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Expression of intermediate filaments and other special markers by testicular germ cell tumors. With reference to embryogenesis

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Summary. Distribution of intermediate filament proteins (IFs) and several special markers was studied in 39 testicular germ cell tumors and 8 embryos and foetuses. The similarity and difference between development of germ cell tumor and embryogenesis were immunohistochemically investigated. Seminoma and embryonal carcinoma, as tumoral counterparts of undifferentiated germ cells, were characterized by little IF expression. This study revealed that the maturing and differentiating process in germ cell tumor is different from normal embryonal development and the tumor cells showed leaping maturing steps in tumorigenesis. Immunostaining for IFs helped to discover the further differentiation occurring in embryonal carcinoma and to demonstrate heterogeneous elements in non-seminoma germ cell tumors, which sometimes might not be apparent by light microscopical observation of H&E staining section. According to the findings, two patterns in mixed germ cell tumors are suggested; i.e., combined and diffuse types. The mechanism of tumorigenesis of the two types is supposed to be different. Clinically, the prognosis of most patients with testicular germ cell tumor is fairly good because of the improved chemotherapies that are dependent on histological diagnosis.

Key words: Testicular germ cell tumors, Intermediate filaments, Embryo and foetus

Introduction

Intermediate filaments (IFs), a class of cytoskeletal proteins, have been considered a powerful tool for objective identification of tumor histogenesis and have gained wide application in differentiation of tumor elements (Gabbiani et al., 1981; Ramaekers et al., 1981; Osborn and Weber, 1983; Lofton et al., 1989). Each IF class has generally been supposed a unique histogenetic marker of cell origin (Franke et al., 1978), therefore, IFs are especially helpful in demonstrating heterogeneity of cell population in tumors. Testicular germ cell tumors are such tumors having the propensity of heterogeneity. Classification and histogenesis of testicular germ cell tumors have been of great interest in the pathologic field (Nistal and Paniagua, 1984; Mostofi et al., 1988), not only for treatment of these tumors, but also for understanding of tumorigenesis. It is believed that identifying individual components of the tumor is a very important step in considering the behaviour of these tumors. Tissue demonstration of IFs in testicular germ cell tumors might provide a good approach to revealing different tumoral components and in making classification of these tumors.

The aim of the present study is to define the expression of IFs by testicular germ cell tumors and to confirm whether these markers could provide more specific information than usual observation with H&E and other routine staining. In relation to the expression of IFs in embryo and foetus, the similarity and difference between the development of germ cell tumor and embryogenesis are investigated, and the pathogenetic way of germ cell tumors is further analyzed.

Materials and methods

Specimens

The material consisted of 39 surgical specimens of germ cell tumor of the testis, which were examined by the department of pathology, Sakai Municipal Hospital (10 specimens) and the department of pathology, the Center for Adult Diseases, Osaka (29 specimens). In addition, tissues from 8 human embryos and foetuses (4 -10 weeks), obtained from abortion materials, were

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Table 1. Antibodies used in this study.

Specificity	Animal source	Supplier	Dilution
Cytokeratin of 45-56kD (including keratin 10, 17, 18)	Mouse	DAKO	1:40
Vimentin	Mouse	DAKO	1:25
Desmin	Mouse	DAKO	1:30
Neurofilaments	Mouse	DAKO	1:50
GFAP	Mouse	DAKO	1:50
CEA	Mouse	DAKO	1:20
EMA	Mouse	DAKO	1:200
HCG	Rabbit	DAKO	1:200
S-100 protein	Rabbit	Ortho	1:300
AFP	Rabbit	Ortho	1:1

Table 2. Clinical data of the patients with testicular germ cell tumors.

Case	Age at surgery	Size of the tumor (and/or weight)	Histological diagnosis	Course of the disease	Treatment	Outcome
1*	26 yr	---	S	Meta. to RPLN	Chemotherapy	Alive 6.7 yr NED
2	57 yr	130g	S	---	Chemotherapy	Alive 1.3 yr NED
3	45 yr	6.5x4.5x3.5 cm, 230g	S	---	Radiation	Alive 2 yr NED
4	24 yr	8x5x4 cm	S	---	Radiation	Alive 8,5 yr NED
5	56 yr	14x10x5 cm, 370g	SS	---	---	Alive 1.7 yr NED
6*	26 yr	---	ECA	Meta. to lung	Chemotherapy	Alive 8 yr NED
7	17 yr	8x5x5 cm, 75 g	ECA	---	---	Alive 8.2 yr NED
8*	40 yr	---	YST	---	---	Alive 7.7 yr NED
9	8 mo	12 g	YST	---	---	Alive 13 yr NED
10	5 mo	3x2.2x2.1 cm, 13.5 g	YST	---	Chemotherapy	Alive 3 yr NED
11	42 yr	7x5x5 cm, 110 g	IT	---	Radiation	Alive 7.5 yr NED
12	19 yr	4x4x3.5 cm, 70 g	IT	---	---	Alive 7.5 yr NED
13	40 yr	4x3x3 cm, 100 g	IT	---	Chemotherapy	Alive 3.2 yr NED
14	27 yr	8x5x4 cm, 145 g	T	---	---	Alive 4.6 yr NED
15	63 yr	25x2x2 cm, 40 g	T	---	---	Alive 8 yr NED
16	28 yr	7x6x5 cm, 115 g	CH	Meta. to lung, PRLN	Chemotherapy	Died 1 mo
17	32 yr	10x8x6 cm, 380 g	CH	Meta. to lung, PRLN	Chemotherapy	Died 2 d
18	31 yr	185 g	S + ST	---	---	Alive 9 mo NED
19	34 yr	10x8x8 cm, 100 g	ECA + S + ST	---	Radiation	Alive 4.3 yr NED
20	20 yr	130 g	ECA + S + ST	---	Chemo. + Radi.	Alive 1.5 yr. NED
21	51 yr	2x2x2 cm, 29 g	ECA + S + ST	---	Chemo. + Radi.	Alive 7.5 yr NED
22	44 yr	80 g	ECA + ST	---	---	Alive 3.9 yr NED
23	27 yr	6x5x3 cm, 65 g	ECA + ST	Meta. to lung	Chemo. + Radi.	Alive 9.9 yr NED
24*	25 yr	---	ECA + ST	Meta. to RPLN	Chemotherapy	Alive 9.7 yr NED
25	26 yr	236 g	ECA + T	---	Chemotherapy	Alive 2.2 yr NED
26*	36 yr	---	ECA + T		Chemotherapy	Alive 6.7 yr NED
27	24 yr	5.5x3.8x3.5 cm, 93 g	ECA + T	---	---	Alive 3.7 yr NED
28	23 yr	---	ECA + T	Meta. to lung, bone	Chemo. + Radi.	Alive 4.1 yr NED
29	27 yr	13x8x7 cm, 320 g	ECA + YST + S + ST	---	Chemotherapy	Alive 6 yr NED
30	35 yr	6x3.5x3.5 cm, 170 g	YST + YST**	---	Radiation	Alive 5.9 yr NED
31	23 yr	180 g	ECA + YST + ST	---	Chemotherapy	Alive 3.4 yr NED
32	34 yr	4.5x3x3 cm, 66 g	YST + T + ST**	---	Chemotherapy	Alive 10 mo NED
33	29 yr	12x7x5 cm, 350 g	ECA + T + S	Meta. to lung, brain, RPLN	Chemo. + Radi.	Alive 6.1 yr NED
34	46 yr	8x7x7 cm, 200 g	ECA + T + CH	---	---	Alive 5.4 yr NED
35	28 yr	8x5x5 cm, 105 g	YST + T + CH	---	---	Alive 4.9 yr NED
36	35 yr	8x7x5 cm, 135 g	ECA + YST + T + S + ST	---	---	Alive 6.8 yr NED
37	17 yr	8x5x5 cm, 75 g	ECA + YST + T	---	---	Alive 8.3 yr NED
38	33 yr	---	ECA + YST + ST	Meta. to RPLN	---	Died 8 mo
39	48 yr	5x3.5x3.5 cm	T + CH	Meta. to RPLN	---	Alive 13 yr NED

S= seminoma, SS= spermatocytic seminoma, ECA=embryonal carcinoma, YST=yolk sac tumor, IT= immature teratoma, T=teratoma. CH=choriocarcinoma, ST= syncytiotrophoblast. RPLN= retroperitonal lymph node, NED=no evidence of disease. * Operation was performed in another hospital. **: There were embryoid bodies in the tumor.

studied together with the tumors. The ages of the embryos or foetuses were estimated from body length measurements with the reference of clinical information.

All the materials were fixed in 10% formalin and embedded in paraffin. One section of each specimen cut from a block was stained with haematoxylin and eosin. Other sections were used for immunostaining.

By using the peroxidase-antiperoxidase (PAP) technique, all the specimens were immunostained for cytokeratin, vimentin, desmin, glial fibrillary acidic protein (GFAP), neurofilaments (NF), S-100 protein, carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), human chorionic gonadotrophin (HCG) and alpha-foetoprotein (AFP). The sections were deparaffinized in xylene, and rehydrated in a graded ethanol series. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. The slides were subsequently incubated at room temperature with two sets of reagents respectively. For monoclonal antibodies; i.e., cytokeratin, vimentin, desmin, GFAP, NF, EMA, CEA, the set included blocking serum, first monoclonal antibodies, second biotinylated anti-mouse antibody, peroxidase reagent, chromogen reagent and haematoxylin. For polyclonal antibodies; i.e., S-100 protein, HCG and AFP, the first antibodies in the set were rabbit antiserum, and the second biotynilated antibody was anti-rabbit antisera. The chromogen reaction was developed in the following incubation medium: 3,3'-diaminobenzidine (DAB), 25 mg; 40 H_2O_2, 50 µl; and PBS, pH 7.3, 50 ml. The slides were then counterstained with haematoxylin, dehydrated, cleared, and mounted with Permount. The characteristics of the antibodies used in this study are shown in Table 1. Moreover, before cytokeratin and desmin immunostaining, sections were first treated with 1 mg/ml trypsin (Difco Laboratories, Detroit, MI, USA), in 0.1% calcium chloride for 1 hr at 37° C.

Clinical data of the patients

The diagnosis of the series and clinical data of the patients are presented in Table 2.

Results

1. Expression of IFs and other special markers in the tumors

The immunostaining results are summarized in Table 3. Grouping is according to the histological components in the tumors. Because some tumors were composed of several components, the total number in the table is greater than the number of the tumors.

Seminoma

Tumor cells in seminoma were negative for most of the antigens. Only occasional cells in the tumors showed weak positivity for vimentin. Endothelial cells of capillary and small vessels were apparently positive for vimentin, and were conspicuous among the tumor cells. HCG-positive trophoblasts were found in 3 of 11 cases.

Spermatocytic seminoma

The tumor cells only showed reactivity with antibody to cytokeratin. The positive staining was found in a scattered collection of the tumor cells with an appearance of submembranous positivity.

Embryonal carcinoma

Except that occasional HCG-positive trophoblasts and AFP-positive tumor cells were found in some of the cases, the tumor cells were seldom positive for any other

Table 3. Intermediate filaments and tissue markers in testicular germ cell tumors

	No.	Cytokeratin	Vimentin	Desmin	NF	GFAP	S-100 protein	HCG	AFP	CEA	EMA
Seminoma	11	±1	±4 +2					+3			
Spermatocytic Seminoma	1	+1									
Embryonal Carcinoma	19	+10	+10[a]	+1[a]				+9	+7		
Choriocarcinoma	5	+3 ++2						+4 ++1			+1
Yolk Sac Tumor	11	+8 ++3	+10 ++1[a]	+1[a]			+1[b]	+4	+10 ++1	+5	±1 +3
Embryoid Body	2	+1 ++1[a]	+1					+2	+2		
Immature Teratoma	13	+7 ++6	+4 ++9[a]	+9 ++2	+1	++1	+10	+5	+4[d]	+11	±1 +11
						+2[c]					++1
Mature Teratoma	3	+1 ++2	+3	+2	+1	+1[c]	+2		+1[d]	+2	+3

a: Positivity in the stroma, b: Positivity in some epithelial cells, c: Positivity in cartilage, d: Positivity in some glandular epithelia, **: more than 50% of tumor cells showed positive activity, +: 5-50% of tumor cells positive, ±: positive staining only in occasional cells of the tumor elements.

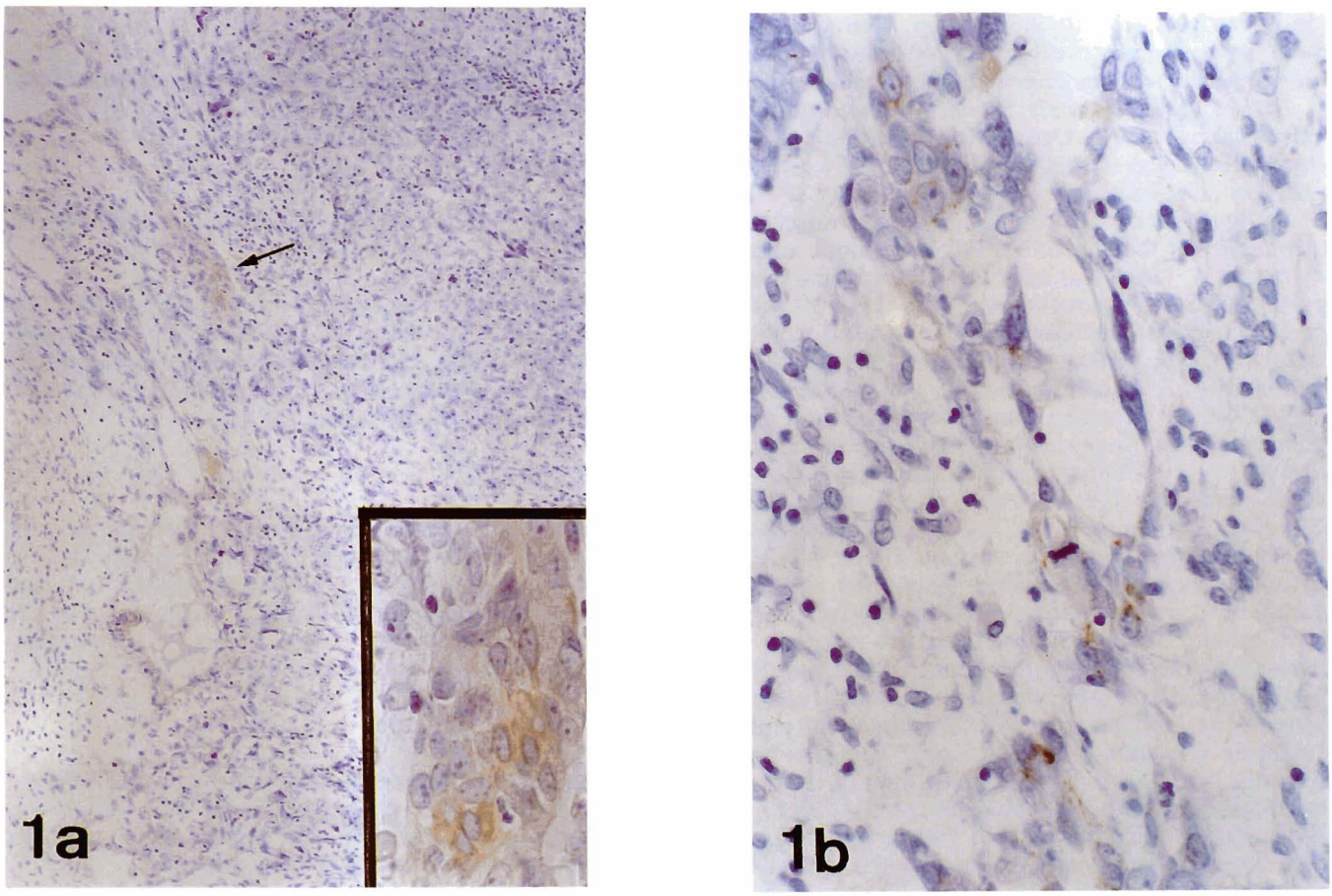


Fig. 1. Cytokeratin positivity is found in a part of a component of embryonal carcinoma **(a)**, which shows early differentiation to yolk sac tumor with AFP-positive staining **(b)**. Inset: Cytokeratin positivity is clearly recognizable in the same part of the tumor shown with arrow in Fig. 1a. a: x 122, b: x 485, inset: x 485

markers. Cytokeratin was detected in some cells of 10 cases of the components, most of which showed further differentiation; e.g., cytokeratin-positive areas in 8 cases showed the early differentiation to yolk sac tumor (Fig. 1a,b) or teratoma or choriocarcinoma, but these elements were very limited and almost could not be separated from the embryonal carcinoma elements. Stromal cells reacted with antibody to vimentin in 10 cases. In one case there was a small focus of immature element mixed with embryonal element, in which primitive mesenchymal tissue could be seen, and some desmin-positive cells were observed.

Yolk sac tumor

All the components were positive for cytokeratin and AFP. Obvious positivity for cytokeratin was revealed in a large part of the tumor cells, which were arranged in glands, solid nests or reticular pattern (Fig. 2). The loose or edematous tumor stroma was positive for vimentin. In one case several desmin-positive spindle cells were found scattered in the stroma. EMA, CEA and S-100 protein were found in some of the components.

Embryoid body

The large, flattened epithelial cells, of which the disc and the amniotic space were made up, were positive for cytokeratin (Fig. 3), and 1 case showed simultaneous positive staining for vimentin in some cells. In the 2 cases, HCG and AFP positive cells were observed.

Choriocarcinoma

All of the 5 choriocarcinoma components showed positive reaction with antibodies to cytokeratin and HCG, but had no reaction with other antibodies.

Immature teratoma

Epithelia in these components frequently showed

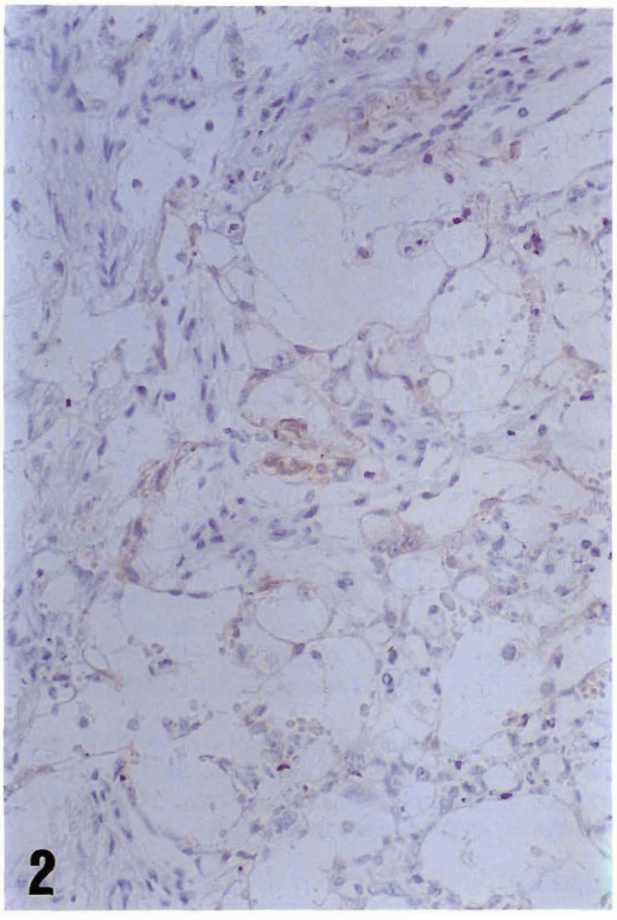


Fig. 2. Cytokeratin positivity is diffusely found in the yolk sac tumor cells which are arranged in reticular pattern. x 243

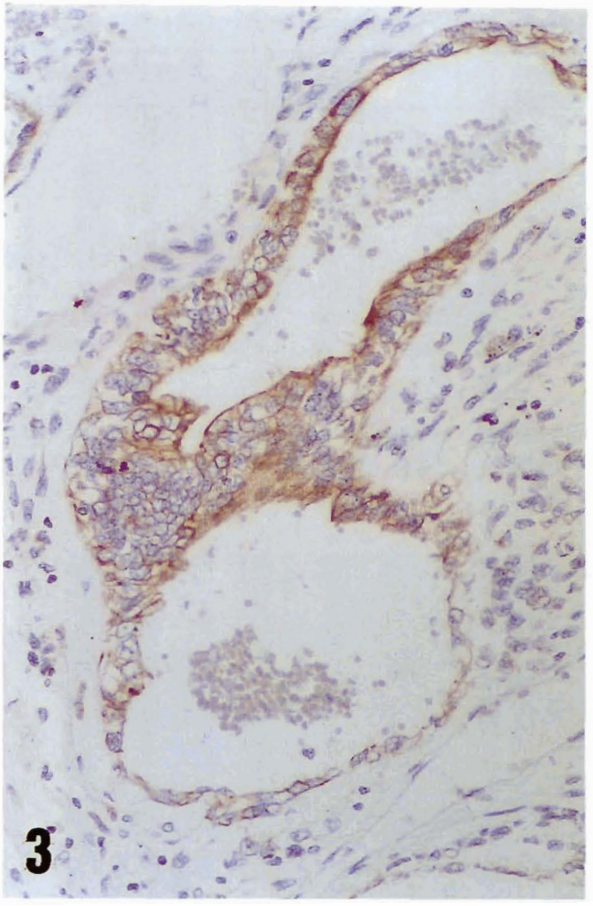


Fig. 3. An embryoid body is strongly positive for cytokeratin. x 243

strong positivity for cytokeratin, and primitive mesenchymal tissue often reacted with antibody to vimentin. Except for stroma, vimentin was also detected in some muscle cells in 2 cases; neural epithelia in 1 case; and tubular epithelia in 1 case. Desmin was often found in the spindle cells located in the stroma (Fig. 4), and in 3 cases desmin was present in both rhabdomyoblasts and spindle cells. NF- and GFAP-positive nervous tissue was revealed in 1 case. S-100 protein was more often found in teratomas than other germ cell tumors, and was seen in cartilages of 5 cases, in some glandular epithelia of 3 cases, in nervous and primitive mesenchymal tissue of 1 case and in tumoral histiocytes of 1 case. Scattered HCG-positive trophoblasts were present in 5 cases. A few epithelia of some single glands showed positive staining for AFP. Positive staining for CEA and EMA could be easily found in most of the tumoral epithelia. In 1 case several neural epithelia were positive for both cytokeratin and NF.

Mature teratoma

All the mature tissue elements were consistently positive for the IF specific to each of them. One of the 3 cases was dermoid cyst, which only consisted of entoderm and ectoderm. One of the other cases had nervous tissue which was positive for NF. S-100 protein was found in cartilages and some glandular epithelia. AFP or CEA positivity was revealed in a few of the glandular epithelia.

Although both cytokeratin and EMA were mainly present in epithelial elements, cytokeratin was far more often seen in all the tumors.

IF proteins and other markers in embryos and foetuses

Table 4 lists the characteristics of the expression of IFs and other markers in embryos and foetuses of 4-10 weeks. The IFs that most early appeared in embryos and foetuses were cytokeratin and vimentin. In 4-week-old embryos a part of the epithelia was weakly positive for both cytokeratin and vimentin. In 5-6 week-old embryos the distribution of cytokeratin and vimentin gradually extended, but some epithelia still had no reaction with antibody to cytokeratin. From 7 weeks desmin and NF began to appear in some cells. After 7.5 weeks IFs, EMA and S-100 protein could be observed in various

Table 4. Expression of intermediate filaments and other markers in embryos and foetus.

Age of embryo or foetus (week)	Epithelia	Mesenchyma				Muscle			Neural tissue	
		Stroma	Cartilage	Endothelia	Notochord	Myocardial	Skeletal	Smooth	Peripheral	Central
4w	$C^{\pm}$, $V^{\pm}$	V^{+}								
5w	$C^{\pm}$	$V^{\pm}$		V+	C^{+}	$V^{\pm}$			$V^{\pm}$	$V^{\pm}$
6w	$C^{\pm}$	$V^{\pm}$		V^{+}						$V^{\pm}$
6-7w	$C^{\pm}$	$V^{\pm}$	V^{+}, S-100^{+}	V^{++}		V^{+}	V^{+}, $D^{\pm}$		NF^{+}, S-100^{+}	$V^{\pm}$, NF$^{\pm}$
7w	C^{+}	V^{+}	S-100^{+}	V^{++}	C^{++}, S-100^{+}		V^{+}, D^{+}		NF^{+}, S-100^{+}	
7-5w	C^{++}, EMA^{+}	V^{++}	V^{+}, S-100^{+}	V^{++}			V^{+}, D^{+}		S-100^{+}	$V^{\pm}$
8w	C^{++}, EMA^{+}	V^{+}	V^{+*}, S-100^{+}	V^{+}			V^{+}, D^{+}	V^{+}	NF^{+}, S-100^{+}	
10w	C^{++}, EMA^{+} $V^{\pm}$	V^{+}	V^{+}, S-100^{+}	V^{++}	C^{++}	D^{+}	V^{+}, D^{+}	V^{+}	NF^{+}, S-100^{+}	$V^{\pm}$

*: Positivity also in some osteoblasts, C: Cytokeratin, V: Vimentin, D: Desmin, NF: Neurofilaments, S-100: S-100 protein.
(The criteria for $^{++}$, $^{+}$, $^{\pm}$ and $^{-}$ are the same as described in Table 3.

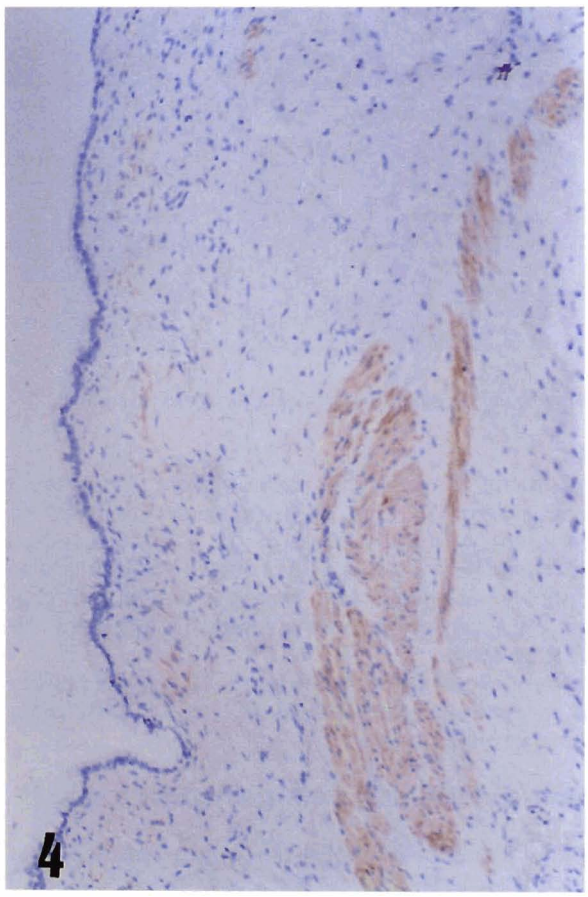


Fig. 4. Spindle cells in an immature teratoma show positive reaction for desmin. x 122

tissues, but no CEA and HCG. However AFP was consistently seen in all of the embryos and foetuses.

In early embryo stage, myoblasts coexpressed vimentin and desmin; however, in these cells vimentin positivity was more intensive and extensive than that of desmin. But latterly, desmin staining became stronger in such cells, and in foetuses desmin was well found in both skeletal and myocardial cells, and was more distinct than vimentin. Whereas spindle cells surrounding gastrointestinal and other tract hardly expressed desmin and vimentin during these weeks of embryos and foetuses.

In all the embryos and foetuses, neural epithelia were more often positive for vimentin than for NF. NF was frequently seen in peripheral nerve fibres. GFAP was not detectable in these embryos and foetuses.

Discussion

In this study we have used antibodies to IFs and other markers to characterize the different tumor components and patterns of testicular germ cell tumors and to compare the tumors with embryos and foetuses. The expression of IF proteins has been studied in ovarian, sacrococcygeal and testicular germ cell tumors before (Miettinen et al., 1985a,b; Ramaekers et al., 1985; Lahdenne et al., 1990), and some characteristics of these tumors were discovered. Especially in heterogeneous tumors, IFs helped to discover various tissue elements because of their high specificity (Puts et al., 1987). Our study was carried out in various types of testicular germ cell tumors and embryos and foetuses, with conception of embryogenesis. The characteristic expression of IFs and its significance in these tumors were reconsidered.

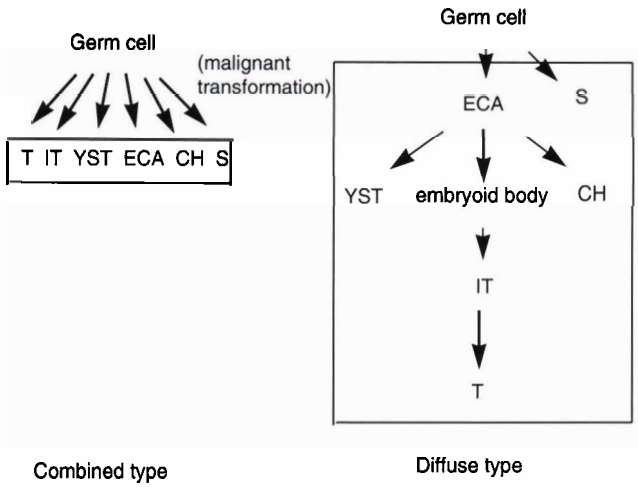
Immunohistochemically IFs have not been found in germ cells, such as spermatocytes and spermatids (Franke et al., 1981), whereas when cell differentiation begins, IFs gradually appeared. IFs have been proposed to possess functions in the maintenance of cell integrity, shape and organelle positioning and in the regulation of the cellular and intracellular movements, but some authors believed that IF expression might be the result of a series of interactions between the microenvironment and synthetic mechanisms rather than as a function of histogenesis (Miettinen et al., 1984; Coggi et al., 1989). In any case, it seems that in undifferentiated stage cells

do not have IFs. In this study, the earliest stage of embryos was 4 weeks; only a few of the cells in it were positive for cytokeratin and vimentin. The counterparts of the undifferentiated stage of embryo in germ cell tumors are seminoma and embryonal carcinoma. Seminoma is believed to be the pure germ cell tumor without differentiation, and embryonal carcinoma is undifferentiated carcinoma with totipotential to differentiate (Cotran et al., 1989); therefore, as revealed in our study, the seminomas were hardly positive for any marker, only occasionally being positive for cytokeratin and vimentin. Some papers report that vimentin positivity is a characteristic of seminoma or dysgerminoma (Miettinen et al., 1985a,b; Ramaekers et al., 1985) but their results also only showed «a minor portion of the tumor cells was positive for vimentin». In normal testis, vimentin was well found in seminiferous tubules; however, positivity was present in Sertoli cells. According to our results and the others, we consider that vimentin positivity is not a differential characteristic of seminoma, and the characteristic of seminomas is that they are often negative for IF proteins. Similarly, cytokeratin positivity has been believed to be a feature of embryonal carcinoma, but in fact cytokeratin was not prominent in this type of germ cell tumor. However, because of the totipotent germ cell in the tumor, it could be possible that differentiation appeared in cells which might show positivity for cytokeratin. In fact, in our study the presence of cytokeratin really helped to distinguish the embryonal carcinoma components from yolk sac tumor components and other components. The latter were often intermingled with embryonal carcinoma and showed positivity for cytokeratin. Non-specific IF expression of seminoma and embryonal carcinoma provided an additional support of their histogenetic entity.

From our results, it can be seen that in embryo and foetus IFs gradually appeared as differentiation proceeded, and the expression pattern altered with age. During differentiation profound changes in IF expression patterns occurred in the different cell type of the developing tissue. For epithelial cell the cytokeratin positivity increased in intensity and extent with increasing age. For mesenchymal and nervous tissues, at an early stage, vimentin was predominant in them, and latterly each cell and tissue tended to express their own type-specific IF. It seems that after 12 weeks almost all the tissues and cells in foetus possess their type-specific IF (Van Muijen et al., 1987). There is a similar finding reported in the lungs of foetuses of 9-10 weeks and 25 weeks (Broers et al., 1989), while in the tumor, although germ cell tumor is recapitulation of embryogenesis (Javadpour, 1985), its development has no age and evolution stage, and often displays aberrant features. Our findings showed that in germ cell tumors IFs seemed to appear as early as the differentiation occurred in the cells. For instance, in the stroma of some embryonal carcinomas and yolk sac tumors a few scattered desmin-positive spindle cells could be seen even when the tumors were at a very immature stage and no organoid tissue could be seen, whereas at an early stage of embryos and foetuses, the spindle cells surrounding gastrointestinal and other tracts that had already developed very well still had no desmin expression. For another instance, cytokeratin could be found in the places of embryonal carcinomas, which showed only early differentiation, and was prominent in embryoid body and other non-seminoma germ cell tumors, whereas in our 4-5 week-old embryo, cytokeratin was seldom detected in the epithelia, although morphologically the cells had already showed certain differentiated types of tissue at that time. Therefore, if the appearance of IF could even be considered as a sign of specific differentiation of cells, the differentiation in tumors was faster than that of normal embryonal development, and it could even be called leap differentiation. This may be an important difference between tumoral and normal development.

WHO classification groups the testicular germ cell tumors under two large headings: tumors of one histological type and tumors of more than one histological type. The histogenesis of these tumor has been in the focus of considerable research interest. Although there were some theories and histogenetic schemes (Damjanov, 1984; Mostofi and Sesterhenn, 1985; Rosai, 1989), it is commonly accepted that the tumors originate from intratubular germ cells that have undergone malignant transformation (Mostofi and Sesterhenn, 1985). A lot of information strongly supports that any type of germ cell tumor may directly arise from germ cells. This hypothesis can explain many types of germ cell tumors, including mixed ones. However, in our observations of the histological arrangement of the mixed type, two patterns among them could be recognized; i.e. combined type and diffuse type. In the former, the components, combined in a patchy and disorderly manner, were separated from each other, while in the latter, the components, intermingled together, often showed transition from one to another. The tumor named «diffuse embryoma» by De Almeida and Scully (1983) is a representative of the latter. Diffuse type often had embryonal carcinoma component. As described before, tumor cells of embryonal carcinoma have been generally believed to be totipotent cells which may progress along the extraembryonic or embryonic route to form further differentiated components. In our findings, the cytokeratin-positive areas in 8 of 10 cytokeratin-positive embryonal carcinoma components showed early differentiation to yolk sac tumor or teratoma or choriocarcinoma. Mostofi et al. (1988) also pointed out that yolk sac tumor elements were present in many embryonal carcinomas; in fact, not only yolk sac tumor but also choriocarcinoma, teratoma, even seminoma could be observed in embryonal carcinoma elements in a diffusely mixed pattern. IF immunohistochemical staining is specially helpful in discovering the early

Diagram 1. Histogenetic scheme of two types of mixed germ cell tumors.



S= seminoma, ECA= embryonal carcinoma, YST= yolk sac tumor, IT= Immature teratoma, T= Teratoma, CH= Choriocarcinoma.

changes in these tumors. Considering tumorigenesis, the developments of two patterns of mixed germ cell tumors are probably different. The tumors with combined pattern may be from more than two groups of germ cells which go in different directions of differentiation and form a random combination, whereas the tumor with diffuse pattern may be a malignant transformation of one group germ cells, which show different stage of differentiation and form diffusely mixed type with transition between them. According to the histogenesis of germ cell tumors, accepted commonly (Damjanov, 1984; Coggi et al., 1989), we have figured out the possible development paths of the two patterns of the mixed type (Diagram 1). Although tumor development is more complicated than this well outlined scheme, and the two patterns may appear in one tumor, we believe that our supposition might explain any bewildering combination of mixed germ cell tumors. It is reported that mixed germ cell tumors constitute a large percentage of testicular germ cell tumors, at least more than 62% (Mostofi et al., 1988). For clinical diagnosis of these tumors, listing all components in the tumor is the best way. However, a given component, partial or at early differentiation stage in diffuse mixed tumors, may be missed, and when any suspicion is aroused, immunostaining for IFs and tumor markers is appropriate.

In addition to IFs, S-100 protein, CEA, EMA as well as AFP and HCG were also tissue-demonstrated in our study. The latter two, established germ cell tumor markers (Jacobsen et al., 1981), were still found useful in classification of germ cell tumors. Although AFP was frequently found in yolk sac tumors, some other tumors could also show positivity for it. It is reported that 19% of teratoma give positive reaction for AFP (Mostofi et al., 1988). In our findings, AFP was revealed in scattered glands in some immature teratoma. This finding enabled us to understand the presence of AFP in some adenocarcinoma (Hanai and Lin, 1990; Lertprasertsuke and Tsutsumi, 1991), and there might be a certain relation between the AFP-positive tumor and AFP-positive embryonic tissue. Our study and others (Carbone et al., 1987) found that antibody to S-100 protein was helpful in revelation of nervous tissue in germ cell tumors, which sometimes was not obvious in H&E staining, although S-100 protein was also present in cartilage and some glandular epithelia. CEA was not found in embryos and early foetuses, but was observed in the tumors. This proved that CEA was only a marker for tumors. EMA was found in epithelia, but not as often as cytokeratin was.

Due to the effectiveness of chemotherapies and combination therapy, the outcome of the patient in this series is fairly good, except for choriocarcinoma. Many other papers also reported that the survival of patients with germ cell tumor has greatly improved over the last decade as a result of newer chemotherapies in the treatment (Brada et al., 1987; Kotake and Miki, 1988; Vugrin et al., 1988; Kobayashi et al., 1989). However, the most essential guideline for treatment is on the basis of clinical diagnosis and histopathological classification of these tumors. Metastases occurred in 10 cases of the series with 6 cases of distant metastases. The 6 cases had choriocarcinoma or embryonal carcinoma components in part. Death occurred in 2 choriocarcinomas and 1 mixed tumor of embryonal carcinoma and yolk sac tumor.

In conclusion, the present study further defines the distribution of IFs in testicular germ cell tumors with embryogenetic consideration. Seminoma and embryonal carcinoma are characterized by lack of IF expression. Moreover, the expression of IFs of testicular germ cell tumors reveals the characteristics of tumoral development; that is, the maturing process in the tumors is faster than normal embryonal development and cells showed leap differentiation with aberrant feature. Immunostaining for IFs helps to discover cell changes in the tumors, which are not apparent by light microscopical observation of H&E stained sections, and two patterns of mixed tumors are distinct, which may assist us in understanding the complicated combination of the germ cell tumors and in making an accurate diagnosis.

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