Biomarker for Thyroid Cancer

Subjects: Oncology | Pathology

Contributor: Guodong Fu

Thyroid cancer has the most rapidly increasing incidence rate among all major cancers, with a triple increase from 4.5 to 14.4 per 100,000 population during 1974–2013. It was estimated 52,890 new cases in the United States in 2020 and contributed to 0.36% of all cancer deaths. Most primary thyroid cancers are follicular cell-derived epithelial tumors, making up four main pathological carcinoma types: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC).

thyroid cancer | Gal-3 expression | NIFTP | EFVPTC | lymphocytic thyroiditis (LT) | immunohistochemical (IHC) analysis

1.Overview

Non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP), which is considered as low-risk cancer, should be distinguished from the malignant invasive encapsulated follicular variant of papillary thyroid carcinoma (EFVPTC). Improved discrimination of NIFTPs from invasive EFVPTCs using a molecular biomarker test could provide useful insights into pre- and post-surgical management of the indeterminate thyroid nodule. Galectin-3 (Gal-3), a β-galactosyl-binding molecule in the lectin group, is involved in different biological functions in well differentiated thyroid carcinomas. The aim of this study was to determine whether Gal-3 expression as a diagnostic marker could distinguish indolent NIFTP from invasive EFVPTC on tissue specimens from surgical thyroid nodules. Methods: immunohistochemical (IHC) analysis of cytoplasmic and nuclear Gal-3 expression was performed in formalin-fixed paraffin-embedded (FFPE) surgical tissues in four specific diagnostic subgroups-benign nodules, NIFTPs, EFVPTCs and lymphocytic/Hashimoto's thyroiditis (LTs). Results: cytoplasmic Gal-3 expression (mean ± SD) was significantly increased in invasive EFVPTCs (4.80 ± 1.60) compared to NIFTPs (2.75 ± 1.58, $p < 0.001$) and benign neoplasms (2.09 ± 1.19, $p < 0.001$) with no significant difference between NIFTPs and benign lesions ($p = 0.064$). The presence of LT enhanced cytoplasmic Gal-3 expression (3.80 ± 1.32) compared to NIFTPs ($p = 0.016$) and benign nodules ($p < 0.001$). Nuclear Gal-3 expression in invasive EFVPTCs (1.84 ± 1.30) was significantly higher than in NIFTPs (1.00 ± 0.72, $p = 0.001$), but similar to benign nodules (1.44 ± 1.77, $p = 0.215$), thereby obviating its potential clinical application. Conclusions: our observations have indicated that increased cytoplasmic Gal-3 expression shows diagnostic potential in distinguishing NIFTP among encapsulated follicular variant nodules thereby serving as a possible ancillary test to H&E histopathological diagnostic criteria when LT interference is absent, to assist in the detection of the invasive EFVPTC among such nodules.

2. Thyroid Cancer

Thyroid cancer has the most rapidly increasing incidence rate among all major cancers, with a triple increase from 4.5 to 14.4 per 100,000 population during 1974–2013 [1]. It was estimated 52,890 new cases in the United States in 2020 and contributed to 0.36% of all cancer deaths [2][3]. Most primary thyroid cancers are follicular cell-derived epithelial tumors, making up four main pathological carcinoma types: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC). Medullary thyroid carcinomas (MTC) originate from thyroid parafollicular (C) cells. PTC and FTC are differentiated thyroid cancers, whereas ATC is undifferentiated. PTC constitutes up to 90% of all thyroid malignancies [4], followed by FTC (5–10%), ATC (<2%, typically occurring in the elder patients) and MTC (2%) [5]. As the most common type of well-differentiated thyroid cancer [6], PTC comprises numerous histopathologic variants that are well validated clinically and biologically, including classic variant, follicular variant (encapsulated/well demarcated, with tumor capsular invasion), follicular variant (encapsulated/well demarcated, noninvasive), follicular variant (infiltrative), tall cell variant, hobnail variant, cribriform-morular variant, columnar cell variant and diffuse sclerosing variant [7]. Among all variants, the follicular variant of papillary thyroid carcinoma (FVPTC) is one of the most common diagnoses, representing up to 30% of all thyroid carcinoma cases [8]. FVPTC is subdivided into two subtypes: the infiltrating/diffuse variant (infiltrative FVPTC), which has the metastatic tendency of the classic papillary thyroid carcinoma, and the encapsulated variant (EFVPTC), which may present with no capsular or angiolymphatic invasion or metastases [9][10]. The microscopic diagnostic criteria for EFVPTC are mainly encapsulation or clear demarcation, follicular growth patterns, and nuclear features of papillary carcinoma (enlargement, crowding/overlapping, elongation, irregular contours, grooves, pseudoinclusions and chromatin clearing) [11][12][13][14]. Controversies have existed for a long time about whether all EFVPTC cases should be classified as malignancy [15][16][17]. Evidence suggests that a subset of non-invasive EFVPTC, earlier considered as conventional thyroid cancer, has been demonstrated to be a highly indolent tumor showing an overall risk of recurrence (local, regional and distant) of less than 1% [15][16][18][19][20]. In 2016, a new terminology Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) has been proposed to name the subset of non-invasive EFVPTC [11] to address the indolent behavior of this tumor and avoid the term "carcinoma" and potential over-treatment.

NIFTP presents the following characteristics: (1) main morphological features, including encapsulation or clear demarcation, follicular growth pattern, no well-formed papillae, no psammoma bodies, <30% solid/trabecular/insular growth pattern, nuclear score 2–3, no tumor necrosis, no high mitotic activity; (2) lack of invasion, which separates this tumor from invasive EFVPTC; (3) a very low risk of the adverse outcome when the tumor is non-invasive [11][21][22]. Histopathological examination of the entire tumor remains the gold standard for NIFTP diagnosis. Of note, the initial exclusionary papillae less than 1% that was subjective to apply has been updated to a 0% cut-off to ensure indolent outcome [22][23]. NIFTP is still an evolving diagnosis, and more data need to be established to substantiate this new entity [24]. As neoplasm develops specific alterations at the proteomic and/or genomic level, molecular marker-based ancillary tests at histological or cytological level may be a great asset in improving the accuracy of the diagnosis of NIFTP [4][11][25][26]. Recently, immunohistochemical test of

Programmed Death-Ligand 1 expression has been shown to distinguish invasive EFVPTC from the indolent NIFTP and benign nodules [25], and recommended as an auxiliary aid to pathologists [27].

Galectin-3 (Gal-3), a member of the beta-galactoside-binding protein family that is predominantly localized in the cytoplasm, may translocate to the perinuclear membrane, nucleus and/or secreted from the cytoplasm [28][29]. This protein is involved in various physiological and pathological processes, such as cell proliferation, apoptosis, inflammation, cell adhesion, cellular transformation, tumor progression and metastasis of cancer cells [28][30]. Overexpression of *galectin-3* cDNA in vitro generates a transformed phenotype [31]; conversely, inhibition of Gal-3 expression has suppressed tumor growth in mice tumor models [32]. Gal-3 is a p53 physiological target mediating p53-induced apoptosis at the molecular level; therefore, the aberrant expression of Gal-3 blocks the apoptotic program, promoting the development of cancer [33]. Gal-3 is overexpressed in a high proportion of carcinomas, especially of the papillary histotype, but weak or absent from normal or benign thyroid tissue [29][30][34], suggesting its potential biological role in the malignant transformation of thyroid cells. Gal-3 immunohistochemistry has been reported to assist the diagnosis of FVPTC [29][35]; however, the role of Gal-3 expression as an ancillary marker in reclassification to NIFTP has not yet been determined.

The aim of the current study was to determine whether Gal-3 subcellular expression in histological surgical tissues could serve as a useful biomarker to distinguish indolent NIFTP from invasive EFVPTC.

3. Discussion

Indeterminate thyroid nodules are highly prevalent in daily clinical practice and represent up to 30% of all clinically assessed thyroid nodules [36], whereas only less than 20% of this group are malignant. Invasive EFVPTC, noninvasive EFVPTC (not meeting criteria for NIFTP diagnosis) and infiltrative FVPTC were designated as malignant lesions, making the diagnosis of thyroid cancer very difficult in cases where histological hallmarks of invasion are not evident [15][16][17][37]. A subset of encapsulated follicular tumors, formerly considered to be noninvasive encapsulated/well demarcated follicular variant of PTC, has been reclassified under a new histological nomenclature, NIFTP [11]. The incidence of NIFTP was as high as 13.3% of all PTC cases in North American and European populations [38] and 16.8% of all well-differentiated thyroid cancers in Ontario, Canada [24]. Histopathological examination of the entire capsulated tumor after its resection according to the rigid diagnostic criteria remains the gold standard for NIFTP diagnosis. Malignant behavior (lymph node and/or distant metastasis) has been reported in NIFTP patients [21][39][40]. NIFTP is not entirely considered as a benign thyroid neoplasm, but correct classification of noninvasive EFVPTC that would qualify NITFP is required to ensure an extremely low rate of adverse oncologic outcomes. Molecular marker testing has risen as an auxiliary tool to distinguish malignant invasive EFVPTCs from more indolent NIFTPs and benign nodules [11][25], potentially assisting pathologists in the management of indeterminate thyroid nodules [27]. Scores on the base of the levels of protein marker expression in thyroid nodules may objectively distinguish malignant lesions from indeterminate thyroid nodules, thereby aiding the correct diagnosis and consequent avoidance of over-treatment of NIFTP lesions when the score is low. Our previous study has shown that the degree of cytoplasmic PD-L1 expression could serve as a useful adjunct to traditional H&E histopathology assessment of such nodules, among which with a low expression of cytoplasmic PD-L1 can be considered as benign nodules or NIFTP [25].

Gal-3 expression has been recognized as a promising diagnostic molecular marker of thyroid malignancy due to its differential expression between thyroid carcinomas and normal or benign thyroid tissues [29][41][42][43]. However, the reclassification of EFVPTC without capsular or vascular invasion to NIFTP not only affects how pathologists evaluate and report this subset of thyroid neoplasms but also raises the need for rebuilding the clinical, histologic, cytologic and molecular parameters for this new entity and accordingly establishing new molecular tests [22]. Therefore, the use of ancillary testing with protein markers previously developed, such as Gal-3 and HBME1, requires to be re-evaluated in the era of NIFTP. In the present study, our data have shown that cytoplasmic Gal-3 expression is significantly increased in invasive EFVPTCs as compared to NIFTPs or benign thyroid nodules in thyroid resection specimens. Concurrently, though there was no significant difference between NIFTPs and benign nodules, cytoplasmic Gal-3 expression in the former was higher than that in the latter, supporting NIFTP cannot be simply considered as a benign lesion. Chronic inflammation can be associated with 30–58% of PTC [44][45]. We also noted that the presence of LT enhances cytoplasmic Gal-3 expression and henceforward the LT increased expression needs to be interpreted with caution. This observation is consistent with the result from another report which showed the increased expression of Gal-3 in an inflammatory environment [46]. Patients with LT were usually under prolonged stimuli from chronic inflammation. The mechanism underlying modulation of Gal-3 expression in thyroid with chronic inflammatory process remains to be determined. Localization of Gal-3 in papillary carcinomas has been reported in both the cytoplasm and nucleus [47][48][49], however, our findings and other's [29] showed predominant expression of Gal-3 in the cytoplasm in PTC rather than the nucleus. Nuclear expression has been observed in some benign thyroid conditions in our study and reports of others [48][49]. The increased cytoplasmic Gal-3 in invasive EFVPTC might contribute to thyroid cancer development through the induction of the capsular, vascular and/or extrathyroidal invasive activity. The detailed correlation between Gal-3 expression and the degree of invasion was not able to be analyzed since most EFVPTC cases were presented with minimal capsular invasion in the current study. Recently, genetic alterations were intensively studied, such as *BRAF*, *RAS* and *TERT* promoter mutations and *RET/PTC* and *PAX8/PPARγ* rearrangements [50][51]. NIFTPs are commonly detected with the frequent occurrence of RAS mutations and lack of BRAF^{V600E} and TERT promoter mutations [23]. Whether the level of Gal-3 expression can be associated with such mutational status for better identifying NIFTP requires further investigation. Our observations have suggested that cytoplasmic Gal-3 expression can be considered as an ancillary aid to H&E diagnostic criteria in distinguishing invasive EFVPTC from NIFTP and benign nodules.

After four decades of steady increase, thyroid cancer incidence rate reached a plateau and possibly started to decline between 2013 and 2020 in the United States [52]. This reverse trend in the incidence of thyroid cancers has been correlating with the increasing understanding of over-diagnosis and the indolent nature of many thyroid nodules that were more likely classified as cancers previously. NIFTP has emerged as a low risk tumor with an indolent clinical course. The present study was focused on evaluating the diagnostic value of the Gal-3 cytoplasmic expression in the histological tissue samples between NIFTP and EFVPTC. To our knowledge, this is the first report showing the diagnostic value of increased cytoplasmic Gal-3 expression in ruling out the indolent NIFTP from invasive EFVPTC. The Gal-3 test proposed here does not replace conventional surgical histopathological examination but represents an auxiliary diagnostic method, especially for cases where morphologic features of invasion are equivocal, that may affect clinical decision-making with regard to completion thyroidectomy, central lymph node dissection, and adjunctive radioiodine therapy. In practice, Gal-3 staining alone add little to histology evaluation when the diagnosis of NIFTP could be achieved via complete resection of the nodules for histological examination of the entire capsule to rule out invasion. However, in pre-surgical fine needle aspiration (FNA) biopsies, NIFTP can belong to any of four categories of the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), including benign, atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm or suspicious for a follicular neoplasm (FN/SFN) and suspicious for malignancy (SFM) [53]. The definitive diagnosis of NIFTP cannot be made based on the observation of the preoperative cytology specimens, while molecular tests would be highly useful to improve the accuracy in the diagnostics of NIFTP in FNA biopsies. Gal-3 test could have clinically significant utility in assisting in preoperative diagnosis if it were successfully applied to cytology specimens [54]. We are aware of the limitation of our study which is based upon a single patient cohort from a single tertiary care center. Future studies in a larger patient cohort from multiple centers are needed to validate our observations and conclusions. Furthermore, the NIFTP cases were re-classified based on a thorough review of pathology reports and assessment of H&E slides in this study. NIFTP diagnosis is challenging for pathologist and a potential misclassification error might exist particularly when specimens were managed in a way the entire tumor capsule could not be fully assessed for invasion based on pathology review of slides. We are also cognizant that the clinical outcome analysis for each subtype was limited due to incomplete follow-up information, hence the possible association of cytoplasmic Gal-3 expression with the long-term prognoses of NIFTP verses invasive EFVPTC remains further investigation.

4. Conclusions

In conclusion, our data have demonstrated that increased cytoplasmic Gal-3 expression can (i) significantly distinguish indolent NIFTP from invasive EFVPTC; (ii) assist in the early detection of thyroid tumors with aggressiveness and potential metastatic spread which can be suspected by the increased cytoplasmic Gal-3 expression; (iii) support its clinical application as a useful ancillary test to H&E histopathological diagnostic assessment in distinguishing invasive EFVPTC from NIFTP when there is no significant interference from LT.

References

1. Lim, H.; Devesa, S.S.; Sosa, J.A.; Check, D.; Kitahara, C.M. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974–2013. JAMA 2017, 317, 1338–1348.
2. The Surveillance E, and End Results (SEER) Program. Cancer Stat Facts: Thyroid Cancer. 2018. Available online: (accessed on 2 June 2020).
3. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. CA A Cancer J. Clin. 2020, 70, 7–30.

4. Xing, M.; Haugen, B.R.; Schlumberger, M. Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013, 381, 1058–1069.
5. Katoh, H.; Yamashita, K.; Enomoto, T.; Watanabe, M. Classification and General Considerations of Thyroid Cancer. Ann. Clin. Pathol. 2015, 3, 1045.
6. Ceresini, G.; Corcione, L.; Michiara, M.; Sgargi, P.; Teresi, G.; Gilli, A.; Usberti, E.; Silini, E.; Ceda, G.P. Thyroid cancer incidence by histological type and related variants in a mildly iodine-deficient area of Northern Italy, 1998 to 2009. Cancer 2012, 118, 5473–5480.
7. Mete, O.; Seethala, R.R.; Asa, S.L.; Bullock, M.J.; Carty, S.E.; Hodak, S.P.; McHugh, J.B.; Nikiforov, Y.E.; Pettus, J.; Richardson, M.S.; et al. Protocol for the Examination of Specimens From Patients With Carcinomas of the Thyroid Gland (Version: Thyroid 4.2.0.0); College of American Pathologists (CAP). Protocol Posting Date: August 2019. Available online: (accessed on 3 June 2020).
8. Burningham, A.R.; Krishnan, J.; Davidson, B.J.; Ringel, M.D.; Burman, K.D. Papillary and follicular variant of papillary carcinoma of the thyroid: Initial presentation and response to therapy. Otolaryngol. Head and NeckSurg. Off. J. Am. Acad. Otolaryngol. -Head and Neck Surg. 2005, 132, 840–844.
9. Gupta, S.; Ajise, O.; Dultz, L.; Wang, B.; Nonaka, D.; Ogilvie, J.; Heller, K.S.; Patel, K.N. Follicular variant of papillary thyroid cancer: Encapsulated, nonencapsulated, and diffuse: Distinct biologic and clinical entities. Arch. Otolaryngol. Head Neck Surg. 2012, 138, 227–233.
10. LiVolsi, V.A.; Baloch, Z.W. Follicular-patterned tumors of the thyroid: The battle of benign vs. malignant vs. so-called uncertain. Endocr. Pathol. 2011, 22, 184–189.
11. Nikiforov, Y.E.; Seethala, R.R.; Tallini, G.; Baloch, Z.W.; Basolo, F.; Thompson, L.D.; Barletta, J.A.; Wenig, B.M.; Al Ghuzlan, A.; Kakudo, K.; et al. Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: A Paradigm Shift to Reduce Overtreatment of Indolent Tumors. JAMA Oncol. 2016, 2, 1023–1029.
12. Kakudo, K.; Bai, Y.; Liu, Z.; Ozaki, T. Encapsulated papillary thyroid carcinoma, follicular variant: A misnomer. Pathol. Int. 2012, 62, 155–160.
13. Liu, J.; Singh, B.; Tallini, G.; Carlson, D.L.; Katabi, N.; Shaha, A.; Tuttle, R.M.; Ghossein, R.A. Follicular variant of papillary thyroid carcinoma: A clinicopathologic study of a problematic entity. Cancer 2006, 107, 1255–1264.
14. Yang, G.C.; Liebeskind, D.; Messina, A.V. Diagnostic accuracy of follicular variant of papillary thyroid carcinoma in fine-needle aspirates processed by ultrafast Papanicolaou stain: Histologic follow-up of 125 cases. Cancer 2006, 108, 174–179.
15. Baloch, Z.W.; LiVolsi, V.A. Encapsulated follicular variant of papillary thyroid carcinoma with bone metastases. Modern Pathol. 2000, 13, 861–865.

16. Daniels, G.H. What if many follicular variant papillary thyroid carcinomas are not malignant? A review of follicular variant papillary thyroid carcinoma and a proposal for a new classification. Endocr. Pract. Off. J. Am. Coll. Endocrinol. Am. Assoc. Clin. Endocrinol. 2011, 17, 768–787.
17. Lloyd, R.V.; Erickson, L.A.; Casey, M.B.; Lam, K.Y.; Lohse, C.M.; Asa, S.L.; Chan, J.K.; DeLellis, R.A.; Harach, H.R.; Kakudo, K.; et al. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. Am. J. Surg. Pathol. 2004, 28, 1336–1340.
18. Piana, S.; Frasoldati, A.; Di Felice, E.; Gardini, G.; Tallini, G.; Rosai, J. Encapsulated well-differentiated follicular-patterned thyroid carcinomas do not play a significant role in the fatality rates from thyroid carcinoma. Am. J. Surg. Pathol. 2010, 34, 868–872.
19. Ganly, I.; Wang, L.; Tuttle, R.M.; Katabi, N.; Ceballos, G.A.; Harach, H.R.; Ghossein, R. Invasion rather than nuclear features correlates with outcome in encapsulated follicular tumors: Further evidence for the reclassification of the encapsulated papillary thyroid carcinoma follicular variant. Hum. Pathol. 2015, 46, 657–664.
20. Rosario, P.W.; Penna, G.C.; Calsolari, M.R. Noninvasive encapsulated follicular variant of papillary thyroid carcinoma: Is lobectomy sufficient for tumours >/=1 cm? Clin. Endocrinol. 2014, 81, 630–632.
21. Cho, U.; Mete, O.; Kim, M.H.; Bae, J.S.; Jung, C.K. Molecular correlates and rate of lymph node metastasis of non-invasive follicular thyroid neoplasm with papillary-like nuclear features and invasive follicular variant papillary thyroid carcinoma: The impact of rigid criteria to distinguish non-invasive follicular thyroid neoplasm with papillary-like nuclear features. Mod. Pathol. 2017.
22. Seethala, R.R.; Baloch, Z.W.; Barletta, J.A.; Khanafshar, E.; Mete, O.; Sadow, P.M.; LiVolsi, V.A.; Nikiforov, Y.E.; Tallini, G.; Thompson, L.D. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: A review for pathologists. Mod. Pathol. 2018, 31, 39–55.
23. Nikiforov, Y.E.; Baloch, Z.W.; Hodak, S.P.; Giordano, T.J.; Lloyd, R.V.; Seethala, R.R.; Wenig, B.M. Change in Diagnostic Criteria for Noninvasive Follicular Thyroid Neoplasm With Papillarylike Nuclear Features. JAMA Oncol. 2018, 4, 1125–1126.
24. Eskander, A.; Hall, S.F.; Manduch, M.; Griffiths, R.; Irish, J.C. A Population-Based Study on NIFTP Incidence and Survival: Is NIFTP Really a “Benign” Disease? Ann. Surg. Oncol. 2019, 26, 1376–1384.
25. Fu, G.; Polyakova, O.; MacMillan, C.; Ralhan, R.; Walfish, P.G. Programmed Death—Ligand 1 Expression Distinguishes Invasive Encapsulated Follicular Variant of Papillary Thyroid Carcinoma from Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features. EBioMedicine 2017, 18, 50–55.
26. Rivera, M.; Ricarte-Filho, J.; Knauf, J.; Shaha, A.; Tuttle, M.; Fagin, J.A.; Ghossein, R.A. Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological

subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. Mod. Pathol. 2010, 23, 1191–1200.

27. Rossi, E.D.; Martini, M. New Insight in a New Entity: NIFTPS and Valuable Role of Ancillary Techniques. The Role of PD-L1. EBioMedicine 2017, 18, 11–12.

28. Nangia-Makker, P.; Nakahara, S.; Hogan, V.; Raz, A. Galectin-3 in apoptosis, a novel therapeutic target. J. Bioenerg. Biomembr. 2007, 39, 79–84.

29. Chiu, C.G.; Strugnell, S.S.; Griffith, O.L.; Jones, S.J.; Gown, A.M.; Walker, B.; Nabi, I.R.; Wiseman, S.M. Diagnostic utility of galectin-3 in thyroid cancer. Am. J. Pathol. 2010, 176, 2067–2081.

30. Cvejic, D.; Savin, S.; Petrovic, I.; Selemetjev, S.; Paunovic, I.; Tatic, S.; Havelka, M. Galectin-3 and proliferating cell nuclear antigen (PCNA) expression in papillary thyroid carcinoma. Exp. Oncol. 2005, 27, 210–214.

31. Yoshii, T.; Inohara, H.; Takenaka, Y.; Honjo, Y.; Akahani, S.; Nomura, T.; Raz, A.; Kubo, T. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. Int. J. Oncol. 2001, 18, 787–792.

32. Honjo, Y.; Nangia-Makker, P.; Inohara, H.; Raz, A. Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells. Clin. Cancer Res. 2001, 7, 661–668.

33. Cecchinelli, B.; Lavra, L.; Rinaldo, C.; Iacovelli, S.; Gurtner, A.; Gasbarri, A.; Ulivieri, A.; Del Prete, F.; Trovato, M.; Piaggio, G.; et al. Repression of the antiapoptotic molecule galectin-3 by homeodomain-interacting protein kinase 2-activated p53 is required for p53-induced apoptosis. Mol. Cell. Biol. 2006, 26, 4746–4757.

34. Kawachi, K.; Matsushita, Y.; Yonezawa, S.; Nakano, S.; Shirao, K.; Natsugoe, S.; Sueyoshi, K.; Aikou, T.; Sato, E. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. Hum. Pathol. 2000, 31, 428–433.

35. Saggiorato, E.; Aversa, S.; Deandreis, D.; Arecco, F.; Mussa, A.; Puligheddu, B.; Cappia, S.; Conticello, S.; Papotti, M.; Orlandi, F. Galectin-3: Presurgical marker of thyroid follicular epithelial cell-derived carcinomas. J. Endocrinol. Investig. 2004, 27, 311–317.

36. 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.; 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 Off. J. Am. Thyroid Assoc. 2016, 26, 1–133.

37. Seethala, R.R.; Asa, S.L.; Bullock, M.J.; Carty, S.E.; Hodak, S.P.; McHugh, J.B.; Nikiforov, Y.E.; Pettus, J.; Richardson, M.S.; Shah, J.; et al. Protocol for the Examination of Specimens from

Patients with Carcinomas of the Thyroid Gland; Version: Thyroid 4.0.0.0; College of American Pathologists (CAP): Northfield, IL, USA, 2017.

38. Bychkov, A.; Jung, C.K.; Liu, Z.; Kakudo, K. Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features in Asian Practice: Perspectives for Surgical Pathology and Cytopathology. Endocr. Pathol. 2018, 29, 276–288.
39. Parente, D.N.; Kluijfhout, W.P.; Bongers, P.J.; Verzijl, R.; Devon, K.M.; Rotstein, L.E.; Goldstein, D.P.; Asa, S.L.; Mete, O.; Pasternak, J.D. Clinical Safety of Renaming Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: Is NIFTP Truly Benign? World J. Surg. 2018, 42, 321–326.
40. Hahn, S.Y.; Shin, J.H.; Lim, H.K.; Jung, S.L.; Oh, Y.L.; Choi, I.H.; Jung, C.K. Preoperative differentiation between noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and non-NIFTP. Clin. Endocrinol. 2017, 86, 444–450.
41. Tang, W.; Huang, C.; Tang, C.; Xu, J.; Wang, H. Galectin-3 may serve as a potential marker for diagnosis and prognosis in papillary thyroid carcinoma: A meta-analysis. OncoTargets Ther. 2016, 9, 455–460.
42. Bartolazzi, A.; Orlandi, F.; Saggiorato, E.; Volante, M.; Arecco, F.; Rossetto, R.; Palestini, N.; Ghigo, E.; Papotti, M.; Bussolati, G.; et al. Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: A prospective multicentre study. Lancet. Oncol. 2008, 9, 543–549.
43. Li, J.; Vasilyeva, E.; Wiseman, S.M. Beyond immunohistochemistry and immunocytochemistry: A current perspective on galectin-3 and thyroid cancer. Expert Rev. Anticancer. Ther. 2019, 19, 1017–1027.
44. Kebebew, E.; Treseler, P.A.; Ituarte, P.H.; Clark, O.H. Coexisting chronic lymphocytic thyroiditis and papillary thyroid cancer revisited. World J. Surg. 2001, 25, 632–637.
45. Tamimi, D.M. The association between chronic lymphocytic thyroiditis and thyroid tumors. Int. J. Surg. Pathol. 2002, 10, 141–146.
46. Ma, H.; Yan, J.; Zhang, C.; Qin, S.; Qin, L.; Liu, L.; Wang, X.; Li, N. Expression of papillary thyroid carcinoma-associated molecular markers and their significance in follicular epithelial dysplasia with papillary thyroid carcinoma-like nuclear alterations in Hashimoto’s thyroiditis. Int. J. Clin. Exp. Pathol. 2014, 7, 7999–8007.
47. Ralhan, R.; Veyhl, J.; Chaker, S.; Assi, J.; Alyass, A.; Jeganathan, A.; Somasundaram, R.T.; MacMillan, C.; Freeman, J.; Vescan, A.D.; et al. Immunohistochemical Subcellular Localization of Protein Biomarkers Distinguishes Benign from Malignant Thyroid Nodules: Potential for Fine-Needle Aspiration Biopsy Clinical Application. Thyroid Off. J. Am. Thyroid Assoc. 2015, 25, 1224–1234.

48. Orlandi, F.; Saggiorato, E.; Pivano, G.; Puligheddu, B.; Termine, A.; Cappia, S.; De Giuli, P.; Angeli, A. Galectin-3 is a presurgical marker of human thyroid carcinoma. Cancer Res. 1998, 58, 3015–3020.

49. Saggiorato, E.; Cappia, S.; De Giuli, P.; Mussa, A.; Pancani, G.; Caraci, P.; Angeli, A.; Orlandi, F. Galectin-3 as a presurgical immunocytodiagnostic marker of minimally invasive follicular thyroid carcinoma. J. Clin. Endocrinol. Metab. 2001, 86, 5152–5158.

50. Nikiforov, Y.E.; Ohori, N.P.; Hodak, S.P.; Carty, S.E.; LeBeau, S.O.; Ferris, R.L.; Yip, L.; Seethala, R.R.; Tublin, M.E.; Stang, M.T.; et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: A prospective analysis of 1056 FNA samples. J. Clin. Endocrinol. Metab. 2011, 96, 3390–3397.

51. Decaussin-Petrucci, M.; Descotes, F.; Depaepe, L.; Lapras, V.; Denier, M.L.; Borson-Chazot, F.; Lifante, J.C.; Lopez, J. Molecular testing of BRAF, RAS and TERT on thyroid FNAs with indeterminate cytology improves diagnostic accuracy. Cytopathol. Off. J. Br. Soc. Clin. Cytol. 2017, 28, 482–487.

52. Powers, A.E.; Marcadis, A.R.; Lee, M.; Morris, L.G.T.; Marti, J.L. Changes in Trends in Thyroid Cancer Incidence in the United States, 1992 to 2016. JAMA 2019, 322, 2440–2441.

53. Brandler, T.C.; Zhou, F.; Liu, C.Z.; Cho, M.; Lau, R.P.; Simsir, A.; Patel, K.N.; Sun, W. Can noninvasive follicular thyroid neoplasm with papillary-like nuclear features be distinguished from classic papillary thyroid carcinoma and follicular adenomas by fine-needle aspiration? Cancer Cytopathol. 2017, 125, 378–388.

54. Trimboli, P.; Guidobaldi, L.; Amendola, S.; Nasrollah, N.; Romanelli, F.; Attanasio, D.; Ramacciato, G.; Saggiorato, E.; Valabrega, S.; Crescenzi, A. Galectin-3 and HBME-1 improve the accuracy of core biopsy in indeterminate thyroid nodules. Endocrine 2016, 52, 39–45.