Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 67 (2007) 105–111
Theriogenology
www.theriojournal.com

Porcine epidermal stem cells as a biomedical model for wound healing and normal/malignant epithelial cell propagation

J. Motlík [a,b,*], J. Klíma [a,b], B. Dvořánková [b,c], K. Smetana Jr. [b,c]

[a] *Institute of Animal Physiology and Genetics, Academy of Sciences, Liběchov, Czech Republic*
[b] *Charles University, 2nd Faculty of Medicine, Center of Cell Therapy and Tissue Repair, Prague, Czech Republic*
[c] *Charles University, 1st Faculty of Medicine, Institute of Anatomy, Prague, Czech Republic*

Abstract

This article summarizes research using cells derived from epidermis of the miniature pigs for use as a cell therapy for skin repair and as a model for squamous carcinoma of the head and neck. Stem cells are an important ''tool'' for biomedical research. Adult stem cells are defined functionally, as cells that have the capacity to self-renew as well as the ability to generate differentiated cells. They are present in defined tissue microenvironments called niches. Asymmetric mitosis allows them to produce one daughter cell with the properties of stem cells (self-renewal) and a second cell with characteristics of progenitor cells, or transit amplifying cells, which proliferate quickly but with a limited number of mitotic divisions. Porcine epidermal stem cells, located in the bulge region of the outer root sheath of hair follicles, migrate in vitro from hair sheaths and because they are resistant to anoikis (detachment induced apoptosis), survive in non-adhesive conditions to form spheroids. These cells express keratins, galectin-1 and their nuclei are rich in ΔNp63α. Interestingly, the multiple phenotype analysis of the human tumor cells in squamous carcinoma of head and neck revealed similarities with epidermal stem cells. These cancer stem cells are usually located on the periphery of the tumor where the invasive front of the tumor responsible for its aggressive behavior is located. In contrast, extensive expression of markers of terminal differentiation such as expression of glycoligands reactive for the endogenous lectin, galectin-3, indicates better tumor prognosis.

Keywords: Pig; Stem cell; Epidermis; Cancer stem cell; Cell therapy

1. Introduction

Rapid progress of biomedical research opens new horizons for the management of many diseases. These decisions ask for new models to better understand the molecular mechanisms of the pathological process and to improve medical technologies. Stem cells are an important tool for biomedical research and have the potential to cure many diseases through their use in cell therapies. Before these can be trialed in humans, it is essential to examine the safety and efficacy of these therapies by transplantation studies in suitable experimental, clinically relevant models. In this article we describe our research using cells derived from epidermis of the miniature pigs.

2. Stem cell characteristics

Stem cells have been studied extensively for use in various cell therapy applications and for research in many diseases such as cancer. Stem cells can be

* Corresponding author at: Institute of Animal Physiology and Genetics, Academy of Sciences, Rumburská 89, 27721 Liběchov, Czech Republic. Tel.: +420 315 639 560; fax: +420 315 639 510.
E-mail address: motlik@iapg.cas.cz (J. Motlík).

0093-691X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.09.018

characterized as totipotent, pluripotent, multipotent and finally monopotent depending on the number of distinct cell types they can give rise to following differentiation [1]. While totipotent cells are represented by the first few blastomers in the course of early embryonic development, pluripotent cells are so-called embryonic stem cells. These can be prepared from the inner cell mass of blastocyst and they can be differentiated to majority of cell lines occurring in the organism [1,2]. Adult (organ/tissue specific) stem cells are multi/monopotent. Adult stem cells are defined functionally as cells that have the capacity to self-renew as well as the ability to generate differentiated cells and they must display sufficient proliferative capacity to last the life time of the animal. The ability of adult stem cells to self-renew and to produce daughter cells that initiate differentiation is the key to tissue homeostasis. They are present in defined tissue microenvironment called niches. In this niche, stem cells are in intimate contact with surrounding support cells that serve as a source of critical signals controlling stem cell behavior. Adhesion between stem cells and either basement membrane or the support cells plays an important role in holding the stem cells within the niche, close to self-renewal signals.

Adult stem cells, which are responsible for self-renewal of tissue, are slowly cycling with non-limited number of divisions. Their mitosis can be characterized as asymmetric, which means that one of two daughter cells retains the property of stem cells (self-renewal) and the second is at the beginning of the differentiation cascade. This cell has characteristics of progenitor cells and is termed a transit amplifying cell. In contrast to stem cells, transit amplifying cells proliferate quickly but with limited number of mitotic divisions [1,3,4].

Transcription profiling of stem cells has revealed that they have the capacity to respond to a broad range of growth factors and signaling molecules and downstream signaling components involved in TGFβ, Notch, Wnt and Jak/Stat signal transduction pathways. The common molecular processes underlying the core stem cell properties of self-renewal and the generation of differentiated progeny are referred to as stemness [5]. Once a detailed understanding of how the choice between stem cell self-renewal and the onset of differentiation is determined, it will help facilitate the expansion of adult stem cells in vitro while maintaining their stemness. Therefore, the progression from a fertilized egg to a differentiated cell via embryonic and adult stem cell may be continuum in which several steps are reversible. For example, human interfollicular keratinocytes are able to transiently reverse phenotypically to a less differentiate state [6]. Recent observations are providing increasing evidence that adult stem cells are part of a natural system for repairing damage to tissue by trauma or disease [7,8].

3. Epidermal stem cell in consequence of functional morphology of squamous epithelium-role in cell therapy

Squamous cell epithelium can be divided into several layers, where only cells of basal layer are able to proliferate. Suprabasal cells are terminally differentiated, highly specialized elements responsible for protecting the skin surface against injury, pathogen colonization and loss of water and crystalloids [9]. Porcine skin is very similar to the human tissue in relation to the morphology and gene expression profile [10].

Epidermal stem cells are present in two sites namely the basal layer and the second so-called bulge region of outer root sheath of hair follicle. Bulge cells possess the ability to reform the hair follicle and to contribute to the re-epithelialisation of the epidermis. While hair follicle epidermal stem cells participate predominantly in the hair cycle, a role for basal layer stem cells in wound healing has also been hypothesized [11–13]. In the squamous epithelia of mucosa (oral cavity, larynx, esophagus), where no hairs are present, stem cells are also present in basal layer of epithelium and their adhesion to the extracellular matrix promotes stem cell identity and prevents their differentiation [11,14].

Similarly to other adult tissue stem cells (neuronal, mammary gland, striated muscle, bone marrow stroma), no specific markers of epidermal stem cells have been discovered but it is known that these cells express a high level of β1 integrin, which is commonly used as a marker for stem cells within the interfollicular epidermis [11]. β1 integrin expression is also enriched in cells within the bulge region of the outer root sheath, which is contiguous with the interfollicular epidermis. Targeted disruption of the β1 integrin gene results in progressive hair loss and interfollicular keratinocytes are significantly reduced [15]. It is plausible that β1 integrin anchors stem cells within the bulge, close to the self-renewal signal. It has been shown that high levels of β1 integrin with signaling through the mitogen-activated protein kinase cascade maintain the epidermal stem cell compartment [16].

Expression of keratin 15 and 19 and of ΔNp63α, respectively was observed in epidermal stem cells [17–19] (Fig. 1). p63 is critical for epithelial development, since knockdown of p63 expression caused down-regulation of cell adhesion-associated genes, cell

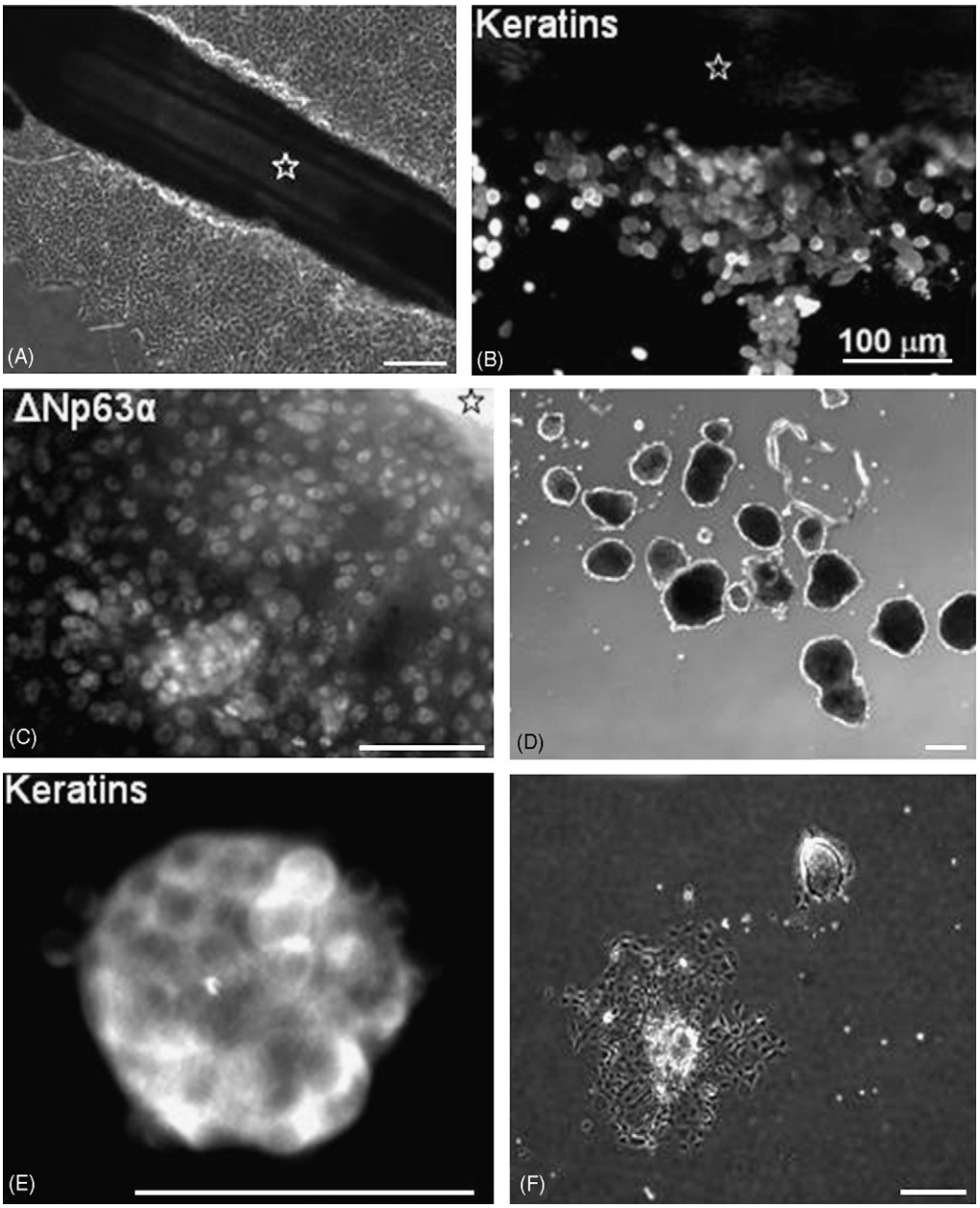

Fig. 1. Porcine epidermal cells which have migrated from hair sheaths (A–C). These cells express keratins (B) and their nuclei are rich in ΔNp63α. These cells form spheroids (D, E) in non-adhesive conditions, which are also rich in keratins (E). These spheroids are able to adhere and form colonies after the transfer to adhesive condition (F). The position of hair is marked by asterisk.

detachment and anoikis in mammary epithelial cells and keratinocytes. In contrast, over-expression of the DeltaNp63alpha isoform of p63 upregulated cell adhesion molecules, increased cellular adhesion and conferred resistance to anoikis [20]. These data implicate p63 a key regulator of cellular adhesion and survival in basal cells of stratified epithelial tissues.

Epidermal cells similar to stem cell elements also express endogenous lectin galectin-1 or epitopes reactive for this lectin in their nuclei [21,22]. Because of their very slow proliferation, they retain labeled precursors of DNA (labeled retaining cells, [23]). Although, this marker is the most reliable, its use is possible only in small laboratory animals [24].

Adult tissue stem cells such as neuronal or mammary gland stem cells appear to be resistant to anoikis (detachment induced apoptosis) and form spheres (neurospheres, mammospheres) [25–27]. Basal layer cells with

phenotype similar to epidermal stem cells with a high clonogenic capacity with extensive resistance to anoikis have been isolated [6,28]. Porcine hair follicle epidermal cells, also phenotypically similar to epidermal stem cells, are also able to survive in non-adhesive condition and form spheroids (Fig. 1). These spheroids adhere after transfer to adhesive surfaces and form large colonies of keratinocytes [19] (Fig. 1).

Keratinocytes need feeder cells such as fibroblasts with arrested mitosis for successful cultivation, although feeder free systems have also been developed [29,30]. Unfortunately, cultured keratinocytes (including stem cells) terminally differentiate and cells with epidermal stem cell properties disappear from the culture. This observation indicates that we are not able to develop culture conditions sufficient for successful function of epidermal stem cell. This phenomenon seems to be very common for many types of adult stem cells in vitro.

Despite this failure to expand epidermal stem cells in vitro, cultured keratinocytes have been used for the treatment of skin defects in the last three decades [31–33]. Success of this technology indicates that function of stem cells after the transfer to the wound bed can be restored. However, it is possible that reconstructed epithelium was repopulated by stem cells from non-affected skin.

4. Porcine skin as a xenograft to treat severe skin defects

Porcine organs are physiologically very similar to those of humans. Therefore, pigs represent a potential source of organs for human transplantation medicine. Unfortunately, two main serious obstructions restrain the use of porcine organs in clinical practice at present. The first of them involves an expression of α-galactoside terminated glycoepitopes (so-called Gal epitop) in cells and tissues namely in vascular endothelium. Humans and Old World monkeys have a significant amount of natural antibodies against the Gal epitop, which is thought to be the major xenoantibody responsible for hyperacute rejection of porcine organs following transplantation [34,35]. The second barrier to xenotransplantation is a hypothetical one, involving the transfer of pathogens, mainly porcine retroviruses to human donors [36]. Though, the use of porcine tissues in human medicine is problematic, the grafting of thin porcine dermoepidermal grafts has been used routinely as a provisional cover of severe skin defects with excellent results [37,38]. This temporary compatibility of porcine provisional graft was probably related by the absence of Gal on the surface of epidermal cells [39]. Recently, Gal-knockout pigs were produced [40]. Use of these genetically manipulated animals as potential donors of skin for provisional cover of extensive skin defects should be possible but their employment as donors of complex vascularized organs needs further research [41].

5. Stem cells and cancer

Solid tumors are characterized by the presence of poorly differentiated cells that originate from the affected tissue. Interestingly when tumor cells are grafted from donor to the host, only around 10% of cancer cells can form ‘‘successful’’ tumors in the recipient animals. The term ‘‘cancer stem cell’’ has been used to describe these cells [42,43]. These cells are very similar to adult stem cells found in almost all tissues [44]. Multiple phenotypic analysis of tumor cells in squamous carcinoma of head and neck in humans has shown that cells located on the periphery of tumors, the so-called invasive front responsible for its aggressive behavior, are similar to epidermal stem cells [45,46]. On the other hand, cells exhibiting markers of terminal differentiation are usually located in the middle of the tumor. Interestingly, human patients suffering form head and neck cancer with extensive expression of markers for terminal differentiation such as expression of glycoligands reactive for the endogenous lectin galectin-3, have better prognosis than patients without expression of galectin-3-reactive glycoligands [47]. This observation seems to be clinically important, because therapy of patients can be individualized according to the biological properties of individual tumors located in a distinct patient.

In normal skin, NF-kappaB signaling is thought to inhibit squamous cell carcinoma development. NF-kappaB activity has been shown to contribute to the survival and growth of cultured non-tumorigenic human keratinocytes [48]. NF-kappaB activation occurs largely through interaction with extracellular matrix components as preventing cell attachment, by forced suspension culture, markedly reduces NF-kappaB signaling associated with cell death (anoikis).

The human cell line FaDu, prepared from squamous cell carcinoma of hypopharynx, expresses many markers observed in epidermal stem cells (for example, keratin 19, nuclear expression of galectin-1) at the beginning of cultivation. During prolonged cultivation these markers disappear indicating some level of differentiation in this tumor line [49]. Many adult stem cell populations contain sub-populations of cells called a side population. These cells actively exclude xenobiotics (dyes) from cytoplasm

using special membrane proteins with pumping activity [50,51]. A similar phenomenon was also observed in many cancer cells. While this mechanism protect adult stem cells from damage, the same phenomenon is thought to be responsible for the multidrug resistance of cancer cells [52,53]. The similarity between cancer and adult tissue stem cells is further underlined by the fact that both these cell types are resistant to anoikis [19,25–28,54]. Resistance to anoikis in adult tissue stem cells can improve survival of these elements in the blood stream and it has been suggested that blood vessels as highways distribute stem cells to areas of the body where they can assist local stem cells in repair [55]. The same phenomenon in cancer stem cells is thought to be responsible for the spreading or metastation of the tumor.

As mentioned above, the niche or microenvironment is critically important for stem cell function. Tumor cells are surrounded by the tumor stroma. According to classical thinking, the stroma is important for the vascularisation of the tumor site and therefore for the nutrition of tumor cells. Many recent findings show that stromal fibroblasts/myofibroblasts can influence functional properties of transformed cells including their local aggressiveness and metastatic potential [56,57]. Many stromal cells exhibit serious genetic alterations improving their protumorigenic function [58]. A very important finding was published from research involving breast cancer, where, due to epithelial-mesenchymal transition, the stromal fibroblasts were developed from transformed glandular epithelium [59]. These data indicate that similarly to the normal adult tissue stem cells, the cancer stem cells need their niche. Even so, the altered regulation of mutual crosstalk between niche and stem cells can induce the cancer formation, as was observed in animal experiments in which introduction of irradiated fibroblasts to the vicinity of mouse mammary gland evoked gland malignisation [60,61].

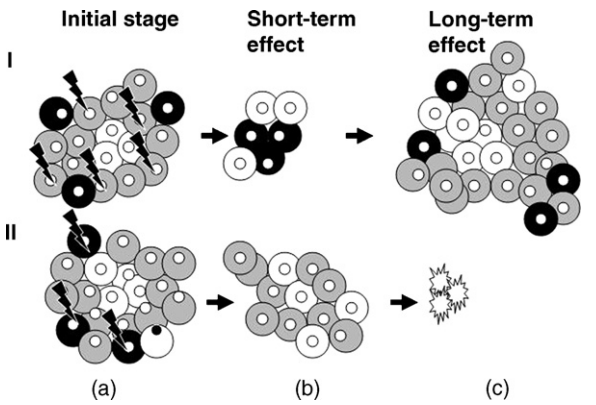

Fig. 2. A hypothetical model of role of cancer stem cell in cancer therapy. The cancer stem cells (white cytoplasm) as slowly proliferating elements are more resistant to chemotherapy (flash) than rapidly dividing tumor progenitor cells (grey cytoplasm). The cancer stem cells and postmitotic cancer cells (black cytoplasm) survive therapy and increase the risk of residual disease as a new source of tumor progression (I). Drugs targeted against cancer stem cells can efficiently destroy the tumor and minimize a risk of its recidive (II).

In summary, some carcinoma including tumors that originate from the squamous epithelia and their appendages can be considered a stem cell disease. They also evoke contemplations about cancer therapy since cytostatic therapy can affect predominantly the cells in S-phase of the cell cycle. The stem cells will be targeted less frequently in comparison to the rapidly proliferating tumor progenitors, which can augment the risk of residual disease (Fig. 2).

6. Concluding remarks

Pigs represent an excellent model for biomedical research due to their physiological similarity including tissues such as epidermis [62]. Data presented here demonstrate that pigs are a suitable model for epidermal stem cell research as well as cancer research with direct therapeutic consequences.

Acknowledgements

Part of the reviewed results were obtained with support of the Ministry of Education, Youth and Sport of the Czech Republic, projects Nos. MSM0021620806 and 1M0021620803, and also from the Grant Agency of the Czech Republic, project No. 304/04/0171.

References

[1] Lanza R, Geahart J, Hogan B, Melton D, Pedersen R, Thomson J, West M, editors. Handbook of stem cells, vols. 1–2. Elsevier, Academic Press; 2004.
[2] Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. Physiol Rev 2005;85:635–78.
[3] Hall PA, Watt FM. Stem cells: the generation and maintenance of cellular diversity. Development 1989;106:619–33.
[4] Smetana Jr K, Plzák J, Dvořánková B, Holíková Z. Functional consequences of glycophenotype of squamous epithelia—practical employment. Folia Biol 2003;49:118–27.
[5] Westphal H. Restoring stemness. Differentiation 2005;73:447–51.
[6] Dvořánková B, Smetana Jr K, Chovanec M, Lacina L, Štork J, Plzáková Z, et al. Transient expression of keratin K19 is induced in originally negative interfollicular epidermal cells by adhesion of suspended cells. Int J Mol Med 2005;16:525–31.
[7] Verfaillie CM. Stem cell research, future implications for internal medicine. Acta Clin Belg 2005;60:277–83.

[8] Roh C, Lyle S. Cutaneous stem cells and wound healing. Pediatr Res 2006;59:100R–3R.
[9] Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol 2002;12:390–9.
[10] Plzák J, Holíková Z, Smetana Jr K, Dvořánková B, Hercogová J, Kaltner H, et al. Differentiation-dependent glycosylation of cells in squamous epithelia detected by a mammalian lectin. Cells Tissues Organs 2002;171:135–44.
[11] Watt FM. The stem cell compartment in human interfollicular epidermis. J Dermatol Sci 2002;28:173–80.
[12] Alfonso L, Fuchs E. Stem cells of the skin. PNAS 2003;100: 11830–5.
[13] Levy V, Lindon C, Harfe BD, Morgan BA. Distinct stem cell populations regenerate the follicle and interfollicular epidermis. Dev Cell 2005;9:855–61.
[14] Seery JP. Stem cells of the oesophageal epithelium. J Cell Sci 2002;115:1783–9.
[15] Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, et al. Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. EMBO J 2000;19:3990–4003.
[16] Zhu AJ, Watt FM. Beta-catenin signaling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. Development 1999;126:2285–98.
[17] Michel M, Török N, Bodnout MJ, Lussier M, Gaudreau P, Royal A, et al. Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. J Cell Sci 1996;109:1017–28.
[18] Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, et al. p63 identifies keratinocyte stem cells. PNAS 2001;98:3156–61.
[19] Liu Y, Lyle S, Yang Z, Cotsarelis G. Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. J Invest Dermatol 2003;121:963–8.
[20] Caroll DK, Caroll JS, Leong CO, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. Nat Cell Biol 2006;8:551–61.
[21] Purkrábková T, Smetana Jr K, Dvořánková B, Holíková Z, Böck C, Lensch M, et al. New aspects of galectin functionality in nuclei of cultured bone marrow stromal and epidermal cells: biotinylated galectins as tool to detect specific binding sites. Biol Cell 2003;95:535–45.
[22] Klíma J, Smetana Jr K, Motlík J, Plzáková Z, Liu F-T, Štork J, et al. Comparative phenotypic characterization of keratinocytes originating from hair follicles. J Mol Histol 2005;36:89–96.
[23] Braun KM, Watt FM. Epidermal label-retaining cells: background and recent applications. J Invest Dermatol Symp Proc 2004;9:196–201.
[24] Bickenbach JR. Isolation, characterization, and culture of epithelial stem cells. Methods Mol Biol 2005;289:97–102.
[25] Grossmann J. Molecular mechanisms of ''detachment-induced apoptosis-anoikis''. Apoptosis 2002;7:247–60.
[26] Reynolds BA, Weiss S. Clonal and population analyses demonstrate than EGF-responsive mammalian embryonic CNS precursors in a stem cell. Dev Biol 1996;175:1–13.
[27] Dontu G, Abdallah W, Foley J, Jackson K, Clarke M, Kawamura M, et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 2003;17: 1253–70.
[28] Dvořánková B, Motlík J, Holíková Z, Vacík J, Smetana Jr K. *Dolichos biflorus* agglutinin-binding site expression in basal keratinocytes is associated with cell differentiation. Biol Cell 2002;94:365–73.
[29] Green H, Kehinde O, Thomas J. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. PNAS 1979;76:5665–8.
[30] Labský J, Dvořánková B, Smetana Jr K, Holíková Z, Brož L, Gabius H-J. Mannosides as crucial part of bioactive supports for cultivation of human epidermal keratinocytes without feeder cells. Biomaterials 2003;24:863–72.
[31] Dvořánková B, Smetana Jr K, Königová R, Singerová H, Vacík J, Jelínková M, et al. Cultivation and grafting of human keratinocytes on poly HEMA support to the wound bed. Biomaterials 1998;19:141–6.
[32] Smetana Jr K, Dvořánková B, Labský J, Vacík J, Holíková Z. Grafting of human epidermal cells, presence and perspectives. Sborník lékařský 2001;102:1–6.
[33] Dvořánková B, Holíková Z, Vacík J, Königová R, Kapounková Z, Michálek J, et al. Reconstruction of epidermis by grafting keratinocytes cultured on polymer support-clinical study. Int J Dermatol 2003;42:219–23.
[34] Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes and Old World monkeys differs from other mammals in the expression of α-galactosyl epitopes on nucleated cells. J Biol Chem 1988;263:17755–62.
[35] Cooper DKC. Xenotransplantation-state of art. Front Biosci 1996;1:248–65.
[36] Fishman JA, Patience C. Xenotransplantation: infectious risk revisited. Am J Transplant 2004;4:1383–90.
[37] Dvořánková B, Matoušková E, Vogtová D. Metabolic activity of cultured skin grafts cryopreserved in different forms. Folia Biol 1994;40:149–59.
[38] Chiu T, Burd A. ''Xenograft'' dressing in the treatment of burns. Clin Dermatol 2005;23:419–23.
[39] Hrdličková E, Smetana Jr K, Plzák J, Holíková Z, André S, Hřebíček M, et al. Cells of porcine epidermis and corneal epithelium are not recognized by human natural anti-α-galactoside IgG. Folia Biol 2001;47:200–5.
[40] Carter DB, Lai L, Park K-W, Samuel M, Lattimer JC, Jordan KR, et al. Phenotyping of transgenic cloned piglets. Cloning Stem Cells 2002;4:131–45.
[41] d'Apice AJ. Is xenotransplantation of vascularized organs just to hard? Xenotransplantation 2006;13:182–5.
[42] Park CH, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. J Natl Cancer Inst 1971;46:411–22.
[43] Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science 1977;197:461–3.
[44] Sell S. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol 2004;51:1–28.
[45] Chovanec M, Smetana Jr K, Betka J, Plzák J, Brabec J, Moya-Álvarez V, et al. Correlation of expression of nuclear proteins pKi67 and p63 with lectin histochemical features in head and neck squamous cell cancer. Int J Oncol 2005;27: 409–15.
[46] Plzák J, Smetana Jr K, Chovanec M, Betka J. Glycobiology of head and neck squamous epithelia and carcinomas. ORL J Otorhinolaryngol Relat Spec 2005;67:61–9.
[47] Plzák J, Betka J, Smetana Jr K, Chovanec M, Kaltner H, André S, et al. Galectin-3: an emerging prognostic indicator in advanced head and neck carcinoma. Eur J Cancer 2004;40:2324–30.
[48] Ren Q, Kari C, Quadros MR, Burd R, McCue P, Dicker AP, et al. Malignant transformation of immortalized HaCaT keratinocytes

through deregulated nuclear factor kappaB signaling. Cancer Res 2006;66:5209–15.
[49] Smetana Jr K, Dvořánková B, Chovanec M, Bouček J, Klíma J, Motlík J, et al. Nuclear presence of adhesion-/growth-regulatory galectins in normal/malignant cells of squamous epithelial origin. Histochem Cell Biol 2006;125:171–82.
[50] Lassalle B, Bastos H, Luis JP, Riou L, Testart J, Duttrilaux B, et al. ''Side population'' cells in adult mouse testis express *Bcrp1* gene and are enriched in spermatogonia and germinal stem cells. Development 2004;131:479–87.
[51] Yano S, Ito Y, Fujimoto M, Hamazaki TS, Tamaki K, Okoch H. Characterization and localization of side population cells in mouse skin. Stem Cells 2005;23:834–41.
[52] Rudas M, Filipits M, Taucher S, Stranzl T, Steger GG, Jakesz R, et al. Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. Breast Cancer Res Treat 2003;81:149–57.
[53] Dean M, Fojo T, Bates S. Tumor stem cells and drug resistance. Nat Rev Cancer 2005;5:275–84.
[54] Wang L-H. Molecular signaling regulating anchorage-independent growth of cancer cells. Mt Sinai J Med 2004;71:361–7.
[55] Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function. Cell 2001;105:829–41.
[56] Kiaris H, Chatzistamou I, Kalofoutis Ch, Koutselini H, Piperi Ch, Kalofoutis A. Tumour-stroma interactions in carcinogenesis: basic aspects and perspectives. Mol Cell Biochem 2004;261: 117–22.
[57] Bhowmick NA, Moses HL. Tumor stroma interaction. Curr Opin Genet Dev 2005;15:97–101.
[58] West RB, Nuyten DSA, Subramanian S, Nielsen TO, Corless CL, Rubin BP, et al. Determination of stromal signatures in breast carcinoma. PLOS Biol 2005;3:1101–10.
[59] Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, et al. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol 2003;162:391–402.
[60] Barcellos-Hoff MH, Ravani SA. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res 2000;60:1254–60.
[61] Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. Cancer Res 2006;66:4553–7.
[62] Vodička P, Smetana Jr K, Dvořánková B, Emerick T, Yingzhi Xu YZ, Ourednick V, et al. The miniature pigs as animal model in biomedicinal research. In: Orednick J, Snyder EY, Ourednick V, editors. Stem cell biology, development and plasticity. Ann New York Acad Sci 2005;1049:161–71.