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Glycophenotype of squamous epithelia: from laboratory to clinical practice

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Summary
The epidermal stem cell plays a pivotal role in the function of squamous epithelia in physiological as well as in pathological conditions such as cancer. This review summarizes data about the glycobiology of normal squamous epithelia and related tumors with respect to epithelial cell differentiation and search for a glycophenotype specific for epidermal stem cells using labeled plant and endogenous lectins. Although the glycophenotype typical for epithelial cells at the stage of low differentiation level were found, no typical cell surface saccharidic markers of stem cells were detected. The nuclear binding of galectin-1 seems to be specific for the keratinocyte population prepared from hair follicles enriched for multipotent stem cells. The close topographical relationship of nuclear galectin-1 binding sites with SC35 splicing factor suggests some role for these glycoepitopes in pre-mRNA splicing. The data shown in this paper can be employed for diagnostic purposes and for cell therapy of skin defects and indicate the importance of the use of endogenous lectins as probes in biology and medicine.

Keywords: epidermal stem cell – glycocode – endogenous lectin-galectin-carcinoma

SQUAMOUS EPITHELIA AS STRATIFIED TISSUES

Squamous epithelium (epidermis, oral mucosa, cornea) is an example of differentiation-dependent and functionally stratified epithelium. Although the structure of epithelium depends on the distinct location, the general structure is the same. The proliferation active cells are located in the basal layer and the direction of differentiation of cells has a gradient from the basal to the last suprabasal layer of epithelium. The basal cells are anchored to the basement membrane by hemidesmosomes and they are able to actively migrate. These cells express receptors of the integrin type and distinct representatives of intermediate filaments as the main cytoskeletal elements. The intercellular contacts of desmosomal type are typical for suprabasal cells. These cells also lack migration potential. They are passively moved to the epithelium surface and cytokeratin 10 represents the main representative of intermediate filaments. The suprabasal cells of cornifying epithelia (epidermis) also express the specific proteins (for example involucrin) forming the cornified envelopes in these cells (for review see Kanitakis 2002).

EPIDERMAL STEM CELL

The epidermal stem cell (ESC) is an example of so called organ stem cells. ESCs have nonlimited mitotic activity. They are located in the bulge region of the sheath of hair follicle and according to other authors also in the basal layer of the interfollicular epidermis. ESCs of corneal epithelium are selectively located in the epithelium covering the limbus. Generally, ESCs are located deeply in the epithelium to be protected against damage of DNA by ultraviolet irradiation (Lavker and Sun 2000, Watt 2002). ESCs seem to be multipotent such was demonstrated using the model of injection of green fluorescent protein transfected newborn ESCs into

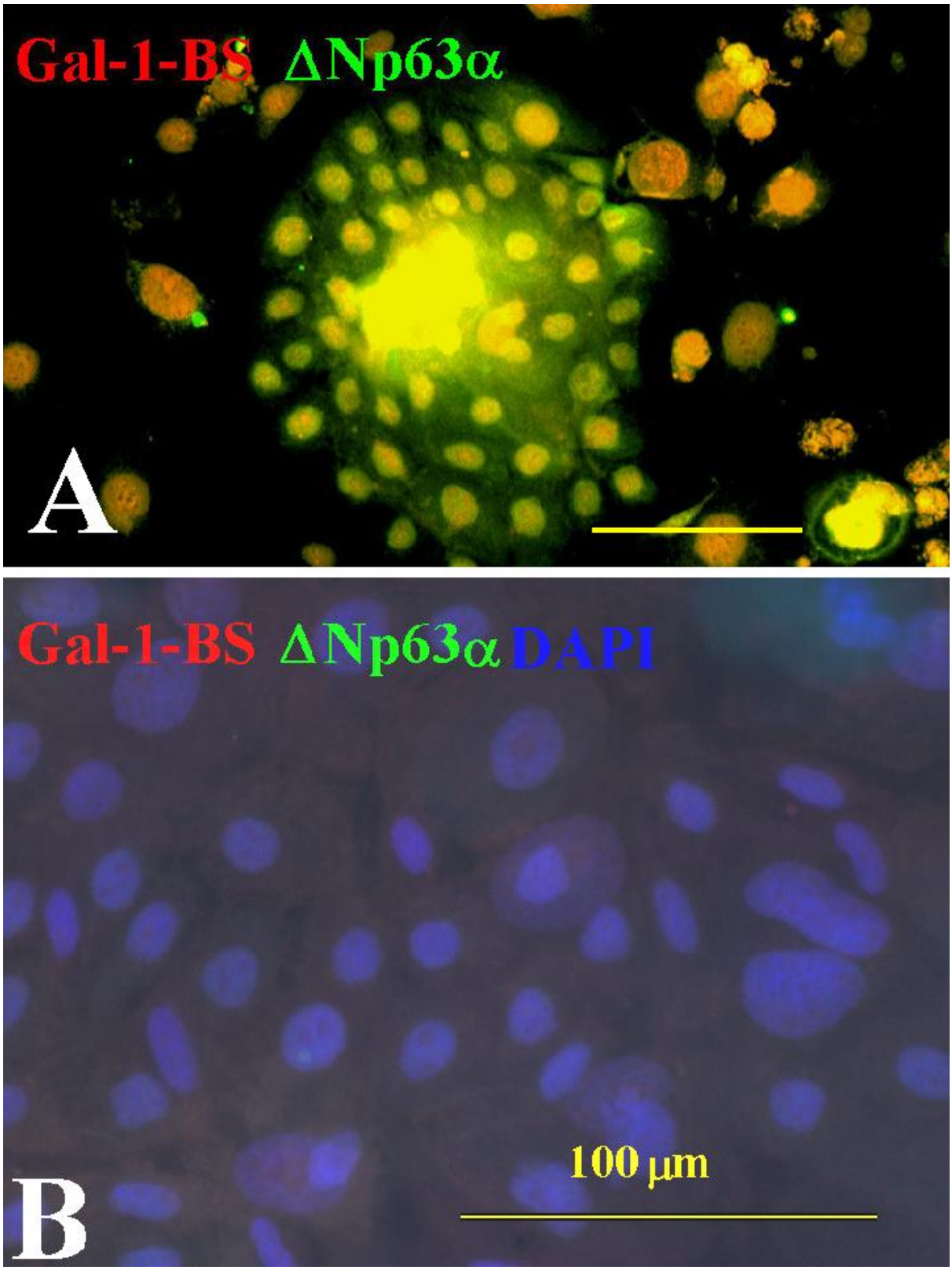

Fig. 1. **Nuclei of cultured keratinocytes of hair follicle origin** (A) **express galectin-1-binding sites** (red signal) **and ΔNp63α** (green signal) **in contrast to cells of interfollicular nature** (B). Colocalization of both markers is yellow. Nuclei in specimen B were counterstained with DAPI. Bar is 100 μm.

the mouse blastocysts (Liang and Bickenbach 2002). ESCs are able to divide by asymmetric mitosis. The first daughter cell has again properties of ESC, the second one (transit amplifying cell) has restricted mitotic activity. The molecular control of the ESC phenotype maintenance is not well understood but the *Sonic hedgehog (Shh)* gene signalization cascade seems to have some role in this mechanism (Chiang et al. 1999). This idea is well supported by observations of *Shh* mutations in basal cell carcinomas of the skin (Fan et al. 1997). In contrary, *c-myc* activity is involved with the transit amplifying cell phenotype (Gandarillas and Watt 1997). No specific phenotypic marker of ESC is known. β_1 integrin highly positive cells seem to have properties of ESC. However, the difference in the positivity of all cells in the basal layer or cells in the bulge region is not so remarkable. The expression of specific cytokeratins (type 15 and 19) is also not so specific (Lavker and Sun 2000, Watt 2002). A member of the protein p53 family known as ΔNp63α expression was described in the nuclei of ESCs (Pellegrini et al. 2001). However, further work in verification of this marker validity is necessary. Some slowly proliferating cells of mouse hair follicle express cytokeratins and CD34 (Trempus et al. 2003). This molecule is, therefore, also a candidate for the marker of ESC. The absence of a specific cell surface ESC marker is a great disadvantage because selective separation and cultivation of ESCs is problematic.

GLYCOCODE

Monosaccharides polymerize into the highly variable chains, although the monomer number is limited. The distinct saccharidic motifs are selectively recognized with lectins, proteins different from enzymes and immunoglobulins. The saccharide oligo-/polymers can be considered for the data storage medium, which is decoded by lectins and the term glycocode was published (Gabius et al. 2002). Higher animals including humans express 5 classes of lectins (endogenous lectins) among them galectins play an important role. This lectin family recognizes β-galactosides and histo-blood A/B trisaccharides (galectin-3). Their activity is not conditioned by the presence of divalent cations. Galectins participate in the intercellular and cell-extracellular matrix interaction. Galectin-1 and -3 have some role in the control of proliferation/apoptosis and splicing of pre-mRNA (Gabius 1997).

GLYCOBIOLOGY OF SQUAMOUS EPITHELIA *in situ* AND *in vitro*

Galectin-3-reactive epitopes are expressed by suprabasal keratinocytes of epidermis, cornea and oral or laryngeal mucosa. These epitopes are localized on the cell surface and colocalize with desmosomal proteins desmoplakin and desmoglein. Galectin-3-binding cells express cytokeratin 10. No expression of the proliferation marker Ki-67 and β_1 integrin was detected in galectin-3-reactive cells. Galectin-1-reactive epitopes expression is not dependent on the level of epithelial cell differentiation, and cytoplasmic signal of galectin-1-binding sites was detected in the cells occurring basally and suprabasally (Plzák et al. 2001, Plzák et al. 2002). Cultured keratinocytes prepared from hair follicle express intranuclear galectin-1-binding sites, which colocalize with ΔNp63α protein. No similar phenomenon was observed in cultured cells of interfollicular origin (Fig. 1). The basal cells of epithelia contain α2,6 isomer of N-acetylneuraminic acid (*Sambucus nigra* agglutinin-reactive) and these cells are reactive for galectin-3 after removal of this moiety by neuraminidase. Expression of α2,3 isomer of N-acetylneuraminic acid (reactive for plant lectin *Maackia amurensis* isoagglutinin 2) has no effect on galectin-3 binding to epithelial cells (Holíková et al. 2002). Expression of α-N-acetylgalactosamine (reactive for plant lectin *Dolichos biflorus* agglutinin) is connected with the accumulation of β_1 integrin in ERGIC (endoplasmic reticulum Golgi intermediate complex), and migration of basal cells suprabasally is connected with this process (Dvořánková et al. 2002). Basal cells of the epidermis recognize mannose-rich oligosaccharides of laminin and immobilization of mannosides to synthetic surfaces is suitable for cultivation of keratinocytes without feeder cells (Labský et al. 2003). The use of this new cultivation support for direct grafting of cultured keratinocytes to the wound bed would be possible. Nuclear expression of galectin-1-reactive epitopes in cultured keratinocytes (Fig. 1) seems to be one of the potential markers of epidermal stem cells and these galectin-1-binding sites have a positional relation to the expression of splicing factor SC35, which indicates some role in pre-mRNA splicing (unpublished results).

GLYCOBIOLOY OF EPIDERMAL AND HEAD AND NECK CARCINOMAS

Basal cell carcinomas exhibited binding sites for galectin-1, but they were not recognized with

galectin-3. Poorly differentiated cells of head and neck squamous cell carcinomas were also negative for the occurrence of galectin-3-binding sites, which were expressed in more differentiated cells (Plzák et al. 2000, Plzák et al. 2001). The binding of galectin-3 to poorly differentiated tumor cells was restored by neuraminidase pretreatment (Holíková et al. 2002). These results indicate the possibility of using labeled endogenous lectins as a tool for diagnostic purposes.

CONCLUSION

Data presented in this review show the glycobiology of epithelial tissue as a highly prospective field of cell biology with the possibility of direct employment in histopathology and cell therapy.

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