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ORIGINAL ARTICLE

Clinical Significance of Hu-Antigen Receptor (HuR) and Cyclooxygenase-2 (COX-2) Expression in Human Malignant and Benign Thyroid Lesions

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Abstract Hu-antigen R (HuR) is considered to play a crucial role in tumor formation and growth by binding to mRNAs encoding proteins such as Cyclooxygenase-2 (COX-2) and inducing their expression via mRNA stabilization and/or altered translation. The present study aimed to evaluate the clinical significance of HuR and COX-2 proteins' expression in human benign and malignant thyroid lesions. HuR and COX-2 proteins' expression was assessed immunohistochemically on paraffin-embedded thyroid tissues obtained from 98 patients with benign (n = 48) and malignant (n = 50) lesions and was statistically analyzed with clinicopathological parameters, follicular cells' proliferative capacity and recurrence risk rate. Enhanced HuR and COX-2 expression was significantly more frequently observed in malignant compared to benign thyroid lesions (p = 0.0073 and p = 0.0016, respectively), as well as in papillary carcinomas compared to hyperplastic nodules (p = 0.0039 and p = 0.0009, respectively). Positive associations of both HuR and COX-2 expression with follicular cells' proliferation rate were also noted (p = 0.0087 and p = 0.0127, respectively). In malignant thyroid lesions, elevated COX-2 expression was significantly associated with female patients' gender (p = 0.0381) and the presence of lymph node metastases (p = 0.0296). The present data support evidence that both HuR and COX-2 may be involved in the malignant state of thyroid neoplasia and may be utilized in the diagnosis of malignant thyroid tumors.

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Introduction

Hu-antigen R (HuR) or ELAV (embryonic lethal, abnormal vision, Drosophila)-like protein 1 (ELAVL1) is an RNA-binding post-transcriptional regulator belonging to the Hu/ELAV family, which consists of the primarily neuronal-specific HuB, HuC and HuD and the ubiquitously expressed HuR protein [1]. The human HUR/ELAVL1 gene is located on chromosome 19 at position 19p13.2 [2], and encodes a 32kD protein containing three highly conserved RNA binding domains belonging to the RNA recognition motif (RRM) superfamily [3]. A HuR-binding RNA motif has been identified, a U-rich sequence approximately 17–20 nucleotides long, preferentially located within the 3′ untranslated region (UTR) of the majority of the target mRNAs [4]. HuR specifically binds to this motif and regulates the stability, translation and intracellular trafficking of target mRNAs [5]. HuR can shuttle between the nucleus and cytoplasm and localization of HuR in the cytoplasm is required for its mRNA stabilizing function [5]. Many HuR targets are mRNAs encoding cytokines, chemokines and proteins involved in the cell cycle progression, senescence and inflammation and stress responses [6, 7]. Notably, HuR can stabilize the mRNA of cyclooxygenase-2 (COX-2), an enzyme that catalyses the synthesis of prostaglandins and is associated with promotion of carcinogenesis and tumor cell resistance to apoptosis [8, 9].

Benign and malignant thyroid lesions constitute the most common neoplasia of endocrine glands with growing rates

during the last two decades [10, 11]. Papillary thyroid carcinoma is the most common amongst thyroid malignancies, accounting for more than 80 % of all thyroid cancers, while together papillary and follicular thyroid carcinoma represent approximately 90 % of all thyroid cancers [12]. Thyroid cancer generally has a favourable outcome; however, a significant proportion of patients ultimately die from the disease due to local recurrences and/or distant metastases [13, 14]. Therefore, it is essential to establish new treatment strategies and find new prognostic markers in order to predict the clinical course for each patient and customize accordingly the available therapeutic modalities.

Changes at HuR levels or its localization have been associated with pathologic inflammation [6], atherosclerosis [15], tissue ischemia [16] and both early and advanced cancer stage [17–19]. HuR has been reported to control gene expression at multiple areas of malignant transformation by regulating the expression of many cancer-relevant genes associated with cell cycle, apoptosis, differentiation, angiogenesis, cell signalling and inflammatory response [17–21]. Notably, HuR has been considered to play a central role in tumor formation, growth and metastasis by binding to mRNAs encoding proteins involved and inducing their expression via mRNA stabilization and/or altered translation [19–21]. Recent clinical studies have indicated that increased HuR expression levels and cytoplasmic HuR staining pattern are correlated with malignant phenotype and poor patient prognosis in several types of human malignancy, including oesophageal, breast, lung, renal cell and urothelial carcinoma [17, 22]. Moreover, it is currently well-established that COX-2 is implicated in tumor proliferation, increases angiogenesis and invasiveness, decreases cell-mediated immunity and promotes tumor cell resistance to apoptosis [8, 9]. Notably, several experimental and clinical studies have documented potent anti-cancer activity of non-steroidal anti-inflammatory drugs (NSAIDs) and other COX-2 inhibitors such as celecoxib [8, 9]. In this aspect, the present study aimed to evaluate the immunohistochemical expression of HuR and COX-2 proteins in benign and malignant thyroid lesions in association with clinicopathological characteristics related to prognosis.

Patients and Methods

Patients

The study group consists of 98 thyroid surgical specimens from an equal number of patients who had undergone thyroid surgery for benign and malignant lesions. Institutional review board approval was obtained to use archived material for research purposes. Forty eight benign (38 hyperplastic nodules and 10 Hashimoto thyroiditis) and fifty malignant (43 papillary and 7 follicular carcinomas) cases were included in the study. Each neoplasm was classified according to the WHO histological classification of thyroid tumors [23]. The risk of recurrence was estimated according to the American Thyroid Association (ATA) staging system [24]. None of the patients had received any kind of anti-cancer treatment prior to surgery and there was no clinical history of head and neck irradiation or of other cancer.

Immunohistochemistry

Immunostainings for HuR and COX-2 were performed on formalin-fixed, paraffin-embedded thyroid tissue sections using commercially available rabbit polyclonal anti-HuR (H-280, sc-20,694) and anti-COX-2 (H-62, sc-7951) IgG antibodies (Santa Cruz Biochemicals, Santa Cruz, CA, USA). Briefly, 4 μm thick tissue sections were deparaffinized, rehydrated, immersed in 3 % H_2O_2 for 30 min and microwaved at 750 W in 0.01 M citrate buffer (pH 6.0) for 15 min and left to cool down in TBS. Sections were incubated with HuR and COX-2 antibodies for 1 h at room temperature (37 °C), at a dilution 1:100 and 1:200, respectively. Immunostaining was performed using the standard two-step peroxidase conjugated polymer technique (DAKO Envision kit, DAKO, Carpinteria, CA, USA) and visualized with diaminobenzidine tetrahydrochloride solution (DAB; Sigma, Saint Louis, MO, USA). Sections were then counterstained with Harris' hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). Appropriate negative controls were performed by omitting the primary antibody and/or substituting it with an irrelevant anti-serum. As positive control, lung cancer tissue sections with known increased HuR and COX-2 expression were used [19]. The tumor cells' proliferative capacity was assessed immunohistochemically, using a mouse *anti*-human Ki-67 antigen; IgG_{1k} antibody (clone MIB-1, Dakopatts, Glostrup, Denmark) as previously described [25–27].

Evaluation of Immunohistochemistry

The immunoreactivity of the tumor cells for HuR and COX-2 was scored according to the percentage of HuR and COX-2 positive tumor cells as 0: negative staining- 0-4 % of tumor cells positive; 1: 5–24 % of tumor cells positive; 2: 25–49 % of tumor cells positive; 3: 50–100 % of tumor cells positive, and its intensity as 0: negative staining, 1: mild staining; 2: intermediate staining; 3: intense staining. Finally, the expression of HuR and COX-2 was classified as negative/weak; if the total immunohistochemical (IHC) score was 0 or 2 and moderate/high; if the total IHC score was ≥3. In this way, we ensure that each group has a sufficient and more homogeneous number of cases in order to be comparable with the other groups [25–27]. Ki-67 immunoreactivity was classified according to the percentage of positively stained follicular

cells exceeded the median percentage value into two categories (below and over mean value), as previously reported [25–27].

Statistical Analysis

Chi-square test was used to assess the difference of HuR, COX-2 and concomitant HuR/COX-2 immunoreactivity between the diverse histopathological entities. Chi-square test was also used assess the associations between HuR, COX-2 and concomitant HuR/COX-2 immunoreactivity and clinicopathological characteristics in the subgroup of patients with malignant thyroid lesions. The association between HuR and COX-2 immunoreactivity and patients' age was assessed by the nonparametric Mann-Witney U test. A 2-tailed $p < 0.05$ was considered statistically significant. Statistical analyses were performed using the software package SPSS for Windows (version 11.0; SPSS Inc., Chicago, IL, USA).

Results

Clinical Significance of HuR Expression in Human Malignant and Benign Thyroid Lesions

HuR positivity (IHC score > 0) was noted in 78 (80 %) out of 98 thyroid lesions. Forty-two (43 %) out of the 98 examined cases presented moderate/high HuR immunoreactivity (IHC score ≥ 3). The subcellular pattern of HuR distribution was predominantly cytoplasmic and occasionally nuclear in malignant and predominantly nuclear and occasionally cytoplasmic in benign thyroid lesions. Normal surrounding areas adjacent to tumor were found negative for HuR. Representative HuR immunostainings for papillary carcinoma and hyperplastic nodules are depicted in Fig. 1a, b, respectively.

In cross-tabulation, HuR immunoreactivity was significantly elevated in malignant compared to benign thyroid lesions (Table 1, $p = 0.0073$). Enhanced HuR expression was significantly more frequently observed in papillary carcinomas compared to hyperplastic nodules (Table 1, $p = 0.0039$). Non significant intergroup differences were observed for all the remaining possible comparisons between the different histopathological entities (data not shown, e.g. papillary vs follicular carcinoma, follicular carcinoma vs hyperplastic nodules, papillary carcinoma vs Hashimoto thyroiditis, follicular carcinoma vs Hashimoto thyroiditis, Hyperplastic nodules vs Hashimoto thyroiditis). A significant positive association between HuR expression and follicular cells' proliferative capacity was also recorded (Table 1, $p = 0.0087$). In the subgroup of malignant thyroid lesions, moderate/high HuR expression was noted in 28 (56 %) out of 50 cases. Elevated HuR expression was associated with increased follicular cells' proliferation rate (Table 2, $p = 0.0060$), while a trend of correlation with the presence of lymphatic invasion was also noted (Table 2, $p = 0.0873$). HuR expression was not associated with either the other clinicopathological parameters examined (Table 2, $p > 0.01$) or the recurrence risk estimated according to ATA staging system ($p = 0.7498$).

Clinical Significance of COX-2 Expression in Human Malignant and Benign Thyroid Lesions

COX-2 positivity (IHC score > 0) was noted in 56 (57 %) out of 98 thyroid lesions. Thirty-eight (39 %) out of the 98 examined cases presented moderate/high COX-2 immunoreactivity (IHC score ≥ 3). The subcellular pattern of distribution was predominantly cytoplasmic and occasionally membranous in both malignant and benign thyroid lesions. Normal surrounding areas adjacent to tumor were found negative for COX-2. Representative COX-2 immunostainings for papillary carcinoma and hyperplastic nodules are depicted in Fig. 1c, d, respectively.

In cross-tabulation, elevated COX-2 expression was significantly more frequently observed in malignant compared to benign thyroid lesions, as well as in papillary carcinomas compared to hyperplastic nodules (Table 1, $p = 0.0016$ and $p = 0.0009$, respectively). A significant positive association between COX-2 expression and follicular cells' proliferative capacity was recorded (Table 1, $p = 0.0128$). Enhanced COX-2 expression was significantly more frequently observed in papillary compared to follicular carcinoma ($p = 0.0231$), whereas non significant intergroup differences for all the remaining possible comparisons between the different histopathological entities were noted (data not shown). In the subgroup of malignant thyroid lesions, moderate/high COX-2 expression was noted in 27 (54 %) out of 50 cases. Elevated COX-2 expression was significantly associated with male patients' gender (Table 2, $p = 0.0381$) and the presence of lymph node metastasis (Table 2, $p = 0.0296$) and borderline with follicular cells' proliferative capacity (Table 2, $p = 0.0548$). COX-2 expression was not associated with either the other clinicopathological parameters examined (Table 2, $p > 0.01$) or the recurrence risk estimated according to ATA staging system ($p = 0.8518$).

Clinical Significance of Concomitant HuR/COX-2 Expression in Human Malignant and Benign Thyroid Lesions

Thyroid lesions' cases were further classified into two groups: a group including cases that presented concomitant moderate/high expression for both HuR and COX-2 proteins and another one including the remaining cases (e.g. moderate/high HuR and negative/weak COX-2 expression; negative/weak HuR and moderate/high COX-2 expression; both negative/weak

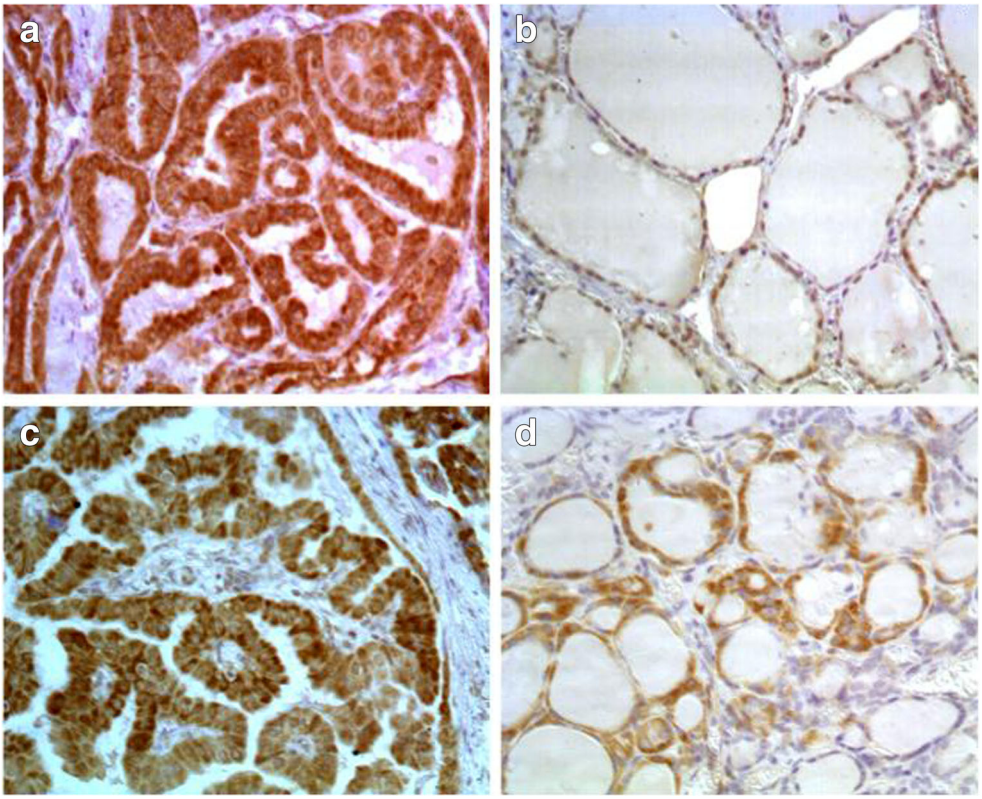

Fig. 1 Representative HuR immunostainings in: **a** Papillary carcinoma and **b** Hyperplastic nodule. Representative COX-2 immunostainings in: **c** Papillary carcinoma and **d** Hyperplastic nodule (original magnification X200)

HuR and COX-2 expression). In this aspect, 26 (27 %) out of 98 thyroid lesions showed concomitant moderate/high HuR/COX-2 expression.

In cross-tabulation, concomitant moderate/high HuR/COX-2 expression levels were significantly more frequently observed in malignant compared to benign thyroid lesions (Table 1, $p = 0.0302$), as well as in papillary carcinomas compared to hyperplastic nodules (Table 1, $p = 0.0226$). Concomitant enhanced HuR/COX-2 expression was significantly more frequently observed in papillary compared to

Table 1 Associations of HuR and COX-2 expression with patients' age and gender, type of histopathology and Ki-67 protein statement in 98 patients with thyroid lesions

Clinicopathological Characteristics	HuR expression			COX-2 expression			Concomitant HuR/COX-2 expression		
	Negative/ Weak	Moderate/ High	*p*-value	Negative/ Weak	Moderate/ High	*p*-value	Negative/ Weak	Moderate/ High	*p*-value
N = 98	56 (57 %)	42 (43 %)		60 (61 %)	38 (39 %)		72 (73 %)	26 (27 %)	
Age (mean ± SD;yrs)	50.9 ± 13.9	46.3 ± 15.9	0.2621	49.5 ± 15.2	48.1 ± 14.6	0.6988	50.9 ± 14.6	47.7 ± 15.1	0.3507
Gender			0.5596			0.1276			0.8518
Female	47 (48 %)	37 (38 %)		54 (55 %)	30 (31 %)		62 (63 %)	22 (23 %)	
Male	9 (9 %)	5 (5 %)		6 (6 %)	8 (8 %)		10 (10 %)	4 (4 %)	
Histopathology (N = 98)			0.0073			0.0016			0.0302
Benign	34 (35 %)	14 (14 %)		37 (38 %)	11 (11 %)		40 (41 %)	8 (8 %)	
Malignant	22 (22 %)	28 (29 %)		23 (23 %)	27 (28 %)		32 (32 %)	18 (19 %)	
Histopathology (N = 50)			0.0039			0.0009			0.0226
Hyperplastic nodules	28 (35 %)	10 (12 %)		29 (36 %)	9 (11 %)		31 (38 %)	7 (9 %)	
Papillary carcinoma	18 (22 %)	25 (31 %)		17 (21 %)	26 (32 %)		25 (31 %)	18 (22 %)	
Ki-67 protein statement			0.0087			0.0128			0.0018
< median value	40 (41 %)	19 (19 %)		42 (43 %)	17 (17 %)		50 (51 %)	9 (9 %)	
≥ median value	16 (16 %)	23 (24 %)		18 (18 %)	21 (22 %)		22 (22 %)	17 (18 %)	

Table 2 Associations of HuR and COX-2 expression with clinicopathological characteristics in 50 patients with malignant thyroid lesions

Clinicopathological Characteristics	HuR expression			COX-2 expression			Concomitant HuR/COX-2 expression		
	Negative/ Weak	Moderate/ High	*p*-value	Negative/ Weak	Moderate/ High	*p*-value	Negative/ Weak	Negative/ Weak	*p*-value
N = 50	22 (44 %)	28 (56 %)		23 (46 %)	27 (54 %)		32 (64 %)	18 (36 %)	
Age (mean ± SD;yrs)	48.6 ± 10.1	44.4 ± 16.7	0.4872	44.3 ± 13.9	47.9 ± 14.5	0.2793	45.7 ± 12.6	46.9 ± 15.1	0.8938
Gender			0.2501			0.0381			0.9231
Female	17 (34 %)	25 (50 %)		22 (44 %)	20 (40 %)		27 (54 %)	15 (30 %)	
Male	5 (10 %)	3 (6 %)		1 (2 %)	7 (14 %)		5 (10 %)	3 (6 %)	
Tumor size (T)			0.6400			0.5361			0.8298
T1	17 (34 %)	20 (40 %)		18 (36 %)	19 (38 %)		24 (48 %)	13 (26 %)	
T2-4	5 (10 %)	8 (16 %)		5 (10 %)	8 (16 %)		8 (16 %)	5 (10 %)	
Lymph node metastasis (N)			0.8493			0.0296			0.2386
N0	20 (40 %)	25 (50 %)		23 (46 %)	22 (44 %)		30 (60 %)	15 (30 %)	
N1	2 (4 %)	3 (6 %)		0 (0 %)	5 (10 %)		2 (4 %)	3 (6 %)	
Capsular invasion			0.9763			0.3998			0.8539
No	18 (36 %)	23 (46 %)		20 (40 %)	21 (42 %)		26 (52 %)	15 (30 %)	
Yes	4 (8 %)	5 (10 %)		3 (6 %)	6 (12 %)		6 (12 %)	3 (6 %)	
Lymphatic invasion			0.0873			0.7766			0.7682
No	20 (40 %)	20 (40 %)		18 (36 %)	22 (44 %)		26 (52 %)	14 (28 %)	
Yes	2 (4 %)	8 (16 %)		5 (10 %)	5 (10 %)		6 (12 %)	3 (8 %)	
Vascular invasion			0.8493			0.2188			0.8442
No	20 (40 %)	25 (50 %)		22 (44 %)	23 (46 %)		29 (58 %)	16 (32 %)	
Yes	2 (4 %)	3 (6 %)		1 (2 %)	4 (8 %)		3 (6 %)	2 (4 %)	
Ki-67 protein statement			0.0060			0.0548			0.0064
< median value	14 (28 %)	7 (14 %)		13 (26 %)	8 (16 %)		18 (36 %)	3 (6 %)	
≥ median value	8 (16 %)	21 (42 %)		10 (20 %)	19 (38 %)		14 (28 %)	15 (30 %)	

follicular carcinoma (p = 0.0323), whereas non significant intergroup differences for all the remaining possible comparisons between the different histopathological entities were noted (data not shown). A significant positive association between concomitant HuR/COX-2 expression and follicular cells' proliferative capacity was also recorded (Table 1, p = 0.0184). In the subgroup of malignant thyroid lesions, concomitant elevated HuR/COX-2 expression was significantly associated with follicular cells' proliferative capacity (Table 2, p = 0.0064), whereas no significant associations with either the other clinicopathological parameters examined (Table 2, $p > 0.01$) or the recurrence risk estimated according to ATA staging system (p = 0.3625) were recorded.

Association Between HuR and COX-2 Expression

A positive association between HuR and COX-2 expression was noted by including in the analysis all the examined malignant and benign thyroid lesions (Table 3, rho = 0.4111, $p < 0.0001$). This positive association was improved when the analysis was restricted to the subgroup of benign thyroid lesions (Table 3, rho = 0.5225, $p < 0.0001$), whereas it was obscured as far as concern the subgroup of malignant thyroid lesions (Table 3, rho = 0.2325, p = 0.0997).

Discussion

A gradually increasing number of studies have currently documented that HuR overexpression and cytoplasmic localization are associated with crucial clinicopathological parameters for patients' management and prognosis in several types of human malignancy [18]. Interestingly, HuR by binding to COX-2 adenine- and uridine-rich elements has been considered to stabilize COX-2 mRNA, leading to increased COX-2 protein expression, which has also been implicated in malignant transformation [8, 9]. However, the currently existing data regarding the exact role of HuR and its possible interrelationship with COX-2 in thyroid malignant transformation remains still scarce.

In this aspect, the present study provided for the first time the clinical evidence that both HuR and COX-2 expression was increased in malignant compared to benign thyroid lesions. Distinct discriminations between papillary carcinomas

Table 3 Spearman's bivariate correlation between HuR and COX-2

All cases; N = 98	HuR expression		rho-value	*p*-value
	Negative/Weak	Moderate/High		
COX-2 expression			0.4111	<0.0001
Negative/Weak	44 (45)	16 (16)		
Moderate/High	12 (12)	26 (27)		
Malignant; N = 50				
COX-2 expression			0.2328	0.0997
Negative/Weak	13 (46)	10 (20)		
Moderate/High	9 (18)	18 (36)		
Benign; N = 48				
COX-2 expression			0.5225	<0.0001
Negative/Weak	31 (65)	6 (12)		
Moderate/High	3 (6)	8 (17)		

and hyperplastic nodules for both proteins were also recorded. HuR subcellular distribution was predominately nuclear in benign and predominately cytoplasmic in malignant thyroid lesions, suggesting that HuR may be translocated from nucleus to cytoplasm during the malignant thyroid transformation process. The observed positive associations of HuR and COX-2 expression with follicular cells' proliferative capacity may further suggest their potential implication in the thyroid tumor phenotype. In the subgroup of malignant thyroid lesions, enhanced COX-2 expression was additionally associated with female patients' gender and the presence of lymph node metastases, while HuR expression showed a trend of correlation with the presence of lymphatic invasion. On the other hand, concomitant HuR/COX-2 expression did not improve the discrimination between malignant and benign thyroid lesions, as well as between papillary carcinomas and hyperplastic nodules, being also not correlated with clinicopathological characteristics. Moreover, the increased expression of HuR and COX-2 might be the cause but also the consequence of the malignant process. Taken together, the present study supported evidence that both HuR and COX-2 may be implicated in the malignant state of thyroid neoplasia. In addition, HuR may be translocated from nucleus to cytoplasm during the malignant thyroid transformation process. The strong positive association of HuR with COX-2 expression, which mostly noted in benign thyroid lesions, further suggests that the cooperation of these molecules could be biologically more important in benign pre-malignant conditions when inflammation is also important [7]. However, the present results could not be considered as conclusive due to the relative low number of sample and should be confirmed by additional larger cohort studies.

In accordance with the present study, there is currently substantial clinical evidence that HuR may be implicated in the malignant transformation process of several types of cancer, being significantly correlated with crucial clinicopathological parameters for patients' management and prognosis [18]. Specifically, in oral carcinoma, elevated HuR expression was associated with male patients' gender, tumor grade and presence of metastasis [28]. Significant associations between enhanced HuR expression and larger tumor size, advanced disease stage and presence of lymphatic invasion in oesophageal carcinoma were also recorded [29]. HuR expression was found to be increased in lung cancer patients presenting advanced disease stage, presence of metastasis, lymphatic and vascular invasion [30]. Elevated HuR expression was also more frequently observed in cervical cancer patients presenting larger tumor size, advanced disease stage and presence of lymphatic and vascular invasion [31]. In urothelial and gallbladder carcinoma, HuR expression was positively correlated with tumor size and grade, as well as the presence of vascular and perineural invasion [32, 33]. Enhanced HuR expression was also associated with advanced disease stage in gastric and colorectal carcinoma [34, 35]. Significant associations between HuR expression and tumor grade in breast and ovarian carcinoma were also noted [36, 37]. Importantly, cytoplasmic HuR expression pattern was shown to be a negative prognosticator for survival in oral, esophangeal, gastric, gallbladder, renal, urothelial, lung, breast and ovarian cancer [18]. On the other hand, in pancreatic ductal adenocarcinoma, cytoplasmic HuR expression pattern was associated with a good prognosis for gemcitabine-treated patients [38].

Recent studies have also shown that COX-2 expression up-regulation was associated with crucial clinicopathological parameters, as well as patients' prognosis in several cancer types, including head and neck squamous cell, colorectal, breast, lung, skin, stomach, liver, pancreas, bladder, ovary and prostate cancers [39]. Contradictory results concerning the clinical impact of COX-2 expression in thyroid neoplasia are currently existed. In fact, in a study conducted on 150 thyroid specimens, papillary and follicular carcinomas were characterized by increased COX-2 expression compared to follicular adenomas and adenomatous nodules [40].

Moreover, elevated COX-2 mRNA expression in well-differentiated carcinomas compared to normal thyroid tissues and follicular adenomas was documented [41]. However, another study conducted on 64 patients with thyroiditis, benign and malignant thyroid lesions with or without metastasis documented no significant correlations between COX-2 protein expression and clinical and/or pathological characteristics [42]. No difference between thyroiditis and thyroid tumors as far as concern COX-2 protein expression was also noted [42]. In accordance, no associations between COX-2 and patients' age, as well as presence of lymph node metastases in papillary carcinomas were recorded [43]. On the other hand, another study showed that COX-2 expression was reduced in papillary carcinoma patients with older age, larger tumor size, advanced stage, satellite tumors and presence of solid, scirrhous or trabecular growth patterns [44]. The above study suggested that COX-2 up-regulation may contribute predominantly in the early phase of papillary carcinoma progression [44]. In contrast, Siironen et al. documented a positive association between COX-2 expression and patients' age in papillary carcinoma [45].

Importantly, because of its ubiquitous expression in malignant clinical samples, as well as its apparently consistent role in tumor formation and progression, HuR has been considered as a potential drug target for cancer therapy. Strategies to decrease HuR expression could be a promising therapeutic approach in controlling tumor progression, taking into account HuR influence on response to anti-cancer therapies. Currently, there is substantial evidence that HuR may be implicated in drug chemoresistance mechanisms, reinforcing its usefulness as potential new target [46, 47]. Moreover, HuR-overexpressing cancer cells were more sensitive to treatment with gemcitabine, the main chemotherapeutic component of treatment regimens for pancreatic ductal adenocarcinoma, compared with control cells [48, 49]. Interestingly, in a recent large phase III adjuvant trial with chemoradiation backbone in pancreatic ductal adenocarcinoma, 5-fluorouracil increased HuR function by enhancing HuR translocation from the nucleus to the cytoplasm [50]. In this aspect, future studies should be orientated to the discovery, development and evaluation of HuR-specific drugs targeting various cancer types, including thyroid neoplasia.

Conclusion

The present study supported clinical evidence that both HuR and COX-2 may be involved in malignant state of thyroid neoplasia and may be utilized in the diagnosis of malignant thyroid tumors. HuR and COX-2 expression showed distinct discriminations between malignant and benign thyroid lesions, being also correlated with clinicopathological parameters crucial for patients' management. The present study further supported that HuR translocation from nucleus to cytoplasm may be a potential event during malignant thyroid transformation process. Further research conducted on larger cohort studies and on each histopathological entity separately is strongly recommended in order to delineate whether HuR and COX-2 could be considered of clinical utility in thyroid neoplasia, evaluating also their usefulness as potential therapeutic targets in this type of neoplasia.

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Compliance with Ethical Standards

Conflict of Interest Statement All authors verify that they have not accepted any funding or support from an organization that may in any way gain or lose financially from the results of the present study. All authors verify that they have not been employed by an organization that may in any way gain or lose financially from the results of the present study. None authors have any other conflicting interest.

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