Research Article

Current and future markers for the diagnosis of thyroid cancer

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Abstract

Today, immunohistochemical markers are routinely used alone or in association to examine thyroid lesions but without sufficient sensitivity and specificity regarding to cancer diagnosis. Additional markers are currently identified among genetic alterations or miRNA panels carrying significant diagnostic values. Combining immunostaining data, mutation status, gene rearrangement and miRNA expression should help to define an integrative signature for the accurate diagnosis of thyroid carcinomas.

Introduction

Whereas thyroid nodules are frequent pathologies of thyroid gland, thyroid cancers are quite rare (<10%) [1-2]. Thyroid FNA and subsequent cytopathological evaluation are the most common preoperative techniques used for the diagnosis of malignant thyroid tumors. However, this invasive procedure showed inconclusive results in 10-20% of cases and many benign lesions with suspicious of cancer are thus abusively referred to surgery [3]. In particular, the diagnosis of follicular lesions is always challenging to clinicians since cytological features overlap between follicular adenoma and carcinoma.

In this context, increasing number of studies had evaluated immunohistochemical markers, such galectin-3, CK19, HBME-1, TPO; DNA alterations, mainly including BRAF and RAS point mutations, RET/PTC and PAX8/PPARg rearrangements; miRNA signatures and circulating tumor cells for diagnosis of thyroid cancers [4-6, 8-12]. While some markers are promising for differential diagnosis, none of them is individually conclusive because of limitations due to significant prevalence in benign thyroid nodules to a notable extent.

Immunohistochemical markers

Among recent evaluations of new IHC markers, galectin-1 was reported to display a higher specificity (97%) than galectin-3 and CK19 which showed higher sensitivity (97% and 98%, respectively), showing complementary diagnosis values [13]. In addition, Ki67 was also found as a suitable single marker for distinguishing carcinomas from benign thyroid diseases [14]. Combinations of markers were evaluated to improve differential diagnosis, especially regarding the classification of thyroid follicular lesions [15-17]. The association of positivity for galectin-3, CK19 and HBME-1 proved to be the most relevant combination (97% specificity, 95% sensitivity) in the distinction between PTC and FA [18]. Additional new marker combinations are currently opening novel opportunities as diagnosis tools. Combined positive emerin (including nuclear level changes) and negative CD56 (lost in cancer) showed 72% sensitivity and 100% specificity and could be additional helpful markers in overlapping cases with high diagnostic validity (high specificity and PPV) [19]. Similarly, the differential expression of TROP-2 and SLP-2 has been evaluated by quantitative PCR and IHC in PTC and FA and results showed that marker expressions were significantly increased in carcinomas. Moreover, when TROP-2...
were combined to CD56, the sensitivity and NPV increased to 100% and had a better diagnostic accuracy, further supporting the interest for CD56 [20]. Again, NRG1 was also recently reported to be highly expressed in PTC compared with adjacent normal tissues [21].

**Genetic alterations**

In parallel to immunoprofiling, significant progress has been made in developing molecular markers for clinical use in FNA materials, including gene mutation panels and gene expression signatures [22]. For examples, TERT promoter and TP53 mutations are more frequent in less differentiated carcinomas, potentially helping in the thyroid cancer classification [23-25]. Clinical tests include 3 approaches to investigate the molecular profile of FNA: (1) the seven genes panel, (2) the Afirma classifier, and (3) the NGS assays [26]. Indeed, a test detecting alterations in seven main genes responsible of 70% of all thyroid cancers was designed, but despite a good sensitivity, the NPV was too low for clinical use [27-28]. In order to assess the risk of cancer in ITN, the Afirma test was introduced to distinguish “benign” from “suspicious” nodules, based on the analysis of a 142 gene expression profile [29].

Finally, the NGS detect various types of genetic alterations in a small amount of cells with high sensitivity [30-32]. In this context, the Thyroseq test identifies several point mutations, hotspot mutations in 14 genes, 42 types of gene fusions and expression levels of 16 genes. Thyroseq is reported to have a NPV of 96-97% and a PPV of 77-83% [33, 34]. Recently, Taye et al. also evaluated the Thyroseq v2 performance in 156 ITN and conclude that it is likely to be an appropriate “rule out” test (NPV >95%) but they warn with the positive results and advice to refine the result based on the mutation and histological type. Indeed, the PPV is dependant of cancer prevalence in patient populations [2]. Their observations agree with those of two other studies that have highlighted an NPV of 91-94% and a PPV of 42-66% [35, 36]. Lastly, a bioinformatic analysis has identified seven-hub genes, including FN1, SERPINA1, ECM1, MMRN1, PROS1, CFD and TIMP1, which may helpful for the development of gene panel for thyroid nodule diagnosis [37].

**Micro RNA panels**

In addition to gene expression signature, miRNA profiling was further developed as an important main for the diagnosis of thyroid carcinomas [38]. The mechanisms of miRNA implication in cancer development are linked to the downregulation of tumor suppressor genes or the upregulation of oncogenes. As for previous markers, a set of multiple miRNAs seems to be more sensitive (87%) than a single miRNA (71%) although there is a discrepancy in terms of set of miRNA proposed among studies [39, 40]. Nevertheless, a set of 15 miRNAs emerge as the more powerful diagnostic panel for indeterminate lesions [26]. Of course, future prospective and retrospective researches are recommended on a larger cohort of indeterminate lesions to validate the diagnostic value of this panel. A meta-analysis reported a significant difference in miRNA-221/222 expression in thyroid cancer compared to normal thyroid or benign thyroid lesions, further supporting miRNAs as promising molecular markers to improve diagnosis of thyroid cancer [41]. In this context, two additional diagnostic tests using miRNAs were introduced to predict malignancy in thyroid nodules. The first one, ThyGenX/ThyraMIR, combines a 7-gene mutation panel and a group of 10 miRNA markers. The second one, Rosetta GX Reveal test, is based on the detection of 24 miRNA markers. In addition, a study aiming to compare the performance of both tests on 10 FNA by correlating the results to histopathology data showed a 100% NPV while RosettaGX disclosed a 75% PPV in comparison to 60% for ThyGenX/ThyraMIR [42]. Finally, a review of the 4 commercially available molecular tests concludes that they offer unique approaches to improve the risk stratification of thyroid nodules [43]. Interestingly, the study of Mazeh et al. reinforces the interest of these technologies combining NGS and miRNA since they were able to classify ITN with the greatest accuracy in comparison with the molecular tests currently marketed [44].

**Conclusions**

Nowadays, novel marker panels/signatures are always under validation step to define additional criteria for the distinction between thyroid lesions, especially regarding the classification of thyroid follicular lesions. Based on meta-analyses, new combinations of markers including immunohistochemical protein detection, genetic alteration evaluations (mutation and rearrangement) and miRNA levels (up/down regulation) should be assessed and validated in a large series of tissues to propose an integrative signature with the highest sensitivity and specificity to improve the diagnosis and the management of thyroid cancer diseases.

**Abbreviations**

- **FNA:** Fine-needle aspiration
- **IHC:** Immunohistochemical
- **HBME-1:** Hector battifora mesothelial-1
- **TPO:** Thyroid peroxidase
- **CK19:** Cytokeratin 19
- **PTC:** Papillary thyroid carcinoma
- **FA:** Follicular adenoma
- **TROP-2:** Trophoblast cell surface antigen-2
- **SLP-2:** Stomatin-like protein-2
- **PCR:** Polymerase cell surface antigen
- **NGR1:** Neuregulin 1
- **miRNA:** micro RNA
- **TERT:** Telomerase reverse transcriptase
- **TP53:** protein 53
- **NGS:** Next generation sequencing
- **NPV:** Negative predictive value
- **PPV:** Positive predictive value
- **ITN:** Indeterminate thyroid nodule
- **FN1:** Fibronectin
- **SERPINA1:** Serpin peptidase inhibitor clade A member 1
- **ECM1:** Extracellular matrix protein 1
- **MMRN1:** Multimerin 1
- **PROS1:** Protein S
- **CFD:** Complement factor D
- **TIMP1:** Tissue inhibitors of metalloproteinases
12) Expression of thyroid nodule cytokeratin 19, located to papillary nodule.

18) The clinicopathological and histochemical Biomarkers -

References

1. Durante C, Costante G, Lucisano G, Bruno R, Meringolo D et al. (2015) The natural history of benign thyroid nodules. JAMA 313: 926-935. [Crossref]

2. Taye A, Garciulio D, Miles BA, Gupta A, Owen RP et al. (2018) Clinical performance of a next-generation sequencing assay (ThyroSeq v2) in the evaluation of indeterminate thyroid nodules. Surgery 163: 97-103. [Crossref]

3. Gharib H, Papini E (2007) Thyroid nodules: clinical importance, assessment, and treatment. Endocrinol Metab Clin North Am 36: 707-735. [Crossref]

4. Mataraci EA, Ozguben BY, Kabakcuoglu F (2012) Expression of cytokeratin 19, HBME-1 and galectin-3 in neoplastic and nonneoplastic thyroid lesions. Pol J Pathol 63: 58-64. [Crossref]

5. Liu Z, Li X, Shi L, Maimaiti Y, Chen T et al. (2014) Cytokeratin 19, thyroperoxidase, HBME-1 and galectin-3 in evaluation of aggressive behavior of papillary thyroid carcinoma. Int J Clin Exp Med 7: 2304-2308. [Crossref]

6. Liu Z, Yu P, Xiong Y, Zeng W, Li X et al. (2015) Significance of CK19, TPO, and HBME-1 expression for diagnosis of papillary thyroid carcinoma. Int J Clin Exp Med 8: 4369-4374. [Crossref]

7. Baloch Z, Mete O, Asa SL (2018) Immunohistochemical Biomarkers in Thyroid Pathology. Endocr Pathol 29: 91-112. [Crossref]

8. Sobrinho-Simões M, Máximo V, Rocha AS, Trovisco V, Castro P et al. (2008) Intragenic mutations in thyroid cancer. Endocrinol Metab Clin North Am 37: 333-362. [Crossref]

9. Xing M (2013) Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 13: 184-199. [Crossref]

10. Saiselet M, Pita JM, Augenlicht A, Dom G, Tarabichi M et al. (2016) miRNA expression and function in thyroid carcinomas: a comparative and critical analysis and a model for other cancers. Oncotarget 7: 52475-52492. [Crossref]

11. Ehlers M, Allelein S, Schwarz F, Hautzel H, Kuebart A et al. (2018) Increased Numbers of Circulating Tumor Cells in Thyroid Cancer Patients. Hormet Metas Rev 50: 602-608. [Crossref]

12. Qiu ZL, Wei WJ, Sun ZK, Shen CT, Song HJ et al. (2018) Circulating Tumor Cells Correlate with Clinicopathological Features and Outcomes in Differentiated Thyroid Cancer. Cell Physiol Biochem 48: 718-730. [Crossref]

13. Arcolia V, Journe F, Wattier A, Letetreue E, Renaud F et al. (2017) Galectin-1 is a diagnostic marker involved in thyroid cancer progression. Int J Oncol 51: 760-770. [Crossref]

14. Tang J, Gui C, Qiu S, Wang M (2018) The clinicopathological significance of Kif67 in papillary thyroid carcinoma: a suitable indicator? World J Surg Oncol 16: 100. [Crossref]

15. Barroeta JE, Baloch ZW, Lal P, Pasha TL, Zhang PJ et al. (2006) Diagnostic value of differential expression of CK19, Galectin-3, HBME-1, ERK, RET and p16 in benign and malignant follicular-derived lesions of the thyroid: An immunohistochemical tissue microarray analysis. Endocr Pathol 17: 225-234. [Crossref]

16. de Matos LL, Del Giglio AB, Matsubayashi CO, de Lima Farah M, Del Giglio A et al. (2012) Expression of CK-19, galectin-3 and HBME-1 in the differentiation of thyroid lesions: Systematic review and diagnostic meta-analysis. Diagn Pathol 7: 97. [Crossref]

17. Dunderović D, Lipkovski JM, Borićić I, Soldatović I, Božić V et al. (2015) Defining the value of CD56, CK19, Galectin 3 and HBME-1 in diagnosis of follicular cell derived lesions of thyroid with systematic review of literature. Diagn Pathol 10: 196. [Crossref]

18. Arcolia V, Journe F, Renaud F, Letetreue E, Gabius HJ et al. (2017) Combination of galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer. Oncol Lett 14: 4183-4189. [Crossref]

19. Abdou AG, Abdelwahed M, Said A, Taie DM, Fahmy S (2018) Evaluation of the diagnostic value of emerin and CD56 in papillary thyroid carcinoma - an immunohistochemical study. J Immunossay Immunochrom 39: 521-537. [Crossref]

20. Yang X, Hu Y, Shi H, Zhang C, Wang Z et al. (2018) The diagnostic value of TROP-2, SLPI-2 and CD56 expression in papillary thyroid carcinoma. Int J Oncol 53: 685-693. [Crossref]

21. Zhang TT, Qu N, Sun GH, Zhang L, Wang YJ et al. (2018) NRG1 regulates redox homeostasis via NRF2 in papillary thyroid cancer. Int J Oncol 53: 685-693. [Crossref]

22. Nikiforov YE (2017) Role of molecular markers in thyroid nodule management: then and now. Endocr Pract 23: 979-988. [Crossref]

23. Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knaul IA et al. (2003) BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab 88: 5399-5404. [Crossref]

24. Vinagre J, Almeida A, Pópolu H, Batista R, Lyra J et al. (2013) Frequency of TERT promoter mutations in human cancers. Nat Commun 4: 2185. [Crossref]

25. Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J et al. (2014) TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab 99: E754-E765. [Crossref]

26. Cantara S, Marzocchi C, Pilli T, Cardinale S, Forleo R et al. (2017) Molecular Signature of Indeterminate Thyroid Lesions: Current Methods to Improve Fine Needle Aspiration Cytology (FNAC) Diagnosis. Int J Mol Sci 18. [Crossref]

27. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopfer JP et al. (2009) Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 94: 2092-2098. [Crossref]

28. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO et al. (2011) 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 96: 3390-3397. [Crossref]

29. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D et al. (2012) Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 367: 705-715. [Crossref]

30. Metzker ML (2010) Sequencing technologies - the next generation. Nat Rev Genet 11: 31-46. [Crossref]

31. Meldrum C, Doyle MA, Tothill RW (2011) Next-generation sequencing for cancer diagnostics: a practical perspective. Clin Biochem Rev 32: 177-195. [Crossref]

32. Le Mercier M, D’Haene N, De Neve N, Blanchard O, Degand C et al. (2015) Next-generation sequencing improves the diagnosis of thyroid FNA specimens with indeterminate cytology. Histopathology 66: 215-224. [Crossref]
33. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U et al. (2014) Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 120: 3627-3634. [Crossref]

34. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U et al. (2015) Impact of the Multi-Gene ThyroSeq Next Generation Sequencing Assay on Cancer Diagnosis in Thyroid Nodules with Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology. Thyroid 25: 1217-1223. [Crossref]

35. Valderrabano P, Khazai L, Leon ME, Thompson ZJ, Ma Z et al. (2017) Evaluation of ThyroSeq v2 performance in thyroid nodules with indeterminate cytology. Endocr Relat cancer 24: 127-136. [Crossref]

36. Toraldo G, Godley FA, Cerda SR (2016) Large independent prospective study to evaluate the performances of ThyroSeq2 multigene next generation sequencing panel analysis on cancer diagnosis in thyroid nodules with indeterminate cytopathology (Abstract). Thyroid 2016: A148.

37. Zhang K, Liu J, Li C, Peng X, Li H et al. (2019) Identification and validation of potential target genes in papillary thyroid cancer. Eur J Pharmacol 843: 217-225. [Crossref]

38. Dom G, Frank S, Floor S, Kehagias P, Libert F et al. (2017) Thyroid follicular adenomas and carcinomas: molecular profiling provides evidence for a continuous evolution. Oncotarget 9: 10343-10359. [Crossref]

39. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE (2008) MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab 93: 1600-1608. [Crossref]

40. Kitano M, Rahbari R, Patterson EE, Xiong Y, Prasad NB et al. (2011) Expression profiling of difficult-to-diagnose thyroid histologic subtypes shows distinct expression profiles and identify candidate diagnostic microRNAs. Ann Surg Oncol 18: 3443-3452. [Crossref]

41. Liang L, Zheng X, Hu M, Cui Y, Zhong Q et al. (2018) miRNA-221/222 in thyroid cancer: A meta-analysis. Clin Chim Acta 484: 284-292. [Crossref]

42. Partyka KL, Randolph ML, Lawrence KA, Cramer H, Wu HH (2018) Utilization of direct smears of thyroid fine-needle aspirates for ancillary molecular testing: A comparison of two proprietary testing platforms. Diagn Cytopathol 46: 320-325. [Crossref]

43. Nishino M, Nikiforova M (2018) Update on Molecular Testing for Cytologically Indeterminate Thyroid Nodules. Arch Pathol Lab Med 142: 446-457. [Crossref]

44. Mazeh H, Deutch T, Karas A, Bogardus KA, Mizrahi I et al. (2018) Next-Generation Sequencing Identifies a Highly Accurate miRNA Panel That Distinguishes Well-Differentiated Thyroid Cancer from Benign Thyroid Nodules. Cancer Epidemiol Biomarkers Prev 27: 858-863. [Crossref]