

Receptor-Binding Cancer Antigen Expression in Thyroid Neoplasms: A Retrospective Study

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Rezumat

Expresia genei RCAS1 (Receptor-Binding Cancer Antigen) în cancerle tiroidiene: un studiu retrospectiv

Context: Gena RCAS1 (Receptor-Binding Cancer Antigen) este o proteină membranară, considerată un antigen asociat tumorilor. Celulele canceroase evită răspunsul imun al organismului cu ajutorul amplificării RCAS1, inducând apoptoza limfocitelor infiltrante ale tumorii. Incidența cancerului tiroidian este în creștere, iar scopul nostru este obținerea unui diagnostic precis în stadii incipiente. Scopul acestui studiu este de a accesa expresia RCAS1 în patologii maligne și benigne ale tiroidei.

Metode: Acesta este un studiu retrospectiv care include 110 pacienți, supuși tiroidectomiei într-un singur centru de referință terțiar în perioada ianuarie 2008 - decembrie 2014. S-a efectuat un studiu imunohistochimic pentru expresia RCAS1 și s-a încercat corelarea cu informațiile clinice și histopatologice.

Rezultate: Imunocolorarea RCAS1 a relevat un rezultat pozitiv în 81 de cazuri din totalul de 110. Rezultatul a fost pozitiv în toate probele de țesut tiroidian malign ($p < 0.001$). Cazurile de tiroidă malignă, mărimea tumorii, invazia capsulei tiroidiene și prezența nodulilor limfatici pozitivi au fost corelate cu o prezență moderată sau puternică a RCAS1. În cazul carcinomului papilar tiroidian, marea majoritate a cazurilor (35/37 cazuri, 94.6%) au avut de asemenea o expresie moderată sau puternică a RCAS1.

Concluzii: Expresia RCAS1 poate ajuta la diagnosticul diferențial între patologia tiroidiană malignă sau benignă, iar prezența puternică poate fi corelată cu caracteristici oncologice grave.

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Cuvinte cheie: RCAS1; neoplazie tiroidiană; cancer tiroidian; biomarkeri tiroidieni

Abstract

Background: Receptor-binding cancer antigen (RCAS1) is a membrane protein, regarded as a tumor-associated antigen. Cancer cells evade immune response with RCAS1 up-regulation, inducing apoptosis to tumor infiltrating lymphocytes. Thyroid cancer incidence is rising and its accurate diagnosis in early stage is targeted. The aim of this study is to access RCAS1 expression in benign and malignant thyroid pathology.

Methods: This is a retrospective study of 110 patients, who had thyroidectomy in a single tertiary referral centre between January 2008 until December 2014. Immunohistochemistry study for RCAS1 expression was carried out and correlation with clinical and histopathological data is attempted.

Results: RCAS1 immunostaining was found positive in 81 out of 110 cases. Notably it was deemed positive in all malignant thyroid tissue samples ($p < 0.001$). In thyroid malignancy, tumor size, thyroid capsule invasion and positive lymph nodes status were positively correlated with moderate and strong expression of RCAS1. For papillary thyroid carcinoma, the vast majority (35/37 cases, 94.6%) were also classified as having moderate or strong RCAS1 expression.

Conclusions: RCAS1 expression can aid in differential diagnosis between benign and malignant thyroid pathology, while its strong expression correlates with worse oncological features.

Key words: RCAS1, thyroid neoplasm, thyroid cancer, thyroid biomarkers

Introduction

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1) was first studied in mice immunized with the human uterine cervical adenocarcinoma cell line SiSo (1). RCAS1 is a recently recognized membrane protein, regarded as a tumor-associated antigen. Cells that express the RCAS1 induce cell-cycle arrest and/or apoptosis (2). Previous studies suggest that lymphocytes such as T, B and NK cells, erythroid leukemia, K562 cells, express RCAS1, which acts as a ligand inhibiting cell proliferation. Cancer cells evade immune response with RCAS1 up-regulation, inducing apoptosis to tumor infiltrating lymphocytes (TILs), giving to this protein an exciting oncogenic role (3). Tumor cells become more tolerant to the immune response, indicating worse oncological features of such malignancies. RCAS1 expression has been studied in various malignancies so far. It is considered to be correlated with higher tumor grade, advanced stage of the disease, positive lymph node

metastases status, and overall with poorer patient's prognosis (4).

Thyroid cancer incidence is increasing worldwide, partly because of higher detection rates of small papillary tumors. Fine-needle aspiration (FNA) samples of thyroid nodules are increasingly utilized in thyroid pathology investigations and novel biomarkers are needed to help differential diagnosis among benign and malignant thyroid lesions (5). Moreover immunohistochemical biomarkers are studied of their prognostic role in established thyroid malignant disease (6).

RCAS1 expression in thyroid neoplasia has been studied scarcely (7). The aim of the current study is to access RCAS1 expression in benign and malignant thyroid neoplasms, as well as its correlation with established adverse prognostic factors of thyroid malignancy.

Material and Method

A total number of 110 patients, who had

thyroid surgery in a single tertiary referral centre between January 2008 until December 2014 were retrospectively studied. All patient's clinical and histopathological data were retrieved from the hospital's medical archives, and informed consent was obtained. Clinical characteristics and thyroid pathology are presented in *Table 1*.

Immunohistochemistry for RCAS1 was carried out on formalin-fixed, paraffin-embedded thyroid tissue sections. Moreover, a mouse monoclonal anti-RCAS1 antibody (ProteinTech Co, USA) was used (8). Negative controls were performed in sections where the primary antibody was either not used and or substituted with an irrelevant anti-serum.

Histopathological sections were reviewed by two independent histopathologists, who were both blinded regarding clinical characteristics of the patients. RCAS1 expression was quantified as 1. Negative, when no cells were stained, 2. Low, when <5% of thyroid follicular cells were stained positive, 3. Moderate, when 5-30% of thyroid follicular cells were stained positive, and 4. High, when >30% of thyroid follicular cells were stained positive (9).

Table 1. Demographics and histopathological characteristics of study sample (n=110)

Age (years), mean $\pm$ SD	50.18 $\pm$ 15.37
Male sex, n (%)	23 (20.9)
Histopathology, n (%)	
Benign	60 (54.5)
- Multinodular hyperplasia	45 (40.9)
- Chronic thyroiditis	10 (9.1)
- Adenoma	3 (2.7)
- Diffuse hyperplasia	2 (1.8)
Malignant	50 (45.5)
- Papillary	37 (33.6)
- Follicular	9 (8.2)
- Myeloid	2 (1.8)
- Anaplastic	2 (1.8)
Malignancy characteristics (n=50)	
- max. diameter (mm), median (IQR)	8.5 (4.75 – 14.0)
- Lobe; Right / left/ bilateral, n (%)	14 (28.0)/15 (30.0) 21 (42.0)
- Multifocal, n (%)	19 (38.0)
- Capsular invasion, n (%)	20 (40.0)
- Positive regional LNs, n (%)	9 (18.0)

n = number; SD= standard deviation; IQR = interquartile range; LNs = lymph nodes

Statistics

Categorical data are presented as absolute and relative frequencies (%). Normality of distribution for quantitative variables was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests. Mean value ($\pm$ standard deviation) was used to describe normally distributed variables, whereas non-normally distributed ones were presented as median [25th – 75th interquartile range (IQR)]. Categorical variables were compared using the Pearson's chi-square test or Fisher's exact test, where appropriate. When statistical significance had been reached in a two by three contingency table, a post hoc analysis was conducted using the Bonferroni adjustment for p-value. Independent samples t-test was used for the comparison of normally distributed continuous data, whereas Mann-Whitney U test was used for non-parametrical variables. Kruskal-Wallis test was used for the comparison of the non-parametric 'maximum cancer diameter' variable between the three subgroups of RCAS1 intensity of expression and multiple post-hoc pairwise analyses using Mann-Whitney U tests were performed, according to the Bonferroni correction for p-value. Finally, the receiver operating characteristics (ROC) curve was used for the calculation of the area under the curve (AUC) as an indicator of RCAS1 diagnostic value and the selection of the optimal cut-off value. All p-values were 2-sided, whereas p-values of < 0.05 were considered statistically significant.

All calculations were performed on SPSS version 23.0 (SPSS, Chicago, IL).

Results

Study's demographic and histopathological characteristics are presented on *Table 1*. The majority of patients that underwent thyroidectomy were females (79.1%), whereas cancer was diagnosed in 50 (45.5%) of thyroid specimens. Papillary cancer was the most frequent type of malignancy (33.6%), whereas anaplastic cancer was diagnosed in only 2 cases.

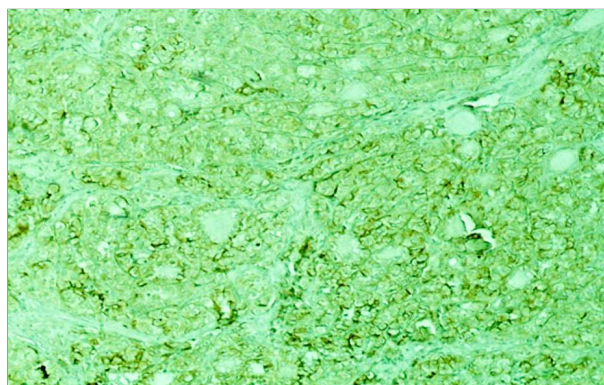
Table 2. Association of clinicopathological characteristics with RCAS1 expression

	RCAS1 (+) (n=81)	RCAS1 (-) (n=29)	p-value
Age (years), mean $\pm$ SD	52.90 $\pm$ 14.82	52.31 $\pm$ 15.91	0.86
Sex, n (%)			0.62
- Male	16 (19.8)	7 (24.1)	
- Female	65 (80.2)	22 (75.9)	
Malignant histopathology, n (%)			<0.001
- Yes	50 (61.7)	0 (0.0)	
- No	31 (38.3)	29 (100.0)	

n = number; SD= standard deviation; IQR = interquartile range; LNs = lymph nodes

RCAS1 immunostaining was found positive in 81 out of 110 thyroid pathology cases studied. Notably it was deemed positive in all malignant thyroid tissue samples (n=50) and in almost 50% (31 out of 60) of benign histopathology specimens (p<0.001, *Table 2*). In more detail, it was characterized as moderate or strong in 48 out of 50 malignant thyroid lesions (*Table 3*). An histopathological immunohistochemistry section of RCAS1 expression is presented in *Fig. 1*.

Myeloid and anaplastic carcinoma cases were found to have strong expression of RCAS1 in total, while all follicular carcinoma cases also categorized as having moderate or strong immunostaining. Moreover, for the more common type of papillary thyroid carcinoma, the vast majority (35/37 cases, 94.6%) were also classified as moderate or strong RCAS1 expression. Intensity of RCAS1 expression did not differ significantly among the different types of thyroid cancer; all specimens which were diagnosed with the

**Figure 1.** Representative immunohistochemistry section of moderate RCAS1 expression in thyroid papillary carcinoma

most aggressive types of thyroid malignancy, myeloid and anaplastic cancer, were stained strongly positive, but statistical significance was not reached possibly because of the small number of such cases (n=4). Similarly, a non-significant trend for more intense immunostaining in multifocal cancer was observed (*Table 3*).

Table 3. Association of histologic type and multifocality with intensity of RCAS1 expression

	RCAS 1 (+)			p-value
	Mild (n=2)	Moderate (n=15)	Strong (n=33)	
Histologic type, n (%)				0.47
- Papillary	2 (100.0)	14 (93.3)	21 (63.6)	
- Follicular	0 (0.0)	1 (6.7)	8 (24.2)	
- Myeloid	0 (0.0)	0 (0.0)	2 (6.1)	
- Anaplastic	0 (0.0)	0 (0.0)	2 (6.1)	
Multifocality, n (%)				0.24
- No	2 (100.0)	11 (73.3)	18 (54.5)	
- Yes	0 (0.0)	4 (26.7)	15 (45.5)	

n = number; SD= standard deviation; IQR = interquartile range; LNs = lymph nodes

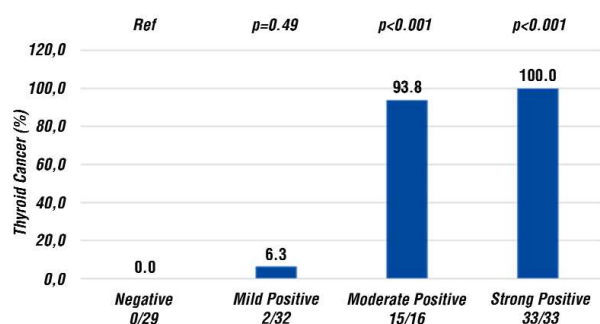


Figure 2. Correlation of RCAS1 intensity of expression with thyroid cancer diagnosis (n=110). All comparisons are versus 'Negative' and p-values are adjusted according to the Bonferroni correction. Overall p-value < 0.001

Moderate and strong positive RCAS1 expressions were significantly correlated with cancer diagnosis, whereas mild staining was not (*Fig. 2*). Interestingly, thyroid capsule invasion, as a known adverse prognostic histopathological characteristic, was detected in 20 out of 33 strong positive cases (p<0.001, *Fig. 3*). On the other hand, although RCAS1 was strongly expressed in all patients with metastatic regional lymph nodes (n=9), a borderline significance was recorded (p=0.059, *Fig. 4*). Another feature indicating poor prognosis which was analyzed was cancer diameter; cancers with strong positive RCAS1 immunostaining had significantly higher median diameter (12 mm) compared to moderate (4 mm) and mild (1 mm) intensity of RCAS1 expression (*Fig. 5*).

Finally, a ROC curve was generated in order to assess the utilization of RCAS1 expression as a diagnostic tool for thyroid cancer. The AUC was high (0.987, 95% Confidence Interval 0.969 – 1.000, p < 0.001), whereas the value of 'moderate expression' was determined as the appropriate cut-off point for cancer diagnosis (sensitivity = 96%, specificity = 83%).

Discussions

RCAS1 expression has an established adverse role in various malignancies, aiding cancer cells to evade immunosurveillance mechanisms (10). In thyroid neoplasia its role has

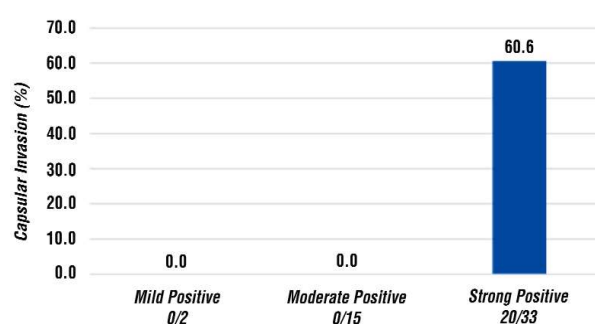


Figure 3. Correlation of RCAS1 intensity of expression with capsular invasion in patients with cancer diagnosis (n=50). Overall p-value < 0.001

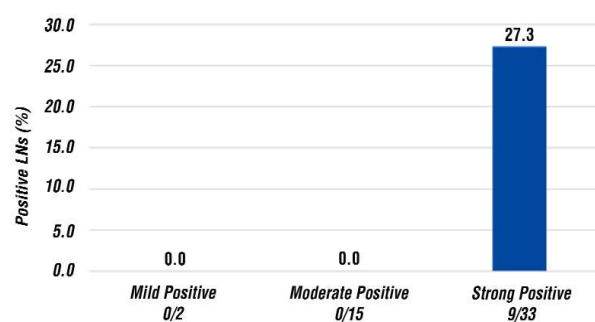


Figure 4. Correlation of RCAS1 intensity of expression with positive lymph nodes in patients with cancer diagnosis (n=50). Overall p-value = 0.059.

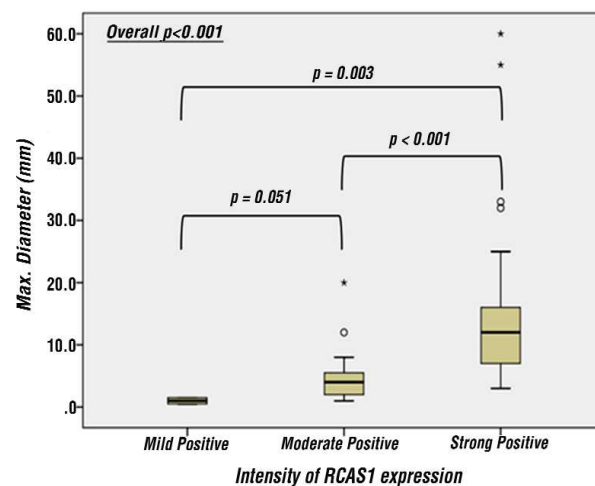


Figure 5. Boxplot diagram for the correlation of RCAS1 intensity of expression with maximum cancer diameter. For pairwise comparisons p-values of < 0.017 were considered statistically significant, according to the Bonferroni correction

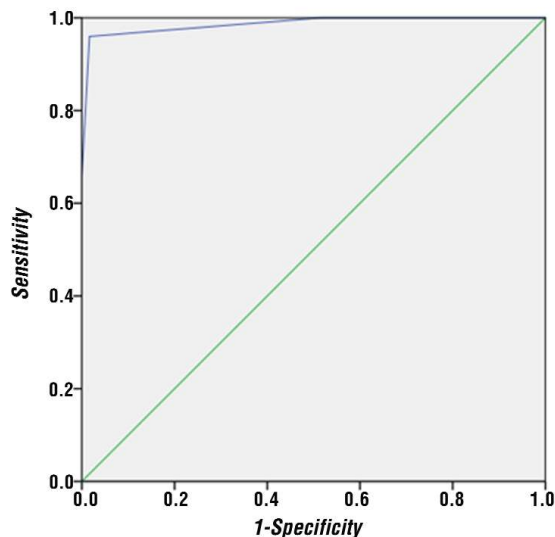


Figure 6. Receiver operating characteristics (ROC) curve. The area under the curve (AUC) is 0.987 (95% Confidence Interval 0.969 – 1.000, $p < 0.001$). The optimal cut-off value is 'Moderate' expression of RCAS1 for cancer diagnosis (sensitivity = 96%, specificity = 83%)

not been extensively studied. The current study suggests that RCAS1 adds important information in differential diagnosis between benign and malignant thyroid lesions, as well as is strongly correlated prognostically with known adverse clinical and histopathological features of malignant thyroid pathology. Large tumors, positive lymph nodes status and capsular invasion, as well as myeloid and anaplastic carcinoma are correlated with RCAS1 strong expression.

In accordance with the above, RCAS1 overexpression was studied in head and neck neoplasms (11,12), indicating advanced disease stage and poor prognosis. Similar findings were published regarding esophageal squamous cell carcinoma (13,14).

In addition, in a study of cervical cancer patients, RCAS1 attributed to cancer cell invasion of uterine connective tissue by induction of stromal tissue remodeling. Anti-apoptotic pathophysiology mechanism inactivating lymphocytes has also been described as adding to the above RCAS1 function (4,15).

The current study has limitations since study's sample was small to reach in statisti-

cally significant results. Despite the above, the trends of RCAS1 expression seem to be in accordance with current literature (16-18). Also, in this study no correlation is made with other molecular or immunohistochemical markers or overall survival, however clinical and histopathological data were used as surrogate parameters. Overall survival in thyroid cancer patients is favorable (19) and a significant correlation among RCAS1 expression and survival would demand a very large sample size.

Conclusions

In conclusion, RCAS1 expression can aid in differential diagnosis between benign and malignant thyroid pathology. Moreover, its strong expression correlates with worse oncological features, adverse prognostic factors and advanced disease stage. Further studies are needed to quantify these statements in more detail. RCAS1 may prove to be a novel biomarker with important clinical significance in cancer diagnosis, prognosis and even used as a cancer recurrence sign since it can be measured in blood serum (20), making its role even more exciting.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Consent

Informed consent from the patient has been obtained.

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