Coll. Antropol. **29** (2005) 1: 189–197
UDC 611-013.39:616-006.2
Original scientific paper

Yolk Sac Carcinoma Derived from the Rat Epiblast as a Renal Isograft

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A B S T R A C T

We report the novel observation that a biphasic, parieto-visceral (PYS/VYS) yolk sac carcinoma can develop from the isolated epiblast of the pre-primitive streak rat embryo in a prolonged cultivation in vivo as a renal isograft. Late 7-day rat egg cylinders were dissected free of the ectoplacental cone and the Reichert's membrane. The middle segment of the cylinder, in which the embryonic and the extraembryonic cell layers partly overlap, were also removed. From the rest of the cylinder the 4 cell layers were isolated and transplanted separately under the kidney capsule of isogenic adult males. After 4 weeks the hypoblast was resorbed, the extraembryonic ectoderm gave rise to hemorrhagic cysts and trophoblastic giant cells, the extraembryonic (visceral yolk sac) endoderm formed benign cystic PYS/VYS tumors, and the epiblast developed into a benign teratoma. After prolonged (7–30 weeks) development of these teratomas as isografts, a malignant yolk sac carcinoma (YSC) developed in 45% of them. It destroyed the teratoma and the recipient's kidney, metastasized to peritoneum and other sites, and caused abundant ascites containing clustered tumor cells. The primary tumor was retransplantable subcutaneously as well as intraperitoneally, and displayed the characteristics of the mixed or biphasic PVYS carcinoma, with a progressive loss of the VYS component with time. Several data are apparently in favor of its origin by transdifferentiation rather than from undifferentiated cells.

Key words: *yolk sac carcinoma, epiblast, primitive ectoderm, rat embryo, transdifferentiation*

Introduction

The yolk sac (YS) of rat and mouse embryos is a topographically, histologically and functionally complex structure[1–5]. As a derivative of the hypoblast (primitive endoderm) its epithelial component displays selective inactivation of the paternal X-chromosome[6]. The involvement of gene products, transcription factors, growth factors and their receptors, and of signaling molecules in development and function of the murine YS epithelium has been partially elucidated[4,7–12].

As a consequence of the peculiar shape of early postimplantation rat and mouse embryos (»egg cylinder« with inverted germ layers), the continuous extraembryonic membrane, which surrounds the YS cavity, consists of two epithelial leaves: the visceral (inner) and the parietal (outer) YS endoderm (Figure 1). They both originate from the hypoblast or primitive endoderm[10,11], but differ strikingly in phenotypic traits and in relationships with adjacent tissues.

The *visceral endoderm* (VE) forms the outer layer of the entire egg cylinder. In the distal (embryonic) segment of the cylinder it surrounds the epiblast (primitive embryonic ectoderm) and consists of one layer of poorly differentiated squamous cells (hypoblast or primitive embryonic endoderm). This is a provisional embryonic structure to be replaced during gastrulation by the definitive embryonic endoderm[13–16]. In the proximal (extraembryonic) segment of the egg cylinder the VE first closely surrounds the extraembryonic ectoderm, and later on (after gastrulation) the extraembryonic mesoderm, with which it forms the *visceral yolk sac* (VYS). The extraembryonic VE forms a continuous epithelial sheet of cuboidal to columnar cells with ultrastructural and enzyme-histochemical characteristics suggesting an absorptive function[5]. The extraembryonic VE cells (VYS endoderm) also display morphological and functional characteristics of a glandular epithelium[17] and

Received for publication November 30, 2004

produce alpha-fetoprotein (AFP) and other fetal serum proteins under appropriate environmental conditions[4,18,19]. Many of the genes later expressed specifically in the adult gut or liver are expressed in the VYS endoderm[4]. Several lines of evidence thus suggest the primary roles of the VYS endoderm as an »early placenta« (nutrient uptake and transport before the formation of the chorioallantoic placenta) and an »early liver« (synthesis of macromolecules prior to the formation of the fetal liver)[7,8,11,20].

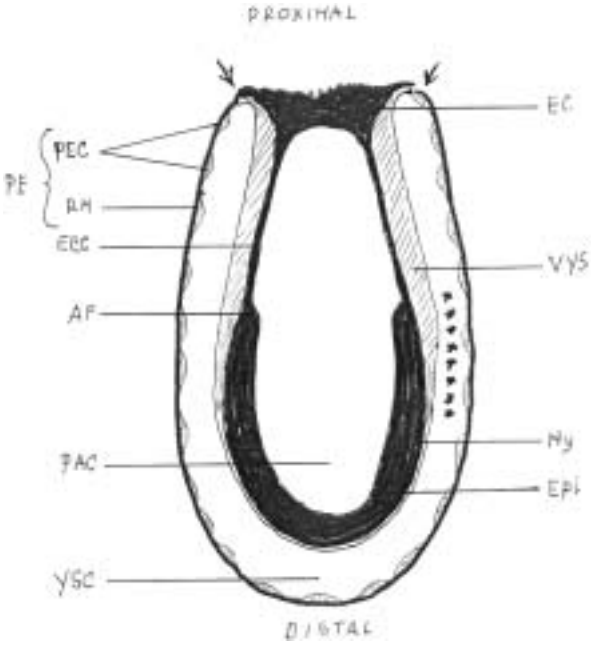

Fig. 1. Anatomy and terminology of the pre-primitive streak (pregastrulation) rat egg cylinder. AF, amniotic fold; EC, ectoplacental cone (cut off); EEC, extraembryonic ectoderm; Epi, epiblast; Hy, hypoblast; PAC, proamniotic cavity; PE, parietal endoderm; PEC, parietal endodermal cells; RM, Reichert's membrane; VE, visceral endoderm; VYS, visceral yolk sac endoderm; YSC, yolk sac cavity; the arrows point to the transitional zone between the visceral and the parietal yolk sacs; the asterix row indicates the overlapping zone of epiblast and the visceral yolk sac endoderm.

The *parietal endoderm* (PE) has no direct topographic relationship with the egg cylinder proper. It lines the inner surface of the mural trophectoderm cells, with which it forms the *parietal yolk sac* (PYS). Its flattened, fibroblast-like cells do not constitute a continuous epithelial layer, but establish only focal contacts. The PE first originates from the epiblast and later from the VYS endoderm; it forms by gradual displacement and cell detachment of VE cells[21,22], as the earliest example of an epithelio-mesenchyme transition[10,12] and transdifferentiation[23]. During this transition a reorganization of the cytoskeleton occurs: the expression of only cytokeratin intermediate filaments in the VE switches to co-expression of cytokeratin and vimentin in the PE[22]. The specific phenotypic trait of the PE is the production of a continuous, thick basement membrane (Reichert's membrane), which contains laminin and type IV procollagen as the major glycoprotein components[24–26]. The Reichert's membrane acts as a selective barrier between the early embryo and the maternal environment[9].

Tumors with phenotypic traits of VYS and/or PYS epithelium were experimentally produced from various sources in the rodent embryo. They have many features in common with the spontaneously arising human yolk sac carcinoma (YSC) or endodermal sinus tumor of Teilum[27–29]. So far the YSC was obtained by using the following tissues of origin and experimental procedures: a) cloning of spontaneous[24,30], and retinoic acid – induced[31,32] mouse teratocarcinoma cells; b) puncturing of 9-day rat embryos through the uterine wall *in situ;* c) isografts of whole pre-streak or early streak stage egg cylinders of the rat[33] and the mouse[34], d) isografts of the extraembryonic portion of the streak stage egg cylinder of the rat[35] and the mouse[36], e) isografts of the embryonic portion of the pre-streak to early-streak stage mouse egg cylinder[37,38], and f) extrauterine displacement *in situ* of fetal membranes (YS and amnion) after fetectomy at somite stage in rat[39], mouse[40] and hamster[41]. The primary tumors were usually benign teratomas in which the YS component occasionally appeared at a later stage. Some of the YSCs metastasized and produced ascites; they were mostly retransplantable and able to produce continuous cell lines *in vitro*[42]. Contrary to some previous assumptions, they could develop without a viral inductor[39] and they did not originate from cells of the germ line[40,42]. AFP-producing VYS cells are also a common component of murine teratocarcinomas[43].

The predominant cellular constituent of the YS tumor displays the PYS phenotype, characterized by large amounts of a hyaline, PAS- and laminin-positive, basement membrane-like extracellular material[24,25], but pleomorphic structures composed of AFP-producing VYS cells are also present[28,42,44]. This compound histological structure has been designated as the biphasic or parietovisceral yolk sac (PVYS) carcinoma[27].

In the present study we report the development of a metastasizing, ascites-forming and retransplantable PVYS carcinoma within long-term renal isografts of the isolated rat primitive embryonic ectoderm (epiblast).

Material and Methods

Experimental procedure

Females of Fischer rats were caged overnight with isogenic males. On the next morning the presence of sperms in the vaginal smear indicated pregnancy and 24 hours thereafter embryos were considered 1 day old. Late in the evening on the 8th day of pregnancy (CCA 7.5-day embryos, pregastrulation stage, less than 24 hrs before the onset of mesoderm formation) the females were sacrificed by cervical dislocation in a slight ether anesthesia. By using watchmaker forcepses the em-

bryos were removed from the decidual tissue in saline. Further dissection was carried out with electrolytically sharpened and polished tungsten needles. After removal of the ectoplacental cone and the PYS endoderm (Reichert's membrane) the whole egg cylinders (embryonic and extraembryonic parts) were treated with a mixture of 0.5% trypsin + 2.5% pancreatin, dissolved in calcium-and magnesium-free (CMF) Tyrode's saline, at +4 °C for 20 minutes. After rinsing in saline (with a few drops of isologous rat serum added to inactivate the trypsin) the germ layers (epiblast and hypoblast, extraembryonic ectoderm and extraembryonic endoderm) were separated from each other with tungsten needles under a dissection microscope[45,46]. Only the proximal (extraembryonic) and the distal (embryonic) segment of the egg cylinder were used for preparation of grafts (Figure 10). A narrow intermediate segment (the transition between the embryonic and the extraembryonic parts at the level of the amniotic fold) was discarded. In this way a »contamination« of the embryonic part with extraembryonic cells and *vice versa* was avoided. Because of difficulties occurring in dissection of so small specimens the two opposite segments were cut off from different egg cylinders. After separation of the two germ layers in each segment, four layers of different cell populations were obtained for transplantation: 1. epiblast (primitive embryonic ectoderm); 2. extraembryonic ectoderm; 3. hypoblast (primitive embryonic endoderm, or the embryonic part of the visceral endoderm) and 4. visceral extraembryonic endoderm (VYS endoderm). These specimens were separately transplanted under the kidney capsule of isogenic adult male recipients. The grafts were checked for the presence of outgrowths by laparotomy in ether anesthesia 4 weeks after transplantation. In animals bearing a visible outgrowth under the kidney capsule the abdominal incision was closed with wound clamps and they were kept in order to observe the further development of the tumor. After different time intervals, ranging from 7 to 30 weeks, the distended abdominal wall of recipient animals indicated the appearance of the ascites. As soon as this was observed, the animals were sacrificed. Fragments of the primary tumor and of the intraperitoneal metastases were subjected to histological analysis, while small pieces of the YS tumor (recognized by its typical macroscopic appearance) were retransplanted under the thigh skin of other recipient animals. The native ascites fluid was microscopically examined and injected (1–2 ml) subcutaneously or intraperitoneally to other recipients. The tumor fragments and the ascites fluid were respectively retransplanted and reinjected at least 6 times at intervals of 3–4 weeks. The rest of the ascites fluid and the serum samples from recipient animals were collected and stored at –70 °C for the electrophoretic demonstration of the AFP.

Histology and immunohistochemistry

All tissue samples were fixed in 4% formaldehyde in phosphate buffer. The paraffin-embedded serial sections were stained either with hematoxylin and eosin or by the periodic acid-Shiff (PAS) method. For the immunohistochemical demonstration of the basement membrane laminin and the intracellular alpha fetoprotein (AFP) 4–6 μm thick paraffin sections were placed on slides coated with poly-L-lysine and hotplated overnight to ensure maximal tissue adhesion. Sections were dewaxed in xylene, rehydrated through graded alcohols and then immersed for 10 minutes in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. After washing in distilled water the sections were immunostained by using the indirect immunoperoxidase method[47–50]. They were incubated with a) the rabbit polyclonal anti-laminin antibody (1:200, Sigma Chemical Co.) overnight at +4 °C, and b) with the goat polyclonal anti-alpha-1-fetoprotein antibody (1:200, DAKO Corporation) for 1 hour at room temperature. Following brief washes in phosphate-buffered saline (PBS containing 1% fetal calf serum) sections were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (swine anti-rabbit and rabbit anti anti-goat, DAKO Corporation) for 1 hour at room temperature. After washing in PBS for 10 minutes, the diaminobenzidine tetrachloride dihydrate (DAB) was used as chromogen. Before mounting, slides were counterstained with Mayer's hematoxylin. Appropriate positive and negative controls were run in parallel. Tissue sections immunolabelled with anti-laminin antibody were pre-digested (30–60 minutes, depending on the section size) with 0.4% pepsin (Sigma Chemical Co.) in 0.01N HCl to maximize immunoreactivity.

Polyacrylamide gel electrophoresis (PAGE)

Samples of serum and ascites fluid from tumor-bearing recipient animals were analyzed for the presence of AFP by polyacrylamide gel electrophoresis in the one-dimensional discontinuous system[51] as modified by Šerman and Škreb[52] to obtain colinearity of protein patterns in separate electrophoretic tubes. The protein titer was determined by the colored complex with Coomassie Brilliant Blue G-250, according to Bradford[53].

Results

During the 4 weeks following transplantation under the kidney capsule the two germ layers, isolated from the embryonic and the extraembryonic portions of the pre-streak rat egg cylinder, underwent different development and were accordingly subjected to different further treatments.

Extraembryonic (VYS) endoderm

The 20 successful grafts of the VYS endoderm appeared as transparent cysts, 2–4 mm in diameter. The external appearance of grafts was pleomorphic and sometimes polycystic. In histological sections epithelial cell layers as well as irregular cell clusters were present. Some of the layered epithelial cells displayed morphological characteristics of the absorptive VYS endo-

derm. The lumen of the cysts usually contained a loose mass resembling the coagulated viscous material. Epithelial cell clusters were invested by or embedded in an abundant hyaline, basement membrane-like eosinophilic material characteristic for a PYS tumor (Figure 2). The tumors were allowed to develop for further 5 months, but even then they did not show any substantial structural changes or signs of malignancy.

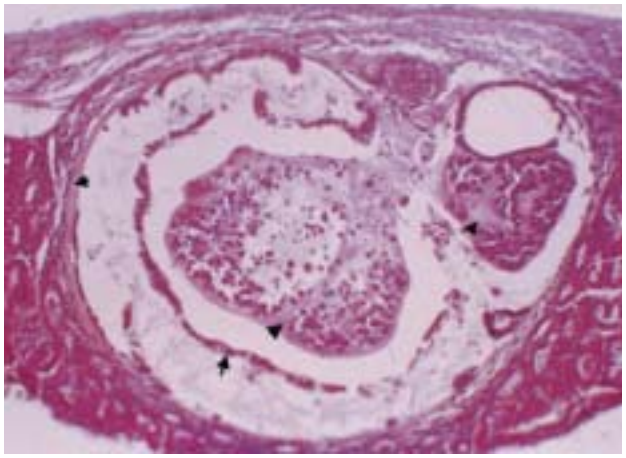

Fig. 2. Graft of the extraembryonic (VYS) endoderm 4 weeks after transplantation. The cystic tumor contains an epithelial layer with characteristics of the VYS epithelium (arrow). Other cell layers and aggregates represent the PYS component, characterized by an abundant amorphous and eosinophilic extracellular material (arrowheads). H and E.

Extraembryonic ectoderm

All 23 grafts transformed into large hemorrhagic cysts surrounded by trophoblastic giant cells. The parenchyma of the recipient's kidney was greatly destroyed.

Hypoblast (primitive embryonic endoderm)

All 20 grafts were completely resorbed.

Epiblast (primitive embryonic ectoderm)

Four weeks after transplantation 22 tumors, 3–7 mm in diameter, were found. They bulged from the surface of the kidney and were sharply outlined. The observation with the dissecting microscope revealed the typical external appearance of a benign embryo-derived teratoma. Some tissues, such as brain, endodermal cysts, (ossifying) cartilage, beating heart, muscle, white and brown adipose tissue etc. were clearly visible when situated close to the surface of the tumor. Neither penetration into the host tissue nor peritoneal metastatic nodules were observed. The tumors were not histologically analyzed, but the laparotomy incision was closed and the recipient animals were surveyed until the distension of the abdominal wall indicated the development of the ascites. This happened in 10 out of 22 operated animals (45.5%) after different time intervals, ranging from 7 to 30 weeks (i.e. 11 to 34 weeks or 77 to 238 days after transplantation). The recipient animals were then re-laparotomized. The rest of the text in this division deals with these tumors of epiblastic origin after prolonged development as renal isografts.

Large (19–64 g), irregularly outlined, grayish-yellow tumorous masses penetrated into the recipient's kidney and destroyed almost completely its parenchyma. Numerous metastatic nodules of various sizes were scattered all over the peritoneum. Mesenteric and paraortal lymph nodes were enlarged. In two animals metastases were also observed in the pleura, lungs and pulmonary lymph nodes. The peritoneal cavity contained up to 90 ml of sanguinolent ascites. In extreme cases the recipient animals were completely exhausted and about to die from progressive emaciation.

The dissection of primary tumors revealed some typical tissues of the initial benign teratoma: cartilage, bone, adipose tissue, epidermal cysts filled with hairs and a caseous mass (desquamated keratinized cells + sebum), and large endodermal cysts filled with a dense, mucous material[46,54,55]. However, the major part of the tumor consisted of an amorphous, soft, grayish-yellow mass containing areas with a spongy structure. Only these portions of the primary tumors, as well as metastatic nodules, were selected for histological and immunohistochemical analysis. They showed the characteristic histological structure of the parietal yolk sac carcinoma (PYSC). Small, pleomorphic cells with a scarce cytoplasm and dark nuclei occurred single, in small clusters, or lining small cysts. They were surrounded by or embedded in an abundant PAS-positive, basement membrane-like material (Figure 3) which reacted with the anti-laminin antibody (Figure 4). In areas with an extraordinary abundant extracellular material the tis-

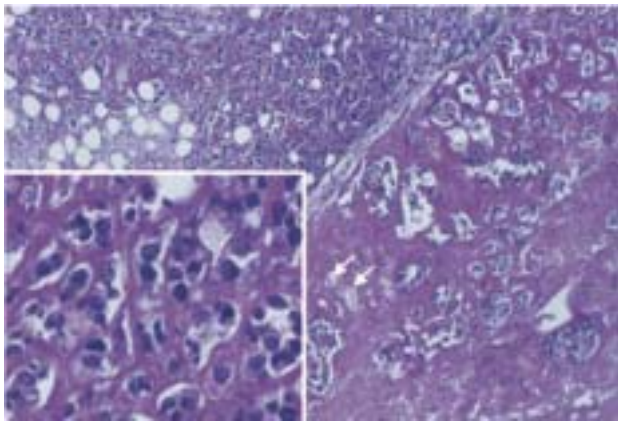

Fig. 3. Graft of the epiblast 20 weeks after transplantation. The two lobules, separated by a connective tissue septum, represent the PYS carcinoma at different stages of aging (different time of origin in the teratoma). Note the difference in the amount of the PAS-positive extracellular material between the early (left, enlarged in the insert) and the advanced stage (right). In the later the PE cells are reduced to small clusters embedded in the abundant extracellular material. Some of these cells undergo necrosis (arrowheads). PAS.

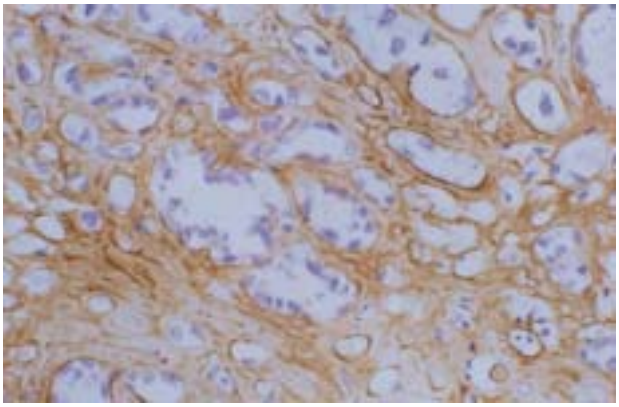

Fig. 4. Laminin in the intercellular matrix of a primary tumor. Laminin immunostaining.

sue had a cartilage-like appearance and some cells underwent necrosis within their »lacunae« (Figure 3). Exceptionally, some portions of the tumor had a papilloma-like structure, in which discontinuous cell rows enveloped the hyaline core (Figure 5).

Small portions of 9 out of 10 tumors consisted of nests of cells without any visible extracellular material.

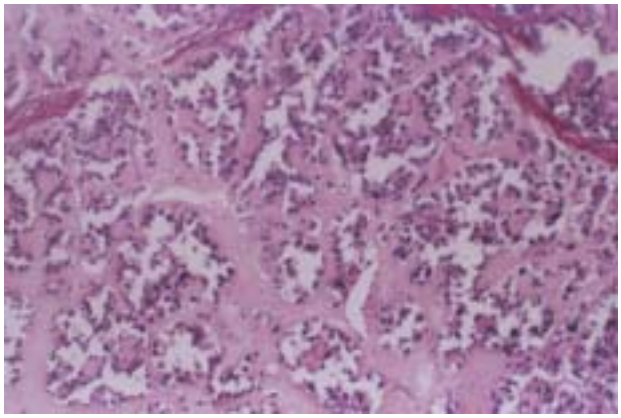

Fig. 5. Papillary form of a primary tumor. H and E.

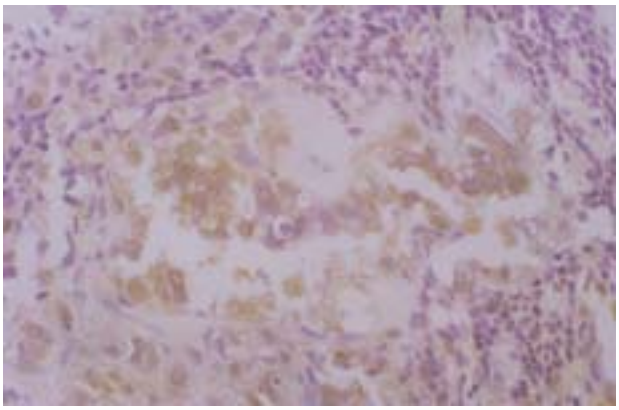

Fig. 6. AFP synthesizing cells in the VYS component of a primary tumor. AFP immunostaining.

The cells were much larger than those of the previously described PYS component. They had pale nuclei and abundant cytoplasm, which reacted positively with the anti-AFP antibody (Figure 6). They were therefore considered as equivalent to endodermal cells of the visceral yolk sac (VYS) though they were never aligned in the form of a surface epithelium. This complex histological structure and immunohistochemical features allow the characterization of the primary tumors as the combined or biphasic parietovisceral yolk sac carcinoma (PVYSC)[27]. They did not contain either mesenchymal tissues or trophoblastic giant cells.

The metastatic nodules displayed the histological structure of the monophasic PYSC (Figure 7).

When observed in the native state after 48 hours *in vitro,* the ascites fluid contained single or clustered cells. The external appearance of some cell clusters suggested the structure of embryoid bodies, but histologic sections of them were not made to confirm this impression (Figure 8).

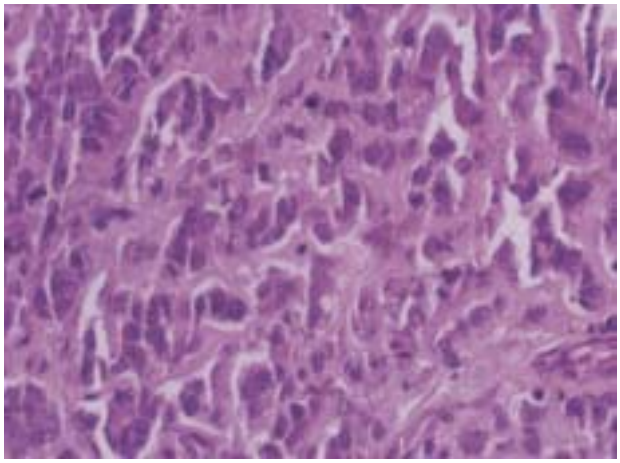

Fig. 7. Metastatic nodule. PYS structure. H and E.

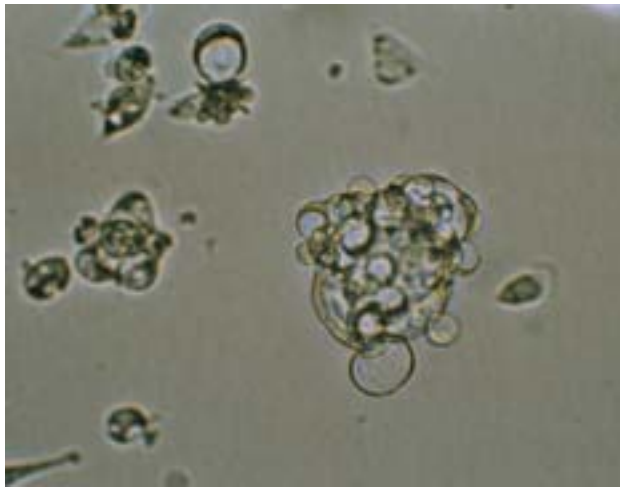

Fig. 8. YSC cells in the ascitic fluid. Note the embryoid body-like structure at the right. Phase contrast micrograph of native cells.

After subcutaneous transplantation and further serial transplantations of the tumor fragments or metastatic nodules, solid PYS tumors grew which did not metastasize or cause ascites. The subcutaneous injection of the ascites fluid gave the same result. The intraperitoneal injection of the ascites fluid into healthy recipients resulted in the development of diffusely scattered peritoneal tumor nodules followed by the appearance of ascites.

The PAGE screening disclosed the presence of the AFP in both the serum and the ascites fluid of recipient animals. The relevant band was expressed with variable intensity, with the tendency of decrease after serial transfers (Figure 9a, b).

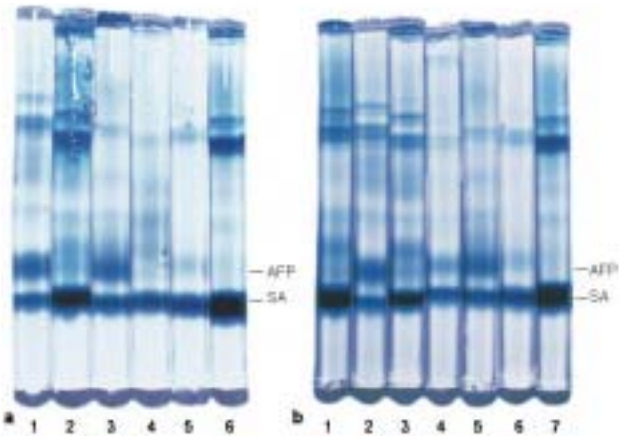

Fig. 9. PAGE of recipient's serum and ascitic fluid. (a) Retransplantations. 1 – primary tumor, serum; 2–6th retransplantation, serum; 3 – primary tumor, ascites; 4–2nd retransplantation, ascites; 5 – newborn rat, serum; 6 – adult (3 month) rat, serum. (b) Primary tumors. 1–20 weeks, serum; 2–26 weeks, serum; 3–22 weeks, serum; 4–26 weeks, ascites; 5–20 weeks, ascites; 6 – newborn rat, serum; 7 – adult (3 month) rat, serum. AFP, alpha fetoprotein; SA, serum albumin.

Discussion

As noted in the introductory part of this paper, tumors composed of visceral and/or parietal yolk sac endodermal cells develop spontaneously in humans and in some mouse strains at gonadal and extragonadal sites, but they can also be induced in laboratory mammals from a variety of sources and by various experimental manipulations. The multitude of results obtained in these experiments can hardly be reduced to a common denominator, particularly with respect to the possible cell type from which the tumor may originate.

In this paper we present the novel observation that a malignant (metastasizing, retransplantable, ascites-forming) biphasic, parietovisceral yolk sac (PVYS) tumor can develop in a long-term renal isograft of the isolated pre-streak epiblast (primitive embryonic ectoderm) of the rat. The various characteristics of this tumor (composition, malignancy, metastasis, transplantability to various sites, ascites form) have been extensively discussed elsewhere[28,42]. We will therefore restrict this discussion to three aspects which we consider to be essential for the evaluation of this investigation: a) design of the experiment; b) biphasic structure of the tumor; and c) origin of the tumor.

Design of the experiment

The correct demarcation of the graft from the rest of the rat (or mouse) egg cylinder seems to be the crucial point of the experiment. This is particularly true in view of considerations on the cell origin of tumors. In previous experiments, using two- or three-layered *embryonic parts* of rat or mouse egg cylinders as grafts, the embryos were »dissected free from extraembryonic tissue«[35,37,38]. The amniotic fold and the amnion itself of the two-layered and the three-layered embryos respectively are conventionally considered as the sharp boundary between the embryonic and the extraembryonic segments of the egg cylinder. In fact, in the two-layered embryo this is only true for the epiblast (primitive embryonic ectoderm). The (visceral) endoderm, however, retains its VYS characteristics (cuboidal absorptive epithelium with vacuolated cytoplasm) over the entire proximal half of the epiblast and shows only a gradual transition into the simple squamous hypoblast (or primitive *embryonic* endoderm) which is restricted to the distal half (or even less) of the embryo proper (embryonic shield) (Figure10). Even at the stage of advanced gastrulation (three-layered embryo) the distal border of the VYS endoderm can still traverse the level of the amnion

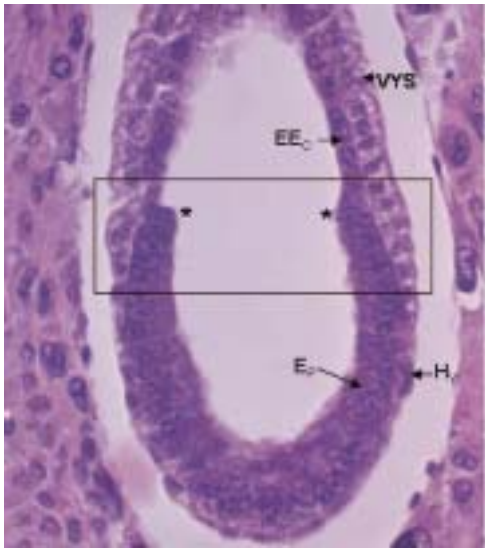

Fig. 10. Cell layers of the pre-primitive streak rat eg cylinder. Ep, epiblast; Hy, hypoblast; VYS, visceral yolk sac endoderm; EEc, extraembryonic ectoderm. Note the abrupt transition between the epiblast (primitive embryonic ectoderm) and the extraembryonic ectoderm at the level of the amniotic fold (asterix), and the wide overlapping of the VYS endoderm and the proximal part of the epiblast. The area within the rectangle was excluded from cell layer separation and subsequent separate transplantation.

and overlap the very proximal part of the embryonic ectoderm. In view of the considerable stage-variability among early embryos of the same litter, even a relatively deep incision distally from the level of the (pro)amnion cannot assure that the separated distal part of the egg cylinder be devoided of any VYS endodermal cells.

In the present study we avoided this problem by isolating the mere epiblast of the pre-primitive streak rat embryo and by removing its very proximal part (close to the proamniotic fold) prior to transplantation.

Biphasic structure of the tumor

In the early reports only the PYS component was described in YS tumors[34,37]. In later studies it became evident that at least the primary tumors – although composed predominantly of the PYS tissue – are as a rule biphasic (parietovisceral)[27] and that the biphasic character is usually retained after retransplantation and in metastases[27,28,38,42]. However, after retransplantation of tumors[38,56] or serial passages of the established YSC cell line[57], only the PYS component could be identified. In the present study the primary tumors were biphasic, with a predominant PYS character. The few examined metastatic nodules and the serial subcutaneous retransplants displayed the monophasic PYS structure. This, however, must be considered with precaution because a) the small specimens for subcutaneous (re)-transplantation were taken at random from a predominantly PYS primary tumor, and b) the PAGE screening of recipients' serum and ascitic fluid demonstrated the presence of small amounts of the AFP even after several retransplantations.

The differentiation of VE vs. PE was reported to depend on the local environment formed by the adjacent cell layers[58], by the type of cell-assemblage (monolayer or aggregate)[59], or by other atypical interactions[60]. These specific environmental conditions cannot be assumed to exist within the complex structure of the embryo-derived teratoma. Bearing in mind that the PYS endoderm is a terminally differentiated epithelium[11] which develops by transdifferentiation of the VYS endoderm[2] and by its epithelio-mesenchyme transition[10,12], it is reasonable to assume that the same course of differentiation also occurs in teratoma: in the absence of the specific environmental control which exists in situ, the VE autonomously follows its developmental path by differentiating into the PE. This could explain the biphasic nature of the primary tumor and the progressive loss of the VE component with time.

Origin of the tumor

The cell of origin of the YSC has not been determined in any of the experiments with various cell sources and experimental procedures. Two main lines of consideration are being proposed: a) re-direction of the differentiation of more primitive (stem) cells, and b) transdifferentiation or re-programming of already overtly differentiated cells[2]. At the first sight, the former possibility appears more plausible, even if one excludes the germline as the possible source. Different hypothetical stem cells have hitherto been proposed: undifferentiated cells within the germ layers[37], embryonal carcinoma cells that underwent extraembryonic differentiation[38], and multipotential cells different from germ cells that arise by dedifferentiation in the displaced yolk sac[28,42].

However, the bulk of evidence seems to add more weight to the alternative possibility of transdifferentiation of mature tissues in teratomas into YS cells. The susceptibility of differentiated cells to re-programming by extrinsic factors has been well documented by observations of transdifferentiation along a reverse pathway: from differentiated extraembryonic tissues (yolk sac[42,61], and amnion[62]) to mature fetal tissues.

In the attempt to consider the results of the present investigation with regard to the possible cell of origin of the YSC, the following data have to be born in mind: a) the graft consisted of the mere epiblast, free of any extraembryonic cells; b) the claims of Pedersen et al.[63] and Dziadek[64] on the ability of the early epiblast to regenerate the previously removed hypoblast cannot be affirmatively considered[1,2,64]; c) the suggestion of Tam and Beddington[65] that at the pre-streak stage of the mouse embryo some endodermal cells arise by focal delamination from the epiblast and contribute to the endoderm, has not been confirmed; d) in diversely designed experiments with tracing single or few labeled epiblast cells at the blastocyst and the early postimplantation stages of the mouse embryo, it was shown that the epiblast contributes cells to fetal tissues, germ line, amnion and extraembryonic mesoderm, but not to the extraembryonic (YS) endoderm[15,66–70]; e) in our previous experiments with grafting whole rat embryonic shields or isolated germ layers under the kidney capsule for 15–30 days, extraembryonic tissues were never found in teratomas[46,54,71]; f) in grafting experiments the benign teratoma always precedes the appearance of the YSC. This holds good even when not the embryo, but the mature YS displaced after fetectomy, is the source of the YSC[28,42]; g) the YSC arises as a late event in the teratoma[38,42], this study).

Taken together, these data lend little support to the presumption that the YSC originated from cells already present in the epiblast at the time of isolation and transplantation, or from any other type of undifferentiated stem cells appearing later on in the graft. We therefore prefer to believe that it developed by transdifferentiation from a mature epithelial constituent of the teratoma. Reinforcement in this belief came from a preliminary observation in a new series of experiments designed identically to the present one. In routinely stained serial sections of a teratoma, 2 months after transplantation of the epiblast, a gradual transition between the ciliated epithelium of an endodermal cyst and an irregularly shaped mass of closely packed cells was observed. At a certain distance from the epithelium of origin, an eosinophilic hyaline material appeared be-

tween these cells, increasing in amount towards the periphery. Although insufficient for any definite interpretation, this single observation is suggestive of the transformation of a differentiated surface epithelium into an irregular cellular mass, which gradually aquires the histological appearance of a PYS tumor.

The other three transplanted embryonic cell layers developed according to expectations based on previous observations. The hypoblast was completely resorbed, the extraembryonic (VYS) endoderm developed into biphasic PYS/VYS benign cystic tumors, while the extraembryonic ectoderm gave rise to trophoblastic giant cells and caused abundant hemorrhage, as reported elsewhere[72,73].

Acknowledgements

The authors are grateful to Drs. S. Audy-Jurković, Z. Smerić, M. Vlahović and A. Šerman for valuable help in different segments of the work, and to Đ. Cesar and R. Delaš for technical assistance. The work was supported by the grant No. 108–219 from the Ministry for Science and Technology of the Republic of Croatia.

This paper is dedicated to the memory of Professor Anton Švajger who inspired generations of scientists with his enthusiasm and scientific wisdom. Work presented here is his last scientific contribution to the field of developmental biology.

Abbreviations:

AFP, alpha fetoprotein
PAGE, polyacrylamide gel electrophoresis
PE, parietal endoderm
PVYS, parietovisceral yolk sac
PYS, parietal yolk sac
VE, visceral endoderm
VYS, visceral yolk sac
YS, yolk sac
YSC, yolk sac carcinoma

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KARCINOM ŽUMANJČANE VREĆE KOJI JE NASTAO OD ŠTAKORSKIH STANICA EPIBLASTA KAO BUBREŽNOG IZOGRAFTA

S A Ž E T A K

U radu se iznosi novo opažanje da se parijetalno-visceralni (PYS/VYS) oblik karcinoma žumanjčane vreće može razviti u kulturi *in vivo* nakon transplantacije predgastrulacijskog primitivnog ektoderma štakorskog zametka pod bubrežnu čahuru izogenične životinje. U sedam dana starih štakorskih zametaka odstranjeni su ektoplancentalni dio embrionalnog cilindra, Reichertova membrana i središnji dio cilindra u kojem se embrionalni i izvanembrionalni slojevi stanica djelomično preklapaju. Iz preostalog dijela cilindra izolirana su četiri stanična sloja te je svaki od njih posebno transplantiran pod bubrežnu čahuru izogenih odraslih mužjaka. Nakon četiri tjedna životinje su žrtvovane i bubrezi s transplantatima su makroskopski pregledani. Endodermalni transplantati su se resorbirali, od izvanembrionalnog ektoderma razvile su se hemoragijske ciste s orijaškim stanicama trofoblasta, od izvanembrionalnog endoderma (visceralnog epitela žumanjčane vreće) nastali su dobroćudni PYS/VYS cistični tumori, dok se primitivni ektoderm razvio u teratome. Nakon dugotrajne kulture (7–30 dana) u 45% teratoma razvio se karcinom žumanjčane vreće. Karcinom je infiltrirao i uništio teratom i parenhim bubrega, metastazirao je u potrbušnicu i uzrokovao stvaranje ascitesa s nakupinama tumorskih stanica. Primarni tumor je bilo moguće dokazati nakon potkožne i intraperitonealne retransplantacije sa značajkama mješovitog ili parijetalno-visceralnog karcinoma kojem je u dugotrajnoj kulturi primjećen gubitak visceralne komponente žumanjčane vreće. Neki podaci govore u prilog da se tumor razvio transdiferencijacijom, a ne od nediferenciranih stanica.