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Histogenetic considerations concerning germ cell tumours

Morphological and immunohistochemical comparative investigation of the human embryo and testicular germ cell tumours

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Summary. Although it is accepted that the different components of germ cell tumours (GCT) imitate the embryonic and extraembryonic structures in early development, various tumour patterns remain to be interpreted in histogenetic terms. In particular, some patterns of embryonal carcinoma (EC) and yolk sac tumour (YST) have not been given a convincing histogenetic explanation. Combined morphological and immunohistochemical studies of GCT in addition to a three-dimensional analysis permit correlations between certain tumour patterns and normal embryonic and extraembryonic structures to be made. The various tumour patterns which reflect various stages of differentiation or maturation of cells and tissues of the normal conceptus may also be placed in chronological order with regard to embryogenesis. On the basis of such considerations a nomenclature using the embryological terms for the various tumour components may be considered, although not recommended as a new system of classification.

Key words: Germ cell tumours – Histogenesis

Introduction

In 1950 Teilum was the first to recognize extraembryonic structures other than the trophoblast in germ cell tumours (GCT). In a later work congruity was shown between characteristic perivascular structures in some of the tumours and well-defined normal yolk sac structures of the rat placenta, described by Duval in 1891 and known as endodermal sinus structures (ESS) (Teilum 1959). Consequently, the tumour was designated endodermal

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Abbreviations: AFP: Alpha-fetoprotein; *CC:* Choriocarcinoma; *CIS:* Carcinoma in situ; *EC:* Embryonal carcinoma; *ESS:* Endodermal sinus structure; *EST:* Endodermal sinus tumour; *GCT:* Germ cell tumour; *HCG:* Human chorionic gonadotropin; *PES:* Pseudoendodermal sinus structure; *STLC:* Syncytiotrophoblast-like cell; *T:* Teratoma; *YST:* Yolk sac tumour; *IP:* Immunoperoxidase technique; *H:* Hematoxylin; *H & E:* Hematoxylin and eosin

sinus tumour (EST) or yolk sac tumour (YST). Since then Teilum (1976) described eight different patterns of EST.

Comparative studies with normal *human* yolk sac were, however, not undertaken until later (Hesseldahl and Larsen 1969; Gonzales-Crussi and Roth 1976) and although the normal human counterpart of the ESS has never been demonstrated (Okamoto 1983), it is generally accepted that the different tumour components in GCT represent different structures occurring during the normal embryogenesis. This concept formed the basis of the classification of GCT later recommended by WHO (Mostofi 1977). Yet, in this classification many problems remain to be elucidated, especially problems of the histogenetic interpretation of tumour patterns occurring in embryonal carcinoma (EC) and in EST.

In recent years early human embryos have become available because of legal abortions. Furthermore, the introduction of immunohistochemical techniques which have made possible the demonstration of various proteins in the tissues has added functional aspects to morphology (Mukai and Rosai 1980). Thus, morphological and immunohistochemical studies of early human embryos and GCT have enabled us to compare various stages of early human development with the different patterns of the diverse tumour components with regard to both structure and function.

In the present paper the results of morphological studies and immunohistochemical investigations of embryos and of GCT, which have been reported previously (Jacobsen et al. 1981; Jacobsen and Jacobsen 1981) are combined in order to identify the possible embryological parallel to some tumour structures which have not yet been given in a final histogenetic interpretation. Finally, a terminology based on these considerations is proposed.

Material and results used for histogenetic interpretation

Normal embryonic tissues. Hertig et al. (1956) described the first 17 days of development in 34 fertilized human ova. The schematic drawings showing the early stages of development (Fig. 1) were made on the basis of these investigations. Studies of yolk sacs in embryos (Fig. 2), twenty days to nine weeks of age, previously reported by Jacobsen and Jacobsen (1983a, c), formed the basis for interpretation of yolk sac components in the tumour, in connection with the morphological studies of the early stages of the yolk sac by Heuser et al. (1945) and by Hesseldahl and Larsen (1969).

The results of immunohistochemical studies of the early conceptus by Jacobsen et al. (1981), which were used as an additional tool in the interpretation of the different tumour components, are included in the schematic drawings of early development in the present paper (Figs. 1 and 10). The immunohistochemical method used to localize alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) in the early conceptus as well as in the tumour tissue is described later. Briefly, AFP was found in the yolk sac endoderm coating the luminal surface as well as in the endodermal cells of the tubular system and in the vacuolated cells present in the yolk sac wall. Mesodermal cells on the outer surface of the yolk sac and the endodermal as well as the ectodermal germ layer of the germ disc were AFP negative. Human chorionic gonadotropin (HCG) was present in the trophoblast of the placenta, but in the syncytiotrophoblast only.

Germ cell tumours (GCT). A series of 189 orchiectomy specimens with GCT formed the basis of morphological and functional comparison with the embryonic tissues. The GCT, which have been presented previously Jacobsen and Jacobsen (1983a), comprised 95 pure

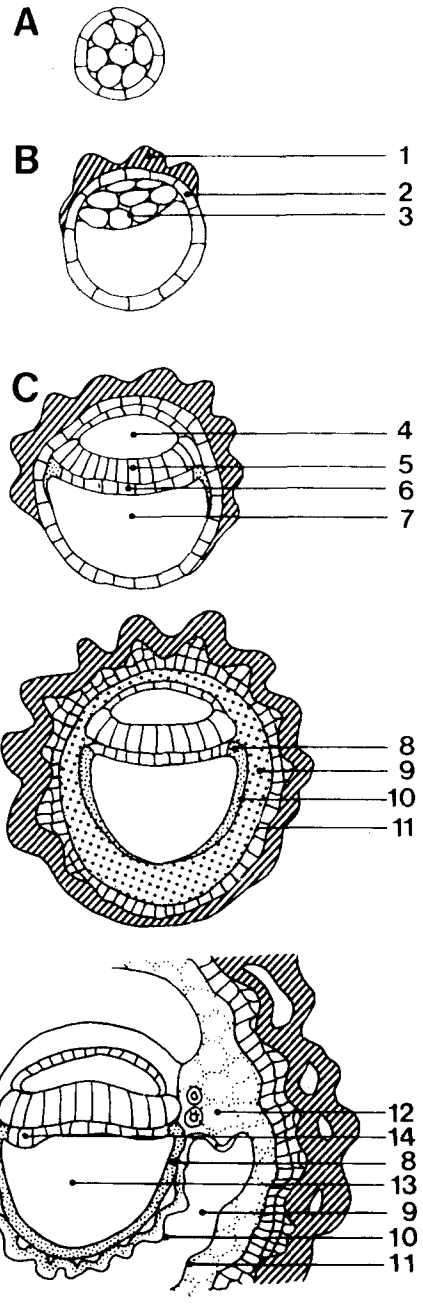

Fig. 1. Schematic drawings showing cross sections of **A** morula with outer cell mass and inner cell mass, **B** blastocyst with outer cell mass or trophoblast (*1*+*2*) and inner cell mass or embryoblast (*3*), and **C** bilaminar germ disc at various stages of development with syncytiotrophoblast (HCG positive) (*1*) and cytotrophoblast (*2*), epiblast or ectodermal germ layer (AFP negative) (*5*), hypoblast or endodermal germ layer (AFP negative) (*6*), amnion cavity (*4*), primary yolk sac (*7*) with inner endodermal layer (AFP positive) (*8*), extraembryonic coelom (*9*) with extraembryonic splanchnopleuric (AFP negative) (*10*) and somatopleuric (AFP negative) mesoderm (*11*), connecting body stalk (*12*), secondary yolk sac (*13*) with inner endodermal (AFP positive (*8*) and outer mesoblastic layer (AFP negative (*10*) and prochordal plate (*14*)

seminomas and 94 non-seminomas of various types, classified according to the WHO typing of testis tumours (Mostofi 1977) and to the concept of Teilum (1959) regarding the EST. Furthermore, 100 testicular GCT (including 85 of the above mentioned tumours and additional 15 cases), containing EC and/or EST were evaluated in regard to the presence of the various histological patterns of EC and YST which are described below and given in Table 1.

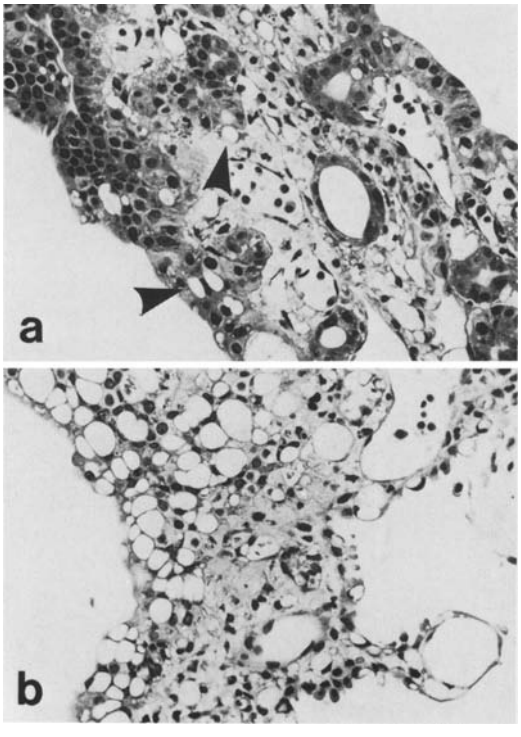

Fig. 2a, b. The normal secondary yolk sac, 9 weeks of age with a well developed tubular system of endodermal cells. The figures show different areas in the same yolk sac. In **a** the wall is folded and thus appear double-layered. The luminal endodermal cells of the yolk sac are seen on the surface on both sides. The endodermal cells on the surface as well as in the tubular system in the wall are slightly vacuolated (*arrows*). In the area of the yolk sac shown in **b** the vacuolization of the endodermal cells is more pronounced. In the wall of the yolk sac many thin walled vessels with erythroblasts are present. In **b** the outer festooned surface of the yolk sac (the extraembryonic splanchnopleuric mesoderm) is seen to the right (H & E ×300)

Table 1. Numbers of various histological patterns present in 100 testicular GCT containing EC and/or EST

	Tumour type	No. of tumour patterns
85	Embryonal carcinoma components	
	Solid	85 (100)
	Papillary and tubular	78 (92)
	Double-layered	7 (8)
	PES	13 (15)
70	Endodermal sinus tumour components	
	Vacuolated network[1]	64 (91)
	Endodermal sinus structure[2]	6 (9)
	Microcystic, honeycomb pattern[3]	47 (67)
	Labyrinthine structure[4]	12 (17)
	Cystic structure[5&6]	31 (44)
	Myxoid stroma[7]	36 (51)
	Solid cell aggregates[8]	19 (27)
	Liver trabecula-like structures	16 (23)
42	Embryoid bodies	

Numbers in brackets are per cent
1–8 indicates the various histological patterns described by Teilum (1976)

Tissues. The orchiectomy specimens were handled according the recommendations of the Danish Testicular Carcinoma (DATECA) Project (DATECA Study Group 1978). This means that at least five blocks were taken from the tumour tissue. The tissues were fixed in 10% buffered formalin in 24–72 h, routinely processed at room temperature and embedded in Paraplast^R at 58–60° C. Haematoxylin and eosin (HE) stained sections were used for classification and evaluation of the various histological patterns.

Immunohistochemical procedure. Two representative tumour blocks were selected for immunohistochemical investigation. The sections were stained for AFP and HCG using the indirect immunoperoxidase technique (Clausen and Thomsen 1978). Specific rabbit antisera against human AFP and HCG were obtained from DAKOPATTS, Denmark. The specificity of the antisera used was verified by performance testing on formalin fixed and paraffin embedded material with known positive reaction before and after absorption with the proper antigen. For absorption human AFP-standard (Beering Werke, Höechst, Denmark) and HCG-standard (LEO, Denmark) were used. Anti-AFP was used in dilution 1:40 and anti-HCG in dilution 1:200. Furthermore, peroxidase labelled swine antirabbit immunoglobulin (DAKOPATTS, Denmark) was used in dilution 1:20 determined by chess board titration. For control staining the immunoglobulin fraction of serum from unimmunized rabbits was used in dilution 1:100. All antisera were diluted in phosphate buffered saline, pH 7.2, containing 10% normal swine serum. For control staining the specific antiserum was replaced by the immunoglobulin fraction of serum from unimmunized rabbits as well as by phosphate buffered saline. All controls were negative. The specimens were counterstained with haematoxylin and mounted in Aquamount^R.

The morphology of the tumours and the immunohistochemical findings are briefly recapitulated.

Morphology. The typical seminomas were composed of large, polygonal, rather uniform cells with clear cytoplasm and distinct cell border. The cells were arranged in cords or groups separated by septae of connective tissue. Eight per cent of the pure seminomas contained syncytiotrophoblast-like cells (STLC).

The distribution of the various tumour types in the non-seminomas was as follows: 78% of the non-seminomas contained EC components, EST was found in 50%, teratoma (T) in 68%, and choriocarcinoma (CC) in 12%. Thirty per cent of non-seminomas contained STLC.

EC components were composed of large poorly differentiated cells arranged in solid, papillary and tubular and double-layered patterns (Fig. 4). Furthermore, special structures, "pseudo-endodermal sinus structures", (PES), previously described by Jacobsen and Jacobsen (1983a), also occurred in the EC components. These structures are papillary and covered with EC cells of high columnar type (Fig. 5). The central part contains a cystic cavity with flattened epithelial cells and a vessel (PES I) (Fig. 5a) and in some cases also areas of vacuolated cells are present (PES II) (Fig. 5b). The numbers of EC components of the various histological patterns present in the tumours are given in Table 1 and 2.

The EST components presented the different patterns described by Teilum (1976), almost always occurring in various combinations: 1) vacuolated network (Fig. 6a, e, f) ESS (Figs. 6b and 7), 3) microcystic honeycomb pattern (Fig. 6a), 4) labyrinthine structure (Fig. 6c, e), 5) cystic structure (Fig. 6c, e), 6) yolk sac vesicles, 7) myxoid stroma ("magma reticulare") (Fig. 6d) and 8) compact cell aggregates (Fig. 6b, f). In addition, liver trabecula-like structures previously described (Jacobsen and Jacobsen 1983b) also occurred in tumours with EST (Fig. 3). The frequencies of the various histological patterns occurring in the tumours with EST components are given in Table 1 and 2.

Embryoid bodies occurring in 42 of the tumours with EC and/or EST represented various more or less well-defined structures of embryos of various stages within the first two weeks of development. Very often the allantois was seen in the vicinity of the yolk sac vesicle.

The T components consisted of immature and mature teratoid structures known to be present in GCT. The most immature components contain primitive endoderm, mesoderm or neuroectoderm. In the more mature components epithelium of various types are present, including epithelium of columnar, often mucus secreting or ciliated, of cuboidal and of squamous type. The mesodermal formations comprise cartilage, bone, smooth and striated muscle.

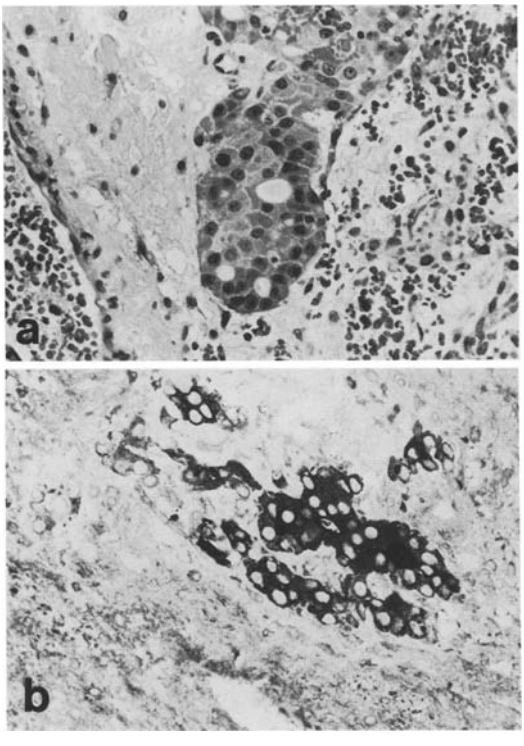

Fig. 3a–b. Epithelial structure in GCT showing morphological resemblance to the endodermal structures in the wall of the secondary yolk sac as shown in Fig. 2a or to liver trabeculae (H & E ×300). **b** AFP demonstrated in similar epithelial structures present in GCT (IP & H ×250)

Table 2. The distribution of the various histological patterns present in 100 testicular GCT containing EC and/or EST components

Tumour type		No. of tumours
85	Embryonal carcinoma	
	Solid only	7
	Solid & papillary-tubular	62
	Solid & papillary-tubular & double-layered	3
	Solid & papillary-tubular & PES	9
	All patterns	4
70	Endodermal sinus tumour components	
	One histological pattern	9
	Two histological patterns	15
	Three histological patterns	18
	Four histological patterns	7
	Five histological patterns	12
	Six histological patterns	6
	Seven histological patterns	0
	Eight histological patterns	0

The mature components often appear as abortive organs. Structures which imitate gastrointestinal and respiratory tract are common, in addition to elements of the central nervous system.

The CC components consisted of elements of syncytiotrophoblast as well as of cytotrophoblast with and without villous-like formations.

The STLC in both seminomas and non-seminomas occurred as single cells or as cell

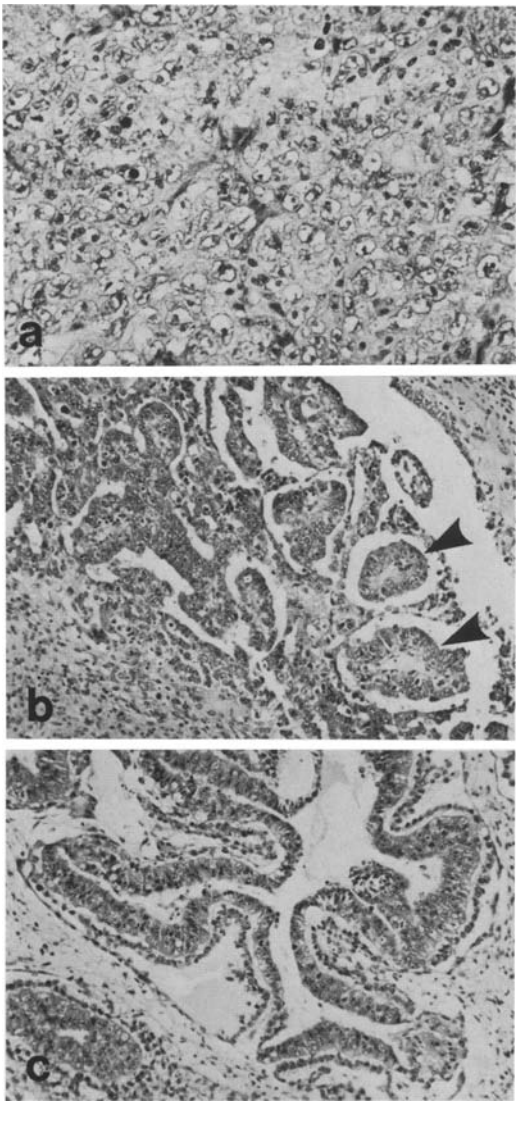

Fig. 4. Patterns of EC. **a** EC of solid type (H & E ×350). **b** EC of papillary and tubular type showing cross-sections of papillary formations (*arrows*), also shown in the schematic drawing in Fig. 10; 1a (H & E ×150). **c** EC of double-layered type (H & E ×150)

groups scattered in the tumour tissue. In non-seminomas STLC were additionally found in close association with embryoid bodies. Compared with the cells of CC no difference was recognized between the appearance of STLC and the cells of the syncytiotrophoblast.

Finally, carcinoma in situ (CIS) (Fig. 9a) diagnosed according to the criteria of Mark and Hedinger (1965) and Skakkebæk (1975) were present in the seminiferous tubules in 64 specimens with seminomas and in 77 with non-seminomas. In three cases of CIS a few STLC were found in a few tubules among the CIS cells.

Distribution of AFP and HCG. Immunohistochemical investigations concerning the occurrence of AFP and HCG in this series of GCT have been reported previously (Jacobsen and Jacobsen 1983a).

In summary, AFP was found in EC of the solid type, but only in some tumours and

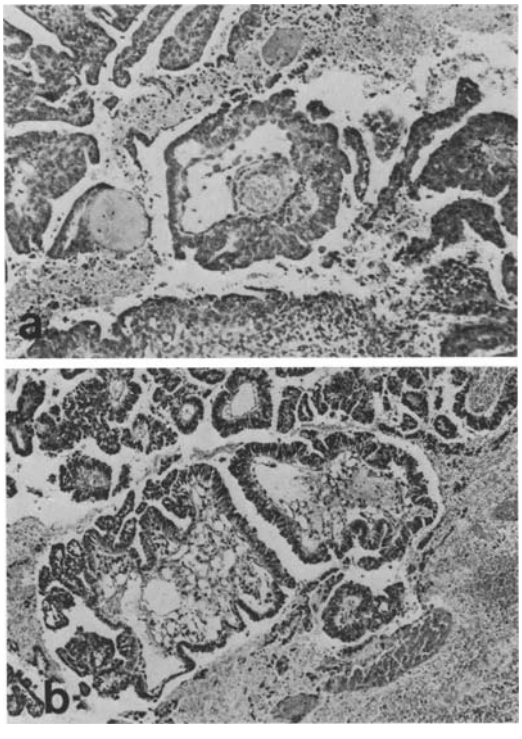

Fig. 5. Patterns of EC. **a** EC with PES of type I (H & E ×100) and **b** PES of type II (H & E ×100) also shown in the schematic drawing in Fig. 10, 1b & c

only in scattered cells or in small cell groups that occasionally showed morphological differentiation towards yolk sac structures. Cells in EC with a papillary or tubular pattern were always AFP negative as were the columnar cells of the double-layered type and the cells on the surface of PES. The flattened cells in the double-layered type were often AFP positive as were the cells lining the cystic cavity of PES.

In EST, AFP was found in all the various patterns, except in the cells in the myxoid stroma pattern and in the cells in the solid aggregates (Fig. 6f). The cells of the vacuolated network, the lining cells of the various cystic patterns and the liver trabecula-like cells were usually heavily stained (Figs. 6e and 3b). Both types of cells of the ESS were occasionally AFP positive (Fig. 7b) as were the cells of the labyrinthine structures.

In embryoid bodies of various stages of maturation AFP was present in the yolk sac endoderm, while the epiblast or ectoderm as well as the hypoblast or endoderm of the germ disc were AFP negative (Fig. 8).

In T, epithelium of various types and various degrees of maturation was occasionally AFP positive, including epithelium of respiratory and gastrointestinal tract-like type, while neuroepithelium was always AFP negative.

CC, seminoma and the cells of the CIS pattern were always AFP negative.

HCG was present in the syncytiotrophoblast of the CC component and in STLC, whether present in seminomas (Fig. 9c), non-seminomas or among the cells in the CIS pattern (Fig. 9b).

Correlations between the various types and subtypes of GCT were made with structures in the developing human embryo.

Histogenetic interpretation

Morphological and functional correlations between GCT components and embryonic structures based on these studies are given below and summarized

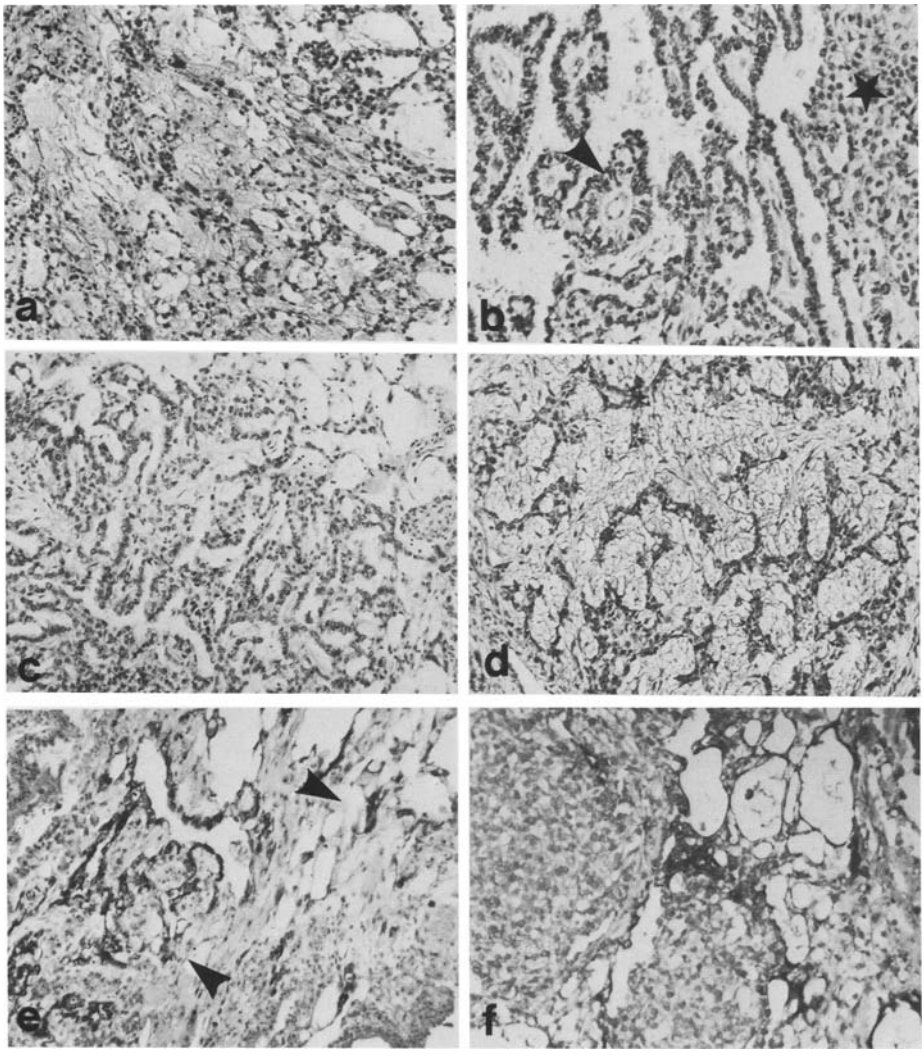

Fig. 6. Patterns of EST. **a** Vacuolated and microcystic pattern (H & E ×100). **b** Endodermal sinus structures (*arrow*) and solid aggregates (*asterix*) (H & E ×100). **c** Cystic and labyrinthine structures (H & E ×100). **d** Myxoid ("magma reticulare") stroma (H & E ×100). **e** AFP in epithelium of cysts and tubules and in vacuolated network (*arrows*) (IP & HE ×150). **f** AFP negative solid aggregates and AFP positive vacuolated network at the periphery (IP & HE ×150)

in Table 3. In addition, a tentative explanation is given to some of the enigmatic structures of the tumours.

Seminomas (S) It is well-known that seminoma cells show resemblance to the germ cells of the early stage of the spermatogenesis (Mostofi 1977). The present study supports the concept that the seminoma cells have no counterpart, neither morphologically nor functionally, among the cells of the various structures of the early human conceptus.

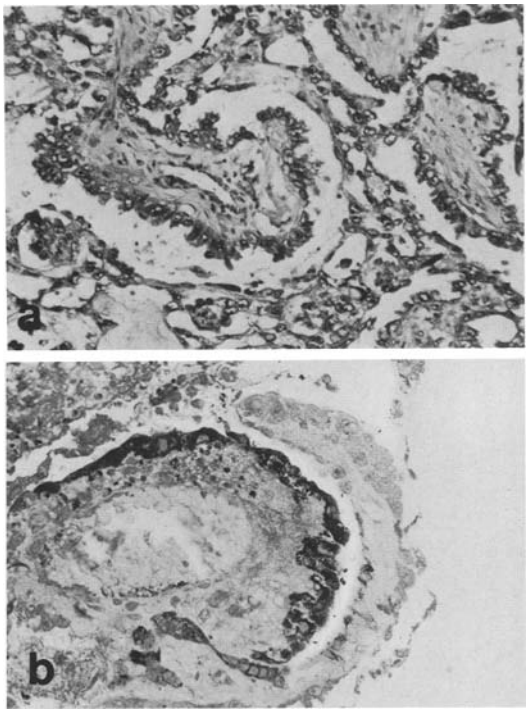

Fig. 7. a ESS in EST (H & E × 250) and **b** AFP in the cells coating a similar structure and in the cells on the luminal surface of the surrounding space (IP & H × 250)

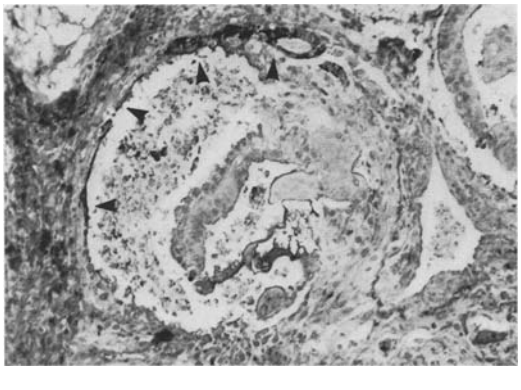

Fig. 8. Embryoid body in GCT with AFP positive cells coating the inner surface of the yolk sac (*arrows*) while the epiblast as well as the hypoblast of the germ disc is AFP negative (IP & H × 100)

Embryonal carcinoma (EC). The EC cells of the solid type correspond to the inner cell mass of the morula and/or to the embryoblasts of the blastocyst.

The EC cells of the papillary (Fig. 4b) and tubular type show resemblance, both morphologically and functionally to the epiblast or ectodermal layer of the germ disc as do the high columnar cells of the double-layered EC (Fig. 4c) and of the PES (Fig. 5). The flattened cells in the double-layered type and in the PES structures may correspond either to the hypo-

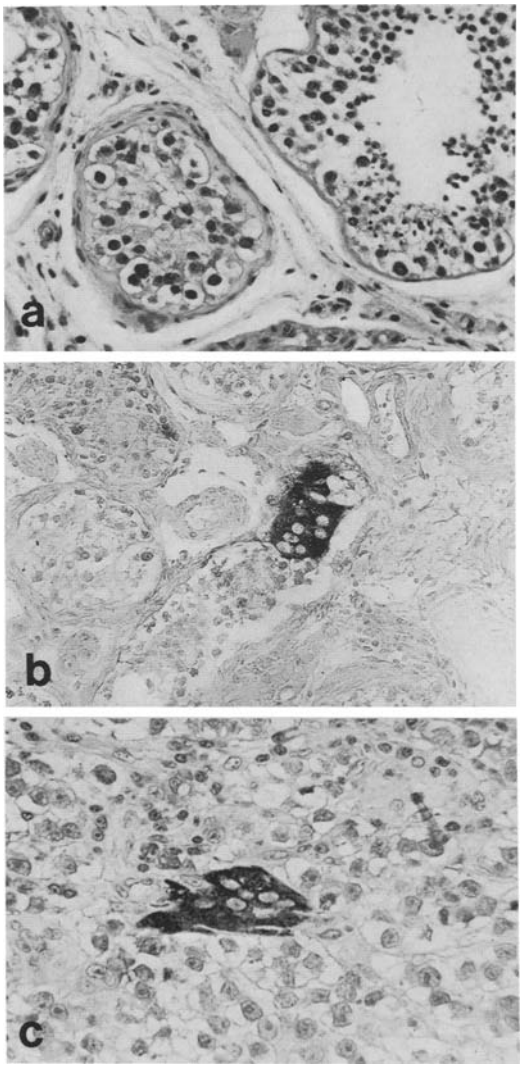

Fig. 9. a CIS pattern of the testis shown in the seminiferous tubule to the left in comparison to a tubule with normal germinal epithelium to the right (H & E ×300). **b** HCG positive STLC in a tubule with CIS (IP & H ×250). **c** HCG positive STLC in seminoma (IP & H ×400)

blast or endoderm (AFP negative) of the germ disc or to the yolk sac endoderm (AFP positive).

The papillary and tubular type of EC is suggested to arise as a result of papillary proliferation of the tumour imitation of the ectoderm of the germ disc, containing vascular structures from the extraembryonic mesoderm (Figs. 10 and 1a). The PES I & II may represent a similar papillary formation comprising ectoderm and mesoderm as well as endoderm of the germ disc or of the yolk sac (Figs. 10, 1b and c). The various structures which occur as a result of tangential cutting of such papillary proliferations are also shown schematically in Figs. 10 and 1a–c and may be compared with the corresponding structures present in the GCT (Figs. 4 and 5).

Table 3. Correlation of various tumour patterns in non-seminomatous germ cell tumours and embryonic structures

Histological tumour patterns	Embryological equivalents
Embryonal carcinoma:	
Solid	Embryoblast
Papillary-tubular	Epiblast or germ disc ectoderm
Double-layered	Epi-hypoblast or ecto-endoderm (germ disc)
PES I	Epi-hypoblast or ecto-endoderm
PES II	Epi-hypoblast or ecto-endoderm & yolk sac
Endodermal sinus tumour:	
Endodermal sinus structure (Teilum type 2)	Hypoblast or germ disc endoderm or yolk sac endoderm
Teilum type 1, 3, 4, 5, 6, 8 & liver trabecula-like	Primary & secondary yolk sac
Teilum type 7	Extraembryonic mesoblast
Teratoma:	
Respiratory & gastrointestinal tract-like structures	Prochordal plate, foregut and allantois
Neuroepithelial structures	Neural plate
Various mesodermal tissues	Body stalk and primitive streak
Embryoid bodies	Embryos (until 15–20 days of age)
Choriocarcinoma	Syncytiotrophoblast & cytotrophoblast
Syncytiotrophoblast-like cells	Syncytiotrophoblast

The double-layered structure of EC is interpreted as an imitation of the bilaminar germ disc.

Endodermal sinus tumour (EST). The endodermal sinus structure (ESS) of the EST may arise as a papillary structure in almost the same way as the papillary type of EC, but from the tumour imitation of the endoderm of the germ disc (AFP negative) or from the yolk sac endoderm (AFP positive) as shown in Fig. 10 (2a and b). Vessels and occasionally also mesodermal tissue join the proliferation. Such vessels and stroma may be imitations of the large vessels present in the wall of the yolk sac proper or in the body stalk which is a dominant structure in early development. The morphology as well as the variation of AFP positivity of the cells coating the papillary structures and lining the inner surface of the surrounding space are in accordance with this suggestion. Alternatively, ESS may occur as a result of a downward growth of the endoderm around vessels in the tumour tissue. Both types of proliferation may, of course, take place.

With regard to the other patterns of EST the vesicles (Teilum type 6) may correspond to the primary yolk sac vesicles (AFP positive), while the vacuolated network (AFP positive) (Teilum type 1) and the microcystic, honeycomb pattern (AFP positive) (Teilum type 3) are found to correspond to similar areas in the wall of the secondary yolk sac (AFP positive) (Fig. 2). The labyrinthine structure pattern (Teilum type 4) is suggested to be an extremely folded tumour imitation of either germ disc endoderm (AFP nega-

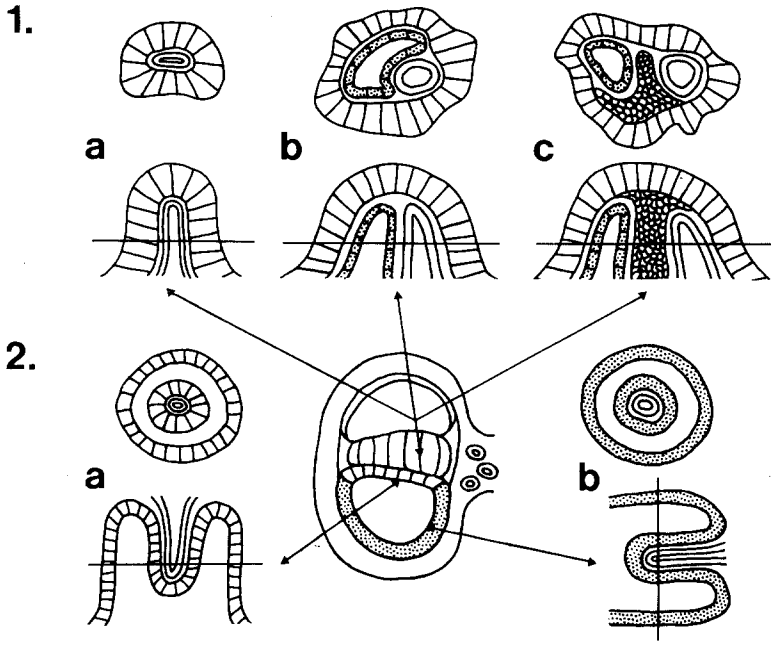

Fig.10. Schematic presentation of the suggested origin of the "pseudoendodermal sinus structures" (PES) in EC and of the endodermal sinus structures (ESS) in EST. The embryo in this drawing corresponds to the bilaminar embryo in Fig. 1 and thus comprises ectodermal germ layer, (high columnar cells, AFP negative), endodermal layer of germ disc, (cuboidal cells, AFP negative), endodermal layer of yolk sac (flattened or cuboidal cells, AFP positive, dots), vessels in the mesoderm of the germ disc periphery or in the connecting body stalk and stroma of the yolk sac wall or the body stalk. Papillary folding is suggested to occur from the various types of epithelium as indicated by the arrows and the results of vertical and horizontal cross sections of the various papillary formations are shown. **1.** Papillary proliferation from ectodermal epithelium joined by **a** a vessel, **b** a vessel and endoderm (dots and hatch) of either germ disc (AFP negative) or yolk sac (AFP positive) and **c** a vessel, endoderm and additional stroma of various types, resulting in **a** papillary structures or **b** PES I or **c** PES II. **2.** Papillary proliferation from endoderm of either **a** germ disc type (AFP negative) or **b** yolk sac origin (AFP positive), resulting in ESS

tive) or yolk sac endoderm (AFP positive). The liver trabecula-like formations (AFP positive) may be tubular and trabeculae structures (AFP positive) of the type present in the wall of the secondary yolk sac in its secondary phase (Fig. 2) and/or imitations of the liver primordium.

A myxoid stromal pattern has previously been interpreted as counterpart of the stroma of the extraembryonic coelom (AFP negative) (Teilum 1976). Solid cellular aggregates (AFP negative) which are usually surrounded by the typical vacuolated network pattern (AFP positive) may represent less differentiated endodermal cells that only show differentiation at the periphery (Fig. 6f).

Teratoma (T). The present interpretation confirms the well-known morphological resemblance between the various teratoid tumour structures and fetal

tissues and organs which are differentiated and mature derivatives of the germ disc proper. In addition, functional similarity of the various epithelial cell types in the tumours and in the fetal tissues in regard to AFP positivity also supports this concept.

Choriocarcinoma (CC) CC is already accepted as a tumour imitation of the trophoblast and this view is supported by both morphological and functional properties.

Syncytiotrophoblast-like cells (STLC). STLC showing morphological as well as functional similarity to syncytiotrophoblast are suggested to be derivatives of the trophoblast.

Comments

The importance of an embryological approach to the interpretation of GCT has been stressed before, especially by Peyron (1941) and Marin-Padilla (1968) who compared the various tumour patterns to structures in embryoid bodies present in the tumour tissue. The present interpretation which is based on a correlation of morphology and functional properties of normal embryonic and extraembryonic structures and tumour patterns in addition to a three-dimensional analysis, confirms previous morphological interpretations, but also presents some new aspects.

The rather varied tumour patterns classified as EC according to the classification of WHO can be correlated to structures present in the first stages of the development of the embryo proper. Morphology and functional properties indicate that they imitate cells and structures in the differentiation from the embryoblast to the epiblast or ectoderm with or without additional differentiation of the hypoblast or endoderm. In addition, papillary structures present in EC, designated PES I & II, can be interpreted as tumour imitations representing transitional structures between EC and EST.

In an extensive study of EST patterns based on polyembryomas Gaillard (1972) demonstrated morphological similarities between the various tumour patterns and the yolk sac with adjoining tissue, but pointed out that ESS is not elucidated and needed a more accurate explanation. The suggested interpretation of ESS in the present study is based on the demonstration of AFP in endodermal derivatives in contrast to ectodermal derivatives which are always AFP negative, in addition to considerations taking into account the third dimension.

Comparing the morphology of the epithelium of the ESS with the epithelium of the allantois of the embroid bodies, it is clear that ESS occurring as the result of proliferation or invagination of the allantois epithelium is less likely, but cannot be excluded with certainty. That the ESS represents an imitation of a normally occurring structure in the human conceptus cannot be confirmed.

The present interpretation of the diverse EST patterns described by Teilum (1976) confirms the suggested yolk sac imitation of most of these.

Furthermore, a new pattern is added that imitates the tubular and trabecular structures of the fully developed secondary yolk sac. These formations may, however, also be interpreted as imitations of the liver primordium in accordance with both morphology and function. This apparent contradiction is explained by the intimate relationship of the trabecular structures of the yolk sac and the trabecules of the liver primordium. Both of them arise from the endoderm although from different areas and at different times (Langman 1976). However, the fact that these structures occur among the other tumour structures that imitate yolk sac components makes it probable that they correspond to the tubular structures in the yolk sac rather than to liver trabecula.

An explanation of the different staining reaction for AFP in the endodermal cells of EST patterns is given as it is pointed out that the endodermal cells of the germ disc is AFP negative, while intimately related and morphologically similar endodermal cells lining the yolk sac are AFP positive. This variation of AFP positivity in endodermal cells is in accordance with the studies of Takada et al. (1982) who found a similar staining pattern in embryoid bodies in GCT of the ovary. Tumour imitations corresponding to the various areas of the endoderm cannot be separated morphologically, while the presence or absence of AFP may point to imitation of either one or the other type.

The predominance of imitations of the foregut and central nervous system in teratomas and the lack of tumour imitations of tissues which occur at later stages of development, such as the kidney, adrenal glands and gonads (Pugh and Cameron 1976) make it probable that the teratoma components imitate tissues and structures already present or present as anlage in the bilaminar germ disc corresponding to 15–20 day of development. This is in accordance with the knowledge that embryoid bodies present in the GCT never develop beyond this point in embryogenesis (Mostofi 1977).

A combined embryogenetic and functional approach to the interpretation of tumour patterns also offers a tentative explanation for the HCG positive STLC which are present in seminomas and non-seminomas as well as among the cells of the CIS pattern without the simultaneous presence of CC components. Current theories of GCT development implies that all the different tumour types except spermatocytic seminoma arise from the atypical cells of the CIS pattern (Skakkebæk and Berthelsen 1981). When these cells develop into tumours they imitate either the primitive spermatogonia and thus form seminomas or they imitate various structures occurring during the embryogenesis resulting in the various other types of GCT. As one of the first steps of differentiation in embryogenesis is formation of the trophoblast it is likely that one of the first steps of germ cell tumour differentiation is also trophoblastic. As tumour development from the atypical cells in addition is believed to be multifocal, the occurrence of STLC in seminiferous tubules with CIS as well as apparently random distribution of STLC within the tumour tissue is not incomprehensible.

As previously suggested on the basis of studies of the embryoid bodies

in the GCT (Marin-Padilla 1968) the different tumour components of GCT seem to reflect various stages of differentiation or maturation of cells or tissues in the conceptus, representing the various stages of embryogenesis from the morula or blastocyst stage to the stage of the bilaminar germ disc. In accordance with this the different tumour components may always be placed in a chronological order in regard to embryological development.

This study has mostly focused on the epithelial components of the tumours because these are prominent and because identification of functional properties of these cells is possible with immunohistochemical techniques. However, more careful studies of the mesenchymal components may provide us with further information in the context of tumour histogenesis and should be recommended. Thus, demonstration of fetal haemoglobin (HbF) in GCT has drawn our attention to an angioblastic component as well as to haemopoietic foci in the stroma (Jacobsen and Jacobsen 1983 b).

In order to help recognize the various structures in the tumours a terminology using embryological terms may be considered (Table 3). The solid pattern of EC may be named *embryoblastic*, the papillary or tubular pattern *epiblastic* or *ectodermal*, while the double-layered configuration and the PES types may be designated *epi-hypoblastic* or *ecto-endodermal structures*. The EST pattern should be named only *endodermal tumour* pattern, while the term *yolk sac tumour* should be reserved for tumours with recognizable yolk sac structures only. The myxoid stroma ("magma reticulare") pattern may be named *extraembryonic mesoblastic*. The STLC should be named *syncytiotrophoblast* without reservation, while tumours containing syncytiotrophoblast in addition to cytotrophoblast should still be designated *choriocarcinoma*.

Such a detailed histological description does not indicate that a new system of classification is necessary as it may be utilized within the current system.

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