EpCAM (MOC-31) – immunohistochemical expression in papillary thyroid carcinoma and non invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

Etude comparative de l'expression du MOC31 dans les carcinomes papillaires de la thyroïde et dans les néoplasmes vésiculaires non invasifs avec atypies papillaires

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Abstract

Introduction : Ep-CAM, is a cell adhesion glycoprotein located on the basolateral cell membrane surface and in the cytoplasm of most normal epithelial cells. It has also been described to be expressed in several malignancies such as lung, digestive, prostate and renal carcinomas suggesting it has a potential role in carcinogenesis. In thyroid carcinoma, Ep-CAM expression has rarely been studied especially in papillary thyroid carcinoma. **Objective:** We sought to describe and compare the immunohistochemical expression of MOC31 in papillary thyroid carcinoma and in non invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).

Methods: We have retrospectively collected 33 cases of PTC diagnosed in the pathology department of the Security forces hospital during a period of 13 years (2008–2021). We have microscopically reviewed all cases and reclassified 9 of 33 cases as NIFTP. An immunohistochemical automated study have been performed with MOC-31 antibody. The immunostaining was considered positive when it was membranous and/or cytoplasmic. The intensity of staining was scored as weak (score 1), moderate (score 2), and strong (score 3). We have used an immunoscore for assessing level of expression of MOC31 as follows: 0 for <5% of positive cells, 1 for 5-30%, 2 for 31-50%, 3 for 51-70%. The total score resulted by summing the percentage score with the intensity score; the final score was varying from 0 to 7, considered low between 1-4 and high 5–7. **Results:** The mean age of patients was 45,2 years-old for PTC cases and 48,1 years-old for NIFTP cases. A net female predominance was found in both groups (male to female ratio of respectively 0,4 and 0,3). MOC31 expression was found in 19 cases of PTC with a percentage of positive cells varying from 5 to 90%. Percentage of positive cells was variable from 5 to 90%. The immunoscore for positive cells was: 0 in 5/24cases, 1 in 4/24cases, 3 in 9/24cases and 4 in 6/24cases. The intensity of staining was assessed score2 (moderate) in 8 cases and score 3 (high) in 7cases (Figure1-2). Final MOC31 staining score was low in 37,5% (9/24) and high in 62.5% (15/24). Patients with advanced pt2-pt3 stages mostly showed high score of MOC31 staining (61,5%).One case was associated with lymph node involvement and was of a high score. 6 cases showed vascular invasion and was of high MOC31 score. MOC31 was expressed in all NIFTP cases with variable proportion of positive cells (5%-80%). The immunoscore for positive cells was: 0 in 1/9cases, 1 in 2/9cases, 2 in 3/9cases, 3 in 1/9cases and 4 in 2/9cases. The intensity of staining was assessed score 1 (weak) in one case, score 2 (moderate) in 6 cases and score 3 (high) in one case (Figure3-4). The final combined score was low in 66,7 (6/9) and high in 33,3% (3/9).

Conclusion: Our study revealed different immunohistochemical profile of MOC31 in benign and malignant tumors. It has somewhat a diffuse and marked staining in the first group. The changes of MOC31 location as well as its score of staining in PTC and NIFTP could hence be helpful in the differential diagnosis. Our findings also support the potential prognostic value of this molecule that deserves further investigations.

Keywords: ,Ep-CAM, papillary, thyroid, NIFTP.

Résumé

Introduction : EpCAM est une glycoprotéine membranaire présente à la surface des cellules épithéliales normales et exprimée par de nombreux carcinomes. Des profils d'expression variable de ce marqueur ont été rapportés dans de nombreux cancers notamment du poumon, du tube digestif et de la prostate suggérant son rôle potentiel dans le processus de carcinogenèse. Très peu d'études ont cependant analysé l'expression de ce marqueur dans le cancer de la thyroïde en particulier de type carcinome papillaire (CPT).

Objectif : Etablir et comparer le profil d'expression du MOC31 dans les CPT et dans les néoplasmes vésiculaires non invasifs avec atypies papillaires (NIFTP).

Méthodes : Il s'agit d'une étude rétrospective, descriptive portant sur 33cas de CPT, colligés au service d'anatomie cytologie pathologique de l'hôpital des Forces de Sécurité Intérieure (2008-2021). Une relecture des lames a été effectuée avec reclassification de 9cas en NIFTP et 24cas en CP selon la dernière édition de l'OMS. Une étude immunohistochimique automatisée a été réalisée à l'aide de l'anticorps EpCAM (MOC31, Leica). L'immunomarquage était considéré positif en présence d'une positivité membranaire et cytoplasmique. Le score d'intensité a été évalué en faible (score1), modéré (score2), intense (score3). Le pourcentage de cellules positives était évalué de manière semi-quantitative avec attribution d'un score de pourcentage : <5% de cellules+(score 0), 5-30% de cellules+(score1), 31-50% de cellules positives(score2), 51-70% (score3) et >70%(score4). Un immunoscore (IS) global a été attribué en combinant le score d'intensité et le score de pourcentage, variable de 0 à 7 et subdivisé : IS faible entre 1 et 4 et élevé entre 5 et 7.

Résultats : L'âge moyen des patients était de 45,17ans pour les CP et de 48,1ans pour le NIFTP. Une prédominance féminine était observée dans les deux groupes avec (ratio H/F respectivement 0,4 et 0,3). L'expression du MOC31 était observée dans 19 cas de CPT avec un pourcentage de positivité variable de 5 à 90%. Le score de positivité était : 0 dans 5/24cas,1 dans 4/24cas,3 dans 9/24 cas and 4 dans 6/24 cas. Dans 79,9% des cas, le pourcentage de cellules positives était >50%. L'intensité du marquage était de score 2 dans 8cas et de score 3 dans 7 cas. L'IS global était faible dans 37,5% des cas et élevé dans 62,5% des cas. Les cas de CPT de stade avancé pt2-pt3 avait un MOC31-IS élevé dans 61,5% des cas. Des métastases ganglionnaires étaient présentes dans un cas correspondant à un IS élevé. Des emboles vasculaires étaient présentes dans 6cas correspondant à un IS élevé. L'expression du MOC31 a été observée dans tous les cas de NIFTP avec un score de positivité à :0 (1/9),1 (2/9),2 (3/9) et 3 (1/9). Le score d'intensité était considéré faible (score1) dans 1cas, modéré (score 2) dans 6 cas et élevé (score 3) dans 1 cas. Concernant les cas de NIFTP, une expression du MOC31 était observée dans tous les cas. L'IS combiné était faible dans 66,7% des cas et élevé dans 33,3% des cas.

Conclusion : Notre étude montre une variabilité d'expression du MOC31 entre les CPT et les NIFTP avec un marquage plus étendu et d'intensité plus marquée dans le premier groupe. Cet anticorps pourrait être ainsi utile pour le diagnostic différentiel dans les cas équivoques. Il serait également judicieux d'analyser les modifications du profil d'expression de ce marqueur dans les CPT en fonction des facteurs histo-pronostiques afin d'établir son valeur pronostique potentielle.

Mots clé : EpCAM, papillaire, thyroide, NIFTP

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INTRODUCTION

MOC-31, also known as Epithelial Specific Antigen/ Ep-CAM, is a cell adhesion glycoprotein located on the basolateral cell membrane surface and in the cytoplasm of most normal epithelial cells. Variable expression levels have been reported in several malignancies such as lung, digestive, prostate and renal carcinomas suggesting it has a potential role in carcinogenesis [1,2]. However, Ep-CAM has rarely been studied in thyroid carcinoma and very few data are available concerning its utility in papillary thyroid carcinoma (PTC). In a recent published study, Andirescu et al [3] reported a significant prognostic role of MOC31 score of staining as a useful tool for the identification of high-risk PT with unfavorable clinical outcome.

In this retrospective study, we sought to describe and compare the immunohistochemical expression of MOC31 in papillary thyroid carcinoma and in non invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).

METHODS

We have retrospectively collected 33 cases of PTC diagnosed in the pathology department of the Security forces hospital during a period of 13 years (2008–2021). The clinical data: age, gender and site were extracted from the patient's medical record. We have collected the histological characteristics: tumor size, encapsulation, invasion, histological variant, vascular invasion, lymph node metastasis from the pathological report exam. We have reviewed all the hematoxylin-eosine stained sections and performed a microscopic reassessment of the lesions according to latest *WHO* Classification of the endocrine system. Among these cases, 9 have been reclassified as NIFTP and 24 as invasive PTC. All cases have been tested with MOC-31 antibody (PA0797, Leica, ready to use form), against the extracellular domain (EpEx-MOC-31) of EpCAM molecule. We have performed the immunohistochemical technique with an automated immunostainer (Leica Bond MAX) according to the manufacturer's protocol. Four-micrometer thick sections were made from the formalin-fixed, paraffin embedded blocks, which were then deparaffinized and rehydrated through three different concentrations of alcohol solutions. For antigen retrieval, paraffin tissue sections were cooked for 2–5 min with 10 mM sodium citrate buffer, pH 6.0, at a sub-boiling temperature for 15 min and cooled for 20 min at room temperature. The sections were treated by the through endogenous peroxidase activity with 3% H2O2 for 10mn and then incubated overnight at 4°C with monoclonal primary antibodies diluted in 1% bovine serum albumin. After washing, the primary antibody was detected with the appropriate secondary antibody for 30 minutes at 37°C. Following washes, slides were incubated in the avidin-biotin complex for 20 minutes at 37°C and visualized using diaminobenzidine as substrate. Afterward, the slides were briefly counterstained with Harris hematoxylin, dehydrated and mounted. Positive control was represented by normal thyroid tissue and the staining was assessed by two pathologists, under a multi-view microscope. The immunostaining was considered positive when it was membranous and/or cytoplasmic. Percentage of staining was scored in a semi-quantitative manner by evaluating the proportion of positive tumor cells over total tumor cells. We have used an immunoscore for assessing level of expression of MOC31 as follows: 0 for <5% of positive cells, 1 for 5-30% cells, 2 for 31-50%, 3 for 51-70% of positive cells and 4 for >70%. The intensity of staining was scored in a semi-quantitative manner as weak (score 1), moderate (score 2), and strong (score 3) in comparison to the internal control intensity of staininag. The total score resulted by summing the percentage score with the intensity score; the final score was varying from 0 to 7, considered low between 1-4 and high 5–7.

RESULTS

Clinical and pathological findings

The average age of patients was 45,2 years-old for PTC cases and 48,1 years-old for NIFTP cases. A net female predominance was found in both groups with a male to female ratio of respectively 0,4 and 0,3. For PTC, the tumor was located in the left lobe in 45,8% of cases, in the right lobe in 41,6%, in the isthmus in 8,3% and multifocal in 4,3%. For NIFTP cases, the tumor was left-sided in 66,7% of cases. The mean tumor size was 2,2cm for PTC and 2,4cm for NIFTP.

For PTC, the histological variants were as follows: follicular variant 54,1%, papillary variant 33,3%, hurthle-cell variant in 4,2%, Whartin-like 4,2% and insular 4,2%. Extension to the adjacent thyroid parenchyma was found in 91,7% of cases. The tumor was classified pt1 in 45,8%, pt2 in 25% and pt3 in 29,2%. Vascular invasion was found in 6 cases and lymph node involvement in 2cases.

Immunohistochemical findings

MOC31 is a membranous adhesion molecule and thus should normally show a membranous staining; however cytoplasmic and nuclear staining has also been described in different tumors [4]. There is not either a codified and universal fixed cut-off for MOC31 expression. However, we have used the same criteria as previously reported studies on MOC31 in thyroid cancers [3]. In our study, circumferential membranous and/or cytoplasmic MOC31 staining was found in 19 cases of PTC. Percentage of positive cells was variable from 5 to 90%. The immunoscore for positive cells was: 0 in 5/24cases, 1 in 4/24cases, 3 in 9/24cases and 4 in 6/24cases (Table1).

Table 1. MOC31 score of percentage of positive tumor cells

Score of %	0 (<5%)	1 (5-30%)	2 (31-50%)	3 (51-70%)	4 (>70%)
Number of cases for PTC	5	4	0	9	6
Number of cases for NIFTP	1	2	3	1	2

79,9% of positive cases showed staining in more than 50% of tumor cells. The intensity of staining was assessed score2 (moderate) in 8 cases and score 3 (high) in 7cases (Figure1-2). Final MOC31 staining score was low in 37,5% (9/24) and high in 62.5% (15/24).

Patients with PTC-MOC31+ were mostly female (13/19) with a median age of 45years-old. The tumor in this group was located in the right lobe (8cases), left lobe (9cases) and in the isthmus (2cases). The median tumor size for PTC-MOC31+ was 2,3cm. Among the MOC31 positive PTC cases, 7/19 cases were of conventional papillary subtype, 11/19 cases were of follicular variant and 1 case was hurthle-cell variant. Two cases in our study were classified Whartin-like and insular variants; both cases showed total loss of MOC31 expression. The tumor was staged pt1 in 9cases, pt2 in 4cases and pt3 in 6cases. Patients with advanced pt2-pt3 stages mostly showed high score of MOC31 staining (61,5%). One case was associated with lymph node involvement and was of a high score. 6 cases showed vascular invasion and was of high MOC31 score.

MOC31 was expressed in all NIFTP cases with variable proportion of positive cells (5%-80%). The immunoscore for positive cells was: 0 in 1/9cases, 1 in 2/9cases, 2 in 3/9cases, 3 in 1/9cases and 4 in 2/9cases. The intensity of staining was assessed score 1 (weak) in one case, score 2 (moderate) in 6 cases and score 3 (high) in one case (Figure3-4). The final combined score was low in 66,7 (6/9) and high in 33,3% (3/9).

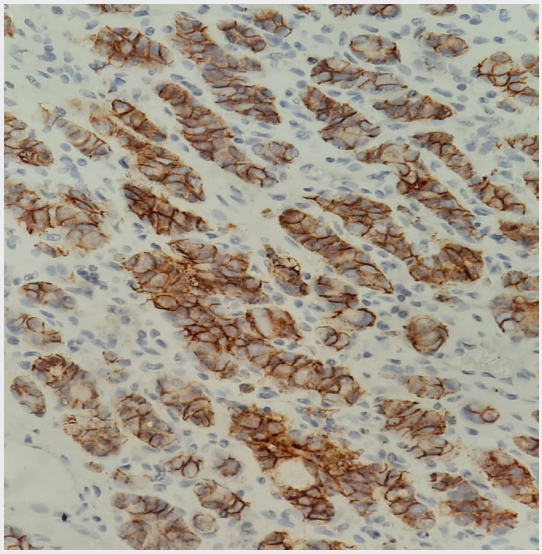

Figure 1. IHC X 40: Score 3 intense and circumferential membranous staining of PTC for MOC31.

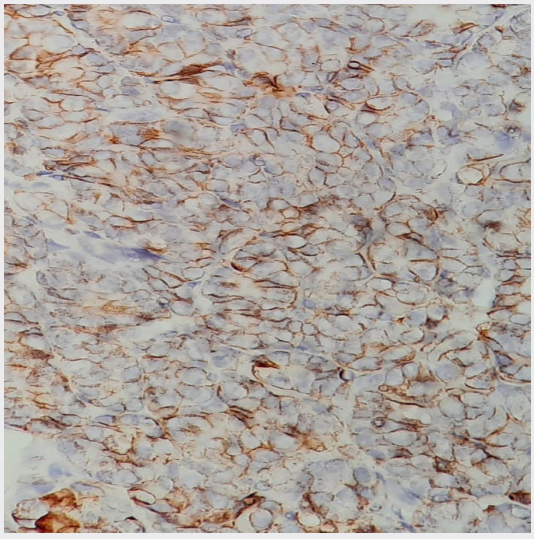

Figure 2. IHC X 40: Score 2 moderate and circumferential membranous staining of PTC for MOC31.

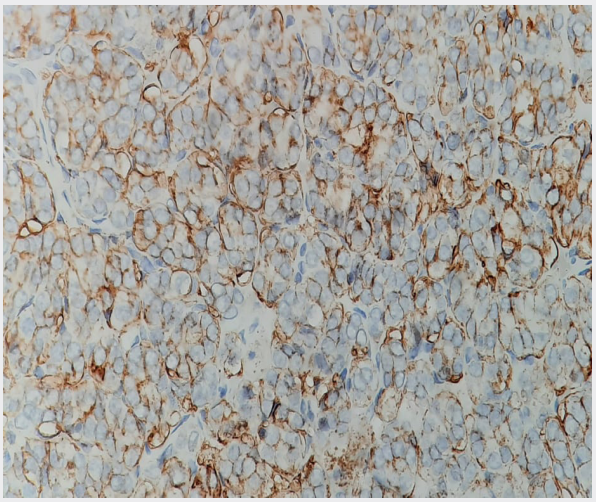

Figure 3. IHC X 40: Score 2 moderate and circumferential membranous staining of NIFTP for MOC31.

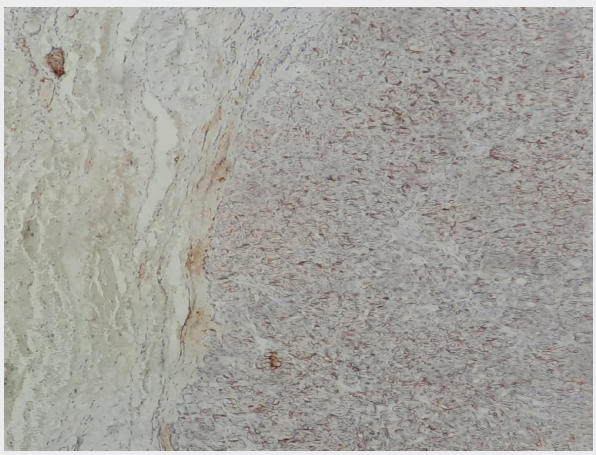

Figure 4. IHC X 20: Score 1 membranous staining of NIFTP for MOC31.

DISCUSSION

Our study showed MOC31 expression in all NIFTP cases and most PTC cases. However, we have found a slightly different immunohistochemical expression profile between these two groups. In fact, the percentage of positive cells was clearly different: in PTC cases, 79,9% of cases showed >50% of positive tumor cells and high score of positivity in 62,5%. In contrast, in NIFT¨P cases, only 33,3% of cases showed >50% of positive tumor cells and high score of positivity in only 3/9cases. The score of intensity was not different between the two groups and was mostly moderate or high. However, the final combined immunoscore was somewhat distinct with a mostly high immunoscore for PTC cases contrasting with a predominantly low score in NIFTP group.

EpCAM is a transmembrane glycoprotein, normally present in most epithelial cells and displays a role in modulating the cadherin-mediated cell adhesion [5]. It has been firstly described in 1970s among the first tumor-associated antigens. Since then, very few papers have focused on its role in the carcinogenesis and mostly supported the involvement of this molecule in cell proliferation and differentiation, migration and metastasis [6,7]. Some studies discussed that activation of Ep-CAM as a key stage in neoplastic transformation such as in digestive, prostate, renal, lung and head and neck carcinomas [1,2,8]. Ep-CAM also shows variable levels of expression in different cancers suggesting a potential prognostic value. Some reports revealed low immunostaining in the gastric carcinoma contrasting with a marked score of staining in colorectal carcinoma as well as in aggressive variants of renal carcinomas [9-10]. In colorectal carcinoma, the loss of membrane expression of EpCAM is associated to the presence of lymph node involvement and positive margins as well as to advanced staging and reduced survival [11]. In contrast, overexpression of this molecule is associated with lymph node metastasis in breast carcinomas [12]. Thus, Ep-CAM profile of expression is widely variable and its prognostic role is controversial.

However, very few reports have tested this molecule expression in thyroid cancer. According to Ralhan et al. (2010), an increased cytoplasmic and nuclear EpCAM expression, in parallel with the loss of membranous one is associated with an unfavorable prognosis and a reduced overall survival in thyroid carcinomas [4]. In the paper published by Andrescieu et al, the authors demonstrated a large variability of MOC-31 expression among PTC histological variants, and highlighted the differences between the low and high MOC-31 score of staining that significantly correlated with aggressive PTC cases and unfavorable clinical outcome [3]. To our knowledge, this is the first study that describes and compares the immunohistochemical profile of expression of MOC31 in PTC and NIFTP.

NIFTP is a non-invasive neoplasm of thyroid follicular cells with a follicular growth pattern and nuclear features of papillary thyroid carcinoma (PTC) that has an extremely low malignant potential [13]. This tumor was formerly classified as the non-invasive encapsulated follicular variant of PTC

or well-differentiated tumour of uncertain malignant potential. However recent studies have proven that the risk of recurrence or other adverse events is extremely low: < 1% within the first 15 years after resection [13]. Thus, according to the latest WHO classification of endocrine organs, tumors that fulfill the following criteria should be reclassified as NIFTP: (1) a complete capsule or clear demarcation of the tumour from adjacent thyroid tissue, (2) the absence of invasion, (3) a follicular growth pattern, and (4) nuclear features of papillary carcinoma. Exclusion features have also been defined and included: a component of solid, trabecular, or insular growth accounting for > 30% of the tumour; high mitotic activity (> 3 mitoses per 10 high-power fields); and tumour necrosis. Although the diagnostic criteria have been clearly defined, some cases are still equivocal and may show overlapping features either with a benign adenoma or with an invasive PTC. In this context, we aimed to describe whether MOC31 has a different profile of expression in NIFTP comparing to PTC.

Our results showed MOC31 expression in 19 cases of PTC (79,2%) and all cases of NIFTP. The staining was circumferential membranous and/or cytoplasmic; contrasting with a mostly basolateral and incomplete membranous staining in the normal thyroid tissue. This finding is consistent with the previously reported changes in the distribution of MOC-31 in the thyroid tumor cells, which is probably related to alterations in the mechanism of cell adhesion. The diffusion of this glycoprotein from its restricted normal location in the adherens junctions to the whole cell membrane and to the cytoplasm may be explained by its potential role in tumorigenesis [3]. Another interesting feature is that in well-differentiated and non aggressive PTC as well as the non invasive follicular thyroid neoplasm with papillary-like nuclear features, the membrane expression of MOC-31 is not lost, but is expanded at the entire cell membrane and cytoplasm.

When comparing the immunohistochemical expression of MOC31 between both groups, the percentage of positive cells and the score of intensity were variable. In PTC cases, 79,9% of cases showed >50% of positive cells and high score of positivity (3-4) in 62,5% (15/24). In contrast to NIFT¨P cases, only 33,3% of cases showed >50% of positive cells and high score of positivity in 3/9cases.

The score of intensity was not different between the two groups and was mostly moderate or high. However, the final combined score was slightly different: final MOC31 staining score for PTC was low in 37,5% (9/24) and high in 62.5% (15/24) contrasting with a predominantly low score in NIFTP 66,7 (6/9). These finding may suggest that the different profile of expression of MOC31 between PTC and NIFTP could be helpful for differential diagnosis in difficult cases. These results suggest that high MOC31 score of staining is more likely associated with malignant lesions. However, it would be interesting to test its expression in benign adenoma and compare it to the NIFTP which is considered of low malignant potential and to PTC which is a malignant tumor.

When considering MOC31 expression in PTC cases, patients with advanced stage pt2-pt3, vascular invasion or lymph node metastasis showed high MOC31 score of staining. This finding supports the previously reported concept that the expression of MOC-31 can become a valuable tool for risk stratification; low score and high score indicate differences in disease progression and aggressiveness [3].

Although our results show evidence of different profile of expression of MOC31 between PTC and NIFTP, our findings are highly limited by the small sample size; further studies are recommended to definitively attest the usefulness of MOC31 in the differential diagnosis of PTC from NIFTP and to define the potential prognostic role of this molecule.

CONCLUSION

MOC31 is an interesting immunohistochemical marker with variable expression in benign and malignant tumors. The changes of MOC31 location as well as its score of staining in PTC and NIFTP could be helpful in the differential diagnosis. Our findings also support the potential prognostic value of this molecule that deserves further investigations.

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