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Increased SOCS1 and SOCS3 expression in papillary thyroid carcinoma and its association with prognosis

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Abstract. – OBJECTIVE: The majority of patients with papillary thyroid carcinoma (PTC) have good outcomes, although the identification of new predictors of a poor prognosis would be beneficial. Chronic thyroiditis is a precancerous condition in which proinflammatory cytokines enhance biologically aggressive features. This study investigated the expression of suppressor of cytokine signaling proteins (SOCS) 1 and 3, which are negative feedback inhibitors, in PTC and benign thyroid nodules (BTN), and analyzed the relations among biomarker expression, pathological prognosis, and clinical features.

PATIENTS AND METHODS: The pathological materials and clinical data of 100 patients with PTC and 40 with BTN were retrospectively analyzed. Immunohistochemical SOCS1 and SOCS3 staining were performed. Besides comparing SOCS1 and SOCS3 expression between PTC and BTN, we analyzed the expression according to pathological factors and clinical variables.

RESULTS: The expression levels of the proteins were significantly higher in PTC than in BTN (p=0.001). SOCS1 expression was higher in older patients with PTC than in younger patients (p=0.001). Unlike SOCS1, SOCS3 was related to the risk group; these groups were distinguished based on the American Thyroid Association (ATA) risk stratification system (p=0.001). SOCS3 was also significantly related to lymph node involvement (p=0.007), capsule invasion (p=0.005), and extrathyroid extension (p=0.009).

CONCLUSIONS: The increased SOCS1 and SOCS3 expression in PTC confirms their roles in thyroid carcinogenesis. Antibodies to both SOCS1 and SOCS3 might aid the diagnosis of PTC through immunohistological staining. SOCS3 provides information on lymph node status and aids risk stratification.

Key Words:
Papillary thyroid carcinoma, Suppressor of cytokine signaling proteins, SOCS1, SOCS3, Prognosis.

Introduction

Papillary thyroid carcinoma (PTC) is the most frequent malignancy of the thyroid gland which originates from follicular cells. PTC is widely diagnosed in younger adults in comparison to other adult cancers. Women are 3 times more likely to develop PTC than men. Despite being malignant cancer, PTC has an excellent overall prognosis. According to the Surveillance Epidemiology, and End Results (SEER) database provided by the National Cancer Institute (NCI), The American Cancer Society (ACS)[1] estimates that 5-year relative survival rates for PTC between 2010 and 2016, are near 100%, 99% and 76% for localized, regional and distant diseases, respectively. However, there are some clinical and pathological features related to poor prognosis, some of which are: older age, male gender, lymph node metastasis, distant metastasis, large tumor size, extrathyroid extension and histologic subtype[2]. Given the high incidence of PTC, a much greater number of patients have apparent symptomatic thyroid nodules, which mostly are benign lesions[3]. In many cases, therapeutic management and long-term follow-up are necessary[4]. In order to manage an appropriate treatment strategy for thyroid diseases with such great prevalence, it becomes essential to understand the underlying mechanisms.

Thyroid diseases are closely related to inflammation, and it has also been suggested[5-9] that thyroiditis could be interpreted as a precancerous condition, due to the increased incidence of thyroid cancer with preexisting thyroiditis. Several studies[10-15] propose that oncogene activation, which increases the production of proinflammatory cytokines, contributes to the establishment and progression of thyroid malignancies. These cytokines

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maintain biologically malignant features such as proliferation, survival, and invasiveness[13,16,17]. But they can also gather inflammatory cells responsible for stroma remodeling and angiogenesis. Thus, inflammatory cells keep releasing these mediators, which enhances tumor progression[18].

Cytokines act as intercellular messengers to control the hematopoietic system, immune system, and inflammatory response. The cytokines consist of a large variety of glycoproteins and have been divided into five groups: a) Tumor necrosis factor-alpha and related molecules, b) Interleukin-1 family members, c) Transforming growth factor-beta molecules, d) chemokines, and e) cytokines that signal through Janus Kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. The latter group could be the largest and consists of hematopoietic growth factors, immunomodulatory cytokines, and inflammatory cytokines[19]. Every cytokine binds to a specific transmembrane receptor located on the surface of the target cell, that contains intracellular domains which are associated with members of the JAK family of thyrosine kinases[20-23]. The establishment of the cytokine-receptor complex activates JAK by transphosphorylation, which in turn phosphorylates a transcription factor, the STAT that is attached to the intracellular part of the receptor[24]. This leads to the disassociation of STAT from the receptor and translocation into the nucleus, where they can interact with cytokine-specific genes and can stimulate transcription[25]. This signaling pathway is a dynamic process and highly organized response controlled by negative feedback by subsequent downregulation and attenuation of the initial signal[26,27]. The suppressor of cytokine signaling (SOCS) proteins are the primary drivers of signal attenuation, which acts as negative-feedback inhibitors due to cytokine exposure[28,29]. There are eight mammalian SOCS family members, SOCS 1-7, and cytokine-inducible SH2 protein (CIS). SOCS proteins can negatively regulate cytokine receptor signaling by several distinct mechanisms. Firstly, they can directly inhibit JAKs by binding to the receptor or to the JAK activation loop[30]. Secondly, they can compete with other signaling molecules containing SH2-domains for binding sites on the receptor[31]. Thirdly, they can target the receptor complex and associated signaling proteins for proteasomal degradation and prevention of nuclear translocation of key signaling molecules[32].

SOCS1 and SOCS3 proteins are unique members of the entire SOCS family, having the ability to directly inhibit the kinase activity of JAK. This activity relies upon a short motif, which is immediately upstream of the SH2 domain, known as the kinase inhibitory region (KIR)[33]. In particular, SOCS1 and SOCS3 can play pivotal roles in inflammation, as well as in the development and progression of cancers. Abnormal expression of SOCS1 and SOCS3 in cancer cells has been reported[34] in human carcinoma associated with dysregulation of signals from cytokine receptors, Toll-like receptors (TLRs), and hormone receptors, resulting in malignancies. According to De Santis at al[35], in PTC, SOCS1 was noticeably downregulated in comparison to thyroid parenchyma. Francipane et al[36] found the expression of SOCS1, SOCS3 and SOCS5 in PTC significantly higher in comparison to thyroid parenchyma. In this regard, our study aims to investigate the expression of SOCS1 and SOCS3 in PTC and compare it with the expression in benign thyroid nodules (BTN) and normohistologic thyroid parenchyma. Besides, we also compared the expression level of either protein against clinical parameters to determine whether it can be utilized as a prognostic and predictive indicator. To the best of our knowledge, among the studies which analyzed SOCS expression in thyroid diseases, our study contains the largest number of cases.

Patients and Methods

Patient Selection

In the current study, we retrospectively retrieved 100 thyroidectomy cases from the archives of Gaziantep University Medicine Faculty Department of Pathology between 2016 and 2021, diagnosed as PTC and 40 thyroidectomy cases diagnosed with BTN, including follicular adenoma and hyperplastic/colloid nodule. All patients were diagnosed, and follow-ups were made in our institute. In addition to histological findings, clinical data were obtained from the medical archives and recorded. Patients whose clinical or histological material was not available were excluded from the current study.

Histological Assessment

For PTC cases, all slides and pathological reports were reevaluated retrospectively by two pathologists for histological subtype, location of

the tumor (bilateral/unilateral and multifocality), thyroid capsule invasion, extrathyroid extension, lymph node involvement, and distant metastasis. During the histological assessment, representative tumor and benign nodule samples were selected for immunohistochemical analysis.

Immunohistochemical Analysis

Immunohistochemical studies were performed on 5 μm sections of formalin-fixed, paraffin-embedded tissues. SOCS1 (GTX100657, Polyclonal Rabbit IgG, 1:100, GeneTex, Irvine, CA, USA) and SOCS3 (SC-518020, Clone: G5 Monoclonal Mouse IgG1, 1:100, Santa Cruz Biotechnology, Heidelberg, Germany) antibodies were performed using an automated immunohistochemistry-staining device (Ventana, Bench Mark Ultra AutoStainer, Roche Diagnostics, Indianapolis, IN, USA).

Semi-Quantitative Evaluation of Immunohistochemical Expression

Staining was evaluated and scored using the immunoreactive score (IRS) system[37] (Table I). The cytoplasmic reaction was evaluated as positive staining for either antibody. Appropriate external positive controls were used: ileum samples for SOCS1 and lung samples for SOCS3 served as a positive control.

Statistical Analysis

The defining statistics of the data obtained from this study were calculated using the mean for numerical variables, frequency, and percent analysis for standard deviation and categorical variables. Clinical parameters were checked with Shapiro-Wilk test to determine whether they were coherent with the normal distribution, which turned out that they were not ($p<0.05$). The Kruskal-Wallis test compared clinical parameters with SOCS1 and SOCS3 expression. The differences between categorical variables were analyzed with the Chi-square test. After variant analysis, Tukey multiple comparison test was used to determine the differences between groups. To compare the epidemiological data with the expression level of SOCS antibodies *t*-test and Chi-square test were used. Analysis has been carried out with SPSS vers. 2.0. (SPSS Inc., Chicago, IL, USA) and $p<0.05$ was evaluated as statistically significant.

Results

Demographic and Histomorphological Findings

One hundred thyroidectomy cases with PTC and 40 thyroidectomy cases with BTN were studied. Of the patients with malignant disease, 83 (83%) were females, 17 (17%) were males, and the female/male ratio was 4.88; whereas of the patients with benign disease, 33 (82.5%) were females, 7 (17.5%) were males, and the female/male ratio was 4.71. The mean age of patients with malignant disease was 40.66±14.99 and 45.5±14.39 in the benign group. Histological subtypes of PTC consisted of 52 classic, 33 follicular, and 15 tall cell variants. The median diameter of tumors was 2.45cm (range 1-6.5). Among all cases, no patient had distant metastasis, whereas lymph node metastasis was evident in 20 cases, multifocality in 39 cases, extrathyroidal extension in 21 cases, capsular invasion in 49 cases, and the presence of tumor in bilateral lobes in 54 cases. Table II shows the results of histomorphological and clinical data.

Expression Rate of SOCS1 and SOCS3 in Benign and PTC Patients

The expression rate of SOCS1 in BTN was negative in 37 cases (92.5%) and had mild reaction (IRS 1) in only 3 cases (7.5%). Expression rate of SOCS3 in BTN was negative in 30 cases (75%) and had mild reaction in 10 cases (25%).

Table I. Immunoreactivity score (IRS) system used for cytokine signaling proteins (SOCS) expression evaluation.

A (percentage of positive cells)	B (intensity of staining)	Final score (multiplication of A and B)
0=no positive cells	0=no color reaction	0-1=negative
1=<10% positive cells	1=mild reaction	2-3=mild
2=10-50% positive cells	2=moderate reaction	4-8=moderate
3=51-80% positive cells	3=intense reaction	9-12=strong reaction
4=>80% positive cells	Final IRS score (A x B): 0-12	

Table II. Comparison of SOCS1 and SOCS3 expression in papillary thyroid carcinoma (PTC) and benign nodules.

		PTC N (%)	Benign disease N (%)	*p*-value
Expression of SOCS1	Negative	38 (38)	37 (92.5)	0.001*
	Mild	33 (33)	3 (7.5)	
	Moderate	26 (26)	0 (0)	
	Strong	3 (3)	0 (0)	
Expression of SOCS3	Negative	2 (2)	30 (75)	0.001*
	Mild	19 (19)	10 (25)	
	Moderate	48 (48)	0 (0)	
	Strong	31 (31)	0 (0)	

*$p<0.05$; Chi-square test; cytokine signaling proteins (SOCS).

SOCS1 expression in PTC was negative in 38 cases (38%), mild in 33 cases (33%), moderate in 26 cases (26%) and strong in 3 cases (3%) (Figures 1-4). SOCS3 expression in PTC was negative in 2 cases (2%), mild in 19 cases (19%), moderate in 48 cases (48%) and strong in 31 cases (31%) (Table II and Figures 5-8). Thyroid follicular epithelium was negative for either protein (Figures 9-10).

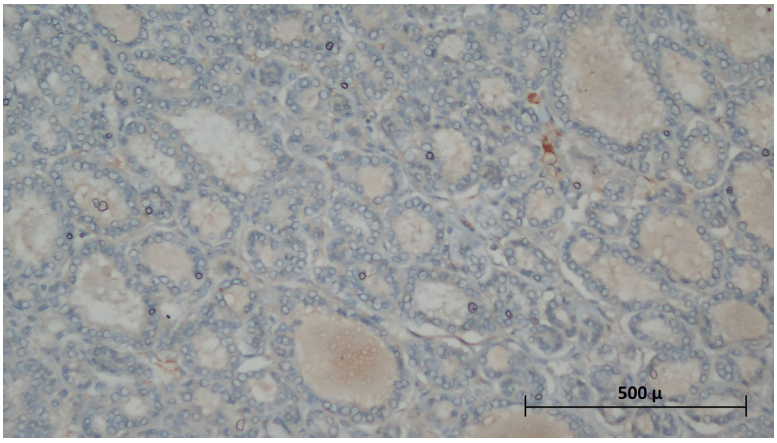

Figure 1. PTC negative for SOCS1 (x200).

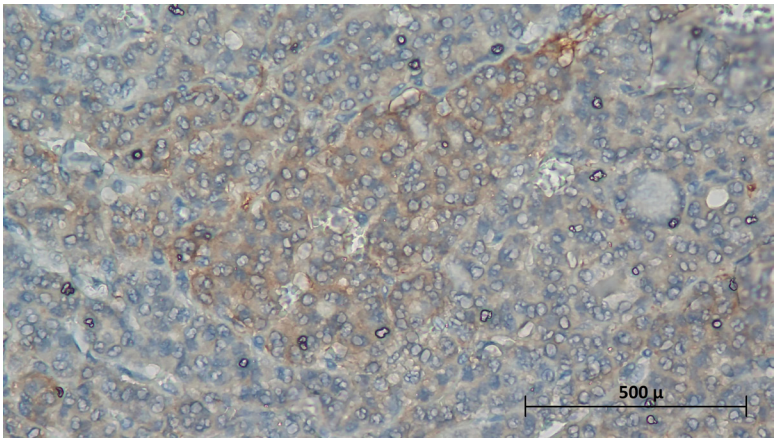

Figure 2. PTC shows mild reaction with SOCS1 (x200).

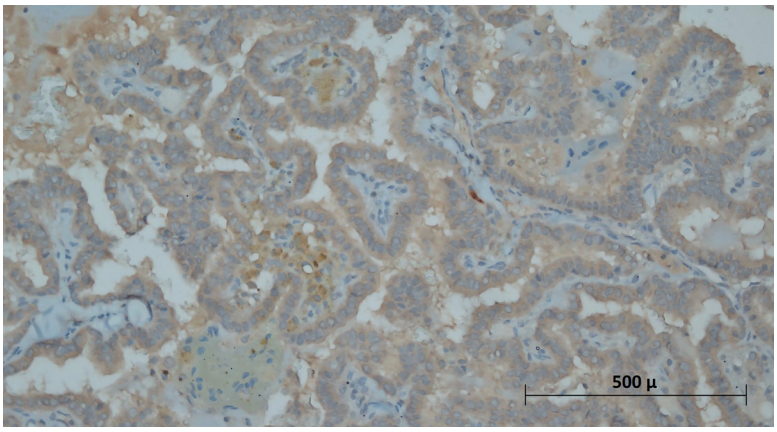

Figure 3. PTC shows moderate reaction with SOCS1 (x200).

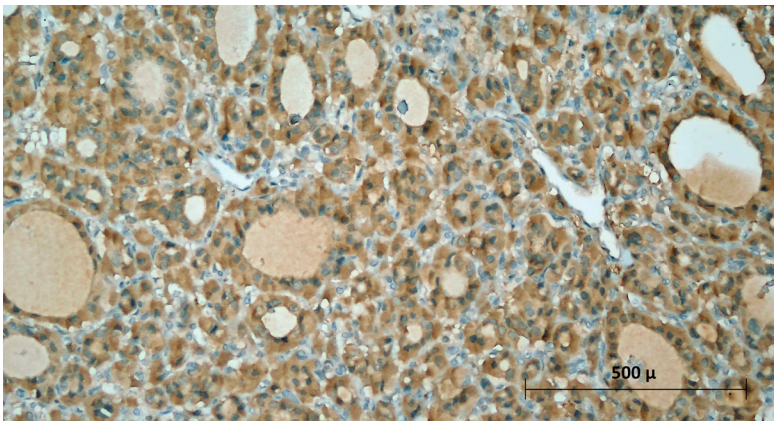

Figure 4. PTC shows strong reaction with SOCS1 (x200).

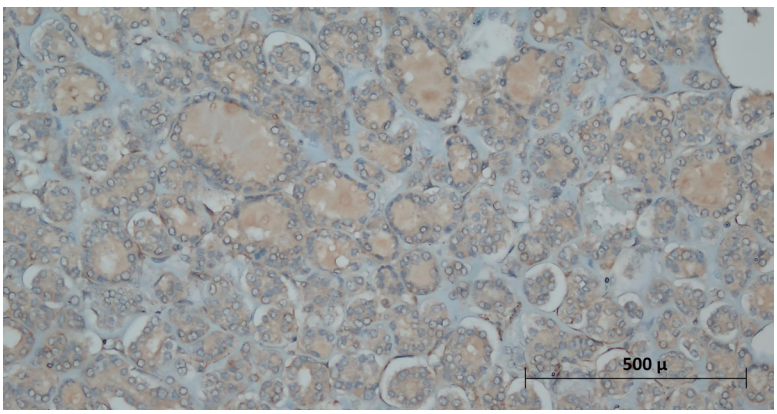

Figure 5. PTC negative for SOCS3 (x200).

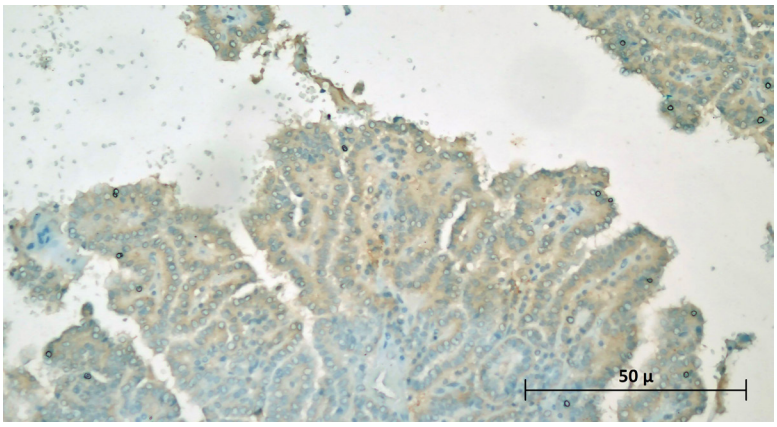

Figure 6. PTC shows mild reaction with SOCS3 (x200).

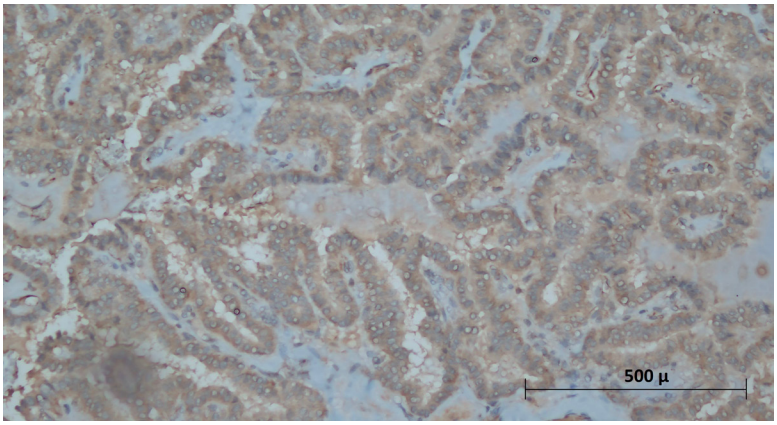

Figure 7. PTC shows moderate reaction with SOCS3 (x200).

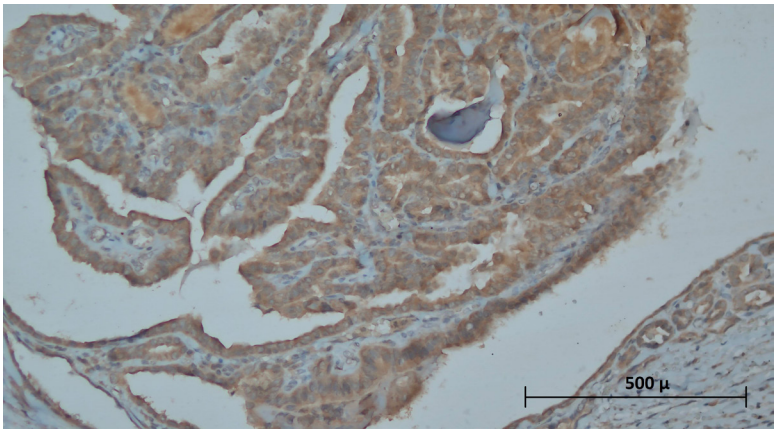

Figure 8. PTC shows strong reaction with SOCS3 (x200).

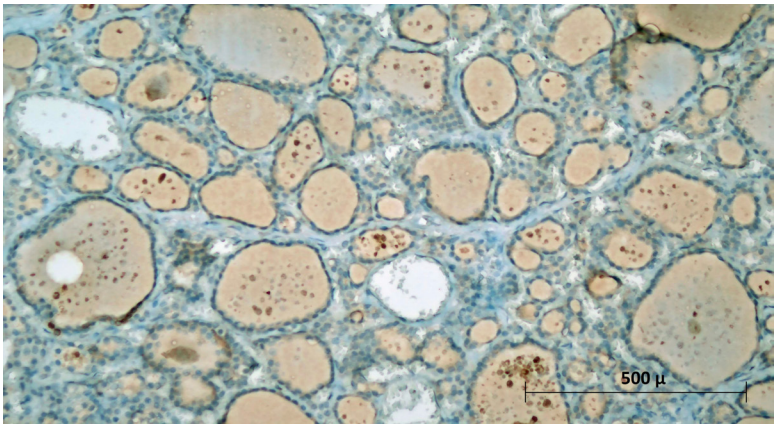

Figure 9. Thyroid follicular epithelium was negative for SOCS1 (x200).

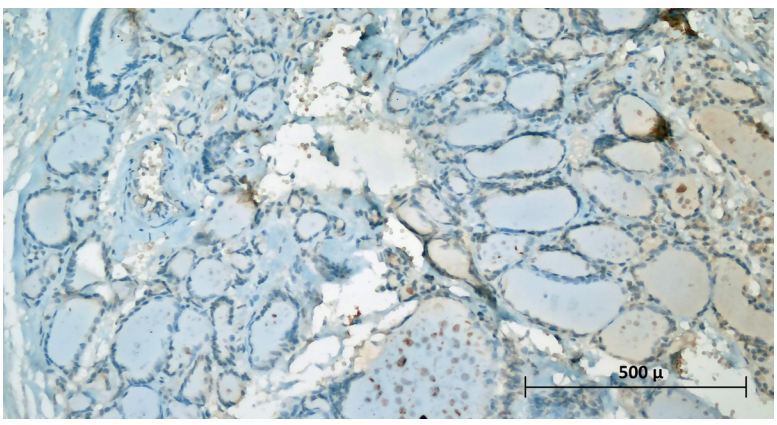

Figure 10. Thyroid follicular epithelium was negative for SOCS3 (x200).

Comparison of SOCS1 and SOCS3 Expression Rate of patients with BTN vs. PTC

Although both BTN and PTC showed immunoreactivity with SOCS1 and SOCS3, the expression rate of these antibodies was significantly higher in PTC than in BTN. The comparison of SOCS1 and SOCS3 expression in PTC *vs.* BTN was statistically significant (p=0.001 and p=0.001, respectively) (Table II).

Comparison of SOCS1 and SOCS3 Expression Rate in PTC with Epidemiological and Clinical Parameters

The propensity of SOCS1 expression in PTC was found to be in older patients rather than younger patients (p=0.001) (Table III), whereas SOCS3 showed relatively equal expression across all ages (p=0.277) (Table IV). Expression of SOCS1 and SOCS3 had no relation with gender comparison (Tables V and VI). Neither of the clinical parameters, including tumor size, serum levels of thyroglobulin and thyrotropin (TSH), stimulated thyroglobulin and TSH, and the Radioactive Iodine doses had no significant statistical relation with the expression of SOCS1 and SOCS3 (Tables VII and VIII). Unlike SOCS1, which showed no relation with the risk groups (p=0.288) (Table IX), SOCS3 was related to the risk groups based on the American Thyroid Association (ATA) risk stratification system (p=0.001) (Table X).

Comparison of SOCS1 and SOCS3 Expression Rate in PTC with Histomorphological Parameters

Regarding histological subtypes, no statistical relation was observed with the expression of SOCS1 and SOCS3 (Table IX). Neither histomorphological parameters, including lymph node involvement, multifocality, unilateral *vs.* bilateral lobe involvement, capsule invasion, and extrathyroid extension, revealed any statistically significant result when compared with SOCS1 expression (Table IX). SOCS3, on the other hand, was significantly related to lymph node involvement, capsule invasion as well as extrathyroid extension (Table X).

Table III. Comparison of SOCS1 expression in papillary thyroid carcinoma (PTC) with age.

	Expression of SOCS1				*p*-value
	Negative Average±SD	Mild Average±SD	Moderate Average±SD	Strong Average±SD	
Age	36.42±12.01[a]	37.76±14.99[a]	49.04±15.58[b]	53.67±13.58[b]	0.001*

*p<0.05; [a]Variant analysis; [b]Represents the difference between groups (Tukey test); cytokine signaling proteins (SOCS).

Table IV. Comparison of SOCS3 expression in papillary thyroid carcinoma (PTC) with age.

	Expression of SOCS3				*p*-value
	Negative Average±SD	Mild Average±SD	Moderate Average±SD	Strong Average±SD	
Age	38.5±20.51	38.47±11.29	38.77±14.31	45.06±17.37	0.277

Variant analysis; cytokine signaling proteins (SOCS).

Table V. Comparison of SOCS1 expression in papillary thyroid carcinoma (PTC) with gender.

		Expression of SOCS1				*p*-value
		Negative N (%)	Mild N (%)	Moderate N (%)	Strong N (%)	
Gender	Male	3 (7.89)	5 (15.15)	8 (30.77)	1 (33.33)	0.095
	Female	35 (92.11)	28 (84.85)	18 (69.23)	2 (66.67)	

Chi-square test; cytokine signaling proteins (SOCS).

Table VI. Comparison of SOCS3 expression in papillary thyroid carcinoma (PTC) with gender.

		Expression of SOCS3				*p*-value
		Negative N (%)	Mild N (%)	Moderate N (%)	Strong N (%)	
Gender	Male	0 (0)	3 (15.79)	8 (16.67)	6 (19.35)	0.907
	Female	2 (100)	16 (84.21)	40 (83.33)	25 (80.65)	

Chi-square test; cytokine signaling proteins (SOCS).

Table VII. Comparison of SOCS1 expression rate in papillary thyroid carcinoma (PTC) with clinical parameters.

	Expression of SOCS1 in PTC				*p*-value
	Negative Median value (Q1-Q3)	Mild N (%) (Q1-Q3)	Moderate N (%) (Q1-Q3)	Strong N (%) (Q1-Q3)	
Tumor size (cm)	2.1 (1.5-2.9)	2 (1.7-2.7)	2.3 (1.6-4)	4.4 (1.3-6.2)	0.569
Thyroglobulin level (ng/mL)	0.05 (0.02-0.2)	0.08 (0.02-0.15)	0.11 (0.04-0.38)	0.04 (0.04-0.2)	0.509
TSH level (mU/L)	0.32 (0.07-0.89)	0.49 (0.2-1.7)	0.88 (0.1-4.2)	0.4 (0.12-3.6)	0.417
Stimulated Thyroglobulin level (ng/mL)	2.4 (0.2-6.05)	2.9 (1.28-17)	1.55 (0.22-9.8)	6 (0.2-512)	0.334
Stimulated TSH level (mU/L)	62.5 (49-83)	64 (41-97)	59.5 (45-80)	50 (50-156)	0.969

Kruskal-Wallis H test; cytokine signaling proteins (SOCS).

Table VIII. Comparison of SOCS3 expression rate in papillary thyroid carcinoma (PTC) with clinical parameters.

	Expression of SOCS3 in PTC				*p*-value
	Negative Median value (Q1-Q3)	Mild N (%) (Q1-Q3)	Moderate N (%) (Q1-Q3)	Strong N (%) (Q1-Q3)	
Tumor size (cm)	2.1 (1.7-2.5)	2 (1.5-2.7)	2 (1.5 -2.5)	2.7 (1.7-4)	0.062
Thyroglobulin level (ng/mL)	0.13 (0.05-0.2)	0.1 (0.03-0.2)	0.04 (0.02 -0.18)	0.2 (0.04-0.55)	0.093
TSH level (mU/L)	0.67 (0.44-0.89)	0.17 (0.1-0.66)	0.6 (0.07 -1.9)	0.88 (0.16-5)	0.085
Stimulated Thyroglobulin level (ng/mL)	12.1 (0.2-24)	1.6 (0.2-4.1)	1.8 (0.24 -9.75)	3.35 (1.8-17)	0.334
Stimulated TSH level (mU/L)	112 (90-134)	59.5 (50-67)	60.5 (47.5 -86.5)	63 (44-79)	0.401

Kruskal-Wallis H test; cytokine signaling proteins (SOCS).

Discussion

The relationship between ongoing inflammation and cancer development has been studied for many years. Thyroid diseases, which tend to affect younger female patients[38], are accompanied by chronic inflammation. The expression of SOCS1 and SOCS3 proteins, which act as negative feedback regulators of cytokine-mediated signaling, is abnormal in several organ malignancies[34]. This study investigated the expression of SOCS1 and SOCS3 in PTC and benign nodules, and their relations with clinical and histomorphological parameters.

Table IX. Comparison of SOCS1 expression with histological features, ATA risk classification and RAI doses.

		Expression of SOCS1				
		Negative N (%)	Mild N (%)	Moderate N (%)	Strong N (%)	*p*-value
Histological type	Follicular	14 (36.84)	7 (21.21)	10 (38.46)	1 (33.33)	0.346
	Classic	20 (52.63)	22 (66.67)	10 (38.46)	1 (33.33)	
	Tall cell	4 (10.53)	4 (12.12)	6 (23.08)	1 (33.33)	
Lymph node metastasis	Absent	33 (86.84)	25 (75.76)	19 (73.08)	3 (100)	0.390
	Present	5 (13.16)	8 (24.24)	7 (26.92)	0 (0)	
Multifocality	Absent	27 (71.05)	20 (60.61)	13 (50)	1 (33.33)	0.272
	Present	11 (28.95)	13 (39.39)	13 (50)	2 (66.67)	
Extrathyroid extension	Absent	33 (86.84)	26 (78.79)	16 (61.54)	3 (100)	0.082
	Present	5 (13.16)	7 (21.21)	10 (38.46)	0 (0)	
Capsule invasion	Absent	22 (57.89)	19 (57.58)	8 (30.77)	2 (66.67)	0.119
	Present	16 (42.11)	14 (42.42)	18 (69.23)	1 (33.33)	
Unilateral/Bilateral	Unilateral	20 (52.63)	15 (45.45)	10 (38.46)	1 (33.33)	0.690
	Bilateral	18 (47.37)	18 (54.55)	16 (61.54)	2 (66.67)	
ATA risk group	(1) Low risk	21 (56.76)	11 (35.48)	8 (33.33)	2 (66.67)	0.288
	(2) Intermediate risk	14 (37.84)	19 (61.29)	16 (66.67)	1 (33.33)	
	(3) High	2 (5.41)	1 (3.23)	0 (0)	0 (0)	
RAI doses (mCI)	<150	31 (83.78)	24 (77.42)	19 (79.17)	3 (100)	0.752
	≥150	6 (16.22)	7 (22.58)	5 (20.83)	0 (0)	

Chi-square test, cytokine signaling proteins (SOCS); Radioactive Iodine (RAI).

Table X. Comparison of SOCS3 expression with histological features, ATA risk classification and RAI doses.

		Expression of SOCS3				
		Negative N (%)	Mild N (%)	Moderate N (%)	Strong N (%)	*p*-value
Histological type	Follicular	1 (50)	7 (36.84)	15 (31.25)	9 (29.03)	0.955
	Classic	1 (50)	9 (47.37)	27 (56.25)	16 (51.61)	
	Tall cell	0 (0)	3 (15.79)	6 (12.5)	6 (19.35)	
Lymph node metastasis	Absent	1 (50)	18 (94.74)	42 (87.5)	19 (61.29)	0.007*
	Present	1 (50)	1 (5.26)	6 (12.5)	12 (38.71)	
Multifocality	Absent	2 (100)	11 (57.89)	31 (64.58)	17 (54.84)	0.550
	Present	0 (0)	8 (42.11)	17 (35.42)	14 (45.16)	
Extrathyroid extension	Absent	2 (100)	18 (94.74)	40 (83.33)	18 (58.06)	0.009*
	Present	0 (0)	1 (5.26)	8 (16.67)	13 (41.94)	
Capsule invasion	Absent	1 (50)	16 (84.21)	24 (50)	10 (32.26)	0.005*
	Present	1 (50)	3 (15.79)	24 (50)	21 (67.74)	
Unilateral/Bilateral	Unilateral	1 (50)	7 (36.84)	25 (52.08)	13 (41.94)	0.665
	Bilateral	1 (50)	12 (63.16)	23 (47.92)	18 (58.06)	
ATA risk group	(1) Low risk	2 (100)	16 (88.89)	19 (42.22)	5 (16.67)	0.001*
	(2) Intermediate risk	0 (0)	2 (11.11)	24 (53.33)	24 (80)	
	(3) High	0 (0)	0 (0)	2 (4.44)	1 (3.33)	
RAI doses (mCI)	<150	2 (100)	16 (88.89)	37 (82.22)	22 (73.33)	0.495
	≥150	0 (0)	2 (11.11)	8 (17.78)	8 (26.67)	

*$p<0.05$; Chi-square test; cytokine signaling proteins (SOCS); Radioactive Iodine (RAI).

The expression of SOCS1 and SOCS3 was significantly increased in PTC compared to benign nodules and non-neoplastic thyroid follicular epithelium, in line with several other studies in literature. Kobawala et al[39] studied the expression of SOCS1-3 in 83 cases with PTC and 45 with benign thyroid diseases and found significantly increased expression of the three markers in PTC. Singh et al[40] investigated SOCS1 expression in neoplastic and non-neoplastic prostate samples and showed that SOCS1 expression was significantly increased in prostate cancer tissues compared to benign prostate hyperplasia. Similarly, Calarco et al[41] reported increased SOCS3 expression in prostate cancer. In a study of 126 prostate cancer and 28 periprostatic non-neoplastic tissue samples, Zhu et al[42] revealed greater SOCS2 expression in the prostate cancer samples. Yang et al[43] reported moderate to very strong SOCS3 expression in 67.8% of hepatocellular carcinoma cases. Moreover, Raccurt et al[44] found elevated expression of SOCS1-3 in tumor cells; in particular, SOCS2 was significantly increased in *in situ* carcinoma. Besides solid organ tumors, studies[45-48] of hematological malignancies also showed increased SOCS expression, mostly using *ex vivo* models. SOCS3 expression was increased in mycosis fungoides[45], chronic myeloid leukemia (CML)[46] and anaplastic large cell lymphoma[47], while SOCS1 and SOCS3 were higher in acute myeloid leukaemia[48].

The increased and consistent expression of SOCS3, in particular, suggests its implementation as an additional useful immune marker to the immunohistochemistry panel used in PTC, which consists of Hector Battifora and Mesothelioma (HBME-1), cytokeratin 19 (CK19) and galectin 3. This utilization of SOCS3 would increase diagnostic accuracy, especially in fine needle aspiration cytology when combined with immunohistochemistry methods.

Contrasting with the increased SOCS protein expression discussed above, several studies[35,36,42,49-55] have reported equal or decreased expression in cancerous tissues compared to their normal histological counterparts. Francipane et al[36] reported decreased SOCS1 and SOCS3 expression in PTC, whereas expression in thyrocytes was normal. De Santis et al[35] reported similar results, i.e., markedly downregulated SOCS1 in PTC compared to surrounding normal tissue. Liu et al[50] found that the expression of SOCS1 and SOCS3 in astrocytomas was higher in the non-cancerous component. Cui et al[51] found downregulated SOCS2 expression in hepatocellular carcinoma, while Deng et al[55] noted significantly lower SOCS3 expression in gastric carcinoma than in normal mucosa. Similarly, Li et al[54] reported that both SOCS1 and SOCS3 mRNA expression were lower in gastric tumor tissues than in normal tissues, while Zhu et al[42] found increased SOCS2 expression in prostate cancer, but downregulated SOCS6 expression. Sasi et al[49] compared the expression of several SOCS proteins between breast cancer and non-cancer breast tissues and found no significant difference in SOCS1-3 and 7 expressions in both tissue types. Marioni et al[52] found no significant difference in SOCS1 expression among laryngeal lesions, including squamous cell carcinoma, hyperplastic benign lesions, and normal laryngeal mucosa. In addition, Ayyildiz et al[53] found equal SOCS1 expression in colorectal carcinoma and a control group.

Dysregulated SOCS proteins during carcinogenesis include both inactivated and upregulated SOCS proteins. For example, the increase in SOCS2 in CML might have negative effects on other SOCS proteins, which would suppress tumor development[56,57]. The continuous effects of cytokines on the JAK-STAT and other pathways may support cancer progression in tumor cells. Rather than being a causal mechanism, SOCS upregulation might result from activating these pathways. Additionally, failure of other negative regulatory pathways acting on the JAK-STAT pathway, or inappropriate oncogene activation, in such tumors could exceed the capacity of SOCS, thereby contributing to STAT activation. Despite the increased SOCS expression, their function as inhibitory proteins remains insufficient to inhibit cancer cell proliferation. As a result, the increased SOCS expression might be a consequence of cancer progression, rather than a contributing factor[58]. This could also explain the elevated levels of SOCS proteins observed herein, which could be interpreted as a consequence of cancer development.

In the present study, the expression of SOCS1 in PTC was significantly higher in older than younger patients, but this was not observed with SOCS3. Zhu et al[42] found that SOCS2 expression in prostate cancer was increased in patients younger than 60 years, while Ayyildiz et al[53] found no significant relationship between age and SOCS1 expression in colon cancer. We found no correlations between the clinical parameters we investigated and SOCS expression, except for the expression of SOCS3 and the clinical risk

group. The ATA risk stratification system allows clinicians to plan treatment by distinguishing low, intermediate, and high-risk groups[59]. Unlike SOCS1, SOCS3 showed intermediate to strong reactivity in the intermediate risk group, which might serve as an immunohistochemistry indicator in addition to the criteria evaluated in this risk group.

Finally, the analysis comparing SOCS3 reactivity with histomorphological parameters revealed a positive correlation with thyroid capsule invasion, but negative correlations with extrathyroidal extension and lymph node metastasis. The relation between deficient SOCS3 expression in advanced cases and extrathyroidal extension and lymph node metastasis suggests a protective role of SOCS3 against carcinogenesis. In this regard, the ability of SOCS3 to inhibit cell growth and migration by blocking the JAK/STAT pathway in oncogenesis might be compromised. These results are in line with several other studies. Deng et al[55] reported decreased SOCS3 expression in advanced gastric carcinoma with lymph node metastasis. Another study found significantly lower SOCS3 mRNA expression in advanced breast cancer patients with greater lymph node involvement than patients with less lymph node involvement or negative lymph nodes[60]. In addition, Ying et al[61] reported relations of decreased SOCS3 expression with lymph node metastasis, blood vessel invasion, and disease-free survival in breast carcinoma. Although many studies in the literature reported a strong negative correlation between SOCS3 expression and advanced disease features, others found the opposite. For example, Yang et al[43] found that SOCS3 expression was positively correlated with tumor vascular invasion.

Conclusions

The expression of SOCS1 and SOCS3 proteins in PTC is significantly increased in malignant thyroid disease compared with benign diseases, suggesting that SOCS proteins have direct and indirect functions in thyroid cancer pathogenesis. Therefore, SOCS expression in thyroid cytology could support the diagnosis of PTC, in addition to immunohistochemical markers and nuclear morphology, and aid the prediction of lymph node involvement. SOCS3 is a promising marker that might predict ATA risk, although this should be verified in larger studies.

Conflicts of Interest

There are no conflicts of interest.

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Ethics Approval

Ethical approval of this study was obtained by the Ethics Committee of Gaziantep University Faculty of Medicine (Number of approval: 2022/478).

Informed Consent

All patients were informed about the study and signed the informed patient consent form.

Authors' Contribution

O. Eronat and Z. Bozdag were involved in patient selection, reevaluation of all cases, pathological data recording, SOCS 1 and 3 immune reaction analysis and scoring. S. Ozturk and E. Akarsu provided clinical information and interpretation of the relation of expression results with clinical data. All authors contributed equally to manuscript drafting, writing and statistics.

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References

1) American Cancer Society. Cancer Facts & Figures 2022; 1-11.c Available at: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2022.html.
2) Londero S, Krogdahl A, Bastholt L, Overgaard J, Pedersen H, Hahn C, Bentzen J, Schytte S, Christiansen P, Gerke O, Godballe C. Papillary thyroid carcinoma in Denmark, 1996-2008: Outcome and evaluation of established prognostic scoring systems in a prospective national cohort. Thyroid 2015; 25: 78-84.
3) Thyroid Association. The American Thyroid Association®, 2017.
4) Hegedus L. The Thyroid Nodule. New Eng J Med 2004; 351: 1764-1771.
5) Bozec A, Lassalle S, Hofman V, Ilie M, Santini J, Hofman P. The Thyroid Gland: A Crossroad in Inflammation-Induced Carcinoma? An Ongoing Debate with New Therapeutic Potential. Curr Med Chem 2010; 17: 3449-3561.
6) Pisanu A, Piu S, Cois A, Uccheddu A. Coexisting Hashimoto's thyroiditis with differentiated thyroid

cancer and benign thyroid diseases: indications for thyroidectomy. Chir Ital 2003; 55: 365-372.

7) Ahn D, Heo S, Park J, Kim J, Sohn J, Park J, Park S, Park J. Clinical relationship between Hashimoto's thyroiditis and papillary thyroid cancer. Acta Oncol 2011; 50: 1228-1234.

8) Alkurt EG, Şahin F, Tutan B, Canal K, Turhan VB. The relationship between papillary thyroid cancer and triglyceride/glucose index, which is an indicator of insulin resistance. Eur Rev Med Pharmacol Sci 2022; 26: 6114-6120.

9) Hodzic-Redzic S, Bumber B, Prgomet D, Rogic D. The role of preoperative levels of serum IL-6, IL-8 and TNF-α and conventional inflammatory parameters in the detection of metastatic forms of papillary thyroid cancer. World Cancer Res J 2021; 8: e1915.

10) Russell JP, Shinohara S, Melillo RM, Castellone MD, Santoro M, Rothstein JL. Tyrosine kinase oncoprotein, RET/PTC3, induces the secretion of myeloid growth and chemotactic factors. Oncogene 2003; 22: 4569-4577.

11) Iwahashi N, Murakami H, Nimura Y, Takahashi M. Activation of RET tyrosine kinase regulates interleukin-8 production by multiple signaling pathways. Biochem Biophys Res Commun 2002; 294: 642-649.

12) Russell JP, Engiles JB, Rothstein JL. Proinflammatory Mediators and Genetic Background in Oncogene Mediated Tumor Progression. J Immunol 2004; 172: 4059-4067.

13) Shinohara S, Rothstein JL. Interleukin 24 is induced by the RET/PTC3 oncoprotein and is an autocrine growth factor for epithelial cells. Oncogene 2004; 23: 7571-7579.

14) Puxeddu E, Knauf JA, Sartor MA, Mitsutake N, Smith EP, Medvedovic M, Tomlinson CR, Moretti S, Fagin JA. RET/PTC-induced gene expression in thyroid PCCL3 cells reveals early activation of genes involved in regulation of the immune response. Endocr Relat Cancer 2055; 12: 319-334.

15) Borrello MG, Alberti L, Fischer A, Degl'Innocenti D, Ferrario C, Gariboldi M, Marchesi F, Allavena P, Greco A, Collini P, Pilotti S, Cassinelli G, Bressan P, Fugazzola L, Mantovani A, Pierotti MA. Induction of a proinflammatory program in normal human thyrocytes by the RETPTC1 Oncogene. Proc Natl Acad Sci 2005; 102: 14825-14830.

16) Melillo RM, Castellone M, Guarino V, De Falco V, Cirafici A, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhoffer M, Orntoft T, Fusco A, Santoro M. The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. J Clin Investig 2005; 115: 1068-1081.

17) Castellone MD, Celetti A, Guarino V, Cirafici AM, Basolo F, Giannini R, Medico E, Kruhoffer M, Orntoft TF, Curcio F, Fusco A, Melillo RM, Santoro M. Autocrine stimulation by osteopontin plays a pivotal role in the expression of the mitogenic and invasive phenotype of RET/PTC-transformed thyroid cells. Oncogene 2004; 23: 2188-2196.

18) Guarino V, Castellone MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. Mol Cell Endocrinol 2010; 321: 94-102.

19) Morris R, Kershaw NJ, Babon JJ. The molecular details of cytokine signaling via the JAK/STAT pathway. Protein Sci 2018; 27: 1984-2009.

20) Velarquez L, Fellous M, Stark GF, Pellegrini S. A Protein Tyrosine Kinase in the Interferon a/p Signaling Pathway. Cell 1992; 70: 313-322.

21) Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zürcher G, Ziemiecki A. Two Novel Protein-Tyrosine Kinases, Each with a Second Phosphotransferase-Related Catalytic Domain, Define a New Class of Protein Kinase. Mol Cell Biol 1992; 11: 2057-2065.

22) Kawamura M, McVicar DW, Johnston JA, Blake TB, Chen Y, Lal BK, Lloyd AR, Kelvin DJ, Staples JE, Ortaldo JR, O'Shea JJ. Molecular cloning of L-JAK, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc Natl Acad Sci U S A 1994; 91: 6374-6378.

23) Wilks AF. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci 1989; 86: 1603-1607.

24) Feng J, Witthuhn BA, Matsuda T, Kohlhuber F, Kerr IM, Ihle JM. Activation of Jak2 catalytic activity requires phosphorylation of Y1007 in the kinase activation loop. Mol Cell Biol 1997; 17: 2497-2501.

25) Chen XP, Losman JA, Rothman P. SOCS Proteins, Regulators Minireview of Intracellular Signaling. Immunity 2000; 13: 287-290.

26) Shuai K, Stark GR, Kerr IM, Darnell Jr. JE. A single phosphotyrosine residue of Stat91 required forgene activation by interferon. Science 1993; 261: 1744-1746.

27) Shuai K, Ziemlecki A, Wilks AF, Harpur AG, Sadowski HB, Gilmann MZ, Darnell Jr. JE. Polypeptide signalling to the nucleus through tyrosine phos-phorylation of Jak and Stat proteins. Lab Mol Cell Biol 1993; 366: 580-583.

28) Naka T, Narazaki M, Hirata M, Matsumoto M, Minamoto S, Aono A, Nishimoto N, Kajita K, Taga T, Yoshizaki K, Akira A, Kishimoto T. Structure and function of a new STAT-induced STAT inhibitor. Nature 1997; 387: 924-929.

29) Starr R, Willson TA, Viney EM, Murray LJL, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ. A familiy of cytokine-inducible inhibitors of signalling. Nature 1997; 387: 917-921.

30) Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S, Yoshimura A. A new protein containing an SH2 domain that inhibits JAK kinases. Nature 1997; 387: 921-924.

31) Matsumoto A, Masuhara M, Mitsui K, Yokouchi M, Ohtsubo M, Misawa H, Miyajima A, Yoshimura A. CIS, a Cytokine Inducible SH2 Protein, Is a

Target of the JAK-STAT5 Pathway and Modulates STAT5 Activation. Hematopoiesis 1997; 89: 3148-3154.

32) Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcale D, Nicola NA. Twenty Proteins Containing a C-Terminal SOCS Box Form Five Structural Classes. Proc Natl Acad Sci 1998; 95: 114-119.

33) Yasukawa H, Misawa H, Sakamoto H, Masuhara M, Sasaki A, Wakioka T, Ohtsuka S, Imaizumi T, Matsuda T, Ihle JN, Yoshimura A. The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. Eur Mol Biol Org J 1999; 18: 1309-1320.

34) Ohara KI, Kondo T, Ito M, Yoshimura A. SOCS, inflammation, and cancer. Landes Biosci 2013; 2: 1-10.

35) De Santis E, Vito MD, Perrone GA, Mari E, Osti M, De Antoni E, Coppola L, Tafani M, Carpi A, Russo MA. Overexpression of pro-inflammatory genes and down-regulation of SOCS-1 in human PTC and in hypoxic BCPAP cells. Biomed Pharmacother 2013; 67: 7-16.

36) Francipane MG, Eterno V, Spina V, Bini M, Scerrino G, Buscemi G, Gulotta G, Todaro M, Dieli F, De Maria R, Stassi G. Suppressor of cytokine signaling 3 sensitizes anaplastic thyroid cancer to standard chemotherapy. Cancer Res 2009; 69: 6141-6148.

37) Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 1987; 83: 138-140.

38) Halfaoui NS, Majda D, Nouria D, Boulenouar H, Behar A, Belhadj M. Dietary and female reproductive risk factors for thyroid cancer: a case-control study in Western Algeria. World Cancer Res J 2021; 8: e1927.

39) Kobawala TP, Trivedi TI, Gajjar KK, Patel GH, Ghosh NR. Significance of expression of suppressor of cytokine signaling proteins: Suppressor of cytokine signaling-1, suppressor of cytokine signaling-2, and suppressor of cytokine signaling-3 in papillary thyroid cancer. J Cancer Res Ther 2017; 13: 337-345.

40) Singh N, Hussain S, Bharadwaj M, Kakkar N, Singh SK, Sobti RC. Overexpression of signal transducer and activator of transcription (STAT-3 and STAT-5) transcription factors and alteration of suppressor of cytokine signaling (SOCS-1) protein in prostate cancer. J Recept Signal Transduct 2012; 32: 321-327.

41) Calarco, A. Pinto F, Pierconti F, Sacco E, Marrucci E, Totaro A, Palermo G, Vittori M, Bassi P. Role of SOCS3 evaluated by immunohistochemical analysis in a cohort of patients affected by prostate cancer: preliminary results. Urologia 2012; 79: 4-8.

42) Zhu JG, Dai QS, Han ZD, He HC, Mo RJ, Chen G, Chen YF, Wu YD, Yang SB, Jiang FN, Chen WH, Sun ZL, Zhong WD. Expression of SOCSs in human prostate cancer and their association in prognosis. Mol Cell Bioch 2013; 381: 51-59.

43) Yang SF, Yeh YT, Wang SN, Hung SC, Chen WT, Huang CH, Chai YH. SOCS-3 is associated with vascular invasion and overall survival in hepatocellular carcinoma. Pathology 2008; 40: 558-563.

44) Raccurt M, Tam SP, Lau P, Mertani HC, Lambert A, Garcia-Cabalerro T, Li H, Brown RJ, McGuckin MA, Morel G, Waters MJ. Suppressor of cytokine signalling gene expression is elevated in breast carcinoma. Br J Cancer 2003; 89: 524-532.

45) Brender C, Nielsen M, Kaltoft K, Mikkelsen G, Zhang Q, Wasik M, Billestrup N, Odum N. STAT3-mediated constitutive expression of SOCS-3 in cutaneous T-cell lymphoma. Neoplasia 2001; 97: 1056-1062.

46) Sakai I, Takeuchi K, Yamauchi H, Narumi H, Fujita S. Constitutive expression of SOCS3 confers resistance to IFN-α in chronic myelogenous leukemia cells. Blood 2002; 100: 2926-2931.

47) Cho-Vega JH, Rassidakis GZ, Amin HM, Tsioli P, Spurgers K, Remache YK, Vega F, Goy AH, Gilles F, Medeiros LJ. Suppressor of cytokine signaling 3 expression in anaplastic large cell lymphoma. Leukemia 2004; 18: 1872-1878.

48) Schuringa JJ, Wierenga, ATJ. Kruijer W, Vellenga E. Constitutive Stat3, Tyr705, and Ser727 phosphorylation in acute myeloid leukemia cells caused by the autocrine secretion of interleukin-6. Hematopoiesis 2000; 95: 3765-3770.

49) Sasi W, Jiang WG, Sharma A, Mokbel K. Higher expression levels of SOCS 1,3,4,7 are associated with earlier tumour stage and better clinical outcome in human breast cancer. BMC Cancer 2010; 10: 1-13.

50) Liu LH, Li H, Cheng XX, Kong QY, Chen XY, Wu ML, Li Y, Liu J, Li C. Correlative analyses of the expression levels of PIAS3, p-SHP2, SOCS1 and SOCS3 with STAT3 activation in human astrocytomas. Mol Med Rep 2017; 15: 847-852.

51) Cui M, Sun J, Hou J, Fang T, Wang X, Ge C, Zhao F, Chen T, Xie H, Cui Y, Yao M, Li J, Li H. The suppressor of cytokine signaling 2 (SOCS2) inhibits tumor metastasis in hepatocellular carcinoma. Tumor Biol 2016; 37: 13521-13531.

52) Marioni G, Agostini M, Cappellesso R, Bedin C, Ottaviano G, Marchese-Ragona R, Lovato A, Cacco T, Giacomelli L, Nitti D, Blandamura S, Stellini E, De Filippis C. miR-19a and SOCS-1 expression in the differential diagnosis of laryngeal (glottic) verrucous squamous cell carcinoma. J Clin Pathol 2016; 69: 415-421.

53) Ayyildiz T, Dolar E, Adim SB, Eminler AT, Yerci O. Lack of prognostic significance of SOCS-1 expression in colorectal adenocarcinomas. Asian Pac J Cancer Prev 2014, 15: 8469-8474.

54) Li G, Xu j, Wang Z, Yuan Y, Li, Y, Cai S, He Y. Low expression of SOCS-1 and SOCS-3 is a poor prognostic indicator for gastric cancer patients. J Cancer Res Clin Oncol 2015; 141: 443-452.

55) Deng J, Jiao X, Liu H, Wu L, Zhang R, Wang B, Pan Y, Hao X, Liang H. Lymph node metastasis is mediated by suppressor of cytokine signaling-3 in gastric cancer. Tumor Biol 2013; 34: 3627-3636.

56) Zheng C, Li L, Haak M, Brors B, Frank O, Giehl M, Fabarius A, Schatz M, Weisser A, Lorentz C, Gretz N, Hehlmann R, Hochhaus A, Seifarth W. Gene expression profiling of CD34+ cells identifies a molecular signature of chronic myeloid leukemia blast crisis. Leukemia 2016; 20: 1028-1034.

57) Schultheis B, Carapeti-Marootian M, Hochhaus A, Weisser A, Goldman JM, Melo JV. Overexpression of SOCS-2 in advanced stages of chronic myeloid leukemia: possible inadequacy of a negative feedback mechanism. Neoplasia 2016, 99: 1766-1775.

58) Sasi W, Sharma AK, Mokbel K. The Role of Suppressors of Cytokine Signalling in Human Neoplasms. Mol Biol Int 2014; 2014: 630797.

59) Ebner S. ATA risk stratification system correctly predicts the chances of thyroid cancer relapse at 1 year. Clinical Thyroidology® for the Public 2020; 13: 11.

60) Nakagawa T, lida S, Osanai T, Uetake H, Aruga T, Toriya Y, Takagi Y, Kawachi H, Sugihara K. Decreased expression ofSOCS-3 mRNA in breaset cancer with lymph node metastasis. Oncol Rep 2008; 19: 9-33.

61) Ying M, Li D, Yang L, Wang M, Wang N, Chen Y, He M, Wang Y. Loss of SOCS3 expression is associated with an increased risk of recurrent disease in breast carcinoma. J Cancer Res Clin Oncol 2010; 136: 1617-1626.