Mouse egg cylinders developed in vitro may form benign and malignant teratoid tumors

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Summary. Egg cylinders developed in vitro from explanted mouse blastocysts were transplanted under the kidney capsules of adult mice to assess their developmental potential. In vitro grown egg cylinders are capable of differentiation into somatic tissues and may give rise to stem cells of teratocarcinoma like the developmentally equivalent 7 day mouse embryos grown in vivo.

The in vitro techniques have been developed for growing mouse blastocysts to early somitic stages of development[1,2]. It has been shown that embryos grown in vitro resemble morphologically embryos grown in vivo[3] but their long term developmental capacity was never tested. Since it has been known that early postimplantation mouse embryos (egg cylinders) transplanted to extrauterine sites give rise to tumors composed of various somatic tissues (teratomas) and malignant teratocarcinomas (tumors composed of benign somatic tissues and malignant stem cells)[4], we wanted to determine whether mouse egg cylinders grown in vitro have the same developmental capacity as the embryos grown in vivo.

Materials and methods. Blastocysts were flushed from the uteri of timed-pregnant inbred BALB/cJ mice during the afternoon h of the 4th day of pregnancy (plug day = 1). Blastocysts were explanted into 35 mm plastic dishes and cultured in a CO_2 incubator according to the specifications given by Hsu[1]. Under these conditions, explanted blastocysts developed into egg cylinders and by day 4 reached stages 9–11 of Witschi[5]. As indicated before[2] egg cylinders developed from blastocysts grow upwards, downwards or laterally, depending on the initial positioning of the inner cell mass of the blastocyst at the time of attachment to the plastic dish. Upward growing egg cylinders, morphologically most alike to the 7 day egg cylinders grown in vivo, were selected on day 4 of culture and transplanted with a micropipette underneath the kidney capsule of syngeneic adult female BALB/cJ mice. Normal invivo grown egg cylinders, removed during the morning h of day 7 of pregnancy served as controls. These embryos were isolated from decidual swellings of timed pregnant BALB/cJ mice, washed in normal saline, cleaned of Reichert's membranes and transplanted in toto in an identical way as the in vitro grown embryos.

Since the egg cylinders developed in vitro were incubated in medium supplemented with fetal calf serum (FCS), another group of 7 day embryos were transferred to FCS, left for 30 min, and transplanted under the kidney capsule of adult recipients to determine if FCS affects the outcome of transplantation. All graft bearing animals were kept in our animal colony for 2 months and sacrificed 2 months after transplantation. The grafts were removed, their largest diameters were measured and they were classified as large (> 10 mm), medium (5–10 mm) or small (< 5 mm). All the grafts were examined histologically and classified as teratomas, teratocarcinomas or yolk sac cysts. The data were statistically evaluated using the χ^2-test.

Results. The data are summarized in the table. Teratomas and teratocarcinomas were identified histologically in all 3 groups, but the incidence of teratocarcinomas was much lower in the experimental group than in the 2 control groups. We were able to obtain only 3 teratocarcinomas from the in vitro grown egg cylinders whereas in the control groups, approximately one half of all teratoid tumors were malignant. Most of the grafts in the experimental group were small and measured less than 5 mm in diameter, whereas medium and large sized tumors predominated in the 2 control groups. Approximately 20% of all embryonic grafts gave rise to yolk sac cysts. Teratomas obtained from in vitro grown egg cylinders contained essentially all the somatic tissues that were identified in the control groups. Teratocarcinomas from all the groups contained approximately similar amounts of embryonal carcinoma cells (ECC) and contained all the somatic tissues seen in the teratomas. Yolk sac cysts were lined by visceral yolk sac epithelium and did not differ from yolk sac cysts obtained by Solter and Damjanov[6] from extraembryonic portions of mouse egg cylinders transplanted to adult kidneys.

Discussion. In this study we have shown that egg cylinders developed in vitro contain embryonic cells that can either differentiate into mature somatic tissues or transform into rapidly proliferating stem cells of teratocarcinomas, equivalent to ECC. A considerable number of grafts (20%) gave rise to yolk sac cysts which were not seen in our previous experiments performed with only the embryonic portions of the egg cylinder[7] or the egg cylinders of another mouse strain (C3H) transplanted in toto[8]. We have no explanation for these differences. It is also not clear why in vitro grown egg cylinders give rise to fewer teratocarcinomas than the in vivo grown embryos of the same developmental age. The initially entertained hypothesis that the bathing of embryos in FCS could be accompanied by an adverse immune response to FCS upon transplantation was made unlikely

Teratomas and teratocarcinomas obtained from in vivo and in vitro grown BALB/c egg cylinders transplanted under the kidney capsule

Grafts	No. of grafts	No. of teratomas	No. of teratocarcinomas	Ratio of malignant teratoid tumors	No. of yolk sac cysts (% of total grafts)	Size of teratoid tumors		
						Small	Medium	Large
Egg cylinder grown in vitro	21	14	3[a]	18%	4 (19%)	13[b]	1	3
Egg cylinder in vivo (N/S)	33	13	13[a]	50%	7 (21%)	5[b]	7	14
Egg cylinder in vivo (FCS)	14	6	5	45%	3 (21%)	1	8	2

(N/S, normal saline; FCS, fetal calf serum.) Small = diameter ≤ 5 mm; medium = 5 mm < diameter ≤ 10 mm; large = 10 mm < diameter. [a] $\chi^2 = 4.6$ ($p < 0.05$); [b] $\chi^2 = 9.15$ ($p < 0.001$).

by the fact that embryos in FCS (2nd control group) did not differ from embryos bathed in saline with regards to their capacity to form teratocarcinomas. The most likely explanation for the low yield of teratocarcinomas from in vitro grown embryos lies in the decreased viability of certain cells in these embryos. We have shown previously that only 27% of all in vitro grown egg cylinders developed into somitic stage embryos if cultured for an additional four days in vitro. Since the number of teratocarcinomas was in the same range it seems that teratocarcinomas develop only from most viable embryos. Although the number of teratocarcinomas was small, it nevertheless proves that the in vitro conditions have not anulled the capacity of the embryonic cells to form teratocarcinomas. On the other hand, this potential for transition into stem cells of teratocarcinomas can be fully realized only upon transplantation into living animals, since we have never seen teratocarcinomas developing in vitro from embryos kept for extended periods of time in culture.

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Streptozotocin induced diabetic nephropathy and renal tumors in the rat

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Summary. Adult Wistar rats rendered diabetic by a single dose of streptozotocin develop renal morphological changes which show subtle differences compared to those seen in human diabetic renal disease. The early tubular degeneration is sited in the distal rather than the proximal convoluted tubule and subsequent glomerular lesion shows linear deposits of IgG and albumin in the basement membrane rather than in the mesangium. The carcinogenicity of streptozotocin in the rat is reconfirmed.

Streptozotocin [2-deoxy-2-(3 methyl-3-nitrosamines)-D-glucopyranose] is a drug produced by the soil organism *Streptomyces achromogenes*[1] which is commonly used experimentally for the induction of diabetes mellitus[3]. It selectively damages pancreatic beta islet cells but in addition to its diabetogenic effect it is a potent carcinogen in many animal species[4–6]. This paper briefly reports the main morphological renal changes in the experimental diabetic rat and draws comparisons with renal lesions of human diabetes.

50 male adult Wistar rats of b.wt 180–225 given a single i.v. dose of 65 mg/kg b.wt of streptozotocin (Upjohn, USA) were compared to control rats treated with normal saline. All rats were housed in galvanized wire cages in air conditioned rooms and fed on a diet of stock pellets (Allied Feeds) with water ad libitum. Following administration of streptozotocin rats were initially tested daily and then at weekly intervals for presence of glycosuria and hyperglycaemia as indicators of diabetes. 30 streptozotocin treated rats were considered diabetic when they showed random urinary glucose levels in excess of 500 mg/dl and blood glucose levels 300–400 mg/dl. Rats became diabetic within 2 weeks following administration of streptozotocin and in addition to glycosuria and hyperglycaemia exhibited polyuria and weight loss but failed to develop hypertension. Groups of diabetic and control rats were therefore sacrificed at intervals from 2 to 30 weeks and examined for evidence of renal diabetic nephropathy and tumor development. Kidney tissues from both groups were processed routinely for examination by light, electronmicroscopy as well as for the localization of immunoglobulins and albumin using a modified immunoperoxidase technique[7].

Morphologically, the earliest visible light microscopic changes in kidneys of diabetic rats in the initial 2 weeks comprised swelling and vacuolation of epithelial cells in the macula densa particularly affecting those cells lying opposite but not immediately adjacent to the glomerular stalk. Subsequently such epithelial degenerative changes became more marked or extensive with progression of diabetes so that by 3–4 weeks almost the entire distal tubular epithelium was affected (fig. 1). Histochemical staining showed that the swelling and vacuolation of epithelial cell cytoplasm was primarily due to an accumulation of fluid and glycogen granules within the cytoplasm rather than to an accumulation of lipid as is the case in human diabetes. The tubular degenerative changes although similar to those reported in man nevertheless differed in that the distal tubule is affected in the rat whereas the proximal tubule is involved in the human. The tubular degenerative changes are considered to be due to the diabetic state rather than to a direct toxic effect of streptozotocin because the changes were not seen immediately following streptozotocin administration.

Various glomerular lesions comparable with those found in human diabetic glomerular disease were noted. These changes included formation of 'fibrinoid caps', linear electron-dense deposits in the basement membrane and focal segmental mesangial sclerosis, however glomerulosclerosis of the nodular (Kimmelsteil-Wilson) type were not found. Fibrinoid caps (fig. 1) seen on light microscopy, corresponded in ultrastructural studies to electron-dense deposits in a subendothelial location adjacent to which there was fusion of foot processes of podocytes. Such lesions first appeared at day 30 and became increasingly more frequent thereafter. The linear deposits in the basement membrane, present after 14 days, were shown by immunoperoxidase staining to comprise both IgG and albumin and contrasted with several previous reports in which the deposits in the experimental rat were localized in the mesangium[8–11].

5 months following administration of streptozotocin 6 of the residual rats developed renal tumors. Tumors were