\mu$g/25 $\mu$l) was trans-
lated using 16 $\mu$l of mRNA dependent reticulocyte
lysate (Promega) (19). The reaction mixture con-
tained 50 $\mu$Ci of [$^{35}$S]methionine (New England
Nuclear, 600 Ci/mMol). Incubations were per-
formed at 30°C for 60 min unless otherwise noted.
With the concentrations of mRNA used, high
molecular weight products (>100000) were trans-
lated. The number of TCA-precipitable counts
ranged from 8 to 22 fold over background. A linear
increase in incorporation usually occurred between
0.1 and 0.4 $\mu$g mRNA/25 $\mu$l reaction mixture. With
higher mRNA concentrations preferential transla-
tion of low molecular weight mRNAs was ob-
erved.

\textit{Immunoprecipitation of translated products}

The Sm-C/IGF-I monoclonal antibody used to
precipitate translation products was produced by \textit{in}
vivo immunization followed by \textit{in vitro} boosting of
cultured spleen cells for 5 days with purified Sm-
C/IGF-I (20). The antibody has 60% crossreactivi-
ty with IGF-II. No crossreactivity was observed
with the following substances in concentrations up
to $10^{-6}$: insulin, human growth hormone, human
thyroid stimulating hormone, mouse epidermal
growth factor, and mouse albumin.

The translation mixture, containing $2 \times 10^6$ cpmp
above background (for translation of reticulocyte
lysate alone), was brought to a volume of 900 $\mu$l
with PBS containing 0.2% Triton X-100 and 0.2%
sodium deoxycholate. This mixture was absorbed
by the addition of 40 $\mu$l of Pansorbin (Calbiochem)
to reduce nonspecific binding of translation
products (21, 22). To assess the specificity of the
immunoprecipitate, identical reaction mixtures
were prepared with Sm-C/IGF-I rich extracts of
acromegalic serum (supplied by the National Hor-
mone and Pituitary Program) a biosynthetic Sm-
C/IGF-I analogue (Thr-59; Amgen Biologicals,
Thousand Oaks, CA) and a highly purified prepa-
ration of human IGF II prepared in our laboratory.
After absorption with Pansorbin and centrifugation,
2 $\mu$l of monoclonal antibody was added to the
pre-absorbed mixture and incubated at 4°C for
16 h. The mixture was then incubated with 20 $\mu$l of
sheep antimouse IgG for 2 h at 4°C, then with
Pansorbin (100 $\mu$l) for one hr. The immuno-
precipitate was pelleted by centrifugation, washed
twice in 1.0 ml of PBS containing 0.2% Triton
X-100 and 0.2% sodium deoxycholate, suspended
in 50 $\mu$l of electrophoresis sample buffer contain-
ing SDS, and heated for 5 min at 90°C to release
the antibody/antigen complex. The undissolved
bacterial residue was separated by centrifugation,
and the supernatant fluid was subjected to elec-
rophoresis.