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Tight junctions in thyroid carcinogenesis: diverse expression of claudin-1, claudin-4, claudin-7 and occludin in thyroid neoplasms

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Claudins and occludin are integral constituents of tight junctions and are deregulated in a variety of malignancies. Their role in thyroid carcinogenesis has not yet been elucidated. This study investigates the expression of occludin and claudin-1, -4 and -7 in thyroid neoplasms. Ninety-one thyroid neoplasms (15 follicular adenomas, 15 follicular carcinomas, 26 papillary carcinomas, 16 papillary microcarcinomas, 8 medullary carcinomas, 3 poorly differentiated carcinomas, 8 undifferentiated carcinomas) were immunostained with antibodies against occludin and claudin-1, -4 and -7. Occludin was mainly expressed in the form of intracytoplasmic vesicles, whereas all claudins tested exhibited membranous immunostaining. Thirteen out of 15 follicular adenomas, 10/15 follicular carcinomas, 24/26 papillary carcinomas, 15/16 papillary microcarcinomas, 1/8 medullary carcinomas, 2/3 poorly differentiated carcinomas and 2/8 undifferentiated carcinomas exhibited claudin-1 expression, whereas claudin-4 was expressed in 13/15, 12/15, 23/26, 13/16, 7/8, 2/3 and 2/8 of the tumors, respectively, and claudin-7 expression was found in 67, 33, 73, 69, 25, 0 and 13% of the cases, respectively. Occludin was expressed in 100% follicular adenomas, 80% follicular carcinomas, 96% papillary carcinomas, 50% papillary microcarcinomas, 50% medullary carcinomas, 33% poorly differentiated carcinomas and 88% undifferentiated carcinomas. Occludin expression was reduced in papillary microcarcinomas, medullary carcinomas and poorly differentiated carcinomas. All claudins exhibited reduced expression in undifferentiated carcinomas. Claudin-1 was additionally reduced in medullary carcinomas and claudin-7 in follicular, medullary and poorly differentiated carcinomas. A correlation between loss of claudin-1 expression and worse disease-free survival was noted on univariate analysis. Dedifferentiation of the thyroid carcinomas is accompanied by reduction in claudin-1, -4 and -7 expression. A differential expression of tight junction proteins in the different histologic types of thyroid gland is noted. Additionally, claudin-1 expression may be an important prognostic indicator of recurrence in thyroid carcinomas.
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Tight junctions are intercellular junctional complexes located at the apical end of the lateral membranous surface of polarized cells. Their main role is the prevention of free diffusion of solutes through paracellular spaces, known as the barrier function. In addition, tight junctions block the free diffusion of proteins and lipids between the apical and basolateral domains of the plasma membrane, therefore maintaining cell polarity (fence function).[1,2]

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Tight junctions are composed of transmembrane and cytoplasmic protein complexes and appear on electron micrographs as series of fusion points between the outer leaflets of plasma membranes of adjacent cells. At these so-called 'kissing points', the intercellular space is eliminated remarkably.[2,3] Up to now, three groups of macromolecules are considered as bona fide integral components of tight junctions: occludin, claudins and junctional adhesion molecule.[2,4]

Occludin represents the first transmembrane protein of tight junctions that was identified and is a 65 kDa protein that spans the membrane four times, thus having two extracellular loops.[5] The cytoplasmic N- and C-terminal domains contain several phosphorylation sites and interact with various proteins.[1,2] The exact role of occludin in

tight junctions function is unclear, although a regulatory function is speculated.[1]

Claudins are integral membrane proteins that have, similar to occludin, four transmembrane domains and two extracellular loops.[6,7] However, unlike occludin, the first loop is longer than the second one.[8] Claudins were named after the latin verb 'claudere', which means 'to close', reflecting their role in tight junctions: they are the key proteins for the sealing of the extracellular space.[7–10] Claudin family consists of 24 members. Different claudins are found at the tight junction of diverse epithelial cells,[11] and this accounts for the observed differences in permeability and electrical resistance of various epithelia.[2,8,10]

Previous studies have shown that occludin and claudin-1, -4 and -7 are deregulated in a variety of malignancies; for example, loss of occludin expression or upregulation of claudin-3 and -4 accompanies the progression of endometrial carcinoma,[12,13] reduced expression of claudin-1 correlates with poorer differentiation, disease recurrence and poor survival in stage II colonic cancer,[14] claudin-7 expression has been found reduced in mammary,[15] esophageal,[16] and head and neck carcinomas,[17] whereas claudin-4 overexpression decreases the invasiveness and metastatic potential of pancreatic cancer cells.[18] On the other hand, various cancers (ie ovarian, colon, cervical cancer) are associated with elevated tight junctions proteins expression levels or protein dislocation to the cytoplasm.[19–21] These reports emphasize the complex and diverse actions of tight junctions proteins in cancer progression.

Follicular cells of the thyroid gland are arranged in a single highly polarized layer and act as a barrier between the lumen of the follicle, where thyroglobulin and thyroid hormones are stored, and the extrafollicular space. Epithelial cell polarity and follicular space entrenchment are due to the presence of firm tight junctions. In a recent study, elevated gene expression of several claudins (ie claudin-3, -4, -7) was found in normal and malignant thyroid tissue.[22] However, the expression of tight junctions proteins in the various histologic types of thyroid neoplasms has not been addressed thoroughly before.

This study investigates the expression of occludin and the claudin-1, -4 and -7 in various thyroid neoplasms and in the adjacent non-neoplastic thyroid tissue by immunohistochemistry, in an attempt to clarify the relationship between tight junctions' protein expression and tumor type.

Materials and methods

Formalin-fixed, paraffin-embedded thyroid tissues of 91 thyroidectomy specimens resected from an equal number of patients for therapeutic purposes, as well as the pathology report for each patient, were retrieved from the files of the Department of Pathology, University Hospital of Patras, Greece. There were 19 males and 72 females, ranging from 19 to 84 years old (mean: 47 ± 14). All available slides were reviewed and the most representative block from each case was selected. Each case was classified according to the WHO histologic classification of thyroid tumors.[23] The material consisted of 15 cases of follicular adenomas, 15 follicular carcinomas, 26 papillary carcinomas, 16 papillary microcarcinomas, 8 medullary carcinomas, 3 poorly differentiated carcinomas and 8 undifferentiated carcinomas. For each tumor, several features were evaluated and finally the tumor size and stage (T)[24] were recorded. Additionally, follow-up data were available for 33 patients (1 patient with papillary microcarcinoma, 13 with papillary carcinoma, 7 with medullary carcinoma, 2 with undifferentiated carcinoma and 10 with follicular carcinoma) and the disease-free survival and overall survival was determined.

Immunohistochemistry

Immunohistochemistry was performed on 4-μm-thick formalin-fixed, paraffin-embedded tissue sections mounted on gelatin-coated glass slides. Deparaffination, rehydration and antigen retrieval were performed in an electric pressure cooker using Trilogy retrieval solution (Cell Marque, AR, USA) for 30 min. Polyclonal antibodies against occludin (1:80), claudin-1 (1:100) and claudin-7 (1:200) and monoclonal antibodies against claudin-4 (3E2C1, 1:100) were used for the primary reaction. All antibodies were purchased from Zymed (CA, USA). The sections were incubated with primary antibodies for 1 (occludin and claudin-1) or 2 (claudin-4 and -7) hours at room temperature, followed by sequential 30 min incubation with Dako EnVision Labelled Polymer (Dako, CA, USA). Diaminobenzidine (Dako) was used as the chromogen. Nuclei were counterstained with Harris hematoxylin. Sections from breast carcinoma were used as positive control for occludin. Normal skin was the positive control for claudin-1 and normal colon for claudin-4 and -7. In negative control slides, the same method was performed and the primary antibody was substituted by 1% TBS. Membranous and cytoplasmic immunostaining of occludin was considered positive. For all antibodies, cases exhibiting $>5\%$ positive cells were considered as positive.[25,26] The adjacent non-neoplastic thyroid tissue was also evaluated.

Statistical Analysis

Results were expressed as number of positive cases per number of total cases. The χ^2-test was used to record any differences between the staining results and the tumor features. The analysis of overall survival and disease-free survival was calculated with the Kaplan–Meier method and the differences in survival between the groups were compared

using the log-rank test. Multivariate analysis including clinicopathologic factors and expression of each protein was performed using Cox proportional hazards method. All data were analyzed with the SPSS program (SPSS® 14.0, Chicago, USA). Any P value <0.05 was considered as significant.

Results

Routine Pathology

Tumor size ranged from 0.3 to 9 cm (2.4 ± 1.9 cm) in greatest diameter. Three tumors exhibited oncocytic differentiation. They were two follicular adenomas (Hürthle cell adenoma) and one papillary carcinoma (oncocytic variant) and were all noted in women with a mean age of 41 ± 16 years.

Regarding tumor stage,[24] 32 tumors were T1, 14 T2, 21 T3 and 9 T4. In 13 cases, lymph node sampling had been performed. Six of them were N0 (two follicular carcinomas, three papillary carcinomas and one papillary microcarcinoma), whereas the remaining 7 (three papillary and four medullary carcinomas) were N1. Table 1 lists the distribution of the different histologic types according to the tumor stage, tumor size and lymph node status.

Follow-up data were available for 33 of the 76 (43%) patients with malignant thyroid neoplasms (including papillary microcarcinomas) with a mean follow-up period of 60 ± 47 months (range 1–144 months). During this period of time, two deaths (one patient with medullary and one with undifferentiated carcinoma) and six relapses (one patient with papillary carcinoma, four patients with medullary and one with undifferentiated carcinoma) were recorded.

Table 1 Tumor stage and size of the 88 thyroid neoplasms

Histologic type	*Pathologic parameter*			
Stage	T1	T2	T3	T4
Papillary microcarcinoma ($N=16$)	16	—	—	—
Papillary carcinoma ($N=26$)	10	4	12	—
Follicular carcinoma ($N=15$)	4	6	5	—
Medullary carcinoma ($N=8$)	2	3	3	—
Poorly differentiated carcinoma ($N=3$)	—	1	1	1
Undifferentiated carcinoma ($N=8$)	—	—	—	8
Size (cm)	1–2	2–4	>4	Unknown
Follicular adenoma ($N=15$)	10	4	1	—
Papillary carcinoma ($N=26$)	13	6	5	2
Follicular carcinoma ($N=15$)	4	5	5	1
Medullary carcinoma ($N=8$)	4	1	1	2
Poorly differentiated carcinoma ($N=3$)	—	1	—	2
Lymph node status	N0	N1		
Papillary microcarcinoma ($N=1$)	1	—		
Papillary carcinoma ($N=6$)	3	3		
Medullary carcinoma ($N=4$)	0	4		

T, tumor stage.

Immunohistochemistry

Claudins expression

In general, positive claudin immunostaining was observed in 91% (83/91) of the cases. Claudins expression in the different histologic types is shown in Table 2. All claudins examined were expressed at the basolateral membrane surfaces in a linear fashion. However, a membranous dot-like pattern was noted quite often and occasionally the immunostaining was in the form of small vesicles located at the membrane. Although dots and vesicles could be found at any part of the basolateral membrane, they were more commonly seen at the apical end of the lateral membrane surface (Figure 1). In non-neoplastic thyroid tissue, claudins were focally expressed, mainly at hyperplastic follicular cells.

Claudin-1

Claudin-1 (Figure 1a) expression was present in 74% (67/91) of the cases, and more specifically in 87% of the follicular adenomas, 67% of the follicular carcinomas, 92% of the papillary carcinomas, 93% of the papillary microcarcinomas, 13% of the medullary carcinomas, 67% of the poorly differentiated carcinomas and 25% of the undifferentiated carcinomas. Statistical analysis revealed a significant reduction of claudin-1 expression in medullary and undifferentiated carcinomas compared to the other histologic types ($P<0.001$).

Table 2 Claudins' and occludin's expression in thyroid neoplasms

	Claudin-1	*Claudin-4*	*Claudin-7*	*Occludin*	P
Follicular adenoma ($N=15$)	13[a] (87%)	13 (87%)	10 (67%)	15 (100%)	0.083
Follicular carcinoma ($N=15$)	10 (67%)	12 (80%)	5 (33%)	12 (80%)	**0.007**
Papillary microcarcinoma ($N=16$)	15 (93%)	13 (81%)	11 (69%)	8 (50%)	**0.036**
Papillary carcinoma ($N=26$)	24 (92%)	23 (88%)	19 (73%)	25 (96%)	0.063
Medullary carcinoma ($N=8$)	1 (12.5%)	7 (87.5%)	2 (25%)	4 (50%)	**0.014**
Poorly differentiated carcinoma ($N=3$)	2 (67%)	2 (67%)	0 (0%)	1 (33%)	0.287
Undifferentiated carcinoma ($N=8$)	2 (25%)	2 (25%)	1 (13%)	7 (88%)	**0.008**
P-value	**<0.001**	**0.01**	**0.002**	**<0.001**	

Bold typing indicate *P*-values <0.05.

[a]No. of positive cases.

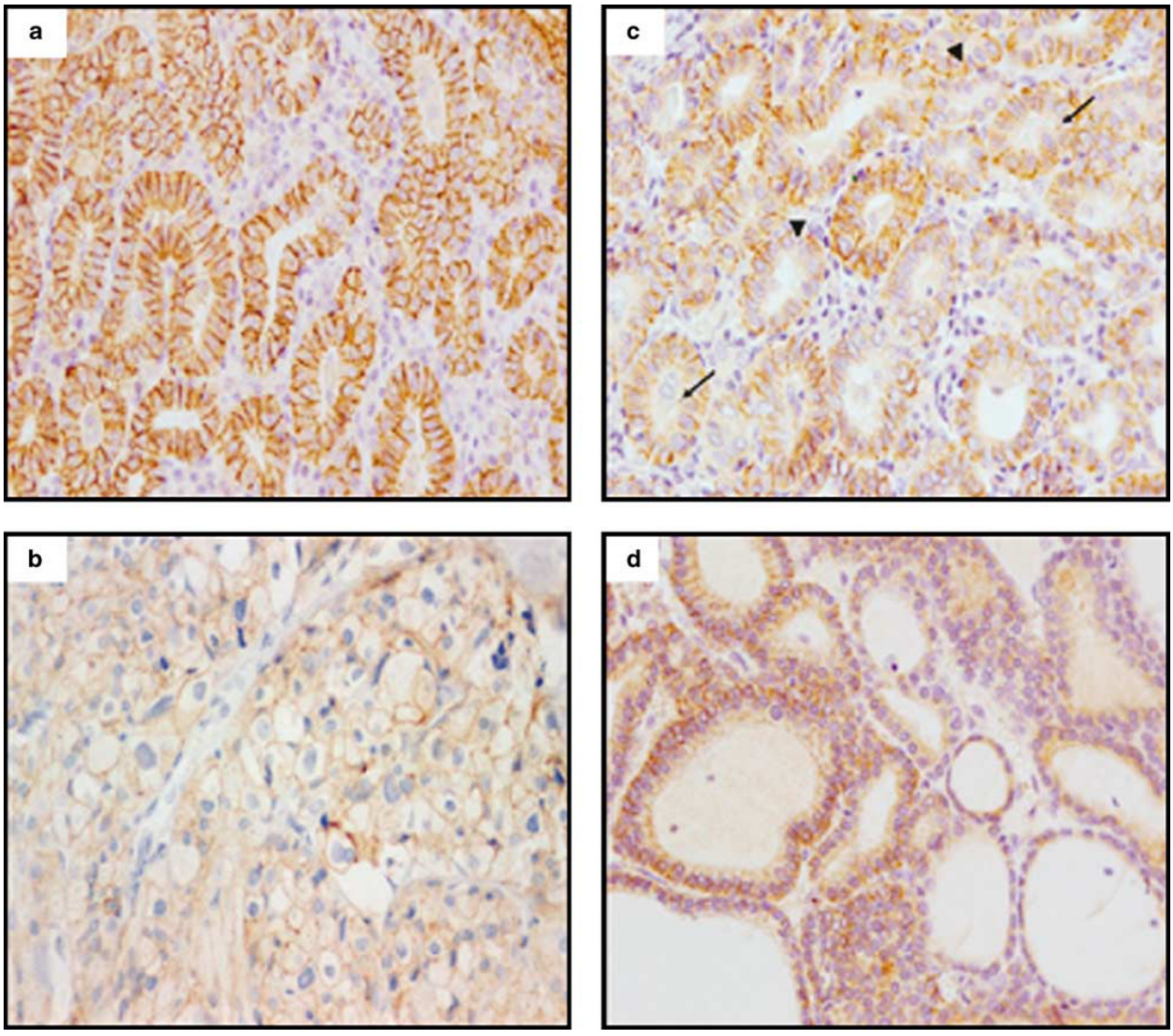

Figure 1 Immunohistochemical analysis of claudins' expression in thyroid neoplasms (streptavidin biotin peroxidase, ×400). (**a**) Strong membranous immunostaining of claudin-1 in a case of papillary carcinoma. (**b**) Cells of medullary carcinoma express claudin-4 in their membranes. (**c**) Continuous and dot-like membranous immunostaining of claudin-7 in papillary carcinoma. Dots are located at the apical end of the lateral membranous surface (arrow) and other parts of the lateral and basal membranous surface as well (arrowhead). (**d**) Continuous and dot-like immunostaining of claudin-7 in a case of follicular carcinoma.

Claudin-4

Claudin-4-positive immunostaining (Figure 1b) was observed in 72 of the 91 cases examined (79%) and in particular in 87% follicular adenomas, 80% follicular carcinomas, 88% papillary carcinomas, 81% papillary microcarcinomas, 88% medullary carcinomas, 67% poorly differentiated carcinomas and 25% undifferentiated carcinomas. Claudin-4 expression was significantly reduced in undifferentiated carcinomas compared to the other histologic types ($P=0.01$).

Claudin-7

Claudin-7 (Figure 1 c and d) was expressed in 53% (48/91) of the tumors. Claudin-7 expression was found in 67% of the follicular adenomas, 33% of the follicular carcinomas, 73% of the papillary carcinomas, 69% of the papillary microcarcinomas, 25% of the medullary carcinomas, 0% of the poorly differentiated carcinomas and 13% of the undifferentiated carcinomas. Statistical analysis revealed that claudin-7 was less frequently expressed in follicular, medullary, poorly differentiated and undifferentiated carcinomas as opposed to papillary carcinomas, papillary microcarcinomas and adenomas ($P=0.002$).

Occludin

Occludin was expressed in 72 of the 91 (79%) cases examined. Occludin's expression in the thyroid tumors examined is shown in Table 2. Of interest, occludin, although a tight junction protein, was only

rarely detected at the membranous surface (17 of the 72 positive cases). In the vast majority of the cases, occludin was detected as intracytoplasmic vesicles either alone (55 of the 72 positive cases) or together with membranous staining (10 cases: three adenomas, three papillary microcarcinomas, two papillary carcinomas, one medullary carcinoma and one undifferentiated carcinoma) (Figure 2) and only in seven cases (three adenomas, two papillary microcarcinomas, one papillary carcinoma and one poorly differentiated carcinoma) exhibited pure membranous staining pattern. In particular, occludin expression was noted in 100% of the follicular adenomas, in 80% of the follicular carcinomas, 96% of the papillary carcinomas, 50% of the papillary microcarcinomas, 50% of the medullary carcinomas, 33% of the poorly differentiated carcinomas and 88% of the undifferentiated carcinomas. Statistical analysis revealed a reduction of occludin expression in papillary microcarcinomas, poorly differentiated carcinomas and medullary carcinomas compared to the other histologic types of thyroid tumors ($P<0.001$). In non-neoplastic thyroid tissue occludin was focally expressed, mainly at hyperplastic follicular cells.

Statistical Analysis

Tight junctions' proteins expression was further correlated with histologic type, tumor size and stage and lymph node status.

Claudin-1 was not expressed in T2 medullary carcinomas in contrast to papillary and follicular carcinomas of the same stage ($P=0.004$). The same applied for occludin expression. A reduction of claudin-1 expression was also noted in T3 medullary and follicular carcinomas compared to T3 papillary carcinomas ($P=0.010$ and 0.011, respectively) (Table 3).

Claudin-4 was the most frequently expressed tight junction protein in medullary carcinomas ($P=0.014$) (Table 2). Moreover, follicular carcinomas greater than 4 cm expressed more frequently claudin-4 and occludin than claudin-1 and 7 ($P=0.04$) (Table 4). The same was true for follicular carcinomas stage T3 ($P=0.04$) (Table 3).

Claudin-7 expression was reduced in follicular carcinomas compared to the other proteins tested ($P=0.007$). Moreover, papillary carcinomas expressed less commonly claudin-7, compared to the other claudins and occludin, although the difference did not reach statistical significance ($P=0.063$) (Table 2).

Occludin was the only tight junction protein frequently expressed in undifferentiated carcinomas ($P=0.008$). The opposite was true for papillary microcarcinomas ($P=0.036$) (Table 2). Regarding tumor size, papillary carcinomas measuring less than 1 cm (microcarcinomas) exhibited reduced expression of occludin compared to papillary carcinomas of size 2–4 cm ($P=0.021$). Surprisingly, occludin expression was also reduced in tumors measuring >4 cm (Table 4).

No correlation between claudins and occludin expression and lymph node status was noted.

Univariate and multivariate survival analysis

On univariate analysis, tumor type (medullary and undifferentiated carcinomas compared to the other neoplasms) and loss of claudin-1 expression correlated with worse disease-free survival ($P=0.01$) (Figure 3).

Tumor type and stage were correlated with overall survival ($P=0.02$ and 0.002, respectively). Additionally, increased expression of claudin-1 was

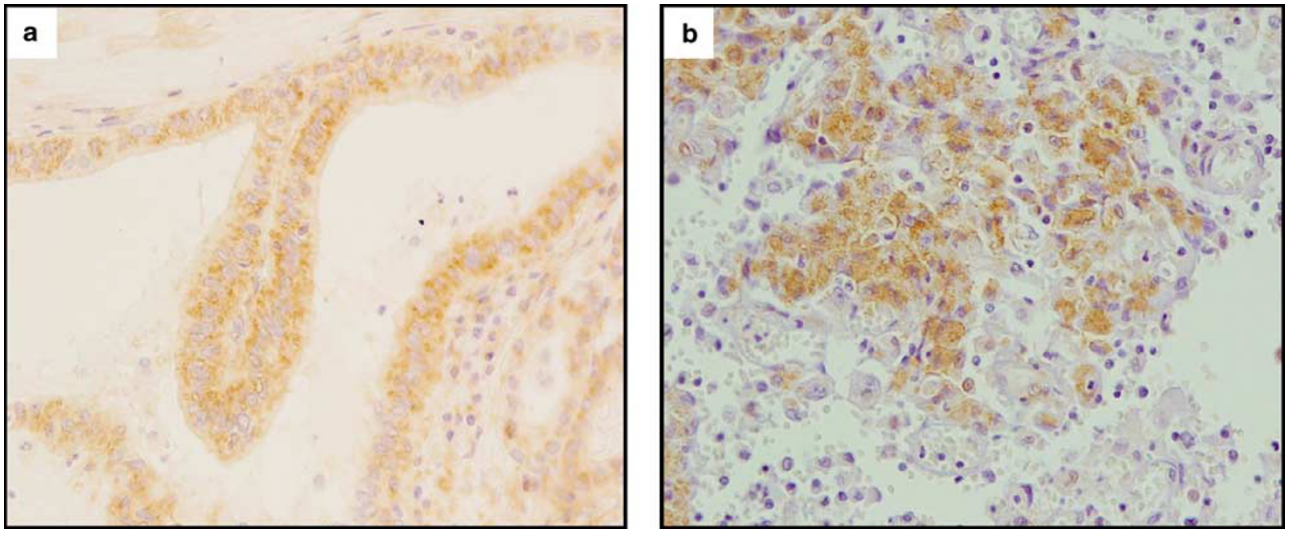

Figure 2 Immunohistochemical expression of occludin in a case of papillary (**a**) and undifferentiated (**b**) carcinoma (streptavidin biotin peroxidase, $\times 400$).

Table 3 Correlation between tumor's stage and claudins' and occludin's expression for papillary carcinomas, follicular carcinomas, medullary carcinomas, poorly differentiated carcinomas and undifferentiated carcinomas

	Stage (T)	*Claudin-1*	*Claudin-4*	*Claudin-7*	*Occludin*
Papillary carcinoma	T1 ($N=10$)	9[a] (90%)	8 (80%)	8 (80%)	10 (100%)
	T2 ($N=4$)	4 (100%)	3 (75%)	3 (75%)	4 (100%)
	T3 ($N=12$)	11 (92%)	12 (100%)	8 (67%)	11 (92%)
Follicular carcinoma	T1 ($N=4$)	3 (75%)	2 (50%)	1 (25%)	3 (75%)
	T2 ($N=6$)	5 (83%)	4 (67%)	3 (50%)	5 (83%)
	T3 ($N=5$)	2 (40%)	5 (100%)	1 (20%)	4 (80%)[b]
Medullary carcinoma	T1 ($N=2$)	1 (50%)	2 (100%)	1 (50%)	2 (100%)
	T2 ($N=3$)	0 (0%)	2 (66.7%)	0 (0%)	0 (0%)
	T3 ($N=3$)	0 (0%)	1 (33%)	0 (0%)	2 (67%)
Poorly differentiated carcinoma	T1 ($N=0$)	—	—	—	—
	T2 ($N=1$)	1 (100%)	1 (100%)	0 (0%)	0 (0%)
	T3 ($N=1$)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	T4 ($N=1$)	1 (1000%)	1 (100%)	0 (0%)	1 (100%)
Undifferentiated carcinoma	T1/T2/T3 ($N=0$)	—	—	—	—
	T4 ($N=8$)	2 (25%)	2 (25%)	1 (123%)	7 (88%)

[a]No. of positive cases.
[b]$P=0.04$.

Table 4 Correlation between tumor's size and claudins' and occludin's expression for papillary carcinomas (including microcarcinomas), follicular adenomas, follicular carcinomas and medullary carcinomas

	Size (cm)	*Claudin-1*	*Claudin-4*	*Claudin-7*	*Occludin*
Papillary carcinoma and microcarcinoma	<1 ($N=16$)	15[a] (94%)	13 (81%)	11 (69%)	8 (50%)[b]
	1–2 ($N=13$)	13 (100%)	12 (92%)	11 (85%)	13 (100%)
	2–4 ($N=6$)	5 (83%)	4 (67%)	4 (67%)	5 (83%)
	>4 ($N=5$)	3 (60%)	4 (80%)	2 (40%)	2 (40%)
Follicular adenoma	<1 ($N=0$)	—	—	—	—
	1–2 ($N=10$)	9 (90%)	9 (90%)	1 (10%)	10 (100%)
	2–4 ($N=4$)	3 (75%)	3 (75%)	3 (75%)	4 (100%)
	>4 ($N=1$)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Follicular carcinoma	<1 ($N=0$)	—	—	—	—
	1–2 ($N=4$)	3 (75%)	2 (50%)	1 (25%)	3 (75%)
	2–4 ($N=5$)	5 (100%)	4 (80%)	3 (60%)	5 (100%)
	>4 ($N=5$)	2 (40%)	5 (100%)	1 (20%)	4 (80%)[c]
Medullary carcinomas	1–2 ($N=4$)	1 (25%)	3 (75%)	1 (25%)	2 (50%)
	2–4 ($N=1$)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
	>4 ($N=1$)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
Poorly differentiated carcinoma					
	<2 ($N=0$)	—	—	—	—
	2–4 ($N=1$)	1 (100%)	1 (100%)	—	—
	>4 ($N=0$)	—	—	—	—

[a]No. of positive cases.
[b]$P=0.021$.
[c]$P=0.04$.

associated with slightly better prognosis, although this was not statistically significant ($P=0.093$).

Cox multivariate analysis of survival revealed that claudins and occludin's expression and the clinicopathologic parameters studied were not independently associated with disease-free or overall survival.

Discussion

This study examined the expression of tight junctions' proteins, occludin and claudin-1, -4 and -7 in thyroid neoplasms. The role of tight junctions' proteins in cancer initiation and progression has been under intense investigation. Reduced expression,

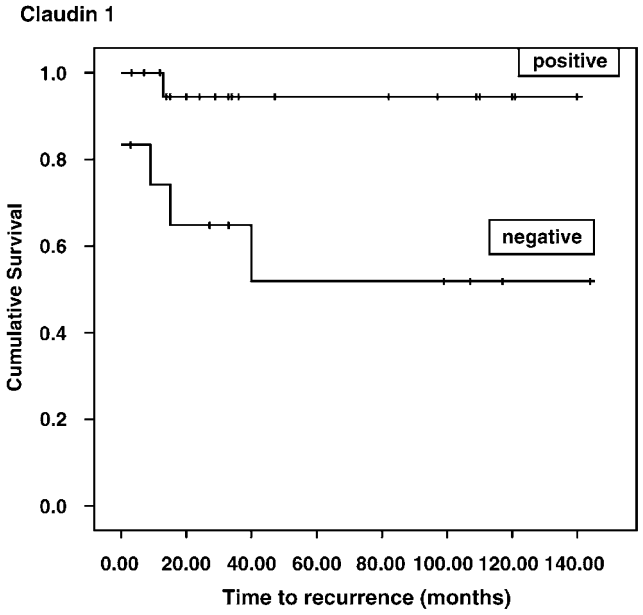

Figure 3 Kaplan–Meier disease-free analysis curve in thyroid carcinomas for claudin-1 expression. A strong association was detected between loss of protein's expression and disease recurrence ($P = 0.01$).

elevated levels or subcellular relocalization of tight junctions proteins have been reported in various human cancers and are variably associated with tumor differentiation and survival.[13–17,19–21,27]

Tight junctions' alterations have been observed in thyroid neoplasms by freeze-fracture analysis.[28,29] In these studies, aberrant tight junction formation characterized poorly differentiated carcinomas or compact oncocytic tumors. Recently, gene expression analysis has shown enhanced expression of claudin-3, -4 and -7 genes in thyroid neoplastic tissues[22] and of claudin-1 and 16 genes in papillary carcinomas of the thyroid.[30] These studies make no specific comment to the different histologic types of thyroid tumors. To the best of our knowledge, the present study is the first one to investigate the expression of tight junction proteins in the various histologic types of thyroid tumors and to correlate them with tumor stage and size and patients prognosis.

In this study, a loss of claudin-1, -4 and -7 expression was frequently seen in undifferentiated thyroid carcinomas. Loss of claudin-7 expression was also noted in poorly differentiated carcinomas. Reduced claudins' expression and associated loss of functional tight junctions are usually a feature of poorly differentiated tumors of various origins (ie breast, esophageal, gastric, colon and thyroid carcinomas).[14–16,30,31] Apparently, loss of claudins expression results in loss of cell-to-cell adhesion, thereby preventing the cells to form organized epithelial structures and leading in an undifferentiated phenotype. Undifferentiated carcinomas of the thyroid gland are the result of anaplastic transformation of a pre-existing well-differentiated tumor.[32] The current study suggests that dedifferentiation of thyroid neoplasms involves tight junction impairment via downregulation of claudin-1, -4 and -7.

Claudin-4 was more frequently expressed in medullary carcinomas than the other proteins tested. Tight junctions in medullary carcinomas of the thyroid, despite their non-epithelial derivation, have been described before and are considered as an evidence of a common stem cell origin of medullary and follicular carcinomas.[33] The protein composition of these tight junctions has not been illustrated so far. The present findings imply that claudin-4 is a basic constituent of tight junctions in medullary carcinomas. The significance of the expression of claudin-4 and the parallel reduced expression of the other claudins and occludin in medullary carcinomas remains unclear. Further studies with a larger series of these tumors are needed to clarify the possible roles of claudin-4 expression in medullary carcinomas and its connection with tumor growth.[18]

A reduction in claudin-7 expression was noted in follicular carcinomas. Moreover, claudin-7 expression, when compared to the other claudins, was found reduced in papillary carcinomas, albeit the difference did not reach statistical significance. Additionally, claudin-7 was never expressed in poorly differentiated carcinomas although no statistical significance was found possibly due to the small number of cases. This can lead to the speculation that claudin-7 loss is an early step in tight junctions protein deregulation in thyroid carcinogenesis, although further studies are needed to validate this observation.

Loss of tight junction integrity leads to an increased influx of growth factors, nutrients and other tumor-promoting molecules, thereby providing an advantage for the development, survival and growth of tumor cells.[17,34] Notably, reduction of claudin-7 was more obvious in follicular carcinomas with large size or advanced stage and was accompanied by claudin-1 reduction. In contrast, T1–T2 tumors or tumors less than 4 cm exhibited a more uniform expression of all three claudins studied. Thus, a possible tight junction impairment in large-sized or in advanced stage follicular carcinomas is suggested through claudin-1 and -7 reduction.

Occludin expression was retained in undifferentiated carcinomas of the thyroid gland. It is worth to note that occludin alone is ineffective in adequate tight junction strands formation.[7] Moreover, an intracytoplasmic localization was noted in the vast majority of the tumors. The intracellular localization of occludin is difficult to explain although some speculations can be attempted, that is formation of intercellular bodies,[35] tight junction remodelling[36] or intracytoplasmic, tight junction unrelated function of occludin.[37] An interesting finding was that occludin was less commonly expressed in papillary microcarcinomas and poorly differentiated carcinomas, compared to other histologic types. Occludin is

considered a feature of organized epithelial structures and is commonly lost in the solid components of various cancers.[38,39] These observations are not in disparity with our finding of reduced occludin expression in papillary microcarcinomas. Membranous pattern of occludin staining was more commonly seen in papillary microcarcinomas and follicular adenomas. On the contrary, papillary, medullary and undifferentiated carcinomas exhibited mainly a cytoplasmic distribution of occludin. Accordingly, poorly differentiated carcinomas displayed a reduced membranous staining (33% of the cases). These findings may imply that occludin is located in the tight junctions of follicular adenomas and papillary microcarcinomas of the thyroid, whereas it is lost from the tight junctions of the apparently malignant forms of thyroid neoplasms.

Interestingly, claudin-1 expression was found to be a prognostic factor for disease-free survival and may prove to be a useful prognostic marker for determining the patients that are in higher risk for recurrence. In line with our observations, reduced claudin-1 expression correlates with disease recurrence and poor survival in various cancers such as colonic and prostatic carcinomas.[14,40]

In conclusion, to the best of our knowledge, this is the first report on claudin-1, -4, -7 and occludin expression in thyroid neoplasms. The present study revealed a differential claudin and occludin expression in the various types of thyroid neoplasms. These findings may represent histogenetic differences (epithelial *vs* neuroendocrine derivation) or differential impact of claudin reduction in neoplastic progression. Reduced claudins' expression correlates with loss of differentiation of thyroid cancer. Membranous occludin expression seems to correlate with thyroid neoplasms of low malignant potential, as it was frequently found positive in follicular adenomas and papillary microcarcinomas. However, the cytoplasmic distribution of this tight junction's protein in thyroid carcinomas is a challenging finding. Further research is warranted in order to elucidate the clinical relevance of aberrant tight junction expression in thyroid neoplasia and the possible prognostic implications of reduced claudin-1 expression.

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