Acta Dermatovenerol Croat 2008;16(3):119-122 MEDICAL RESEARCH

Development of Epithelia in Experimental Teratomas Derived from Rodent Embryos

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SUMMARY Investigation of developmental potential of various embryonal tissues is important for design of new approaches to regenerative medicine aimed at supplementing tissues damaged by trauma or disease. Rodent embryos have been extensively used in experiments designed for investigation of developmental potential to give rise to various types of epithelia such as superficial epithelia, neuroepithelium and sometimes even malignantly transformed epithelium in teratoma-like structures. These experiments have been done *in vitro*, in transplants *in vivo* and by combined *in vitro-in vivo* methods.

KEY WORDS: teratoma, embryo, rodent, epithelium, differentiation, development

INTRODUCTION

Considering that there is a shortage of organ donors, the aim of tissue engineering is to develop substitutes for the replacement of wounded or diseased tissues. For the production of such substitutes, it is essential to control the culture conditions and histological organization and functionality of reconstructed tissues which must approach those of native organs. It is important to preserve stem cells during *in vitro* expansion and control cell differentiation as well as tissue organization to ensure quality and functionality of tissue-engineered organs such as skin (1).

Experimental teratomas grown *in vitro*

An original method appropriate also for investigation of development of different kinds of epithelia has been established at Department of Biology, School of Medicine, University of Zagreb, and used extensively in basic research for several decades. Rodent embryos are explanted at the time of gastrulation and grown at the air-liquid interface *in vitro* for two weeks. During this period, germlayers (ectoderm, mesoderm and endoderm) are able to give rise to various tissues in a teratoma-like structure (2).

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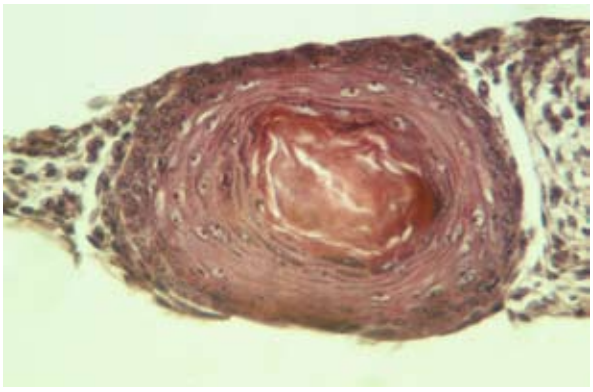

Figure 1. Stratified squamous epithelium in an embryo cultivated *in vitro*. (HE, X500)

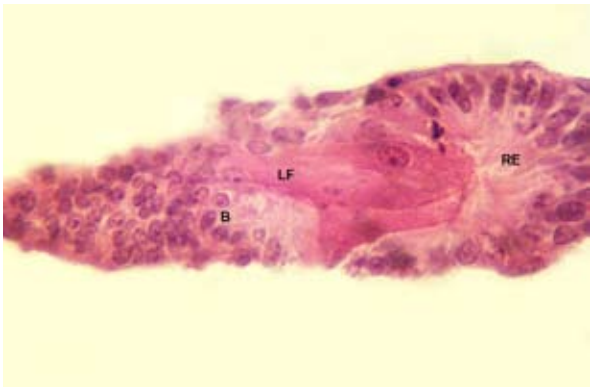

Figure 2. Lens fibers in an explant cultivated with 50 µg/mL transferrin. B=brain-like tissue, LF=lens fibers, RE=retinal epithelium. (HE, X570)

Epithelia that develop in a disorganized mixture of tissues are epidermis (Fig. 1), gut epithelium, respiratory epithelium and neuroectoderm. It has been found that epidermis is a tissue most frequently found even without the usual serum-supplementation to the simple chemically defined medium during the first or second week only or during two weeks (3). This means that differentiation of the epidermis is a process independent of the addition of classic polypeptide growth factors. The addition of retinoic acid to such a defined medium without any protein supplements leads to suppression of this differentiation and an endoderm-like gut epithelium is found in almost all explants instead (4). On the other hand, gut epithelium is more prone to respond to the lack of growth factors. Cultivation of embryos in a simple defined medium without proteins impairs columnar gut epithelium differentiation in a two-week serum-free culture in such a deficient medium. In this system differentiation of ectodermal derivatives such as neuroblasts is severely impaired (3). However, transferrin applied to the serum-free medium induced differentiation of neural tissue and even transdifferentiation of the neural epithelium to the typical cells of ocular lens elongated in lens fibers (Fig. 2) (5,6).

In a similar *in vitro* culture system, epithelium such as the undifferentiated neural retina was able to survive and differentiate further on its typical photoreceptors to full extent (7-9).

Experimental teratomas grown *in vivo*

Rodent embryos or their parts are able to give rise to various epithelia after *in vivo* transplantation under the kidney capsule. In such a nutritionally rich microenvironment, epidermis has usually differentiated together with its appendages (hair and skin glands) (Fig. 3). The pseudostratified ciliated columnar epithelium with goblet cells is usually associated with the hyaline cartilage as in the respiratory tube *in situ*, and epithelium of the digestive tract is surrounded by smooth muscle cells (10,11). In the mouse, pluripotent embryonal carcinoma (EC) cells can be found in experimental teratomas, meaning that the differentiation of all ectodermal cells was not completed. These tumors are called teratocarcinomas and are malignant although differentiated epithelia can also be found (12). Transplantation of the rat fetal epiglottis revealed that the immature epithelium differentiates mainly towards ciliated pseudostratified epithelium with goblet cells and stratified squamous epithelium is hardly differentiating at all, which leads to a conclusion that epiglottal epithelia cannot reach the ratio found in the adult (dominance of stratified squamous epithelium) (13).

In some experiments rat fetal eyeballs were lensectomized and transplanted under the kidney capsule of adult rats for 5 to 66 days. Transdifferentiation of the neuroepithelium to the lens cells

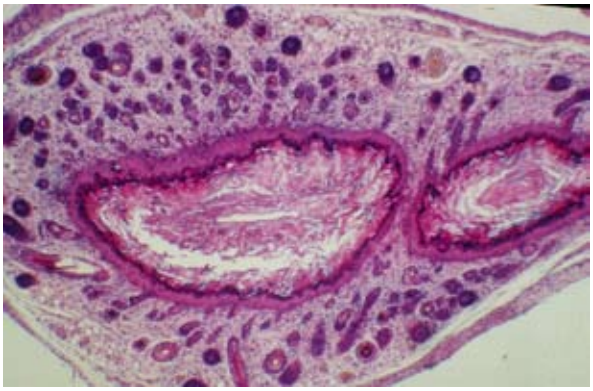

Figure 3. Differentiation of epidermis and skin appendages in a transplant under the kidney capsule. (HE, X300)

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was detected and documented (Fig. 4) in these experiments (14-16).

Experimental teratomas *in vivo* derived from *in vitro* cultivated embryos

A series of experiments was done with subsequent transplantation of *in vitro* cultivated gastrulating embryos. It was discovered that regardless of the medium used *in vitro* and even after spending two-weeks in the simple protein-free medium, the embryos retained the developmental potential for differentiation of skin and its appendages (hair and sebaceous glands). On the other hand, these experiments revealed that the restriction of differentiation discovered in simple defined media without proteins was similar for the neuroectodermal derivatives. Although brain-like tissue had differentiated well, its incidence was significantly lower than in controls cultivated with serum or transferrin. This was also true for neural crest derivatives because the differentiation of peripheral ganglia was impaired in transplants precultivated in a protein-free medium (17-19). This all means that superficial epithelia seem to be more independent in their development than neural ectodermal and neural crest derivatives.

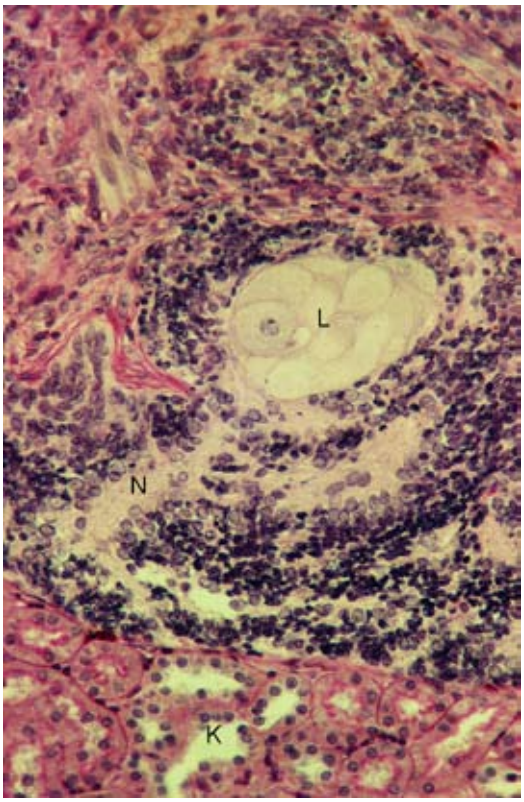

Figure 4. Transdifferentiation of the neuroepithelium to lens cells in a transplant of lensectomized fetal rat eyeball under the kidney capsule. L=lens cells, N=neural retina, K=kidney. (PAS, X350)

CONCLUSION

In experimental embryonic teratomas it is possible to investigate the development of epithelia of different kinds. In the defined *in vitro* system the main types of epithelia differentiate readily and are easily recognized. Extraneously added substances (e.g., proteins, RA) are able to exert their specific influence upon development, to modulate or even drastically change the course of differentiation, as in case of RA. Although differentiation is richer in the *in vivo* environment because organotypic structures are developing and epithelia are found in relationships typical for the adult organism (e.g., epidermis with its appendages), the sensitivity of neuroectodermal derivatives to the *in vitro* manipulation remains obvious in transplants.

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