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Research Article

Prognostic Value of CD10 among Tunisian Patients with Nasopharyngeal Carcinoma

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Abstract

Introduction: CD10 expression was identified as a marker of poor prognosis in several types of cancer. However, its impact on the survival of Tunisian NPC patients has not been discussed. So, our objective was to confirm the prognostic value of this marker, in addition to its relationship to clinicopathologic parameters.

Materials and Methods: Imunohistochemistry method was performed on formalin-fixed paraffin-embedded sections of 66 cases of NPC, 6 patients with inflammatory nasopharynx and 20 normal mucosa tissues.

Results: CD10 expression was detected in 66.66 % of the NPC with predominant staining in stromal cells (81.81%). While, no expression of CD10 was noted in control group (inflammatory nasopharyngeal lesions and normal nasopharynx mucosa).

CD10 positive cases were correlated with increasing tumor size (p=0.001) and lymph node metastasis (p=0.034). Furthermore, with the Kaplan-Meier test, a strong association was observed between the expression of CD10 and the recurrence status (p = 0.003). Multivariate Cox proportional hazard regression analysis showed that CD10 and lymph node metastasis factors were independent predictors of time to recurrence among NPC patients with respectively p=0.001 and p=0.044.

Conclusion: The present study confirms the prognostic value of CD10 expression and suggests its strong effect on aggressive behavior of nasopharyngeal carcinoma.

Keywords: CD10; Immunohistochemistry; Nasopharyngeal carcinoma (NPC); Survival

Introduction

Nasopharyngeal Carcinoma (NPC) is a rare pathology, which is endemic in southeastern China and southeastern Asia with an annual incidence rate of approximately 20 per 100,000 people [1]. Although NPC is not as frequent in North Africa as it is in Asia, it remains the most frequent head and neck cancer in Tunisia, Algeria, and Morocco [2]. Despite significant radiosensitivity and chemosensitivity compared to other head and neck cancers, metastatic development of nasopharyngeal cancers (upper cervical lymphadenopathy) is common. Due to the anatomical situation of the nasopharyngeal cancer, tumor development can be asymptomatic for a long time. This often makes the diagnosis late and the prognosis relatively unfavorable.

EBV is one of the etiologic factors of NPC, particularly the undifferentiated form (UCNT) [3,4]. As previously described, EBV DNA has been detected in plasma, serum [5-7] or unfractionated whole blood [8-10] of NPC patients and suggested to be a sensitive and specific molecular marker for both diagnosis and prognosis. Until now, EBV remains the only biomarker used for clinical NPC patients follow-up. So the identification of new markers could be helpful for better diagnosis and prognosis. CD10 is the marker chosen for this study. In fact, it is a 90-110 kDa cell surface zinc-dependent metallo-protease, which possesses a well-defined enzymatic activity. It has also been called neutral endopeptidase and Common Acute Lymphoblastic Leukemia Antigen (CALLA) [10]. Expression of CD10 was observed on some normal and neoplastic hematopoietic, lymphoid and stromal epithelial cells [11,12]. It was detected in stroma of various malignancies including gastric, lung, breast, prostate and colorectal carcinomas [12-15]. On nasopharyngeal carcinoma, the only one study concerning CD10 expression published by Braham et al. [16] demonstrated the correlation of CD10 with tumor progression. We assumed that this result is not conclusive on the prognostic value of this marker. So, we proposed in the present study, to evaluate the association of CD10 expression and the survival of the NPC patients and to confirm the clinical impact of this marker.

Materials and Methods

Clinical and pathological data

This study was performed at Immuno Histo Cytology department in the Salah Azaiez Cancer Institute. The biopsy specimens were taken from 66 patients with Nasopharyngeal Carcinoma (NPC), diagnosed between 2008 and 2011. They were represented by 49 males and 17 females with age ranging from 13 to 79 years. As control group, we also included 6 cases with inflammatory nasopharyngeal lesions, and 20 samples from patients with normal nasopharynx mucosa.

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Immunohistochemical Staining for CD10

We used 5µm slices for the immunohistochemical staining. The sections were deparaffinized in xylene, dehydrated in alcohol and washed in Phosphate Buffered Saline (PBS) and antigenic retrieval was done by incubation with 1% citrate buffer (pH=6) in a microwave oven for 40min . The endogenous peroxidase activity was blocked using 3% H_2O_2 for 5 minutes. Thereafter, the sections were incubated with primary anti-CD10 monoclonal antibody ($56C_6$, Novo Castra, Newcastle, United Kingdom) as primary antibody for 60 minutes followed by incubation with post primary block for 30 minutes. Then, the sections were incubated with Novo Link polymer anti mouse/rabbit IgG-Poly-HRP for 30 minutes. Between each step, the sections were washed twice in Tris Buffer Saline (TBS). Sections were further incubated with the substrate, 3,3'-diaminobenzidine (DAB) chromogen prepared according to the manufacturer's recommendations. Reactions with peroxidase produced a visible brown precipitate at the antigen site. Sections were counterstained with hematoxylin, rinsed in running tap water, dehydrated in ethanol (70%, 90%, and 100%, consecutively) and cleared with xylene.

Evaluation of immunostaining

The degree of staining was determined by two independent observers. CD10 was expressed on the apical membrane or in the cytoplasm. CD10 expression was highlighted in stromal lymphocytes. Staining was scored semi-quantitatively as negative (0), (no staining), and positive if more than 10% of the cells exhibited CD10 immunostaining. Positive immunostaining was categorized as weak (if less than 30% of the cells were positive), and as strong (when staining was more than 30% of the studied cells).

Statistical analysis

Data analyses were performed using the SPSS Statistics 20 software package.

Associations between the biomarker (CD10) and clinico pathological features of NPC patients and the survival or recurrence status, were analyzed using the Chi-square or Fisher's exact test.

Association between the time to recurrence and stromal CD10 expression was performed using the Kaplan-Meier test. The log-rank test was used to evaluate statistical significance of the differences between the curves.

Cox proportional hazards regression model was used for multivariate analysis.

P-values <0.05 were considered as being statistically significant.

Results

Expression of CD10 was determined by immunohistochemistry and results were shown in the Table 1.

CD10 expression was highlighted in stromal lymphocytes cells (Figure 1).

CD10 immunostaining was observed in 44 of 66 NPC cases (66.66 %). Among them 20 cases (45.45%) showed weak immunoreactivity and 24 cases (54.54 %) showed strong immunoreactivity. while, no staining of CD10 was noted in the control group (inflammatory nasopharyngeal lesions and normal nasopharynx mucosa).

Table 1: Correlation between CD10 expression and clinico pathological parameters/survival of NPC.

Parameters	CD10 expression		p value
	Positive	Negative	
All (n=66)	44 (66.66%)	22 (33.33%)	
Age (years)			
>30	37 (56.06%)	21 (31.81%)	0.37
<30	7 (10.60%)	1 (1.52%)	
Gender			
Female	11 (16.66%)	5 (7.58%)	0.83
Male	33 (50%)	17 (25.76%)	
Tumor size			
T1+T2	7 (10.60%)	17 (25.76%)	0.001
T3+T4	37 (56.06%)	5 (7.58%)	
Lymphe node Metastasis			
N0+N1	14(21.21%)	13(19.70%)	0.034
N2+N3	30(45.45%)	9 (13.64%)	
Recurrence			
No	11 (16.66%)	22 (33.33%)	0.002
Yes	27 (40.91%)	6 (9.09%)	
Survival status			
Alive	39 (59.09%)	19(28.79%)	0.75
Dead	5 (7.58%)	3(4.54%)	

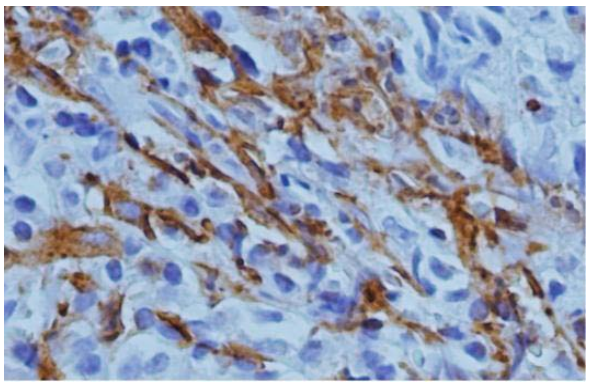

Figure 1: CD10 expression in stromal fusiform cells (immunohistochemistry for CD10 antibody, original magnification X 40).

Correlation of CD10 expression with clinicopathological features of NPC patients

CD10 expression was more frequently detected in high tumor size (37 cases/56.06%) than in low tumor size (7 cases/10.60%) with a significant difference (p=0.001).

In terms of overall lymph node status, a significant correlation was observed between CD10 expression and the presence of metastatic lymph node (p=0.034). But, no significant difference was noted according to age and gender (Table 1).

Prognostic significance of CD10 expression

A significant association was observed between the recurrence

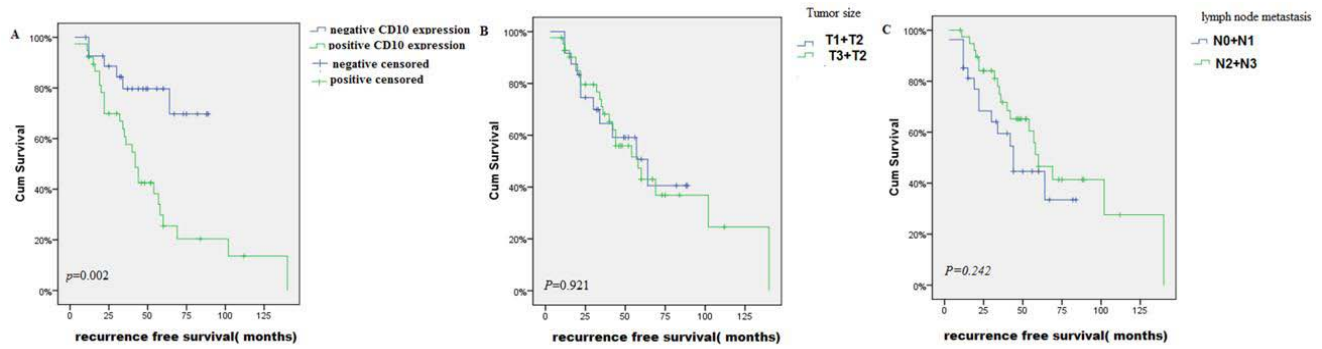

Figure 2: Kaplan-Meier univariate survival curves of patients with NPC and different clinicopathological characteristics. A: Stromal CD10 expression and time to recurrence (positive *vs.* negative), B: Tumor size (T1+T2 *vs.* T3+T4), C: Lymph node metastasis (N0+N1 *vs.* N2+N3).

Table 2: Multivariate Cox regression analysis for prognosis of NPC patients.

	B	SE	Wald	Sig	Exp(B)	95.0% CI for Exp (B)	
						Lower	Upper
CD10	1.49	0.465	10.34	0.001	4.46	1.794	11.1
Lymph node metastasis	-0.744	0.37	4.052	0.044	0.475	0.23	0.981

status and time of recurrence (Fig) with expression of CD10 (p=0.003). Recurrence was observed in 27 of 44 (61.36%) patients with positive CD10 expression and in 6 of 22 (27.27%) patients with negative CD10 expression. Furthermore, the odds of recurrence were estimated to be 9 times higher (odds ratio = 9, 95% CI: 2.89.28.22) for NPC patients with positive CD10 than for those not expressing CD10. Positive CD10 expression was also associated with shorter time to recurrence (54.83 months) (95% CI 39.55 to 70.12) than the negative CD10 expression which recurrence time was 71.46 months (95% CI 60.07 to 82.86) (Figure 2).

On the other hand, there was no significant difference of CD10 expression with overall survival rates of NPC patients. We also, plotted the survival curve for NPC patients with clinical parameters, which were correlated with CD10 expression including Tumor size and lymph node metastasis. As shown in Figure 2, NPC patients with advanced lymph node metastasis (N2 and N3) had shorter time of recurrence than that patient with early stage (N0 and N1). But the difference is not statistically significant (p=0.242) On the other hand, tumor size has no influence on survival or risk of recurrence.

Multivariate Cox proportional hazard regression analysis showed that CD10 and lymph node metastasis factors were independent predictors of time to recurrence among NPC patients with respectively p=0.001 and p=0.044. Furthermore, positive expression of CD10 showed the significantly higher risk assessment of Exp (B) = 4.46 which was compared with lymph node metastasis status (Table 2).

Discussion

This study was designed to explore the prognostic impact of CD10 in nasopharyngeal carcinoma among Tunisian patients. Several studies have assessed the CD10 expression in several types of cancers [13,17,18]. According to many findings [14,18,19], CD10 plays an important role in tumor progression by degrading the extracellular matrix and promoting the remodalization of the stroma. Furthermore, the CD10 expression was noted mainly in stromal cells and was considered probably as a prognostic parameter in breast [19,20] prostate [21,22] lung [23] and colorectal cancer [24]. In oral cancer and particularly in nasopharyngeal carcinoma, the CD 10 prognostic value was not assessed until now in the literature. Therefore, our findings were compared to the only study conducted by Braham et al concerning CD 10 expression in nasopharyngeal expression in nasopharyngeal carcinoma [16].

In this study, we used immunohistochemical staining to investigate the expression of CD10 in NPC patients. Stromal CD10 expression was predominant in our NPC cases (36 / 44 positive cases; 81.81%). The positive CD10 expression was correlated with increasing tumor size (p=0.001) and lymph node metastasis (p=0.034). Braham et al. [16] revealed expression of CD10 in stromal cells in 22 of 47 NPC cases (46.8%), and a significant correlation of CD10 expression with advanced clinical stage (p=0.04) and in patients older than 25 years (p=0.05). According to their findings, there is no significant difference between CD10 expression and tumor size and lymph node metastasis. The difference between our results and Braham's results may be due to their small number of NPC.

In terms of the significance of the correlation of CD10 expression with the tumor size and lymph node metastasis, our results are in agreement with several studies conducted in other cancers such as colorectal showing a correlation with the tumor size only [15] and in gastric, pancreatic and oral cancers showing a correlation with only lymph node metastasis [14,29,30]. However, in breast cancer, many studies demonstrated a strong correlation of CD10 staining with both tumor size and lymph node metastasis [18,19,25-28] suggesting that CD10 expression in these tumors could be considered as a marker that facilitates tumor invasion and tumor progression by degrading extracellular matrix proteins.

By contrast with our findings, conflicting data were reported by other studies, showing that no statistically significant difference was detected between CD10 expression and any clinical parameters in

lung cancer [23,24,31].

Furthermore, we demonstrated that CD10 expression is predictor of NPC recurrence. Indeed, positive CD10 staining is associated with short time to recurrence (p=0.003), conferring a high risk of recurrence in NPC patients. This information was supported by Cox regression analysis that proved that CD10 was an independent factor-influencing patient's prognosis added to lymph node metastasis risk to recurrence.

The prognostic value of CD10 has been mentioned in several studies including different types of cancer. Some of them are consistent with our findings and have shown a positive significance between CD10 expression and the time to recurrence in breast cancer [18,19,20], prostate cancer [21,22], lung cancer [23,31], pancreatic cancer [29], oral cavity squamous cell carcinoma [30] and renal carcinoma [32] but, other studies reported that patients whose CD10 is positive have higher 5-year survival than those having no CD10 staining [33,34]. According to our results, supported by many studies, we can suggest that stromal CD10 expression is a new marker of invasion, metastasis and increased risk for NPC recurrence.

The exact role of CD10 in carcinogenesis is currently unknown. CD10 appears to have opposite enzymatic functions. In addition to its proteolytic activity, Sumitomo et al., have suggested an inhibitory role of CD10 in the migration of prostate cancer cells through a non-enzymatic protein-protein interaction [35].

Several studies have shown that CD10 levels are influenced by several factors. Some cytokines such as interleukin-1α, tumor necrosis factor and interleukin-6 and granulocytic macrophage colony stimulating factor increase CD10 in the inflammatory process [36,37]. On the other hand, transforming growth factor-β1 could decrease CD10 activity by reducing gene transcription or mRNA stability [38]. In addition, CD10 activity can be regulated by prostaglandin synthesis and Alpha-methyl-p-tyrosine (AMPT) [37]. These factors secreted by tumor cells can stimulate or inhibit stromal CD10 expression. It can be speculated that differences in the combination of these factors secreted by different types of tumors could contribute to the variable expression and activity of CD10 in different tumors. Further studies, on molecular basis of CD10 expression on stromal and carcinomatous cell interaction are necessary to confirm the specific role of CD10 as a predictor for adjuvant therapy and new therapeutic strategies in NPC.

Conclusion

Finally, our results in parallel with those by other investigators confirm the value of CD10 as a pronostic marker and as a risk factor for tumor cell invasion. This finding open new horizons for therapeutic strategies in future. Treatment targeted to decrease the role of CD10 positive stromal component in aggressive behavior of nasopharyngeal carcinoma may be promising in this regard.

Authors' Contribution

Mokni-Baizig N: 1st responsible for this study and drafting of the manuscript; Mhamdi H: Technical manipulation (Immunohistochemical test); El Amine El Hadj O: slide reading and assessment of CD10 staining scores; Goucha A: Slide reading and assessment of CD10 staining scores; Hsairi M: Statistical interpretation of data; Touati S: Clinical data. The manuscript has been read and approved by all the authors.

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