Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 6s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Correlation Between CD44 and CDK4 Expression with T Stage of Papillary Thyroid Carcinoma

Debby Victoria Arief[1,2], Nila Kurniasari[1,2], Ridholia[1,2]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Email ID: debbyvarief@gmail.com

[2]Dr. Soetomo General Academic Hospital Surabaya, Indonesia

Email ID: nila-k@fk.unair.ac.id , dr.ridholia@yahoo.com

Cite this paper as: Debby Victoria Arief, Nila Kurniasari, Ridholia, (2025) Correlation Between CD44 and CDK4 Expression with T Stage of Papillary Thyroid Carcinoma. *Journal of Neonatal Surgery*, 14 (6s), 799-809.

ABSTRACT

Background: The most common endocrine malignancy in the world is papillary thyroid carcinoma, with rising numbers worldwide. The prognosis of this tumor is determined by age, size, local invasion and metastasis. Cell proliferation determined tumor size, where cancer stem cell such as CD44 becomes the main driver. Numbers of pathways will affect cell proliferation, CD44 could impact protein such as cyclin dependent kinase 4 (CDK4) which will affect cell cycle, causing increased proliferation which in turn, altering disease progression.

Objective: To analyze correlation between CD44 and CDK4 expression in various T stages of papillary thyroid carcinoma

Methods: This research is observational analytical cross-sectional research. Samples of paraffin blocks from patients diagnosed with papillary thyroid carcinoma from January 2019 to December 20202, who underwent lobectomy, isthmolobectomy and thyroidectomy. Samples then were categorized into groups according to their pathological T (pT) stage. Immunohistochemistry staining was done using CD44 and CDK4 antibodies. Differences of expression across different pT stage groups was done using statistics.

Results and Discussion: No differences in CD44 expression in various pT stage of papillary thyroid carcinoma (p=0.335), no correlation found (p=0.703). No differences in CDK4 expression in various pT stage of papillary thyroid carcinoma (p=0.904), no correlation found (p=0.752). Positive correlation was found between CD44 and CDK4 expression (p=0.0000849, r=0.518).

Conclusion: Various CD44 and CDK4 expression was found in various pT stage of papillary thyroid carcinoma, with positive correlation between CD44 and CDK4.

Keywords: *CD44, CDK4, papillary thyroid cancer, T stage, thyroid cancer*

1. INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, 90% of them is papillary thyroid carcinoma [1]. Papillary thyroid carcinoma happens in adult as well as children (90% of the childhood thyroid malignancy), the incidence of papillary thyroid carcinoma increased fourfold since 1975 to 2016 in the United States [2]

Most common staging system used in papillary thyroid carcinoma is the tumor-node-metastasis (TNM) classification as per recommendations from the American Joint Committee on Cancer (AJCC) with the 8th edition currently used [3].

Pathologic T (pT) stage of papillary thyroid carcinoma is determined by the size of the largest tumor and the invasion to the adjacent organs, pT1 is tumor sized 2 cm or less, pT2 is tumor sized 2-4 cm, pT3 is tumor sized 4 cm or more, limited to the thyroid and/or gross extension to the strap muscles, pT4 is not defined by size but to the extension to the subcutaneous tissue, larynx, esophagus, laryngeal recurrent nerve, prevertebral fascia or vascular tissue of mediastinum or carotid arteries [4].

Factors that contributed to the prognosis and disease progression are age, size, local invasion and metastases. Tumor size (pT stage) correlates with mortality, research done by Konturek et al (2012) showed that pT1 stage have a mortality rate of 4,8% that increased to 52,5% for pT3-T4. Mortality risk increased almost elevenfold in patients with pT3 stage compared to patients with pT1 stage [5].

Various researches were conducted to diagnose a cancer and its causes, the presence of cancer stem cells (CSC)—a group of cells that are very malignant and is related to resistance to chemotherapy and radiotherapy—in a cancer, is still controversial, with *cluster of differentiation* 44 (CD44) as the most researched CSC [6,7].

Activation of CD44 by ligand such as hyaluronan, osteopontin, fibronectin, serglycin/sulfated proteglycan induced various pathways in cell proliferation such as Ras, mitogen-activated protein kinase (MAPK) and phospoinositide (PI3K), all associated with cell cycle [6,7,8].

Cell proliferation is associated with cell cycle, where cyclin D1 and cyclin dependent kinases 4/6 (CDK4/6) complexes will assist mitoses phase G1/S. Normal cell proliferation is usually arrested in phase G1/G0, in cancer, there is hyperactivity of CyclinD1-CDK4/6 that causes uncontrolled cell proliferation [9].

This research aims to analyze the correlation between CD44 and CDK4 expression in various T stage of papillary thyroid carcinoma.

2. MATERIALS AND METHODS

Research Design

52 paraffin block samples from patients with papillary thyroid carcinoma that were diagnosed by histopathology in Dr. Soetomo General Hospital Surabaya from January 2019-December 2022 were chosen. Inclusion criteria are paraffin blocks with papillary thyroid carcinoma from lobectomy, isthmolobectomy and total thyroidectomy. Paraffin blocks are good in quality and there were adequate tumor cells for immunohistochemistry staining.

Immunohistochemistry

Paraffin blocks were cut with thickness of 3-5 microns and made into slides. Deparaffination was done using xylol and then rehydrated with 96%, 90% and 80% alcohol each for 2 minutes. The specimen then rinsed with running water for 5 minutes and distilled water for 5 minutes. Slides then submerged in 3% hydrogen peroxide in methanol for 15 minutes in room temperature and then rinsed with distilled water for 5 minutes. Slides were then warmed with target retrieval solution (TRS)/citrate buffer with a pH of 6 in decloaking chamber for 20 minutes in 95^{o}C. Slides were rinsed with PBS for 5 minutes. Background snipper then pipetted for 15 minutes, then primary antibodies (CD44 mouse monoclonal antibody (DF1485) (1:200 dilution. Santa Cruz Biotechnology, Inc), (CDK4 mouse monoclonal antibody (DCS-35) (1:100 dilution. Santa Cruz Biotechnology, Inc). Slides then were incubated in room temperature for 60 minutes and rinsed with PBS for 5 minutes. Secondary antibody (Trekkie link) then pipetted for 20 minutes. Second secondary antibody (HRP label) pipetted for 10 minutes. Rinsing with PBS then done for 5 minutes. Diaminobenzidine (DAB) pipetted and incubation in room temperature for 5 minutes. Slides then rinsed under running water for 5 minutes and then soaked in mayer hematoxylin in room temperature for 10 minutes. Slides rinsed with running water. Dehydration with alcohol 80%, 90% and 96% were done, each for 2 minutes. Last step was to soak the slides in xylol and cover with cover glass [10]. The slides examination was done by two anatomical pathologists using light binocular microscope Olympus CX31.

Assessment

CD44 expression is positive by membranous and/or cytoplasmic staining. CDK4 expression is positive by cytoplasmic and/or nuclear staining. Immunoreactivity score was assessed using histochemical scoring assessment (H-score) [11]. The assessment is a semi-quantitative scoring that grades intensity score in stained tumor cells (negative = 0, weak = 1, moderate = 25, strong = 3) that will be multiplied by the percentage of the stained tumor cells (0-100%) the results will yield H-score ranging from 0 to 300. The assessment will be done by two anatomical pathologist and if the score difference was 50 or more, a reassessment will be done, and the average score would be calculated.

3. RESULTS AND DISCUSSION

This research results included expression of CD44, CDK4 and information of sex distribution, age group, pT stadium and tumor subtypes.

Patients and Tumor Characteristics

Research sample size was 52 samples, with 39 female patients (75%) and 13 male patients (25%) diagnosed with papillary thyroid carcinoma that underwent surgery for thyroid lobectomy, isthmolobectomy and total thyroidectomy.

Patients mean age is 46.82 years old with youngest age of diagnosis was 19 years old and oldest age was 73 years old. Largest age group was 41-50 years old (30.79%), and the smallest age group was 10-20 years old (1.92%).

Consecutive sampling was done and yielded pT1 group with 17 samples (32.62%), pT2 group with 17 samples (32.63%) and pT3 group with 18 samples (32.62%). pT4 group was not available because of limitations in radiologic information and other clinical features to determine extrathyroid extension.

Most common papillary thyroid carcinoma subtype in this research is the classic subtype with 35 samples (67.35%), followed by follicular subtype with 15 samples (28.84%). Less common subtypes such as hobnail cell with 1 sample (1.92%) and follicular oncocytic with 1 sample (1.92%) were also included this research.

Table 1. Research Characteristics

Characteristic		**Total**
Population		52
	Sex	
Female		39 (75%)
Male		13 (25%)
	Age	
10-20 years		1 (1.92%)
21-30 years		9 (17.30%)
31-40 years		7 (13.4%)
41-50 years		16 (30.76%)
51-60 years		8 (15.38%)
61-70 years		6 (11.53%)
71-80 years		5 (9.61%)
	Stage	
pT1 (≤2 cm)		17 (32.62%)
pT2 (2-4 cm)		17 (32.62%)
pT3 (>4 cm)		18 (34.69%)
	Subtype	
Classic		35 (67.35%)
Follicular		15 (28.84%)
Other (Hobnail cell, Oncocytic, etc)		2 (3.84%)

The most common endocrine malignancy is papillary thyroid carcinoma [12] accounting for 90% of thyroid malignancy [1]. Papillary thyroid carcinoma is also the most common thyroid malignancy across all age group [2,13].

Age is one of the most significant prognoses, with older patients having worse prognosis and mortality rates compared to younger patients. Age also determined the TNM staging of papillary thyroid carcinoma, with 55 years as the cut off age [3,14]. Mean age from various literatures and researches was 48 years old [13], in this research the largest age group is 41-

50 years old with mean age of 46.82 years old. Older age patients have higher distributed concentrations of thyroid stimulating hormone (TSH) that are correlated with relative thyroid organ's TSH resistance or central resistance of pituitary organ to thyroid hormones. Higher TSH levels correspond to higher risk of thyroid cancer [15,16].

This research had 39 females (75%) and 13 males (25%), this is consistent with the literature that stated papillary thyroid carcinoma is more common in females than males with the ratio of 3:1 [12]. The correlation between female sex and risk of thyroid cancer was thought because of the estrogen hormone and estrogen hormone reseptors [17]. Some research showed that male patients have worse prognosis and more aggressive disease progression compared to their female counterparts [18].

There are about 15 subtypes of papillary thyroid carcinoma, with the classic subtype as the most common subtype, followed by follicular subtype. This research most common subtype is the classic subtype with 35 samples (67.35%), followed by follicular subtype with 15 samples (28.84%). Lesser common subtypes such as hobnail cell and follicular oncocytic subtype also included in this research (Figure 1.A-D).

Subtypes of papillary thyroid carcinoma correlates to the mutation of the disease itself, with the most common mutation is BRAF mutation (55-65%), followed by RAS mutation (10%), the latter being less common and found almost exclusively in the follicular subtype [19]. The mutations in papillary thyroid carcinoma are important because some mutations harbor more aggressive disease progression and less favorable prognosis.

Pathological stage (pT) stage of papillary thyroid carcinoma was one of the prognoses of the disease. Research from Konturek et al showed that there was correlation between tumor size and mortality. Increase in mortality rates were showed from 4.8% for pT1 stage patients to 52.5% for pT3-4 stage patients, while patients with pT3 stage showed increased mortality rates almost eleventh folds compared to patients with pT1 stage ($p<0.001$) [5].

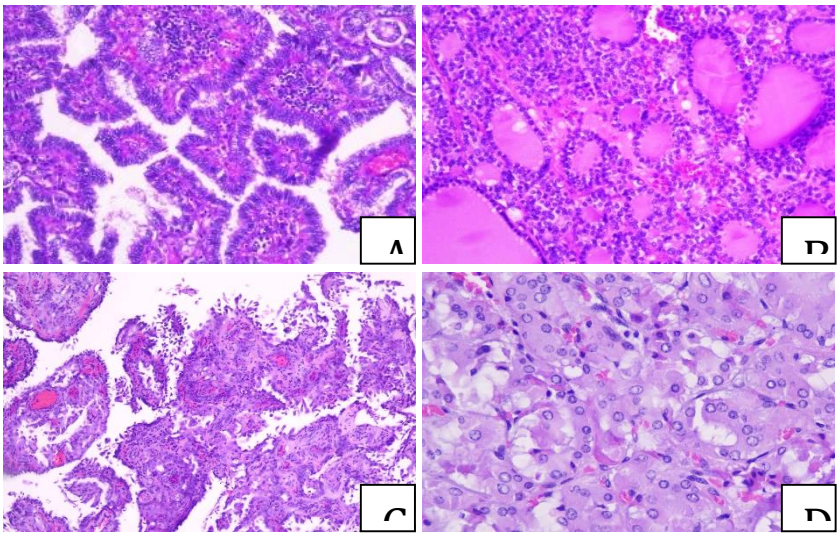

Figure 2. Subtypes of papillary thyroid carcinoma, A. Classical subtype papillary thyroid carcinoma (HE, 200x), B. Follicular subtype papillary thyroid carcinoma (HE, 200x), C. Hobnail cell papillary thyroid carcinoma (HE, 100x), D. Follicular oncocytic subtype (HE, 400x).

CD44 Expression

Expression of CD44 in this research is observed in all three pT stage groups papillary thyroid carcinoma samples. The assesment of CD44 expression was done using semiquantitative histochemical scoring assessment (H-score) that assess the intensity of the staining (negative, weak, moderate and strong) and the percentage of the staining (0-100%) [11]. CD44 expression was assessed by membranous and/or cytoplasmic staining (Figure 2.A-C).

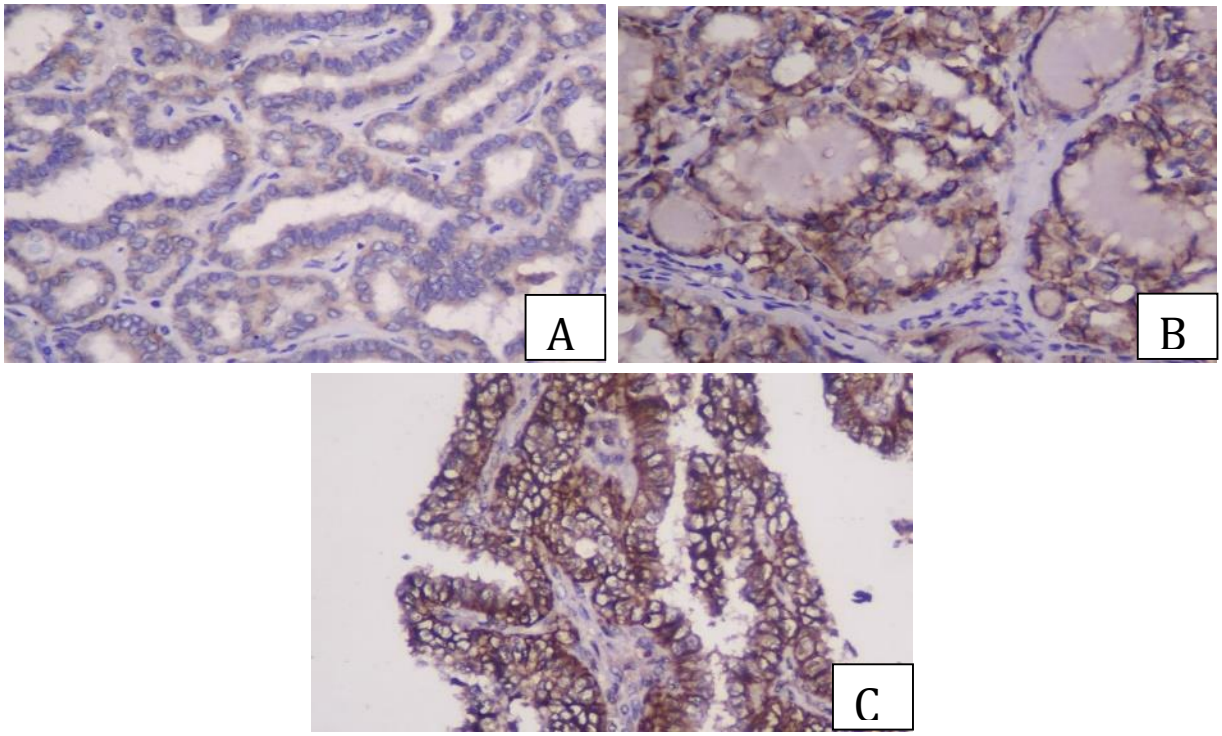

Figure 2. Immunostaining of CD44 in papillary thyroid carcinoma, A. Weak CD44 expression in papillary thyroid carcinoma, B. Moderate CD44 expression in papillary thyroid carcinoma, C. Strong CD44 expression in papillary thyroid carcinoma.

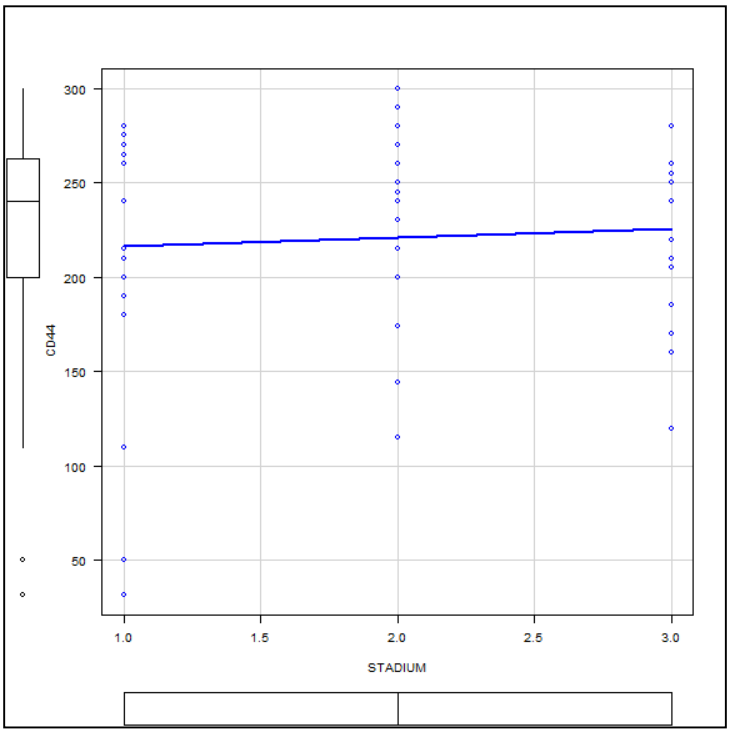

Figure 3 Scattered plot of Spearman correlation of CD44 and pT stage

CD44 expression was observed in all papillary thyroid carcinoma samples with lowest H-score of 32 and highest H-score of 300. The average H-score in pT1 stage group was 208.64. Average H-score in pT2 stage group was 236.05. Average H-score in pT3 stage group was 218.33.

Table 2 CD44 expression

CD44	Category	pT1 (n=17)	pT2 (n=17)	pT3 (n=18)	Kruskal-Wallis *p*
H-score	0-50	2 (11,74%)	0	0	0.335
	51-100	0	0	0	
	101-150	1 (5,88%)	2 (11,74%)	1 (5,55%)	
	151-200	3 (17,64%)	2 (11,74%)	2 (11,11%)	
	201-250	4 (23,52%)	5 (29,41%)	10 (55,55%)	
	251-300	7 (41,17%)	8 (47,05%)	5 (27,7%)	

A normality test was done using Kolmogorov-smirnov and found that the data was not distributed normally, a non-parametric testing using Kruskal-wallis was done. Data analytics showed that there are no differences in CD44 expression across different pT stages of papillary thyroid carcinoma (Table 2).

Additionally, a correlation test using Spearman correlation test was performed and showed non-significant results (p=0.335). It was concluded that there were no differences in CD44 expression across all pT stage groups and there were no correlations between CD44 expression and pT stage (Figure 3).

The existence of cancer stem cells (CSC) in a tumor is still an ongoing debate, CSCs are a population of malignant cells that are chemo resistant and radiotherapy resistant [6,7]. As of today, the most researched CSCs are cluster differentiation 44 (CD44) and its isoforms.

The role of CD44 in cancer is still not clear to this day, there are still debates on whether higher levels or lower levels of CD44 expression that leads to the progression of cancer (higher differentiation grades, progressive stage and survival rates) but consistently, expression of CD44 correlates with the increase of tumorigenesis such as proliferation, metastasis, invasion and also migration [7].

Previous research about CD44 expression in papillary thyroid carcinoma were not as abundant. Research that was done by Han et al in 2017 showed that there were no differences in CD44 expression and tumor size (p=0.162) [20], while the research that was done by Ryu et al in 2020 showed that there were no significant correlations between CD44 and tumor size in papillary thyroid carcinoma (p=0.608), while proving that tumors that were more than 2 cm correlated to recurrence rate (p=0.015), showing that there are other factors beside CD44 that contributed to tumor size and recurrence [21]. Other research that is consistent with these results is the research done by Rohani et al that proved here was no correlation between CD44 and tumor size in colon cancer (p=0.629) [22].

One of the research that investigated the relationship between CD44 and its isoforms expression and papillary thyroid carcinoma was the research done by Kawai et al in 2019, which proved the presence of CD44, especially the CD44v8-10 isoform in papillary thyroid carcinoma cells [23]. Different isoforms of CD44 had been linked to various cancer and its different mechanisms, perhaps a further investigation is necessary to link CD44 and its various isoforms with thyroid cancer.

As tumor progressed, some tumor regained epithelial-mesenchymal transition (EMT) that were linked with the transformation of one CD44 isoform to another [24]. More research is needed to determine the relationship between CD44 and its various isoforms with papillary thyroid cancer and other factors that contributed to tumor progression.

CDK4 Expression

Expression of CDK4 in this research is observed in all three pT stage groups papillary thyroid carcinoma samples. The assessment of CDK4 expression was done using semiquantitative histochemical scoring assessment (H-score) that assess the intensity of the staining (negative, weak, moderate and strong) and the percentage of the staining (0-100%) [11]. CDK4 expression was assessed by nuclear and/or cytoplasmic staining (Figure 4)

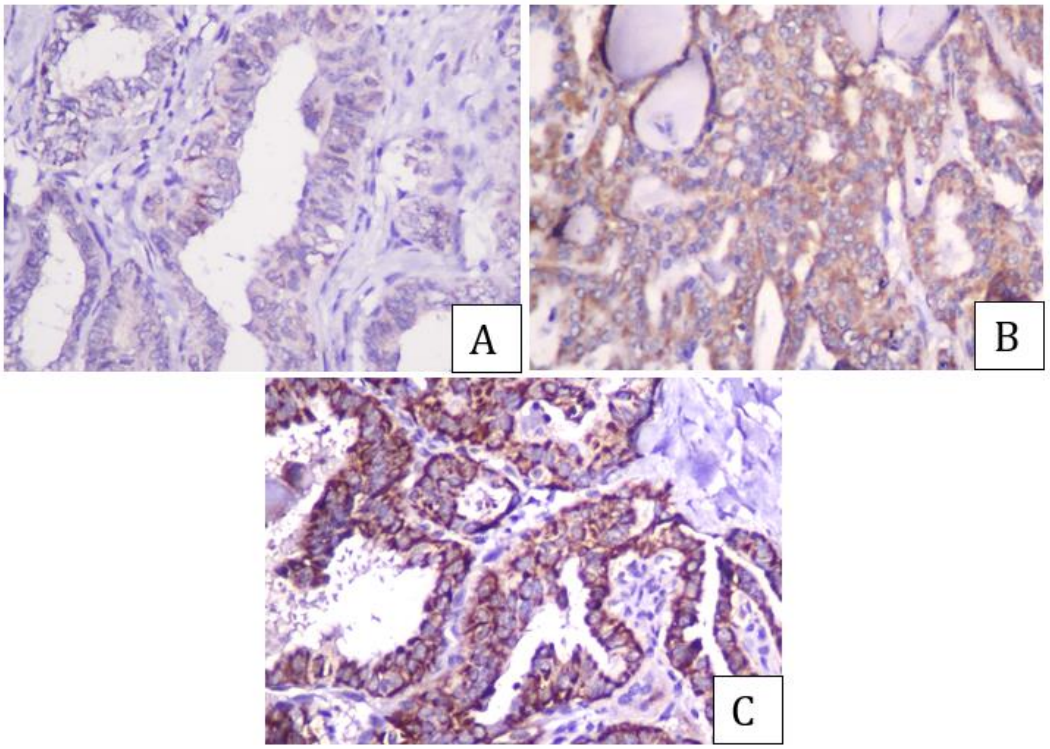

Figure 4. Immunostaining of CDK4 in papillary thyroid carcinoma, A. Weak CDK4 expression in papillary thyroid carcinoma, B. Moderate CDK4 expression in papillary thyroid carcinoma, C. Strong CDK4 expression in papillary thyroid carcinoma.

CDK4 expression was observed in all papillary thyroid carcinoma samples with lowest H-score of 25 and highest H-score of 290. The average H-score in pT1 stage group was 174.58. Average H-score in pT2 stage group was 179.25. Average H-score in pT3 stage group was 166.88.

Table 3. CDK4 Expression

CDK4	**Category**	**pT1** (n=17)	**pT2** (n=17)	**pT3** (n=18)	***Kruskal-Wallis*** ***p***
H-score	0-50	3 (17,64%)	1 (5,88%)	3 (16,66%)	0.904
	51-100	1 (5,88%)	4 (23,52%)	4 (22,22%)	
	101-150	3 (17,64%)	1 (5,88%)	0	
	151-200	2 (11,76%)	3 (17,64%)	2 (11,11%)	
	201-250	3 (17,64%)	4 (23,52%)	5 (27,77%)	
	251-300	5 (29,41%)	4 (23,52%)	4 (22,22%)	

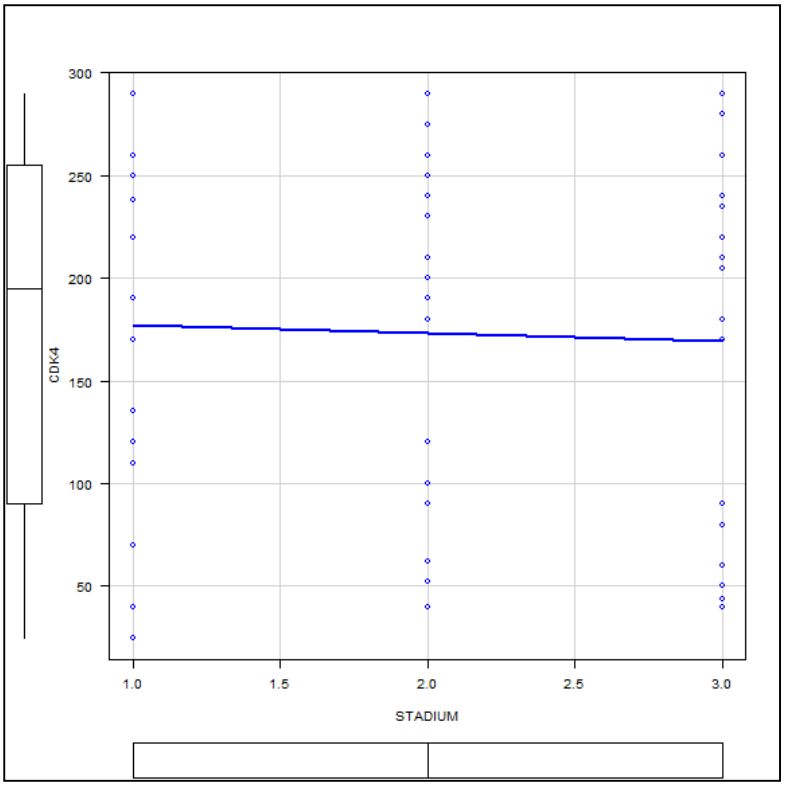

Figure 5. Scattered plot of Spearman correlation of CDK4 and pT stage

A normality test was done using Kolmogorov-smirnov and found that the data was not distributed normally, a non-parametric testing using Kruskal-wallis was done. Data analytics showed that there are no differences in CDK4 expression across different pT stages of papillary thyroid carcinoma (Table 3).

Additionally, a correlation test using Spearman correlation test was performed and showed non-significant results (p=0.752). It was concluded that there were no differences in CDK4 expression across all pT stage groups and there were no correlations between CDK44 expression and pT stage (Figure 5).

CDK4 is linked to cyclin D1, its role is phosphorylation of Rb, which partially inactivates RB1 and releasing E2F and upregulation of E2F transcriptional targets which in turn facilitate the cell entry to S-phase. Normal cells have mechanisms to keep this cycle in homeostasis and stopping the cell cycle. In various cancer, there are higher cyclin D1 and CDK4 expression [9].

One must remember natural inhibitors of CDK4, such as from the INK4 protein (such as $p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, $p19^{Ink4d}$) that specifically inhibits CDK4 and CDK6 activity. Protein family that inhibits CDK, CDK interacting protein/kinase inhibitory protein (CIP/KIP) such as p21Cip1, p27Kip1 dan p57Kip2, could bind to cyclins and CDK subunits. These inhibitors block cell cycle in transition phase G1 to S and arrest cell cycle [25].

Research about the correlation between CDK4 and papillary thyroid carcinoma was not abundant, research done by Pita et al in 2023 showed that phosphorylated CDK4 was not found or very weak in normal thyroid cells, while being expressed abundantly in thyroid cancer cells. It is thought that normal thyroid follicle cells don't have phosphorylated CDK4 because of the absence of mitogenic stimulation. Tumor cells with absent phosphorylated CDK4 were also found (even when tumor proliferation rate was high), these findings could be considered when giving inhibitors of CDK4/6 as therapy. The absence of phosphorylated CDK4 in tumor cells showed high levels of $p16^{CDKN2A}$ [26]

Other factors such as other cyclins and/or CDKs had to be considered as factors contributing to cell cycle.

CD44 and CDK4 Expression

Correlation test between CD44 and CDK4 expression in various pT stage of papillary thyroid carcinoma was done using Spearman correlation test, the result yielded significant result (p=0.0000849) (table 4) (Figure 6).

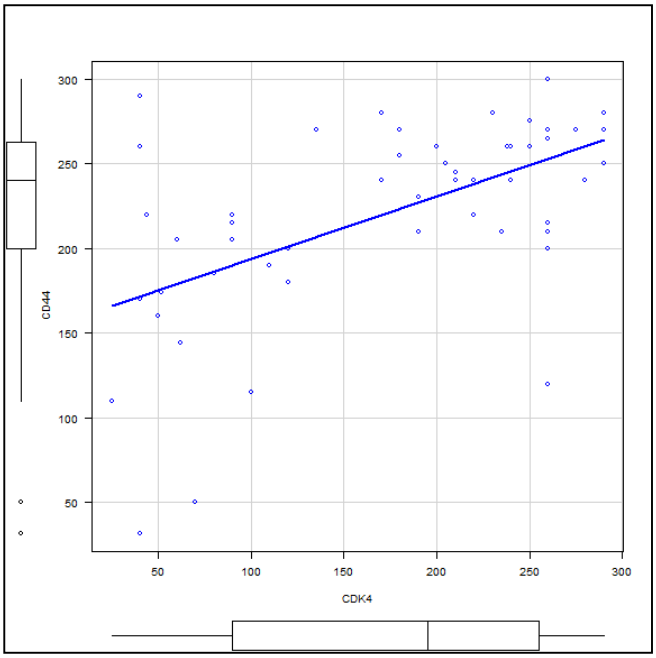

Table .CD44 and CDK4 expression

		CDK4 expression
CD44 expression	r_s	0.518
	p	0.0000849
	n	52

Figure 6. Scattered plot of Spearman correlation test of CD44 and CDK4

CD44 is one of the most researched CSC. The mechanism of CD44 and tumorigenesis was theorized by four known mechanisms, the vascular endothelial growth factor receptor (VEGFR) that contributed to angiogenesis, then by activating AKT to promote phosphorylation and nuclear translocation of MDM2 and blocking p53, then by activating Ras and FAK which activated MAPK/ERK signalling, also promoting Pl3K/Akt signalling [7,8].

One of the researches concluding the correlation between CD44 and CDK4 is the research from Dai et al in 2016 which showed CDK4 and CD44 correlation in breast cancer, in which the increase of CDK4 expression correlate with increased CD44 expression [27]

This research proved that CD44 itself is a factor contributing to cell cycle because of its correlation with CDK4, thus this finding needs to be further investigated.

4. CONCLUSION

Thyroid cancer is one of the most common cancers in the world, with rising numbers around the world. While the most common type, papillary thyroid carcinoma has good prognosis, factors such as age, sex, pathological stage (pT), lymph node metastasis and metastasis could increase mortality rates.

The presence of cancer stem cells until this day is still controversial and not well known, multiple attempts had been done to understand the nature of CSC and its relation to tumorigenesis. CD44 is one of the most researched CSC today.

CD44 is known to activate multiple pathways in intracellular signalling, one of them in proliferation, in cell cycle itself, one of most important protein is the cyclin protein. Cyclin dependent kinase protein such as CDK4/6 phosphorylates Rb protein and activate the E2F protein and progressing cell cycle from G1 into S phase. The correlation between CD44 and CDK4 is not well understood.

This research showed that there are no correlations between CD44 and pT stage, which could be caused by the absence of

specific isoforms of CD44 that were researched. Epithelial mesenchymal transition (EMT) as tumor progressed could also cause the change from one isoform to another. Further research is needed to find out the correlation between various isoforms of CD44 and tumor stage in papillary thyroid carcinoma.

No correlation between CDK4 and pT stage also suggested further investigation. Different inhibitory factors such as p16, different proteins that also contribute to cell cycles such as other cyclins and CDKs, could be other factors that could be researched.

Positive correlation between CD44 and CDK4 showed the role of CD44 and cell proliferation, although other intracellular factors should be researched further.

ACKNOWLEDGMENTS

The authors are grateful for the support from the Dean of Medical Faculty at Universitas Airlangga and Director of Research and Development Unit at Dr. Soetomo General Academic Hospital.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors. Funding of this research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

REFERENCES

[1] Rossi, E. D., & Pantanowitz, L. (2023). Typical "BRAFV600E-Like" atypia in papillary thyroid carcinoma: A morphologic sign of a driver mutation. *Endocrine Pathology*, *34*(2), 265–266. https://doi.org/10.1007/s12022-023-09765-1

[2] Ghossein, Ronald A, Asa, Sylvia L, Baloch, Zubair Wahid, et al. Papillary Thyroid Carcinoma, in: WHO Classification of Tumours Editorial Board. Endocrine and neuroendocrine tumours [Internet]. Lyon (France): International Agency for Research on Cancer; 2022. (WHO classification of tumours series, 5th ed.; vol. 10). Available from: https://tumourclassification.iarc.who.int/chapters/53.

[3] Tuttle, R. M., Haugen, B., & Perrier, N. D. (2017). Updated American joint committee on cancer/tumor-node-metastasis staging system for differentiated and anaplastic thyroid cancer (Eighth Edition): What changed and why? *Thyroid*, *27*(6), 751–756. https://doi.org/10.1089/thy.2017.0102

[4] Bai, Y., Kakudo, K., & Jung, C. K. (2020). Updates in the pathologic classification of thyroid neoplasms: A review of the world health organization classification. *Endocrinology and Metabolism*, *35*(4), 696–715. https://doi.org/10.3803/ENM.2020.807

[5] Konturek, A., Barczyński, M., Nowak, W., & Richter, P. (2012). Prognostic factors in differentiated thyroid cancer - A 20-year surgical outcome study. *Langenbeck's Archives of Surgery*, *397*(5), 809–815. https://doi.org/10.1007/s00423-011-0899-z

[6] Guo, Q., Yang, C., & Gao, F. (2022). The state of CD44 activation in cancer progression and therapeutic targeting. *FEBS Journal*, *289*(24), 7970–7986. https://doi.org/10.1111/febs.16179

[7] Mesrati, M. H., Syafruddin, S. E., Mohtar, M. A., & Syahir, A. (2021). CD44: A multifunctional mediator of cancer progression. *Biomolecules*, *11*(12). https://doi.org/10.3390/biom11121850

[8] Xu, H., Niu, M., Yuan, X., Wu, K., & Liu, A. (2020). CD44 as a tumor biomarker and therapeutic target. *Experimental Hematology and Oncology*, *9*(1), 1–14. https://doi.org/10.1186/s40164-020-00192-0

[9] Fassl, A., Geng, Y., & Sicinski, P. (2022). CDK4 and CDK6 kinases: From basic science to cancer therapy. *Science*, *375*(6577). https://doi.org/10.1126/science.abc1495

[10] Magaki, S., Hojat, S. A., Wei, B., So, A., & Yong, W. H. (2019). An introduction to the performance of immunohistochemistry. *Methods in Molecular Biology*, *1897*, 289–298. https://doi.org/10.1007/978-1-4939-8935-5_25

[11] Ruengwanichayakun, P. (2021). Histochemical scoring assessment (H-score). *Asian Archives of Pathology*, *3*(1), 13.

[12] Rashid, F. A., Munkhdelger, J., Fukuoka, J., & Bychkov, A. (2020). Prevalence of BRAFV600E mutation in Asian series of papillary thyroid carcinoma—a contemporary systematic review. *Gland Surgery*, *9*(5), 1878–1900. https://doi.org/10.21037/gs-20-430

[13] Lim, H., Devesa, S. S., Sosa, J. A., Check, D., Cari, M., Branch, E., & Cancer, N. (2021). Trends in thyroid cancer incidence and mortality in the United States 1974-2013. *Journal of the American Medical Association*, *317*(13), 1338–1348. https://doi.org/10.1001/jama.2017.2719.Trends

[14] Haugen, B. R., Alexander, E. K., Bible, K. C., Doherty, G. M., Mandel, S. J., Nikiforov, Y. E., Pacini, F.,

Randolph, G. W., Sawka, A. M., Schlumberger, M., Schuff, K. G., Sherman, S. I., Sosa, J. A., Steward, D. L., Tuttle, R. M., & Wartofsky, L. (2016). 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*, *26*(1), 1–133. https://doi.org/10.1089/thy.2015.0020

[15] Golbert, L., De Cristo, A. P., Faccin, C. S., Farenzena, M., Folgierini, H., Graudenz, M. S., & Maia, A. L. (2017). Serum TSH levels as a predictor of malignancy in thyroid nodules: A prospective study. *PLoS ONE*, *12*(11), 1–12. https://doi.org/10.1371/journal.pone.0188123

[16] Haymart, M. R. (2009). Understanding the Relationship Between Age and Thyroid Cancer. *The Oncologist*, *14*(3), 216–221. https://doi.org/10.1634/theoncologist.2008-0194

[17] Derwahl, M., & Nicula, D. (2014). Estrogen and its role in thyroid cancer. *Endocrine-Related Cancer*, *21*(5), T273–T283. https://doi.org/10.1530/ERC-14-0053

[18] Ding, J., Wu, W., Fang, J., Zhao, J., & Jiang, L. (2020). Male sex is associated with aggressive behaviour and poor prognosis in Chinese papillary thyroid carcinoma. *Scientific Reports*, *10*(1), 1–8. https://doi.org/10.1038/s41598-020-60199-9

[19] [19] Tang, K. T., & Lee, C. H. (2010). BRAF Mutation in Papillary Thyroid Carcinoma: Pathogenic Role and Clinical Implications. *Journal of the Chinese Medical Association*, *73*(3), 113–128. https://doi.org/10.1016/S1726-4901(10)70025-3

[20] Han, S. A., Jang, J. H., Won, K. Y., Lim, S. J., & Song, J. Y. (2017). Prognostic value of putative cancer stem cell markers (CD24, CD44, CD133, and ALDH1) in human papillary thyroid carcinoma. *Pathology Research and Practice*, *213*(8), 956–963. https://doi.org/10.1016/j.prp.2017.05.002

[21] Ryu, Y. J., Choe, J. Y., Lee, K., & Ahn, S. H. (2020). Clinical prognostic significance of cancer stem cell markers in patients with papillary thyroid carcinoma. *Oncology Letters*, *19*(1), 343–349. https://doi.org/10.3892/ol.2019.11087

[22] Rohani, P., Noroozinia, F., Modarresi, P., & Abbasi, A. (2017). CD44 standard isoform; Not a good marker for colon cancer. *International Journal of Cancer Management*, *10*(9). https://doi.org/10.5812/ijcm.9166

[23] Kawai, T., Iwata, K., Shinotsuka, Y., Kubo, S., Masuoka, H., Yabuta, T., Hirokawa, M., Nakamura, H., Miyauchi, A., & Komai, K. (2019). CD44v8-10 and CD44s are age-dependently expressed in primary cultured papillary thyroid carcinoma cells and are associated with cell proliferation. *Kobe Journal of Medical Sciences*, *65*(1), E1–E9.

[24] Miyazaki, H., Takahashi, R., Prieto-vila, M., & Kawamura, Y. (2018). *CD44 exerts a functional role during EMT induction in cisplatin- resistant head and neck cancer cells*.

[25] Sobhani, N., Angelo, A. D., Pittacolo, M., Roviello, G., Miccoli, A., Corona, S. P., Bernocchi, O., Generali, D., & Otto, T. (2019). Combinations in Breast Cancer. *Cells*, *8*(4), 1–24

[26] Pita, J. M., Raspé, E., Coulonval, K., Decaussin-Petrucci, M., Tarabichi, M., Dom, G., Libert, F., Craciun, L., Andry, G., Wicquart, L., Leteurtre, E., Trésallet, C., Marlow, L. A., Copland, J. A., Durante, C., Maenhaut, C., Cavaco, B. M., Dumont, J. E., Costante, G., & Roger, P. P. (2023). CDK4 phosphorylation status and rational use for combining CDK4/6 and BRAF/MEK inhibition in advanced thyroid carcinomas. *Frontiers in Endocrinology*, *14*(October), 1–24. https://doi.org/10.3389/fendo.2023.1247542

[27] Dai, M., Zhang, C., Ali, A., Hong, X., Tian, J., Lo, C., Fils-, N., Burgos, S. A., Ali, S., & Lebrun, J. (2016). CDK4 regulates cancer stemness and is a novel therapeutic target for triple-negative breast cancer. *Nature Publishing Group*, *October*, 1–15. https://doi.org/10.1038/srep35383