Detection of New Diagnostic Markers in Pathology by Focus on Growth-regulatory Endogenous Lectins. The Case Study of Galectin-7 in Squamous Epithelia

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Abstract: Lectins represent one of pivotal regulators of the cell proliferation. The potential of galectin-7 as a new prognostic marker was studied in normal and transformed squamous epithelia of both ectodermal (epidermis, cornea vs. trichoepithelioma, basal and squamous cell carcinoma) and endodermal (vocal fold epithelium vs. carcinoma) origin. Studies on the cultured cells were also performed. Expression of galectin-7 seems to be connected to the process of stratification, no matter of origin of epithelium. Its expression is significantly reduced in malignant cells, thus galectin-7 might be a differentiation marker of epithelial malignancies.

Key words: Carcinoma – Basal cell carcinoma – Galectin-7 – Lectin – Squamous cell – Sugar code

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Introduction

Squamous epithelium originates from the ectoderm (epidermis, corneal and conjunctival epithelium, oral mucosa) or endoderm (vocal cords of larynx, oesophagus). The major structural role of this tissue compartment is to add an active protective layer to various tissue architectures. In the mentioned cases, the epithelium is morphologically and functionally stratified with proliferatively active cells in the basal layer. The postproliferative cells migrate to suprabasal layers, where their firm adhesion established by a panel of intercellular contacts contributes markedly to the mechanical stability of the tissue. The most superficial cells are prone to die and to be replaced by continuous regeneration. The zonal occurrence encompassing the mentioned functional differences between the cells is not only the attribute of the normal cells but it also gives rise to characteristic biochemical features in malignancy [for review see 1 and 2]. In this context, one distinct aspect is gaining increasing attention, i.e. the realization of the so far generally neglected role of the glycan determinants of cellular glycoconjugates as biochemical signals [3–5]. In fact, the multifarious roles of oligosaccharides in information storage and transfer have shaped the concept of the sugar code [6]. The analysis of the way this information system is decoded is sure to provide a new insights into the molecular mechanisms of cell adhesion and growth regulation with the impact on the devising innovative diagnostic and therapeutic procedures.

Information transfer from the glycan determinants often starts with specific recognition by receptor proteins [7]. These endogenous (animal) lectins represent carbohydrate-binding proteins different from carbohydrate-utilizing enzymes, immunoglobulins specifically recognizing distinct saccharidic motifs and transport proteins [for review see 6, 8]. One family of endogenous lectins has especially developed to bind β-galactosides at branch ends of glycan antennae, and like the enzymes responsible for the diversity of such structures intrafamily diversity is also aparent in this lectin category, a strong argument for a complex system for

Table 1 Expression of galectin-7 in squamous epithelia

Tissue	Number of samples	Gal-7 Intensity/Number
Normal skin	4	++/4
BCC	5	–/5
Follicułlar trichoepithelioma	1	+/1
SCC of skin	4	±/4
Cornea	5	+/5
Larynx	3	+/3
SCC of larynx	5	±/4
		+/1
SCC of larynx- lymph node metastasis	3	±/2
		+/1

signal recognition [5, 9]. Of note, they can even read changes in glycan conformation introduced by common substitutions [10]. Reflecting their functionality, they have already become targets of drug design [11]. In addition to their specificity to glycans they also home in on peptide/lipids motifs in intracellular sites, hereby contributing to growth regulation or pre-mRNA splicing [8, 12, 13]. In fact, galectins are known to harbor pro- and anti-apoptotic activities linked to growth and malignancy [8, 14]. In this report, we focus on a homodimeric galectin present in the tissue compartment mentioned above, i.e. galectin-7 (Gal-7). This galectin is characteristic for squamous stratified epithelia, where it appears to be connected with the program of tissue stratification [15]. It figured prominently as p53-induced gene-1 in a colon cancer line, and initial reports indicate that expression of Gal-7 is actually downregulated in basal and squamous cell carcinomas of the skin [16, 17].

To illustrate our activity in the delineation of the functionality of the sugar code the histochemical monitoring of Gal-7 in normal and cancer squamous cell epithelia of different histogenetic origin and the comparison of these *in situ* results with observations performed under *in vitro* conditions are the objective of the presented study.

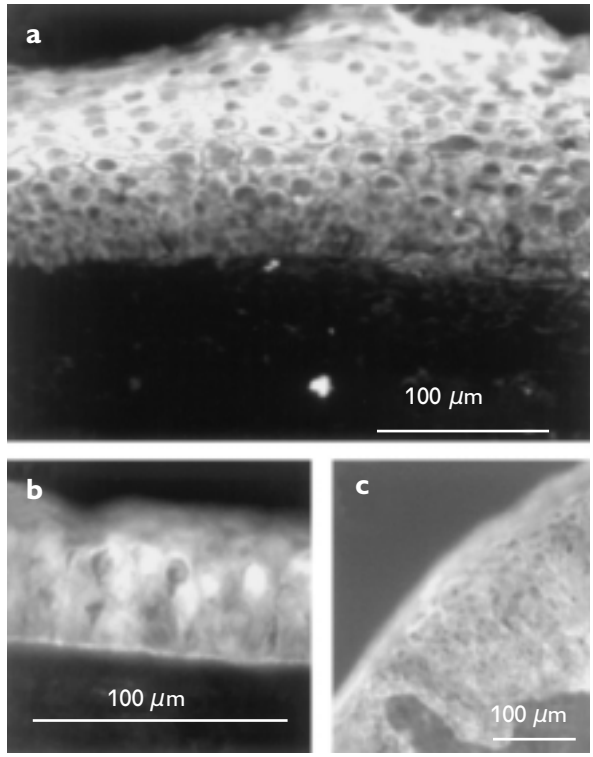

Figure 1 – Detection of galectin-7 in human epidermis (a), cornea (b) and vocal cord epithelium of larynx (c).

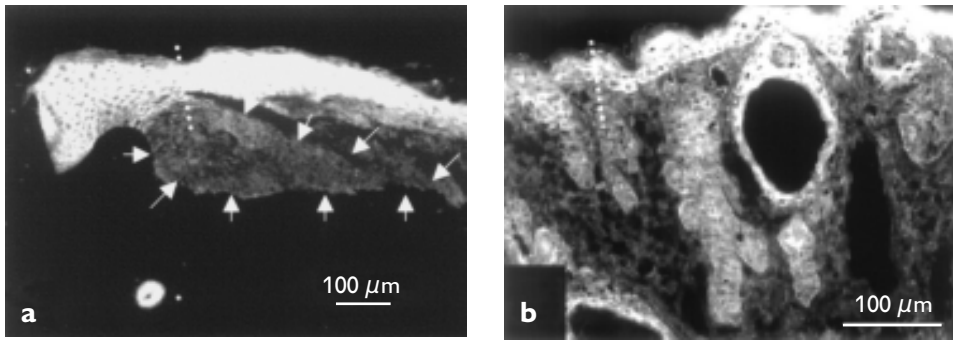

Figure 2 – Detection of galectin-7 in basal cell carcinoma (surrounded by arrows) (a) and follicular trichoepithelioma (b) dashed line marks the site of the measurements of fluorescence profile.

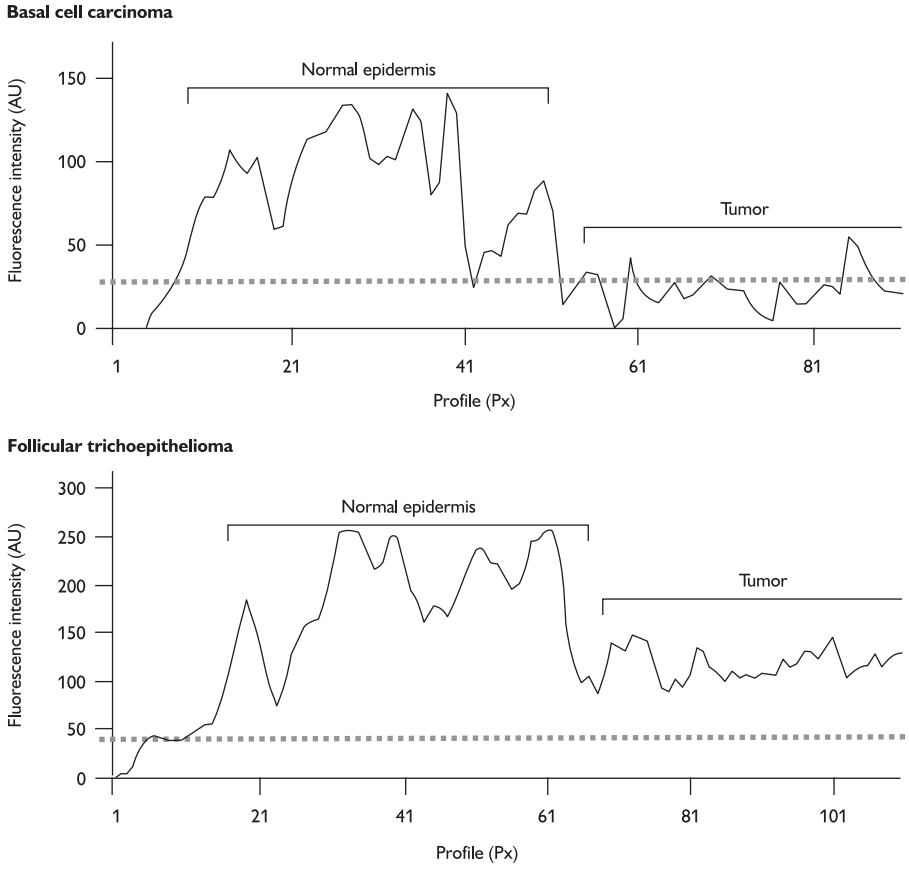

Figure 3 – Fluorescence profile of galectin-7 expression in basal cell carcinoma and follicular trichoepithelioma. See the significant difference of expression on non-affected surface epidermis and tumor site.

Material and methods

The specimens of human tissue (Table 1) were obtained with the explicit informed consent of patients according to the Helsinki Declaration. The tissue samples were frozen in liquid nitrogen using Tissue-Tek (Sakkura, Zoeterwoude, The Netherlands) and stored deeply frozen until further processing started. 7μm thin frozen sections were prepared using Cryocut E (Reichert, Vienna, Austria). Tissue-Tek was removed by PBS immediately before immunohistochemical processing. Interfollicular epidermis and epithelium from the periphery of cornea containing limbus was used for cultivation experiments as described [18].

Gal-7 was detected by a routine immunohistochemical procedure using rabbit polyclonal antibody free of cross-reactivity against any other galectin diluted 1:50. FITC-labeled swine-anti rabbit antibody (AlSeVa, Prague Czech Republic) diluted 1:50 was used as the second-step reagent. Substitution of anti Gal-7 antibody by

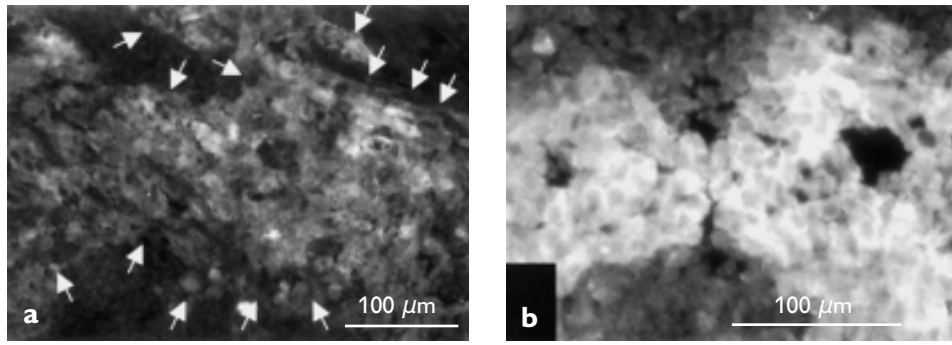

Figure 4 – Expression of galectin-7 in squamous cell carcinoma of larynx (a) and of lymphatic metastasis of the carcinoma of the same origin (b).

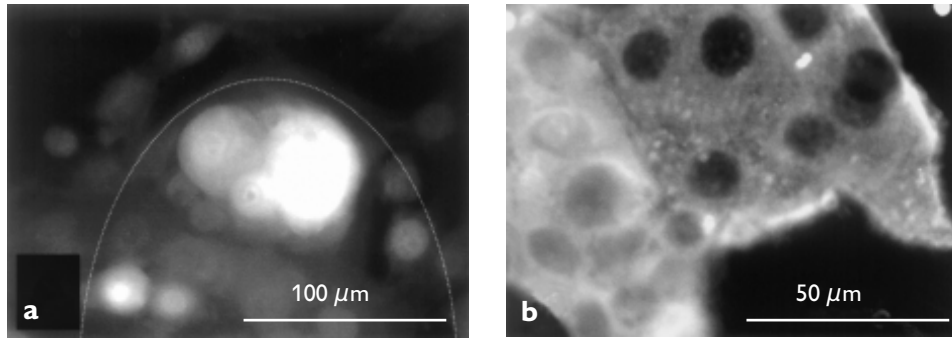

Figure 5 – Expression of galectin-7 in cultured cells of corneal epithelium at the 5th day of cultivation (the group of cells is surrounded by dashed circle) (a) and of cultured epidermis at the 10th day of cultivation (b). The second layer of differentiated cells is positive only.

non-immune rabbit serum was used as a control of the specificity of reaction, as was saturation of antibody by Gal-7. The specimens were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and inspected employing an Optiphot-2 (Nikon, Praha, Czech Republic) fluorescence microscope equipped with a CCD camera Cohu and a computer-assisted image analysis system LUCIA (Laboratory Imaging, Prague, Czech Republic).

Results

Cells of the layers of the different types of studied epithelia, i.e. of epidermis (Figure 1a), cornea (Figure 1b) and larynx (Figure 1c), expressed Gal-7 (Table 1). While the cells of basal cell carcinomas were devoid of Gal-7 expression (Figure 2a), cells of follicular trichoepithelioma were positive (Figure 2b), although the extent of signal was lower than in the non-affected epidermis (Figure 3) as is visible from the measurements of fluorescence profiles. The samples of squamous cell carcinoma (SCC) of epidermis and a majority of samples of the same type of tumor but from the laryngeal epithelium exhibited significant regulation of galectin expression (Figure 4a). A similar phenomenon was also observed in lymph node metastases of the laryngeal SCC (not shown). Interestingly, cells of one sample of laryngeal SCC and its lymphatic metastasis presented Gal-7 expression in tumor cells (Figure 4b).

The initial step of the cultivation of epidermal cells and corneal epithelium is associated with absence of Gal-7 expression. At the beginning of the formation of multilayered colonies Gal-7 expression is apparently initiated (Figure 5a and b). Two-weeks-old confluent culture of human keratinocytes constitutes a mosaic of monolayer and multilayered regions. Expression of Gal-7 reflects this growth of culture, where the cell multilayer is only positive for the studied lectin (Figure 6).

Discussion

Our results support the notion that Gal-7 is involved in the negative growth regulation of epithelial tumor cells. Fittingly, the follicular trichoepithelioma,

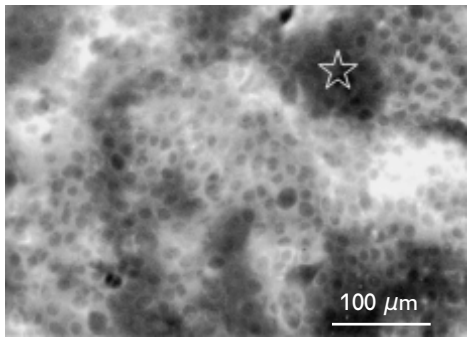

Figure 6 - Expression of galectin-7 in cultured epidermal cells at the 2nd week of cultivation. Cells of the monolayer (asterisk) are weekly positive in comparison with multilayered formation.

a rare benign tumor of the skin with high degree of tumor cell differentiation, showed only small reduction of Gal-7 expression in comparison with the epidermis. However, recent results by proteomics demonstrate an increase of Gal-7 expression in buccal squamous cell carcinoma in comparison with normal epithelium [19]. Evidently, cell biological studies are required to clarify the role of Gal-7 in each cell type under study. Looking at the colon cancer (DLD-1) cells, galectin-7 offers the potential for application as suppressor [16, 20], as was similarly reported for neuroblastoma cells by virtue of cell surface binding to ganglioside GM_1 [21]. In epithelia it is reasonable to assume that Gal-7 is linked to the program of stratification in squamous type of epithelia arising from both the ectoderm and entoderm. This process is under the control of transcription factor $\Delta Np63\alpha$. Decrease of Gal-7 expression in epithelial tumors can thus serve to indicate the lack to develop an organized multilayered tissue. These tumors present a high level of expression of $\Delta Np63\alpha$ and decrease or absence of adhesion molecules responsible for interacellular contacts typical for suprabasal cells in squamous cell carcinomas. At this stage, further study of Gal-7 might be instrumental to define a diagnostic marker for this type of malignancy. Having developed a panel of antibodies for galectins, it will be a next step to comparatively map the network and relate its status to the level of differentiation and tumor progression. A new perspective is opened by the introduction of labeled galectins to localize endogenous binding sites, recently reported [22]. Especially, galectins-1 and -3 and their reactive ligands have role in biology and spreading of squamous cell carcinomas [23]. The comparison of expression of these galectins and their ligands with Gal-7 could be suitable for better characterization of malignant epithelial tumors.

Conclusion

Galectin-7 expression represents the specific feature of squamous epithelia of different nature. Monitoring of this endogenous lectin can be employed in the histopathology of tumors originating from this type of epithelium.

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