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REVIEW ARTICLE

Molecular biomarkers involved in the tumor dedifferentiation process of thyroid carcinoma of epithelial origin: perspectives ☆

Cintia González Blanco [a,b,*], **Eugenia Mato Matute** [b,c], **Alberto de Leiva Hidalgo** [a,b,c]

[a] *Servicio de Endocrinología y Nutrición, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*
[b] *Centro de Investigación Biomédica en Red (CIBER-BBN) ISCIII, Ministerio de Ciencia e Innovación, Madrid, Spain*
[c] *Universidad Autónoma de Barcelona, Spain*

Abstract   Although papillary or follicular well-differentiated thyroid carcinoma usually has a good prognosis, a proportion of well-differentiated thyroid carcinomas show a more aggressive behavior with local recurrence and metastases, either at diagnosis (in less than 5% of cases) or over time. Although there are several scoring systems to assess prognosis of well-differentiated thyroid carcinoma, mainly based on clinical and pathological data, there is currently no valid criterion to define an adequate, differential treatment for patients with low risk carcinomas as compared to those with more aggressive tumors. Identification of patients with a high risk at the time of diagnosis would be essential to develop new therapeutic strategies and to improve follow-up, and molecular biomarkers could be a highly useful tool for this purpose.
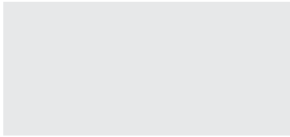
Biomarcadores moleculares implicados en el proceso de desdiferenciación tumoral del carcinoma de tiroides de origen epitelial: perspectivas

Resumen   Aunque el carcinoma diferenciado de tiroides, papilar o folicular, tiene habitual-mente un buen pronóstico, existe un porcentaje de casos que presentan un comportamiento más agresivo con recurrencias locales y metastatización, ya sea en el momento del diagnos-tico (en menos de un 5% de los casos) ya en el seguimiento. A pesar de que existen diferentes sistemas de evaluación del pronóstico del carcinoma diferenciado de tiroides, basados espe-cialmente en datos clínicos y patológicos, no hay en la actualidad un criterio válido que permita definir un tratamiento diferencial entre los pacientes con carcinomas de bajo riesgo y aquellos con carcinomas más agresivos. La identificación de los pacientes de riesgo en el momento del

diagnóstico sería clave para desarrollar nuevas estrategias terapéuticas y mejorar el seguimiento, siendo en este sentido los biomarcadores moleculares una herramienta de gran valor.

Introduction

Differentiated thyroid carcinoma is the most common endocrine neoplasm, with an increasing incidence in recent years.[1,2] More than 90% of these tumors have their origin in follicular cells, and they are classified as well differentiated carcinoma (DTC), including the papillary (PTC) and follicular (FTC) variants, and undifferentiated or anaplastic carcinoma (ATC). The latter is one of the most aggressive tumors, with a mean 5-month survival rate from the time of diagnosis and a one-year survival rate of only 20%.[3]

Most well differentiated tumors have a favorable clinical course, with survival rates close to 90% 10 years after diagnosis.[4–6] There is however a proportion of tumors with more aggressive behavior including local recurrence and metastases either at diagnosis (in less than 5% of cases) or follow-up, and it has been estimated that approximately 10–15% of patients with differentiated carcinoma will develop local or distant metastases.[7,8] Survival rates ranging from 49% to 68% have been reported 10 years after the occurrence of cervical metastases, which are responsible for one third of deaths related to the disease. Distant metastases occur in lung (50%), bone (25%), lung and bone (20%), and other sites (5%). The survival rate following the diagnosis of distant metastases decreases to 25–42%.[9]

The treatment of low-risk differentiated thyroid carcinoma has not changed substantially over the past decades and is based on surgery, ablation with ^{131}I, and suppressant treatment with levothyroxine,[10] although there is an increasing trend to base both treatment and follow-up recommendations on individualized risk assessment.[7,11] Most evaluation systems are based on clinical and pathological data, and different risk factors have been identified, including age at diagnosis, sex, size, the presence of metastasis, and the initial treatment used.[6,12,13]

The response rate to the standard treatment is high, with overall survival rates higher than 75%. However, patients with dedifferentiation processes (poorly differentiated thyroid carcinoma [PDTC]) who have iodine refractory disease (the presence of at least one lesion with no ^{131}I uptake or progression within one year of ^{131}I ablation) show a mean survival of 3–6 years after the diagnosis of distant metastases, and although growth is slow, most metastases progress. These patients are therefore candidates for other treatment modalities,[14] and one of the main objectives of clinicians is to distinguish this subgroup from DTCs of good prognosis.

It is therefore essential to know the process of dedifferentiation or anaplastic transformation of these tumors. Our understanding of the molecular biomarkers related to this process, and thus with prognosis and survival, has increased in recent years, so allowing for new therapeutic targets to be established.

Biomarkers: Definition

Biomarkers are defined as biological parameters that may be measured or detected and may in turn be correlated to a pathological process. However, for these parameters to be considered as true biomarkers, they must meet the following criteria, described by Herberman in 1977: (1) their measurement should be simple, reproducible, and easily available; (2) they should discriminate between normal and pathological states; (3) they should be highly sensitive and specific; and (4) they should be able to monitor recurrent disease processes.[15]

In recent years, the development of new technologies applied to the molecular understanding of disease has significantly contributed to the identification of new biomarkers, called molecular biomarkers. These new biomarkers have undoubtedly become highly relevant because they have made possible a deeper understanding of the etiopathogenesis of many diseases by identifying, at least partly, the molecular mechanisms involved, especially in neoplastic conditions, and have provided valuable diagnostic, prognostic, and therapeutic information. However, there are many factors, not only genetic or epigenetic, but also environmental, directly or indirectly involved in the development and metastatic progression of tumors, which often makes selection of these new biomarkers difficult. This is one of the most significant limitations in clinical practice. The different types of biomarkers in thyroid tumor disease are discussed below.

Serological markers

In epithelial thyroid tumors, thyroglobulin (TG), a glycoprotein produced by thyroid follicular cells, was one of the first tissue-specific biomarkers used. However, although this is a helpful serum marker for assessing the presence of residual or metastatic tumor in patients undergoing total thyroidectomy, many studies have reported that it is of no use in detecting populations at high risk of developing this type of tumor.[16] In addition, TG tests may not be reliable in patients on suppressing levothyroxine therapies and in patients who develop some types of benign conditions such as thyroiditis, thyrotoxicosis, thyroid adenoma, or iodine deficiency. In these latter cases, false positive results are frequently seen because of increased serum TG levels.[17]

Genomic and transcriptomic markers

It is well known that the process of carcinogenesis results from the random accumulation of genetic and epigenetic aberrations in tissue. At least 10–20% of gene expression

changes have been reported in tumor cells. These changes are directly induced by changes in cell DNA, significantly contributing to the oncogenetic process in virtually all human cancers.[18,19] Early studies focused on the identification and subsequent analysis of either one or of several genes implicated in tumor start, development, and metastases. Thus, in the specific case of the thyroid gland and because of the discrepancies found by different authors in serum TG measurements in patients, one of the first choices proposed was the detection of the TG transcript in the blood of these patients instead of protein. However, the results obtained showed a great variability, and no differences in expression could be seen between the control subjects used in the studies and those with disease.[17,20–22] The transcript of the thyroid-stimulating hormone (TSH) receptor was another marker proposed. This receptor acts as the main regulator, through TSH, of the differentiation and division process in thyroid follicular cells, but not in pathological follicular cells, which are insensitive or refractory to such stimulation. However, although different gene expression studies showed total or partial modification of gene expression in differentiated thyroid carcinomas, the results were not conclusive because neither TG nor TSH alone allowed for discrimination between the different degrees of malignancy of tumors or their progression to more aggressive tumors.[23–25]

The most relevant findings in terms of the expression of genomic and transcriptomic markers in tumors of different histological characteristics are discussed below.

Well differentiated tumors: Papillary and follicular variants of differentiated thyroid carcinoma

Early changes reported included mutations in *RAS* family oncogenes affecting both GTP in codons 12, 13 and the GTPase domain in codon 61 of the protein, with a similar prevalence in benign and malignant tumors. Some authors found these mutations in approximately 50% of FTCs analyzed, which may suggest their implication in associated early changes in the transformation process of thyroid follicular cells.[26,27]

The *RET* proto-oncogene located in chromosome 10q112 has been reported in 50% of PTCs diagnosed, and is called *RET/PTC*. Active forms of the RET proto-oncogene result from oncogenic rearrangements and fusions of the tyrosine kinase domain of the RET gene with the 5′ of the domain of different genes, leading to different variants (RET/PTC1, –2, –3, –4, and –5).[28] However, while there are studies identifying RET/PTC as a good genetic marker in PTCs, others have found the mutations in benign nodules.[29,30]

Tyrosine kinase, which may be inadequately activated in PTCs, has also been studied. However, the molecular mechanism is unknown, and no validation exists in benign tumors.[31]

Angiogenic factors identified in DTC thyrocytes include VEGF (vascular endothelial growth factor), whose expression has been correlated to the degree of tumor dedifferentiation. It has therefore been proposed as a poor prognostic marker involved in metastatic processes in PTC.[32,33] An additional growth factor implicated in tumorogenesis is EGF (epidermal growth factor), which in addition to promoting cell proliferation in the thyroid gland, acts by inhibiting specific thyroid functions such as iodine transport and organification and peroxidase and TG synthesis. That is to say, EGF promotes thyroid cell proliferation, but not differentiation. Finally, TGF-alpha (tumor growth factor-alpha) interacts with the EGF receptor (EGFR) and contributes with EGF to stimulate cell proliferation. However, the expression of both EGF and its receptor (EGFR) in the nucleus is not only detected in FTC, but also in Graves' disease and adenomas.[34]

However, it was not until the advent of mass sequencing of human genoma and the subsequent technological advances, such as array and/or microarray techniques, that new molecular targets involved in key cell regulation processes (signaling pathways, transcription factors, changes in adhesion molecules as markers of tumor aggressiveness) and specific molecular profiles (also called genetic signatures) associated with a variety of clinical patterns could be identified. In many cases, advances made in the search for these specific genetic profiles have been crucial for improving our ability to ascertain differences in the biological behavior of closely related conditions which have no differential histological patterns but show major differences in their clinical course. In the thyroid gland, comparative tests performed in follicular adenomas or benign tumors and carcinomas have allowed for the identification of a number of differential genetic changes such as different galectin-3 gene expression, differences in telomerase activity, and detection of PAX8/PPARγ gene translocation, among others. However, none of these markers have proved sufficiently reliable to allow for the safe differentiation of benign and malignant tumors in cytological samples.[35,36]

These new diagnostic approaches were simultaneously used to study PTC.[37–40] Thus, Huang et al. reported in 2001 one of the first studies using array techniques in this type of tumor. The authors were able to identify 220 differentially expressed genes. However, some of these could not subsequently be validated in a new PTC cohort, which is a critical point in the study.[41]

Subsequent studies by Giordano et al. in 2005 identified a genetic signature associated with mutations in *BRAF*, *RAS*, and *RET/PTC* genes in PTC. Testing for this signature allowed for the differentiation of tumors of epithelial origin which showed different histological patterns (classical papillary pattern and its different variants). This was thus the first study that identified gene expression patterns and their mutational correlation. This allows not only for the prediction of the potential success of the treatment used, but also for contemplating new cellular targets that allow for designing new treatment strategies.[42] Thus, Capella et al.[43] detected *RAS* gene mutations in different types of thyroid lesions, including ATC, thus confirming their role in tumorogenesis, although the relationship with prognosis could not be determined. Subsequent detection of hypermethylation of the *PTEN* promoter, especially in follicular tumors, suggested its potential role during tumor development or course.[44]

An increasing number of studies aimed at the identification of new clinically useful markers and genetic signatures were subsequently conducted on larger series. Recent studies including significant numbers of patients

Table 1 Genetic signatures identified by Montero et al. which are being analyzed in a new patient cohort.

Alias	Name of gene	Biological function
ANLN	*Actin binding protein (ANLN)*	Necessary for cytokinesis
APLP2	*Amyloid beta (A4) precursor-like protein 2*	Regulation of hemostasis
ASPM	*Abnormal spindle-like microcephaly-associated protein*	Mitotic spindle regulation, mitotic process coordination
BIRC5	*Survivin*	Member of the family of apoptosis inhibitory (API) genes
CCNB2	*G2/mitotic-specific cyclin-B2*	Cell cycle control in the G2/M transition
CDH1	*E-cadherin*	Cell-to-cell regulation of epithelial cell adhesion, motility, and proliferation
CENPA	*Centromere protein A*	Mitotic progression and chromosome segregation
CEP55	*Centrosomal protein 55 kDa*	Mitotic death and cytokinesis
DIAPH3	*Diaphanous homolog 3 (Drosophila)*	Promotes actin polymerization, cytokinesis, stress fiber formation
IL1RN	*Interleukin 1 receptor antagonist*	Related to immune and inflammatory responses
NUSAP1	*Nucleolar and spindle associated protein 1*	Promotes the organization of microtubules in mitotic spindle
ORMDL3	*ORM1-like 3 (S. cerevisiae)*	Regulation of Ca-mediated signaling in endoplasmic reticulum
PDK2	*Pyruvate dehydrogenase kinase, isozyme 2*	Regulation of glucose metabolism
PPAP2B	*Phosphatidic acid phosphatase type 2B*	May be implicated in cell adhesion and cell interaction
PRC1	*Protein regulator of cytokinesis 1*	Cytokinesis
PTPRN2	*Protein tyrosine phosphatase, receptor type, N polypeptide 2*	Development of the nervous system and endocrine pancreatic cells
RRM2	*Ribonucleotide reductase M2*	Precursor needed for DNA synthesis. Inhibits the Wnt signal
SDK2	*Sidekick homolog 2 (chicken)*	Cell adhesion protein
SH3BGRL2	*SH3 domain binding glutamic acid-rich protein like 2*	Apoptosis, tumor suppression, cell cycle, TNF-α signaling
SIAH1	*Seven in absentia homolog 1 (Drosophila)*	Proteosomal degradation of target proteins
TWIST1	*Twist homolog 1 (Drosophila)*	Transcriptional regulator
UBE2 C	*Ubiquitin-conjugating enzyme E2 C*	Cell cycle regulation by ubiquitin ligase progression through mitosis

Source: Montero-Conde et al.[56]

(n = 1168) confirmed that *BRAF*[V600E] gene mutations were relevant in PTC and were associated with greater increases in recurrence, mortality, and loss of the capacity to take up [131]I. They could therefore be used as a biomarker for these tumors. However, they had little prognostic power in sporadic tumors that lacked the mutation. Thus, their value as a diagnostic and prognostic test was not conclusive.[45,46] A recent study examined the relationship of the presence of the *BRAF*[T1799A] mutation to PTC recurrence or persistence, and was unable to conclude that its presence confers a poorer prognosis.[47]

A meta-analysis by Griffith et al. of 21 published studies using the same Affimetrix technology found that among the 1785 differentially expressed genes, 39 were the most significant, including 12 candidate genes, of which six had previously been reported (*MET, TFF, SERPINA1, TIMP1, FN1,* and *TPO*) and six were candidate genes (*TGFA, QPCT, CRABP1, FCGBP, EPS8,* and *PROS1*) identified for the first time in the thyroid gland, which could represent, together with the previous ones, a valid diagnostic tool in a clinical setting.[48]

Dedifferentiated and anaplastic tumors (undifferentiated or anaplastic carcinoma and poorly differentiated carcinoma)

Undifferentiated or anaplastic carcinoma
Few gene expression studies have been conducted in more aggressive tumors, the etiology of which continues to be highly controversial. No key specific agent for the development of such tumors has been identified yet (a role for TSH and/or external irradiation in the promotion of the development of these tumors has been suggested).[49]

A correlation exists between the frequency of a loss of heterozygosity and a prognosis of thyroid carcinoma. A loss of heterozygosity occurs more frequently in ATC as compared to PTC and FTC, where it is found in 19p in up to 36% of cases.[26] Localized loss in the short arm of chromosome 16 has also frequently been noted. This would suggest the probable lack of a suppressor gene at that location. This observation is supported by the fact that ATC cell lines have been seen to frequently have losses in 16p as compared to cell lines of differentiated carcinoma, which suggests the

presence at this location of some gene that may be associated with the transformation from well differentiated to undifferentiated carcinoma.[50]

On the other hand, microarray studies in these tumors have identified groups of genes related to different signaling pathways, including transcription factors, mitosis, cell proliferation and differentiation, apoptosis, cell adhesion molecules, cytoskeleton, etc.,[45,46,51–53] but have found no consistent genetic signature. As in differentiated tumors, mutations have been identified in *RAS* and *BRAF* genes, and this has led to the hypothesis that they could be the first steps in the dedifferentiation process and that they could allow in turn for the acquisition of new mutations (late mutations), including those identified in *TP53*, catenin (cadherin-associated protein), beta 1, and *PIK3CA*. It has been suggested that all these cumulative mutations in these types of tumors contribute to their extremely aggressive behavior. By contrast, rearrangements in the *RET/PTC* gene identified in childhood and in post-radiation PTC or fusion protein PAX8/PPARγ detected in FTC are not identified in poorly differentiated or anaplastic tumors.[54] From this postmalignant transformation associated with genetic mutations arises the interesting but controversial concept of ''anaplastic transformation''.[55]

Poorly differentiated carcinoma

PDTCs, recognized in 2004 by the World Health Organization as a separate pathological condition, have a differential molecular expression as compared to all other tumors described. Thus, our group[56] studied in 2008 these types of tumors (n = 6) and compared their differential expression from ATCs (n = 6) and a group of well differentiated tumors (n = 31). This study identified 1031 differential genes implicated in different cell-signaling pathways: MAPKinases, genes involved in cell cycle regulation, cell adhesion, cytoskeleton, and TGFβ pathways. Of all these genes, 23 represented a genetic signature conferring a poor prognosis (Table 1) which is currently being validated in a new patient cohort. To date, 22 of the 23 genes identified have been analyzed using low density array technology in a total of 19 well differentiated tumors at the time of diagnosis, of which 7 were FTCs and 12 PTCs. The data analyzed identified differential gene groups between the two types of tumors. Specifically, *PTPRN2*, *TWIST1*, *ANLN*, *PRC1*, and *RRM2* were found in PTCs, and *APLP2*, *PPAP2B*, *SIAH1*, and CEP55 were found in FTCs.[48] The next step will be a prospective analysis of the different expression of these gene groups in tumors which experience a dedifferentiation process during their course and those which do not.[57]

Conclusion

Thyroid carcinoma is the most common endocrine tumor, accounting for 2% of all human cancers, and its incidence has constantly increased over the past three decades (3% annually), especially at the expense of papillary microcarcinoma.[1] Ninety percent of thyroid carcinomas arise in follicular cells, and most of them are considered well differentiated and have an excellent prognosis. DTC usually has an indolent course, with a mean 10-year survival close to 90%. By contrast, ATC cause 50% of deaths related to thyroid cancer, with a mean survival of 6 months,[3,58] and some patients with DTC have an unfavorable prognosis mainly related to dedifferentiation processes. An understanding of the different processes involved in carcinogenesis and in dedifferentiation processes would allow for a better understanding of the disease and of the potential uses of targeted therapies. In addition, the possibility of recognizing the genetic signatures associated with a poor prognosis among all the biomarkers identified would represent a tool of great diagnostic and prognostic value that would help us to better stratify the risks. This would in turn be very helpful for the application of adequate treatment strategies and for developing new pharmacological alternatives. In this regard, the availability of an animal model of undifferentiated/anaplastic carcinoma would be of great value for in vivo research of the biological mechanism of these tumors. Such a model would also allow for the testing of new treatment alternatives, such as the application of nanoparticles targeted to the tumor cell able to release drugs and gene therapies using cells carrying suicide genes. This work is currently being performed by our group in cooperation with other institutions within the CIBER-BBN project (*Cell-Nano-Thyroid* [website caber-bbn.es]).

Conflicts of interest

The authors state that they have no conflicts of interest.

References

1. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. JAMA. 2006;295:2164–7.
2. Riego-Iraeta A, Pérez-Méndez LF, Mantinan B, García-Mayor RV. Time trends for thyroid cancer in northwestern Spain: true rise in the incidence of micro and lager forms of papillary thyroid carcinoma. Thyroid. 2009;19:333–40.
3. Smallridge RC, Copland JA. Anaplastic thyroid carcinoma: pathogenesis and emerging therapies. Clin Oncol (R Coll Radio). 2010;22:486–97.
4. Randolph GW, Daniela GH. Radioactive iodine lobe ablation as an alternative to completion thyroidectomy for follicular carcinoma of the thyroid. Thyroid. 2002;12:989–96.
5. Toniato A, Boschin I, Casara D, Mazzarotto R, Rubello D, Pelizzo M. Papillary thyroid carcinoma: factors influencing recurrence and survival. Ann Surg Oncol. 2008;15:1518–22.
6. Ito Y, Miyauchi A. Prognostic factors and therapeutics strategies for differentiated carcinomas of the thyroid. Endocrine J. 2009;56:177–92.
7. Cooper DS, Doherty GM, Haugen 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;19:1167–214.
8. Pacini F, Schlumberger M, Dralled H, Elisei R, Smit JWA, Wiersinga W, The European Thyroid cancer Taskforce 2006. European consensus fort he management of patients with differentiated Thyroid cancer of the follicular epithelium. Eur J Endocrinol. 2006;154:787–803.
9. Schlumberger M, Sherman SI. Clinical trials for progressive differentiated thyroid cancer: patient selection, study design, and recent advances. Thyroid. 2009;19:1393–400.
10. Vini L, Harmer C. Management of thyroid cancer. Lancet Oncol. 2002;3:407–14.
11. Mazzaferri EL. Management of low-risk differentiated thyroid cancer. Endocr Pract. 2007;13:498–512.

12. Pelizzo M, Boschin IM, Toniato A, Piotto A, Pagetta C, Gross MD, et al. Papillary thyroid carcinoma: 35 ear outcome and prognostic factors in 1858 patients. Clin Nucl Med. 2007;32:440–4.

13. Lang BH, Lo CY, Chan WF, Lam KY, Wan KY. Staging systems for papillary thyroid carcinoma. A review and comparison. Ann Surg. 2007;245:366–78.

14. Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, et al. Long term outcome of 444 patients with distant metastases from papillary and follicullary thyroid carcinoma: benefits and limits of radioiodine therapy. J Clin Endocrinol Metab. 2006;91:2892–9.

15. Herberman RB. Immunologic tests in diagnosis of cancer. Am J Clin Pathol. 1977;68 Suppl. 5:688–98.

16. Van Herle AJ, Uller RP, Matthews NI, Brown J. Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest. 1973;52:1320–7.

17. Refetoff S, Lever EG. The value of serum thyroglobulin measurement in clinical practice. JAMA. 1983;250:2352–7.

18. Cahill DP, Kinzler KW, Vogelstein B. Lengauer C genetic inestability and Darwian selection in tumours. Trends Cell Bio. 1999;9:M57–60.

19. Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, et al. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acd Sci USA. 2002;99:12963–8.

20. Gupta M, Chia SY. Circulating thyroid cancer markers. Curr Opin Endocrinol Diabetes Obes. 2007;14:383–8.

21. Haugen BR, Pacini F, Reiners C, Schlumberger M, Ladenson PW, Sherman SI, et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab. 1999;84:3877–85.

22. Chinnappa P, Taguba L, Arciaga R, Faiman C, Siperstein A, Mehta AE, et al. Detection of thyrotropin-receptor messenger ribonucleic acid (mRNA) and thyroglobulin mRNA transcripts in peripheral blood of patients with thyroid disease: sensitive and specific markers for thyroid cancer. J Clin Endocrinol Metab. 2004;89:3705–9.

23. Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, et al. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab. 2007;92:2840–3.

24. Potter E, Horn R, Scheumann GF, Dralle H, Costagliola S, Ludgate M, et al. Western blot analysis of thyrotropin receptor expression in human thyroid tumours and correlation with TSH-binding. Biochem Biophys Res Commun. 1994;205: 361–7.

25. Bruno R, Ferretti E, Tosi E, Arturi F, Giannasio P, Mattei T, et al. Modulation of thyroid-specific gene expression in normal and nodular human thyroid tissues from adults: an in vivo effect of thyrotropin. J Clin Endocrinol Metab. 2005;90:5692–7.

26. Russo D, Arturi F, Pontecorvi A, Filetti S. Genetic analysis in fine-needle aspiration of the thyroid: a new tool for the clinic. Trends Endocrinol Metab. 1999;10:280–5.

27. Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. Clin Endocrinol (Oxf). 1999;50:529–35.

28. Suarez HG. Genetic alterations in human epithelial thyroid tumours. Clin Endocrinol (Oxf). 1998;48:531–46.

29. Elisei R, Romei C, Vorontsova T, Cosci B, Veremeychik V, Kuchinskaya E, et al. RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. J Clin Endocrinol Metab. 2001;86:3211–6.

30. Santoro M, Papotti M, Chiappetta G, Garcia-Rostan G, Volante M, Johnson C, et al. RET activation and clinicopathologic features in poorly differentiated thyroid tumors. J Clin Endocrinol Metab. 2002;87:370–9.

31. Jhiang SM, Sagartz JE, Tong Q, Parker-Thornburg J, Capen CC, Cho JY, et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology. 1996;137:375–8.

32. Viglietto G, Maglione D, Rambaldi M, Cerutti J, Romano A, Trapasso F, et al. Upregulation of vascular endothelial growth factor (VEGF) and downregulation of placenta growth factor (PlGF) associated with malignancy in human thyroid tumors and cell lines. Oncogene. 1995;11:1569–79.

33. Soh EY, Duh QY, Sobhi SA, Young DM, Epstein HD, Wong MG, et al. Vascular endothelial growth factor expression is higher in differentiated thyroid cancer than in normal or benign thyroid. J Clin Endocrinol Metab. 1997;82:3741–7.

34. Marti U, Ruchti C, Kämpf J, Thomas GA, Williams ED, Peter HJ, et al. Nuclear localization of epidermal growth factor and epidermal growth factor receptors in human thyroid tissues. Thyroid. 2001;11:137–45.

35. Barden CB, Shister KW, Zhu B, Guiter G, Greenblatt DY, Zeiger MA, et al. Classification of follicular thyroid tumors by molecular signature: results of gene profiling. Clin Cancer Res. 2003;9:1792–800.

36. Mora J, Lerma E, Thyroid Neoplasia Study Group. Telomerase activity in thyroid fine needle aspirates. Acta Cytol. 2004;48:818–24.

37. Riesco-Eizaguirre G, Santisteban P. New insights in thyroid follicular cell biology and its impact in thyroid cancer therapy. Endocr Relat Cancer. 2007;14:957–77.

38. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1955. Cancer. 1998;83: 2638–48.

39. LiVolsi VA. Well differentiated thyroid carcinoma. Clin Oncol (R Coll Radiol). 1996;8:281–8.

40. Giuffrida D, Gharib H. Anaplastic thyroid carcinoma: current diagnosis and treatment. Ann Oncol. 2000;11: 1083–9.

41. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, et al. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci USA. 2001;98:15044–9.

42. Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, et al. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene. 2005;24:6646–56.

43. Capella G, Matias-Guiu X, Ampudia X, de Leiva A, Perucho M, Prat J. Oncogene Mutations in thyroid tumors. Diagn Mol Pathol. 1996;5:45–52.

44. Alvarez-Nuñez F, Bussaglia E, Mauricio D, Ybarra J, Vilar M, Lerma E, the Thyroid Neoplasia Study Group. PTEN promoter methylation in sporadical thyroid carcinomas. Thyroid. 2006;16:17–23.

45. Chevillard S, Ugolin N, Vielh P, Ory K, Levalois C, Elliott D, et al. Gene expression profiling of differentiated thyroid neoplasms: diagnostic and clinical implications. Clin Cancer Res. 2004;10:6586–97.

46. Aldred MA, Huang Y, Liyanarachchi S, Pellegata NS, Gimm O, Jhiang S, et al. Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. J Clin Oncol. 2004;22:3531–9.

47. Cañadas Garre M, López de la Torre Casares M, Becerra Massare P, López Nevot MÁ, Villar Del Moral J, Muñoz Pérez N, et al. BRAF(T1799A) mutation in the primary tumor as a marker of risk, recurrence, or persistence of papillary thyroid carcinoma. Endocrinol Nutr. 2011;58:175–84.

48. Griffith OL, Melck A, Jones SJ, Wiseman SM. Meta-analysis and meta-review of thyroid cancer gene expression profiling

studies identifies important diagnostic biomarkers. J Clin Oncol. 2006;24:5043–51.

49. Aldinger KA, Samaan NA, Ibanez M, Hill Jr CS. Anaplastic carcinoma of the thyroid: a review of 84 cases of spindle and giant cell carcinoma of the thyroid. Cancer. 1978;41: 2267–75.

50. Komoike Y, Tamaki Y, Sakita I, Tomita N, Ohue M, Sekimoto, et al. Comparative genomic hybridization defines frequent loss on 16p in human anaplastic thyroid carcinoma. Int J Oncol. 1999;14:1157–62.

51. Onda M, Emi M, Yoshida A, Miyamoto S, Akaishi J, Asaka S, et al. Comprenhensive gene expression profiling of anaplastic thyroid cancer with cDNA microarray of 25344 genes. Endocr Relat Cancer. 2004;11:843–54.

52. Salvatore G, Nappi TC, Salerno P, Jiang Y, Garbi C, Ugolini C, et al. A cell proliferation and chromosomal inestability signature in anaplastic thyroid carcinoma. Cancer Res. 2007;67:10148–58.

53. Pallante P, Federico A, Berlingieri MT, Bianco M, Ferraro A, Forzati F, et al. Loss of the CBX7 gene expression correlates with a highly malignant phenotype in thyroid cancer. Cancer Res. 2008;68:6770–8.

54. Nikiforv Y. Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. Endocr Pathol. 2004;15:319–27.

55. Wiseman SM, Loree TR, Rigual NR, Hicks WL, Douglas WG, Anderson GR, et al. Anaplastic transformation of thyroid cancer: review of clinical pathologic and molecular evidence provides new insights into disease biology and future therapy. Head Neck. 2003;25:662–70.

56. Montero-Conde C, Martín-Campos JM, Lerma E, Giménez G, Martínez-Guitarte JL, Combalía N, et al. Molecular profiling related to poor prognosis in thyroid carcinoma. Combining gene expression data and biological information. Oncogene. 2008;27:1554–61.

57. González C, Mato E, Bell O, Lerma E, Moral A, Perez JI, et al. Predictive value of molecular markers that involved in a genetic signature in thyroid tissue and their involvement in the tumour dedifferentation process. P 151 ETA; 2011.

58. Kosary CL. Cancer of the thyroid. In: Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner MJ, editors. SEER survival monograph: cancer survival among adults. U.S. Seer program, 1988–2001, patient and tumor characteristics. Bethesda, MD: National Cancer Institute, SEER, Program, NIH; 2007.