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ID Proteins May Reduce Aggressiveness of Thyroid Tumors

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Abstract

ID genes have an important function in the cell cycle, and ID proteins may help identify aggressive tumors, besides being considered promising therapeutic targets. However, their role in thyroid tumors is still poorly understood. We examined *ID* expression and their correlation with diagnostic and prognostic features aiming to find a clinical application in differentiated thyroid carcinoma (DTC) cases. mRNA levels of *ID1*, *ID2*, *ID3*, and *ID4* genes were quantified and their expression was observed by immunohistochemistry in 194 thyroid samples including 68 goiters, 16 follicular adenomas, 75 classic papillary thyroid carcinomas, 18 follicular variants of papillary thyroid carcinoma, 5 follicular thyroid carcinomas, and 1 anaplastic thyroid cancer, besides 11 normal thyroid tissues. DTC patients were managed according to standard protocols and followed up for $M = 28 \pm 16$ months. *ID2*, *ID3*, and *ID4* mRNA levels were higher in benign (2.0 ± 1.9; 0.6 ± 0.6; and 0.7 ± 1.0 AU, respectively) than those in malignant nodules (0.30 ± 0.62; 0.3 ± 0.3; and 0.2 ± 0.3 AU, respectively, $p < 0.0001$ for all three genes) and were associated with no extra thyroid invasion or metastasis at diagnosis. *ID3* nuclear protein expression was higher in benign than that in malignant cells (5.2 ± 0.9 vs 3.0 ± 1.8 AU; $p < 0.0001$). On the contrary, the cytoplasmic expression of *ID3* was higher in malignant than that in benign lesions (5.7 ± 1.5 vs 4.0 ± 1.4 AU; $p < 0.0001$). Our data indicate that *ID* genes are involved in thyroid tumorigenesis and suggest these genes act impeding the evolution of more aggressive phenotypes. The different patterns of their tissue expression may help identify malignancy and characterize thyroid lesion aggressiveness.

Keywords ID genes · bHLH · Thyroid cancer · Aggressiveness

Introduction

According to the Surveillance, Epidemiology, and End Results (SEER), Program of the National Cancer Institute, approximately 1.2% of North American men and women will be diagnosed with thyroid cancer at some point during their lifetime [1]. Most of these tumors have an indolent progression and the majority of patients with differentiated thyroid cancer (DTC) present good outcome when treated appropriately; however, recurrences occur in a large percentage of cases and some patients eventually stop responding to conventional treatment and die [1]. In fact, age-adjusted rate of death from DTC, based on 2010–2014 data, was calculated to be 0.5 per 100,000 individuals per year [2]. In addition, the increasing use of some inadequately indicated image scans of the neck, cervical ultrasounds in particular, has strongly contributed to an epidemic of small tumor diagnostics [2]. Hence, the current challenge in clinical practice is to identify which individuals need a more aggressive approach. However, the recognition of most patients, who may be spared from unnecessary treatments and procedures, is even more important [2–4].

ID proteins have long been considered as promising targets in cancer therapy because of their role inhibiting helix-loop-helix transcription factors family [5, 6]. There are four recognized members of ID family in vertebrates: *ID1*, *ID2*, *ID3*, and *ID4*, whose main characteristic is their ability to bind to bHLH transcription factors, subsequently binding with the DNA [7, 8]. Therefore, when ID proteins are present, transcription factors are unable to bind to DNA and activate transcription [9].

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These proteins usually have increased expression between G1 and S phases in the cell cycle. Since ID genes control the transcription of other genes, their function in the cell cycle and expression must play different roles and vary depending on the tissue analyzed [10].

Working with thyroid cancer cells, Kebebew et al. suggested that *ID1* could have an important role in the metastatic process [11]. However, a possible role in human thyroid cells is uncertain [12]. Moreover, other IDs have not been better investigated, to the best of our knowledge, in thyroid tumors. Looking for a possible utility of ID identification in the clinical setting, this study aimed to examine ID expression correlating it with diagnostic and prognostic features observed in DTC cases.

Materials and Methods

Patients and Samples

This study was approved by the Research Ethics Committees of the institutions involved (38462414.2.0000.5404/948.744). Quantitative real-time PCR (qPCR) was made in 194 samples, including 174 fresh frozen tissues and 20 formalin-fixed paraffin-embedded (FFPE) tissues (142 women and 36 men, 45.2 ± 14.9 years old). There were 16 follicular adenomas (FA), 68 goiters, 5 follicular thyroid carcinomas (FTC), 75 classic papillary thyroid carcinomas (CPTC), 18 follicular variants of papillary thyroid carcinoma (FVPTC), 1 anaplastic thyroid carcinoma, and 11 normal thyroid tissues. In addition, we performed immunohistochemistry in 113 samples (97 women and 16 men, 45.2 ± 14.9 years old), including 86 fresh frozen tissues and 27 FFPE tissues. There were 85 goiters, 1 FA, 17 CPTC, 4 FVPTC, and 6 FC.

All samples were submitted to histological analysis by an experienced pathologist in the moment of diagnosis, and two experts in thyroid pathology (ISB and AHJMF) further confirmed the diagnostic. Fresh samples were frozen in liquid nitrogen right after surgery and stored at − 80 °C for mRNA quantifications. Only samples with at least 70% of neoplastic cells were included in this study. Normal samples were obtained from the contralateral lobe of benign and small nodules.

Patients' clinical information were obtained from their charts. All patients were submitted to total thyroidectomy, and then to periodic total body scans with 131I, serum TSH and thyroglobulin (Tg) measurements, computed tomography scan, and other eventual procedures to detect distant metastasis, according to a follow-up protocol based on current guidelines [13]. Patients who were pregnant, underage, and experienced accidental or medical exposure to ionizing radiation were excluded from this study.

Patients were followed up for at least 12 months after surgery ($M = 28 \pm 16$ months). Since patients with indeterminate response, or with persistent abnormal thyroglobulin values, or rising anti-thyroglobulin antibody levels in the absence of localizable disease (biochemical incomplete response) were not included in any statistical evaluation involving patients' outcome, only 67 data were analyzed.

Quantitative Real-Time PCR

RNA was extracted from all 194 samples. RNA extraction of fresh frozen samples was performed using TRIzol reagent protocol as previously described [14]. RNA was extracted from FFPE samples with Recover All Total Nucleic Acid Isolation FFPE kit (Thermo Fisher Scientific, Waltham, MA, USA), following manufacturer instructions. Reverse transcription assays were performed using Superscript First-Strand – Synthesis System for RT-PCR kit (Thermo Fisher Scientific, Waltham, MA, USA), also following manufacturer instructions. Commercially available TaqMan gene expression assays (Applied Biosystems, Foster City, USA) were used for *ID1* (Hs03676575_s1), *ID2* (Hs04187239_m1), *ID3* (Hs00954037_g1), and *ID4* (Hs02912975_g1), relative to the internal reference gene *GAPDH* (Hs03929097_g1). TaqMan gene expression PCR master mix 2× (Applied Biosystems) was used in the reactions according to the manufacturer protocol. Analyses were performed for each sample in triplicate, with 7500 RT-PCR system (Applied Biosystems) using a four-stage program: 50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, and 60 °C for 1 min. The sequence detection software (Applied Biosystems SDS v1.3 Software) to obtain the threshold cycle (Ct) was employed. ΔΔCT was used, in which the amount of target (*ID1*, *ID2*, *ID3*, and *ID4*) normalized to an endogenous reference and relative to the calibrator is given by $2^{-\Delta\Delta CT}$. Levels of expression were given in arbitrary units (AU).

Immunohistochemistry

After reviewed by the pathologist (ISB or AHJMF), the two most representative areas with 2 mm of each sample were selected in order to build a tissue microarray (TMA) for immunohistochemical detection of *ID1*, *ID2*, *ID3*, and *ID4*.

TMA slides were deparaffinized in hot xylene and in decreasing concentrations of alcohol. Peroxide activity was blocked with H_2O_2 for 12 min. All slides were subjected to heat-induced antigen retrieval, with 10% citrate buffer in a steamer (90 °C for 40 min). The slides were embedded in 3% milk solution for 30 min to block nonspecific reactions. Slides were then incubated at 37 °C for 30 min and overnight at 4 °C with 1:2000 diluted anti-*ID1* rabbit monoclonal EPR7098 antibody (ab134163) solution, 1:200 anti-*ID2* mouse monoclonal 4E12G5 antibody (ab166708) solution, 1:300 anti-*ID3* rabbit monoclonal 10C3 antibody (ab90055) solution, and 1:50 anti-*ID4* rabbit polyclonal antibody (ab49261) solution, all from ABCAM (Cambridge, MA, USA). Advanced biotin-free polymer detection system

was used (DAKO, Santa Clara, CA, USA), and DAB (3,3-diaminobenzidine tetrahydrochloride; Sigma-Aldrich, St. Louis, MA, USA) was applied. Slides were counterstained with hematoxylin.

All slides were quantified using the Allred score by a pathologist (ISB or AHJMF) blinded to the prior evaluations and to tumor features.

Statistical Analysis

Statistical analysis was performed through Statistical Analysis System (SAS System for Windows) v9.4 (SAS Institute Inc., Cary, NC, USA). Groups were compared by Fisher's exact test, Mann-Whitney test, and chi-square test. The correlation between proteins and tumor size was evaluated by Spearman's coefficient. To demonstrate the accuracy of gene expression studies to predict malignancy, the receiver operating characteristic (ROC) curve was used. All tests were conducted with a $p = 0.05$ significance level.

Results

qPCR

As represented in Table 1, the group of 99 patients with malignant thyroid nodules did not differ from the 84 patients with benign nodules concerning gender (79 females and 19 males vs 63 females and 17 males, respectively, $p = 0.7583$), presence of encapsulated nodules, multinodularity, and presence of concomitant chronic lymphocytic thyroiditis. The two groups are different concerning age at diagnosis (49.1 ± 13.6 years vs 42.0 ± 15.1 years respectively $p = 0.0002$). Hence, age factor was corrected in all statistical analyses where it could be a confounding factor.

All *ID*s, aside from *ID1* (0.3 ± 0.4 AU vs 0.5 ± 0.9 AU, respectively, $p = 0.2780$), were able to differentiate benign from malignant nodules, as presented in Fig. 1. *ID2*, *ID3*, and *ID4* showed significantly higher levels of mRNA in benign (2.0 ± 1.9; 0.6 ± 0.6; and 0.7 ± 1.0 AU, respectively) than those in malignant nodules (0.30 ± 0.62; 0.3 ± 0.3; and 0.2 ± 0.3 AU, respectively, $p < 0.0001$ for all three genes) as shown in Fig. 1.

Aiming to investigate whether *ID1*, *ID2*, *ID3*, and *ID4* expression values could predict malignancy, a ROC analysis based on predicted probabilities from logistic regression models was further performed. *ID1* was not able to predict malignancy, but *ID2* was able to identify malignant nodules with an 80.92% accuracy, 84.70% sensibility, 76% specificity, 82.15% positive predictive value (PPV), and 79.21% negative predictive value (NPV), using a cutoff of 0.939 AU. *ID3* was able to identify malignant nodules with 83.70% sensibility, 43.20% specificity, 65.35% accuracy, 64.02% PPV, and 68.70% NPV, using a cutoff of 0.454 AU. *ID4* was able to identify malignant nodules with 75.17% accuracy, 77.60% sensibility, 72% specificity, 78.33% PPV, and 71.14% NPV, using a cutoff of 0.267 AU.

The comparison of *ID* expression with clinical and pathological features showed that *ID2* was not associated with any of the characteristics of aggressiveness analyzed, as demonstrated in Table 2. The mRNA levels of *ID1*, *ID3*, and *ID4* were lower in samples of patients that presented metastasis at diagnosis, concomitant chronic lymphocytic thyroiditis, and extra thyroid invasion (Table 2). The larger the tumor, the lower the mRNA levels of both *ID3* and *ID4* ($p = 0.0056$ and $p = 0.217$, respectively). The anaplastic carcinoma presented low mRNA levels: *ID1* = 0.01 AU; *ID2* = 0.263 AU; *ID3* = 0.026 AU; and *ID4* = 0.003 AU.

Correlation between *ID* expression and patients' clinical outcome was not found.

Table 1 Clinical and pathological features of the 84 patients with benign thyroid nodules and the 99 patients with malignant thyroid nodules

Clinical and pathological features	Benign (*N*)	Malignant (*N*)	Total (*N*)	*p*
Age at diagnosis	49.1 ± 13.6 (82)	42.0 ± 15.1 (100)	45.2 ± 14.9 (182)	*0.0002**
Tumor size	2.5 ± 2.1 (63)	2.2 ± 1.9 (99)	2.3 ± 2.0 (162)	0.4086
Gender				
Female	78.8% (63)	80.6% (79)	79.8% (142)	0.7583
Male	21.3% (17)	19.4% (19)	20.2% (36)	
Tumor multinodularity				
No	40.6% (28)	52.5% (52)	47.6% (80)	0.1272
Yes	59.4% (41)	47.5% (47)	52.4% (88)	
Encapsulated nodule				
No	91.2% (52)	86.1% (62)	88.4% (114)	0.3679
Yes	8.8% (5)	13.9% (10)	11.6% (15)	
Concomitant chronic lymphocytic thyroiditis				
Absent	71.0% (44)	64.9% (50)	67.6% (94)	0.4499
Present	29.0% (18)	35.1% (27)	32.4% (45)	

*$p < 0.05$

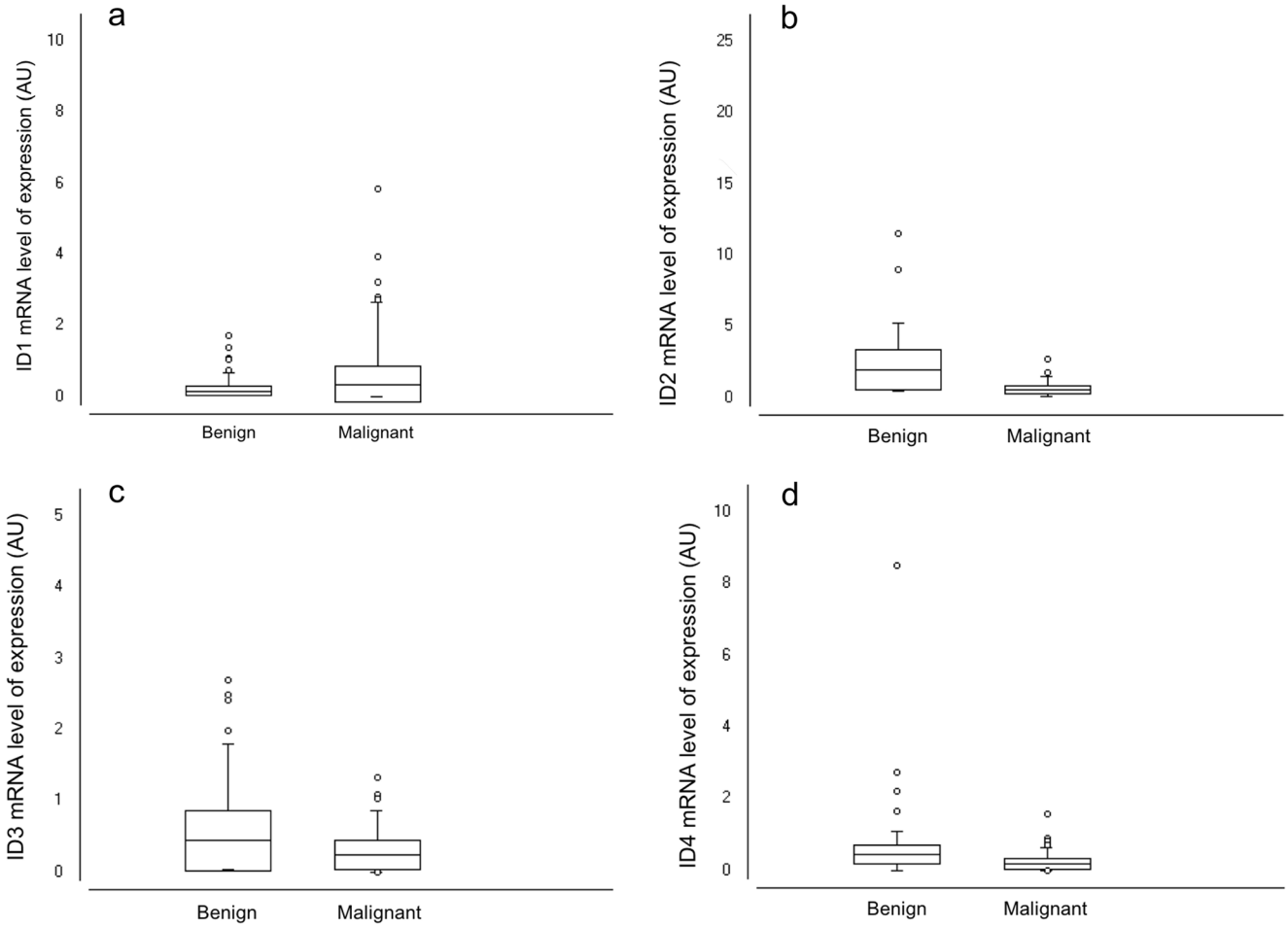

Fig. 1 Thyroid-specific transcription factor mRNA levels in 99 benign and 84 malignant thyroid lesions. **a** *ID1* mRNA expression by quantitative PCR in benign and malignant thyroid lesions ($p = 0.2780$). **b** *ID2* mRNA expression by quantitative PCR in benign and malignant thyroid lesions ($p < 0.0001$). **c** *ID3* mRNA expression by quantitative PCR in benign and malignant thyroid lesions ($p < 0.0001$). **d** *ID4* mRNA expression by quantitative PCR in benign and malignant thyroid lesions ($p < 0.0001$)

Immunohistochemistry

Unfortunately, results with *ID2* and *ID4* antibodies were not satisfactory.

ID1 was positive in both the nucleus and the cytoplasm. A higher *ID1* expression was observed in the nucleus of benign cells than in malignant thyroid cells (4.6 ± 1.8 vs 0.7 ± 1.6 AU, $p < 0.0001$). However, there was no difference

Table 2 Clinical and pathological features regarding *ID1*, *ID2*, *ID3*, and *ID4* expression

Clinical and pathological features	*ID1* (*N*)	*p*	*ID2* (*N*)	*p*	*ID3* (*N*)	*p*	*ID4* (*N*)	*p*
Metastasis at diagnosis								
Absent	0.70 ± 1.11 (47)	*0.0012**	0.83 ± 1.21 (46)	0.4499	0.41 ± 0.36 (47)	*< 0.0001**	0.29 ± 0.30 (46)	*0.0070**
Present	0.30 ± 0.62 (50)		0.57 ± 0.43 (50)		0.17 ± 0.19 (49)		0.17 ± 0.19 (50)	
Concomitant chronic lymphocytic thyroiditis								
Absent	0.60 ± 0.87 (50)	0.0507	0.67 ± 0.61 (50)	0.8011	0.38 ± 0.33 (50)	*0.0431**	0.30 ± 0.29 (50)	0.0752
Present	0.39 ± 0.63 (27)		0.82 ± 1.49 (26)		0.26 ± 0.20 (27)		0.23 ± 0.22 (26)	
Extra thyroidal invasion								
Absent	0.62 ± 1.04 (64)	*0.0027**	0.79 ± 1.07 (63)	0.2026	0.37 ± 0.33 (64)	*< 0.0001**	0.30 ± 0.29 (63)	*< 0.0001**
Present	0.29 ± 0.54 (34)		0.51 ± 0.35 (34)		0.13 ± 0.17 (33)		0.10 ± 0.09 (34)	

*$p < 0.05$

between benign and malignant nodules regarding *ID1* cytoplasmic expression (4.4 ± 1.8 vs 5.3 ± 1.5 AU, $p = 0.1637$), as shown in Fig. 2.

ID3 was also expressed both in the nucleus and in the cytoplasm but presented a different pattern of expression in benign and malignant cells: *ID3* had a higher nuclear expression in benign cells than in malignant cells (5.2 ± 0.9 vs 3.0 ± 18 AU, $p < 0.0001$). On the contrary, *ID3* cytoplasmic expression was higher in malignant cells than that in benign thyroid cells (5.7 ± 1.5 vs 4.0 ± 1.4 AU, $p < 0.0001$), as shown in Fig. 3.

A ROC analysis of *ID1* nuclear expression showed that it distinguished malignant from benign thyroid tissues with 90.13% accuracy, 89.5% sensibility, 90.30% specificity, 70.91% PPV, and 97.02% NPV, with a cutoff of 2.0 AU. Cytoplasmic expression distinguished malignant from benign cells with 39.13% accuracy, 80% sensibility, 27.8% specificity, 23.49% PPV, and 83.38% NPV, with a cutoff of 5.0 AU.

ID3 nuclear expression distinguished malignant from benign thyroid cells with 83.30% accuracy, 75% sensibility, 86.5% specificity, 68.14% PPV, and 89.99% NPV, with a cutoff of 4.0 AU. *ID3* cytoplasmic expression distinguished malignant from benign cells with 85.11% accuracy, 63.6% sensibility, 94.2% specificity, 82.25% PPV, and 85.97% NPV, with a cutoff of 4.0 AU.

ID1 and *ID3* expressions were not associated with any of the clinical or pathological parameter of tumor aggressiveness analyzed or with patients' clinical outcome.

ID1 and *ID3* qPCR × IHC Expression

The expression of *ID1* and *ID3* qPCR and immunohistochemistry (IHC) expression results complement and confirm each other. Both *ID1* and *ID3* were more expressed in benign than in malignant thyroid tumor cells, as shown in Table 3. ID3 is the only ID protein to present a higher cytoplasmic expression in malignant cells, being therefore able to distinguish malignant from benign thyroid cells.

Discussion

The data of this study showed lower mRNA levels of *ID1*, *ID2*, *ID3*, and *ID4* genes in malignant thyroid samples and in tumors with less features of aggressiveness. Kebebew et al. 2003, 2004 also described a decreased level of *ID1* in malignant thyroid tissues compared to benign tissues [11, 15]. This must be due to different methodologies of analysis, types, and numbers of samples [16]. Deleu et al. 2002 confirmed diminished levels of *ID3* mRNA in papillary cancer tissue versus normal tissue, showing that in papillary thyroid carcinomas, *ID3* mRNA was downregulated and suggesting a possible role for *ID* genes in thyrocyte differentiation rather than in proliferation [17].

Contradicting our results, Kebebew et al. 2003, 2004 found higher expression of *ID1* in more aggressive variants of thyroid nodules, including anaplastic and medullary thyroid

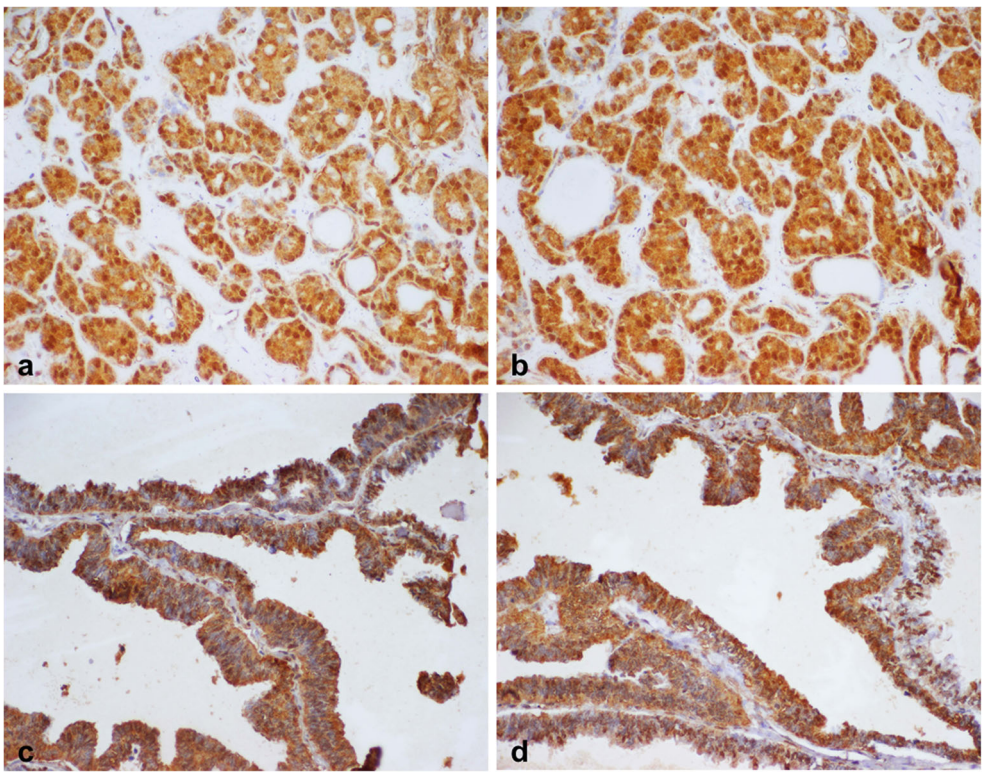

Fig. 2 **a**, **b** *ID1* nuclear and cytoplasmic expression in goiter. **c**, **d** *ID1* nuclear and cytoplasmic expression in PTC

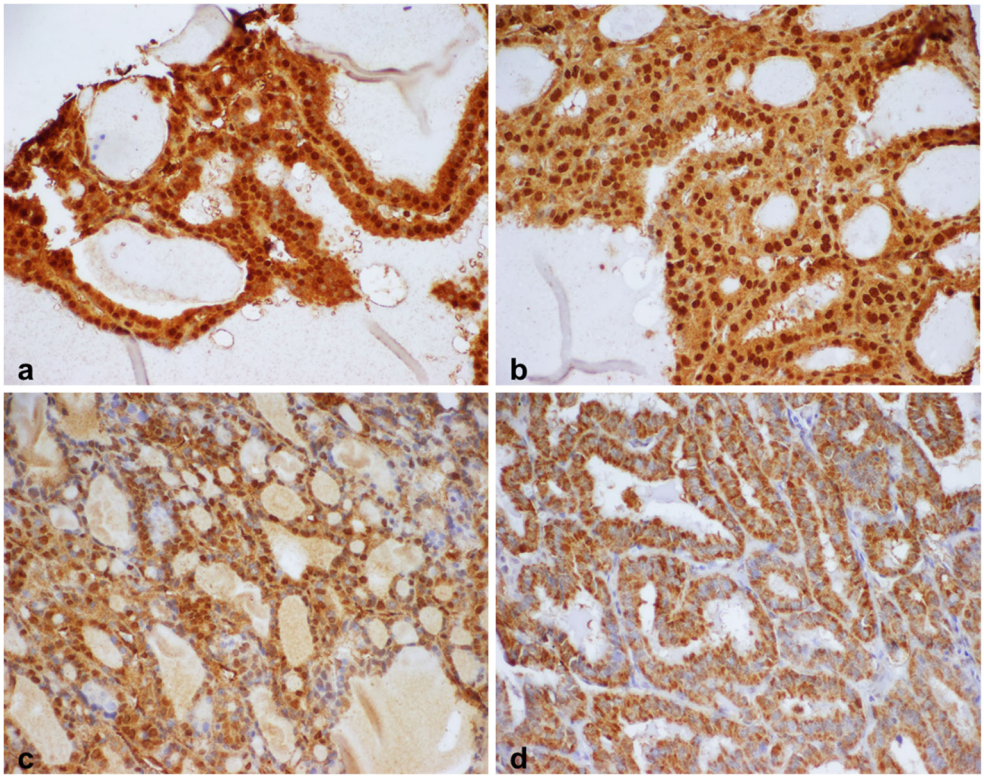

Fig. 3 **a**, **b** *ID3* nuclear expression in goiter. **c**, **d** *ID3* cytoplasmic expression in papillary PTC and FTC

cancer [11, 15]. Yokota et al. 1999 demonstrated that rats with *ID2* gene silenced did not develop lymph nodes and presented a reduced number of natural killer cells [18]. Pan et al. 1999 and Riviera et al. 2000 silenced *ID3* in rats and observed a lower production of B cells and defects in the development of T cells [19, 20]. In addition, Morse D demonstrated that the *ID3* silenced rats presented a defect in MHC classes I and II [21]. All these results point to a direct relationship between the *ID* genes and the immunological system, suggesting that *ID* expression may collaborate for a normal function of the immunological system. Therefore, if these genes are downregulated in a tissue, the defense cells would be less efficient in preventing the dedifferentiated process. In fact, the results support this assumption since it demonstrated lower expression of *ID* genes in malignant—compared to benign—nodules and normal thyroid tissues. The low expression associated to more aggressive tumors observed also indicates a less effective immune system protection that may have been important in the appearance of metastasis and determinant in tumor extra thyroidal invasion.

In addition, a different pattern of *ID1* and *ID3* expression was observed in benign and malignant cells at immunohistochemistry. ID proteins are known to be small enough to transit between the nucleus and cytoplasm of cells. It was found that, while *ID1* was expressed homogeneously in both the nucleus and cytoplasm of the benign and malignant cells, *ID3* was expressed more frequently in the nucleus of benign cells and in the cytoplasm of the malignant thyroid cells. Kebebew et al. 2005 also described a more intense *ID1* expression in the nucleus and cytoplasm of thyroid cells, with *ID1* protein being more expressed in the cytoplasm of malignant tissue than in the benign ones, much likely our own findings with *ID3* [12].

In conclusion, this study showed that *ID1*, *ID2*, *ID3*, and *ID4* genes are involved in thyroid tumorigenesis, and data suggest these genes act impeding the evolution of more aggressive phenotypes. In addition, the different patterns of their tissue expression may help identify malignancy of thyroid nodules.

Table 3 Comparison of *ID1* and *ID3* qPCR and Immunohistochemistry (IHC) nuclear and cytoplasmic expression

Thyroid Tumor	*ID1* qPCR (N)	*ID1* IHC Nuclear (N)	*ID1* IHC Cytoplasmic (N)	*ID3* qPCR (N)	*ID3* IHC Nuclear (N)	*ID3* IHC Cytoplasmic (N)
Benign	0.3 ± 0.4 (N=87)	4.6 ± 1.8 (N=75)	4.4 ± 1.8 (N=75)	0.6 ± 0.6 (N=89)	5.2 ± 0.9 (N=55)	4.0 ± 1.4 (N=55)
Malignant	0.5 ± 0.9 (N=100)	0.7 ± 1.6 (N=19)	5.3 ± 1.5 (N=20)	0.3 ± 0.3 (N=99)	3.0 ± 1.8 (N=20)	5.7 ± 1.5 (N=21)
p	0.2780	*<.0001**	0.1637	*<.0001**	*<.0001**	*<.0001**

*$p < 0.05$

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Authors' Contributions Laís Helena Pereira Amaral performed and designed the research, analyzed the data, and wrote the paper. Natássia Elena Bufalo contributed with the research design and development. Karina Colombera Peres contributed with data of the samples and analysis. Icleia Siqueira Barreto performed immunohistochemistry and data analysis. Antonio Hugo José Marques Fróes contributed with all the samples and their data. Laura Sterian Ward idealized and designed the study, analyzed data, and reviewed the paper.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent For this type of study, formal consent is not required.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Surveillance Epidemiology and End-Results Cancer Registries Program [Available from: http://seer.cancer.gov/]
2. Lim H, Devessa SS, Sosa JÁ, Check D, Kitahara CM (2017). Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974–2013. JAMA. Apr 4;317(13):1338–1348.
3. Sanabria A, Kowalski LP, Shah JP, Nixon IJ, Angelos P, Williams MD, Rinaldo A, Ferlito A. (2017). Growing incidence of thyroid carcinoma in recent years: Factors underlying overdiagnosis. Head Neck.
4. Lin JS, Bowles EJA, Williams SB, Morrison CC. (2017). Screening for Thyroid Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. May 9;317(18):1888–1903.
5. Bellido M, Aventin A, Lasa A, Estivill C, Carnicer MJ, Pons C, et al. (2003). Id4 is deregulated by a t(6;14)(p22;q32) chromosomal translocation in a B-cell lineage acute lymphoblastic leukemia. Haematologica. 88(9):994–1001.
6. Fong S, Debs RJ, Desprez PY (2004). Id genes and proteins as promising targets in cancer therapy. Trends Mol Med. 10(8):387–392.
7. Massari ME, Murre C (2000). Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol. 20(2):429–440.
8. Perk J, Iavarone A, Benezra R (2005). Id family of helix-loop-helix proteins in cancer. Nat Rev Cancer. 5(8):603–614.
9. Iavarone A, Lasorella A (2006). ID proteins as targets in cancer and tools in neurobiology. Trends Mol Med. 12(12):588–594.
10. Ruzinova MB, Benezra R (2003). Id proteins in development, cell cycle and cancer. Trends Cell Biol. 13(8):410–418.
11. Kebebew E, Peng M, Treseler PA, Clark OH, Duh QY, Ginzinger D, Miner R (2004). Id1 gene expression is up-regulated in hyperplastic and neoplastic thyroid tissue and regulates growth and differentiation in thyroid cancer cells. J Clin Endocrinol Metab. 89(12):6105–6111.
12. Rao AS, Goretzki PE, Köhrle J, Brabant G (2005). Letter re: Id1 Gene Expression in Hyperplastic and Neoplastic Thyroid Tissues. J Clin Endocrinol Metab. 90(10):5906.
13. Heugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. (2006). 2015 American Thyroid Association Management Guidelines for adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and differentiated Thyroid Cancer. Thyroid. Jan; 26(1):1–33.
14. Batista, F.A., Ward, L.S., Marcello, M.A., Martins M.B., Peres K.C., Torricelli C., Bufalo N.E., Soares F.A., da Silva M.J., Assumpção L.V.M. (2016). Gene expression of thyroid-specific transcription factors may help diagnose thyroid lesions but are not determinants of tumor progression. J Endocrinol Invest 39: 423. https://doi.org/10.1007/s40618-015-0386-4, 429
15. Kebebew E, Treseler PA, Duh QY, Clark OH (2003). The helix-loop-helix protein, Id-1, is overexpressed and regulates growth in papillary thyroid cancer. Surgery. 134(2):235–241.
16. Kebebew E, Treseler PA, Duh QY, Clark OH (2000). The helix-loop-helix transcription factor, Id-1, is overexpressed in medullary thyroid cancer. Surgery. 128(6):952–957.
17. Deleu S, Savonet V, Behrends J, Dumont JE, Maenhaut C (2002). Study of gene expression in thyrotropin-stimulated thyroid cells by cDNA expression array: ID3 transcription modulating factor as an early response protein and tumor marker in thyroid carcinomas. Exp Cell Research. 279: 62–70.
18. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, et al. (1999). Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. Nature. 397(6721):702–706.
19. Pan L, Sato S, Frederick JP, Sun XH, Zhuang Y (1999). Impaired immune responses and B-cell proliferation in mice lacking the Id3 gene. Mol Cell Biol. 19(9):5969–5980.
20. Rivera RR, Johns CP, Quan J, Johnson RS, Murre C (2000). Thymocyte selection is regulated by the helix-loop-helix inhibitor protein, Id3. Immunity. 12(1):17–26.
21. Morse D (2006). Inhibition of differentiation (id) proteins. Elsevier. 5.