Expression patterns of α2,3-Sialyltransferase I and α2,6-Sialyltransferase I in human cutaneous epithelial lesions

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Abstract

Skin tumors have become one of the most common cancers in the world and their carcinogenesis is frequently associated with altered glycosylation patterns. The aberrant sialylation, a type of glycosylation, can mediate pathophysiological key events during various stages of tumor progression, including invasion and metastasis. Sialyltransferases play a key role in a variety of biological processes, including cell-cell communication, cell-matrix interaction, adhesion, and protein targeting. In this study, it was evaluated the expression of ST3Gal I and ST6Gal I in cutaneous epithelial lesions that include actinic keratosis (n=15), keratoacanthoma (n=9), squamous cell carcinoma (n=22) and basal cell carcinoma (n=28) in order to evaluate if sialyltransferases expression is different in premalignant and in malignant tumors. The expression of ST3Gal I was observed in actinic keratosis (53%), keratoacanthoma (78%), squamous cell carcinoma (73%) and basal cell carcinoma (32%) with statistic differences between basal cell carcinoma and keratoacanthoma (P=0.0239) and basal cell carcinoma and squamous cell carcinoma (P=0.0096); for ST6Gal I, cytoplasmic expression was noted in actinic keratosis (40%), heterogeneous and cytoplasmic expression was noted in keratoacanthoma (67%), squamous cell carcinoma (41%) and basal cell carcinoma (7%) with statistic differences between basal cell carcinoma and squamous cell carcinoma (P=0.0061) and basal cell carcinoma and keratoacanthoma (P=0.0008). In summary, our results showed that the high expression of ST3Gal I and ST6Gal I, in skin tumors, is associated with tumors with greater potential for invasion and metastasis, as in the case of squamous cell carcinoma, and this may be related to their behavior.

Introduction

Skin tumors have become one of the most common cancers in many countries, with rapid increasing incidence during the last half century.[1] The cutaneous epithelial lesions, also called non-melanoma skin cancer (NMSC), are the most common cancers in the Caucasian population. The most common skin lesions are actinic keratosis (AK), keratoacanthoma (KA), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Molecular, genetic and biochemical changes occur in the development of skin malignancies but they are only partially understood leading to the determination of the genetic basis of skin lesions to explain their phenotypes, biological behaviors and different metastatic potential.[2]

Of all the molecular research involving skin lesions glycobiology, including cancer, are scarcer. The phenotypic alterations in cell surface glycans provide malignant features to cells.[3] It is well known that glycans on a cell surface or in extracellular space play important roles in cellular differentiation, adhesion and proliferation[4] and that tumors aberrantly express glycans.[5] Glycosylation is determined by the expression and relative activities of glycosyltransferases in particular tissues.[6-9] Most of the changes in glycosylation pattern that occurs during oncogenesis is associated with increased sialylation of glycoconjugates in the end-forming sialylconjugates.[10]

Abnormally high levels of sialylated tumor associated carbohydrate antigens are frequently described at the surface of cancer cells and/or secreted in biological fluids. It is now well established that this over-expression may result from deregulation in sialyltransferases activity involved in their biosynthesis but the precise molecular mechanisms remain unknown.[11] The sialylation is one of the critical mechanisms for the regulation of various biological processes. In fact, inhibition of sialyltransferases[12] or gene targeting of sialyltransferases such as β-galactoside α-2,6 sialiltransferase (ST6Gal-I),[13] revealed that sialylation of glycoproteins or glycosphingolipids is very important in tumour development, neuronal development, nerve repair, immunological processes and regulation of hormone sensitivity.[14] Sialic acids are one of the most important monosaccharide being expressed as terminal sugars with a shared nine-carbon backbone in several classes of cell surface and secreted glycan molecules.[15] In addition to providing negative charge and hydrophilicity to vertebrate cell surfaces, to masking subterminal galactose residues from recognition by certain receptors, and to acting as receptors for pathogens and toxins, sialic acids play an important role during development, include early embryonic development in mice.[16]

The study of changes in the structure of oligosaccharide in surface cell, promoted by sialyltransferases, has been frequently associated to human cancer. This is important because they may be related to a precise stage of the disease and its detection with lectins or monoclonal antibodies may provide useful information for diagnosis or prognosis, or both, and in many cases, they directly contribute to cancer biology. However, there are no studies that are dedicated to evaluate the expression of these enzymes associated with altered patterns of sialylation (glycophenotype) in epithelial lesions. To better understand the role of sialyltransferases in cutaneous epithelial tumors, this study was undertaken to evaluate and compare the expression and distribution of β-galactoside α-2,3 sialiltransferase (ST3Gal I) and β-galactoside α-2,6 sialiltransferase (ST6Gal I) in benign (keratoacanthoma, KA), premalignant (actinic

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keratosis, AK) and malignant skin lesions (squamous cell carcinomas, SCC, and basal cell carcinomas, BCC).

Materials and Methods

Samples

Paraffin-embedded tumor biopsies (actinic keratosis (AK) n=15, keratoacanthoma (KA) n=9, squamous cell carcinoma (SCC) n=22, and basal cell carcinoma (BCC) n=28) were obtained from the Tissue Bank of the Clinic Hospital at the Federal University of the State of Pernambuco (UFPE), after Ethical Committee approval. The diagnosis of skin cancer was based on hematoxylin and eosin (H&E) histopathology (Figure 1). The histologic sections of all cases were re-reviewed and the diagnoses confirmed by an independent dermatopathologist.

Immunohistochemistry

Tissue slices (4 μm) were deparaffinized in xylene and hydrated with decreasing ethanol concentration. Antigen retrieval that was performed with a in citrate buffer (pH 6.0) in steamer for 30 min followed by endogenous peroxidase blocked (H_2O_2 0.3% in Methanol) and then block of nonspecific sites were done with 1% PBS-BSA for 1 hour at room temperature. Slices were incubated with rabbit policlonal antibody anti-ST3Gal I (1:200, Sigma-Aldrich, St. Louis, MO, USA) and mouse monoclonal antibody anti-ST6Gal I (1:200, Sigma-Aldrich) overnight at 4ºC. After washing with Phosphate Buffered Saline (PBS) sections were incubated with biotin-free polymer (ADVANCE™ HRP KIT, DAKO, Carpinteria, CA, USA). According manufacture instructions, samples were incubated with ADVANCE™ HRP LINK (containg secondary antibodies) for 30 min and then with ADVANCE™ HRP Enzyme (containing antibodies conjugated to horseradish peroxidase, HRP) for 30 min. Finally the reaction was reveled with 3,3'-Diaminobenzidine (DAKO) and counterstaining with hematoxylin. All washes between the steps were performed with PBS. Negative controls were obtained by replacing primary antibodies with bloking solution.

Image analysis and semi-quantification

Tissue images were acquired using a video camera system coupled to an Eclipse 50i microscope (Nikon, Melville, NY, USA). Random areas (μm^2) were analyzed taking into account the number of stained cells per area. Quantitative analysis of stained cells was measured using the automatic system (three areas in each case). Staining intensity was measured according to Dornelas[17] as: 0, negative staining; 1+, low staining for up to 1/3 of cells stained; 2+, moderate staining for up to 2/3 of cells stained; and 3+, intense staining for more than 2/3 of cells stained. Three different areas (1 cm^2) per tissue were analyzed (magnification 100×).

Statistical analysis

The statistical association ST3Gal I and ST6Gal I were analyzed using the χ^2-test or Fisher's Exact test (GraphPad Software) to determine whether there were differences of significance in expression of the different sialyltransferases amongst the categories studied. A two-tailed P value of less than 0.05 was considered to be statistically significant.

Results

ST3Gal I

The immunoreactivity of ST3Gal I was observed in a basal pattern, defined by staining of the basal cell layer of AK, KA, SCC, BCC and normal skin. AK presented a diffuse cytoplasmic staining (Figure 2A), while KA and SCC presented diffuse cytoplasmic and membrane staining (Figure 2B,C respectively). In most cases of BCC was negative staining with rare nuclear and perinuclear staining (Figure 2D).

Immunopositivity to ST3Gal I was observed in 8/15 (53%) (4 cases: 1+; 3 cases: 2+; 1 case: 3+) of AK, 7/9 cases (78%) (2 cases: 1+; 4 cases: 2+; 1 case: 3+) of KA, 9/28 (32%) (3 cases: 1+; 5 cases: 2+; 1 case: 3+) of BCC and 16/22 cases (73%) (7 cases: 1+; 4 cases: 2+; 5 cases: 3+) of SCC. Significant differences in immunostaining of ST3Gal I were noted between KA and BCC (P=0.0239) and between SCC and BCC (P=0.0096). Immunostaining differences between all positive and negative lesions are summarized in Table 1.

ST6Gal I

Immunopositivity was heterogeneous and predominantly diffuse in cytoplasm in most of the samples of squamous cell carcinoma (Figure 3A,B), keratoacanthoma (Figure 3C) and actinic keratosis and absent, nuclear and/or perinuclear in basal cell carcinoma.

The immunopositivity to ST6Gal I was observed in 6/15 (40%) (2 cases: 1+; 3 cases: 2+; 1 case: 3+) of AK, 6/9 cases (67%) (2 cases: 1+; 2 cases: 2+; 2 cases: 3+) of KA. BCC samples were characterized by positivity to anti-ST6Gal I in 2/28 (7%) (2 cases: 1+) and 9/22 cases (41%) (2 cases: 1+; 2 cases: 2+; 5 cases: 3+) of SCC. Significant differences in expression of ST6Gal I were observed between KA and BCC (P=0.0008) and between SCC and BCC (P=0.0061). Immunostaining differences between all positive and negative lesions are summarized in Table 1.

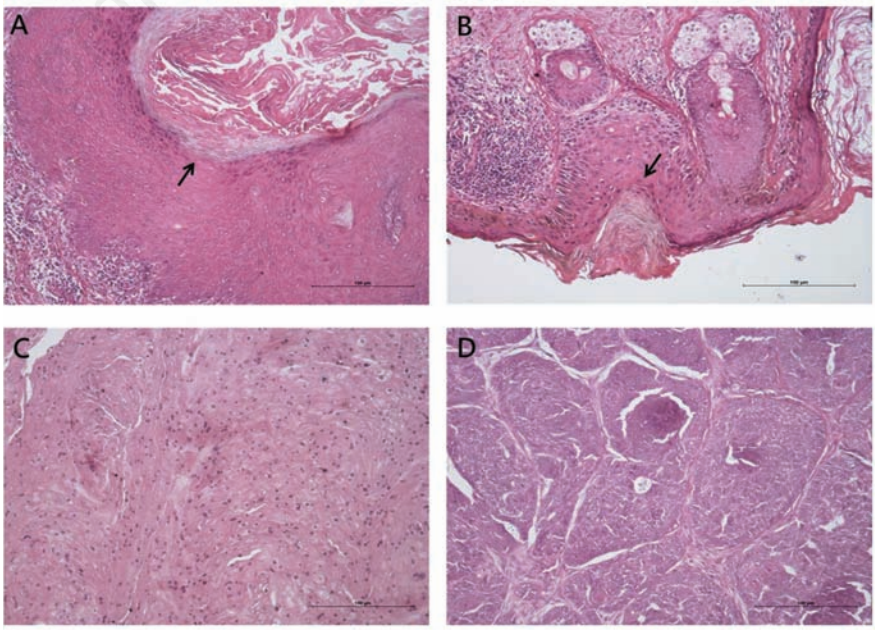

Figure 1. Skin lesions stained with haematoxylin-eosin. A) Keratoacanthoma with squamoproliferative nodules and a central keratin plug (arrow). B) Actinic keratosis with focal parakeratosis (arrow), acanthosis and basal squamous atypia overlying a dense lichenoid inflammatory infiltrate. C) Squamous cell carcinoma with abundant eosinophilic cytoplasm and atypical mitosis. D) Basal cell carcinoma with groups of atypical basaloid cells and peripheral palisading in a nodular pattern. Scale bar: 100 μm.

Discussion

Studies indicate that skin carcinogenesis is associated with altered glycosylation patterns.[8,18,19] In normal squamous cell epithelia, the glycophenotype is characterized by α2,6-linked sialic acid expression in epithelium cells of the basal layer and α2,3-linked sialic acid in basal as well as suprabasal layers.[19]

Sialic acids (Sia) is found in cellular secretions and on the outer surface of cells, mostly as terminal components of glycoproteins and glycolipids.[20] Terminal sugars play an important role in the function of glycoconjugates and it has been recognized that this sugar may somehow modulate the adhesion of cancer cells to extracellular matrix components.[21] Changes in the expression of sialic acid have been correlated with changes in gene expression of sialyltransferases.[22] Sialyltransferases participates of many biological processes, including cell-cell communication, cell-matrix interaction, adhesion, and protein targeting.[23]

In this study it was observed that in most of the lesions analyzed, ST6Gal I and ST3Gal I presented a diffuse pattern in cytoplasm and membrane. Although sialyltransferases are expected to be located in the *trans* Golgi and *trans* Golgi network, there are reports about post-Golgi localizations, one report showed a plasma membrane association of ST6Gal I by using protein-specific antibodies.[24,25] According to Burger and colleagues,[25] sialyltransferases in the region of the luminal membrane of kidney proximal tubular cells might have a function in the re-sialylation of recycling cell surface glycoproteins.

In present study, all the cutaneous epithelial lesions studied exhibited an increased expression of ST3Gal I when compared with ST6Gal I. This pattern was previously observed by our group (*data not shown*) using lectin histochemistry, with greater expression of α2,3-linked Sia residues than α2,6-linked Sia. In bladder cancer, the ST3Gal I plays a major role in the sialylation of the T antigen and its overexpression appears to be part of initial oncogenic transformation.[26]

Beside this, our results showed a higher expression of ST6 Gal I in tumors with high invasive potential as SCC. Clinical and experimental studies suggest a positive correlation between high ST6Gal I levels and the invasive behavior of cancer cells.[27] Using immunohistochemistry Cao *et al.*[28] showed that expression levels of ST6Gal I was lower in poor-differentiated hepatocellular carcinoma. The same was observed in the studies by Poon *et al.*[29] These studies conclude that the role of this enzyme differ from tumor to tumor.

No significant difference was observed in pattern of expression of ST3Gal I and ST6Gal I in cases of SCC, KA and AK. Some authors consider that both KA and AK tumors are pre-

Table 1. ST3Gal I and ST6Gal I immunoexpression in cutaneous epithelial lesions.

	ST3Gal I (%)			ST6Gal I (%)		
	(+)	(-)	P	(+)	(-)	P
Actinic keratosis	53	47		40	60	
Keratoacanthoma	78	22	0.0239*	67	33	0.0008*
Basal cell carcinoma	32	68		7	93	
Squamous cell carcinoma	73	27	0.0096**	41	59	0.0061**

*Fisher's exact test (keratoacanthoma vs basal cell carcinoma); **Fisher's exact test (basal cell carcinoma *vs* squamous cell carcinoma).

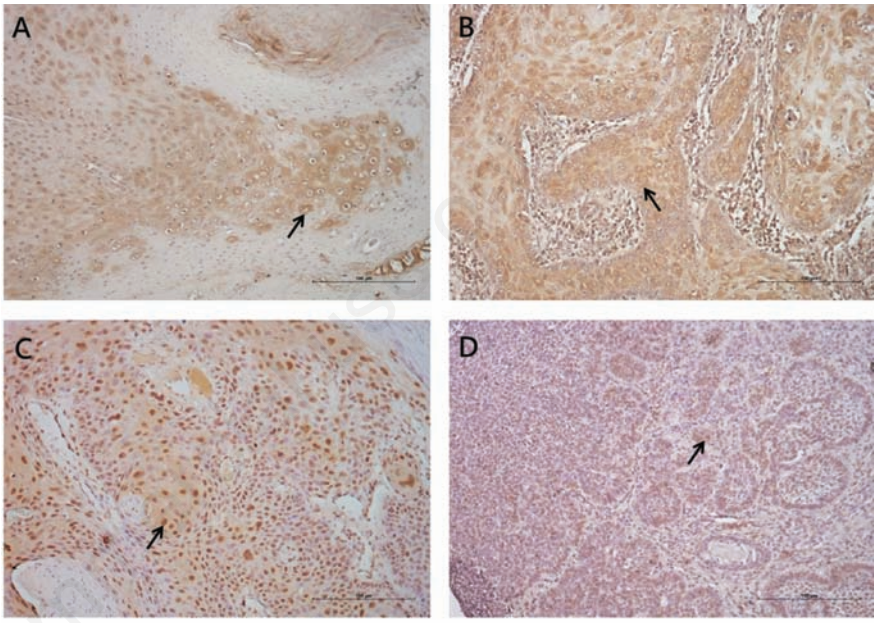

Figure 2. Immunohistochemical expression of ST3Gal I in human cutaneous epithelial lesions. A) Actinic keratosis with diffuse cytoplasmic staining (arrow), grade 3+. B) Keratoacanthoma with diffuse cytoplasmic staining (arrow), grade 3+. C) Squamous cell carcinoma with diffuse cytoplasmic and nuclear (arrow) staining, grade 3+. D) Basal cell carcinoma with nuclear staining (arrow), grade 1+. Staining patterns: 1+, weak; 2+, moderate; 3+, intense positivity. Scale bar: 100 μm.

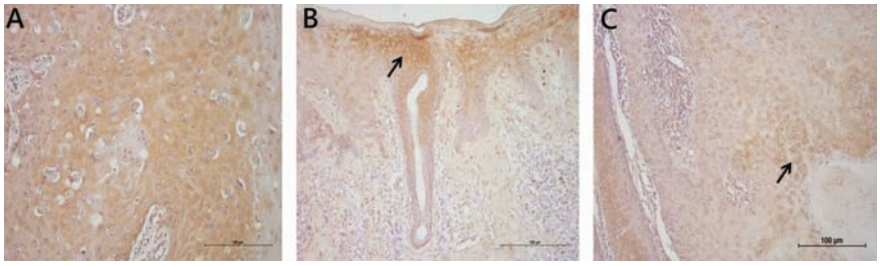

Figure 3. Immunohistochemical expression of ST6Gal I in human cutaneous epithelial lesions. A) Squamous cell carcinoma with diffuse cytoplasmic staining, grade 3+. B) Squamous cell carcinoma with diffuse cytoplasmic staining, grade 1+. C) Keratoacanthoma with diffuse cytoplasmic staining, grade 3+. Staining patterns: 1+, weak; 2+, moderate; 3+, intense positivity. Scale bar: 100 μm.

malignant lesions that precede the SCC. AKs are considered premalignant as they may develop into invasive SCC.[3,30] It is suggested that 10% of these sun-induced lesions will develop into SCC.[31] Whether ST3Gal I and ST6Gal I plays a causal role in the progression of AK to SCC remains to be addressed.

Although many immunohistochemical studies have claimed to be helpful in the distinction between KA and SCC, the results have not confirmed these so far.[32] Indeed some authors believe that these lesions (KA) are SCC,[33] others support that KA are benign squamous proliferation.[34] Our results showed that immunohistochemical staining for ST3Gal I and ST6 Gal I it is no able to distinguish clearly between KA and SCC. However, these tumors have a different pattern of sialylation (*data not shown*) that is related to tumor behavior. KA tends to regress spontaneously indicating a biologically benign course in distinction from the SCC.

This work showed a significant difference in expression pattern of sialyltransferases between BCC and SCC, and between BCC and KA. The differential expression of ST3Gal I and ST6Gal I between SCC and BCC is curious. The fact that both ST3Gal I as ST6Gal are consistently expressed in most SCC cases, which are capable of metastasis, and not expressed in BCC, which have no potential of metastasis although often locally aggressive,[35] suggest the potential role of these sialyltransferase in facilitating metastasis. Recent studies support this possibility in others tumor, both ST6Gal I and α2-6 sialylconjugates play important roles in oncogenic transformation and metastasis in hepatocellular carcinoma[29] and A549 lung cancer cell line.[33] The overexpression of ST6Gal I is well documented in several types of cancers as ovarian cancer cells resulting in a phenotype consistent with aggressive metastasis.[34,36,37] In the case of ST3Gal I some studies demonstrated that its over-expression is functionally involved in oncogenesis, suggesting that it is not just a collateral effect of carcinogenesis but may provide some advantages to tumor development in breast cancer. The results also suggested that ST3Gal I exerts its effect early in tumor development.[9]

ST3Gal I and ST6Gal I expression is important, since the aberrant sialylation can mediate pathophysiological key events during various stages of tumor progression, including invasion and metastasis.[17,26] Our study was the first in the literature which aimed to analyze the expression of sialyltransferases in NMSC and results showed that the high expression of ST3Gal I and ST6Gal I, in skin tumors, is associated with tumors with greater potential for invasion and metastasis, as in the case of squamous cell carcinoma, and this may be related to their behavior.

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