IMMUNOHISTOCHEMICAL LOCALIZATION OF BASIC FETOPROTEIN IN GYNECOLOGICAL MALIGNANCIES

Keiko MURAKAMI and Morimasa MATSUTA

Department of Obstetrics and Gynecology, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate 020

Received for publication November 20, 1991 and in revised form December 19, 1991

Localization of basic fetoprotein (BFP) as well as its dynamics in malignant, normal tissue and precancerous lesions of the female genital organs were investigated. For light microscopy, conventionally processed paraffin sections were stained by the avidin-biotin peroxidase complex method. For electron microscopy, 4% paraformaldehyde fixed frozen sections were subjected to an indirect enzyme antibody method. As the results, BFP positive cases were 74% in ovarian carcinomas, 77% in endometrial carcinomas and 100% in cervical carcinomas. With respect to the localization of BFP in malignant, normal and precancerous cells, diffuse granular localization was observed in the cytoplasm of BFP positive cells. BFP was localized in the ribosomes of cells with well-developed organella, electron microscopically, and figures of dialyzing secretion were observed. Based on these findings, BFP was found in dedifferentiated cells still retaining the original structure and/or properties. This suggests BFP is a functional protein secreted by dialyzing pattern, and may become a good tumor marker of gynecological malignancies.

In recent years, knowledge concerning tumor markers has markedly increased. These markers have been divided into those with reactivity to relatively limited tumors such as α-fetoprotein (1, 9) and CA125 (10), and those with relatively low organ specificity such as carcinoembryonic antigen (CEA) (11). Basic fetoprotein (BFP) has been reported to increase in the blood of patients suffering from various malignant tumors (3–7). There have been a few reports investigating the localization of this marker in tumors, and there has been no report concerning its electron microscopic localization. We investigated the light and electron microscopic localization of BFP in gynecological malignant tumors. At the same time, dynamics of BFP in the tissues and its secreting pattern were investigated. The significance of BFP appearing in the blood is discussed.

MATERIALS AND METHODS

Specimens were extracted from malignant tumor lesions obtained from 124 patients suffering from gynecological malignant tumors including 47 cases of ovarian cancers, 49 cases of endometrial carcinomas, and 28 cases of infiltrating cancers in the uterine cervix.

The clinical stage of disease and histological patterns were determined by the International Federation of Gynecology and Obstetrics (FIGO) classification and Wentz and Reagan’s classification (16), respectively. Compilation of patients supplying the specimens for this study is summarized in Table 1.

Ovarian cancers were classified into the following histological types: serous cystadenocarcinoma (28 cases), mucinous cystadenocarcinoma (4 cases), endometrioid carcinoma (7 cases), clear cell carcinoma (3 cases), and undifferentiated carcinoma (4 cases). Endometrial carcinomas were classified into the following histological types: adenocarcinoma (7 cases of well differentiated adenocarcinoma, 33 cases of moderately differentiated adenocarcinoma and 7 cases of poorly differentiated adenocarcinoma) and adenoacanthoma (2 cases). Carcinomas of the uterine cervix were all squamous cell carcinomas including 22 cases of large cell non-keratinizing type and 14 cases of the keratinizing type. With respect to the clinical stage of disease, ovarian carcinomas ranged from stage I–IV, endometrial carcinomas ranged from stage I–III, and carcinomas of the uterine cervix ranged from stage I–IIIb.

For ovarian cancers, a sliced specimen with the
largest area of tumor was selected among those with tissues showing their natural characteristic structures. For cancers of the uterine body and cervix, a sliced specimen with the largest area of tumor was selected among those showing the longitudinal section of the extracted uterus. As for controls, 5 formalin-fixed paraffin sections of each of the following specimens were used: serous cystadenoma, mucinous cystadenoma, and mucinous cystadenoma of low potential malignancy of the ovary; proliferative and secretory phases of the endometrium; cystic, adenomatous, and atypical hyperplasia of the endometrium; normal squamous epithelium of the uterine cervix; mild, moderate, and severe dysplasia of the squamous epithelium, reserve cell hyperplasia and carcinoma in situ of the squamous epithelium.

In order to confirm the preservation of antigenicity of BFP during fixation and embedding of the tissues, extracted tumor tissues of a patient with ovarian cancer (serous cystadenocarcinoma) and a high BFP level in the blood were fixed overnight in 4% paraformaldehyde/0.1 M phosphate buffer (PB) at pH 7.4 at 4°C. The frozen sections were stained with the indirect peroxidase method (14). Adjacent tumor tissues were fixed in 10% formalin solution, embedded in paraffin conventionally and stained by the avidin-biotin-peroxidase complex (ABC) method (2). Results of the two methods were compared.

**Immunohistochemical observation of BFP by light microscopy**

The extracted tissues were fixed in 10% formalin solution and embedded in paraffin conventionally, and paraffin sections were stained by Hematoxylin-Eosin (H.E.) staining. Adjacent sections were stained by the ABC method. This method was performed as follows: Deparaffinized sections were incubated for 30 min in a 0.3% solution of hydrogen peroxide in methanol and were rinsed with 0.01 M phosphate buffered saline (PBS) at pH 7.4. They were covered with 10% normal goat serum for 10 min, then incubated for 30 min at room temperature with rabbit anti-BFP serum (Nihon Kayaku Ltd., Tokyo) diluted 500 times in PBS. Incubation was then performed with biotinized anti-rabbit IgG and finally with avidin-biotin-peroxidase complex (Vectastain, Elite, ABC kit, Vector, USA). Following staining with dianinobenzidine (DAB), the sections were counterstained with hematoxylin or methylgreen cases in which more than 30% of epithelial or tumor nuclei were stained were considered positive.

**Immunoelectron microscopic observations**

For the immunoelectron microscopic observations of BFP, frozen sections of the following tumor tissues were used: 7 ovarian cancers (consisting of 5 serous cystadenocarcinomas and 2 clear cell carcinomas), 5 cancers of the uterine body (moderately differentiated adenocarcinoma), and 5 cancers of the uterine cervix (squamous cell carcinoma, large cell non-keratinizing type) (16). The tumor tissues were fixed overnight in 4% paraformaldehyde/PB at 4°C and were rinsed for 2 days at 4°C with PBS containing 20% sucrose. They were then frozen, sectioned, and attached to albumin-coated slide glasses. Sections were pretreated with 10% normal goat serum and incubated for 48 hr at 4°C in anti-BFP serum (Nippon Kayaku Ltd., Tokyo). They were further incubated for 12 hr in anti-rabbit IgG (goat IgG/(Fab)2) conjugated with horseradish peroxidase (Amersham, USA). Sections were then fixed in 1% glutaraldehyde and stained by the DAB reaction. They were further fixed in 2% osmic acid solution, dehydrated with graded alcohols, and embedded in Epon 812 by the inverted capsule method. The ultra-thin sections were examined with an Hitachi H-700H type electron microscope with or without lead staining.

**RESULTS**

Immunohistochemical observation of antigenicity of BFP

In frozen sections, BFP was detected in the cytoplasm of tumor cells. Since similar positive findings were obtained in the formalin-fixed paraffin sections, the ABC method was employed for staining of paraffin sections and the indirect method for frozen sections.

1. **BFP staining for light microscopy**

   a) Localization of BFP in ovarian cancer

   Positive ratios for each histological type were as follows: 82% in serous cystadenocarcinomas, 75% in mucinous cystadenocarcinomas, 71% in endometrioid carcinomas, 100% in clear cell carcinomas, and 0% in undifferentiated carcinomas (see Table 1).

   In serous cystadenocarcinomas, the apical portions of the papillary structures showed a high incidence of positive cells (23/28). Although positive cells were observed in parts where tumor cells showed substantial proliferation, the incidence was low and psammoma bodies showed negative reactions. In positive cells, the cytoplasm was diffusely positive (Fig. 1-A).

   In mucinous cystadenocarcinomas, positive cells were found sporadically or in clusters. In the glandular structures, mucin was not stained and the cytoplasm compressed by the mucin showed a positive reaction. In mucin-free cells, a diffuse positive reaction was observed in the whole cytoplasm. The reac-
A. Serous cystadenocarcinoma. The apical portions of the papillar structure showed a high incidence of positive cells. In positive cells, the cytoplasm was diffusely positive. ABC method. Counterstain with hematoxyline.  × 100

B. Mucinous cystadenocarcinoma. Mucin in the cells was not stained and the cytoplasm compressed by the mucin showed a positive reaction. In mucin free-cells, a diffuse positive reaction was observed in the cytoplasm. ABC method. Counterstain with hematoxyline.  × 250

C. Endometrioid carcinomas. In the glandular structure and squamous metaplasia, positive reactions were seen in cells. ABC method. Counterstain with hematoxyline.  × 100

D. Clear cell carcinomas. Cells with clear plasma under H.E. stain were negative or showed positive reaction in their marginal plasm. Tumor cells with eosinophilic plasm showed a diffuse positive reaction. ABC method. Counterstain with methylgreen.  × 250
tion was marked, especially in the parts of substantial proliferation of tumor cells. Secretions in the glan-
dular cavity were negative (Fig. 1-B).

In endometrioid carcinomas, tumor cells in the glandular structures consisted of pseudostratified epithelium which showed a diffuse positive reaction in the cytoplasm. Apical portions showed a stronger positive reaction. In portions indicating squamous metaplasia, positive reactions were seen in cells with relatively abundant cytoplasm and were located from the margin to near the center of squamous epithelial cell clusters. Where tumor cells showed substantial proliferation, there were sporadic positive cells (Fig. 1-
C).

In clear cell carcinomas, positive cells located sporadically. No specific differences were noted even between portions with glandular proliferation and substantial proliferation. Cells with clear cytoplasm stain were negative or showed positive reaction in their marginal cytoplasm. Tumor cells with eosinophilic plasm by H.E. staining showed a diffuse positive reaction (Fig. 1-D).

In undifferentiated carcinomas, no positive reaction was noted in any case.

For all the above cases, the interstitium showed no positive reaction. In serous cystadenomas, there were sporadic cells with cytoplasm showing a diffuse positive image. In low potential malignancy of mucinous cystadenocarcinoma, many cells showed a diffuse positive reaction in their cytoplasm and formed stratified structures. In mucinous cystadenomas, cytoplasm compressed by mucin to the basal portion showed a positive reaction.

b) Endometrial carcinomas

The BFP positive ratios were 71% in cases of well differentiated adenocarcinoma, 78% in cases of moderately differentiated adenocarcinoma, and 71% in cases of poorly differentiated adenocarcinoma. The total positive ratio of endometrial carcinomas was 77%.

In cases of well differentiated adenocarcinoma, BFP positive cells were located sporadically along the glandular structures in general, although some variations were noted in individual carcinoma foci. Many cells in the glandular cavity showed a positive reaction. In positive cells, a part of or the entire cytoplasm was stained diffusely with no consistent tendency in the staining pattern (Fig. 2-A).

In cases of moderately differentiated adenocarcinoma, the staining tendency was similar to that in cases of well differentiated adenocarcinoma. Localization of positive cells was sporadic both in the glandular structures and in the portions of substantial proliferation.
In cases of poorly differentiated adenocarcinoma, a small number of positive cells were present sporadically. Their cytoplasm showed diffuse staining (Fig. 2-B).

In adenoacanthomas, some of the tumor cells in the glandular portions showed granular or diffuse staining in the cytoplasm. In the portions of squamous metaplasia, metaplastic cells with abundant cytoplasm in the center of the glandular foci showed a diffuse positive reaction of the cytoplasm.

The endometrium in the proliferative phase showed diffuse staining of almost all the glandular epithelium. When vacuoles appeared above the nuclei and cells shifted generally to the climax of the secretory phase, the positive area in cytoplasm above the nuclei decreased and positive areas were exclusively retained in the basal portions of these cells. In cystic hyperplasia, only a part of glandular epithelial cells showed granular positive parts. On the contrary, adenomatous hyperplasia showed an accumulation of glandular cells with a diffuse or granular positive reaction or mixtures of positive and negative cells in various proportions. Such a tendency was more marked in atypical hyperplasia.

c) Carcinomas of the uterine cervix

All of the cases examined showed positive cells. Irrespective of the large cell non-keratinizing (LNK) type and keratinizing (K) type, many tumor cells were BFP positive. LNK cases sometimes contained cells with a strong positive reaction in the apical portions of tumor tissues (Fig. 3-B). However, positive cells were frequently observed adjacent to the cancer pearl in K type cases. In these cases, thin disconnected ring-like layers of positive keratinizing cells were sometimes observed in the cancer pearl itself or parts indicating hyper-parakeratosis (Fig. 3-C). Large cells with abundant cytoplasm showed spot or diffuse positive figures and monocellular keratinizing cells showed positive figures in a part of the cytoplasm.

Localization of BFP was also observed in non-cancerous squamous epithelium of the uterine cervix or vagina. BFP was observed spottily or diffusely in cells of stratum germinaticum and stratum granulosum of normal epithelium. In dysplastic epithelium, almost all dysplastic cells showed a diffuse stain of the cytoplasm, although there were few differences between normal epithelium and dysplastic ones in the DAB coloration. Large cells with abun-
dant cytoplasm showed a diffuse or partial positive reaction in the cytoplasm. In reserve cell hyperplasia, almost all cells were stained positive (Fig. 3-A). In the parakeratosis portions, negative or spotted positive figures were observed.

2. Immunoelectron microscopic findings

BFP was observed in the cytoplasm and ribosome on the rough endoplasmic reticula (RER).

Serous cystadenocarcinoma of the ovary showed diffuse reaction products in both the cytoplasm and the

Figs. 3A-C. Localization of basic fetoprotein in reserve cell hyperplasia and cervical cancer.

A. Reserve cell hyperplasia.

Hyperplastic reserve cells showed a positive image (RH), and squamous metaplastic cells became partly positive (SQ) and dysplastic cells enhanced the epithelium (DY). ABC method. Counterstain with methylgreen. ×100.

B. Cervical cancer (squamous cell carcinoma, large cell non-keratinizing type)

Cells with a strong positive reaction in the apical portions of the tumor tissues. ABC method. Counterstain with hematoxyline. ×250

C. Cervical cancer (squamous cell carcinoma, keratinizing type)

Positive cells were frequently observed adjacent to the cancer pearl. Thin disconnected ring-like layers of positive keratinizing cells were sometimes observed in the cancer pearl itself or parts indicating hyperparakeratosis. ABC method. Counterstain with hematoxyline. ×250
ribosomes of cells with well-developed organelles such as RER and mitochondria. These products were also observed even beneath the microvilli and apical membrane in some cases. There was no morphological difference between adjacent negative and positive cells (Fig. 4-A).

In clear cell carcinomas the BFP was observed in the cytoplasm and did not show their localization on glycogen or smooth surfaced endoplasmic reticula (Fig. 4-B).

In endometrial carcinomas, positive images were located similarly in the cytoplasm and ribosome of RER and showed a spotted pattern (Fig. 4-C).

In carcinomas of the uterine cervix, positive cells

---

**Figs. 4A-D.** Ultrastructural localization of basic fetoprotein.

A. Serous cystadenocarcinoma. Reaction products are distributed in cytoplasm and ribosomes on rough-surfaced endoplasmic reticula (RER). MT: Mitochondria. With lead citrate staining. ×20,000

B. Clear cell carcinoma. Reaction products in cytoplasm. Note no positive image on smooth-surfaced endoplasmic reticula (SER). N: Nucleus. Without electron staining. ×18,000

C. Endometrial carcinomas (moderately differentiated adenocarcinoma.) Diffuse or spotty positive image in cytoplasm. With lead citrate staining. ×20,000

D. Cervical carcinoma (squamous cell carcinoma, large cell non-keratinizing type) Spotty decorations were observed just beneath the endoplasmic membrane (arrow). N: Nuclei. Without electron staining. ×25,000
had well developed Golgi apparatus and BFP was seen spotted in cytoplasm beneath the membranes. (Fig. 4-D).

**DISCUSSION**

BFP is a basic protein with a molecular weight of 55,000 and was discovered in the human fetal serum and supernatant of homogenate prepared from the intestine or brain (3-7, 11). In order to determine the localization of BFP in tissues, the first step should be to investigate the degree to which the antigenicity of BFP was preserved during the fixation and embedding processes of tissues. Protein type antigens are said to preserve sufficient antigenicity in an aldehyde type fixative. In this study, an antigenicity of BFP was preserved in frozen sections fixed in 4% paraformdehyde, as well as formalin-fixed paraffin sections. Localization of BFP was thus observable both under light and electron microscopy. Localization of BFP in the cytoplasm was confirmed by electron microscopy. Although there was a report regarding the localization of BFP in the nucleus, it was considered to be an artifact accompanying the fixation process (13).

In light microscopy, BFP was observed diffusely or sometimes granularly in the cytoplasm. In the glandular structures of serous cystadenocarcinomas and endometrial carcinomas, cells with a strong positive reaction were observed frequently in the apical portions of the papillary proliferation.

However, in mucinous cystadenocarcinomas, cells containing mucin showed a positive reaction only in a limited part of the cytoplasm. On the other hand, cells not containing mucin showed diffuse localization of BFP in their cytoplasm and were located rather abundantly in the area adjacent to the interstitial tissues.

In clear cell carcinomas, the majority of cells containing glycogen was negative. On the other hand, cells poor in glycogen showed a diffuse positive reaction. Furthermore, in carcinomas of the uterine cervix, cells with an abundant cytoplasm and keratinizing tendency were BFP positive at a high incidence. However only traces of positive reaction were observed in the parakeratotic foci and cancer pearl. In normal squamous epithelium of uterine cervix, localization of BFP was observed only from stratum germinaticum to stratum granulosum.

Based on the above observations, localization of BFP was considered to be decreased or absent in cells containing mucin, glycogen, or keratin which accumulated in the cytoplasm accompanying the maturation and differentiation of cells. Electron-microscopical reaction products located along the glandular cavity of the papillary proliferation in serous cystadenocarcinoma cells, which were rich in organella. Similarly, positive cells in carcinomas of the uterine body or cervix showed well-developed organella. In addition, BFP positive cells were frequently observed in the apical portions of the tumors. Based on these findings, the appearance of BFP in tumor cells was considered to be derived and accompany the active cellular actions under dedifferentiation rather than the parameter of cellular differentiation or maturation.

By electron microscopy, reaction products were found in ribosomes on RER and in cytoplasm. The localization was considered to indicate the protein synthesizing process in which BFP was synthesized in ribosomes on RER and distributed to cytoplasm. BFP was thus regarded as a functional protein. The layers of squamous cells in the maturing process gradually lost BFP staining. BFP decreased or disappeared in cells of mucinous cystadenocarcinoma, clear cell carcinoma, or squamous cell carcinoma, which accumulated their characteristic substances completely. It is suggested that BFP provides a necessary field in synthesizing a substance which is characteristically seen in those cells, and plays the role of a catalyzer. Since BFP appears at the period when the cells actively proliferate, it would play an enzymatic part of the metabolic system which is necessary for proliferation and growth of cells.

It is interesting to know what the mechanism is for transportation of BFP synthesized in cells into the blood. Kurosumi et al. classified the excretion pattern of substances into the following: namely, type I, whole excretion type; type II, apocrine type; type III, open port excretion type; and type IV, dialyzing type (12). The blood concentration of BFP is thus expected to increase during necrosis or inflammation by whole excretion, because BFP existed in the cytoplasm. Since no limiting membrane around BFP was observed and the molecular weight of BFP was small, dialyzing excretion was considered to play an important role in the transportation of BFP into the blood.

In order to understand the significance of localization of BFP in malignant tumors, it is necessary to compare the staining characteristics of normal tissue, precancerous lesions, and cancer. BFP was detected in the cytoplasm of normal, precancerous and tumor cells. There was no difference of DAB reaction seen among these cells. Finally it was considered that BFP is a parameter of cellular activity and indicates a certain degree of their differentiation.

Immunohistological findings and concentration of
BFP in blood showed an interesting relationship. Ishii et al. reported that BFP level in the blood increased in 17% and 19% of benign tumors of the ovary and uterus (8), respectively. The data were thus supported morphologically by our results. There was no difference in the BFP positive ratio in tissues of ovarian cancers, endometrial carcinomas, or carcinomas of the uterine cervix in clinical stages or among histological types. BFP is thus considered to be applicable as a nonspecific marker for gynecological tumors.

ACKNOWLEDGEMENT

The authors express special thanks to Dr. M. Ishii (Saitama Cancer Center), Prof. H. Nagura (Department of Pathology, Tohoku University), Prof. I. Nishiya Associated Prof. T. Kagabu and Assistant Prof. T. Izutsu, (Department of Obstetrics and Gynecology, Iwate Medical University) for their information and useful advice.

Thanks are also due to Miss S. Tomite, Miss. N. Satoh, Mr. H. Sotoyanagi and Mr. Y. Yoshida for technical assistance.

REFERENCES