Original Article
Middle East Journal of Cancer; January 2023; 14(1): 29-40

Expressions of YAP-1, ANXA10, and UNC5D in Tissues of Papillary Thyroid Carcinoma; Prognostic, Pathological, and Clinical Significance

Walid S. H. Elsayed*, MD, Ola A. Harb*♦, MD, Ahmed A. Obaya, MD, Dalia Hamouda Elsayed***, MD, Loay M. Gertallah****, MD, Walid A Mawla****, MD, Ahmed Raafat Abd El Fattah****, MD**

*Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
**Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt
***Department of Medical Oncology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
****Department of General Surgery, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Please cite this article as: Elsayed WSH, Harb OA, Obaya AA, Elsayed DH, Gertallah LM, Mawla WA, et al. Expressions of YAP-1, ANXA10, and UNC5D in tissues of papillary thyroid carcinoma; prognostic, pathological, and clinical significance. Middle East J Cancer. 2023;14(1):29-40. doi: 10.30476/mejc.2022.89869.1551.

Abstract

Background: There is an ongoing need for targeted therapy and systemic treatment protocols for papillary thyroid carcinoma (PTC) patients. Yes activated protein-1 (YAP-1) has been found to control many targets of Hippo pathway. Annexin family is a huge family playing many roles in cellular processes. Annexin A10 (ANXA10) is a member of annexin family. Uncoordinated-5D (UNC5D), a recently discovered Unc5 family member which is found in normal tissues and downregulated in cancer cell lines and tissues. Aim of the study was to assess the expression of YAP-1, ANXA10, and UNC5D in PTC and in non-neoplastic tissues of thyroid gland using immunohistochemistry to evaluate their clinical significance and prognostic values in PTC patients.

Method: In the present prospective study, we took samples from 60 patients with PTC and 30 samples from non-neoplastic thyroid tissues for YAP-1, ANXA10, and UNC5D immunohistochemistry. All the patients were followed up for assessment of the prognostic, clinical, and pathological of their expression.

Results: Upregulation of YAP-1 and ANXA10 in addition to downregulation of UNC5D was found in PTC tissues more than non-neoplastic thyroid tissues.

In PTC tissues, there were positive associations between high YAP-1 and ANXA10 expression, low UNC5D expression, tumor size (P = 0.022, 0.011, 0.014), presence of lymph node metastases (P = 0.005, < 0.001, 0.008), and inferior disease-free survival rate (P = 0.003, 0.01, 0.03).

Conclusion: Upregulation of YAP-1 and ANXA10 expression and downregulation of UNC5D was associated with bad clincopathological criteria, disease progression, high incidence of disease recurrence, and poor survival.

♦Corresponding Author:
Ola A. Harb, MD
Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Email: olaharb2015@gmail.com

Keywords: YAP-1 protein, ANXA10, UNC5D, PTC, Immunohistochemistry, Prognosis

Received: January 23, 2021; Accepted: July 04, 2022

Introduction

Thyroid cancer incidence has increased recently forming the fourth most prevalent cancer worldwide.[1] Papillary thyroid carcinoma (PTC) forms the most common histopathological variant forming about 85% of thyroid malignancies.[2] PTC is usually diagnosed in the early stages, which has a good prognosis, while a small percentage of patients experience disease progression, recurrence, and dismal outcome.[3] Thus, there is a need for targeted therapy and systemic treatment protocols for those patients.

Hippo signal transduction pathway plays an important role in modulating organs size through the regulation of cell proliferation and apoptosis balance.[4]

Yes activated protein-1 (YAP-1) has been found to control numerous targets of Hippo pathway.[5] Disturbances in YAP-1 expression have been detected in many malignancies.[5, 6]

Annexin family is a huge family containing about 160 members with more than 65 variable species which play many roles in cellular processes.[7] Annexin A1 (ANXA1) is a member of annexin family.[8] The oncogenic roles of Annexin A10 are found in many cancer types.[9]

Uncoordinated-5 (Unc5) receptors, which include four homologues (Unc5A-D), are found in many human tissues and have roles in many cell processes.[10] UNC5D, a recently discovered Unc5 family member,[11] was found in normal tissues and downregulated in cancer cell lines and cancer tissues.[12]

However, to our knowledge, YAP-1, ANXA10, and UNC5D expressions and roles in PTC have not been sufficiently studied.

Aim of the study was to assess the expressions of YAP-1, ANXA10, and UNC5D in PTC and in non-neoplastic tissues of thyroid gland with immunohistochemistry (IHC) to evaluate their clinical significance and prognostic values in PTC patients.

Materials and Methods

In the current retrospective report, we collected 60 patients with PTC that were surgically managed between May 2014 and January 2020 at the Department of General Surgery. We collected clinicopathologic and prognostic data from files of all the patients. We assessed persistence,

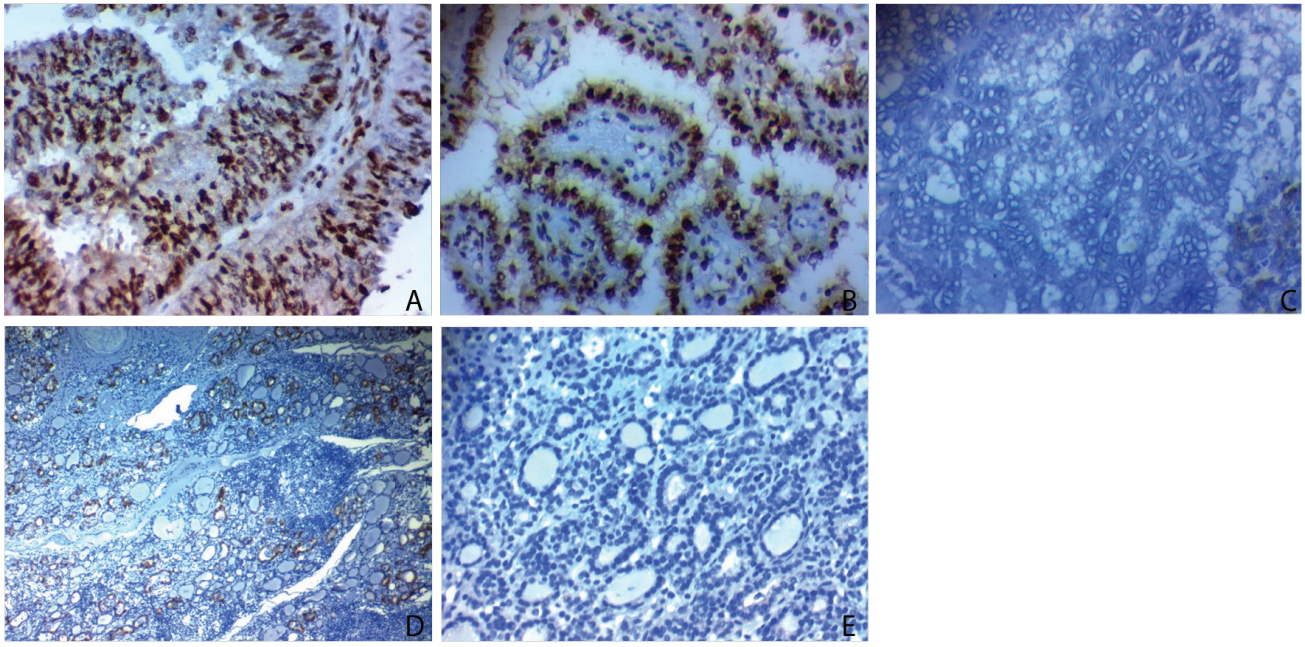

Figure 1. Expression of YAP-1 in tissues of papillary thyroid carcinoma and benign tissues of the thyroid gland: (A) increased nuclear expression in high-grade PTC stage IV (IHC, ×400), (B) increased nuclear expression in high-grade PTC stage III (IHC ×400), (C) decreased nuclear expression in low-grade PTC stage II (IHC, ×400), (D) decreased nuclear expression in low-grade PTC stage I (IHC, ×400), and (E) negative nuclear expression in benign tissues of the thyroid gland (IHC, ×400).
YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D

Table 1. Clinicopathological and baseline features of the included samples

		No.	%
Patient age	**Mean ± Std. deviation (38.9 ± 10.4) median (range) 40 (21-53)**		
Age group	<40y	36	60%
	≥40y	24	40%
Sex	Male	13	21.6%
	Female	47	78.3%
Histopathological subtype			
	Conventional PTC	51	85.0%
	follicular variant PTC	9	15.0%
	non-neoplastic thyroid tissue	30	50%
Group	Case	60	100.0%
	Control	30	50.0%

No.: Number; Std.: Standard deviation; PTC: Papillary thyroid carcinoma

progression, or recurrence of the PTC via imaging. We received ethical approval for our study from the Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University (ethics code: Zag IRB. No 19-2020) and obtained informed consents from the included patients. Once the surgical samples were sent to Pathology Department, they were routinely processed, diagnosed, graded and staged. We classified the pathological stages of PTC using TNM staging system of AJCC/UICC 7th edition. We took samples from 60 patients with PTC and 30 samples from non-neoplastic tissues for IHC.

All the patients were followed for about 35 months (range 12-55 months), Departments of Clinical Oncology and Nuclear Medicine, Zagazig University, and Department of Medical Oncology, Zagazig University, and we assessed the overall survival (OS) and disease-free survival (DFS) rates.

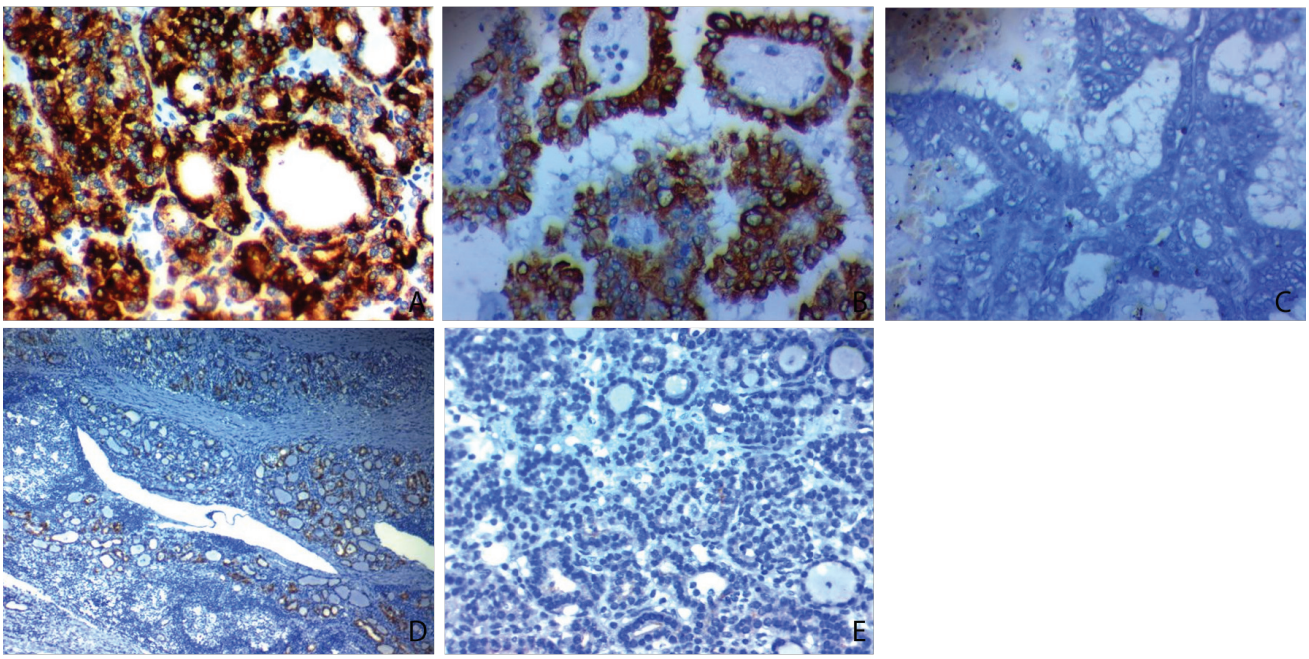

Figure 2. Expression of ANXA10 in tissues of papillary thyroid carcinoma and benign tissues of the thyroid gland: (A) increased cytoplasmic expression in high-grade PTC stage IV (IHC, ×400), (B) increased cytoplasmic expression in high-grade PTC stage III (IHC, ×400), (C) decreased cytoplasmic expression in low-grade PTC stage II (IHC, ×400), (D) decreased cytoplasmic expression in low grade PTC stage I (IHC, ×400), and (E) negative cytoplasmic expression in benign tissues of the thyroid gland (IHC, ×400).
YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D

IHC

For IHC: The following sections were incubated with primary mouse monoclonal overnight at 41C: anti-YAP-1 antibody (clone: EP1674Y, ab52771, dilution, 1:1000), primary Anti-Annexin A10/ANXA10 antibody [clone: EPR19342, ab214486, dilution, 1:100), and primary anti- UNC5D/UNC5H4 (clone: BS-11494R-A750, Bioss, ALEXA FLUOR 750 Conjugated, dilution, 1:200).

IHC scoring

IHC staining was evaluated by two experienced pathologists. We assessed the staining intensity and extent of stain for all the markers and reached the final stain score through the summation of values of both scores. Extent scores were classified as follows: 0, 0% to 5%; 1, 6% to 25%; 2, 26% to 50%; 3, 51% to 75%; and 4, 76% to 100%. Intensity scores were classified as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. Finally, we considered the values less than 4 to be low expression and more than or equal to 4 to be high expression for statistical analysis.

Statistical analysis

We employed EpiData software (version 3.1; EpiData Association, Odense, Denmark) for entry of collected clinical and pathologic data. Statistical analyses were performed using SPSS software (version 13.0; IBM SPSS Inc., Chicago, IL).

We used Kaplan-Meier survival curves and log-rank statistics for evaluating OS and DFS rates. Univariate and multivariate regression analyses were carried out via Cox proportional

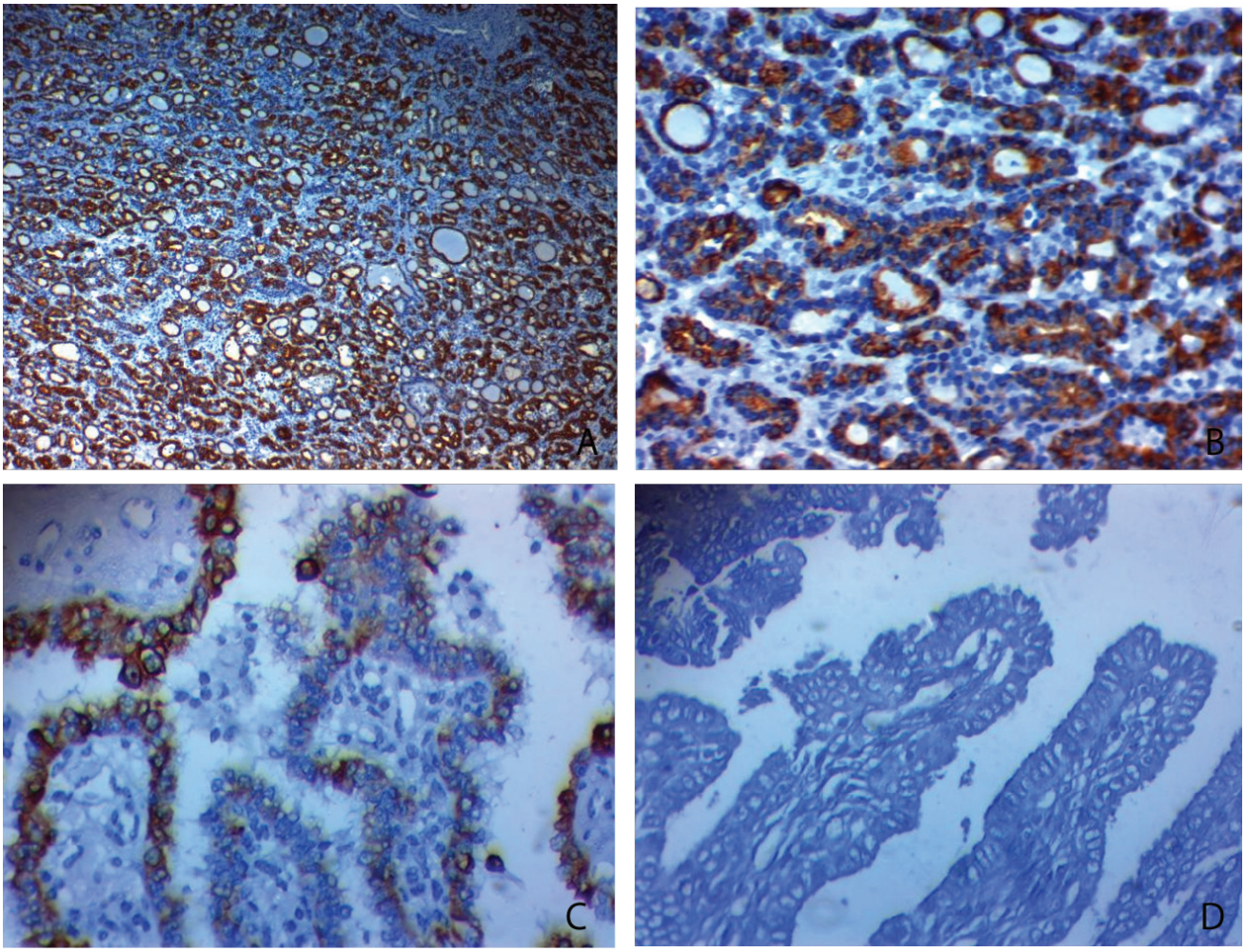

Figure 3. Expression of UNC5D in tissues of papillary thyroid carcinoma and benign tissues of the thyroid gland: (A) increased nuclear expression in benign tissues of the thyroid gland (IHC, ×400), (B) increased nuclear expression in benign tissues of the thyroid gland (IHC, ×400), (C) decreased nuclear expression in high-grade PTC stage III (IHC, ×400), (D) Negative nuclear expression in high grade PTC stage IV (IHC, ×400).

YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D

Table 2. YAP-1, ANXA10, and UNC5D expression in all the studied samples

	Case N= 60		Control N= 30		Total N= 90		*P*
	No.	%	No.	%	No.	%	
YAP-1							
Low	25	41.6%	26	86.7%	61	67.7%	< 0.001
High	35	58.3%	4	13.3%	39	43.3%	
ANXA1							
Low	31	51.6%	22	73.3%	53	58.8%	0.009
High	29	48.3%	8	26.7%	37	41.1%	
UNC5D							
Low	35	58.3%	4	13.3%	39	43.3%	0.002
High	25	41.6%	26	86.7%	51	56.7%	

YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D; No.: Number

hazards models. All the tests were two-sided and *P*-values <0.05 were considered statistically significant.

Results

Clinicopathologic and demographic results of the included PTC are detailed in table 1 and figures 1-3.

YAP-1 expression in the included tissue samples and association with clinicopathological findings (Tables 1-3; Figure 1)

Upregulation of YAP-1 was found in PTC tissues more than non-neoplastic tissues of the thyroid gland.

In PTC tissues there were significant positive correlations between YAP-1 expression and tumor size ($P = 0.022$), advanced stage ($P < 0.001$), presence of lymph node metastases ($P = 0.005$), presence of extra-thyroid extension ($P = 0.007$), and presence of distant metastases ($P = 0.012$).

Correlations between YAP-1 expression and follow-up findings (Tables 4 and 5; Figures 4 and 5)

In PTC tissues, there were significant positive correlations among YAP-1 expression, disease recurrence ($P = 0.001$), progression, and inferior disease-free survival (DFS) ($P = 0.003$).

ANXA10 expression in the included tissue samples and association with clinicopathological findings (Tables 1-3; Figure 2)

Up-regulation of ANXA10 was found in PTC tissues more than the non-neoplastic tissues of the thyroid gland.

In PTC tissues, there were significant positive correlations between ANXA1 expression and tumor size ($P = 0.011$), presence of lymph node metastases ($P < 0.001$), presence of extra-thyroid extension ($P = 0.01$), advanced stage ($P < 0.001$), and presence of distant metastases ($P = 0.042$).

Correlations between ANXA10 expression and follow-up findings (Tables 4 and 5; Figures 4 and 5)

In PTC tissues, there were significant positive correlations among ANXA10 expression, disease recurrence ($P = 0.006$), progression, and inferior DFS ($P = 0.03$).

UNC5D expression in the included tissue samples and association with clinicopathological findings (Tables 1-3; Figure 3)

Downregulation of UNC5D was found in PTC tissues more than the non-neoplastic tissues of the thyroid gland.

In PTC tissues, there were significant inverse correlations between UNC5D expression and tumor size ($P = 0.014$), presence of lymph node metastases ($P = 0.008$), presence of extra-thyroid extension ($P = 0.045$), advanced stage ($P = 0.044$), and presence of distant metastases ($P = 0.014$).

Correlations between UNC5D expression and follow-up findings (Tables 4 and 5; Figures 4 and 5)

In PTC tissues, there were significant inverse correlations between UNC5D expression, disease recurrence ($P = 0.02$), progression, and inferior DFS ($P = 0.03$).

In univariate and multivariate Cox regression analyses and expressions of YAP-1 and ANXA10

were the most important prognostic factors for unfavorable DFS (Tables 4 and 5).

High expressions of YAP-1 and ANXA10 along with the low expression of UNC5D was associated with unfavorable OS rate, but the results were not statistically significant.

We found a direct association between the expressions of YAP-1 and ANXA10. Spearman's rho correlation coefficient was equal to r = +0.530 which detects an inverse association between the expressions of YAP-1 and UNC5D. Spearman's rho correlation coefficient: r = -0.362 which detects an inverse association between the expressions of ANXA10 and UNC5D. Spearman's rho correlation coefficient: r = -0.637 ($P < 0.001$).

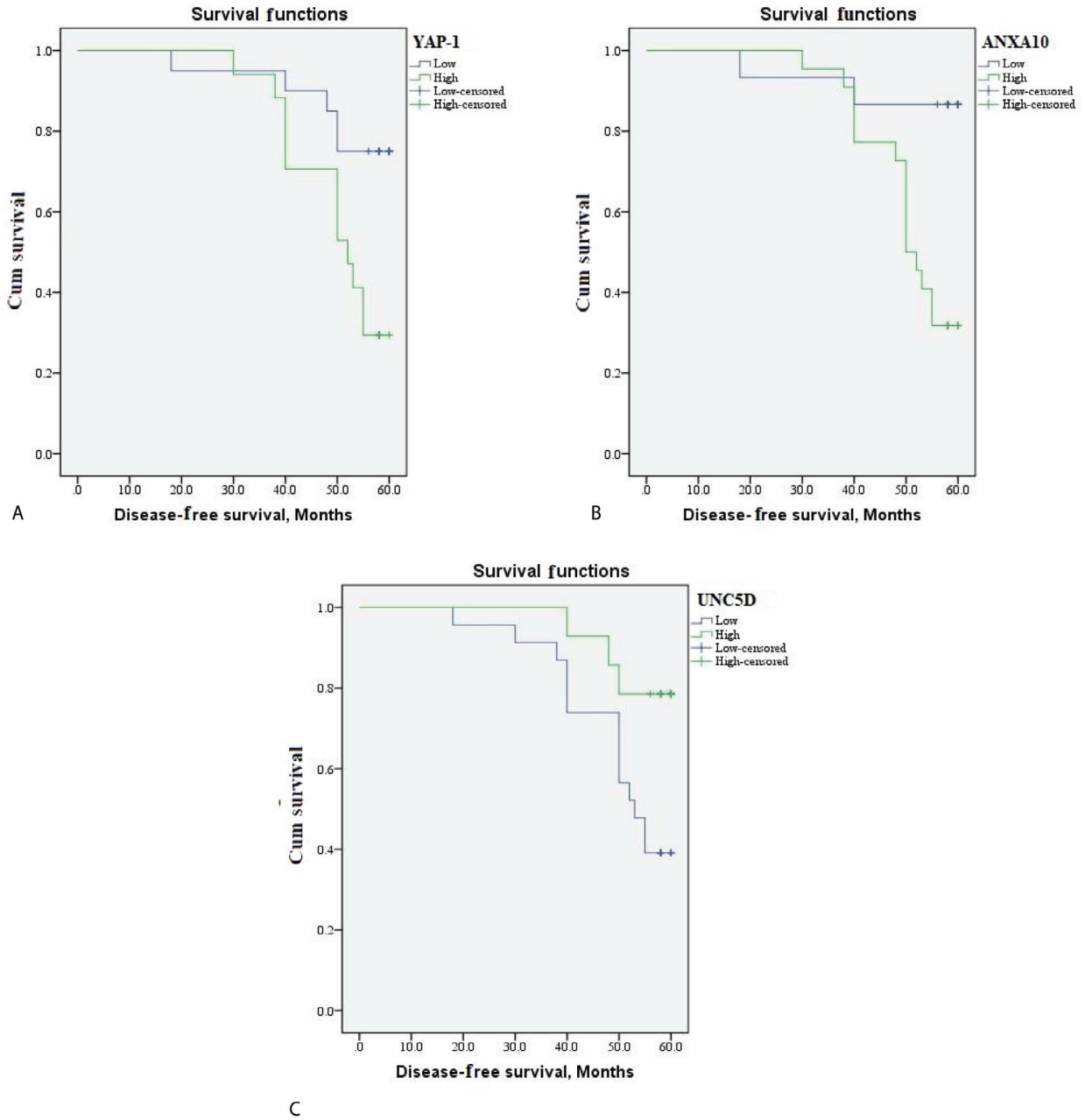

Figure 4. (A-C) Disease-free survival and Kaplan Meir survival curves of patients with papillary thyroid carcinoma stratified according to the expression of YAP-1, ANXA10, and UNC5D, respectively.

YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D, Cum: Cumulative

Table 3. Correlations between YAP-1, ANXA10, and UNC5D expressions in tissues of PTC and clinicopathological features of patients

	YAP-1				*P*	ANXA1				*P*	UNC5D				*P*
	Low N=25		High N=35			Low N=31		High N=29			Low N=35		High N=25		
Age group															
<40y	16	64.0%	24	68.6%	0.327	17	54.8%	23	79%	0.027	25	71%	5	20%	0.102
≥40y	9	36.0%	11	31.4%		14	45%	6	21%		10	28.5%	20	80%	
Sex															
Male	5	20%	6	17%	0.522	6	19%	5	17%	0.873	7	20.0%	4	16%	0.927
Female	20	80%	29	83%		25	81%	24	83%		28	80%	21	84%	
Histo-pathological subtype															
Conventional PTC	24	96%	32	91%	0.586	24	77.4%	26	90%	0.246	32	91%	24	97%	0.586
Follicular variant of PTC	1	4%	3	9%		7	22.6%	3	10.5%		3	8.5%	1	4%	
Stage															
I	16	64%	1	3%	<0.001	17	22%	0	0.0%	<0.001	3	8.5%	14	56%	0.044
II	7	28%	4	11%		9	29%	2	7%		14	40%	7	28%	
III	1	4%	13	37%		2	6%	12	42%		12	34%	2	8%	
IV	1	4%	17	49%		3	9%	15	51.7%		6	17%	2	8%	
Tumor size															
<4cm	18	72%	12	34%	0.022	27	87%	8	27.5%	0.011	12	34%	23	92%	0.014
≥4cm	7	28%	23	65.7%		4	13%	21	53.8%		23	66%	2	8%	
Surgery (thyroidectomy)															
Total	16	64.0%	14	40%	0.303	20	65%	10	34%	0.121	14	40%	16	64%	0.094
Subtotal	5	20%	2	6%		6	19%	1	3%		12	34%	5	20%	
Total +block neck dissection	4	16%	18	51%		4	13%	18	62%		9	25.7%	3	12%	
lobectomy	0	0.0%	1	3%		1	3%	0	0.0%		0	0.0%	1	4%	
Multifocality	2	8%	15	43%	0.003	4	13%	13	44.8%	0.002	14	40%	3	12%	0.026
Lymph node involvement	4	16%	20	57%	0.005	16	52%	18	62%	<0.001	19	54.7%	5	20%	0.008
Vascular invasion	1	4%	19	54%	0.026	3	10%	17	59%	0.1	18	51.4%	2	8%	0.187
Capsular invasion	1	4%	14	40%	0.004	4	13%	11	38%	0.011	12	34%	3	12%	0.077
Extrathyroid extension	1	4%	12	34%	0.007	3	10%	10	34%	0.01	11	31.4%	2	8%	0.045
Distant metastasis	1	4%	17	48.5%	0.002	13	41%	15	51%	0.042	16	45.7%	2	8%	0.014
Lung metastasis	1	4%	3	8%	0.559	2	6%	2	6%	0.871	3	8%	1	4%	0.559
Bone metastasis	1	4%	11	31%	0.731	1	3%	1	3%	0.911	2	5%	0	0.0%	0.251
Brain metastasis	0	0.0%	1	2%	0.423	0	0.0%	1	3%	0.274	1	2.5%	0	0.0%	0.423
Mediastinal metastasis	1	4%	0	0.0%	0.2	0	0.0%	1	3%	0.274	1	2.5%	0	0.0%	0.423
Nodal metastasis	1	4%	22	63%	0.005	2	6%	21	72%	0.001	21	60%	12	48%	0.036

YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D; PTC: Papillary thyroid carcinoma

Discussion

Herein, we revealed that YAP was elevated in PTC tissues more than non-neoplastic tissues of the thyroid gland and was related to tumor progression, higher incidence of recurrence, and dismal outcome. Similarly, Liu et al.[4] found that YAP upregulation was found in many epithelial cell tumors and soft tissue sarcoma. PTC patients have an accepted survival rates following curative surgical treatment, but there is still the risk of recurrence worsening the patients' prognosis.[12] It is important to detect predictive factors for tumor recurrence which help detect high-risk patients that would need more frequent follow-ups.

Moreover, Boin et al.[13] demonstrated that YAP could control a signaling pathway responsible for cell proliferation in schwannomas. Additionally, Bai et al.[14] showed that YAP enhances apoptosis by controlling p53 during chemotherapy. YAP was found to be responsible for increasing tumor cells migration and invasion, inhibiting response to chemotherapy, and increasing tumor growth.[15]

YAP-1 expression was revealed to be related to the larger size of the tumor. This might be explained with the fact that YAP increases the size of organs in Drosophila and size of the liver in transgenic mice due to its role in cell proliferation.[16-18] YAP was found to increase organ proliferation through the interaction with many factors responsible for cells proliferation and finally, induce epithelial mesenchymal transition (EMT) in tumor cells responsible for cancer metastases.[19]

Xia et al.[20] has illustrated that YAP promoted the proliferation of ovarian cancer cells and it was associated with ovarian cancer progression which was in line with our findings in PTC.

Moreover, we showed that high YAP

expression was related to the aggressive features of PTC, therefore indicating its role in cancer progression and dismal outcome which is similar to previous findings.[4]

Recurrence of PTC and recurrence free survival (RFS) were found to be the most important predictive factors of patients' prognosis. We found that high YAP-1 expression was related to the high incidence of recurrence and unfavorable RFS rate which highlighted its oncogenic role. Similar findings were reported in many malignancies as ovarian cancer, colon cancer, and bladder cancer.[21-23]

Serrano et al.[24] pointed to a recent cancer molecular targeted therapy, dasatinib, that antagonized YAP/TAZ and inhibited YAP-1

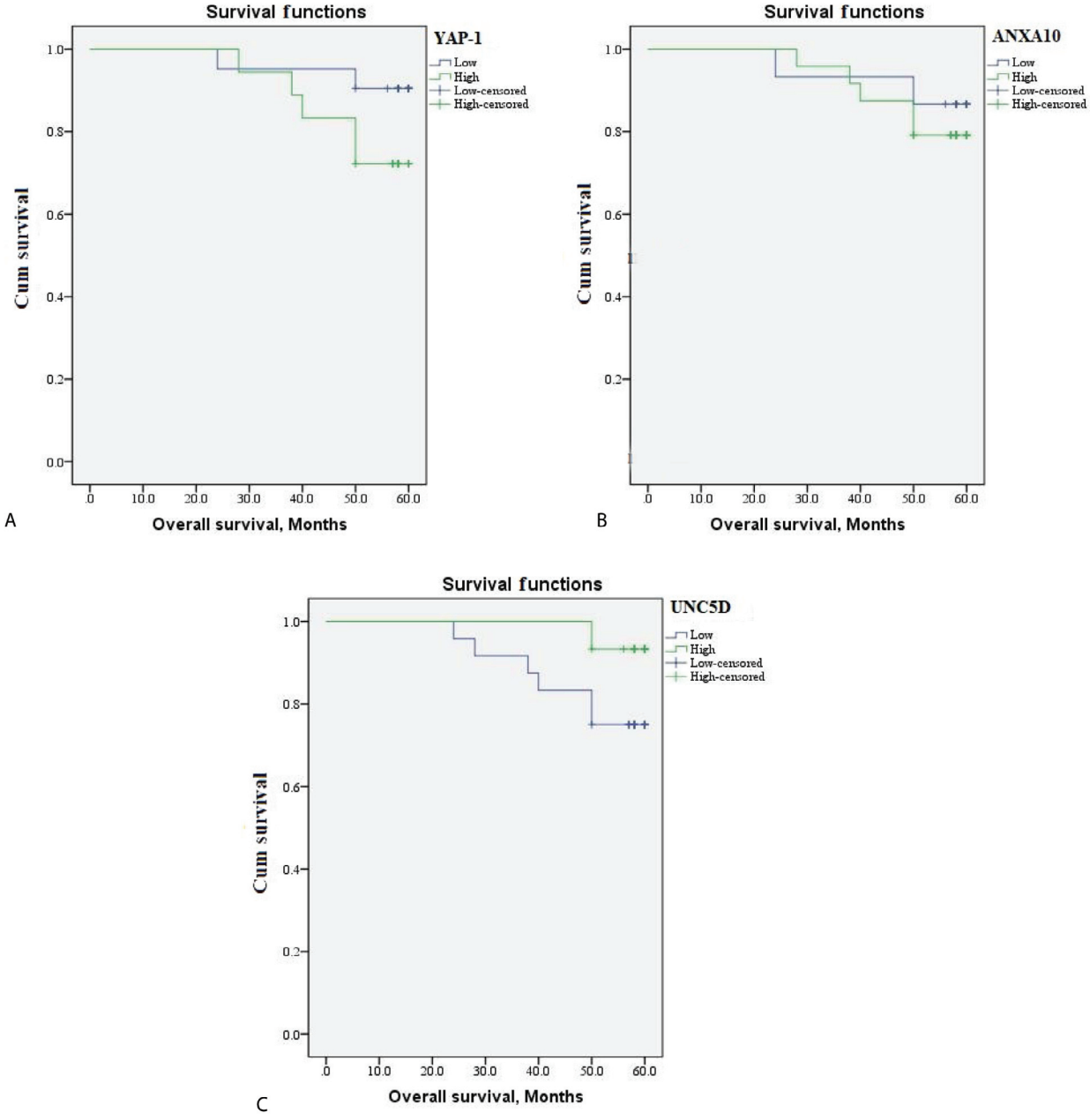

Figure 5. (A-C) Overall survival Kaplan Meir survival curves of patients with papillary thyroid carcinoma stratified according to the expression of YAP-1, ANXA10, and UNC5D, respectively.

YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D; Cum: Cumulative

Table 4. Correlations between YAP-1, ANXA10, and UNC5D expressions in tissues of papillary thyroid carcinoma (PTC) and the outcome of patients

		YAP-1				*P*	ANXA1				*P*	UNC5D				*P*
		Low		High			Low		High			Low		High		
	All	N=25		N=35			N=31		N=29			N=35		N=25		
Response																
PD	0	0	0.0%	0	0.0%	0.378	0	0.0%	0	0.0%	0.564	0	0.0%	0	0.0%	0.692
SD	4	0	0.0%	4	11%		0	0.0%	4	13.7%		4	11.4%	0	0.0%	
PR	9	0	0.0%	9	26%		1	4.8%	8	27.5%		8	22.8%	1	4%	
CR	47	25	100.0%	22	63%		20	95.2%	27	93%		23	65.7%	24	96%	
Overall response																
NR	13	0	0.0%	13	37%	0.163	1	4.8%	12	41%	0.489	12	34%	1	4%	0.877
OAR	47	25	100.0%	22	63%		20	65%	27	93%		23	65.7%	24	96%	
Recurrence																
No	32	13	52%	9	26%	0.001	15	43%	5	17.2%	0.006	9	25.7%	21	84%	0.02
Yes	15	2	8%	13	37%		5	16%	22	76%		2	5%	13	52%	
Outcome																
Censored	43	23	92%	20	57%	0.591	27	77%	16	55%	0.163	29	82.8%	14	56%	0.162
Died	17	2	8%	15	43%		4	11%	13	45%		6	17%	11	44%	

PD: Progressive disease; SD: Stable disease; PR: Partial response; CR: Complete response; NR: No response; OAR: Overall response; YAP-1: Yes activated protein-1; ANXA10: Annexin A10; UNC5D: Uncoordinated-5D

through the activation of the core kinases that subsequently could inhibit the occurrence and progression of malignant tumors.

In the present report, we implied that high ANXA10 expression was associated with high YAP-1 expression and was related to larger tumor sizes and high grades of PTC, proving their roles in PTC progression and poor prognosis which was in line with that of the study by Liu et al.[7]

Previous reports have shed light on the increased expression of ANXA10 in many cancers.[25-27]

ANXA10 role in cancer progression was performed with Stat3 activation and EMT induction.[28] Consequently, increased ANXA10 and YAP-1 expressions in PTC have similar roles in cancer progression through EMT induction, highlighting that the targeted therapies against them might be considered novel therapies to PTC. As the exact molecular mechanisms of ANXA10 in PTC oncogenesis and progression were not sufficiently clarified, there were no targeted therapies until now to inhibit ANXA10 in PTC.

We assessed the expression of a novel biomarker in PTC tissues as its roles were not sufficiently clear and showed that its expression was downregulated in the malignant thyroid tissue of PTC than non-neoplastic tissues of the thyroid gland. Additionally, high expression of UNC5D was associated with good prognosis, favorable outcome, low expressions of YAP-1 and ANX10A.

Similar results were found by Zhang et al.,[29] who focused on the role of UNC5D in PTC for the first time and revealed that its expression was markedly reduced or even lost in PTC and confirmed its possible cancer suppressor roles in thyroid tissues.

Although the mechanisms of action of UNC5D were not sufficiently clarified, previous studies showed that its expression was related to favorable outcome and longer survival in patients with neuroblastomas[11]

Moreover Lu et al.[30] and Zhu et al.[31] stated that UNC5D was absent or downregulated in cancer cell lines and tissues of renal cell carcinoma (RCC) and bladder cancer and it could inhibit tumor cells invasion and migration, highlighting its tumor suppressor role in RCC and bladder cancer.[30-31]

UNC5D was found to be resulted from DNA damage-mediated apoptosis as a P53 transcriptional target.[32] UNC5D was found to be mutated and downregulated in colon cancer and lung cancer as a late stage of cancer progression and metastases.[29, 33]

High expression of UNC5D decreased the capacity of cells to proliferate, proving its tumor suppressor role. Additionally, overexpressed UNC5D induced arrest at G2-M cell-cycle arrest in PTC cells, which was in line with the results reported in RCC and bladder cancer cells.[29, 30]

Previous reports have shed light on the

Table 5. Correlations between YAP-1, ANXA10, and UNC5D expressions in papillary thyroid carcinoma patients and survival analysis

	Survival time (Months)		Survival rate (%)		*P*
	Means ± SD (months) (95% CI)	Median ± SD (months) (95% CI)			
5-year overall survival					
YAP-1					
Low	56.9 ± 2.4	(52.3-61.56)	NR	86.7	0.571
High	56.1 ± 1.7	(52.68-59.49)	NR	79.2	
ANXA10					
Low	57.8 ± 1.7	(54.45-61.17)	NR	90.5	0.149
High	54.8 ± 2.2	(50.4-59.16)	NR	72.2	
UNC5D					
Low	54.6 ± 2.2	(50.34-58.83)	NR	75	0.142
High	59.3 ± 0.6	(58.07-60.6)	NR	93.3	
Overall	56.4 ± 1.4	(53.66-59.16)	NR	82.1	
5-year disease-free survival					
YAP-1					
Low	55.9 ± 2.9	(50.16-61.57)	NR	86.7	0.003
High	51 ± 1.8	(47.4-54.51)			
	50 ± 1.7	(46.72-53.28)		31.8	
ANXA10					
Low	55.3 ± 2.3	(50.85-59.75)	NR	75	0.01
High	50.2 ± 2.2	(45.85-54.5)			
	52 ± 5.4	(41.51-62.49)		29.4	
UNC5D					
Low	50.5 ± 2.3	(45.95-55.01)			
	53 ± 3	(47.13-58.87)		39.1	0.03
High	57 ± 1.6	(53.81-60.19)	NR	78.6	
Overall	52.9 ± 1.6	(49.72-56.17)	NR	54.1	

NR: Not reached; YAP-1: Yes activated protein-1; ANXA10: Annexin A10, UNC5D: Uncoordinated-5D; CI: Confidence interval

association between UNC5D and cell cycle and proliferation, but no previous reports have assessed the association with EMT. We detected an inverse association between UNC5D expression and YAP-1 and ANXA10 expressions, which were previously shown to induce EMT in PTC and resulted in its progression. These findings showed novel roles of UNC5D overexpression in cancer progression with EMT induction.

Limitations of the study

First, this is a retrospective single-center study which included a small number of patients. Second, we assessed the expressions of YAP-1, ANXA10, and UNC5D through only IHC without genetic analysis, which stands against the accurate detection of the levels of markers and association with prognosis.

Additionally, we could not detect a logical explanation for the high expressions of YAP and ANXA and low expression of UNC5D in some samples of the control group.

Thus, we recommend performing an extended prospective multicenter cohort study with a long-period follow-up to confirm the roles of YAP-1, ANXA10, and UNC5D genetic and protein expressions in PTC patients to explain these points and clarify our findings.

Conclusion

In the current work, we found that YAP-1 and ANXA10 expressions was significantly increased in PTC tissues more than the non-neoplastic tissues of the thyroid gland whereas UNC5D expression was decreased in PTC. Upregulation of YAP-1 and ANXA10 expressions and downregulation of UNC5D was associated with bad clincopathological criteria, disease progression, high incidence of disease recurrence, and poor survival.

Our findings suggest that YAP-1, ANXA10, and UNC5D might be considered promising targets for individualization of PTC. Moreover, the levels of expressions of those markers might categorize patients who need more aggressive radical therapy.

Conflict of Interest

None declared.

References

1. Singh Ospina N, Iñiguez-Ariza NM, Castro MR. Thyroid nodules: diagnostic evaluation based on thyroid cancer risk assessment. *BMJ.* 2020;368:l6670. doi: 10.1136/bmj.l6670.
2. Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. *Nat Rev Endocrinol.* 2016;12(11):646-53. doi: 10.1038/nrendo.2016.110.
3. Vuong HG, Odate T, Duong UNP, Mochizuki K, Nakazawa T, Katoh R, et al. Prognostic importance of solid variant papillary thyroid carcinoma: A systematic review and meta-analysis. *Head Neck.* 2018;40(7):1588-97.
4. Liu Z, Zeng W, Maimaiti Y, Ming J, Guo Y, Liu Y, et al. High expression of yes-activated protein-1 in papillary thyroid carcinoma correlates with poor prognosis. *Appl Immunohistochem Mol Morphol.* 2019;27(1):59-64.
5. Ye S, Eisinger-Mathason TS. Targeting the Hippo pathway: Clinical implications and therapeutics. *Pharmacol Res.* 2016;103:270-8. doi: 10.1016/j.phrs.2015.11.025.
6. Wang Z, Wu Y, Wang H, Pajonczyk D, Rescher U. Interplay of mevalonate and Hippo pathways regulates RHAMM transcription via YAP to modulate breast cancer cell motility. *Proc Natl Acad Sci USA*. 2014;111:89-98. doi: 10.1073/pnas.1319190110.
7. Liu X, Yang M, Guo Y, Lu X. Annexin A10 is a novel prognostic biomarker of papillary thyroid cancer. *Ir J Med Sci.* 2021;190(1):59-65. doi: 10.1007/s11845-020-02263-x.
8. Schloer S, Pajonczyk D, Rescher U. Annexins in translational research: Hidden treasures to be found. *Int J Mol Sci.* 2018;19(6):1781. doi: 10.3390/ijms19061781.
9. Foo SL, Yap G, Cui J, Lim LHK. Annexin-A1 - A blessing or a curse in cancer? *Trends Mol Med.* 2019;25(4):315-27. doi: 10.1016/j.molmed.2019.02.004.
10. Engelkamp D. Cloning of three mouse Unc5 genes and their expression patterns at mid-gestation. *Mech Dev.* 2002;118(1-2):191-7. doi: 10.1016/s0925-4773(02)00248-4.
11. Zhu Y, Li Y, Haraguchi S, Yu M, Ohira M, Ozaki T, et al. Dependence receptor UNC5D mediates nerve growth factor depletion-induced neuroblastoma regression. *J Clin Invest.* 2013;123(7):2935-47. doi: 10.1172/JCI65988.
12. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 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.* 2016;26(1):1-133. doi: 10.1089/thy.2015.0020.
13. Boin A, Couvelard A, Couderc C, Brito I, Filipescu D, Kalamarides M, et al. Proteomic screening identifies a YAP-driven signaling network linked to tumor cell proliferation in human schwannomas. *Neuro Oncol.* 2014;16(9):1196-209. doi: 10.1093/neuonc/nou020.
14. Bai N, Zhang C, Liang N, Zhang Z, Chang A, Yin J, et al. Yes-associated protein (YAP) increases chemosensitivity of hepatocellular carcinoma cells by modulation of p53. *Cancer Biol Ther.* 2013;14(6):511-20. doi: 10.4161/cbt.24345.
15. Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, et al. Yes-associated protein (YAP) functions as a tumor suppressor in breast. *Cell Death Differ.* 2008;15(11):1752-9. doi: 10.1038/cdd.2008.108.
16. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev.* 2007;21(21):2747-61. doi: 10.1101/gad.1602907.
17. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, et al. YAP1 increases organ size and expands undifferentiated progenitor cells. *Curr Biol.* 2007;17(23):2054-60. doi: 10.1016/j.cub.2007.10.039. Erratum in: *Curr Biol.* 2007;17(23):2094.
18. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a universal size-control mechanism in Drosophila and mammals. *Cell.* 2007;130(6):1120-33. doi: 10.1016/j.cell.2007.07.019.
19. Zhao B, Lei QY, Guan KL. The Hippo-YAP pathway: new connections between regulation of organ size and cancer. *Curr Opin Cell Biol.* 2008;20(6):638-46. doi: 10.1016/j.ceb.2008.10.001.
20. Xia Y, Chang T, Wang Y, Liu Y, Li W, Li M, et al. YAP promotes ovarian cancer cell tumorigenesis and is indicative of a poor prognosis for ovarian cancer patients. *PLoS One*. 2014;9(3):e91770. doi: 10.1371/journal.pone.0091770. Erratum in: *PLoS One*. 2016;11(3):e0152712.
21. Jeong W, Kim SB, Sohn BH, Park YY, Park ES, Kim SC, et al. Activation of YAP1 is associated with poor prognosis and response to taxanes in ovarian cancer. *Anticancer Res.* 2014;34(2):811-7.
22. Wang L, Shi S, Guo Z, Zhang X, Han S, Yang A, et al. Overexpression of YAP and TAZ is an independent predictor of prognosis in colorectal cancer and related to the proliferation and metastasis of colon cancer cells. *PLoS One.* 2013;8(6):e65539. doi: 10.1371/journal.pone.0065539.
23. Liu JY, Li YH, Lin HX, Liao YJ, Mai SJ, Liu ZW, et al. Overexpression of YAP 1 contributes to progressive features and poor prognosis of human urothelial carcinoma of the bladder. *BMC Cancer.* 2013;13:349. doi: 10.1186/1471-2407-13-349.
24. Serrano I, McDonald PC, Lock F, Muller WJ, Dedhar S. Inactivation of the Hippo tumour suppressor pathway by integrin-linked kinase. *Nat Commun.* 2013;4:2976.

doi: 10.1038/ncomms3976.
25. van der Heijden AG, Mengual L, Lozano JJ, Ingelmo-Torres M, Ribal MJ, Fernández PL, et al. A five-gene expression signature to predict progression in T1G3 bladder cancer. *Eur J Cancer.* 2016;64:127-36. doi: 10.1016/j.ejca.2016.06.003.
26. Zhu J, Wu J, Pei X, Tan Z, Shi J, Lubman DM. Annexin A10 is a candidate marker associated with the progression of pancreatic precursor lesions to adenocarcinoma. *PLoS One.* 2017;12(4):e0175039. doi: 10.1371/journal.pone.0175039.
27. Macaron C, Lopez R, Pai RK, Burke CA. Expression of Annexin A10 in serrated polyps predicts the development of metachronous serrated polyps. *Clin Transl Gastroenterol.* 2016;7(12):e205. doi: 10.1038/ctg.2016.60.
28. Sun R, Liu Z, Qiu B, Chen T, Li Z, Zhang X, et al. Annexin10 promotes extrahepatic cholangiocarcinoma metastasis by facilitating EMT via PLA2G4A/PGE2/STAT3 pathway. *EBioMedicine.* 2019;47:142-55. doi: 10.1016/j.ebiom.2019. 08.062.
29. Zhang MM, Sun F, Cui B, Zhang LL, Fang Y, Li Y, et al. Tumor-suppressive function of UNC5D in papillary thyroid cancer. *Oncotarget.* 2017;8(56):96126-38. doi: 10.18632/oncotarget.21759.
30. Lu D, Dong D, Zhou Y, Lu M, Pang XW, Li Y, et al. The tumor-suppressive function of UNC5D and its repressed expression in renal cell carcinoma. *Clin Cancer Res.* 2013;19(11):2883-92. doi: 10.1158/1078-0432.CCR-12-2978.
31. Zhu Y, Yu M, Chen Y, Wang Y, Wang J, Yang C, et al. Down-regulation of UNC5D in bladder cancer: UNC5D as a possible mediator of cisplatin induced apoptosis in bladder cancer cells. *J Urol.* 2014;192(2):575-82. doi: 10.1016/j.juro.2014.01.108.
32. Wang H, Ozaki T, Shamim Hossain M, Nakamura Y, Kamijo T, Xue X, et al. A newly identified dependence receptor UNC5H4 is induced during DNA damage-mediated apoptosis and transcriptional target of tumor suppressor p53. *Biochem Biophys Res Commun*. 2008; 370(4):594-8. doi: 10.1016/j.bbrc.2008.03.152.
33. Liu P, Lu Y, Liu H, Wen W, Jia D, Wang Y, et al. Genome-wide association and fine mapping of genetic loci predisposing to colon carcinogenesis in mice. *Mol Cancer Res.* 2012;10(1):66-74. doi: 10.1158/1541-7786.MCR-10-0540.