Iranian Journal of Pathology | ISSN: 2345-3656

Immunohistochemical Evaluation of β- Catenin Marker in Papillary Thyroid Cancer: Clinicopathologic Significance

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KEYWORDS

Thyroid Cancer, Papillary; Immunohistochemistry; β-Catenin

Article Info

Received 08 April 2017;
Accepted 05 April 2018;
Published Online 17 July 2018;

ABSTRACT

Background & objective: papillary thyroid cancer is the most common cancer of thyroid accounting for 75%-85% of all thyroid malignancies. Recently, β-catenin has been determined to play a role in clinical course of human epithelial cancers. This study was designed to reveal the association of β-catenin marker and papillary thyroid carcinoma behavior.

Methods: 63 paraffin blocks of papillary thyroid carcinoma were stained with ready to use monoclonal β-catenin antibody according to manufacturer's instructions. Memberanous, cytoplasmic and nuclear staining was scored according to intensity of immunoreactivity. β-catenin immunostaining association with clinical parameters like number of recurrences and cumulative dose of radioiodine therapy were analyzed using SPSS version 15. Histopathologic parameters like tumor stage, grade, capsular invasion, lymphovascular invasion, lymph node involvement, distant metastasis and other variables were also evaluated for association with β-catenin immunoreactivity.

Results: 77.8% of papillay thyroid carcinoma were well differentiated and the remaining were poorly differentiated. Loss of β-catenin membrane immunostaining depicted correlation with number of recurrences (P=0.023% , Pearson correlation= -0.285). Its loss of memberanous staining correlated similarly with cumulative dose of radioiodine (P= 0.046, Pearson correlation = -0.253). Loss of membranous β-catenin was significantly associated with some histopathologic findings like nodal involvement (P<0.001), distant metastasis (P=0.003) and tumor dedifferentiation (P< 0.001).

Conclusion: Loss of β-catenin membranous staining and its cytoplasmic accumulation were associated with aggressive clinicopathologic behavior. The exact effect of radioiodine exposure on β-catenin pathway remained to be determined in future.

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Introduction

Thyroid cancer constitutes 1% of all human cancers and is the most frequent endocrine neoplasm. Papillary thyroid carcinoma is the most prevalent cancer of thyroid accounting for 75-85% of all thyroid cancers (1).

Tumor recurrence occurs in 5-20% of all papillary thyroid carcinoma and usually results from inadequate treatment or aggressive histology. It sometimes happen 20 years after initial diagnosis (2). Distant metastasis usually happens in 10-15% of all papillary thyroid carcinoma and reduces 10 years survival to 40 % (3).

Genetic alterations like point mutation in BRAF lead to an aggressive course and a more invasive form of papillary thyroid carcinoma in more than 70% of cases. Other gene mutations like RAS and structural changes including insertion/deletion and translocations of RET, PAX8/PPAR have been determined to play a role in clinical course of papillary thyroid carcinoma (4-6). In recent decade proto-oncogene effect of wnt/ β-catenin pathway and its role in human cancers have been determined (7-10). Role of

β-catenin in propagation, renewal and regeneration of epithelial tumors have also been highlighted.

Thyroid cancers have different clinical course in such a way that some of them require surgical treatment and radioiodine therapy once while others may need several courses of surgical treatment and radioiodine therapy due to disease recurrence. On the other hand, with respect to lack of β-catenin overexpression in 33% of papillary thyroid carcinoma and possible role of radioiodine exposure in aggressive behavior of thyroid cancer, this question strikes mind whether β-catenin plays a role in recurrence , clinical course and prognosis of papillary thyroid carcinoma.

Material and Method

Tumor Specimens

We selected 63 patients with the diagnosis of papillary thyroid carcinoma from 2009 to 2015 in an endocrine research center and obtained their paraffin blocks from pathology ward. Patients clinical data such as number of surgeries, number of radioiodine therapies, cumulative dose radioiodine were retrieved from medical files. All histologic sections were reviewed by two separate pathologists to confirm the diagnosis and reevaluate histologic subtype, tumor grade, multifocality, encapsulation, capsular invasion, extrathyroid extension, lymphovascular invasion, lymph node involvement, distant metastasis, additional pathologic findings and tumor stage based on American Joint Commission on Cancer 7th edition.

Immunohistochemistry

Paraffin blocks were cut into 4μm sections and were deparaffinized in for 15 min. A 3% hydrogen peroxide was utilized for blocking endogenous peroxide activity. Subsequently, antigen retrieval was fulfilled by using EDTA buffer (pH=8). Then sections were incubated with ready to use monoclonal antibody reactive with β-catenin for 1 hour. Envision secondary antibody was used to react with 3,3-diaminobenzidine (DAB) to visualize immunostain.

Marker Scoring

β-Catenin immunoreactivity with membrane, cytoplasm and nucleus were evaluated separately. A histological score (H score) was defined for membranous staining which was calculated multiplying percent of stained cells by the intensity of staining (0= no stain, 1+=weak but detectable, 2+=distinct, 3+= intense). Cytoplasmic β-catenin staining was scored as nil, mild, moderate and severe. Nuclear β-catenin staining was simply reported as negative versus positive (11). Due to paucity of tumor cells with cytoplasmic and nuclear β-catenin reaction, H score system was not applied for these variables. Memberanous staining of normal follicular epithelial cells of thyroid gland was considered as internal control and stromal cells' reaction in the background was considered as non-specific reaction. In addition, a case of fibromatosis was also used as the positive control in each immunohistochemistery run ro reaasure the correct procedure and lack of inhibibitors.

Statistical Analysis

β-Catenin cytoplasmic and nuclear immunostaining were regarded as categorical data , thus chi square , fisher exact test and one way ANOVA or its equivalent for non-parametric variables, Kruskal Wallis test, were used to analyze relevant data. On the other hand, β-catenin immunoreactivity was considered as a continuous numerical variable, therefore student t test, one way analysis of variance and Pearson correlations were applied for statistical assessment. Commercially available statistic software, SPSS version 15, was utilized to perform statistical analysis. P value less than 0.05 was considered to be statistically significant.

Results

In the 63 papillary thyroid carcinoma cases, female predominance with male to female ratio of 1:6.8. Classic papillary thyroid carcinoma was the most frequent histological subtype (73%) followed by follicular variant (11.1%), papillary microcarcinoma (6.3%), solid variant (4.8%), oncocytic type (3.2%), and tall cell variant (1.6%). Most of them fell in well differentiated category (77.8%) and the remaining were poorly differentiated (table 1).

Table 1. Frequencies of demographic and other variables

Variable	Frequency	
sex	Male (12.7%)	Female (87.3%)
Histologic subtype of papillary thyroid carcinoma	Classic (73%)	Non-classic (27%)
Tumor differentiation	Well differentiated (77.8%)	Poorly differentiated (22.3%)
Concomitant non-neoplastic finding	Hashimoto thyroiditis (38.1%)	Nodular goiter (34.9%)

In terms of β-catenin membranous immunoreactivity, there was a significant correlation between loss of membranous stain and number of tumor recurrences (*P*=0.023, Pearson correlation=-0.285). There was a similar trend between number of surgeries and loss of β-catenin membranous stain intensity (*P*=0.03, Pearson correlation = -0.365). The correlation was also similarly significant between cumulative dose of radioiodine required for post-operative treatment and the loss of membranous β-catenin immunoreactivity (*P*=0.046, Pearson correlation= - 0.253). There was a significant relationship between some histopatholgic parameters and memberanous staining of β-catenin which are summarized in table 1.

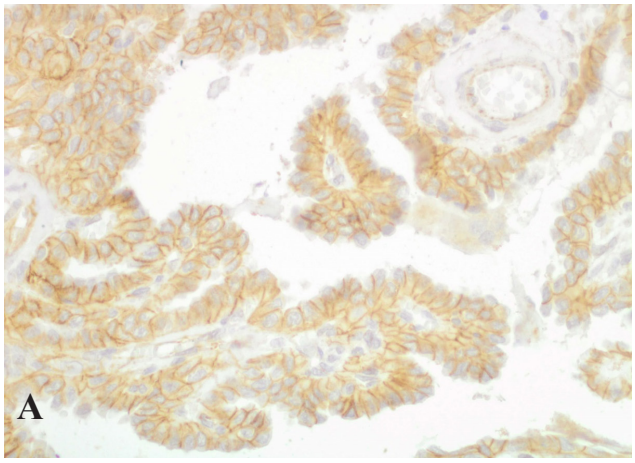
This relationship was not significant for tumor size, histopathologic subtype and non-tumoral concomitant pathologic findings.

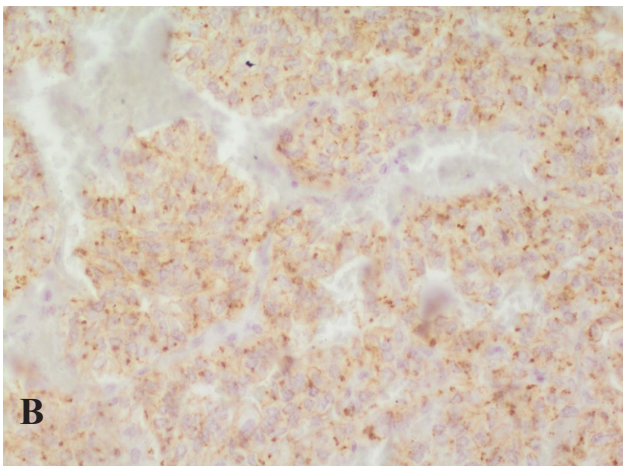
Table 2. β-Catenin memberanous immunoreactivity relationship with prognostic histopathologic parameters.

Histopathologic Parameter	β-Catenin membranous immunoreactinity(H score mean ±SD in paranthesis)		P value
Nodal Involvement	Yes (133.5±45.7)	No(175.4±29.8)	*P*< 0.001
Metastasis	Yes (102±21.6)	No (161.5±40.9)	*P*=0.002
Lymphovascular invasion	Yes (139.2±43.7)	No (170.8±37.1)	*P*=0.003
Tumor differentiation	Well differentiated tumor(168.7±29.5)	Poorly differentiated tumor(115±55.8)	*P*<0.001

With regard to cytoplasmic β-catenin immunostainig, there was significant statistical relationship between intensity of β-catenin cytoplasmic immunoreactivity and number of surgeries (0.005) number of recurrences (*P*=0.008) number of involved lymph nodes (*P*=0.003) distant metastasis (*P*<0.001) and tumor dedifferentiation (*P*=0.005).

None of the cases showed true nuclear staining for β-catenin, however nuclear pseudoinclusions were remarkably immunoreactive for β-catenin in several cases. No significant relationship between this pattern of staining and clinicopathologic parameters was found.





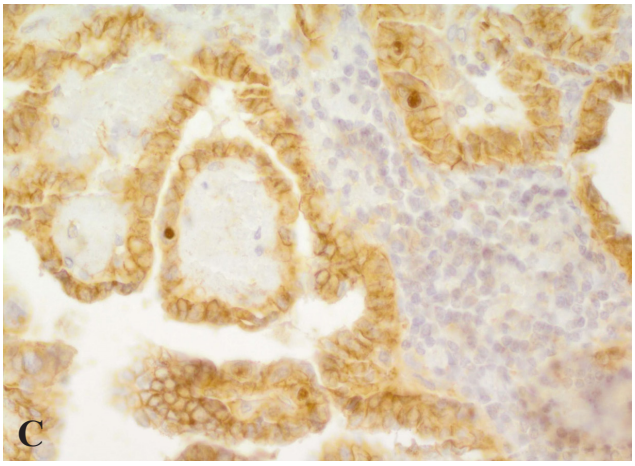


Figure1.

A- strong memberanous β-catenin immunoreactivity in a primary well differentiated papillary thyroid carcinoma without lymph node and distant metastasis (x400 counterstained by hematoxyline).

B- Moderate to severe dot like cytoplasmic β catenin immunostaining and loss of its membranous reaction in a case of poorly differentiated papillary thyroid carcinoma with trabecular pattern and distant metastasis(x400 counterstained by hematoxyline).

C- Strong pseudonuclear inclusion staining in a case of typical well differentiated papillary thyroid carcinoma (x400 counterstained by hematoxyline)

Discussion

In this study, there was a female to male predominance. Classic papillary thyroid carcinoma was the most frequent subtype followed by follicular variant, papillary microcarcinoma, solid type, oncocytic and tall cell variants. Loss of memberanous β-catenin imunoreactivity was correlated with number of recurrences, number of surgeries and cumulative dose of radioiodine. There was a significant relationship between loss of memberanous β-catenin staining and histopathologic parameters like lymphovascular invasion, lymph node involvement, distant metastasis, and tumor dedifferentiation. Cytoplasmic β-catenin immunostaining nearly led to similar results.

Wnt signaling pathway consists of three different components: one canonical or β-catenin dependent on and two β-catenin independent ones. The former, Wnt/β-catenin, plays an important role in cancer initiation and propagation. β-catenin is normally localized in cell adherens junctions which are bound to E cadherin (12-14). When Wnt pathway is activated, β-catenin is released and accumulates in cytoplasm, then it can enter the nucleus and regulate genes involved in proliferation such as cyclin D1 and c-myc (15).

In this study, loss of membranous β-catenin and cytoplasmic accumulation was significantly associated with clinical and histopathologic prognostic factors such as number of recurrences, lymph node involvement and distant metastasis. No significant nuclear staining was detectable in this study. Thus it could be concluded that initial part of β-catenin pathway, E-cadherin bound part, plays a more remarkable role in papillary thyroid cancer progression. Cellular discohesiveness is underlying cause of cancer dissemination. On the other hand E-cadherin and β-catenin binding is important in cell to cell adhesion. Logically, the loss of this complex leads o cancer propagation. In a recent survey by Zhang et al,β-catenin expression was higher in nodal metastasis compared to primary tumors, however this difference did not reach a statistical significant level (16). In a study which was conducted by Gracia-Rostan et al, it was showed that memberanous β-catenin was significantly lower in thyroid carcinomas comparing to benign thyroid lesions such as follicular adenomas. Similarly, they observed that loss of memberanous β-catenin was associated with tumor dedifferentiation (11). Membranous H-score in their study for well differentiated carcinoma (199 ± 90) was higher comparing to poorly differentiated carcinoma (114 ± 70). Although, their H score for well differentiated carcinoma was higher comparing to present study (168), this score for poorly differentiated carcinoma was close to current study (115) and both of them showed a significant reduction of the memberanous β-catenin H score during transition of thyroid carcinoma from a well differentiated tumor to a poorly differentiated neoplasm.

Present study findings exhibited loss of membrane β-catenin staining in poorly differentiated tumor and lack of nuclear staining in any of them. In a recent study by Sethi K et al, it was claimed β-catenin could be a diagnostic and prognostic factor for papillary thyroid carcinoma (17). In a recent study by Rossi

et al, it was observed that HBME-1 and Galectin-3 were expressed in poorly differentiated thyroid carcinomas, they also found that β-catenindid not express in thyroid tumors without differentiation and it was associated with vascular invasion, distant metastasis and higher mortality.Their findings implied to β-catenin prognostic significance (18). Present study findings supported prognostic role of β-catenin but did not suggest its diagnostic utility with exception of its typical pseudoinclusion staining pattern in some cases.

Number of recurrences and subsequent requirement for surgery and radioiodine therapy are considered to be clinical indicators of a papillary thyroid carcinoma behavior. Recurrent disease necessitates reoperation with multiple radioiodine ablative therapy with I^{131} (19-20).Specific biomarker evaluation in lymphatic invasion could predict future papillary thyroid carcinoma recurrences and allow its prevention by applying more aggressive surgery and radiotherapy at the beginning of disease (21-22). There was a significant association between membranous β-catenin loss, then its cytoplasmic accumulation and number of recurrences, number of surgeries and cumulative dose of radioiodine in present study. With regard to radioiodine therapy, its exact role is not determined whether loss of β-catenin demands more doses of radioiodine or its higher cumulative dose could reduce expression of β-catenin. In a cohort study by Zalboska B et al it was revealed that external and internal exposure to radioiodine per se could lead to papillary thyroid carcinoma (23). Thus it is recommended that to design a study in order to evaluate radioiodine exposure impact on β-catenin expression.

In conclusion, loss of membranous β-catenin immunostainig and its accumulation in cytoplasm led to aggressive clinicopathologic behavior especially more recurrences, lymphovascular invasion, lymph node involvement and distant metastasis. Although there was a significant statistical relationship between β-catenin immunoreactivity and radioiodine exposure, their exact interaction remained to be determined in future.

Conflicts of interest

The authors declared no conflict of interest.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. CA Cancer J Clin. 2010;60(5):277-300. https://doi.org/10.3322/caac.20073 PMID:20610543
2. Rotstein L. The role of lymphadenectomy in the management of papillary carcinoma of the thyroid. J Surg Oncol. 2009;99(4):186-8. https://doi.org/10.1002/jso.21234 PMID:19170045
3. Lee JH, Lee ES, Kim YS. Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid. Cancer. 2007;110(1):38-46. https://doi.org/10.1002/cncr.22754 PMID:17520704
4. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol. 2008;21(S2):S37-43. https://doi.org/10.1038/modpathol.2008.10 PMID:18437172 PMCid:PMC2673022
5. Witt RL, Ferris RL, Pribitkin EA, Sherman SI, Steward DL, Nikiforov YE. Diagnosis and management of differentiated thyroid cancer using molecular biology. Laryngoscope. 2013;123(4):1059-64. https://doi.org/10.1002/lary.23838 PMID:23404751
6. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High Prevalence of BRAF Mutations in Thyroid Cancer Genetic Evidence for Constitutive Activation of the RET/PTC-RAS-BRAF Signaling Pathway in Papillary Thyroid Carcinoma. Cancer Res. 2003;63(7):1454-7. PMID:12670889
7. Polakis P. The many ways of Wnt in cancer. Curr Opin Genetics Dev. 2007;17(1):45-51. https://doi.org/10.1016/j.gde.2006.12.007 PMID:17208432
8. Chiu CG, Chan SK, Fang ZA, Masoudi H, Wood-Baker R, Jones SJ, et al. Beta-catenin expression is prognostic of improved non–small cell lung cancer survival. Am J Surg Pathol. 2012;203(5):654-9. https://doi.org/10.1016/j.amjsurg.2012.01.002 PMID:22402266
9. Zaid KW. Immunohistochemical assessment of E-cadherin and β-catenin in the his-

tological differentiations of oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2014;15(5):8847-53. https://doi.org/10.7314/APJCP.2014.15.20.8847 PMID:25374218

10. Zhang DP, Li XW, Lang JH. Prognostic Value of β-catenin Expression in Breast Cancer Patients: a Meta-analysis. Asian Pac J Cancer Prev. 2014;16(14):5625-33. https://doi.org/10.7314/APJCP.2015.16.14.5625
11. Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL, Tallini G. β-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol. 2001;158(3):987-96. https://doi.org/10.1016/S0002-9440(10)64045-X
12. Kikuchi A, Yamamoto H. Tumor formation due to abnormalities in the β-catenin-independent pathway of Wnt signaling. Cancer Sci. 2008;99(2):202-8. https://doi.org/10.1111/j.1349-7006.2007.00675.x PMID:18271916
13. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009;17(1):26-9. https://doi.org/10.1016/j.devcel.2009.06.016 PMID:19619488 PMCid:PMC2861485
14. Reya T, Clevers H. Wnt signaling in stem cells and cancer. Nature. 2005;434(7035):843-50. https://doi.org/10.1038/nature03319 PMID:15829953
15. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature. 1999;398(6726):422-6. https://doi.org/10.1038/18884 PMID:10201372
16. Zhang J, Gill AJ, Issacs JD, Atmore B, Johns A, Delbridge LW, et al. The Wnt/β-catenin pathway drives increased cyclin D1 levels in lymph node metastasis in papillary thyroid cancer. Hum Pathol. 2012;43(7):1044-50. https://doi.org/10.1016/j.humpath.2011.08.013 PMID:22204713
17. Sethi K, Sarkar S, Das S, Rajput S, Mazumder A, Roy B, et al. Expressions of CK-19, NF-kappaB, E-cadherin, beta-catenin and EGFR as diagnostic and prognostic markers by immunohistochemical analysis in thyroid carcinoma. J Exp Ther Oncol. 2011;9(3):187-99. PMID:22070050
18. Rossi ED, Straccia P, Palumbo M, Stigliano E, Revelli L, Lombardi CP, et al. Diagnostic and prognostic role of HBME-1, galectin-3, and β-catenin in poorly differentiated and anaplastic thyroid carcinomas. Appl Immunohistochem Mol Morphol. 2013;21(3):237-41. PMID:23235344
19. Cooper DS, Doherty GM, Hauger BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2009;19(11):1167-214. https://doi.org/10.1089/thy.2009.0110 PMID:19860577
20. Udelsman R. Treatment of persistent or recurrent papillary carcinoma of the thyroid—the good, the bad, and the unknown. J Clin Endocrinol Metab. 2010;95(5):2061-3. https://doi.org/10.1210/jc.2010-0583 PMID:20444934
21. Machens A, Hauptmann S, Dralle H. Lymph node dissection in the lateral neck for completion in central node-positive papillary thyroid cancer. Surgery. 2009;145(2):176-81. https://doi.org/10.1016/j.surg.2008.09.003 PMID:19167972
22. Melck A, Masoudi H, Griffith OL, Rajput A, Wilkins G, Bugis S, et al. Cell cycle regulators show diagnostic and prognostic utility for differentiated thyroid cancer. Ann Surg Oncol. 2007;14(12):3403-11. PMID:17882495
23. Zablotska LB, Ron E, Rozhko AV, Hatch M, Polyanskaya ON, Brenner AV, et al. Thyroid cancer risk in Belarus among children and adolescents exposed to radioiodine after the Chornobyl accident. Br J Cancer. 2011;104(1):181-7. https://doi.org/10.1038/sj.bjc.6605967 PMID:21102590 PMCid:PMC3039791

How to Cite This Article

Ziari K, Sanjari M, Safavi M. Immunohistochemical Evaluation of β-catenin Marker in Papillary Thyroid Cancer Clinicopathologic Behavior. Iranian Journal of Pathology, 2018; 13(2): 151-156.