Neuroectodermal immunophenotype in uterine malignant mullerian tumors (MMT): Comparative immunohistochemical analysis with embryonal uterine development

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Summary

Multidirectional differentiation of neoplastic cells in uterine MMT is still a subject of controversy. The present study was designed to assess the immunophenotype of 15 uterine MMT paying special attention to the markers of neural (neuroendocrine) differentiation. In addition, the same immunohistochemical study was performed on 20 human fetal specimens in order to establish possible relationships between the immunophenotype of MMT and the expression of the corresponding antigens in the fetal tissues of the embryonal female genital tract. Besides the typical immunohistochemical patterns in three cases the epithelial component showed simultaneous coexpression of vimentin and desmin, EMA and cytokeratin, whereas epithelial markers were coexpressed with vimentin in the sarcomatous component of one adenosarcoma. Moreover, both components were immunoreactive to the markers of neural differentiation (PGP 9.5, GFAP, HNK-1, N-CAM, HBA71). This aberrant expression was not correlated with morphological signs of neural differentiation at either light microscopy or ultrastructural levels.

Regarding the analysis of fetal tissues, both epithelial and mesenchymal elements in the fetal genital tract expressed the above-mentioned neural markers at different dates of gestation. The intensity of this expression diminishes as the fetus matures and at the end of antenatal life the immunophenotype characteristic for adult life is established.

Taking into consideration the capacity of uterine tissue to reproduce embryonal phenotype during neoplastic transformation, we studied this abnormal immunoprofile and its hypothetic value for the diagnosis and prognosis of MMT.

Key words: Uterine MMT; Immunophenotype of carcinosarcomas; Antigen coexpression; Neural differentiation; Immunophenotype of embryonal uterus.

Introduction

Malignant mixed mullerian tumors (MMT) of the uterus represent common biphasic neoplasms of the female genital tract. The majority of them comprise both malignant components (carcinosarcomas, CS) or less frequently benign epithelial and malignant mesenchymal components (adenosarcomas).

Traditionally, uterine carcinosarcomas have been classified into two major groups. The main criterion for such a division is the structure of the sarcomatous component. Tumors containing sarcomatous elements indigenous to the uterus (smooth muscle, fibromatous, cells of endometrial stroma) are called homologous, whereas those comprising tissues not native to the uterus (striated muscle, cartilage, bone) are called heterologous carcinosarcomas or mixed Mullerian mesodermal tumors. This classification has no prognostic significance [1].

Several theories have been proposed to explain the genesis of CS. The collision theory suggests that CS is a mixture of two independent neoplasms. The conversion theory assumes the transmutation of one type of neoplastic cell to the other. The combination theory in which both components of CS arise from the common stem cell precursor is widely accepted and supported by molecular genetic analysis [2-4].

Contrary to the assumption of the dominant role of the sarcomatous component in the progression of the disease, recent data favor the importance of the epithelial component as "driver" of the process. Furthermore, carcinoma cells constitute the major invading part of CS, and are associated with extrauterine spread and lymphatic or vascular invasion [5-7].

Advances in immunochemistry and molecular biology have significantly improved our understanding of some of the mechanisms of the histogenesis of CS. The simultaneous expression of epithelial and mesenchymal markers (keratin and vimentin, respectively) in both components of the tumors favors a monoclonal origin [6]. Also, immunohistochemical analysis is useful in order to define heterologous components of MMT, such as rhabdomyoblastic cells (positive for desmin and myogenin) or neuroendocrine elements that express either chromogranin or other neural markers [8, 9]. The presence of neuroectodermal traits of differentiation, represented by morphological structures such as pseudorosettes, melanosomes, fibrillary cytoplasmic processes, reinforced with the expression of neural markers, has been

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reported in some cases of MMT [10-12]. In the present paper, we undertook the immunohistochemical investigation of the uterine tumours which according to the traditional histological criteria fell into the category of MMT with special emphasis on the expression of neural antigens. To clarify the mechanisms of their expression we also studied the immunophenotype of the cellular elements of the human female genital tract at different stages of embryonal development.

Material and Methods

We selected from our files (2 laboratories), 15 tumors displaying convincing signs of dimorphic differentiation and diagnosed as carcinosarcomas or adenosarcomas. In addition, 20 human fetal genitalia were obtained from aborted embryos and fetuses of 10-40 weeks of gestation. The gestational ages in weeks: 10, 12, 13, 14 (2 cases), 15 (2 cases), 16, 17, 18 (2 cases), 20, 22 (2 cases), 26, 30 (2 cases), 35, 40 (2 cases) were calculated from the dates of the last menstruation periods of the mothers and crown-rump lengths of the fetuses.

Tissues were fixed in 10% neutral-buffered formalin, paraffin embeded and cut at 5 microns. For electron microscopical studies, the tissues were fixed in 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide and embedded in epon 812. Thick sections were stained with toluidine blue, thin sections with uranyl acetate and lead citrate and examined in a JEM-1200 EX-11 transmission electron microscope. Immunohistochemical analysis was performed using the ABC peroxydase method. Antigen retrieval was obtained by heating in autoclave (1.5 atm, 3 minutes), using buffer citrate pH 6. Antibodies, dilutions and sources are listed in Table 1.

Clinical data

The age of the patients ranged from 53 to 78 yrs, mean, 57 yrs. Clinical manifestation of the disease was mainly bloody discharge and abdominal swelling. In three patients the tumor was an incidental finding at gynecological examination.

In all patients the diagnosis of malignant neoplasm was rendered after curettage.

Table 1. — *Antibodies used.*

	Antibody	Dilution	Source
1	Vimentin	1/40	Dako
2	Desmin	1/100	Dako
3	Actin	1/20	Biogenex
4	Myogenin	1/100	Santa Cruz, Biotech
5	CK	1/20	Dako
6	Cam5.2	1/20	Biomeda
7	EMA	1/200	Dako
8	S-100	1/200	Dako
9	CD34	1/20	Biogenex
10	Chromogranin	1/50	Biomeda
11	GFAP	1/200	Dako
12	PGP9.5	1/100	Biomeda
13	HNK	Undiluted	ATCC
14	NCAM	1/50	Sigma
15	HBA71	1/20	Signet
16	NSE	1/50	Biogenex
17	Trk A	1/50	Santa Cruz Biotech
18	C-kit	1/50	Biogenex

All patients underwent radical hysterectomy with bilateral salpingo-oophorectomy and omentectomy. In two cases the surgical intervention was incomplete. Postoperatively all patients received irradiation as well as multiple courses of chemotherapy. In addition to metastatic deposits observed at admittance, in some cases subsequent metastases were found predominantly in the abdomen, liver, kidneys, bones, mediastinal and retroperitoneal lymph nodes.

Necropsy was performed in two cases; the metastases were extensive and involved the peritoneal and pleural cavities, liver, kidneys, retroperitoneal and mediastinal lymph nodes.

Ten patients died of disease, one patient died of cardiovascular complications, two are alive with no evidence of disease. No follow-up was available on the two remaining cases.

Pathological findings

Grossly, the tumors were polypoid masses or nodes of up to 7 cm in maximum diameter. In two cases the tumors enlarged diffusely within the uterus and involved all genitalia, measuring 30-40 cm and reaching the diaphragm. Except in one intramucosal case, the tumors invaded deeply into the uterine wall, and adjacent pelvic fat.

Microscopical findings of all tumors are summarised in Table 2. Three cases were typified as adenosarcomas represented by a benign epithelial component (2 of them endometrial and one serous type) and malignant sarcomatous elements consisting of rhabdomyosarcoma (1 case) and leiomyosarcoma (2 cases). Twelve cases were carcinosarcomas in which the epithelial component was endometrioid (9 cases), serous (2 cases) and clear cell carcinoma (2 cases), whereas the sarcomatous component was homologous in seven cases (endometrial stromal sarcoma, leiomyosarcoma and spindle cell fibrosarcoma) and heterologous in five patients (rhabdomyosarcoma in 4 cases and chondrosarcoma in 1 case, respectively).

The cellular composition of the invading component of the tumors and their metastasis was different. We observed invading carcinomatous elements in three cases and sarcomatous in five cases. Both components invaded in six cases. Vascular emboli were both of carcinomatous and sarcomatous nature with slight predominance of the carcinomatous type. In the metastatic foci, we observed monophasic as well as biphasic elements.

Electron Microscopy

Ultrastructural studies of ten CS and two adenosarcomas revealed epithelial cells with desmosomes or tight junctions, apical microvilli, agglomeration of glycogen, secretory type granules and lysosomes. The cells of the sarcomatous component contained filamentous material usually with dense bodies and typical structures of rhabdomyoblastic differentiation with parallel arrays of thin and thick filaments and Z bands.

The cells which labelled to chromogranin contained dense core granules. However, we did not observe specific structures in the cells labelled with other neural markers.

In the cells coexpressing vimentin and keratin we observed fibrillary structures characteristic of both types mentioned.

Immunohistochemistry

The results of the immunohistochemical findings are listed in Table 2.

Table 2. — *Immunohistochemical results.*

Tumor type	No.	Component of the tumor (epithelial/ mesenchymal)	EMA	CK	VM	Actin	Desmin	Myogenin	S-100	Chromogr	HBA 71	NSE	NCAM	HNK	GFAP	PGP 9.5	Trk A	c-kit
Adenosarcoma	1	Endometrial	+	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–
		Homologous	+	+	+	+	+	–	–	–	+	+	–	–	–	+	–	–
	2	Endometrial	+	+	–	–	+	–	–	–	+	–	–	–	–	+	+	–
		Homologous	–	–	+	–	–	–	–	–	+	–	–	–	–	+	+	–
	3	Serous	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		Heterologous	–	–	+	+	+	+	–	–	–	+	–	–	+	+	–	–
Carcinosarcomas	4	Endometrial	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–
		Homologous	–	–	+	+	–	–	–	–	+	–	–	–	–	+	–	–
	5	Clear cell	+	+	–	–	–	—	–	–	–	–	–	–	+	+	+	–
		Heterologous	–	–	+	+	+	–	+	–	–	–	–	–	+	+	–	+
	6	Endometrial	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–
		Heterologous	–	–	+	+	+	+	–	–	–	+	–	–	–	+	–	–
	7	Clear cell	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
		Heterologous	–	–	+	+	+	+	–	–	–	–	–	–	–	+	+ –	–
	8	Endometrial	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–
		Homologous	–	–	+	–	–	–	–	–	–	–	–	+	–	+	+	–
	9	Serous	+	+	–	–	–	–	–	–	–	–	–	+	–	+	–	–
		Heterologous	–	–	+	+	+	+	–	–	–	–	–	+	–	+	–	–
	10	Endometrial	+	+	+	–	–	–	+	+	–	–	–	+	–	–	–	–
		Homologous	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–
	11	Endometrial+ clear cell	+	+	–	–	–	–	–	–	–	+	–	+	+	+	–	–
		Homologous	–	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–
	12	Endometrial	+	+	+	–	–	–	–	–	–	+	–	+	+	–	–	–
		Homologous	–	–	+	+	+	–	–	–	–	+	–	+	–	+	–	–
	13	Endometrial	+	+	–	–	–	–	–	+	–	+	–	+	–	+	–	–
		Homologous	–	–	+	+	–	–	–	–	–	+	–	–	–	+	–	–
	14	Endometrial	+	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–
		Heterologous	–	–	+	+	+	+	–	–	–	+	–	–	–	+	–	–
	15	Endometrial+ clear cell	+	+	–	–	–	–	–	+	–	–	–	–	–	+	–	–
		Homologous	–	–	+	–	—	–	–	–	–	–	–	–	–	+	–	–

Epithelial component

EMA and CK were uniformly positive in the cells of the epithelial component. Two CS containing endometrioid type adenocarcinoma showed vimentin positivity (Figure 1a). Actin and myogenin were negative, as was desmin except in one case of benign cystadenomatous areas where the lining cells showed strong desmin positivity. The epithelial component was S-100 positive in two cases. One was endometrioid carcinoma and the second adenosarcoma.

Small papillary structures were chromogranin positive in three cases of endometrial type carcinomas and one clear cell carcinoma (Figure 1b).

N-CAM positivity was slight and focal. HNK was positive in seven carcinomatous componenets irrespective of their type. The staining was predominantly apical, though in one case the glandular-cribrous structures gave a very intensive positive reaction. The cells of normal adjacent endometrium showed HNK positivity in several cases.

HBA-71 was usually negative, only small foci of benign adenomatous structures in one adenosarcoma showed immunopositivity for this marker. Two cases, one endometrial and one clear cell, labelled with Trk A.

The cells of the endometrial carcinomatous component of three patients and one clear cell carcinoma expressed focal immunostaining for GFAP (Figure 1c). On the contrary, carcinomatous cells in seven cases demonstrated a marked immunopositivity to PGP 9.5.

NSE labelled in the six endometrioid-type carcinomas and in the epithelial component of one adenosarcoma.

Sarcomatous component

The cells of the sarcomatous component were usually negative for EMA and CK. Only in one case of adenosarcoma were both markers positive in the mesenchymal and sarcomatous component (Figure 1d).

Immunopositivity for vimentin was of various intensity, from the fields of strongly positive reacting cells to comparatively low intensive foci. It is also worth noting that the neoplastic cells in blood vessels gave much stronger reaction to vimentin than the surrounding cells outside the vessels.The highest intensity of vimentin staining was encountered in the multinucleated giant cells.

Desmin was positive in nine cases, predominantly in the foci of rabdomyoblastic differentiation, and also in multinucleated giant cells as well. Staining for actin was less intensive and

Fig. 1a

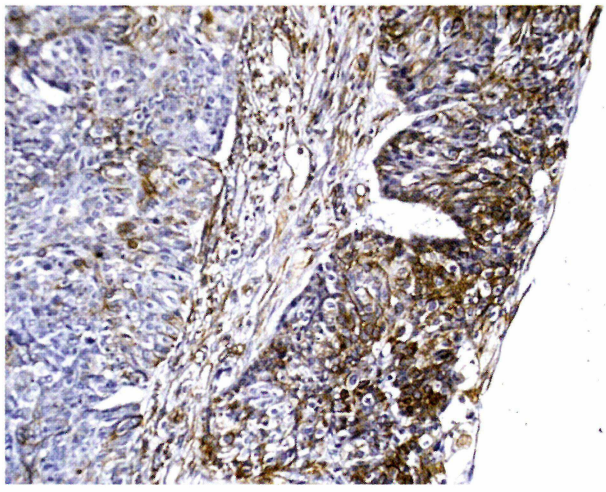


Fig.

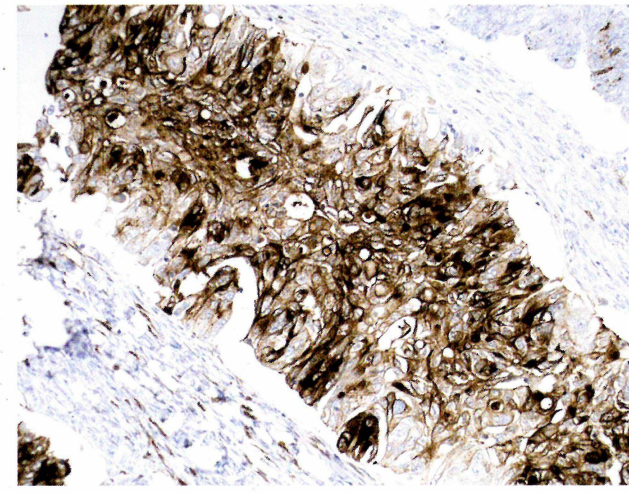
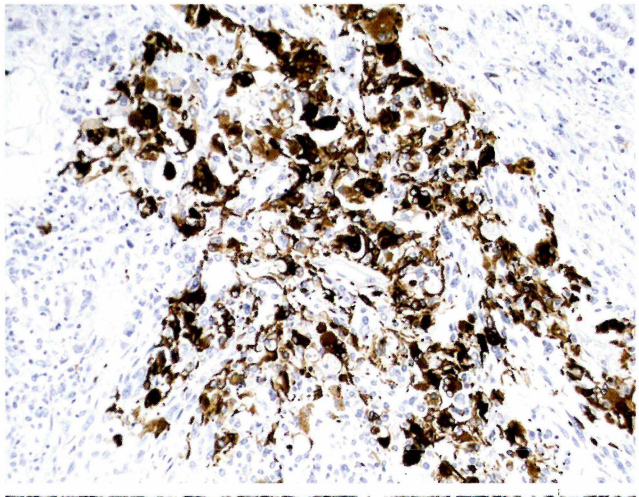


Fig. 1c



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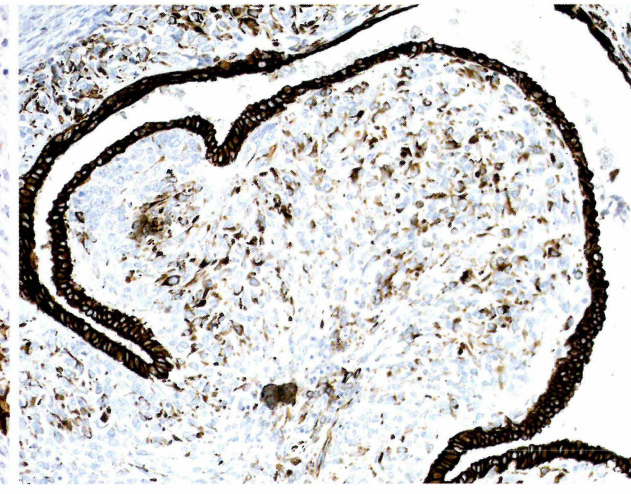
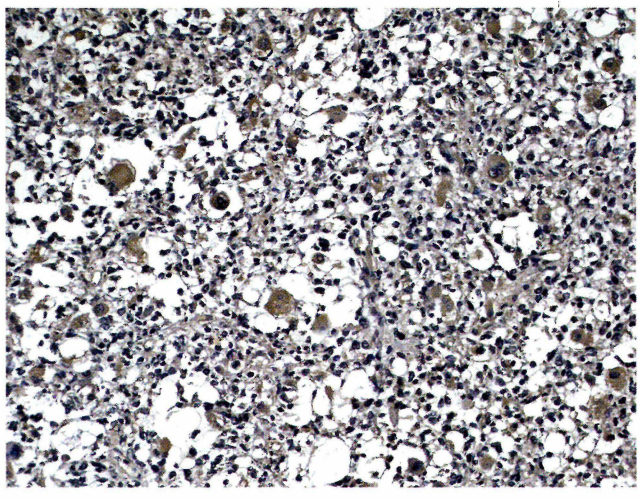


Fig. 1e



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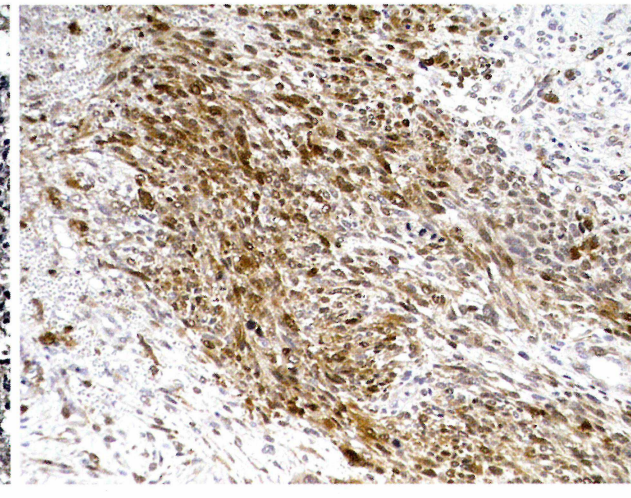
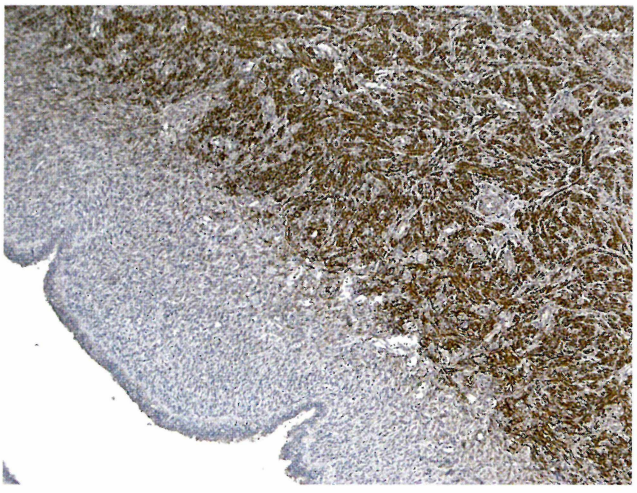


Figure 1. — Immunohistochemical results - uterine carcinosarcomas:
a) Vimentine positivity in the epithelial component of MMT;
b) Chromogranin expression in carcinomatous structures;
c) Positive reaction to GFAP in carcinomatous component of MMT;
d) CK is positive in both epithelial and sarcomatous components of adenosarcoma;
e) GFAP positivity in multinucleated giant rhabdomyoblasts;
f) PGP 9.5 staining in the sarcomatous cells.

g. 2a



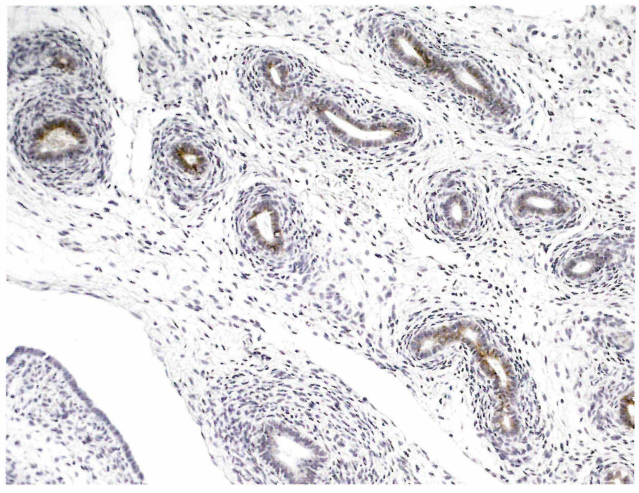


Fig. 2b

g. 2c

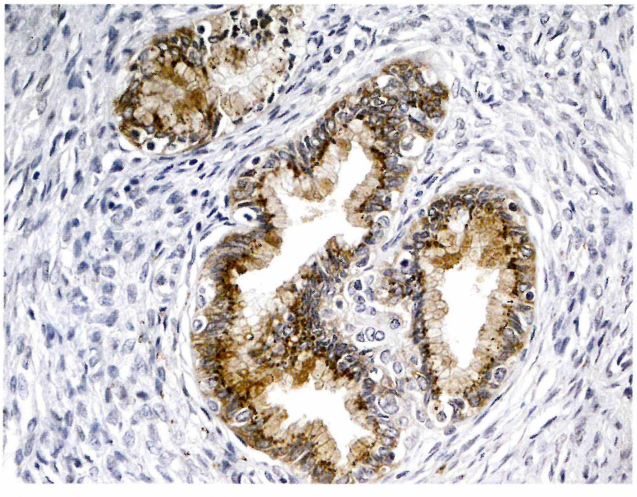


Fig. 2d

Figure 2. — Immunohistochemical results - embryonal uterus:
a) Subepithelial cells negative for desmin in the fetal uterine wall compared with the high expression of adjacent myometrium;
b) Staining for GFAP in the tubular structures of the regressing mesonephros;
c) PGP 9.5 staining in the glands of the fetal uterus;
d) PGP 9.5 staining in the muscle of the fetal uterus.

uniform. Myogenin labelling exhibited all rhabdomyosarcomas and one chondrosarcoma in the poorly differentiated mesenchymal component of the tumor. Sarcomatous cells were always immunonegative for NCAM, chromogranin and S-100, except the chondrosarcomatous areas which showed intensive positive immunoreactivity for S-100.

HNK was positive in four cases, usually focally in small groups of cells, but highly intensive in homologous as well as in heterologous sarcomas.

Cells with immunopositive staining for HBA-71 were disclosed in three cases - two adenosarcomas and one CS. All of them contained the homologous sarcomatous component.

Strong GFAP immunopositivity was observed in two cases. In one case the marker was positive in the rhabdoid giant cells (Figure 1e), and in the second, in the immature blastemic cells around the chondrosarcomatous nests.

The cells of the sarcomatous component of all tumors showed strong immunopositivity for PGP 9.5 (Figure 1f), though in homologous CS the reaction was weaker than in heterologous CS, and intense in the multinucleated giant cells. NSE labelled the sarcomatous cells of all six types of tumors including two adenosarcomas. Trk A immunopositivity exhibited small foci in the mesenchymal component of one adenosarcoma (homologous) and two CS. Finally, patchy labelling of c-kit was revealed in the chondrosarcomatous cells of one CS.

Immunohistochemistry of fetal tissues

The microscopical structure of the developing uterus represents stages of proliferation of mesenchymal cells around the Mullerian duct. Gradually the monolayer epithelial lining of the duct invaginates into the underlying tissues forming the glands. The caudal portion of the duct undergoes intensive keratinisation characteristic of the ectocervix.

Our studies have revealed that the expression of various antigens during antenatal development differs in the cranio-caudal direction as well as from outer to inner surfaces of the presumptive uterus (serosal-endometrial direction).

EMA was positive in the ten-week embryos throughout the whole length of the future genital tract. Intensity of labelling was highest at 22 weeks in the ectocervix. At 35 weeks,

labelling arose in the invaginates of future glands especially at the junction of the endometrial and endocervical epithelia. Expression of CK labelling was similar except in its comparative low intensity.

Vimentin was strongly expressed not only in mesenchymal cells, but in the epithelium of the presumptive fallopian tubes, endometrium and the cells of mesonephric rests. Gradually vimentin labelling of the epithelial cells diminished and by 26 weeks disappeared.

Immunopositivity to desmin emerged early and was extensive. The intensity of labelling decreased near the epithelial lining and by 20-22 weeks subepithelial layers were always desmin immunonegative (Figure 2a).

The first actin labelled cells were also found in the outer subserosal layers of the future uterus but unlike vimentin and desmin, actin expression was low and actin labelled cells were never encountered in the inner third of the organ.

Concerning neural markers, S-100 labelled only vascular elements, ganglion cells and nerves. Chromogranin immunopositivity was disclosed in the cells of future endocervical glands at 14-17 weeks of development. HNK-1 labelled the ectocervical epithelium at 14-17 weeks of gestation and nerves and vessels throughout the whole period of development. HBA-71 immunopositivity appeared in the apical part of the endometrial epithelium by 20-22 weeks of gestation, and in the future endocervical glands at 40 weeks of gestation. GFAP positive cells were never encountered in the uterus. Only the cells of degenerating mesonephros at the early stages of development showed marked immunopositivity for GFAP (Figure 2b), and this reaction was maintained up to and including 22 weeks. Both mesenchymal and epithelial cells showed high expression of PGP 9.5 (Figures 2c, d). Gradually, intensity of staining and areas of labelled cells decreased. In the fetuses of 30 weeks' gestation PGP 9.5 labelled only apical surfaces of the lining epithelium and the muscle cells of the outer part. The muscular cells of the inner part of the uterus after 26 weeks of gestation did not contain PGP 9.5 positive cells.

Discussion

We did not find confirmation of the dominating role of carcinomatous components of CS as "drivers" of aggressive behavior. According to our observations the front of invasion was represented both by carcinomatous as well as sarcomatous components, though the emboli in the blood and lymph vessels more frequently displayed epithelial phenotype. Neither could we demonstrate the dependence between the predominant cellular structures and the anatomical shape of the sites of dissemination. According to Sreenan and Hart [7], CS grows in anatomical sites with hollow spaces as sarcoma. However, we encountered all types of structures – dimorph, isolated carcinomatous or isolated sarcomatous in all locations – abdominal cavity, vagina, etc.

Among the results of our investigations which merit special attention are some specific patterns of immunostaining in MMT – a coexpression of epithelial and mesenchymal markers and aberrant ectopic staining of so-called neural antigens.

We found such patterns in both epithelial and mesenchymal components of MMT. In several cases the cells of normal endometrial glands showed immunoreactivity to desmin, S-100, and HNK.

The distribution of ectopic reactions lacked systemic character. Desmin and S-100 labelled benign epithelium in adenosarcoma but on the other hand S-100 positivity was disclosed in endometrial type adenocarcinoma. PGP 9.5 labelled different types of structures in eight cases.

Coexpression of epithelial and mesenchymal antigens in uterine and ovarian MMT has been previously described. De Brito *et al.* [13] found immunoreactivity with an epithelial marker in the stromal component of 60% uterine and 43% ovarian carcinosarcomas. They confirmed these results with ultrastructural studies revealing in EM the cells of hybrid, epithelial/stromal characteristics. Geisinger *et al.* [14] demonstrated cytokeratin and vimentin coexpression in both components of uterine MMT. Ramadan and Goudie [15] showed immunoreactivity of the stromal cells in uterine MMT with antibodies to EMA and cytokeratins. In spite of the similarity of these findings their interpretation is different. De Brito and Costa [13, 16] considered such a coexpression as an argument for metaplasia of the epithelial cells into sarcomatous ones. George *et al.* [8] believe it to be proof of a common cell origin of CS. Some authors are of the opinion that the labelling of neoplastic cells with epithelial markers is inconsistent with the diagnosis of CS and reclassify such tumors as carcinomas. More controversy arose in classifying the MMT after communications on expression of so-called neural (neuroendocrine), markers. A good deal of uterine and ovarian MMTs were classified as "neuroectodermal" and "neuroendocrine" [8, 11]. Later it turned out that the mentioned markers are expressed in non-neural neoplasms: PGP9. 5 in prostatic cancer neuroendocrine cells [17], GFAP in renal carcinomas [18], NCAM in adenoid cystic carcinoma cell lines [19], embryonal rhabdomyosarcoma [20], GFAP and Leu-7 in fetal rhabdomyoma [21] or PGP 9.5 in prostatic glands [22].

Immunohistochemical studies of the antenatal development of the female genital tract have demonstrated that a broad spectrum of antigens is expressed in the embryonal Female Genital Tract (FGT) and this process has several spatiotemporal peculiarities. It is evident to embryologists that the distal parts of the Mullerian canal matures faster than the proximal [23-25]. Some of our data on immunoreactivity with different antigens confirm this postulation. For example, future ectocervix labels with cytokeratins much earlier than the tubal or uterine fundal epithelium. Maturation of muscle cells – labelling to actin and desmin – begins from the outer serosal part of the uterus and proceeds in an internal endometrial direction. This fact is in accordance with the data from electron microscopical (EM) investigations which show the same sequence of events. Although ultrastructurally miofilamentous material is detectable at 18 weeks of gestation and mature smooth muscle structures at 31 weeks, we had already found strong immunoreactivity to actin and desmin by 13-14 weeks of gestation. Most probably synthesis of muscle contractile proteins proceeds their structural shaping.

The rate of expression of several antigens decreases as

the embryo matures. Some of them completely disappear or are preserved in fewer structures. Vimentin labels all cellular elements throughout the entire Mullerian canal but gradually its expression is maintained only in the mesenchymal cells. Such transitory immunopositivity displayed chromogranin by 14-17 weeks in the future cervical glands, HBA-71 by 20-22 weeks in the endometrial and at 40 weeks in the cervical epithelium. GFAP labels the cells of degenerating mesonephros, and canalicular structures of the future rete ovarii from 14-15 up to 22 weeks inclusive. PGP 9.5 immunoreactivity is strongly positive until 24-26 weeks both in epithelial and mesenchymal cells. Some of our results are in accordance with the observations of other authors. Aumuller and Haley [17, 26] found marked PGP 9.5 immunopositivity in the cells of human embryonal lung, Wolffian duct and mesonephros. Goos *et al.* [27], documented GFAP immunopositivity in embryonal kidney and epithelium of the lung.

We are of the opinion that the pathologist has to assess the immunopositivity of each antigen, carefully taking into consideration its structural and functional properties. In our material we could clearly distinguish two types of so-called "neural" antigens. Some of them are matrical proteins and have their own structural analogues in the cell. For example chromogranin is associated with neurosecretory granules and can be regarded as absolutely specific for neuroendocrine differentiation [9]. Others have no ultrastructural parameters and may be of temporary functional character. For example PGP 9.5 removes ubiquitin, an intermediate filament, from other proteins and protects them from degeneration by proteases. NCAM neural cell adhesion molecule is a cell membrane protein that has a role in the cohesiveness of cells in NS, etc. Part of these antigens can be expressed in non-neuronal tissues as well [9].

We think that an additional type of differentiation should be established in those cases when the positive staining antigen is a matrical protein, and has a relevant light morphological and ultrastructural image, as for instance, chromogranin, neurofilaments etc. Such examples of diagnostic approaches are well-known in the literature, as in the case of Fukunaga *et al.* [10]. They established the extensive neuroectodermal differentiation in conventional carcinosarcoma. This included small to medium sized cells characterized by fibrillary cytoplasmic processes, rosette-like formations and immunopositivity to GFAP, synaptophisin, Leu-7 and NSE. Another example is the case of Amant *et al.* [28], a uterine carcinosarcoma with melanotic differentiation in which the cells immunopositive with S-100 and HMB-45, contained giant cytoplasmic melanosomes at the ultrastructural level. Moreover, a high expression of neuroendocrine markers, correlated with the ultrastructural demonstration of neurosecretory granules, has been reported in both carcinomatous and sarcomatous areas in one case of mesenteric MMT [29].

In all other cases immunopositivity to any antigen can be noted in the report but without the necessity of ascribing divergent differentiation to the tumor. This will help to avoid unnecessary complications of the antigenical profile of the tumors which adds nothing to the clinical implication of the pathologic diagnosis.

Such an approach to the problem is of course temporary. The prospects for using molecular biological attributes [2, 3] in the diagnostic process will lead us in the foreseable future to a more rational classification of the neoplasms, including those tumors which exhibit various types of differentiation.

Finally, we report the aberrant expression of neural antigens in MMT without an ultrastructural correlation. The significance of this finding is unknown but we note that such expression is also observed in fetal tissues throughout the development of the female genital tract.

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