

## Endodermal origin of yolk-sac-derived teratomas

H. SOBIS, A. VERSTUYF and M. VANDEPUTTE

*Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium*

### Summary

Mutant mice deficient in glucose-6-phosphate dehydrogenase were used to induce teratomas. This enzyme is linked to the X chromosome, which can be inactivated in female embryo. The differences in the enzyme activity between yolk sac mesoderm and embryo *versus* yolk sac endoderm can be detected in female concepti by using appropriate crosses of wild-type and G6PD-deficient mice. Histochemical study showed that the dual cell population was observed in heterozygous embryos and in the embryomas derived from them. The teratomas derived from the corresponding yolk sac, however, were

G6PD-positive from wild-type and G6PD-negative from homozygous enzyme-deficient mothers. We conclude that yolk-sac-derived teratomas are of endodermal origin because of the fact that the paternal X chromosome is inactivated in the yolk sac endoderm, whereas in the yolk sac mesoderm, as in the embryo, the inactivation is at random.

Key words: teratoma, yolk-sac, endoderm, transdifferentiation, mouse embryo, glucose-6-phosphate dehydrogenase.

### Introduction

After displacement, the rodent visceral yolk sac differentiates into benign teratomas containing derivatives of all three germ layers. This differentiation was observed in the following: (1) the visceral yolk sac pulled outside the uterus after fetectomy (Sobis and Vandeputte, 1974, 1977, 1979); (2) the visceral yolk sac transplanted into the peritoneum (Payne and Payne, 1961; Sobis *et al.* 1986) and (3) this membrane cultured *in vitro* as organ culture (Lu *et al.* 1984). These observations indicate the presence or appearance in the displaced visceral yolk sac of multipotential stem cells capable of differentiating into derivatives of all three germ layers. These stem cells might correspond to the poorly differentiated cells observed in the early stages of teratoma formation (Sobis *et al.* 1982). Numerous experiments indicate that these poorly differentiated cells are not germ cells (Sobis and Vandeputte, 1976, 1979; Sobis *et al.* 1982). Hence, the stem cells have to originate from mesoderm or from endoderm as these are the only two layers present in the visceral yolk sac. We used polyclonal antiserum against endodermal antigen(s) (Van Hove *et al.* 1978), monoclonal antibodies against visceral endoderm (Van Hove *et al.* 1984) and various lectins reacting specifically with the visceral endoderm (Sobis *et al.* 1989) in order to distinguish between these two possibilities. The results of all these experiments indicate that the yolk-sac-derived teratomas are most probably of endodermal origin. However, definite proof is still lacking. Only a marker that distinguishes endodermal from mesodermal derivatives during embryogenesis and fetal development

can provide such proof. Therefore, an X-linked enzyme marker would be very helpful. In the female embryo, one of the X chromosomes is inactivated (Lyon, 1961, 1972). This inactivation is at random in embryonal ectoderm but the paternal X chromosome (Xp) is preferentially inactivated (Takagi and Sasaki, 1975; Wake *et al.* 1976; Frels and Chapman, 1980; Harper *et al.* 1982; West, 1982) in the trophectoderm and in the yolk sac endoderm. The maternal X chromosome (Xm) should be expressed in teratomas when of endodermal origin since the yolk sac mesoderm, similarly to primary germ cells, derive from embryonal ectoderm (Gardner *et al.* 1985). In the embryos corresponding to this membrane, the expression of X chromosomes may be partially of maternal and partially of paternal origin. If the inactivation of X chromosome is at random in the yolk-sac-derived teratomas, as in the embryo, this would indicate a mesodermal origin of the tumour.

Mutant mice deficient in glucose-6-phosphate dehydrogenase (G6PD) (Pretsch *et al.* 1988), recently described, make it possible to verify from which of the tissues present in the visceral yolk sac (endoderm or mesoderm) the teratomas develop. This X-linked enzyme expressed in very low amounts in hemizygous G6PD-deficient males (20% *versus* 100% in wild-type mice) will be fully expressed in yolk sac endoderm belonging to heterozygous female embryos developed after mating wild-type females with the hemizygous enzyme-deficient male. In contrast, the homozygous G6PD-deficient female, after copulation with wild-type males will develop female concepti which are composed of heterozygous embryos and enzyme-negative yolk sac endoderm. These results were confirmed by the

histochemical technique (Sobis and Vandeputte, unpublished data).

In this report, we describe the differences in G6PD activity observed between the female embryos and the teratomas developed from their corresponding visceral yolk sacs.

## Materials and methods

G6PD-deficient males and syngeneic C3H/E1 wild-type females were obtained from Dr W. Pretsch and were bred in the Proefdierencentrum, Leuven.

As anticipated, the crosses between wild-type  $G6pd^a/G6pd^a$  ( $a/a$ ) females with hemizygous  $G6pd^{a-m1Neu}/-$  ( $a-m1Neu/-$ ) males and between homozygous  $G6pd^{a-m1Neu}/G6pd^{a-m1Neu}$  ( $a-m1Neu/a-m1Neu$ ) females with wild-type  $a/-$  males all resulted in heterozygous female embryos (Sobis and Vandeputte, unpublished results). For this reason, these were the only two combinations used in our experiment (Fig. 1). The yolk sac endoderm of female fetuses from wild-type mothers will be G6PD-positive and this same tissue from homozygous females will be enzyme-negative (Fig. 1). Teratomas were obtained by the method described previously by Sobis and Vandeputte, 1976. Briefly, at day 11 of pregnancy, fetectomy was performed and the visceral yolk sac was pulled outside the uterus. After removal the fetuses were frozen separately or transplanted under the kidney capsule of a syngeneic host to develop into embryomas. In order to be able to match at autopsy each operated site with the corresponding

fetus, threads of different colours were sewn at the sites of the uterus, where the fetal membrane was left outside. The mice were killed 3 weeks after operation and the teratomas or embryomas were frozen in liquid nitrogen. Pieces of skin, gut, oesophagus and trachea from 2-week-old homozygous G6PD-deficient, heterozygous and wild-type female mice were also frozen.

## Histochemistry

Fresh frozen sections of embryos, teratomas, embryomas and tissues taken from young female mice were tested for the G6PD activity by the method described by Thomas *et al.* (1988) with some modifications. The sections were incubated in media comprising 5 mM nitroblue tetrazolium (NBT), 0.8 mM NADP, 10 mM monosodium glucose-6-phosphate (G6P), 0.32 mM phenazine methosulfate (all from Sigma Ltd, Poole, Dorset), 5 mM  $NaN_3$ , 4 mM  $MgCl_2$  and 20% polyvinyl alcohol GO4/140 (Wacker Ltd, Walton-on-Thames, Surrey) in 0.05 M Tris-HCl buffer at pH 7.5. We found that an incubation time of 20 min at 37°C enabled us to distinguish, under optimal conditions, between the different phenotypes in the various tissues present in the teratomas and embryomas. Control sections were incubated in media devoid of either G6P alone or both G6P and NADP. Sections were post-fixed in 4% formol saline, counterstained with carmin or methyl green and mounted in glycerine jelly or Canada balsam.

## Results

All fetectomized mice developed tumours attached to the uterine horns. Their number was approximately half the number of yolk sacs left outside the uteri during operation. All embryos transplanted under kidney capsule developed into tumours (embryomas). The histology was identical to that previously described (Sobis and Vandeputte, 1974, 1979, 1982) for the yolk-sac-derived benign teratomas. The tissues most frequently found were endodermal and ectodermal cysts, gut, skin with its appendages, trachea and cartilage. The tumours obtained after transplantation of the embryos (embryomas) were composed of various tissues. Among these, gut, skin and cartilage were regularly observed.

Tissues from 2-week-old female mice, corresponding to those most frequently found in the teratomas and embryomas, were examined for G6PD activity. In wild-type  $a/a$  mice, the skin, trachea, gut and oesophagus were strongly positive. In the organs from the heterozygous  $a/a-m1Neu$  females, a dual cell population was observed. The enzyme could not be detected in tissues from homozygous  $a-m1Neu/a-m1Neu$  females, with the exception of skin. The epithelium of this organ was slightly positive for G6PD.

The crosses between  $a/a$  wild-type females and hemizygous  $a-m1Neu/-$  males produced 26 teratomas on 47 operated sites where fetectomy and displacement of the visceral yolk sac had been performed. The G6PD activity in embryonal tissues was strong in 24 embryos and weaker in 23. 26 teratomas developed at the site of fetectomy (64) from homozygous  $a-m1Neu/a-m1Neu$  mothers mated with wild-type  $a/-$  males. 36 embryos were G6PD-negative while in the other 28 enzyme

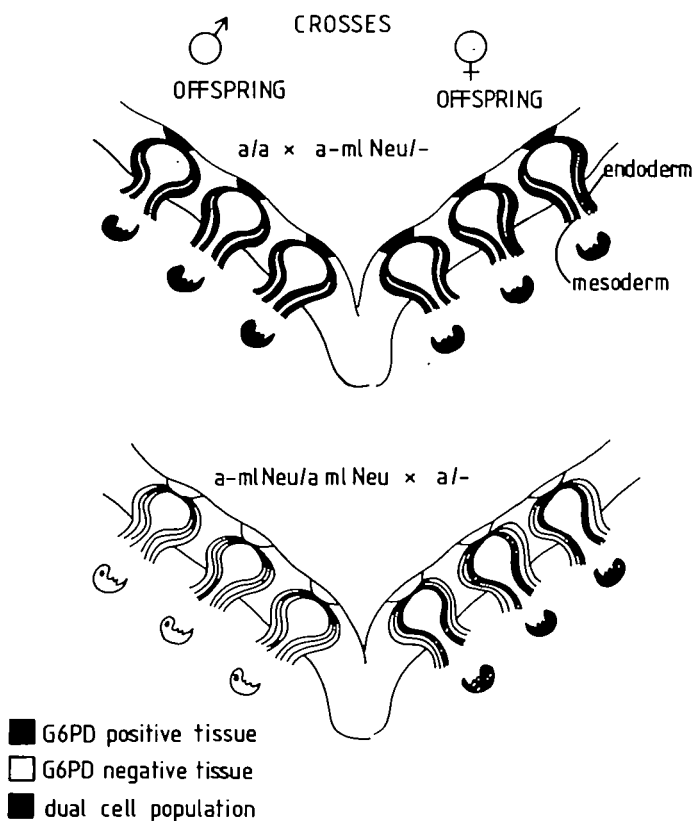


Fig. 1. Expected G6PD activity in fetuses and fetal membranes of fetectomized wild-type or homozygous enzyme-deficient females mated with hemizygous G6PD deficient or wild-type male.

**Table 1.** G6PD activity in embryos and corresponding teratomas

Crosses	Embryo	Teratoma	No.
$a/a \times a\text{-mlNeu}/-$	+/-*	+	7
	+	+	9
$a\text{-mlNeu}/a\text{-mlNeu} \times a/-$	+/-	-	6
	-	-	8

+, presence of G6PD activity.  
 -, absence of G6PD activity.  
 \* +/-, dual cell population.

activity was present. Embryos with high G6PD activity from the first crosses ( $a/a \times a\text{-mlNeu}/-$ ) and without the enzyme from the second crosses ( $a\text{-mlNeu}/a\text{-mlNeu} \times a/-$ ) are expected to be males. To the contrary, the female embryos from both crosses are heterozygous and should display a dual population of G6PD-positive and -negative cells. The endoderm of the visceral yolk sac should be G6PD-positive for the first crosses and G6PD-negative for the second. It was, however, very difficult to obtain frozen sections of sufficient quality from the isolated embryos always to be able to distinguish the dual cell population from wild-type and hemizygous enzyme-deficient. Therefore, we only report on the teratomas corresponding to the embryos in which a clear-cut diagnosis could be made (Table 1).

From the  $a/a \times a\text{-mlNeu}/-$  crosses, all teratomas showed strong G6PD activity which was most pronounced in skin and its appendages (Fig. 2A), gut (Fig. 2B) and in epithelium of endodermal and epidermal cysts. From the  $a\text{-mlNeu}/a\text{-mlNeu} \times a/-$  crosses, all teratomas were G6PD-negative (Fig. 3). Only in the skin a weak reaction was seen. Similar results were obtained in the skin of 2-week-old homozygous enzyme-deficient females.

We did not obtain absolutely convincing results with the experiments on embryos due to technical difficulties. Therefore we considered the possibility of replacing the isolated embryos by the tumours derived from them (embryomas). This experiment enabled us to compare the presence of G6PD in tissues differentiated from the visceral yolk sac and from the corresponding embryos. The tumours obtained, after transplantation of the embryos under the kidney capsule, showed either high or very low enzyme activity in all cells of organoid structure or G6PD-positive and -negative dual cell population (Fig. 4A-C). Only the latter was developed from the heterogenous female embryos. In the cells of the teratomas, corresponding to these embryos, the enzyme was present in the first crosses (Fig. 5A,B) and absent in the second crosses (Fig. 6A,B, Table 2).

## Discussion

Our results show that teratomas derived from visceral yolk sac are of endodermal origin. The detection of differences in X-linked G6PD activity between yolk sac mesoderm and endoderm in female concepti using

**Table 2.** G6PD activity in embryomas and corresponding teratomas

Crosses	Embryo	Teratoma	No.
$a/a \times a\text{-mlNeu}/-$	+/-*	+	10
	+	+	11
$a\text{-mlNeu}/a\text{-mlNeu} \times a/-$	+/-	-	9
	-	-	8

\* +/-, dual cell population.

appropriate crosses of wild-type and G6PD-deficient mice led to the above conclusion. It is known that, in the yolk sac endoderm, the paternally derived X chromosome is preferentially inactivated. However, in the embryo proper and the yolk sac mesoderm, this inactivation is random (Takagi and Sasaki, 1975). We did observe differences in the enzyme activity between the embryos and the corresponding teratomas. However, the observations made on cryostat sections of isolated 11-day-old embryos were not always of sufficient quality to give reliable results. Moreover, in spite of the stability of the G6PD activity described during embryogenesis and in adult life (Luzzatto and Battistuzzi, 1985), we wanted to verify if, in embryo-derived tumors (embryomas), a dual cell population could eventually be shown. For these reasons, we performed the second experiment in which we compared the G6PD activity in the tissues, differentiated from the yolk sac pulled outside the uterus, with the tissues developed from the corresponding embryos transplanted under the kidney capsule. The fetectomized and transplanted mice were killed at the same time. The histochemical tests were performed under the same conditions. The results clearly show that nearly half of the embryomas and all teratomas from crosses of wild-type females with hemizygous enzyme-deficient males contain tissues uniformly positive for G6PD. The other half of the embryomas are composed of tissues containing a dual (G6PD+ and -) cell population. The latter are expected to develop from female embryos. The corresponding teratomas are G6PD-positive. The enzymatic reaction in the organoid structures of the teratomas is similar to that in organs from wild-type females, while the tissues in embryomas can be compared to the tissues from heterozygous mice. In the second combination, composed of homozygous G6PD-deficient females and wild-type males, all teratomas and half of the embryomas are nearly devoid of the enzyme. The other half of the embryomas are composed of dual cell population. These results clearly indicate that the teratomas develop from a tissue in which the Xp chromosome is inactivated and therefore originates from the endoderm of the visceral yolk sac. Moreover, the obtained data exclude a germ cell origin of the yolk-sac-derived tumour. It is known that the germ cells develop from ectoderm in which inactivation of one of the X chromosomes is random (McMahon *et al.* 1981; Gardner *et al.* 1985).

Previously, we have shown that the yolk-sac-derived teratomas develop from poorly differentiated cells that

appear during the first days after displacement of the fetal membrane (Sobis *et al.* 1982). These cells show the characteristics of endodermal cells and have dedifferentiated from yolk sac endoderm (Sobis *et al.* 1989). Since they proliferate and differentiate into derivatives of all three germ layers, this phenomenon has to be considered as transdifferentiation (Eguchi, 1976). It has still to be determined by which mechanism the mature endodermal yolk sac cells can switch to form ectodermal and mesodermal organoid structures.

We thank Dr W. Pretsch for providing G6PD-deficient mice. This work was supported by the Belgian ASLK Cancer Foundation. We are indebted to Miss Ria Vanlaer, Mr L. Bassi, Mr G. Hermans and Mr C. Seghers for skilful technical assistance. The editorial assistance of Miss Christiane Callebaut is appreciated.

## References

- EGUCHI, G. (1976). Transdifferentiation of vertebrate cells in cell culture. In *Ciba Foundation Symposium* no. 40. *Embryogenesis in Mammals*, pp. 241–258. Amsterdam: Elsevier.
- FRELS, W. I. AND CHAPMAN, V. M. (1980). Expression of the maternally derived X chromosome in the mural trophoblast of the mouse. *J. Embryol. exp. Morph.* **56**, 179–190.
- GARDNER, R. M., LYON, M. F., EVANS, E. P. AND BURTEENSHAW, M. D. (1985). Clonal analysis of X-chromosome inactivation and the origin of the germ line in the mouse embryo. *J. Embryol. exp. Morph.* **88**, 349–363.
- HARPER, M. I., FOSTEN, M. AND MONK, M. (1982). Preferential paternal X inactivation in extra-embryonic tissues of early mouse embryos. *J. Embryol. exp. Morph.* **67**, 127–135.
- LU, Y. L., SOBIS, H., VAN HOVE, L. AND VANDEPUTTE, M. (1984). Differentiation potentiality of rat visceral yolk sac in organ culture. *J. Embryol. exp. Morph.* **80**, 127–136.
- LUZZATTO, L. AND BATTISTUZZI, G. (1985). Glucose-6-phosphate dehydrogenase. In *Advances in Human Genetics* (ed. H. Harris and K. Hirschorn), pp. 217–329. New York: Plenum Press.
- LYON, M. F. (1961). Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* **190**, 372–373.
- LYON, M. F. (1972). X-Chromosome inactivation and developmental patterns in mammals. *Biol. Rev.* **47**, 1–35.
- MCMAHON, A., FOSTEN, M. AND MONK, M. (1981). Random X-chromosome inactivation in female primordial germ cells in the mouse. *J. Embryol. exp. Morph.* **64**, 251–258.
- PAYNE, J. M. AND PAYNE, S. (1961). Placental grafts in rats. *J. Embryol. exp. Morph.* **9**, 106–116.
- PRETSCH, W., CHARLES, D. J. AND MERKLE, S. (1988). X-Linked glucose-6-phosphate dehydrogenase deficiency in *Mus musculus*. *Biochem. Genet.* **26**, 89–103.
- SOBIS, H., GOEBELS, J. AND VANDEPUTTE, M. (1986). Histochemical and autoradiographic study of the cultured rat visceral yolk sac. *J. Embryol. exp. Morph.* **97**, 169–176.
- SOBIS, H. AND VANDEPUTTE, M. (1974). Development of teratomas from displaced visceral yolk sac. *Int. J. Cancer* **13**, 444–453.
- SOBIS, H. AND VANDEPUTTE, M. (1975). Sequential morphological study of teratomas derived from displaced yolk sac. *Devl Biol.* **45**, 276–290.
- SOBIS, H. AND VANDEPUTTE, M. (1976). Yolk sac-derived rat teratomas are not of germ cell origin. *Devl Biol.* **51**, 320–323.
- SOBIS, H. AND VANDEPUTTE, M. (1977). Yolk-sac-derived teratomas and carcinomas in hamsters. *Eur. J. Cancer* **13**, 1175–1181.
- SOBIS, H. AND VANDEPUTTE, M. (1979). Teratoma induction in mice and rats in relation to the age of the visceral yolk sac. *Eur. J. Cancer* **15**, 143–151.
- SOBIS, H. AND VANDEPUTTE, M. (1982). Development of teratomas from yolk sac of genetically sterile embryos. *Devl Biol.* **92**, 553–556.
- SOBIS, H., VAN HOVE, L. AND VANDEPUTTE, M. (1982). Cellular events during early formation of yolk-sac-derived teratomas. *J. Embryol. exp. Morph.* **70**, 225–240.
- SOBIS, H., VERSTUYF, A. AND VANDEPUTTE, M. (1989). Nature of the multipotential cells in the displaced rat yolk sac. *Tumour Biol.* **10**, 140–148.
- TAKAGI, N. AND SASAKI, M. (1975). Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature* **256**, 640–642.
- THOMAS, G. A., WILLIAMS, D. AND WILLIAMS, E. D. (1988). The demonstration of tissue clonality by X-linked enzyme histochemistry. *J. Pathol.* **155**, 101–108.
- VAN HOVE, L., DELACOURT, M., PARK, B., SOBIS, H. AND VANDEPUTTE, M. (1978). Presence of common surface antigen(s) on endodermal tumours and embryonal tissues of rats, hamsters and mice. *Int. J. Cancer* **21**, 731–740.
- VAN HOVE, L., SOBIS, H., LU, Y. L. AND VANDEPUTTE, M. (1984). A rat yolk-sac antigen defined by monoclonal antibodies. *Int. J. Cancer* **33**, 851–858.
- WAKE, N., TAKAGI, N. AND SASAKI, M. (1976). Non-random inactivation of X chromosome in the rat yolk sac. *Nature* **262**, 580–581.
- WEST, J. D. (1982). X Chromosome expression during mouse embryogenesis. In *Genetic Control of Gametic Production and Function*, pp. 49–91. New York: Academic Press.

(Accepted 27 September 1990)