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EVALUATION OF TYPE IV COLLAGEN EXPRESSION IN SQUAMOUS CELL CARCINOMA OF THE LARYNX

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Abstract: *The aim of this study was an immunohistochemical assessment of the basement membrane antigen collagen IV (Col IV) in squamous cell carcinomas of the larynx and their respective nodal metastases. Forty radical laryngectomy specimens from 2009 to 2011 were randomly selected for this study. Tumor blocks were immunostained using the monoclonal antibody CIV 22 (Dako, Denmark). Col IV was evaluated using a semiquantitative morphometric method and graded according to its quantity and continuity. Col IV expression and epithelium-stroma interface (TESI) showed high significant differences between surface and deepness of larynx tumors. As for TESI, there was a strong relation between tumors with and without lymph node metastases. There was no correlation between desmoplasia and TESI disarrangement, but the latter had a high significant relation with histological grade. Col IV expression was independent of histological grading of the tumor. The results of the present study show an alterated distribution of collagen type IV with a significant loss in all cases of laryngeal cancer, reflecting tumor aggressiveness.*

Key words: *laryngeal cancer, collagen IV, immunohistochemistry, metastases, prognosis.*

1. Introduction

Squamous cell carcinoma (SCC) is one of the most common malignant cancers of the larynx. Despite improved diagnostic and therapeutic methods over the 20 last years, this tumor is still characterized by a high rate of mortality [5].

Carcinomas are characterized by invasion of malignant cells into the underlying connective tissue and migration of malignant cells to form metastases at distant sites. These processes require alterations in cell-cell and cell-extracellular matrix interactions [12]. Loss of basement membrane has been associated with many types of carcinomas [9].

Basement membranes are complex structures composed of collagen, glycoproteins, and proteoglycans. Four major molecules are present in most basement membranes: collagen (type IV), laminin, perlecan, and entactin [8]. Among them, type IV is important as a structural backbone of the basement membrane [14].

Breakdown of basement membrane by tumor cells has been related to their invasive potential, and used as an aid in the early diagnosis and prediction of the biological behavior in various tumors [1], [3], [6], [10], [11], [13], [15].

The aim of this study was to investigate type IV collagen expression at the

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basement membrane in the tumor tissue of laryngeal cancer by immunohistochemical staining. The degree of expression was correlated with histological grade, tumor epithelium – stroma interface disarrangement, and stromal desmoplasia.

2. Material and Methods

In this retrospective study, we investigated 40 samples with primary and metastatic laryngeal cancer randomly selected from the Clinical Counting Hospital Braşov between 2009 and 2011. All tumor specimens were routinely fixed in 10% formalin, and paraffin embedded. The tumor was examined microscopically to assess its histological type and grade. Grading was done according to the World Health Organization (WHO) grading system [16].

Immunohistochemical staining using the standard avidin–biotin–peroxidase complex (ABC) technique was performed. Tumor-tissue nodal sections (4 μm) were deparaffinized and stained with the type IV collagen monoclonal antibody CIV 22 (DAKO, Denmark). The dark brown immunostaining was developed with 3,3′-diaminobenzidine chromagen. Slides were then counterstained with hematoxylin-eosin, dehydrated and mounted.

Collagen IV was evaluated using a semiquantitative morphometric, relatively subjective method, as described by Hewitt *et al.* [4]. Collagen IV (Col IV) expression was graded according to its quantity and continuity, both at the tumor surface (S) and invasive front (F). Three grade of expression were obtained. In grade 1 Col IV was present, without discontinuities around tumor nests; in grade 3 Col IV was present in small quantities and discontinuous, often absent, and grade 2 was attributed to intermediary aspects. Col IV was assessed in 5 microscopic fields for each case (x200). In the same areas, tumor epithelium – stroma interface (TESI) disarrangement degree was evaluated.

In the present study, specimens were classified as group A+B (all laryngeal cancers), group A (primary laryngeal cancers with negative lymph node metastases), group B (primary laryngeal cancers with positive lymph node metastases), and group B-LN (matched lymph node metastases).

Data were analyzed by StatSoftInc 4.3 software. The Pearson correlation was used to assess the relationship between two continuous variables. Mean differences between counts were compared with the use of *t* tests; the calculated p-value below 0.05 was considered statistical significant.

3. Results and Discussions

Col IV expression around tumor nests and at the invasive front is illustrative in figures 1 and 2.

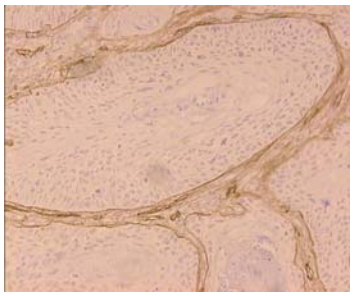

Fig. 1. *Col IV expression in a well differentiated SCC (Col IV,20x).*

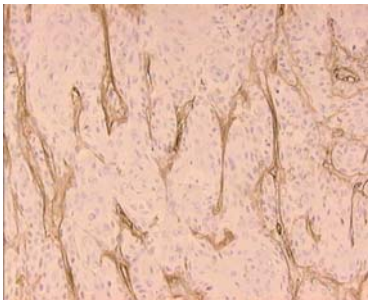

Fig. 2. *Col IV expression in a medium differentiated SCC (ColIV, 20x).*

In the present study, collagen IV was considered an indicator of basement membrane disruption.

Col IV expression (quantity and continuity) showed high significant differences between tumor surface and invasive front (Table 1), reflecting an increase of tumor anaplasia and, in this way, tumor progression.

Table 1

Col IV expression (mean number, SD) at the tumor surface (S) and invasive front (F) in all tumors (A+B).

Col IV	**Group (A+B)**			No.
	Mean±SD	t test	p	
S F	1.17±0.44 2.10±0.74	-8.92	0.00	40

Larynx SCC distribution (mean number and standard deviation) according to collagen IV expression (graded from 1 to 3) at the surface and profoundness of the tumors showed a high significant correlation (Pearson şi M-L Chi-square) (Table 2 and 3).

Table 2

Col IV expression at the surface (S) and invasive front (F) of the tumors

Col IV S	**Col IV F**			Total
	1	2	3	
1	9	18	7	34
2	0	0	5	5
3	0	0	1	1
Total	9	18	13	40

Table 3

Col IV S versus F

Col IV S vs. F	Chi-square	df	p
Pearson Chi-square	14.66	4	0.005
M-L Chi-square	15.87	4	0.003

The epithelium – stroma interface (TESI), in which epithelium is represented by tumor nests, and stroma by stromal desmoplastic reaction, showed no significant differences betweeen the surface tumor and invasive front (Table 4).

Table 4

The epithelium-stroma interface (TESI) at the surface tumor (S) and invasion front (F).

	Group (A+B)			No.
	Mean ±SD	t test	p	
TESI S	1.12±0.40			
TESI F	2.15±0.92	-7.27	0.19	40

In lymph node metastases, there is no relation between Col IV expression in the center and at the periphery of metastasis (Table 5).

Table 5

Col IV expression in the center (C) and at the periphery (P) of the metastasis

COL IV	**Group B-LN**			No.
	Mean ±SD	t test	p	
C	1.30±0.57			
P	2.55±0.69	-8.75	0.18	20

Even so, metastases show high significant correlations (Table 6) (M-L Chi-square) in respect of epithelium-stroma interface of tumor nests versus tumor periphery.

Table 6

The epithelium–stroma interface (TESI) at the center (C) and periphery (P) of metastases.

TESI	**Group B-LN**			No.
	Mean ±SD	t test	p	
C	1.50±0.68			
P	2.55±0.68	-6.84	0.000 002	20

Col IV expression was strongly related to epithelium-stroma interface from the surface to the depth of the tumor (Table 7 and 8).

Table 7

Correlation between col IV expression and epithelium-stroma interface at tumor periphery (S)

Col IV S	**TESI S**			Total
	1	**2**	**3**	
1	33	1	0	34
2	3	1	1	5
3	0	1	0	1
Total	36	3	1	40

Table 8

Col IV S vs.*TESI S*

Col IVS / TESI S	Chi-square	df	p
Pearson Chi-square	21.98	4	0.0002
M-LChi-square	11.97	4	0.017

Our results showed that there was a strong relation between tumors with and without lymph node metastases, suggesting the metastastic potential of larynx tumors. Tumors with metastases showed more often grade 3 of TESI disarrangement at the invasive front and grade 2 of collagen IV expression.

There was no relation between stromal desmoplasia and histological grade of laryngeal tumors according to the WHO system. Correlation between epithelium-stroma interface and histological grade was strong, unlike col IV expression at the invasion front, independent of grade (Table 9).

Table 9

Correlation between stromal desmoplasia, Col IV expression, epithelium-stroma interface at the invasive front and grade of histologic differentiation

Desmoplasia /G OMS	Chi-square	df	p
Pearson Chi-square	2.05	4	0.72
M-L Chi-square	2.04	4	0.72
Col IV F / G OMS	Chi-square	df	p
Pearson Chi-square	2.44	4	0.65
M-L Chi-square	2.60	4	0.62
TESI F / G OMS	Chi-square	df	p
Pearson Chi-square	10.22	4	0.03
M-L Chi-square	12.20	4	0.01

There was no correlation between desmoplasia and TESI disarrangement, but the latter had a high significant relation with histological grade.

In our study, col IV expression was independent of histological grading of the tumor, in disagreement with Krecicki *et al*, who found a significant correlation between the two parameters [7] and suggested that collagen IV pattern in the basement membrane surrounding nests of carcinoma is an important prognostic factor [2], [7].

4. Conclusions

Type IV collagen is an indicator of basement membranes disruption and therefore an indicator of invasion and metastasis. Likewise, disarrangement of the area surrounding tumor nests, named "tumor epithelium-desmoplastic stroma interface (TESI)" is an important marker of tumor invasiveness. These two parameters increase in parallel with tumor profoundness, suggesting an increase of tumor anaplasia and progression. Contrary, stromal desmoplasia is a barrier for metastasis.

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