PROLACTIN SYNTHESIS AND TROPHOBLASTIC DIFFERENTIATION IN METASTATIC CHORIOCARCINOMA IN THE LIVER

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Prolactin synthesis was investigated in trophoblastic cells in the metastatic tumors of choriocarcinoma of the liver by means of the fluorescent antibody technique and electron microscopy. Of interest is the morphological function of the trophoblast in choriocarcinoma which is presumably free from decidual interaction whereas the morphology of the tumor is very similar to that of the cell column in the normal placenta. In the tumor tissue, trophoblasts were observed, though randomly arranged, in gradations with their cell differentiation from cytotrophoblast to syncytium. The intermediate cell of the trophoblasts was demonstrated to be capable of synthesizing prolactin by fine granular distribution in cytoplasm while syncytium in the metastatic tumors was characterized by fluorescence and consisted of fine granules mixed with network formation.

The production site of human prolactin (PRL) has been disputed to be other than the pituitary gland. Some evidence has stipulated the synthesis to be in the decidual cells during normal pregnancy (17) and in the decidualized endometrium in the normal menstrual cycle (11). In fact, though the ultrastructure of the decidual cells demonstrates the secretory granules in the cytoplasm (20), they are possibly concerned not with peptide hormone synthesis but with the production of acid phosphatase (22). Previously the authors described immunohistological evidence to demonstrate that syncytium played a role of PRL synthesis in normal chorionic villi (7, 8).

It is recognized that the morphology of choriocarcinoma is very similar to that of the cell column in normal placenta where various trophoblasts are randomly arranged except in the gradations of their cell differentiation (1, 2, 10, 19). Of interest is the PRL synthesis in trophoblasts without either morphological or functional interaction of the decidual cells. If PRL is synthesized in either the decidual cells or in syncytium with an interaction of the decidual cells in normal placental tissue, PRL would not be synthesized in syncytium in the metastatic tumors of choriocarcinoma in the liver where the interaction of the decidua is morphologically negligible.

In this paper, the authors describe the synthesis and distribution of PRL in the trophoblasts of the metastatic tumors of choriocarcinoma in the liver in relation to the functional morphology of trophoblasts, especially of the intermediate trophoblast, by means of the fluorescent antibody technique and electron microscopy.

MATERIAL AND METHODS

I. *Material*

A 43 year old female patient was used who had received a panhysterectomy with bilateral salpingo-oophorectomy at a hospital in Shiga Prefecture because of invasive choriocarcinoma in the uterus one year prior to autopsy. Tissue pieces of the liver which contained multiple tiny metastatic tumors of choriocarcinoma were obtained at autopsy. Plasma concentration of PRL was not measured before autopsy.

II. *Methods*

a) Fluorescent antibody technique

i) Tissue pieces of the liver were immersed in 95.0% ethanol with 1.0% acetone solution immediately after dissection at autopsy. The well fixed tissue pieces were dehydrated in a graded series of ethanol solution and embedded in paraffin.

They were sectioned to 3 μ m in thickness. The sections were deparaffinized with xylol and washed in a series of graded ethanol solutions. They were washed with cold staining phosphate buffer solution (PBS, pH 7.20) at staining.

ii) Human prolactin and other placental peptide hormones

Anti-PRL antiserum was obtained by the immunization of albino rabbits with human antigen PRL. Thorough purification was done in this preparation for the purpose of immunization by the procedures that the authors previously reported with hCG (5). The antibody was extracted from the antiserum and purified according to modified Johnson and Holborow's procedures (4). Immunological specificity was confirmed according to the description by Hamashima & Kyogoku (3). A drop of highly purified anti-PRL antibody solution which contained 6.03 mg/ml equivalent to protein was put on the tissue section, which was kept in a moist chamber for 36 hr and then washed with cold PBS. A drop of FITC conjugated anti F(ab)₂ of rabbit-IgG antiserum was put on the same tissue section to keep it for 36 hr again in a moist chamber. A microscopic examination was done.

Subsequent to the completion of PRL staining, a drop of Rhodamin B conjugated antiserum of either anti-hCG- β or anti-hPL was put on the same tissue section, which was kept in a moist chamber for 48 hr. Microscopic examination was done thereafter.

All of the staining procedures were carried out at a temperature of below 10°C.

b) Electron microscopy

Small pieces of the liver tissue in which tiny metastatic choriocarcinoma was involved were fixed with an equal volume of 2% paraformaldehyde in cacodylate buffer (pH 7.4) with sucrose and 2% glutaraldehyde, and washed with cacodylate buffer. Then the specimens were incubated for one hr with Gomori's medium and fixed with 1% OsO₄. They were embedded in Epon 812 following the dehydration in a graded series of ethanol solutions. Ultrathin sections were observed by a Hitachi H-500 electron microscope.

RESULTS

1. *By fluorescent antibody technique*

Fluorescence specifically reactive to human prolactin (PRL) was observed in the cytoplasm of the intermediate cell type of the trophoblasts as well as of the syncytium. Both were low in population among the metastatic tumors. The former contained usually one nucleus which was irregular in shape, with figures suggesting deep cytoplasmic protrusions into the nucleus. The cell border seemed to be mostly smooth. Intracytoplasmic distribution of PRL was observed by fluorescence of fine granules disseminated all over the cytoplasm including the area of irregular nucleus border (Fig. 1). In the latter, cells contained mostly one but sometimes more than two nuclei with a usually smooth and regular shaped border. Cytoplasmic protrusion was rarely observed. The cytoplasm became enlarged with many vacuoles in different sizes the content of which might have been dissolved during the tissue fixation. Fluorescence was observed by fine granules in the cytoplasm except in the area occupied by vacuoles (Fig. 2). Fluorescence was also observed on the surface of blood corpuscles in the area of normal liver tissue adjacent to the tumor tissue (Fig. 3).

2. *By light and electron microscopic observations*

By light microscopy, several different types of trophoblasts were observed in

FIG. 4. Prolactin synthesizing large cytotrophoblastic cell with irregular nucleus border (arrow). Hematoxylin and eosin. $\times 1,500$

FIG. 1. Diffuse distribution of PRL is observed in cytoplasm and nucleus area with irregular shape of projecting fluorescence. $\times 1,200$

FIG. 2. Among trophoblasts which contain mostly fine granular fluorescence of PRL, syncytium (left) and cytotrophoblastic cell (right) are distinguishable by light microscopy. The latter has usually mononuclear with irregular nucleus border. $\times 1,200$

FIG. 3. Intravascular PRL is observed on the surface of blood corpuscle and vascular wall in normal liver tissue which locates in the area very close to a focus of choriocarcinoma. $\times 1,200$

the tumor tissue which were randomly arranged without formation of villous pattern. This morphology is very similar to that of the cell column of the anchoring villi in an early placental tissue. The size, shape and number of nuclei was different among trophoblasts. The size of cell was usually larger than that observed in an early stage of normal chorionic villi. Remarkable irregularity of the nucleus border was observed in mostly mononuclear cytotrophoblast-like cells (Fig. 4). By electron microscope, syncytium was characterized by abundant rough endoplasmic reticulum (rER), many filaments and small mitochondria in the cytoplasm as well as microvilli on the cell surface while poor rER, large mitochondria and glycogen granules in the cytoplasm were the characteristic organelles to cytotrophoblast. In addition to these two trophoblasts, the intermediate cell was observed which was characterized by a different amount of rER with gradations between cytotrophoblast and syncytium as well as by abundant fine filaments, by scanty glycogen granules and large mitochondria in the cytoplasm. Microvilli were rarely observed in this cell (Fig. 5). Most of the trophoblasts contained one nucleus but more than two nuclei were found in some syncytium.

DISCUSSION

According to present concepts, cells capable of protein synthesis are related to abundant ER and prominent Golgi complex (14, 16). However, the morphology of cytotrophoblast in normal chorionic villi as well as in the tumor tissue of choriocarcinoma has revealed sparse intracytoplasmic organelles endowed with protein synthesis (13, 21). Thus, syncytium is regarded as having the capability of synthesizing peptide hormones as was demonstrated by the authors in hCG- β by means of the fluorescent antibody technique (6). In this regard, the authors have previously demonstrated that PRL is synthesized in the syncytium in normal chorionic villi. Microscopic findings have substantiated that syncytium is derived from cytotrophoblast through the cell differentiation (12, 15, 18). Of interest is the functional morphology of the intermediate trophoblast in the production of PRL. In this study, the metastatic tumor tissue of choriocarcinoma in the liver contained cells in gradations with the development of trophoblasts, of which morphology is very similar to that of the placental cell column which is regarded as the progenitor of the villous pattern formation (12). In the cell column, decidual elements cannot always be excluded in the functional development of the trophoblasts. Of additional interest in this study is that the metastatic tumor trophoblasts are independent of decidual elements in their morphological development.

Fluorescence specific to PRL was not observed in those cells which by light microscopy and ultrastructurally belonged to cytotrophoblasts. On the contrary, fluorescence was demonstrated in syncytium though in a low cell population among the metastatic tumor tissues. Cells which were ultrastructurally regarded as the intermediate trophoblasts also demonstrated the capability of PRL synthesis by fine

FIG. 5. Intermediate trophoblast: cytotrophoblastic cell by light microscopy but it has characteristics of syncytial trophoblast, i.e. moderate amount of rough ER (arrow head), disseminating fine filaments (F). Emphasis is in remarkable indentation (arrow) where rough ER is observed.

N: nucleus, Nc: nuclear corpuscle. $\times 20,000$

granular distribution of intracytoplasmic fluorescence involving the area of nuclear indentations.

These cells were observed also in a low population among the tumor tissues but not so low as that of syncytium. Though there has been some evidence to substantiate that the decidual tissue and the decidualized endometrium initiate the synthesis of PRL in either normal pregnancy or normal menstrual cycle (11, 17), and despite an experiment carried out by Knoth *et al.* (9), the authors presume that PRL is synthesized in a trophoblast which acquires morphological characteristics of syncytium including the intermediate cell during trophoblastic differentiation irrespective of an association of the decidual elements (22).

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