Expression of the Carbohydrate Tumor Marker Sialyl Lewis a (Ca19-9) in Squamous Cell Carcinoma of the Larynx

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Abstract. *Background: The clinical relevance of the carbohydrate antigen Sialyl Lewis a (SLea) as a serum tumor marker in diagnosis and follow-up treatment is unquestioned in a broad spectra of human carcinomas. Overexpression of this antigen is combined with poor prognosis and malignant relapse. The aim of our study was the systematic investigation of SLea expression in squamous cell carcinoma of the larynx versus normal and phlogistic tissue. Materials and Methods: Paraffin-embedded sections of normal, phlogistic and squamous cell carcinoma tissue were incubated with a monoclonal antibody against SLea. The staining reaction was performed using ABC-Peroxidase and DAB. As a positive control tissue of breast cancer was used and the negative control was performed with unspecific mouse IgM. Semiquantitative evaluations were carried out double-blinded by two independent investigators, including a pathologist. Results: A very faint expression of SLea (Ca19-9) in normal laryngeal tissue, a moderate upregulation in phlogistic tissue and a dramatic upregulation in some types of squamous cell carcinoma of the larynx were observed. Laryngeal cancer is the most common cancer of the upper aerodigestive tract. Most cases of laryngeal cancer are squamous cell carcinoma and can be classified into: well differentiated (more than 75% keratinization), moderately differentiated (25-75% keratinization), and poorly differentiated (<25% keratinisation) carcinomas. Conclusion: The results of this study indicate that SLea is a potential tumor marker in carcinoma of the larynx.*

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Laryngeal cancer is the most common head and neck cancer (1). It occurs mostly in people over the age of 50 and men are suffering considerably more often than women (2). In Germany, 3000 men and 400 women are diagnosed with larynx cancer every year (3-5). A clear association has been made between smoking, excess alcohol ingestion, and laryngeal cancer and the combined exposure to alcohol and tobacco has a multiplicative effect on carcinogenesis (6-15).

The larynx consists of three subsites: the supraglottis, consisting of the false vocal cords, the epiglottis and the aryepiglottic folds; the glottis or true vocal cords and the subglottis, consisting of the area bounded by the under-edge of true vocal cords and the top of the cricoid cartilage.Supraglottic laryngeal carcinoma occures in 35% of cases, glottic carcinoma in 60% and subglottic carcinoma in 5% (16). More than 95% of all primary laryngeal cancers are squamous cell carcinomas, with the remainder being sarcomas, adeno-carcinomas, neuroendocrine tumors and, rarely, metastasis from renal cell, breast, lung, prostate and gastrointestinal cancers (17-19). For the diagnosis a physical examination, indirect and direct laryngoscopy with biopsies should be done (20). If the histology reveals a carcinoma further examinations (i.e ultrasound, CT-scan) are necessary depending on the size of the tumor, in order to schedule the respective treatment which includes surgery, radiation, chemotherapy or combinations of these three (20). The relative 5-year survival rate of patients with larynx carcinoma averages approximately to 61% in men and 62% in women (21-25). Despite advances in treatment, improvement in survival and quality of life of patients still remains a challenge. Specific tumor markers, which are widely used for diagnosis and follow-up are missing.

The aim of this study was the systematic investigation of SLea (CA19-9) expression in squamous cell carcinoma of the larynx versus normal and phlogistic tissue.

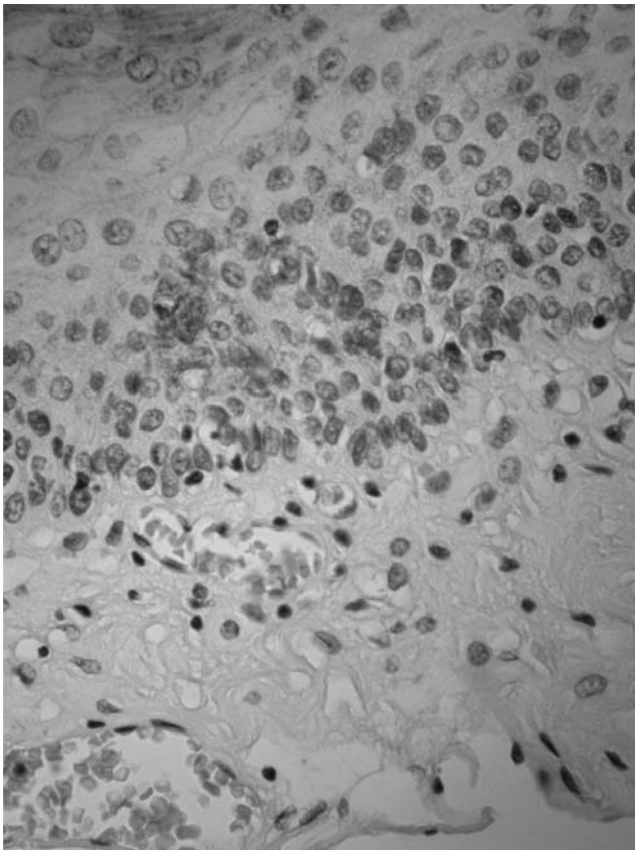

Figure 1. *Expression of SLea in normal squamous epithelial tissue, ×40.*

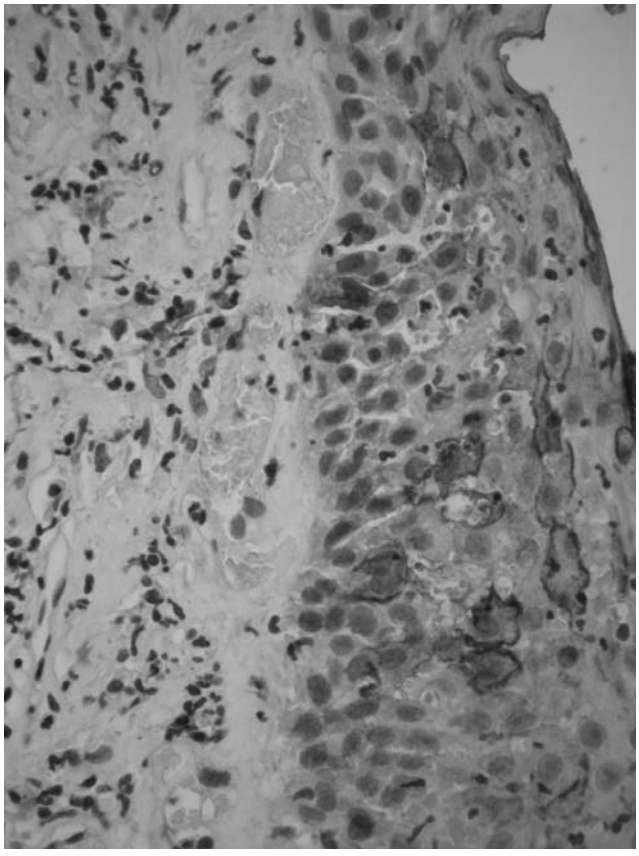

Figure 2. *Expression of SLea is elevated in phlogistic tissue, ×40.*

Materials and Methods

Samples of five normal, five phlogistic and five squamous cell carcinoma were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections of 3 μm were prepared.

Immunohistochemistry. Sections were dewaxed in xylol twice for 10 min and rehydrated in a descending set of alcohol. After inhibiting endogenous peroxidase with methanol/H_2O_2 for 30 min slides, sections were washed in PBS (phosphate-buffered saline, pH 7.4) and incubated with normal goat-serum for 30 min at room temperature (RT) to reduce unspecific background. Incubation with the monoclonal antibody against Sialyl Lewis a (KM231, Calbiochem, San Diego, USA) at a concentration of 2 μg/ml was done overnight at 4°C. After acclimation for 30 min at RT slides were washed twice in PBS for 10 min and then incubated with the biotinylated secondary anti-mouse (Vectastain, Vector laboratories, Burlingame, USA) antibody for 1 h at RT. After washing the slides again in PBS, they were incubated with the avidin-biotin peroxidase complex (Vectastain-Elite, Vector Laboratories, UK) for 45 min at RT. Slides were visualised with the chromogen diaminobenizidine DAB (Dako, Glostrup, Denmark) and counterstained with Mayer´s hematoxylin. Then slides were washed in an ascending set of alcohol, transferred to xylene and coverslipped.

Controls. Sections of breast-carcinoma were used as the positive control. Negative control slides were performed by replacing the primary antibody by isotype control mouse IgM at the same concentration compared to the primary antibody.

Statistical analysis. Statistical analysis was performed using the Wilcoxon's signed rank tests for comparison of the means. A $p<0.05$ value was considered statistically significant. The intensity and distribution patterns of the specific immunohistochemical staining was evaluated using a semi-quantitative method (IRS score) as previously described (26). The IRS score was calculated as follows: IRS = SI × PP, where SI is the optical staining intensity (graded as 0, no staining; 1, weak staining; 2, moderate staining and 3, strong straining) and PP the percentage of positively stained cells. The PP was estimated by counting approx. 100 cells and it was defined as 0, no staining; 1, <10% staining; 2, 11-50% staining; 3,51-80% staining and 4, >80% staining. The Mann-Whitney rank-sum test was used to compare the means of the different IRS scores (27, 28).

Results

Only a faint expression of SLea was observed in normal squamous epithelial tissue (Figure 1). Expression of SLea

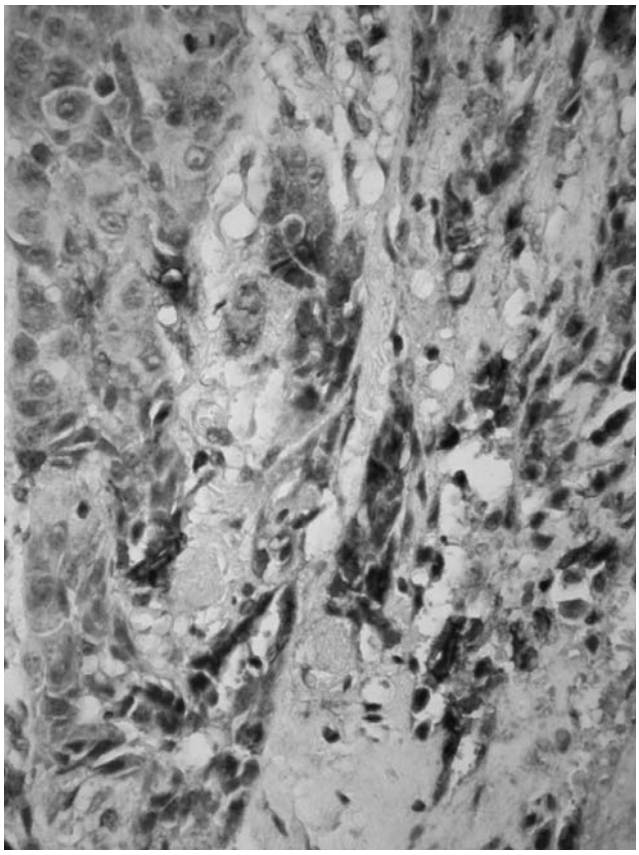

Figure 3. *Up-regulation of SLea in squamous cell carcinoma tissue, ×40.*

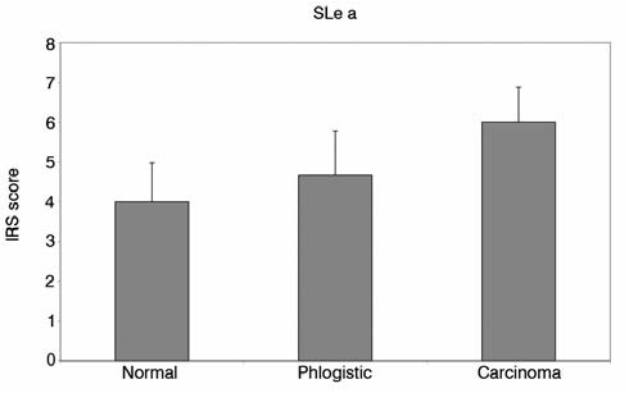

Figure 4. *Summary of staining results of SLea in normal, phlogistic and carcinoma squamous epithelial tissue.*

was elevated in phlogistic tissue (Figure 2). A significant upregulation was found in squamous cell carcinoma tissue (Figure 3, $p<0.05$).

A summary of the staining results is shown in Figure 4.

Discussion

The results obtained in this study showed that SLea (Ca 19-9) is only faintly expressed in normal laryngeal tissue. A moderate up-regulation was found in phlogistic tissue and a dramatic up-regulation was shown in some types of squamous cell carcinoma of the larynx.

Sialyl Lewis antigens play a major role as ligands on tumor cells that are involved in interactions between E-selectin on endothelial cells. This interaction is the first step of building metastases by floating tumor cells sticking to the endothelium (29-31). Malignant transformation is associated with abnormal glycosylation, resulting in synthesis and expression of altered carbohydrate determinants including SLea and SLeX. These determinants appear in sera of patients with cancer, and are extensively utilized for serum diagnosis of cancer in Japan. SLea and SLex are involved in selectin-mediated adhesion of cancer cells to vascular endothelium, and these determinants are thought to be closely associated with hematogenous metastasis of cancer (30).

Selectins are glycoproteins bearing a lectin domain that is specifically recognized by the tetrasaccharides SLeX or SLea (32). Depending on individual conditions, selectins are permanently or temporarily expressed on leucocytes (L-selectin) (33), platelets (P-selectin) (34)and activated endothelial cells (E-selectin, P-selectin) (35). They are involved in initial steps of the attachment of circulating cells to activated vascular endothelial cells (36). Blocking the lectin domain by exogenous probes could offer a way to interfere pathological processes caused by the adhesion of circulating cells from the blood stream to vessel walls (37). Efficient blocking probes should bear the minimum recognized tetrasaccharide structure SLeX or SLea in a pattern that fits to the cellular accommodation of selectins (38). This pattern is unknown. Expression of SLeX or SLea is not compelling connected to an inhibitory function of the probe. Moreover, a multivalent binding of the probe to the receptor pattern is necessary to reach high efficacy, because of the low affinity of a single carbohydrate–lectin interaction (39).

In addition, in previous studies we have shown that sialyl Lewis carbohydrate antigens and amniotic fluid glycoproteins containing SLeX modulate the endocrine function of trophoblasts in culture by up-regulating progesterone production (40, 41).

There is a focus on Lewis a and Lewis b antigens as they are the only two components of the Lewis blood group system. But fucosylation of these antigens produces Lewis isomers which include Lewis Y (LeY). Numerous α-(1, 3)-fucosyltransferase genes have been characterized that can perform this function (42). This oligosaccharide structures

may play a key role in lymphocyte traffic and inflammatory responses (43, 44). The Lewis Y (LeY) antigen, which is one of the Type 2 human blood group-related antigens, is also thought to behave as an onco-developmental cancer-associated antigen (45).

In this study we found a very faint expression of SLea (Ca19-9) in normal laryngeal tissue, a moderate upregulation in phlogistic tissue and a dramatic upregulation in some types of squamous cell carcinoma of the larynx. The results indicate that SLea (Ca19-9) may be a potential tumor marker in carcinoma of the larynx.

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