Original Article

Potentially Important Markers in Thyroid Neoplasia: Claudin-1 and MMP-7

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Abstract

Background: Thyroid carcinomas are the most common malignant endocrine tumors, and various immunohistochemical markers are tested in routine practice to reduce diagnostic differences, as well as to elucidate carcinogenesis and detect malignancy. Disruption of basement membranes and the extracellular matrix is an important step in tumor carcinogenesis and progression. The claudin and matrix metalloproteinase families are also thought to be effective in this process. **Aim:** In this retrospective study, the comparative expression of claudin-1 and MMP-7 immunomarkers in normal tissues and thyroid neoplasia were investigated. **Materials and Methods:** Immunohistochemical staining was performed for claudin-1 and matrix metalloproteinase 7 (MMP-7) in 112 sections, including 24 follicular adenomas, 22 follicular carcinomas, 24 medullary carcinomas, 24 papillary carcinomas, and 18 single dominant nodules from thyroid lesions. **Results:** A significant staining difference for claudin-1 was observed in follicular carcinoma and medullary carcinoma, papillary carcinoma, and single dominant nodules compared to normal thyroid tissue. A statistically significant staining difference was observed for MMP-7 in follicular adenoma, medullary carcinoma, and papillary carcinoma compared to normal thyroid tissue. **Conclusions:** These results indicate that claudin-1 and MMP-7 are important in the diagnosis, differential diagnosis, and carcinogenesis of follicular adenoma, follicular carcinoma, medullary carcinoma, papillary carcinoma, and single dominant nodules.

Keywords: *Claudin-1, MMP-7, thyroid neoplasm*

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Introduction

In the thyroid, follicular adenoma, follicular carcinoma, and papillary carcinoma are formations of follicular cells originating from medullary carcinoma (MC) and single dominant nodules (SDNs) is of parafollicular C-cell origin. Follicular cell-derived thyroid neoplasms comprise an extremely complex spectrum and attempt to classify them based on both morphology and molecular genetics have been made. Papillary thyroid carcinoma (PC) is the most common thyroid carcinoma. PC contains the true papillary pattern and distinctive cellular features. Even if strong immunohistochemical markers are used in the differential diagnosis, there are situations where they are not sufficient and it is sometimes necessary to use double or triple markers. follicular adenoma (FA), follicular carcinoma (FC), or SDNs are formations that appear as nodular well-circumscribed lesions and sometimes cause problems in histopathological diagnosis. In addition, MC does not originate from the follicular epithelium, unlike other thyroid carcinomas, but from parafollicular C-cells that secrete calcitonin.[1]

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The expression of various immunohistochemical markers in benign and malignant thyroid cases has been discussed comparatively in many studies.[1] In thyroid neoplasms, the immunohistochemical expression of claudin-1 and matrix metalloproteinase 7 (MMP-7) have been investigated in relatively few studies.[2-8] Claudin-1 is a member of the claudin family, which is defined as a tight junction (TJ) component.[9] These TJs are transmembrane and cytoplasmic proteins that prevent the diffusion of solutes and provide cell polarity.[10-13] MMP-7 is a member of MMPs. MMPs are zinc-containing, calcium-dependent endopeptidases that play a role in the extracellular matrix degradation and tissue remodeling and increase hemostasis in normal tissue under physiological conditions.[14,15] It has been reported that the disruption of basement membranes and the extracellular matrix is an important step in tumor carcinogenesis and progression. The claudin and MMP families are also effective components in this process, and overexpression or loss of expression of claudin-1 and MMP-7 varies depending on the type of cancer.[2-8]

Immunohistochemical analyses of thyroid neoplasms guide both diagnosis and oncogenesis studies. In our study, we examined the comparative expression of claudin-1 and MMP-7 immunomarkers in normal tissues and FA, FC, MC, PC, and SDNs. In addition, we investigated whether there was a difference in staining with these markers between the groups.

Materials and Methods

A total of 112 patients who underwent thyroidectomy at Erzincan Mengucek Gazi Training and Research Hospital were included in this study. These cases included 24 FA, 22 FC, 24 MC, 24 PC, and 18 SDNs. The original diagnoses of all pathological sections were confirmed. Paraffin blocks containing normal and pathological thyroid tissue were selected, and 4 µm thick sections were taken from these paraffin blocks and fixed on lysine slides for immunohistochemical staining. These slides were then stained with claudin-1 (Abcam, dilution ratio 1/100) and MMP-7 (Abcam, dilution ratio 1/100) markers in a fully automated immunohistochemical staining device (Leica Bond-Max, Melbourne, Australia) and processed.

Evaluations were carried out semi-quantitatively. An Olympus BX53 microscope (Tokyo, Japan) was used for microscopic evaluation. According to the amount of complete membranous staining for claudin-1, those with <5% were 0: negative staining; those with 5–25% uptake were evaluated as +; those with 26–50% uptake were ++; and those with more than 50% uptake were +++.[3] According to the percentage of cytoplasmic staining with MMP-7, 0 indicated negative staining. Samples with 1–10% uptake were considered +, those with 11–50% uptake were marked ++, and those with 51–100% uptake were marked +++. When comparing the groups, the final scoring for the statistical analysis was negative if the immunoreactivity of tumor cells was 1 or less, and +1 if the total score was 2 and more.[7]

Statistical analysis

IBM SPSS 22 (IBM Corp., Armonk, NY) was used for all statistical analyses. The staining scores were summarized categorically and continuously. Categorical variables are expressed as *n* (%), and continuous variables are expressed as mean ± standard deviation median (min - max) value. The assumption of normal distribution was checked with the Kolmogorov–Smirnov test, and tests were selected according to the distribution type. A *t*-test was applied to the dependent and independent groups for the normally distributed variables. The Mann–Whitney *U* and Wilcoxon tests were used for scores that did not show normal distribution. Cases with $P < 0.05$ in the evaluation were considered statistically significant.

Table 1: Average age within the groups

	Average age			
Tumor types	Mean	Std. deviation	Minimum	Maximum
FA	46.17	12.52	30	77
FC	51.46	12.17	31	65
MC	45.50	14.80	18	69
PC	52.83	12.71	34	75
SDNs	56.11	13.72	34	83
Total	50.09	13.56	18	83

FA: follicular adenoma, FC: follicular carcinoma, MC: medullary carcinoma, PC: papillary thyroid carcinoma, SDNs: single dominant nodules

Table 2: Claudin-1 staining scores in the study groups

		CLAUDİN 1			
Tumor Types	Group	Median	Minimum	Maximum	*P*
FA	Control	1	0	1	0.414
	Tumor	1	0	2	
FC	Control	1	0	1	0.023
	Tumor	1	0	3	
MC	Control	1	0	1	0.002
	Tumor	0	0	1	
PC	Control	1	1	1	<0.001
	Tumor	3	2	3	
SDNs	Control	1	1	1	0.046
	Tumor	1	1	2	

FA: follicular adenoma, FC: follicular carcinoma, MC: medullary carcinoma, PC: papillary thyroid carcinoma, SDNs: single dominant nodules

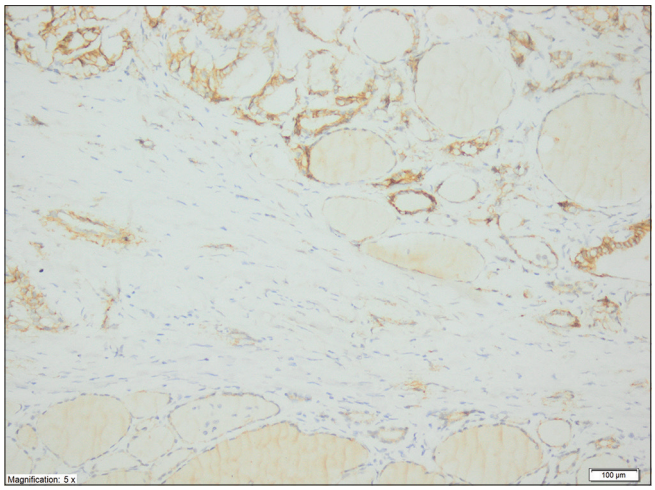

Figure 1: Increased FC staining with claudin-1 in the upper right and normal thyroid tissue in the lower half (×200)

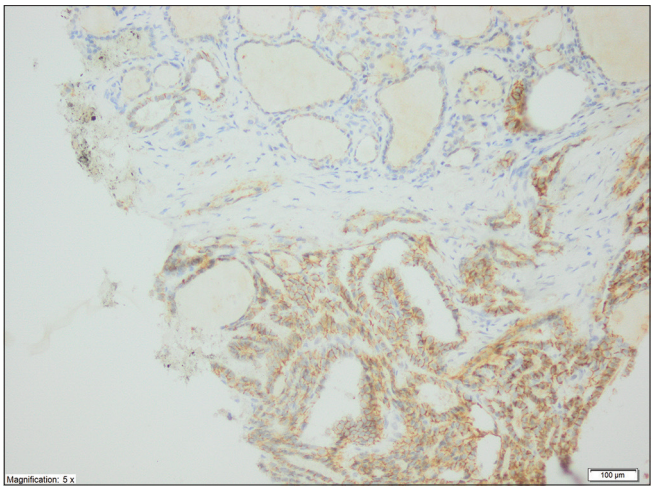

Figure 2: Increased membranous staining in PC with claudin-1 at the bottom and normal thyroid tissue at the top (×200)

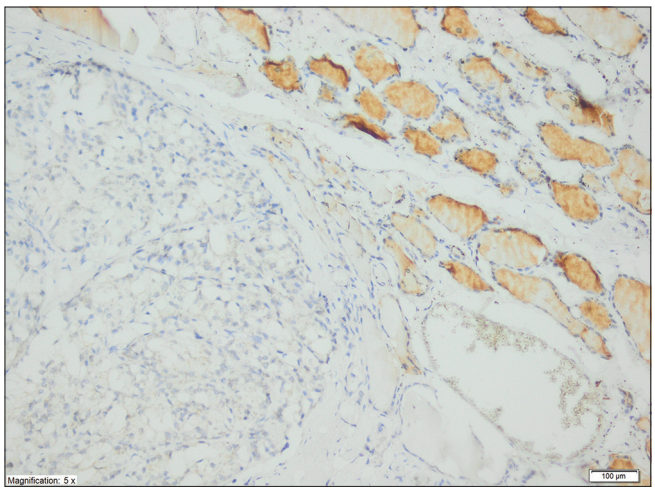

Figure 3: Loss of expression in MC with claudin-1 on the left and normal thyroid tissue on the right (×200)

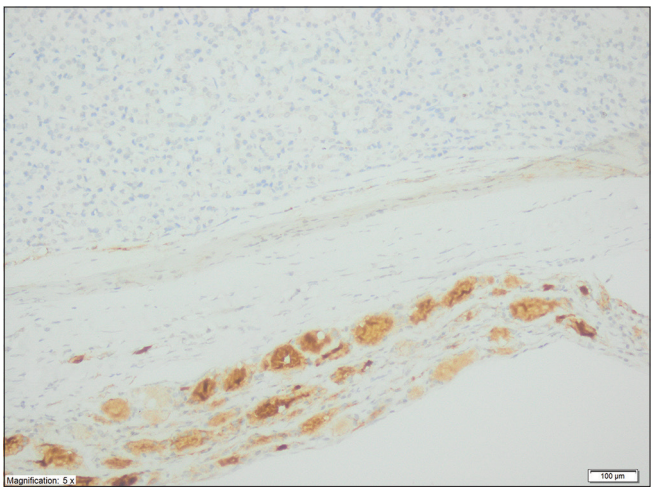

Figure 4: Loss of expression in FA with MMP-7 at the top and normal thyroid tissue at the bottom (×200)

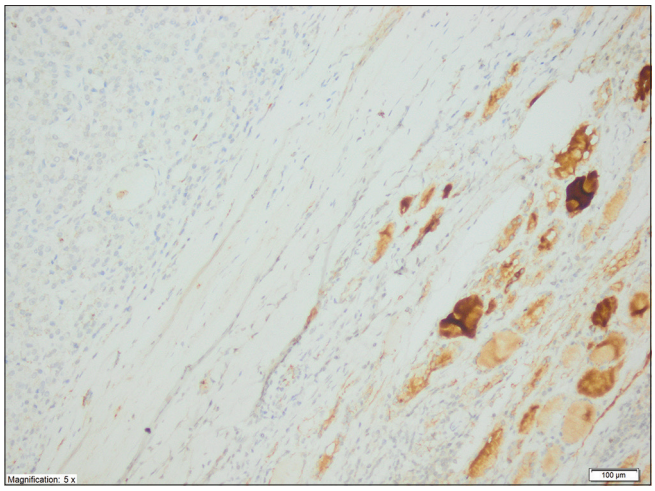

Figure 5: Loss of expression in PC with MMP-7 on the left and normal thyroid tissue on the right (×200)

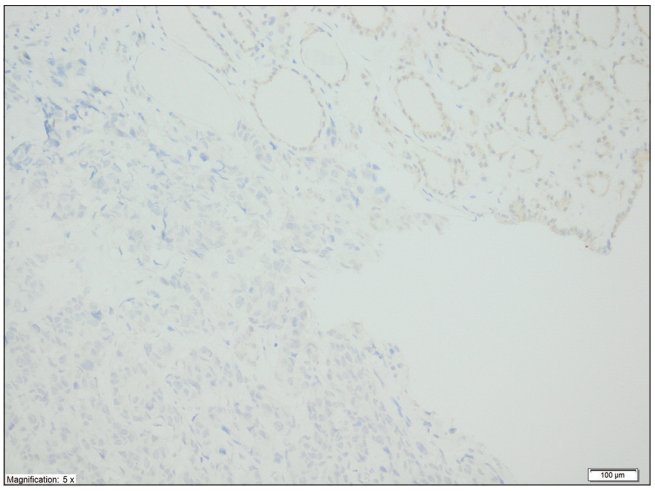

Figure 6: Loss of expression in MC with MMP-7 at the bottom, normal thyroid tissue at the top (×200)

Results

Of the 112 patients who underwent thyroidectomy, 70 were women and 42 were men, with a mean age of 50 ± 13.6 years. The distribution of cases was 21.4%

Table 3: MMP-7 staining scores in the study groups

		MMP-7			
Tumor Types	**Group**	**Median**	**Minimum**	**Maximum**	***P***
FA	Control	1	0	1	0.014
	Tumor	0.5	0	1	
FC	Control	1	0	1	0.157
	Tumor	0	0	1	
MC	Control	0.5	0	1	0.005
	Tumor	0	0	1	
PC	Control	1	0	1	<0.001
	Tumor	0	0	1	
SDNs	Control	0	0	1	0.999
	Tumor	0	0	1	

FA: follicular adenoma, FC: follicular carcinoma, MC: medullary carcinoma, PC: papillary thyroid carcinoma, SDNs: single dominant nodules

FA, 19.6% FC, 21.4% MC, 21.4% PC, and 16.1% SDNs. The mean patient ages for each tumor type are given in Table 1.

In FA, the number of cases not stained with claudin-1 was six, the number of + staining cases was 16, and the number of ++ staining cases was 2. The number of cases with no staining in FC was 2, the number of cases with + staining was 14, the number of cases with ++ staining was 4, and the number of cases with +++ staining was 2 [Figure 1]. Although the number of cases with + staining was 14 in SDNs, the number of cases with ++ staining in SDNs was 4. Although four of the cases were stained with ++ in PC, 20 of them were stained with +++ [Figure 2]. No staining was observed in 16 of the cases in MC [Figure 3], and + staining was observed in 8 cases.

When claudin-1 was compared with normal thyroid tissue and pathological tissues, a significant staining difference was observed in FC, MC, PC, and SDNs; however, this difference was not observed in FA. The staining values in these groups with claudin-1 are given in Table 2. In addition, a statistically significant difference in staining was observed when claudin-1 was compared with PC and other groups ($P < 0.05$). In addition, a significant staining difference was observed between FC and MC, SDNs, and MC ($P < 0.05$).

The number of cases that did not stain with MMP-7 in FA was 12, and the number of cases that stained + was 12 [Figure 4]. The number of unstained cases in FC was 12, and the number of stained cases was 10. There were 12 unstained cases and 6+ stained cases in SDNs. Twenty unstained cases and 4+ stained cases were found in PC [Figure 5]. In MC, no staining was observed in 20 cases, whereas + staining was observed in 4 cases [Figure 6]. When MMP-7 was compared with normal thyroid tissue and pathological tissue, a significant staining difference was observed in FA, MC, and PC; however, this difference was not observed in FC and SDNs. The staining values in these groups with MMP-7 are given in Table 3. When the groups were compared with each other, a statistically significant staining difference was observed between FC and PC and between FC and MC ($P < 0.05$).

Discussion

One of the highlights of our immunohistochemical study is that claudin-1 contains important clues for FC, MC, and PC, as well as MMP-7 for FA, MC, and PC in carcinogenesis. Another important highlight is that claudin-1 may be useful in the differential diagnoses of PC and other groups. Furthermore, MMP-7 may be useful in the differential diagnoses of FC, MC, and PC.

The roles of the claudin family in the diagnosis and carcinogenesis of thyroid neoplasms have not yet been fully determined. It has been observed in several immunohistochemical studies that claudin-1 expression is increased in PC, whereas the membranes of normal and benign thyroid lesions are either unstained or stained weakly.[2-5] In addition, it has been claimed that claudin-1 is a new marker in PC.[7] In our study, membranous claudin-1 expression increased in PC when compared to both normal tissues and other groups. These results show that claudin-1 is diagnostic for PC with Hector Battifora mesothelial 1 (HBME 1), galectin 3, and cytokeratin 19 and that it can work as a routine marker. There may also be morphological overlaps in the differential diagnosis of MC and PC. The loss of claudin-1 expression in MC cases and the significant increase in the membranous expression with claudin-1 in PC may facilitate the differential diagnosis.

Significant increases in membranous staining with claudin-1 in lesions in PC were observed when compared with normal tissue. However, the decrease in staining in MC indicates that claudin-1 may be effective in PC carcinogenesis by increasing and, on the contrary, decreasing MC carcinogenesis. Although there was no significant increase in PC, the increase of claudin-1 in FC and SDNs lesions was also an important result, as it shows that claudin-1 may also be effective in the carcinogenesis of FC and SDNs.

The disruption of basement membranes and the extracellular matrix is an important step in tumor formation and invasion. It has been reported that MMP-7 is associated with the development of carcinoma, is mainly expressed by tumor cells, and contributes to invasion.[16,17] In a similar study, it was reported that MMP-7 is more expressed in thyroid malignant neoplasms.[8] In our study, on the contrary, a significant

loss of MMP-7 expression was observed in FA, MC, and PC when compared to normal tissues, whereas no statistically significant change was observed in the expression in FC and SDNs. These data suggest that decreased MMP-7 expression in FA, MC, and PC may cause tumor formation and progression. However, more staining was observed in FC when compared with MC and PC separately. These results show that FC can be useful in the differential diagnosis in cases where FC overlaps with MC and FC overlaps with PC.

Morphological overlaps are common between FA, FC, and SDNs. In these cases, morphological evaluation alone is insufficient for the realization of an objective and consistent differential diagnosis. Moreover, to differentiate FC, which is a malignant and aggressive tumor, from FA, it is necessary to deepen the histopathological examination. There is almost no illuminating information on this subject within current immunohistochemical studies. The apparent loss of MMP-7 expression in FA when compared with normal tissues in our study, unlike in FC and SDNs, shows that it may be useful in the differential diagnosis of these cases.

In conclusion, the differential expression of claudin-1 and MMP-7 in normal and neoplastic tissues may facilitate the differential diagnosis of FA, FC, PC, and SDNs immunohistochemically. It may also guide the elucidation of the carcinogenicity of MC and PC and provide new opportunities for targeted cancer therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lloyd RV, Osamura RY, Klöppel G, Rosai J, editors. WHO Classification of Tumours of Endocrine Organs. 4th ed. Lyon, France: IARC; 2017.
2. Abd El Atti RM, Shash LS. Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma and solitary follicular thyroid nodules. J Egypt NatlCancInst 2012;24:175-84.
3. Németh J, Németh Z, Tátrai P, Péter I, Somorácz A, Szász AM, *et al*. High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. PatholOncol Res 2010;16:19-27.
4. Tzelepi VN, Tsamandas AC, Vlotinou HD, Vagianos CE, Scopa CD. Tight junctions in thyroid carcinogenesis: Diverse expression of claudin-1, claudin-4, claudin-7 and occludin in thyroid neoplasms. Mod Pathol 2008;21:22-30.
5. Liang HS, Zhong YH, Luo ZJ, Huang Y, Lin HD, Luo M, *et al*. Comparative analysis of protein expression in differentiated thyroid tumours: Amulticentre study. J Int Med Res 2009;37:927-38.
6. Buergy D, Weber T, Maurer GD, Mudduluru G, Medved F, Leupold JH, *et al*. Urokinase receptor, MMP-1 and MMP-9 are markers to differentiate prognosis, adenoma and carcinoma in thyroid malignancies. Int J Cancer 2009;125:894-901.
7. Süren D, Yildirim M, Sayiner A, Alikanoğlu AS, Atalay I, Gündüz UR, *et al*. Expression of claudin 1, 4 and 7 in thyroid neoplasms. OncolLett 2017;13:3722-6.
8. Cho Mar K, Eimoto T, Tateyama H, Arai Y, Fujiyoshi Y, Hamaguchi M. Expression of matrix metalloproteinases in benign and malignant follicular thyroid lesions. Histopathology 2006;48:286-94.
9. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 1998;141:1539-50.
10. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2001;2:285-93.
11. Oliveira SS, Morgado-Díaz JA. Claudins: Multifunctional players in epithelial tight junctions and their role in cancer. Cell Mol Life Sci 2007;64:17-28.
12. Schneeberger EE, Lynch RD. The tight junction: Amultifunctional complex. Am J Physiol Cell Physiol 2004;286:1213-28.
13. Cereijido M, Valdes J, Shoshani L, Contreras RG. Role of tight junctions in establishing and maintaining cell polarity. Annu Rev Physiol 1998;60:161-77.
14. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, *et al*. Matrix metalloproteinases: Areview. Crit Rev Oral Biol Med 1993;4:197-250.
15. Freije JM, Díez-Itza I, Balbín M, Sánchez LM, Blasco R, Tolivia J, *et al*. Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J BiolChem 1994;269:16766-73.
16. Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: Role for cell migration. PatholInt 2002;52:255-64.
17. Ma H, Xu S, Yan J, Zhang C, Qin S, Wang X, *et al*. The value of tumor markers in the diagnosis of papillary thyroid carcinoma alone and in combination. Pol J Pathol 2014;65:202-9.