RESEARCH ARTICLE

Stromal Modulation and its Role in the Diagnosis of Papillary Patterned Thyroid Lesions

Sahar Aly Daoud[1], Reham Shehab El Nemr Esmail[2]*, Amal Ahmed Hareedy[3], Abdullah Khalil[3]

Abstract

The papillary patterned lesion of thyroid may be challenging with many diagnostic pitfalls. Tumor stroma plays an important part in the determination of the tumor phenotype. CD34 is thought to be involved in the modulation of cell adhesion and signal transduction as CD34(+) fibrocytes are potent antigen-presenting cells. Smooth muscle actin (SMA) positivity could be diagnostic for fibroblast activation during tumorigenesis. We aimed to examine the expression of CD34 and alphaSMA in the stroma of papillary thyroid hyperplasia, papillary thyroid carcinoma and papillary tumors of uncertain malignant potential in order to elucidate their possible differential distribution and roles. A total number of 54 cases with papillary thyroid lesions were studied by routine H&E staining, CD34 and ASMA immunostaining. ASMA was not expressed in benign papillary hyperplastic lesions while it was expressed in papillary carcinoma, indicating that tumors have modulated stroma. Although the stroma was not well developed in papillary lesions with equivocal features of uncertain potentiality, CD34 was notable in such cases with higher incidence in malignant cases. So ASMA as well as CD34 could predict neoplastic behavior, pointing to the importance of the stromal role. Differences between groups suggest that the presence of CD34 + stromal cells is an early event in carcinogensis and is associated with neoplasia, however ASMA+ cells are more likely to be associated with malignant behavior and metastatic potential adding additional tools to the light microscopic picture helping in diagnosis of problematic cases with H&E.

Keywords: Papillary lesions - malignancy - stroma - ASMA - CD34

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Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy worldwide accounting for more than 70% of follicular cell derived thyroid malignancies (Song et al., 2011; Guo et al., 2014). The incidence is progressively increasing in many countries according to the majority of tumor registries (Giusti et al., 2010), the observation that may be attributed to the recent better diagnostic tools - the high resolution ultrasound and the fine needle aspiration cytology (Alam et al., 2014).

Besides PTC, the papillary pattern in thyroid could be seen in a variety of lesions including; florid untreated hyperplasia which may develop as a physiological response of follicular epithelium to hormonal changes, in some adenomas, degenerated colloid nodules and in Hashimoto thyroiditis (Baloch and LiVolsi, 2006a) and in some cases the diagnosis may be challenging with many diagnostic pitfalls being seen. According to the World Health Organization WHO, PTC is defined as a malignant epithelial tumor showing evidence of follicular cell differentiation and characterized by distinctive nuclear features (Livolsi et al., 2004) These distinctive nuclear features are defined by most authors as; overlapping nuclei, nuclear atypia, polar disorder, ground glass nuclei, nuclear grooves and nuclear pseudoinclusions (Tong et al., 2011), however, none of these features appear to be peculiar for PTC, and a list of mimics is regarded including; autoimmune thyroiditis, hyperplasia (diffuse and adenomatous), Hashimoto's disease and some adenomas particularly trabecular type (Gorla et al., 2012). In some cases of nodular goiter, the nuclear clearing and inclusions are so pronounced as to be present in every cell (Baloch and Livolsi, 2006b; LiVolsi., 2011), the facts which led Liu et al. to doubt that these features are the golden standard of malignancy, although the majority of PTC do have them (Liu et al., 2011). Even the psammoma bodies may rarely found in benign thyroid conditions (<1% of these bodies are in benign glands) (LiVolsi., 2011).

In 2000, Williams proposed a new diagnostic terminology describing the papillary tumors with equivocal nuclear features, that is, well differentiated tumor of uncertain malignant potential WDT-UMP

[1]*Department of Pathology, Faculty of Medicine, Beni Sueif University,* [2]*Department of Pathology, National Research Center,* [3]*Department of Pathology, Cairo University, Cairo, Egypt *For correspondence: rehamelnemr@yahoo.com*

(Williams, 2000). These borderline lesions do not completely fulfill the different morphological criteria established, making their diagnosis and prognosis difficult and despite all recent progress in immunohistochemistry as well as in molecular biology in identifying relevant biomarkers , robust diagnostic tools predicting the degree of malignancy of these borderline tumors are not available (Lassalle et al., 2011).

Many studies tried to solve this diagnostic dilemma, however to date no definite satisfactory results. During the last 10 years it is has been increasingly recognized that tumor stroma plays an important part in the determination of the tumor phenotype and that the stroma compartment is essential for the outgrowth. This knowledge has initiated new fields of research that might improve cancer diagnosis (Lammerts, 2001; Min, 2005).

CD34 is a transmembrane glycoprotein expressed by hematopoietic stem cells, endothelial cells and mesenchymal cells in different tissues including thyroid, which is thought to be involved in the modulation of cell adhesion and signal transduction (Kuroda et al., 2005) they constitute a population of stromal cells that are capable of synthesizing connective tissue matrix. Moreover, CD34 fibrocytes are potent antigen-presenting cells and they may play a role in host response (Quan et al., 2006; Catteau et al., 2013). Batistatou et al. (2002) suggested that these CD34+ cells in the thyroid stroma are dendritic interstitial cells. It is well established that during carcinogenesis stromal fibroblasts undergo certain changes, and in their transition from normal into cancer associated stroma they are subjected to a myofibroblastic differentiation program that is exemplified by the expression of smooth muscle actin (SMA). So some authors consider that SMA positivity could be diagnostic for fibroblasts activation during tumorogenesis (Wang et al., 2013).

The aim of the work is to examine the expression of CD34 and alpha smooth muscle actin ASMA in the stroma of papillary thyroid hyperplasia, papillary thyroid carcinoma and papillary tumors of uncertain malignant potential in order to elucidate their possible differential distribution and roles.

Materials and Methods

A total number of 54 cases of thyroidectomy specimens' paraffin blocks were studied from Egyptian patients collected from El-Kasr El-Aini Hospital, Cairo University. Fifty two cases were females while only 2 cases were males, the age of the patients ranged between 27-54y. All the cases were studied by H&E stained slides for histopathological assessment and immunohistochemical staining for ASMA and CD34.

The information extracted from the patient records and consultation correspondence included; sex, age, clinical presentation and lymph node status.

Microscopically, papillary carcinomas share certain features; the neoplastic papillae contain a central core of fibrovascular (occasionally just fibrous) tissue lined by one or occasionally several layers of cells with crowded, overlapped, optically cleared oval nuclei (DeLellis et al., 2004) .Cases with papillae lacking all nuclear features were considered benign, while cases with equivocal features were considered papillary tumors of uncertain potentiality (PTUP) (Williams, 2000).

Immunohistochemical staining

5 μm sections of formalin-fixed paraffin-embedded tissues were mounted onto ChemMate capillary gap slides (Dako, Glostrup, Denmark), dried in a slide oven at 60°C for 1h, deparaffinized with xylene, and rehydrated with ethanol to distilled water. The staining procedures were performed on an automated immunostainer (TechMate 1000; Dako) using the biotin-streptavidin detection system (ChemMate-HRP/DAB; Dako). The primary antibody was diluted in ChemMate diluent, and incubation performed overnight at 4°C. All following procedures were carried out at room temperature in accordance with the ChemMate protocol. Each TechMate holder included a positive and negative control slide. The results of this analysis revealed that the optimal procedure was epitope retrieval in microwave heating/TEG buffer with anti-ASM, and anti CD34 antibodies. We determined the cytoplasmic expression for both ASMA and CD34.

Immunohistochemical study

Immunohistochemical staining for alpha smooth muscle actin and CD34 was assessed, cases were considered positive if more than 5% of cells were positive. Vascular smooth muscle cells and endothelial cells were also used as positive controls. The slides were reviewed blindly by two independent observers, both pathologists (D. E).

Statistical analysis

Data was statistically analyzed by the Statistical Package of Social Science Software program (SPSS), version 21, summarized using frequency and percentage for qualitative variables. Comparison between groups was done using chi square test as well as Fisher's exact test. P values less than 0.05 were considered statistically significant and less than 0.01 were considered highly significant.

Results

The present study included a total number of 54 cases ranging in age between 27-54y with mean age 38y with female to male ratio 6.7:1. Cases were diagnosed by H&E which all the cases showed papillary growth. Twenty two cases (40.74%) were benign papillary thyroid hyperplasia (PTH) all were females, 7 cases (12.96%) "3 males, 4 females" were considered papillary thyroid tumor with uncertain potentiality (PTUP), follow up of such cases showed positive lymph node metastatic deposit in one case. 25 cases (46.30%) were diagnosed as papillary thyroid carcinoma (PTC) 4 of 25 cases were males, assessment of lymph node status was available in 12/25 cases, of the 12 cases 8 showed positive metastatic deposits; they were all females, while 4 cases (2 males, 2 females) were negative for metastatic deposits, the remaining PTC (2 males, 11 females) at which lymph node status could not be assessed.

Immunohistochemical results

Cases of PTH showed negative immunostaining for ASMA and showed only one positive case (4.5%) for CD34 these results were increasing in PTUP; at which ASMA was positive in one case (14.3%) and CD34 was positive in 85.7% of cases.

While for PTC 68% and 80% of cases were positive for ASMA and CD34 respectively with highly statistical significant value (p value <0.001).

There was a highly statistically significant difference between PTC and PTH groups in expression of actin and CD34 (p value <0.001) as 100%, 95.5% of the cases of PTH showed negative immunostaining for ASMA (Figures 1, 2).

CD34 showed highly statistical significant difference between PTH and group of uncertain malignant potential (PTUP) figure 3 (p value <0.001), while ASMA was not significant as only one case showed positive immunostaining shown in figure, 4 (Table 1).

Alpha smooth muscle actin was significantly higher in PTC relative to the group of uncertain malignant potential follow up of the latter showed positive lymph node metastatic deposit 2 years later, (p value <0.03) and CD 34 was positive in both groups although it was slightly lower in PTC, statistically insignificant (Table 2) (Figures 3,5,7). The distribution of ASMA was mainly subepithelial and in the core of the papillae (Figures 4, 6, 8).

Assessment of lymph node status was available in 12/25 cases of PTC, of the 12 cases 8 showed positive metastatic deposits while 4 cases were negative for metastatic deposits, cases positive for ASMA showed positive lymph node metastatic deposits.

Table 1. Comparison between PTH and Uncertain Malignant Groups Regarding ASMA as Well as CD34.

	PTH (n=22)		PTUP (n=7)		p value
	N	%	N	%	
ASMA					
+	0	0.0	1	14.3	0.2
-	22	100.0	6	85.7	NS
CD34					
+	1	4.5	6	85.7	<0.001
-	21	95.5	1	14.3	HS

*HS=Highly Significant

Table 2. Comparison between PTC and uncertain groups regarding ASMA as well as CD34

	PTH (n=25)		PTUP (n=7)		p value
	N	%	N	%	
ASMA					
+	17	68.0	1	14.3	0.03
-	8	32.0	6	85.7	S
CD34					
+	20	80.0	6	85.7	1.0
-	5	20.0	1	14.3	NS

*NS=nonSignificant, S= significant.

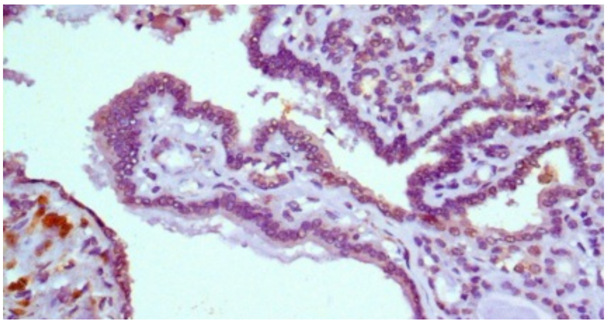

Figure 1. A Case of PTH Negative for CD34

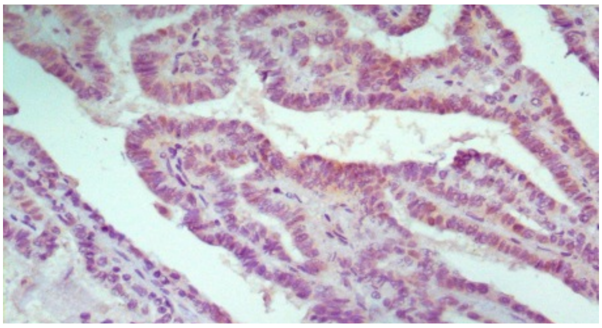

Figure 2. A Case of PTH Negative for ASMA Immunostaining (HP)

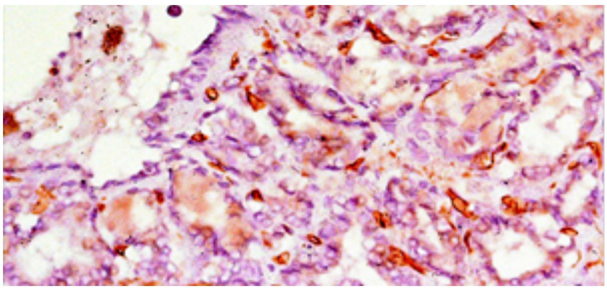

Figure 3. PTUP with Equivocal Nuclei (Irregular, Overlapped at the Bottom), Stromal Cells Show Positive CD34 Immunostaining (HP)

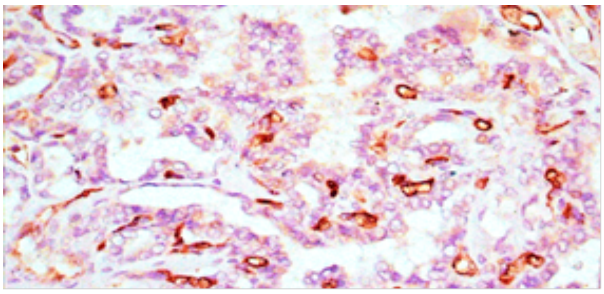

Figure 4. PTUP Positive for ASMA Immunostaining, Some of Nuclei Show Clearing and Overlapping (at the Bottom) While Papillae at the Top Lacking these Features (HP)

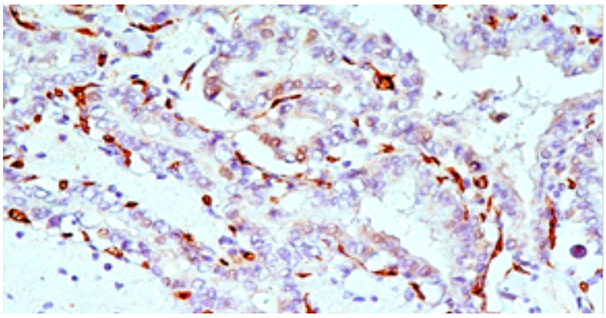

Figure 5. Figure 5, PTC Stromal Cells Show Positive CD34 Immunostaining (HP)

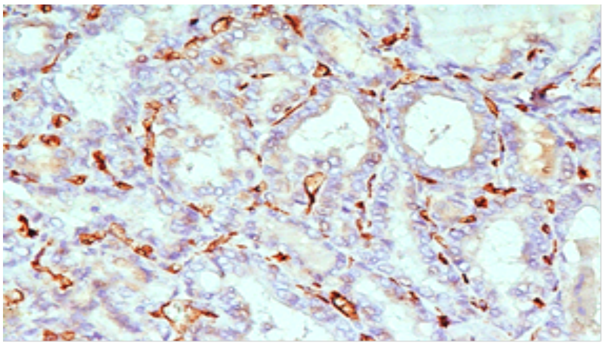

Figure 6. PTC, Showing Subepithelial Positive ASMA Stromal Cells (HP)

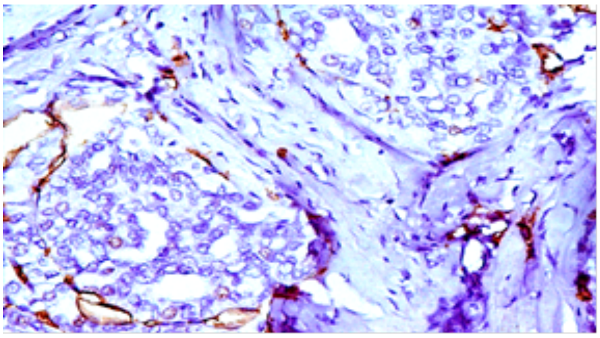

Figure 7. A Case of PTC Showing-ve Stromal Cells for CD34 while Positive Blood Vessels as an Internal Control (HP)

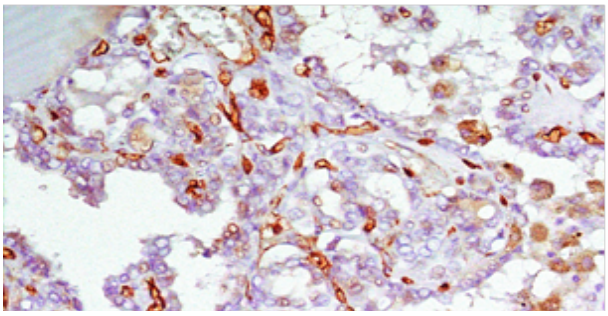

Figure 8. A Case of PTC Showing Positive Stromal Cells for ASMA(HP)

Discussion

It is known that papillary lesions in thyroid are sometimes challenging; although papillary thyroid carcinoma can be usually differentiated based on some histologic and cytologic features including well-developed papillary fronds with fibrovascular cores, presence of Psammoma bodies, large vesicular nuclei and the peculiar nuclear cytology. The distinction between papillary lesions in some cases may still be difficult and additional diagnostic methods would be useful (Erickson et al., 2000).

El Demellawy et al. (2008) found that some cases do raise controversy as being PTC or non PTC, when some of the nuclear diagnostic criteria for PTC are occasionally present and that such controversy exists between expert thyroid pathologists. Some authors have highlighted the importance of stromal interaction with epithelial cells in embryonic development and tumorogenesis; the precancerous epithelial cells may acquire multiple genetic mutations and the associated stroma is then becoming "activated", commonly expressing myofibroblastic markers (Cimpean et al., 2005). It was only recently recognized that reactive stroma co-evolves with cancer, exhibiting tumor-promoting properties, however, the specific cell types of origin and the spatial/temporal patterns of reactive stroma initiation are poorly understood (San Martin et al., 2014). Therefore, in this study we examined the stroma of thyroid papillary patterned lesions using ASMA and CD34 aiming at finding out special characterizations which may help in definite differentiation between benign, border line, and malignant thyroid papillary lesions.

In the present study, we studied a total number of 54 cases ranged in age between 27-54y with mean age 38y with female predominance (F:M ratio 6.7:1) which was also observed by many other authors (Bhargava et al., 2012) (Parikh et al., 2012).

Cases were diagnosed by H&E where all the cases showed papillary patterned lesions; 22 cases (40.74%) were PTH, 25 cases (46.30%) were diagnosed as PTC, 7 cases (12.96%) were considered PTUP at which the nuclear features were equivocal; follow up of such cases showed positive lymph node metastatic deposit in one case. Cases of PTH did not show myofibroblastic differentiation as they were negative for ASMA and showed only one positive case (4.5%) for CD34 positive, the immunostaining expression was increased in PTUP at which ASMA was positive in one case presented by 14.3% and CD34 was positive in 85.7%, on the other hand PTC cases showed positive stromal reaction in 68% and 80% of cases for ASMA and CD34 respectively with highly statistical significant value (p value<0.001); the positive expression for both CD34 and ASMA was significantly associated with the diagnosis of malignancy.

To the best of our knowledge no published studies examined the status of both CD34 and ASMA in thyroid stroma as a differential point in papillary lesions, therefore few data were found in this regard. Kuroda et al. (2005) studied both markers in breast lesions and in their study they found that in benign cases there was no stromal cells expressing both CD34 and ASMA while stromal cells were positive for ASMA, negative for CD34 in malignant cases, they proposed that this finding may imply that the phenotypic switching in stromal cells occurs rapidly in malignant cases. Tumor cells may recruit circulating CD34-positive cells derived from myeloid precursors and convert them into myofibroblasts expressing ASMA (Ruiter et al., 2002; Kojc et al., 2005).

CD34 is thought to be involved in the modulation of cell adhesion and signal transduction. CD34+ fibrocytes/fibroblasts derive from myeloid precursors, invade sites of tissue damage and are capable of connective tissue matrix synthesis, claimed that CD34+ may play a role in host response to tissue damage (Chesney et al., 1997) (Moore and Lee, 2001).

In agreement of our results, San Martin et al. (2014), evaluated human tumor tissue arrays by using multiple labeled, quantitative, spectral deconvolution microscopy. In their study they reported novel CD34/vimentin dual-positive reactive stromal fibroblastic cells observed in the cancer microenvironment of human breast, colon, lung, pancreas, thyroid, prostate, and astrocytoma.

This is also may be matching with Yong et al. (2013) who stated that papillary thyroid carcinoma (PTC) is known to have several morphologic variants with extensive proliferation of the stroma, resembling fibroblastic/myofibroblastic proliferative lesion in the soft tissue.

Within the cases of PTC, those with positive nodal deposits showed significant ASMA stromal cell positivity, similarly, the PTUP case that showed positive nodal deposit was as well positive for ASMA. These findings were in concordance with the recent concept that the desmoplastic stroma was significantly associated with lymph node metastases (Koperek et al., 2011).

As a conclusion, The difference between groups may point to that the presence of CD34 + stromal cells is an early event in carcinogensis and is associated with neoplasia, however ASMA+ cells is more likely to be associated with malignant behavior and metastatic potentials adding additional tools to the light microscopic picture helping in diagnosis of problematic cases with H&E. We therefore, join the recommendation that a more clear understanding of the nature and origin of reactive stroma is needed for better disclosure of the tumorogensis, better solving of the challenging diagnostic cases and to identify novel therapeutic targets in cancer and fibrosis.

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