Acta Dermatovenerol Croat 2008;16(2):55-59 MEDICAL RESEARCH

Development of Epithelia in the Ectopic Transplant of the Fetal Rat Epiglottis

Sandra Marinović Kulišić[1], Gordana Jurić-Lekić[2], Floriana Bulić-Jakuš[3], Vedran Radujković[2], Ida Parčetić[2], Maja Vlahović[3], Ana Katušić[3], Nino Sinčić[3], Ljiljana Šerman[3]

[1]University Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine; [2]Department of Histology and Embryology; [3]Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia

Corresponding author:
Sandra Marinović Kulišić, MD, MS
University Department of Dermatology and Venereology
Zagreb University Hospital Center and School of Medicine
Šalata 4
HR-10000 Zagreb
Croatia
sandra.marinovic@zg.htnet.hr

Received: March 4, 2008
Accepted: May 5, 2008

SUMMARY Embryonic *in situ* development is strictly regulated within the specific microenvironment of developing tissues. However, for regenerative medicine purposes (supplementation of damaged tissues/organs), transplantation to ectopic sites has been considered. To investigate developmental potential of fetal epiglottic epithelia at an ectopic site, fetal epiglottis was transplanted under the kidney capsule and its development compared to fetal and adult epiglottis. Seventeen-day-old Fischer rat epiglottides were microsurgically isolated under a dissecting microscope and transplanted under the kidney capsule of adult males. After 14 days, classic histology and immunohistochemical detection of the Proliferating Cell Nuclear Antigen (PCNA) were done in isolated and accordingly fixed transplants. The 17-day-old fetal epiglottis and adult epiglottis were processed in the same way. The 17-day-old fetal epiglottides were covered with immature epithelium expressing PCNA in almost all cells. Adult epiglottis was covered with two types of epithelia (stratified squamous epithelium and ciliated pseudostratified epithelium). In the stratified squamous epithelium PCNA was abundantly expressed in the basal cell layer and absent from more superficial and more differentiated cells. Transplants survived well during the experimental period. On their surface ciliated pseudostratified epithelium could be easily recognized, but squamous epithelium was almost absent. PCNA was expressed in basal cells of the ciliated pseudostratified epithelium and was absent from the more differentiated superficial cells. It seems that at this ectopic site further differentiation of the epiglottic epithelia can proceed but differentiation of squamous epithelium seems not to be favored. It seems that this ectopic site is optimal for further differentiation of the epiglottic epithelium towards ciliated pseudostratified epithelium.

KEY WORDS: epiglottis, fetus, rat, transplant, epithelium

INTRODUCTION

For regenerative medicine purposes (supplementation of damaged tissues/organs), a variety of different cells and organs have been either considered or already used (1). Regenerative medicine also deals with immature organisms as a source for material used in therapy (2). Because

Acta Dermatovenerol Croat
2008;16(2):55-59

embryonic *in situ* development is strictly regulated within the specific microenvironment of developing tissues, it is important to fully understand the developmental potential of undifferentiated tissues to avoid all kinds of possible side effects at the site of transplantation (3-5).

Adult epiglottis has already been used for autologous transplantation to reconstruct the eyelid (6), and it has been shown that mammalian fetal epiglottis can survive for 14 days in ectopic transplants preserving its typical shape (7).

To investigate the development of fetal epiglottic epithelia at an ectopic site in the adult, fetal epiglottis was transplanted under the kidney capsule and after a period of two weeks compared with the epithelia from the initially isolated fetal epiglottis and adult epiglottis.

MATERIAL AND METHODS

Concerning ethical issues, animal experiments were done accordingly to Croatian legislation. Fischer rats were mated overnight and embryos were designated as 0-day-old in the morning finding of the sperm in vaginal smear. Microsurgical isolation of epiglottis from 17-day-old fetus was performed under a dissecting microscope by fine watchmaker's forceps, Graefe's knives and tungsten needles.

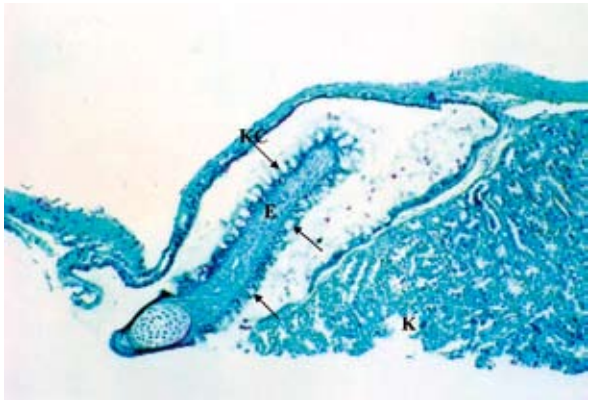

Figure 1. 17-day-old fetal epiglottis in a transplant. KC = kidney capsule; K = kidney; E = epiglottis; → ciliated psudostratified epithelium with goblet cells. (Masson trichrome, X40)

Adult Fischer males were anesthetized and the skin and muscle cut to approach the kidney. A small "pocket" was made under the kidney capsule to place the transplant, where it was left for 14 days. Transplants were fixed in St Marie's solution (1% acetic acid in 96% ethanol, +4 ºC), dehydrated and embedded in paraffin. Uninterrupted serial sections (5 μm) or semi-thin sections (1 μm) were used for histological analysis and stained by HE, Masson trichrome stain, Verhoeff's iron hematoxylin or toluidine blue.

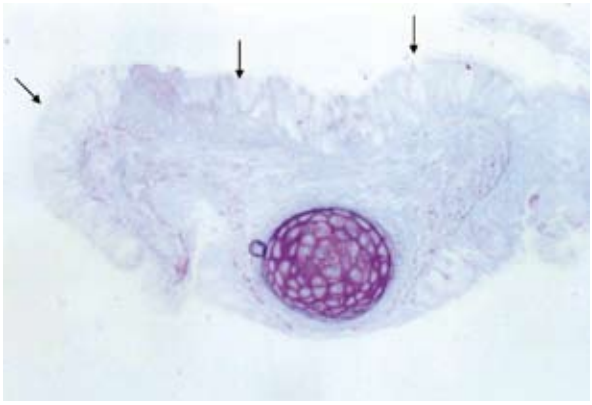

Figure 2. 17-day-old fetal epiglottis in a transplant. Cross-section; → ciliated psudostratified epithelium with goblet cells. (Toluidine blue, X100)

For immunohistochemical analysis, mild fixation during 24 hours with St Marie's solution (1% acetic acid in 96% ethanol, +4 °C) was used. Explants were dehydrated and embedded in paraffin at 56 °C. Serial sections (5 μm) were put on silanized slides (S3003; DAKO, Glostrup, Denmark) and air-dried for 24 hours at room temperature. Sections were deparaffinized in xylene (2x5 min), treated with absolute ethanol and 96% ethanol (2x3 min) and H_2O (30 seconds). Sections were placed in retrieval solution (S2031, DAKO) in a plastic covered jar, heated in a microwave oven for 5 minutes at 700 W and cooled in the buffer for 1 minute at room temperature. This procedure was repeated three times. Finally, sections were cooled for 30 minutes and transferred to PBS for 5 minutes. Monoclonal mouse anti-Proliferating Cell Nuclear Antigen

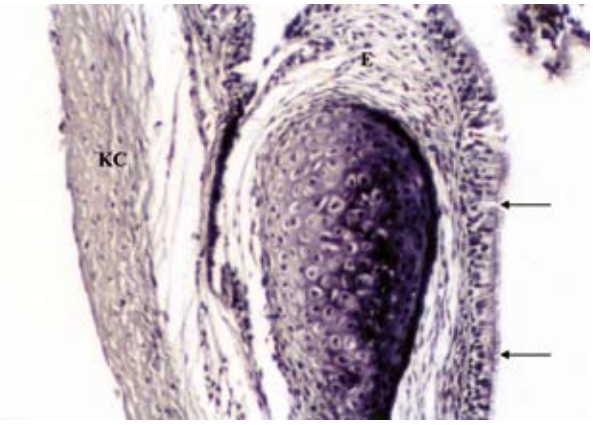

Figure 3. 17-day-old fetal epiglottis in a transplant. E = epiglottis; cilia of the psudostratified epithelium with goblet cells; KC = kidney capsule. (Verhoeff's iron hematoxylin, X100)

Acta Dermatovenerol Croat
2008;16(2):55-59

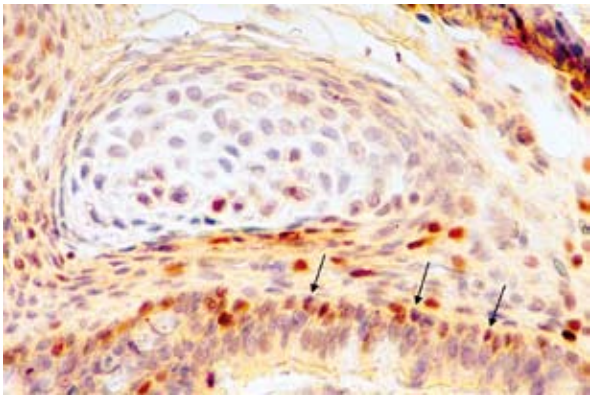

Figure 4. 17-day-old fetal epiglottis in a transplant. → Proliferating Cell Nuclear Antigen (PCNA). (DAB, X200)

(PCNA), Clone PC 10 (M0879, DAKO), was diluted to 1:100. Negative controls were treated with an unspecific antibody (DAKO, V1617). Primary antibodies were labeled with a modified biotinylated anti-mouse immunoglobulin. The Peroxidase Block was applied for 5 minutes and biotin labeled primary antibodies for 15 minutes. Streptavidin-peroxidase complex was applied for 15 minutes, followed by DAB or Fast Red and chromogen substrate complex for visualization of primary antibodies (DAKO Animal Research kit, peroxidase K 3954). Hematoxylin counterstaining and mounting in 50% glycerol:PBS (1:1) was done.

RESULTS

Twenty two 17-day-old fetal epiglottides were transplanted under the kidney capsule. After 14 days under the kidney capsule, all transplants survived and retained their shape (Fig. 1). On both oral and laryngeal surface, ciliated pseudostratified epithelium could be easily recognized (Figs. 1, 2 and 3). However, squamous epithelium was almost absent. PCNA was expressed in basal cells of the ciliated pseudostratified epithelium and was absent from the more superficial cells (Fig. 4). In the 17-day-old fetal epiglottis, epithelium was immature pseudostratified without cilia and goblet cells. PCNA was expressed in almost all cells (Fig. 5). In the adult epiglottis, stratified squamous epithelium covered the oral and laryngeal surface of the epiglottis and ciliated pseudostratified epithelium was only present at the basis of the laryngeal surface. PCNA was expressed in the cells of the basal layer (Fig. 6).

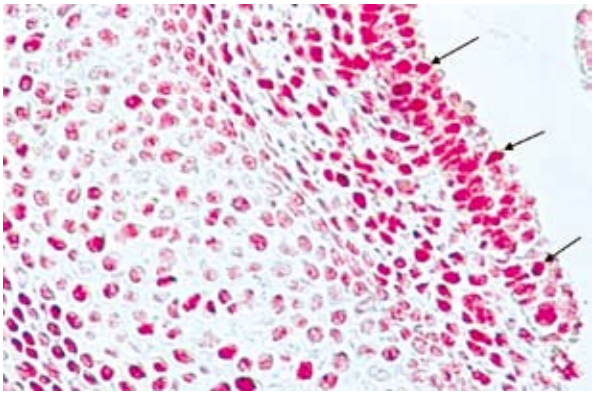

Figure 5. 17-day-old fetal epiglottis. → Proliferating Cell Nuclear Antigen (PCNA). (Fast Red, X200)

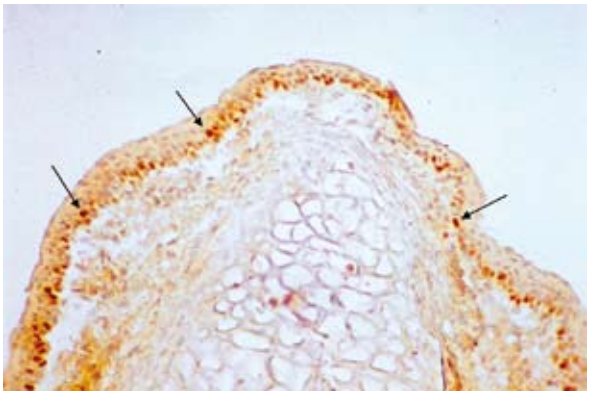

Figure 6. Adult epiglottis. → Proliferating Cell Nuclear Antigen (PCNA). (DAB, X400)

DISCUSSION

Our results first showed that fetal rat epiglottis survived well for 14 days under the kidney capsule, which was in concordance with our previous results (3) and once again showed that the ectopic subcapsular kidney space was a well chosen ectopic site for the experiments dealing with the investigation of developmental potential of various immature tissues and organs (8,9).

So far, epiglottis was investigated mainly considering the development of the supportive tissue (10,11) and little is known about the morphology and distribution of epithelia in mammalian fetal epiglottis. It is known that during the organogeny of the respiratory system in the mouse epiglottis swellings first appears at 11.5 days of the development. At 13 days, the epiglottis grows separately from the lower larynx. By day 16 epiglottis projects into the nasopharyngeal meatus and by day 17 it remains as a nasopharyngeal duct. At day 19, just before birth, the mouse epiglottis is covered by the soft palate, the nares are open, so the air passage to the lung is now complete and the lungs can become inflated with air (12). Epiglottis is probably about to close the entrance to the respiratory system, which is its main role in the adult. In adult

mammals, stratified squamous epithelium covers the oral and laryngeal surface of the epiglottis and ciliated pseudostratified epithelium is only present at the basis of the laryngeal surface (13), which is in concordance with our findings on the adult rat epiglottis.

The stage of development of the 17-day-old rat fetal epiglottis, used in this study, can be compared to the 15-day mouse developmental stage (12). This was the earliest stage to easily isolate it and use it for transplantation. In the 17-day-old rat fetal epiglottis extensive proliferation shown by the wide expression of the proliferating marker PCNA was expected in the morphologically undifferentiated epithelium found. It is known that proliferation is restricted to actively cycling compartments of the tissues and is absent from the terminally differentiated cells (8,14).

In two-week old transplants of the 17-day-old fetal epiglottis ciliated pseudostratified epithelium with nuclear proliferation marker expressed only in basal cells was found, which is in concordance with the above mentioned tissue distribution of PCNA.

In the human embryo (7-week-old) it seems that epiglottis is covered by a two-layered epithelium and at birth ciliated pseudostratified epithelium is dominating over the stratified squamous epithelium (our unpublished results). The reason for this could be the lack of airflow, which should stimulate change to stratified squamous epithelium. It is possible that in the subcapsular kidney space of the adult rat, the lack of airflow is responsible for the predominance of the ciliated pseudostratified epithelium too.

CONCLUSIONS

We can conclude that our experimental system under the kidney capsule was sufficient for the development of the ciliated pseudostratified epithelium from the immature fetal epithelium. However, it was not optimal for differentiation of the stratified squamous epithelium, which is covering the major part of adult epiglottis.

ACKNOWLEDGMENTS

This research was supported by a grant Experimental embryonic tumors and development of the mammalian embryo *in vitro* and *in vivo* number 108-1080399-0335 and Male and female reproductive organs: development, normal histophysiology and infertility 108-1080399-0383 from the Ministry of Science, Education and Sports of the Republic of Croatia. We thank Mrs. Jasna Ljubek, Mirela Vranić and Željka Punčec for their skilled technical assistance.

References

1. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006;367:1241-6.
2. Wu DC, Boyd AS, Wood KJ. Embryonic stem cell transplantation: potential applicability in cell replacement therapy and regenerative medicine. Front Biosci 2007;12:4525-35.
3. Katušić A, Jurić-Lekić G, Jovanov-Milošević N, Vlahović M, Ježek D, Šerman Lj, *el al*. Development of the fetal neural retina in *in vitro* and in ectopic transplants *in vivo*. Coll Antropol 2008;32:315-9.
4. Solter D, Škreb N, Damjanov I. Extrauterine growth of mouse egg-cylinders result in malignant teratoma. Nature 1970;227:503-4.
5. Arnhold S, Klein H, Semkov I, Addicks K, Schraermeyer U. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. Invest Ophthalmol Vis Sci 2004;45:4251-5.
6. Adams JL, Olson NR, Siders DB. The use of the epiglottis as an autologous composite graft in eyelid reconstruction. Ophthal Plast Reconstr Surg 1993;9:206-11.
7. Bulić-Jakuš F, Jurić-Lekić G, Vlahović M, Katušić A, Marinović-Kulišić S, Šerman Lj, *el al*. Survival of rat embryonic parts after ectopic transplantation. Acta Clin Croat 2005;44:23-7.
8. Bulić-Jakuš F, Strahinić-Belovari T, Marić S, Ježek D, Jurić-Lekić G, Vlahović M, *el al*. 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.
9. 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. Croat Med J 2001;42:611-7.

Acta Dermatovenerol Croat
2008;16(2):55-59

10. Egerbacher M, Krestan R, Bock P. Morphology, histochemistry and differentiation of the cat's epiglottic cartilage: a supporting organ composed of elastic cartilage, fibrous cartilage, myxoid tissue and fat tissue. Anat Rec 1995;242:471-82.
11. Niellsen EH. The elastic cartilage in the normal rat epiglottis. Cell Tissue Res 1976;173:179-91.
12. Rugh R. The Mouse, Its Reproduction and Development. Minneapolis: Burgess Publishing Comp; 1968.
13. Duančić V, Posinovec J. Osnove histologije čovjeka. Beograd – Zagreb, Medicinska knjiga; 1986.
14. Marić S, Bulić-Jakuš F, Ježek D, Jurić-Lekić G, Kos M, Vlahović M. Expression of the proliferating cell nuclear antigen and protein products of tumor suppressor genes in the human foetal testis. Andrologia 2004;36:1-7.

Learn how to sunbathe properly! Before sunbathing use Nivea cream or Nivea oil; year 1935. (from the collection of Mr. Zlatko Puntijar)