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SURVIVAL OF RAT EMBRYONIC PARTS AFTER ECTOPIC TRANSPLANTATION

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SUMMARY – Investigation of the developmental potential of embryonic cells is important in designing novel approaches in the field of regenerative medicine. The purpose of our experiments was to compare the survival of different embryonic tissues transplanted at an ectopic site *in vivo*. Embryo proper (9.5-day-old), neural retinas (20-day-old), lensectomized eyes (14- and 18-day-old), epiglottis (17-day-old), mandible (13- and 14-day-old), lacrimal gland (17- and 20-day-old) were microsurgically isolated from rat embryos and transplanted under the renal capsule. The embryo-proper survived in transplants for at least 60 days. Fetal rat retina survived in transplants for 180 days forming rosettes. Neural and glia cells with abundant neuropil as well as plexiform layers were found by electron microscopy. The lensectomized eye survived in transplants for 66 days. Transdifferentiation of the retina to lens cells was discovered. The epiglottis, mandible and lacrimal gland survived in transplants for at least 14 days. In the mandible, fully structured teeth developed. This study showed not only the early postimplantation embryo but also various more developed tissues and organs to be able to survive under the renal capsule for at least 14 days. This ectopic site has therefore proved to be a very convenient environment for transplantation experiments.

Key words: *Embryo; Transplantation; Renal capsule; Survival; Differentiation*

Introduction

Basic research in mammalian developmental biology has been recognized to be of utmost importance for the development of new therapies such as *in vitro* fertilization[1]. Today, the accent in clinically applicable basic research is put on investigation of the developmental potential of different cells and tissues, which is important in designing novel approaches to tissue replacement therapy aimed to cure damaged parts of the body[2]. Pluripotential embryonic cells can also be used in gene therapy as well as in the studies of embryotoxic substances[3-5]. The purpose of the present study was to compare the survival of various embryonic tissues after transplantation at an ectopic site under the renal capsule.

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Material and Methods

Fischer rats were mated overnight and the morning finding of the sperm in vaginal smear indicated that embryos were 0.5-day-old. Microsurgical isolation of different embryonic tissues and organs was done under a dissecting microscope by fine watchmaker's forceps, Graefe's knives and tungsten needles. From 9.5-day-old embryos, embryonic shield containing three germ layers (embryo-proper) was isolated and extraembryonic parts were discarded. In 20-day-old embryos, enucleation of the eye was done and neural retina was isolated. From the eyes of 14- and 18-day-old embryos, the eye lens was extracted and discarded. The epiglottis was isolated from 17-day-old, the mandible from 13- and 14-day-old, and lacrimal gland from 17- and 20-day-old embryos.

Adult Fischer males were anesthetized with ether, and the skin and muscle cut to approach the kidney. A small "pocket" was made under the renal capsule to place the transplant where it spent various periods of time (14-180 days).

Transplants were fixed in St. Marie's solution (1% acetic acid in 96% ethanol, +4 °C), dehydrated and paraffin embedded. Uninterrupted serial sections (5 mm) were used for histologic analysis and stained by HE or PAS.

Some specimens were fixed for TEM in 4% buffered glutaraldehyde and postfixed in 1% OsO_4. After dehydration, embedding in Durcopan was done and ultrathin sections were contrasted with lead citrate and uranyl acetate, and examined by transmission electron microscopy.

Results

Transplants of the whole gastrulating embryo-proper were shown to survive for 60 days under the renal capsule. Different tissues such as epidermis with its appendages

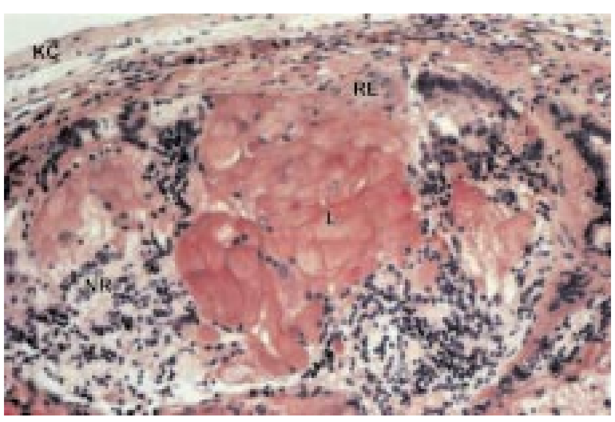

Fig. 1. Lensectomized embryonic eye (18-day-old) transplant after 57 days.
Note typical lentoids in close proximity to retinal epithelium. RE, retinal epithelium; NR, neural retina; L, lentoid; KC, kidney capsule; X200.

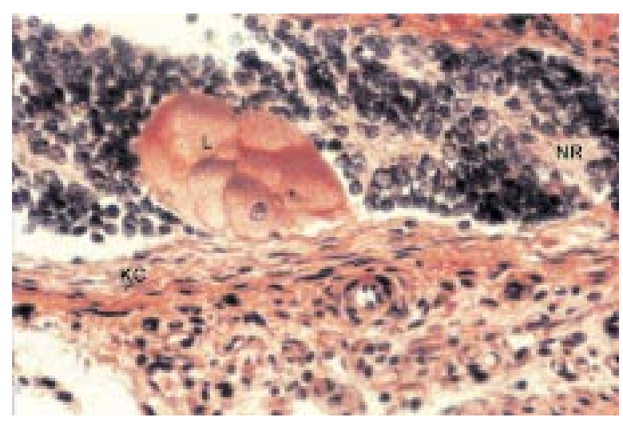

Fig. 2. Lensectomized embryonic eye (18-day-old) transplant after 14 days. Note typical lens cells with granular eosinophilic cytoplasm and a big light nucleus with nucleoli. The lentoid is positioned between neural retina cells and vascularized renal capsule. NR, neural retina; L, lentoid; KC, kidney capsule; X400.

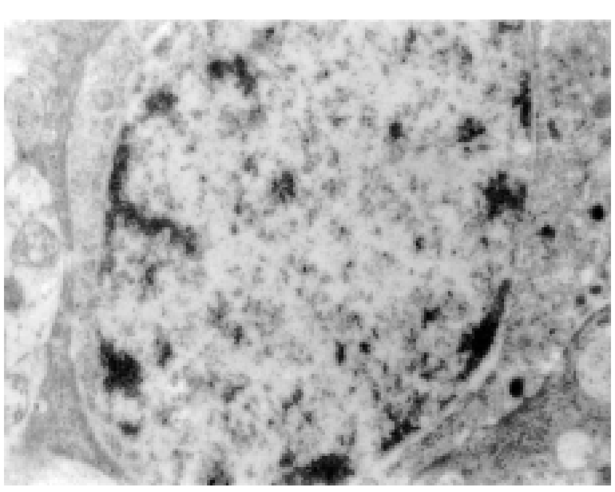

Fig. 3. Rat embryonic retina transplant after 120 days. Note a typical neural cell nucleus with abundant euchromatin and adjacent neuropil; TEM X7000.

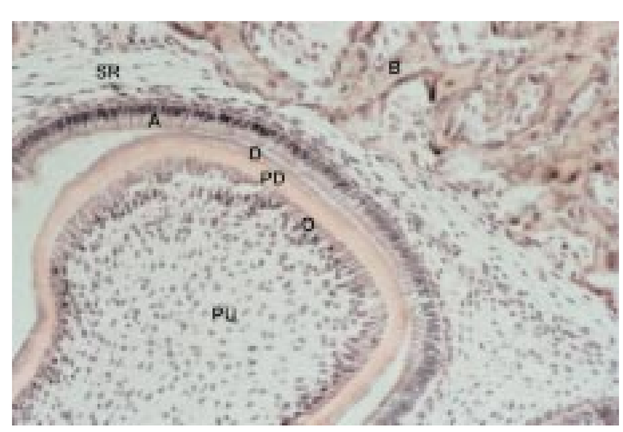

Fig. 4. Bell differentiation stage of tooth development in a transplant of embryonic mandible (13-day-old). SR, stellate reticulum; A, ameloblasts; D, dentin, PD, predentin; O, odontoblasts; PU, pulp; B, bone; X200.

(hair, sebaceous glands), brain tissue, retinal epithelium, vegetative ganglia, smooth and striated muscle, cartilage, bone, epithelium of the digestive and respiratory tracts, and glandular tissue (e.g., thyroid) were present in transplants organized in a teratoma-like pattern.

Neural retina survived under the renal capsule for as long as 180 days. Its orderly structure was lost and rosettes were formed. Neural (Fig.1) and glia cells with abundant neuropil as well as plexiform layers were found by transmission electron microscopy. However, typical photoreceptors have not yet been detected.

Transplants of the enucleated eye survived for 66 days under the renal capsule. Staining by PAS method specific for the basement membrane showed that no remainings of the lens capsule were present and that the lens was extracted *in toto*. However, in transplants typical lens cells were found adjacently to the retinal cells (Figs. 2 and 3).

The epiglottis survived in transplants for 14 days. Its typical shape was well preserved under the renal capsule. Cartilage was enveloped in perichondrium and two different epithelia were present at its surface. Stratified squamous epitehlium was found on the one side, and columnar epithelium on the other side.

The mandible also preserved its shape well under the renal capsule. In both kinds of transplant, originating from either 13- or 14-day-old embryo, teeth in the bell differentiating stage of development were detected (Fig. 4).

Lacrimal glands survived in transplants for 14 days. In transplants, lacrimal gland epithelial cells and excretory ducts as well as epidermis were found.

Discussion

Study results indicated that several different mammalian embryonic tissues and organs were able to survive under the renal capsule of a male syngeneic animal for at least 14 days. The epiglottis and the mandible preserved their shape under the renal capsule, which was not the case with softer explants such as the embryo-proper or the retina that showed a disorganized structure.

In case of lacrimal gland, some cells were still in the cycling compartment and therefore still able to divide[6]. It was also found that the inductive interactions and subsequent differentiation could have proceeded on, so that the gastrulating embryo developed into a teratoma containing various derivatives of the three germ layers. At the same ectopic site, the mouse embryo was found to give rise to a teratocarcinoma[7]. Other pluripotential cells such as embryonic germ cells derived *in vitro* from primordial germ cells (PGCs) of the mouse formed differentiated tumors[8]. Investigation of the developmental potential of the rat embryo-proper precultivated *in vitro* in chemically defined media showed the degree of differentiation to be higher in transplants than in explants cultivated *in vitro*[9]. However, the same restriction of differentiation potential for specific tissues observed after *in vitro* culture was also found in transplants[10]. A combination of *in vitro* cultivation with subsequent transplantation to this ectopic site enabled investigation of the development of definitive endoderm[11], which did not develop at all under the renal capsule[12]. The subcapsular kidney space seems to be more favorable for investigation of the developmental potential of embryonic pluripotent cells than other ectopic sites such as the anterior chamber of the eye or chorioallantoic membrane of the chick embryo, where the differentiation potential appears to be more restricted and does not result in a wide variety of cell types or in the formation of complex structures resembling morphogenesis[12,13].

In case of the fetal mandible transplant, we demonstrated that complete morphogenesis of an organ was possible at the rat subcapsular kidney space. The rat mandible was isolated at an early stage just before and at the moment of the appearance of dental lamina (several layers of cuboidal cells), which is the first indication of odontogenesis[14]. After 14 days, well developed teeth were always found (Fig. 3). The subcapsular kidney space in the mouse was used for investigation of compromised odontogenesis in Msx1-deficient mice because it allowed completion of organogenesis and terminal differentiation of BMP4 rescued tooth germs in cell culture[15], and investigation of odontogenesis in mouse embryonic lethal knockouts such as mice deficient for Pdgfra[16].

The subcapsular kidney space was also favorable for the study of transdifferentiation. In transplanted 14- and 18-day-old rat lensectomized eyes, retinal cells transdifferentiated to lentoids[17]. In corneal epithelium the ability of transdifferentiation to typical epidermis with hairs was detected not only in embryonic but also in 12-day-old offspring corneas transplanted under the renal capsule of athymic mice[18].

We found that neural retina survived for as long as 180 days under the renal capsule although photoreceptor differentiation could not be proven. That is a very long period of culture for a primary explant which usually survives *in vitro* for only few days[19,20]. Such a prolonged *in vivo* culture period could be important for future investigation of the positive activity of various neurotrophic factors over a long period of time.

Conclusion

This investigation showed that various embryonic tissues, organs and even the whole postimplantation mammalian embryo could survive and differentiate further on after transplantation under the renal capsule. Moreover, the subcapsular kidney space at which a prolonged cultivation can be exerted is superior to *in vitro* culture of primary explants.

References

1. BULIĆ-JAKUŠ F. Nikola Škreb Memorial Symposium: Experimental Mammalian Embryology at the Turn of the Century. Period Biol 2004;106:313-6.
2. GLASER V. Regenerative medicine R&D comes of age. Gen Eng News 2003;23:24-5.
3. BULIĆ-JAKUŠ F, VLAHOVIĆ M, JURIĆ-LEKIĆ G, CRNEK-KUNSTELJ V, ŠERMAN D. Gastrulating rat embryo in a serum-free culture model: changes of development caused by teratogen 5-azacytidine. ATLA 1999;27:925-33.
4. BULIĆ-JAKUŠ F, VLAHOVIĆ M, CRNEK V, ŠERMAN D. Model kulture *in vitro* zametka štakora u razvojnom stadiju najosjetljivijem na vanjske teratogene faktore. In: RADAČIĆ M, BAŠIĆ I, ELJUGA D, eds. Pokusni modeli u biomedicini. Zagreb: Medicinska naklada, 2000:215-22.
5. WOBUS AM, BOHELER KR. Embryonic stem cells: prospects for developmental biology and cell therapy (review). Physiol Rev 2005;85:635-78.
6. KATUŠIĆ A, ŠERMAN Lj, BULIĆ-JAKUŠ F, JURIĆ-LEKIĆ G, VLAHOVIĆ M, KRATOCHWIL K. The expression of proliferating cell nuclear antigen and retinoblastoma protein in transplanted fetal rat lacrimal gland. Acta Clin Croat 2004;43:69-73.
7. ŠKREB N, SOLTER D, DAMJANOV I. Developmental biology of the murine egg cylinder. Int J Dev Biol 1991;35:161-76.
8. DURCOVA-HILLS G, WIANNY F, MERRIMAN J, ZERNICKA-GOETZ M, McLAREN A. Developmental fate of embryonic germ cells (EGCs), *in vivo* and *in vitro*. Differentiation 2003;71:135-41.
9. BULIĆ-JAKUŠ F, STRAHINIĆ-BELOVARI T, MARIĆ S, JEŽEK D, JURIĆ-LEKIĆ G, VLAHOVIĆ M, ŠERMAN D. Chemically defined protein-free *in vitro* culture of mammalian embryo does not restrict its developmental potential for differentiation of skin appendages. Cells Tissues Organs 2001;169:134-43.
10. BELOVARI T, BULIĆ-JAKUŠ F, JURIĆ-LEKIĆ G, MARIĆ S, JEŽEK D, VLAHOVIĆ M. Differentiation of rat neural tissue in a serum-free embryo culture model followed by *in vivo* transplantation. Croatian Med J 2001;42:401-5.
11. KUBO A, SHINOZAKI K, SHANNON JM, KOUSKOFF V, KENNEDY M, WOO S, FEHLING HJ, KELLER G. Development of definitive endoderm from embryonic stem cells in culture. Development 2004;131:1651-62.
12. LEVAK-ŠVAJGER B, KNEŽEVIĆ V, ŠVAJGER A. Development of separated germ layers of rodent embryos on ectopic sites: a reappraisal. Int J Dev Biol 1991;35:177-89.
13. GAJOVIĆ S, GRUSS P. Differentiation of the mouse embryoid bodies grafted on the chorioallantoic membrane of the chick embryo. Int J Dev Biol 1998;42:225-8.
14. RUGH R. The mouse: its reproduction and development. Minneapolis: Burgess Publ. Co., 1968:227-37.
15. BEI M, KRATOCHWIL K, MAAS RL. BMP 4 rescues a non-cell-autonomous function of Msx1 in tooth development. Development 2000;127:4711-8.
16. XU X, BRINGAS P, SORIANO P, CHAI Y. PDGFR-alpha signaling is critical for tooth cusp and palate morphogenesis. Dev Dyn 2005;232:75-84.
17. JURIĆ-LEKIĆ G, BULIĆ-JAKUŠ F, KABLAR B, ŠVAJGER A. The ability of the epithelium of diencephalic origin to differentiate into cells of the ocular lens. Int J Dev Biol 1991;35:231-7.
18. FERRARIS C, CHALOINDUFAU C, DHOUAILLY D. Transdifferentiation of embryonic and postnatal rabbit corneal epithelial cells. Differentiation 1994;57:89-96.
19. BULIĆ-JAKUŠ F, JURIĆ-LEKIĆ G, BELOVARI T, MARIĆ S, JEŽEK D, VLAHOVIĆ M, ŠERMAN D. Survival of rat foetal neural retina in serum-free media *in vitro* and in subcapsular kidney transplants *in vivo*. Vet Arch 2000;70:321-9.
20. IWASAKI Y, ICHIKAWA Y, IGARASHI O, IKEDA K, KINOSHITA M. Influence of termocapril on cultured ventral spinal cord neurons. Neurochem Res 2003;28:711-4.

Sažetak

PREŽIVLJAVANJE EMBRIJSKOG TKIVA ŠTAKORA NAKON EKTOPIČNE TRANSPLANTACIJE

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Istraživanje razvojnoga potencijala embrijskih stanica osnova je novih pristupa terapiji u području regenerativne medicine. U našim istraživanjima proučavali smo preživljenje različitih embrijskih tkiva nakon ektopične transplantacije *in vivo*.

Zametak u užem smislu starosti 9,5 dana, neuralna mrežnica (20 dana), lensektomirano oko (14 i 18 dana), epiglotis (17 dana), mandibula (13 i 14 dana) te suzna žlijezda (17 i 20 dana) mikrokirurški su izolirane iz štakorskih zametaka navedenih starosti i transplantirane pod bubrežnu čahuru odraslih štakora. Zametak je u transplantatu preživio 60 dana. Fetalna mrežnica preživjela je čak 180 dana stvarajući rozete u transplantatu. Elektronskom mikroskopijom u njoj su dokazane živčane i glija stanice s obilnim neuropilom i mrežastim slojem. Lensektomirano oko preživjelo je 66 dana, a u transplantatu je otkrivena transdiferencijacija mrežnice u stanice leće. Epiglotis, mandibula i suzna žlijezda preživjele su 14 dana u transplantatu. U mandibuli su se razvili dobro formirani zubi. Pokazali smo, dakle, da uz rani poslijeimplantacijski zametak štakora, različita druga diferenciranija tkiva i organi zametka mogu također preživjeti pod bubrežnom čahurom najmanje 14 dana. Ovo ektopično mjesto ponovno se je dokazalo kao vrlo pogodno za pokuse transplantacije.

Ključne riječi: *Embrij; Transplantacija; Bubrežna čahura; Preživljenje; Diferencijacija*