Review

Functional Consequences of the Glycophenotype of Squamous Epithelia – Practical Employment

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Abstract. Squamous epithelia represent a morphologically and differentiation-dependent stratified tissue. The stem cells are located in the bulge region of hair follicles or in the basal layer of interfollicular epidermis and in the limbus of the cornea. This article summarizes the data about the glycobiological aspects of squamous epithelia cell differentiation under physiological as well as pathological conditions in relation to the function of this epithelial tissue. The entries about the LC, Merkel cells and melanocytes are also mentioned. The employment of the described data in the diagnostics of carcinomas derived from this type of epithelium as well as in the cell therapy of skin defects are shown.

Definition of squamous epithelia

The squamous epithelia are usually located on the surface of the body areas affected by mechanical stress (epidermis, oral mucosa, vocal cord of larynx, oesophagus, conjunctiva, cornea, and so on). They also protect our body against the evaporation of water, chemical injury and microorganism invasion. Moreover, they produce a wide panel of cytokines and growth factors (Uchi et al., 2000). Epidermis as a squamous epithelium prototype is composed of the *stratum basale* (basal layer), the *stratum spinosum* (spinous = prickle cell layer), the *stratum granulosum* (granular layer) and the *stratum corneum* (cornified layer). However, the detailed histological structure of squamous epithelium is strongly influenced by the site of location (for example a difference between cornea and epidermis covering the plantar region of the foot). The deepest layer, which is in contact with the basement membrane (BM) through hemidesmosomes, is the basal layer. More superficially, the cells of the spinous layer with abundant intercellular contacts of the desmosome type are located. The cells of the cornified layer are present over the spinous layer and the death cells desquamate (for review see Kanitakis, 2002). The majority of squamous epithelia are of ectodermal origin (e.g. epidermis); however, squamous epithelia of endodermal origin are also known (oesophagus, larynx) (for review see Seery, 2002). The different developmental origin of the same tissue is a good evidence of the influence of the body environment on the tissue appearance and function.

Except for keratinocytes of different stages of differentiation, other elements are present in the epithelium. Melanocytes are of neural crest origin and protect the cells of the basal layer of epidermis against UV-light injury (for review see Kanitakis, 2002). Merkel cells are located in epidermis in mammals and these elements form a functional unit with axon terminal; they can be characterized as mechanoreceptors (for review see Iggo and Findlater, 1984). However, their neuroendocrine function is also hypothesized (for review see Tachibana et al., 1995). Merkel cells were recently shown to originate from the neural crest (Grim and Halata, 2000; Szeder et al., 2003), but prevailed opinion is their

Received March 3, 2003. Accepted April 10, 2003.

The part of results summarized in this review was received in projects supported by the Ministry of Education, Youth and Sport of the Czech Republic Nos. MSM 111100005 and LN0A0065, the Ministry of Health of the Czech Republic No. ND 6340-3, the Grant Agency of the Czech Republic No. 203/00/1310 and No. 304/02/0463, and the Academy of Sciences of the Czech Republic No. S4050005.

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Abbreviations: BM – basement membrane, ConA – concanavalin A, DBA – *Dolichos biflorus* agglutinin, ESC – epidermal stem cell, Gal – galectin(s), LC – Langerhans cells, MAL – *Maackia amurensis* isolectin 2, Man – α-D-mannosides, NeuNAc – N-acetylneuraminic acid, SNL – *Sambucus nigra* lectin, TAC – transit amplifying cell.

epithelial origin (for review see Halata et al., 2003). Langerhans cells (LC) of haematopoietic origin are professional antigen-presenting cells. They colonize squamous epithelia and participate in the immune surveillance against pathogens and cancer cells (for review see Kanitakis, 2002).

Phenotypic characterization of squamous epithelia

The proliferative cells of the basal layer can be discriminated from the postmitotic cells of suprabasal layers according to the expression of distinct phenotypic markers. The pattern of cytokeratins is highly specific for both basal and suprabasal compartments. While basal cells express cytokeratins K5 and K14/15, cytokeratins of type K1, K10, K2e and K11 (granular layer) are expressed suprabasally (Moll et al., 1982). The cells of the basal layer express integrin receptors employed for their contact with BM (Adams and Watt, 1990). P-cadherin, a molecule participating in the intercellular contact formation, is also expressed by basal cells only (Fujita et al., 1992). Because basal cells can proliferate, their nuclei are frequently positive for proliferation marker Ki-67 and proliferating cell nuclear antigen (Plzák et al., 2002c). The highly specific proteins connected with the late terminal differentiation such as involucrin are expressed suprabasally and reflect formation of the cornified envelope in the suprabasal-keratinized cells (Ishida-Imamoto and Iizuka, 1998). The suprabasal cells have no migration activity and they express proteins participating in the formation of desmosomes (for review see Kanitakis, 2002). Because it is known that the cell differentiation continues from the basal layer to the surface of the epithelium and many markers characteristic for the cell at distinct differentiation levels are known, the differentiation of epithelial cells can be easily estimated not only in normal epithelia, but also in tumours and under *in vitro* conditions when the normal architecture of the epithelial sheet is heavily altered.

Epidermal stem cell, topology and function

The part of basal layer cells and cells of the so-called bulge region of the hair follicle are able to proliferate. Some of these cells retain much more extensively labelled precursors of DNA (label-retaining cells) (Bickenbach and Mackenzie, 1980). This phenomenon can be explained by their slow proliferating activity. The number of mitotic divisions of these cells is not limited and because these cells are also able to form colonies, they were determined as the epidermal stem cells (ESC). However, their characterization is approximate only, because no specific marker of ESC is known, although some markers seem to be promising. ESC highly express $\alpha_6\beta_4$ and β_1 integrins (for review see Watt, 1998; Kaur and Li, 2000). The fundamental role of β_1 integrin in the process of wound repairs was demonstrated in a knock-out mice model (Grosse at al., 2002). Some observations also indicate that ESC express cytokeratins type K15 (Lyle et al., 1998) and K19 (Commo et al., 2000). However, the validity of these markers is somewhat problematic. Encouraging results were obtained by employment of the antibody against protein p63, which is a member of the p53 family. ESC seem to express isoform ΔNp63α of the p63 protein intranuclearly (Pellegrini et al., 2001). The positive cells were even found in the corneal limbus, highly restricted part of the periphery of cornea, where the corneal epithelium stem cells are located only (Pellegrini et al., 2001).

ESC divide into two daughter cells. One of them has the properties of a stem cell again and the second, so-called transit amplifying cell (TAC), represents the first member of the differentiation cascade. This element is quickly cycling, but the number of divisions is restricted contrary to ESC with unlimited number of mitoses (for review see Watt, 1998; Lavker and Sun, 2000; Watt 2002). Information about the control of this process is very limited. However, the transition between ESC and TAC seems to be dependent on the activity of *c-myc* and *Notch* genes (Gandarillas and Watt, 1997; Niemann and Watt, 2002). On the other hand, the ΔNp63α and namely *sonic hedgehog* and *Wnt* (including control of β-catenin) signalization cascade (for details see Alberts et al, 2002) seem to be important for correct function of ESC and maintenance of the stem cell phenotype (Fig. 1) (Chiang et al., 1999; Widelitz et al., 2000; Huelsken et al., 2001; Pellegrini et al., 2001; Little and Jochemsen, 2002). Aberrant function of *sonic hedgehog* seems to be related to the formation of basal cell carcinomas (Fan et al., 1997). However, β-catenin seems not to be directly required for proliferation and differentiation of mouse keratinocytes under *in vitro* conditions (Posthaus et al., 2002).

The topic of the potency and distinct location of ESC is under discussion at present. Some data suggest that ESC will be multipotent similarly to the bone marrow stromal stem cells (Liang and Bickenbach, 2002). The data about the epidermal stem cell position/localization are still controversial and some people believe that the real ESC are located in the bulge region of the hair follicle and basal cells of the interfollicular epidermis are progenitor cells only. Additional data are necessary to decide on this problem.

Although the definition of ESC needs additional data, it is clear that this element exists and is responsible for the self-renewal of epithelia as well as for the healing of epithelial defects. Moreover, the basal as well as squamous cell carcinomas can be defined as stem cell diseases. These data predestine ESC as an attractive object of research with perspective of tumour therapy and cell therapy of skin defects.

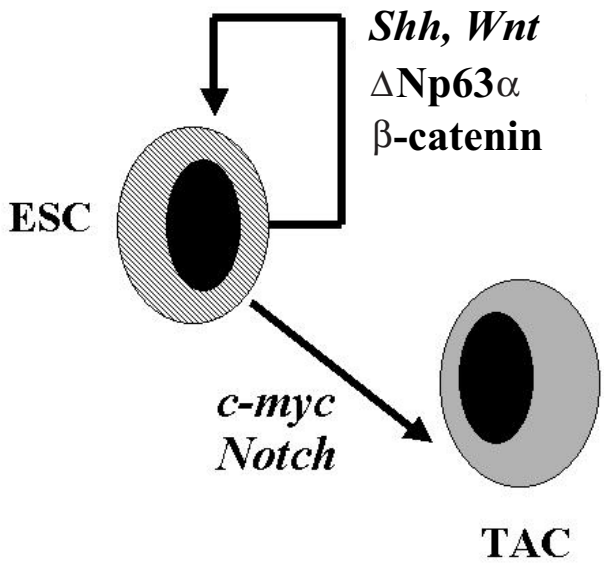

Fig. 1. Schematic presentation of the control of asymmetric mitosis of an epidermal stem cell (ESC), where the first daughter cell has again the properties of ESC with an unlimited number of mitoses and the second, so-called transit amplifying cell (TAC), with a restricted number of divisions can be considered for the determined progenitor element. Other terms are explained in "Epidermal stem cell, topology and function".

Glycobiology of normal squamous cell epithelia

Glycocode

Saccharides represent one of the basic building blocks of living organisms. They are able to form oligopolymers representing a favorable medium for the storage of biological information. Comparing the theoretical number of distinct oligomers synthesized from the same number of amino acids and monosaccharides, the number of saccharides is significantly higher than the number of peptides (Laine, 1997). It is not surprising that the receptor molecules able to decode the information stored in saccharides were developed during the phylogeny. These receptor molecules are called lectins and they can be defined as proteins (glycoproteins) different from enzymes or immunoglobulins which are able to recognize specific saccharidic motifs. Because of this complexity and potential biological importance some authors use the term glycocode (for review seeVillalobo and Gabius, 1998; Gabius et al., 2002).

Lectins isolated from plants and invertebrates have been used for the detection of distinct saccharidic epitopes for a long time. Many plant lectins are able to aggregate erythrocytes and other cells (agglutinins) and they have distinct biological properties (stimulation of mitotic division, toxins). The lectin molecules were also discovered in higher vertebrates such as mammals, including humans. Five families of animal lectins (so-called endogenous lectins) are present in these species. They have numerous important intracellular functions (participation in the control of pre-mRNA splicing, in proliferation and apoptosis and glycoprotein addressing) and extracellular functions (intercellular interaction, interaction with the extracellular matrix, immune recognition of non-self cells) (for review see Gabius, 1997).

Glycobiology at the cellular level

Labelled plant and invertebrate lectins are widely used to determine the presence of distinct saccharidic epitopes in cells and tissues. The animal (endogenous) lectins have also been used as probes for several last years (for review see Gabius, 2001). Their employment in monitoring the cell glycophenotype is very important as the positive reaction allows to also interpret the function of observed glycoligands, since the lectin probe occurring naturally in the tissue was used. The expression of endogenous lectins is detected by convential immunocytochemistry. These two methods are refilled with detection of carbohydrate-binding sites (lectin reactivity) by labelled (neo)glycoconjugates containing a distinct saccharidic epitope. This complexity permits to detect endogenous lectin, carbohydrate ligand reactive for this lectin and the reactivity of this lectin for glycoconjugates in cells and tissues (Froňková et al., 1999).

The normal squamous epithelia make it possible to interpret very roughly the glycobiological observation in relation to differentiation and function of cells because this tissue is morphologically and differentiation-dependently stratified (Nemanic et al., 1983). However, this interpretation is not precise because as was mentioned above, the basal layer contains ESC, TAC and terminally differentiated postmitotic elements before their suprabasal movement. Moreover, if the typical epithelial architecture is altered (*in vitro* cultured cells, malignant tumours), the differentiation must be estimated by the detetection of well-defined markers. The multiple labelling technology at the single-cell level, where the studied glycobiological parameters were assigned to the defined differentiation marker, seems to be a favourable procedure for this purpose. The fluorescence methods seem to be better than others because the multiple fluorescence labelling (eg. green signal+red signal=yellow signal) is very important to interpret the colocalization of two (or more) detected markers (Froňková et al., 1999).

Functional glycobiology of squamous epithelia

Galectins. Galectins (Gal) (former S-lectins) represent a family of minimally 12 endogenous lectins with a conservative carbohydrate-recognition domain. They are reactive for β-galactosides and Gal-3 also recognizes trisaccharides of the histo-blood group A and

B antigens. Gal are located intracellularly or secreted extracellularly. No representative of this endogenous lectin family has a hydrophobic domain which could be included to the cell membrane, and therefore no transmembrane Gal is known. Gal are widely distributed in cells and tissues and their function is very complex, ranging from the participation in the pre-mRNA splicing to the cell-extracellular matrix interaction (for review see Gabius, 1997). Ligation of glycoligands located on the cell surface by Gal seems to be biologically highly active and this mechanism participates in the control of biologically important processes such as proliferation or apoptosis (Brewer, 2002). Gal-1, -3 and -7 seem to have some relation to squamous epithelia structure and function.

Gal-1 and Gal-1-reactive glycoligands are expressed in the cytoplasm of cells of basal and suprabasal layers of all types of squamous epithelia (Plzák et al., 2000). Similarly, expression of Gal-7, lectin specific for this epithelial tissue, is detected in both basal and suprabasal layers (Magnaldo et al., 1998). A significantly different situation is observed in the case of Gal-3. This lectin and, similarly, glycoligands reactive for Gal-3 are expressed by suprabasal cells (Smetana et al., 1999; Plzák et al., 2001). No expression of proliferation markers such as Ki-67 is observed in cells positive for Gal-3 and reactive glycoligands (Plzák et al., 2002c). These cells also never express the β_1 chain of the integrin receptor (Holíková et al., 2002). The Gal-3-reactive glycoligand expression is restricted to the intercellular contacts of suprabasal cells (Plzák et al., 2001; Plzák et al., 2002c). These binding sites precisely colocalize with desmosomal proteins desmoplakin and desmoglein (Plzák et al., 2001; Plzák et al., 2002c). The cells recognized by Gal-3 strongly express cytokeratin type 10 (Plzák et al., 2002c). Labelled neoglycoligands with covalently linked defined saccharidic motifs reactive for Gal-3 recognize the cells of suprabasal layers of the studied epithelia. These findings from histological sections are very similar to observations received in colocalization experiments using cultured cells (Plzák et al., 2002c). Gal-3 is also produced by leukocytes located in the tear fluid of the inflamed human eye and this Gal seems to be able to interact with corneal and conjunctival epithelium (Hrdličková-Cela et al., 2001b). The role of Gal-3 as agent preventing the adsorption of pathogenic microorganisms to the cell surface by lectin mechanims can be speculated.

From the functional point of view, Gal-1 participates in the inhibition of proliferation and induction of apoptosis (Kopitz et al., 2001) and Gal-3 should be pro-proliferative and anti-apoptotic as was investigated in other cell types (Moon et al., 2001). Gal-3, Gal-3-reactive glycoligands and binding sites for saccharides reactive for Gal-3 are under differentiation-dependent control and these markers are in the non-proliferative cell compartment of squamous epithelia. No differentiation-dependent control is observed in the case of Gal-1, where this molecule and Gal-1-reactive glycoligands are detected in basal and suprabasal layers of epithelium, i.e. in cells which are able to proliferate as well as in postmitotic elements. These observations indicate the cell type-specific function of Gal-1 and -3. Gal-7, expressed in basal and suprabasal cells, has some relation to the induction of apoptosis (Bernerd et al., 1999; Kuwabara et al., 2002). However, data about the function of this Gal need further specification (Fig. 2A).

Dolichos biflorus agglutinin (DBA)

A plant lectin prepared from *Dolichos biflorus* recognizes α-N-acetylgalactosamine, including the histo-blood group A epitope. Therefore, one glycoligand recognized by this lectin is also reactive for Gal-3. In agreement with expression of Gal-3-reactive glycoligands, this lectin recognizes some suprabasal cells of squamous epithelia. However, part of basal cells are also positive. These basal cells are negative for expression of the Ki-67 proliferation marker and expression of β_1 integrin in site of their contact with the basement membrane is lower than in the DBA negative basal cells (Hrdličková-Cela et al., 2001a; Dvořánková et al., 2002). Mitotic cells and interphasic cells with high expression of Ki-67 are never recognized by DBA *in vitro*. The depression of Ki-67 signal is connected with perinuclear binding of DBA (Plzák et al., 2002c). The DBA positive cisternae and granules also contain β_1 integrin and p58 protein, marker of ERGIC (endoplasmic reticulum Golgi intermediate compartment) (Dvořánková et al., 2002; Plzák et al., 2002c). The loss of anchor of epidermal cells known as inductor of differentiation significantly elevated DBA binding to these elements (Dvořánková et al., 2002). ERGIC is known as the cell compartment where β_1 integrin is accumulated in cellular mutants with affected adhesion (Martel et al., 2000). Glycosylation is the common posttranslational modification and it influences protein intracellular addressing. The glycosylation of β_1 integrin with DBA-reactive glycoligand may have some role in the retention of this integrin in ERGIC. Therefore, the DBA positive basal cells can be interpreted as elements at the beginning of the differentiation cascade migrating to the first suprabasal layer of stratum spinosum (Fig. 3).

Sialylation detected by the Maackia amurensis isolectin II (MAL) and Sambucus nigra lectin (SNL)

Representatives of the sialic acid family (mostly N-acetylneuraminic acid, NeuNAc) are usually located in the end-position of the oligosaccharidic chain, where they are linked as α2,3-NeuNAc or α2,6-NeuNAc. α2,3-NeuNAc is specifically recognized with MAL and SNL is reactive for α2,6-NeuNAc. While α2,3-NeuNAc expression seems to be more common, α2,6-NeuNAc

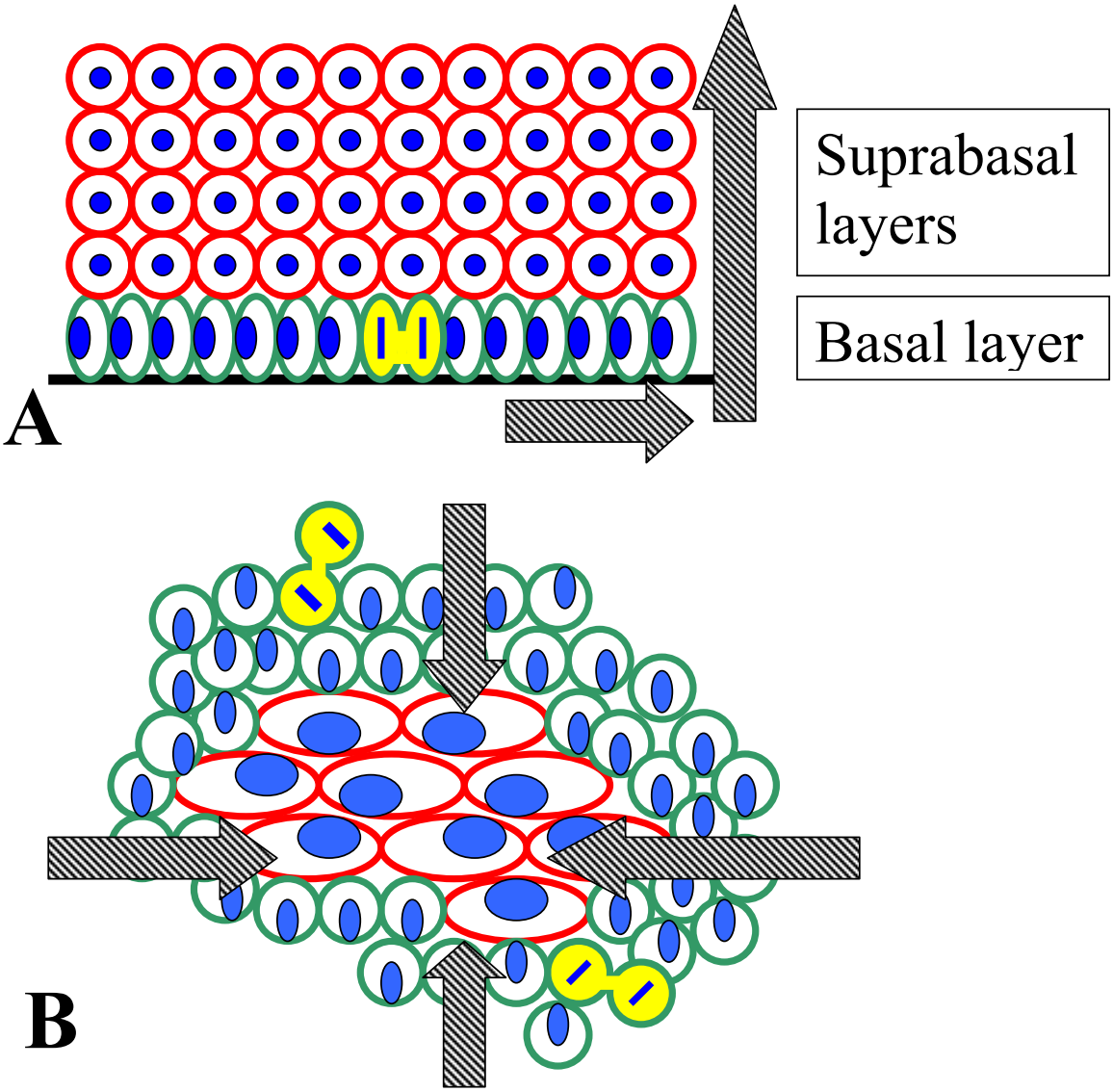

Fig. 2. Schematic presentation of squamous epithelium such as epidermis or oropharyngeal epithelium (A) and squamous cell carcinoma (B) showing expression of selected markers without pretreatment with neuraminidase. The mitotic cells are labelled with yellow and the arrows indicate the direction of differentiation. **A/green color:** integrin receptors, P-cadherin, α2,6NeuNAc, α2,3NeuNAc (epidermis only), Gal-1, glycoligands reactive for Gal-1, Gal-7; **A/red color:** desmosomal proteins, α2,3NeuNAc, Gal-1, glycoligands reactive for Gal-1, Gal-3, glycoligands reactive for Gal-3, Gal-7; **B/green color:** desmosomal proteins (in some tumours only), α2,6NeuNAc, Gal-1, glycoligands for Gal-1; **B/red color:** desmosomal proteins, α2,3NeuNAc, Gal-1, glycoligands for Gal-1, Gal-3, glycoligands for Gal-3.

seems to be regulated oncodevelopmentally, namely in case of enterocytes and colon carcinoma (Vierbuchen, 1995). In squamous epithelia, α2,6-NeuNAc is detected in cells of the basal layer in the epidermis, cornea and vocal cord laryngeal epithelium. α2,3-NeuNAc is expressed suprabasally or in basal as well as suprabasal layers of the epithelium (Holíková et al., 2002) (Fig. 2A). Interestingly, the removal of sialic acids by neuraminidase made the cells of the basal layer accessible for the Gal-3 binding. Since the Gal-3 binding to the basal layer of neuraminidase-treated epithelium is sensitive to lipid extraction, the role of glycolipids as counterparts of Gal-3 in the basal layer can be hypothesized. The same observation was performed in young colonies of cultured epidermal cells (Holíková et al., 2002)

The sialic acids are known as masking agents that prevent the binding of other lectins to the glycoepitope (Smetana et al., 1992; Takahashi, 1992). Data received from the squamous epithelia suggest that α2,6-NeuNAc is a more potent masking agent than α2,3-NeuNAc concerning the binding of Gal-3 and also indicate the differentiation-dependent control of α2,6-sialylation of the basal layer cells' surface. As was mentioned above, Gal-3-binding sites seem to have some role in the intercellular contact of desmosomal type formation. The cells of the basal layer are able to migrate laterally in

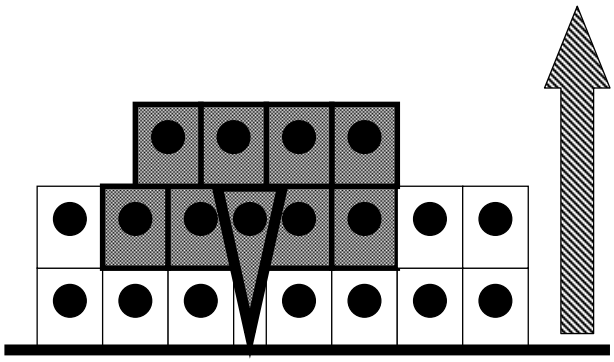

Fig. 3. Basal cells positive for expression of binding sites for DBA (grey) migrate to the first suprabasal layer of the spinous cell layer. These cells are also characterized by a lower expression of β1 integrin in the site of contact with the basement membrane. The direction of cell differentiation and movement is indicated by an arrow.

contrast to suprabasal cells. Perhaps the masking of the glycoepitope reactive for Gal-3 with α2,6-NeuNAc has some role in the mobility of these elements.

Canavalia ensiformis lectin – concanavalin A (ConA)

Plant lectin ConA recognizes α-D-mannosides (Man) and, to a lower extent, glucose. The literature data about the squamous epithelia cell reactivity for this lectin are highly controversial. Laminins as a principal component of BM exhibit unique saccharidic epitopes (Man-rich oligosaccharides) (Fujiwara et al., 1988). BM from many locations are therefore reactive for ConA. Interestingly, BM of epidermis is very weakly reactive or areactive for this lectin (Labský et al., 2003). One of interpretations should be that the Man-rich oligosaccharides of BM are occupied by lectin of basal cells reactive for this glycoepitope. Neoglycoligands containing mannose clusters immobilized to a cultivation support enable growth of keratinocytes without feeder cells under *in vitro* conditions by a specific mechanism (Labský et al., 2003). This observation indicates some role of Man in the function of ESC and suggests participation of these saccharides in formation of a niche important for stem cell behaviour (Spradling et al., 2001).

Glycobiology of carcinomas derived from the squamous epithelia

According to a study of the inactivation of X chromosomes in female patients with carcinomas, tumours represent clones based on the transformation of one cell, probably caused by severe mutations of a stem cell (for review see Reya et al., 2001). There are many data demonstrating some glycobiological abnormalities of tumour cells. This review will stress the markers whose role was defined in normal epithelia.

The basal cell carcinoma glycobiological profile is very similar to cells of the basal layer of epidermis and mucosa covered with squamous epithelia (Plzák et al., 2001). These cells are recognized by Gal-1 and they express α2,6-NeuNAc and no α2,3-NeuNAc. Pretreatment of sections by neuraminidase makes the tumour cells also accessible for Gal-3, which is not able to interact with basal cell carcinomas in the native form (Holíková et al., 2002). A limited number of cells are also reactive for DBA (Plzák et al., 2002c). The mentioned glycobiological markers colocalize with β_1 integrin in part of tumour cells (Holíková et al., 2002; Plzák et al., 2002c).

The squamous cell carcinomas strongly express Gal-1 in almost all cells and Gal-3 in highly differentiated parts of the tumour (Plzák et al., 2000; Plzák et al., 2001). While all cells are positive for Gal-1-reactive glycoligands, Gal-3-reactive glycoligands are expressed in the highly differentiated areas of carcinomas only. The cells that are recognized by Gal-3 express the desmosomal proteins (Plzák et al, 2000; Plzák et al., 2001). Interestingly, cells of regional lymph node metastases of squamous cell carcinomas of the oropharynx are usually Gal-3-reactive glycoligand-free (Plzák et al., 2001). The poorly differentiated tumour cells are characterized by α2,6-linked NeuNAc expression and the differentiated squamous cell carcinoma cells are positive for α2,3-linked-NeuNAc. Similar results were obtained by Wang and coworkers in squamous cell carcinoma of uterine cervix and cell lines derived from aggressive types of these tumours (Wang et al., 2002) (Fig. 2B). The removal of α2,6-linked NeuNAc by sialidase makes tumour cells accessible for Gal-3 (Holíková et al., 2002). Almost all DBA-reactive tumour cells are free of nuclear expression of Ki-67 (Plzák et al., 2002c). The differentiated cells of craniopharyngioma, tumour developed from the Rathke's pouch originating from the embryonic oral cavity also covered with squamous cell epithelium, exhibit the binding of Gal-3 to intercellular contacts among differentiated cells in the late postnatal period (Plzák et al. 2002a).

In conclusion, the glycophenotype of cells of basal cell carcinomas is almost identical with that of the basal cell layer of normal epidermis. The cells of squamous cell carcinoma exhibit signs of differentiation. However, the glycobiological pattern of the tissue organization is highly aberrant namely in case of Gal-3-binding site expression.

Cell therapy of skin defects

Approximately 10 years ago, the progress of cell biology and tissue cultivation brought new possibilities for the employment of cultured tissue in biomedicine. It was connected with introduction of the term tissue engineering (for review see Langer and Vacanti, 1993). The skin defects of traumatic as well as trophic nature represent an extensive medical as well as social and

economical problem (for review see Smetana et al., 2001). One of the possibilities how to help these patients is employment of the cultured epidermis-like sheet prepared according to the procedure of Rheinwald and Green (Rheinwald and Green, 1975). Because of the technical complication of the fragile epithelial sheet transfer in clinical practice, many centres are active in the development of a procedure more suitable for clinical employment. The complete tissue-engineered skin (Dermagraft) has been prepared and it is even commercially available. Because this device is allogenic, it can be used as biologically active provisional cover only. The technology of the transfer of cultured cells directly on a cultivation support at the stage of subconfluent growth seems to be encouraging, including the favourable results of clinical trials (Dvořánková et al., 1998; Dvořánková et al., 2003). However, this procedure also needs the feeder cells as a fundamental condition of epidermal keratinocyte cultivation. The employment of feeders is not very suitable from the clinical point of view. Keratinocytes can also be cultured without feeder cells in highly defined serum-free media (Daley et al., 1990). Unfortunately, use of these media for cultivation of keratinocytes is also problematic in clinical practice because of their price. Employing the glycobiological procedure, synthetic polymer sheets with immobilized Man clusters permitting to culture keratinocytes without feeder cells were prepared (Labský et al., 2003) and the use of this system in cell therapy of skin defects would be possible.

The employment of animal xenogenic cells for the cell therapy and tissue engineering in human medicine is widely discussed. The presence of the so-called Galili antigen (Galα1,3Gal-) seems to be a serious barrier of the animal cell use in human medicine. This glycoepitope is widely expressed in the animal kingdom except for the Old World monkeys and humans. These species with no expression of this antigen have natural anti-Galili antibodies responsible for a hyperacute rejection of xenograft by human and Old World monkey hosts (for review see Cooper, 1996). Thin dermo-epidermal porcine xenografts are widely used as a provisional biological cover of extensive burns and they are well tolerated (Moserová and Housková, 1989). The finding of the absence of Galili antigen in porcine epidermal cells is in good agreement with these clinical data and shows the possibility to use the cultured porcine keratinocytes in the preparation of tissue-engineered provisional bioactive covers without necessity of genetic manipulation of porcine cells (Hrdličková-Cela et al., 2001c). Another possible problem of the animal graft use is a risk of transfer of the animal pathogens or pathological prions to the human host (Stoye et al., 1998), although the risk of transfer of porcine endogenous retroviruses to a human host seems to be minimal (Tacke et al., 2001). However, preparation of cloned pigs can, probably, minimize this danger (Onishi et al., 2000).

Melanocytes

Melanocyte precursors emigrated from the neural crest. They are present in the basal layer of the epidermis. Their specific product melanin in melanosomes is transported to the basal keratinocytes, where melanosomes protect DNA against UV-light damage and malignization of cells via serious mutations of ESC (for review see Lavker and Sun, 2000; Kanitakis, 2002). The passage of melanosomes from melanocytes to keratinocytes seems to be dependent on the lectin-carbohydrate interaction mechanism (Minwalla et al., 2001). The extensive literature about malignant melanoma is available, but it is out of the limits of this article.

Langerhans cells

LC are professional antigen-presenting dendritic cells of myeloid lineage occurring in epidermis and other epithelia delineating the external as well as internal body surfaces (for review see Holíková et al., 2001). Contrary to other types of dendritic cells, LC don't express the macrophage tandem repeat 175 kDa mannose receptor, although it is known that this lectin is very important in antigen uptake and presentation. However, LC also recognize mannosides (Mommaas et al., 1999). New protein Langerin important for the formation of LC-specific organelles – Birbeck granules – was discovered and it is also a C-type lectin recognizing mannosides (Valladeau et al., 2000; Plzák et al., 2002b). LC express Gal-3, although mRNA for this lectin was not detected in these elements, it is produced by keratinocytes (Smetana et al., 1999). Gal-3 is present in active form in Birbeck granules, organelles participating in the antigen processing (Smetana et al., 1999; Holíková et al., 2000). This phenomenon can be explained by the collaboration of keratinocytes with LC in the course of non-self discrimination. Interestingly, the binding reactivity of LC cells for Gal-3-reactive epitopes and mannosides is dramatically reduced in LC infiltrating malignant tumours (Plzák et al., 2002b).

Merkel cells

Merkel cells are present in the basal layer of the mammalian epidermis (Kanitakis et al., 2002). These cells represent a mechanoreceptor unit with axonal termination and some endocrine function can also be hypothesized (Tachibana et al., 1995; Leonard et al., 2002). They are of the neural crest origin (Grim et al., 2000; Szeder et al., 2003), but their origin in epithelial cells is also hypothesized (Moll et al., 1984; for review see Halata et al., 2003). α-Fucose-binding isolectin I isolated from the *Ulex europeus* seems to be highly reactive for Merkel cells in the rat skin (Rosati et al., 1984). On the contrary, cells of human Merkel cells carcinoma are negative for this lectin binding and they are recognized by mannose/glucose specific ConA (Sames et al., 2001).

Conclusions

The glycobiological approach to the cell biology of squamous epithelia brings new data, which make it possible to better understand the biology of this tissue under physiological as well as pathological conditions. Data received by these studies can be employed in carcinoma diagnostics and cell therapy of skin defects.

Acknowledgments

The authors are grateful to Miloš Grim for helpful discussion about Merkel cell origin and function.

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