Gene expression profile of human thyroid cancer in relation to its mutational status

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Abstract

This review describes the gene expression profile changes associated with the presence of different mutations that contribute to thyroid cell carcinogenesis. The results are discussed in the context of thyroid cancer biology and of the implications for disease prognosis, while the diagnostic aspect has been omitted. For papillary thyroid cancer (PTC), the most characteristic gene expression profile is associated with the presence of BRAF mutation. BRAF-associated PTC differ profoundly from RET/PTC or RAS-associated cancers. Simultaneously, they retain many characteristic gene expression features common for all PTCs, induced by the alternative mutations activating MAPK pathway. Although the difference between papillary and follicular thyroid cancer (FTC) is significant at the gene expression profile level, surprisingly, the RAS-related signature of FTC is not well specified. PAX8/peroxisome proliferator-activated receptor γ (PPARγ) rearrangements, which occur in FTC as an alternative to the RAS mutation, are associated with specific changes in gene expression. Furthermore, the difference between well-differentiated thyroid cancers and poorly differentiated and anaplastic thyroid cancers is mainly a reflection of tumor degree of differentiation and may not be attributed to the presence of characteristic mutations.

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Introduction

Gene expression profiling of thyroid tumors has expanded our knowledge of the molecular biology of thyroid cancer. Several recent reviews addressed the biological and diagnostic aspects of microarray-based studies of thyroid cancer (Eszlinger et al. 2007, Handkiewicz-Junak et al. 2010, Kouniavsky & Zeiger 2010). However, this field remains open and new reports continue to appear, thus, updating its present status is necessary. The aim of this review is to describe the gene expression profile changes associated with different mutations that contribute to thyroid carcinogenesis (see Kondo et al. (2006) for an overview of genetic alterations involved in the development of thyroid cancer). The results will be discussed in the context of thyroid cancer biology and of the implications for disease prognosis, while the diagnostic aspect will be omitted. Only follicular cell-derived neoplasms will be considered and emphasis will be placed on papillary thyroid cancer (PTC), the most frequent histotype, since it has been the subject of the majority of microarray-based studies published to date.

Transcriptome of papillary thyroid cancer

Papillary thyroid cancer develops directly from the thyroid cells and does not have a benign intermediate. The majority of PTCs exhibit the BRAF V600E activating point mutation, which is observed in 40–70% of cases, and is more frequently found in older patients with more aggressive disease (see Handkiewicz-Junak et al. (2010) for review). Chromosomal rearrangements involving receptor tyrosine kinase genes such as RET/PTC or TRK rearrangements, or rarely, activating RAS point mutations, are other molecular events involved in the activation of the MAPK–ERK cascade (mitogen activated protein kinases cascade) and in the development of PTC (Kimura et al. 2003, Fagin 2005).

The BRAF V600E mutation and the gene expression profile of papillary thyroid cancer

BRAF mutation is associated with the morphological and functional alterations characteristic of the PTC phenotype. The detection of BRAF mutation is a specific but not sensitive feature of papillary thyroid
cancer, since this mutation has not been observed in benign thyroid lesions (Eszlinger & Paschke 2010, Soares & Sobrinho-Simoes 2011). The most distinct phenotypic feature of BRAF-positive tumors is the presence of papillary structures that determine the diagnosis of the classical variant of PTCs (Nakamura et al. 2005, Xing et al. 2005, Frasca et al. 2008). Notwithstanding, this may be a secondary phenomenon. Jakubowski & Hunt (2009) compared the frequency of BRAF mutation in PTC showing a mixed papillary and follicular growth pattern and observed no difference, the BRAF mutation status was always concordant, in both areas showing papillary and follicular growth type, at the overall rather high BRAF mutation frequency.

Functionally, the presence of the BRAF oncoprotein results in the inhibition of membrane sodium–iodide symporter (NIS) expression. This is the most often evoked explanation for the clinically well-known tendency of BRAF-positive PTCs to be non-radioiodine avid, to have a more aggressive clinical course, and to have a poorer prognosis (Xing et al. 2005, Handkiewicz-Junak et al. 2010). However, a deeper insight into the molecular profile of PTC and the presence of different mutations reveals a more complex network of molecular interactions that should be further elucidated both on the basis of gene expression research and on the basis of prospective clinical studies. This controversy cannot be clarified without integrating PTC variants with tumor invasiveness (Soares & Sobrinho-Simoes 2011). In addition, without neglecting the role of the BRAF mutation in PTCs with poor prognosis, there are many other genetic alterations known to be associated with the progression of papillary thyroid cancer to more aggressive phenotypes, including mutations of TP53 (Fagin et al. 1993, La Perle et al. 2000), under-expression of E-cadherin, or dysregulation of catenin signaling (Karim et al. 2004).

The first study devoted to the comparison between PTC with BRAF mutations or RET and TRK rearrangements was published by Frattini et al. (2004), who stressed similarities in the gene expression profiles of PTC with various mutations. These authors were the first to detect a difference in the gene expression between BRAF-positive PTCs and those with receptor tyrosine kinase-related rearrangements (Frattini et al. 2004). Giordano et al. (2005) showed that the gene expression profile of the BRAF-associated PTC differs profoundly from the RET/PTC or RAS-associated PTC. Using a significance criterion of \( P < 0.01 \), these authors found over 3800 genes differentially expressed in PTC with BRAF compared to those with RET alterations. They also indicated that tumor mutational status was more strongly correlated with gene expression profile than its morphology. In opposition to Frattini et al. and Giordano et al., hypothesized that these mutations signaled through alternative pathways and might be related to tumors with discrete mutation-specific phenotypic and biological features. One of the groups of genes most distinctly over-expressed in BRAF-mutated PTCs was related to the regulation of the immune response (TM7SF4, CLECFS2, STAT1, and LY75). The proposed unique role of the BRAF mutation in initiating a specific immune response in PTC was supported by further SAGE data (Oler et al. 2008).

Comparing the BRAF status in a set of metastatic and non-metastatic PTCs with their gene expression profiles, Oler et al. found that cystatin 6 (CST6) and the chemokine gene CXCL14 were over-expressed and positively correlated with the BRAF V600E mutation, which was in agreement with the data of Giordano et al. Both CST6 and CXCL14 are putative downstream genes in the BRAF/MEK/ERK signaling pathway, CXCL14 being involved in the homeostasis of macrophages. Furthermore, the in vitro studies of Mesa et al. (2006), who focused on the early molecular consequences of different mutations leading to PTC, showed that BRAF-induced genes included many involved in the immune response, mostly in the innate response, such as the chemokine (C–C motif) ligands 2, 7, 15, and granulocyte–macrophage colony-stimulating factor 2 (MCP1, MCP3, CCL15, and GM-CSF respectively). In this study, a preferential induction of MMP3 and MMP9 and the exclusive activation of MMP13 were observed in PCCL3 cells with the BRAF mutation compared to a cell line with RET/PTC rearrangement. The authors related these differences to ERK activation, which was much higher because of the BRAF mutation. The association of MMPs (matrix metalloproteinases), which are able to degrade all components of the extracellular matrix, with the invasive phenotype and poor prognosis is well known in PTC (Klopfleisch et al. 2011, Yang et al. 2010, Horndler et al. 2011). The activation of BRAF in PCCL3 cells downregulated the mitochondrial respiratory chain (MRC) complex 1 genes. There are contradictory data in the literature concerning the influence of the inhibited MRC complex 1 genes on cell survival, but the most predominant studies indicate that it protects the cells from apoptosis (Mesa et al. 2006).

Although Giordano et al. (2005) identified many BRAF-related genes showing differential expression between RET/PTC-positive or RAS-positive PTCs, these authors did not analyze other factors that could differ in the population of BRAF-positive and BRAF-negative tumors that could cause gene profile differences (e.g. age, disease stage, or other still unknown mutations that BRAF may co-segregate with). In addition, an independent validation of the BRAF-related gene list was not performed. The multifactorial nature of gene expression changes in malignant tumors, which is well known from the studies of other cancers (Rhodes et al. 2004), underscores the need for multivariate analysis of the association between gene expression profile and different mutations. In univariate
approaches, the risk of incorrectly attributing an observed change in gene expression to the presence of the BRAF mutation is high. Our own analysis of the BRAF-related gene expression profile, which was performed on 49 PTC patients, confirmed the differences between BRAF-positive and both RET/PTC-positive and all no-BRAF cancers; the meta-analysis of our and Giordano’s study is presented in Fig. 1. Surprisingly, a comparison of the genes selected on the basis of our study and those listed by Giordano et al. showed only 11 common genes in the BRAF versus RET comparison (D Rusinek, M Oczko-Wojciechowska, M Kowalska, M Świernak, M Jarząb, A Czarniecka, J Wloch, S Szpak-Ulczok, E Chmielik, D Lange, M Kowal, M Wiench, D Handkiewicz-Junak & B Jarząb 2007, unpublished observations). This lack of overlap clearly indicates that large numbers of cases are needed for multivariate analysis.

Currently, the only multivariate analysis carried out considering the influence of the BRAF V600E mutation on PTC gene expression was performed by Jo et al. (2006). Upregulation of the vascular endothelial growth factor (VEGF) gene was observed in BRAF-positive PTCs even after adjustment for extrathyroidal PTC invasion, tumor stage, and nodal and distant metastasis ($P=0.035$). The authors concluded that the over-expression of VEGF in association with the BRAF mutation might account for the high rate of recurrences and aggressive phenotype of PTC as well as its poor outcome.

Platelet-derived growth factor (PDGF; Wang et al. 2008) is among the highly upregulated genes in papillary thyroid carcinomas induced by the BRAF V600E mutation, and its over-expression in thyroid neoplasms (Yano et al. 2004) and its presence in other tumors (Terrile et al. 2010) have been reported earlier. The receptor for PDGF – PDGFRB – is able to activate the ERK1/2 signaling through cross talk with other PDGFRB-induced pathways (Jurek et al. 2011). Moreover, Wang et al. (2008) observed a correlation between the presence of the BRAF V600E mutation and high platelet count, which is similar to the BRAF mutation itself, and was significantly associated with extrathyroidal invasion.

Many other genes were also associated with the presence of mutated BRAF. Franzoni et al. (2009) showed that prohibitin (PHB) was over-expressed only in PTCs harboring the BRAF mutation. Transfection of a BRAFV600E construct resulted in an increase in PHB promoter activity and higher expression of the protein product. PHB may act as tumor suppressor or exhibit a permissive influence on tumor growth.

In their search for BRAF-related transcriptome changes, Nucera et al. (2010) attempted to identify a PTC progression-related gene signature. Using a gene set enrichment analysis, they selected 18 gene sets significantly associated with the presence of

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**Figure 1** Comparison of gene expression profiles of BRAF(+) PTCs to RET(+) PTCs (analysis performed by combining our data and those of Giordano et al.).
BRAF mutation and identified one set of downregulated genes involved in cell polarity, indicating the importance of BRAF/V600E in cell dedifferentiation. Comparison of BRAF-positive PTCs with controls led to the identification of many extracellular matrix genes related to the presence of BRAF. In their study, Nucera et al. focused on thrombospondin 1 (TSP1), a multifunctional matricellular protein that binds to many integrins and other matrix proteins, one among them being fibronectin 1, which is abundant in tumor stroma. Knockdown of the BRAF V600E gene led to a decrease in the TSP1 expression that was associated with a reduction in the migration/invasion phenotype of tumor cells both in vitro and in vivo.

The mechanism of cancer invasion in PTC is not well understood. Riesco-Eizaguirre et al. (2009) concluded that PTC invasion, which is more distinct in BRAF-positive PTCs, is TGFβ/Smad dependent. Gene expression analysis (Vasko et al. 2007) of microdissected intratumoral samples from central and invasive regions of invasive PTCs and normal thyroid tissue showed an upregulation of TGFβ, NFKB, integrin pathway members, and CDC42 in the invasive regions. Interestingly, the downregulation of a subset of mRNAs encoding proteins related to the epithelial to mesenchymal transformation (EMT) and involved in cell–cell adhesion and communication was observed in invasive PTCs. Vasko et al. proposed an association between CDC42/PAK signaling and the mechanisms of PTC invasion. In opposition to their results, Watanabe et al. (2009) performed a QPCR analysis to show a positive correlation between the BRAF V600E mutation and over-expression of vimentin, which was confirmed using NPA cells (inhibition of BRAF expression decreased vimentin levels). These authors also showed the BRAF V600E-dependent upregulation of fibronectin and CITED1, both of which have been identified as PTC markers. The role of fibronectin 1 as a biomarker was first shown by QPCR and immunohistochemistry (Takano et al. 1998) and later by microarray analysis (Huang et al. 2001, Jarzab et al. 2005), while the role of CITED1 as a biomarker was first shown by microarray analysis (Huang et al. 2001) followed by immunohistochemistry (Prasad et al. 2004). Fibronectin 1, an adhesion-related gene, is considered a part of the EMT signature (Huang et al. 2001). CBP/p300-interacting transactivators with glutamic acid (E)'/aspartic acid (D)-rich C-terminal domain (CITED1) is a transcriptional coactivator initially identified in melanoma and known to interact with SMAD4C, CPB/p300, and the estrogen receptor (de Caestecker et al. 2000, Yahata et al. 2000).

In this context, it is noteworthy that mutations of the BRAF gene, the V600E mutation being the most frequent, are not the only characteristics of PTC. They are found in about 60% of melanomas (also in benign nevi), in a subset of lung, ovarian, and colon cancers and in childhood astrocytomas (Thomas 2006, Estep et al. 2007, Pratilas et al. 2008, Yousem et al. 2008, Farina-Sarasqueta et al. 2010, Schiffman et al. 2010). Interestingly, in nearly all these tumor localizations, the presence of the BRAF mutation is related to a rather better prognosis. Comparative studies of the BRAF gene expression signature in various tumor types may help to elucidate the relationship between the transcriptional consequences of its presence and the invasive tumor potential.

**The miRNA expression profile of papillary thyroid cancer and its relation to BRAF mutations**

Differences in gene expression profile related to the mutational status of PTC may be visible not only at the mRNA level but also at the level of the non-coding RNAs. In this context, Yoon et al. (2007) identified a novel non-coding RNA gene called NAMA, which is associated with the MAP kinase pathway and growth arrest, physiologically expressed in the testis and under-expressed in the BRAF-positive PTC.

Several authors have examined the differences in the levels of miRNAs between BRAF-positive and BRAF-negative PTCs and have shown contradicting results. Cahill et al. (2007) reported a unique miRNA expression signature in the cell lines with BRAF mutation in comparison to the normal thyroid cell line, showing 15 upregulated miRNAs and 23 down-regulated miRNAs. Sheu et al. (2009), however, did not detect any significant differences between BRAF-positive and BRAF-negative human PTCs, although their study was limited to a subset of five miRNAs. Chou et al. (2010) compared the levels of three miRNAs known to be over-expressed in PTC. A significantly higher expression of miR-146b was detected in BRAF-positive PTC, while miR-221 and miR-222, also shown to be related to a more aggressive tumor behavior, did not exhibit any difference in comparison to BRAF-negative tumors. Aherne et al. (2008) analyzed different fragments of one multifocal thyroid cancer and described miRNA profiles distinguishing tumors containing the BRAF mutation from other tumor types (with insular or anaplastic phenotype).

**RET/PTC rearrangements and their relation to gene expression in PTC**

RET/PTC rearrangements arise from the fusion of the 3' end of the RET gene that encodes the tyrosine kinase domain with the 5' domain of one of the several constitutively expressed genes. To date, 12 fusion genes and 15 different RET/PTC rearrangements have been described (Mishra et al. 2009), and the RET/PTC1 and RET/PTC3 are the most frequent rearrangements.
**RET/PTC1** and **RET/PTC3** are paracentric inversions because both **RET** and its respective fusion partner **H4** or **NCOA4** (also called **ELE1**, **RFG** and **ARA70**) are localized on the long arm of chromosome 10 (Grieco et al. 1990, Santoro et al. 1994). **RET/PTC3** rearrangements were initially thought to be related to radiation-induced thyroid cancer (Rabes et al. 2000, Maenhaut et al. 2011). Now it is clear that they are characteristic of short latency PTC that develops in young persons, while **RET/PTC1** prevails in long latency PTC (Williams et al. 2004). In fact, **RET/PTC** rearrangements are quite frequent both in radiation-induced and in sporadic pediatric PTC. They are found in 50–70% of cases of children with sporadic PTC, while they are rare in older PTC patients, especially after the 45th year of age. The unique **RET/PTC**-related gene expression profile is less well characterized than that related to the **BRAF** status. Frattini et al. (2004) were the first to identify a subset of genes differentially expressed in PTCs carrying receptor tyrosine kinase-related rearrangements by analyzing **RET/PTC** and **NTRK1**-mutated PTCs and comparing them to tumors with **BRAF** mutation. Giordano et al. (2005), who constructed a simple multigene classifier, were able to recognize an additional **RET/PTC**-positive tumor sample without knowing its mutational status by its gene expression profile only. The gene expression signatures that differed in **BRAF**, **RET/PTC**, and **RAS** tumors included genes whose changed expression was a direct consequence of mutation (of **RET/PTC** rearrangement) as well as other genes involved in the immune response, signal transduction, and other processes. The hypothesis that different downstream pathways are activated by **RET/PTC** was supported by Miyagi et al. (2004), who used cell culture experiments to show that **RET/PTC3** mutants signaled preferentially through the PI3K pathway compared to MAPK. Among the genes involved in signal transduction, besides **ERBB3**, **MET**, or **DAPPI**, **VAV3** desired attention as a phosphoinositol-3-kinase pathway-related gene responsible for **AKT** activation that was preferentially expressed in PTCs with **RET** and **RAS** alterations.

Interestingly, the above-mentioned study of Mesa et al. (2006) on the early consequences of the **BRAF** V600E mutation and **RET/PTC3** rearrangement activation in rat thyroid PCCL3 cells revealed that the number of **RET/PTC3**-dependent genes was almost twice as high as that of genes activated by the **BRAF** mutation. In total, only 25% of genes, all related to the MAPK pathway, were co-regulated by these two genetic alterations. Among those genes regulated by **RET/PTC** that did not need the activation of the **BRAF** gene, there were many genes associated with the immune response, including the IFN (interferon) pathway genes. This was not the conclusion in Giordano’s paper or in our own **BRAF**-related expression analysis of PTC (D Rusinek, M Oczko-Wojciechowska, M Kowalska, M Świernak, M Jarząb, A Czarnecka, J Włocho, S Szpak-Ulczok, E Chmielik, D Lange, M Kowal, M Wiench, D Handkiewicz-Junak & B Jarząb 2007, unpublished observations), but the number of **RET/PTC**-dependent genes could be underestimated because of the lower number of **RET/PTC**-positive than **BRAF**-positive tumors.

**Expression of thyroid-specific genes in relation to different mutations in PTC**

The expression of key genes involved in thyroid hormone biosynthesis is known to be deregulated in thyroid cancer compared to normal thyroid tissue. The downregulation of the TSH receptor (**TSHR**), **NIS**, apical iodide transporter (**SLC5A8**), thyroperoxidase (**TPO**), and thyroglobulin as well as impaired NIS trafficking to the cell membrane was observed in papillary thyroid carcinomas, both with and without the **BRAF** mutation (Durante et al. 2007, Espadinha et al. 2009b). Simultaneously, the degree of dedifferentiation was higher in the **BRAF**-positive tumors. The loss of thyroid hormone production ability in PTC with the **BRAF** mutation was accompanied by the increased expression of the glucose transporter 1 (**GLUT1**), which reflects the V600E potential to induce the dedifferentiation processes (Durante et al. 2007, Puxeddu & Moretti 2007).

When comparing the wild-type **BRAF** PTCs with papillary thyroid carcinomas harboring the V600E mutation, the **NIS** transcript levels in the **BRAF**-positive PTC were about five times lower than those observed in the **BRAF**-negative tumors, with even more distinct differences at the level of protein expression (almost ten times lower; Durante et al. 2007). Romei et al. (2008) observed that the number of **NIS**-positive cells was significantly lower in **BRAF**-positive tumors (53.5 vs 72-6%), while at the mRNA level, the difference was seen both in the **NIS** and in the **TPO** genes. Investigating the differences related to the **BRAF** mutation in PTC, Riesco-Eizaguirre et al. (2009) analyzed the **BRAF**-induced secretion of functional TGFβ, which repressed the **NIS** expression by the activation of Smad. This mechanism has been shown to be MAPK–ERK kinase independent. In the interpretation of these results, it is important that not only the expression level but also the localization of the transporter is crucial for its function. In both **BRAF**-positive and **BRAF**-negative PTCs, the expression of the **NIS** protein was mainly limited to the cytoplasm and was less observed at the cell membrane.

The lower **SLC5A8** expression in the **BRAF**-associated PTCs is due to gene methylation (Porra et al. 2005). This thyroid-specific gene, unlike pendrin and **NIS**, is not TSH dependent. It is involved in the passive transport of iodide from the thyrocytes to the follicle lumen and was shown to be downregulated (40-fold) in the classical variant of papillary thyroid carcinoma.
suggests that methylation occurs as a secondary event in the presence of the
BRAF V600E mutation and silencing of the BRAF gene is related to the later stages of cancer progression. A significant correlation between the presence of the BRAF mutation and silencing of the SLC5A8 gene is also observed in colon cancer, where it occurs in the early stage of cancer development (Li et al. 2003). In thyroid carcinoma, methylation of SLC5A8 is related to the later stages of cancer progression. A significant correlation between the presence of the BRAF V600E mutation and silencing of the SLC5A8 gene is also observed in colon cancer, where it occurs in the early stage of cancer development (Li et al. 2003). In thyroid carcinoma, methylation of SLC5A8 is related to the later stages of cancer progression.

Gene expression changes common to all papillary thyroid cancer cases

The differences in gene expression between primary PTC and normal thyroid tissue are significant and encompass thousands of genes (Jarzab et al. 2005, Fujarewicz et al. 2007). The rather homogenous gene expression profile of PTC surprised the authors of the first microarray-based investigation (Huang et al. 2001). However, it seems less unexpected if one considers that different mutations activate the MAPK–ERK cascade and cause similar downstream molecular and functional effects. Many gene expression changes triggered by neoplastic transformation are not only common to all PTC but are also seen in follicular thyroid cancer (FTC; Polanski et al. 2007) and adenocarcinomas in general or even in all malignant tumors (Rhodes et al. 2004, Polanski et al. 2007).

Transcriptome of FTC

The biology of FTC and its gene expression profile is much less known in comparison with PTC (Cerutti et al. 2003, Weber et al. 2005, Cerutti 2007, Hinsic et al. 2009). The underlying molecular alterations of FTC encompass either activating RAS mutations (present in most cases already on the adenoma level) or PAX8/PPARγ rearrangements, which are more frequent at the carcinoma level (Knauf et al. 2006, Placzkowsi et al. 2008). The oncotypic variant of follicular carcinoma expresses a very characteristic gene expression profile related to the presence of abundant mitochondria (Barden et al. 2003, Finley et al. 2004b, Baris et al. 2005).

RAS-related changes in the gene expression of FTC and other thyroid tumors

RAS point mutations that activate the protein product are typical of follicular thyroid adenomas (FA) and FTC, showing a frequency range nearly 50% in both types of tumors. Because they are found at equal frequency in FAs and FTCs, RAS mutations are considered an early event in follicular neoplasia (Garcia-Rostan et al. 2003). There is no unequivocal proof of a larger tendency of RAS-positive follicular adenomas to progress to follicular carcinomas, especially since nothing is known about the molecular events responsible for the further malignant transformation of a RAS-positive follicular adenoma. PAX8/PPARγ rearrangements occur preferentially in RAS-negative follicular tumors (Nikiforova et al. 2003b).

Surprisingly, the RAS-related signature of FTC is not well specified, possibly because it is not easily detected at the mRNA level. The impact of the RAS mutation on the gene expression of thyroid cells was studied using the FRTL-5 rat thyroid epithelial-derived cell line infected with the Kirsten murine sarcoma virus carrying the v-Ki-Ras oncogene (Visconti et al. 2007). Genes that were found to be significantly altered in RAS-transfected cells were validated by QPCR and studied in thyroid cancer cell lines of follicular cell carcinoma origin (WRO), papillary carcinoma origin (FB-2, NPA, TPC-1, and B-CPAP), and from anaplastic cancer (FRO and ARO). Annexin A2 and RALA (v-ras simian leukemia viral oncogene homolog A, RAS related) were upregulated in the RAS-transfected rat cells and positively validated in all seven cancer lines. Dual-specificity phosphatase 1 (DUSP1) and small cell lung carcinoma cluster 4 antigen (CD24) were downregulated by RAS in rat cells and also in all studied cancer cell lines (Rodrigues et al. 2007a).

RAS mutations are found in 0–15% of PTCs (Vasko et al. 2003, Abrosimov et al. 2007, Hou et al. 2007), mostly as activating mutations of codons 12 and 13 of the NRAS gene, and showing higher incidence in follicular variant of PTC (Vasko et al. 2003, Zhu et al. 2003, Castro et al. 2006, Di Cristofaro et al. 2006, Goutas et al. 2008). RAS mutations have also been reported to be frequent in poorly differentiated thyroid cancer (PDTC; Garcia-Rostan et al. 2003, Riesco-Eizaguirre & Santisteban 2007, Volante et al. 2009). However, the distinct gene expression profile differences between PTC and PDTC are related to the grade of differentiation itself rather than to the presence of different initiating mutations (Fluge et al. 2006). However, Pita et al. (2009) reported similarities between the follicular variant of PTC and the RAS-mutated PDTC.

Gene signature of the PAX8/PPARγ rearrangement

PAX8/PPARγ translocations (PAX8/PPARγ fusion protein, PPFP) are present in 26–56% of FTCs and also in a number of FAs. This kind of translocation generates a fusion oncoprotein that acts as a dominant negative inhibitor of wild-type PPARγ and has unique transcriptional activity when compared with wild-type PAX8 or PPARγ. Lacroix et al. (2005) revealed a pattern...
of 93 genes that discriminated FTCs with and without translocation, which at the morphological level were indistinguishable. PPARγ-dependent genes were over-expressed in FTCs bearing the translocation, including angiopoietin-like 4, aquaporin 7, or a potential target – FGD3. No differences in the expression of thyroid-specific genes were noted. In addition, Giordano et al. (2006) reported that the presence of this balanced translocation is associated with a specific gene expression signature, which is composed of known PPAR target genes involved in fatty acid metabolism such as carnitine/acylcarnitine translocase (SLC25A20) and acetyl-CoA acyltransferase 1 (ACAA1), amino acid and carbohydrate metabolism, and specific micro-RNA target genes. Among the top, 20% of genes highly upregulated in PPFP-positive FTCs were genes detected on chromosome 3p (95 from 341 genes). Four miRNAs were strongly over-expressed in FTCs harboring PAX8/PPARγ rearrangement: miR-101, miR-30A-3P, miR-200A, and miR-199A. The authors found 21 upregulated genes that were potential targets for at least three of the above-mentioned miRNAs, including oncogenes like RUNX1/AML1 and SS18.

Other molecular events characterizing FTC and not related to its mutational status

The majority of the gene expression analyses of FTC (Barden et al. 2003, Cerutti et al. 2004, Finley et al. 2004a, Weber et al. 2005, Hirsch et al. 2009) have considered the diagnostic aspect and have searched for gene signatures or genes differentiating FTC from FA. Among the genes listed are cyclin D2, protein convertase 2 (PCSK2), and prostate differentiation factor (PLAB; Borup et al. 2010). Borup et al. performed a global expression profiling of follicular tumors and found expression changes in genes involved in DNA replication and mitosis, loss of growth arrest, and proapoptotic factors to be characteristic of FTC, among them being NR4A1 and A3, encoding orphan nuclear receptors involved in the regulation of proliferation and apoptosis, as well as FOSB and JUN. Loss of apoptosis and growth arrest factors occurred during malignant transformation and was prominent in all samples, implying that this event preceded proliferation. The coordinated downregulation of the expression of NR4A1 and A3, JUN, FOSB, and CITED2 was a striking observation for these authors because it was contrary to their increased expression in other cancers. Interestingly, NR4A1 and A3 translocate to the mitochondria, where they stimulate the release of cytochrome C in a BLC2-dependent manner (Borup et al. 2010). The frequent loss of expression had been reported as a characteristic feature of FTC previously. The self-regulation of caveolin-1, both at the mRNA and protein levels, was one of the first expression changes described in FTCs by Aldred et al. (2003), who also considered insular-type and oxyphilic (Hurthle-type) thyroid cancers.

Transcriptome of PDTC and undifferentiated thyroid cancer in relation to their mutational profile

The PDTC and undifferentiated anaplastic thyroid carcinomas (ATC) respectively present a much more aggressive clinical course than papillary or follicular thyroid cancers, which are frequently described as well-differentiated cancers (DTC). Their origin is not well defined but the prevalence of BRAF, RAS mutations, or genetic alterations of PIK3CA as well as the presence of pre-existing PTC areas observed in some PDTCs suggest that at least some of them may originate from DTCs (Garcia-Rostan et al. 2003, 2005, Nikiforova et al. 2003a). In addition, RET/PTC rearrangements were detected in PDTCs at a frequency of 10%, and the proportion increased to 20% when only PDTCs with areas of well-differentiated thyroid carcinoma were considered (Santoro et al. 2002). Although a mouse model of papillary thyroid carcinoma induced by RET/PTC3 showed the formation of a solid PTC variant with metastases (Powell et al. 1998), there is no evidence of the association between RET/PTC and poor prognosis-associated PDTCs (Santoro et al. 2002). Wresmann et al. (2002) noticed an increase in the incidence of DNA copy number changes from DTC to PDTC, both at the level of number of patients with abnormalities and number of abnormalities per case. Saltman et al. (2006) suggested an intermediate position for PDTC between the well-differentiated and anaplastic thyroid carcinomas, with the most significant changes represented by the Ki-67 protein, which was present in 5-8% of well-differentiated PTC, 48-88% of PDTCs, and 81-8% of ATCs. Nevertheless, the hypothesis that PDTC and ATC arise de novo cannot be excluded.

The deregulation of TP53 (tumor protein p53) and Wnt pathways and mutations of β-catenin are common in both PDTC and ATC (Donghi et al. 1993, Garcia-Rostan et al. 1999). Lavra et al. (2009) showed a positive correlation between galectin-3 expression and p53 mutations. Gal-3, an adhesion-related and simultaneously anti-apoptotic molecule, was upregulated in most ATCs and thyroid carcinoma cell lines harboring the most frequently detected p53 mutation, R273H. Over-expression of the p53 protein (TP53) was also found to be correlated with the upregulation of its antagonist p63, a member of the p53 protein family (Malaguarnera et al. 2005) and the GLUT1 (Kim et al. 2006). Schwartzengren-Bar-Yoseph et al. (2004) demonstrated that GLUT1 and GLUT4 are repressed by wild-type p53, and mutations within the DNA-binding
domain of TP53 disable this interaction. These data help to explain the findings of Kim et al. (2006) showing over-expression of TP53 and GLUT1 in ATC. Guida et al. suggested dependency between mutated TP53 and upregulation of minichromosome maintenance protein 7 (MCM7), a protein required for DNA replication, which was reported to be highly over-expressed in ATC but absent in normal thyroid and PTC (Guida et al. 2005).

Salvatore et al. (2007) presented a gene signature of anaplastic thyroid carcinoma characterized by the upregulation of genes involved in the regulation of cell cycle progression and chromosome segregation. The authors demonstrated that the Polo-like kinase 1 (PLK1), previously included in the ‘proliferation cluster’ and ‘chromosomal instability 70 cluster’ (Tabach et al. 2005, Carter et al. 2006, Whitfield et al. 2006), was required for ATC cell proliferation and cell survival, and this dependence was not detected in normal thyroid cells, in agreement with the data of Nappi et al. (2009). Pita et al. (2009) found a number of upregulated cell cycle-related genes in PDTC. Among them were genes associated with mitosis such as CDC28 protein kinase regulatory subunit 2 (CKS2) and cyclin E2 (CCNE2), which were previously reported as upregulated in many other types of tumors (Guida et al. 1999, Scrideli et al. 2008). The authors stressed the over-expression of the ubiquitin-like, containing PHD and ring finger domains (UHFR1) gene in PDTC. This gene encodes a protein involved in processes of breaking and rejoining of DNA strands through the regulation of the TOP2A enzyme and is involved in the DNA damage response and, through the regulation of methyltransferase 1 (DNMT1), in VEGF regulation. The UHFR1 gene is located within the chromosome region 19p13, in which Lee et al. (2008) by CGH-array reported gains in 67% of ATCs. Microarray analysis also showed a strong upregulation of PKB (v-AKT murine thymoma viral oncogene homolog 1), which encodes a serine/threonine kinase of the MAPK pathway, in PDTC. The over-expression of PKB in poorly differentiated thyroid carcinomas was first demonstrated by Rodrigues et al. (2007b), who emphasized the importance of the MAP kinase pathway in the progression of thyroid carcinomas. Montero-Conde et al. (2008) investigated differentiated and undifferentiated thyroid tumors by using cDNA microarrays and also reported significant changes in the expression of genes of the MAPK pathway, as well as those of the TGFβ pathway, focal adhesion, and activation of actin polymerization and cell cycle.

Ito et al. (2009) reported the co-expression of S100A8 and A100A9, which represent the S100 calcium-binding proteins A8 and A9, in undifferentiated thyroid carcinomas, and the lack of S100A8 expression and a weak expression of S100A9 in PDTC, and lack of these proteins in well-differentiated DTC. Similar gradation was reported by Aratake et al. (2010) who examined the expression of extracellular matrix metalloproteinase inducer (EMMPRIN) and found a weak expression level in well-differentiated DTC (4.9) and high levels in ATC (245.7).

With regard to genes downregulated (lost) in ATC, Rodrigues et al. (2007b) reported the downregulation of pleomorphic adenoma gene-like 1 (PLAGL1) and described under-expression of CDH1 (cadherin 1, type 1, E-cadherin (epithelial)) in PDTC. Most downregulated genes described by Pita et al. in PDTC in comparison to normal thyroid tissue were related to cell adhesion. Deregulation of cell adhesion genes may promote tumor invasion and metastasis. However, there were only two genes differentially expressed when PDTC was compared to DTC, and 3-phosphoinositide-dependent protein kinase-1 gene (PDK1) was the most interesting gene with regard to its function in AKT activation. Downregulation of the epithelial cell adhesion molecule (EpCAM) was reported by Ensinger et al. (2006), who reported its absence from undifferentiated thyroid carcinomas and high level of EpCAM expression in well-differentiated DTC and PDTC. Previous studies indicated the dual role of EpCAM, suggesting its association with tumor formation and oncogene characteristics (Spizzo et al. 2004). However, another study also described EpCAM as a tumor suppressive protein (Gosens et al. 2007). Because of the adhesive properties of EpCAM (Litvinov et al. 1994), a role of this protein in the inhibition of invasion was proposed.

Espadinha et al. (2009a) reported an association between the downregulation of PPARγ and lower differentiation of thyroid follicular cells. These authors focused on the effect of PPARγ on the function of E2F/DP, which regulates the expression of genes involved in entry into S phase and DNA synthesis and whose decrease was observed during the differentiation of several types of cells (La Thangue and Rigby 1987, Hara et al. 1993, Melamed et al. 1993).

With reference to miRNA expression in undifferentiated thyroid cancers, Visone et al. (2007) suggested that the deregulation of miRNA might be an important step in thyroid carcinogenesis based on the detection of a decrease in miR-30d, miR-125b, miR-26a, and miR-30a-5p in ATC compared with normal thyroid tissue. Not unexpectedly, the array-based analyses showed a marked under-expression of thyroid-specific genes in anaplastic thyroid carcinoma (Onda et al. 2004, Rodrigues et al. 2007b, Matsumoto et al. 2008).

Final remarks

The relationship between the presence of thyroid cancer histotype-specific mutations and the gene expression profiles of tumors is complex, and many factors related to cancer invasiveness and host...
responses have to be considered. However, studies on the association of gene signatures with mutational events associated with carcinogenesis may help to elucidate the molecular consequences of the activation of oncogenes and the loss of tumor suppressors and thus improve our understanding of tumor biology and facilitate the search for prognostic and predictive markers and new therapy targets. The gene expression profile differences between papillary thyroid cancer and FTC and PDTC and undifferentiated thyroid cancer mainly reflect the differences in the degree of tumor differentiation. However, the observed profound differences between BRAF-positive and BRAF-negative PTCs, much more distinct than the respective differences in the gene expression signatures of FTCs with different mutations, are indicative of profound changes in biology related to the appearance of this particular oncogene.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References


Chou CK, Chen RF, Chang HW, Chen YJ, Yang KD, Cheng JT, Huang CC & Liu RT 2010 miR-146b is highly expressed in papillary thyroid carcinomas with high-risk features including extrathyroidal invasion and the BRAF(V600E) mutation. Thyroid 20(5):489–494.


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