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Review

Molecular prognostic markers in papillary and follicular thyroid cancer: Current status and future directions

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A B S T R A C T

Gene expression profiling shows that, by gene signature, the difference between BRAF-positive and BRAF-negative PTC is so distinct that BRAF-positive cancer may be regarded as a molecular subtype of papillary thyroid cancer (PTC). Since much enthusiasm surrounds the BRAF-oncogene as a molecular prognostic factor, a central focus of our consideration is to weigh the current arguments for and against applying BRAF mutation status of the tumor in clinical practice. The frequency of BRAF mutation in PTC is high—45% on average, with values over 70–80% in some populations. This will mean that implementing BRAF mutation as a factor of poor prognosis will shift many PTC patients, considered up to now as low risk ones, to the more extensive treatment. We estimate that 31% of all PTC patients and 39% of those diagnosed with stage I–II disease will face the risk of overtreatment if the decision will be based on the BRAF-positivity of their tumors. Also, the risk of undertreatment in the young patients with BRAF-negative tumors is evaluated with 26%. We think that, as of now, the evidence-based support for such consequences is still weak. Thus, there is urgent need to look for genes or gene signatures which will be helpful in the stratification of BRAF-positive tumors to specify these with poor prognosis with higher accuracy, needed for clinical decisions. Considering this, in the review we summarize the present status of knowledge on other prognosis-related gene expression changes in papillary and follicular cancer and relate them to he tumor’s biology.

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1. Introduction

Papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) are both described clinically as differentiated thyroid cancers (DTCs) due to their tumor cells structurally and functionally resembling normal follicular cells and due to their sharing a relatively indolent natural history and good responsiveness to surgery and radioiodine in most — but far from all — cases. DTC constitutes the commonest endocrine malignancy, and among neoplastic diseases, possesses the most favorable overall prognoses. However, over 10% of patients eventually die of DTC and an even greater proportion faces the morbidity of recurrences (Eustatia Rutter et al., 2006; Mazzaferri and Kloos, 2001; Sherman, 2003). In these respects, little to nothing has improved in DTC management in the last twenty years (Clark and Duh, 1991).

As in other cancers, numerous studies in DTC have concentrated on relatively simple clinicopathological variables, e.g., primary tumor size, extent of disease, pathological histotype, patient’s age at diagnosis or sex, to formulate risk group stratification or staging systems. The goal has been to select patients who will do well without further therapy (i.e., to identify prognostic factors) or who will do well with some treatments but not others (i.e., to identify predictive factors).

In recent years, the impact of molecular diagnosis is becoming even more substantial in cancer care. This trend is already evident in many hematological malignancies, where the presence of particular chromosomes rearrangement influences the natural history and the choice of treatment, i.e., is not only of prognostic significance but also of predictive significance (Haferlach et al., 2003; Jabbour et al., 2008; Meijerink et al., 2009). A similar phenomenon is also occurring with solid tumors such as breast cancer, where the division into two molecular subtypes — luminal and basal — is slowly gaining influence in formulating disease management strategies (Sorlie et al., 2001; van’t Veer et al., 2005).

In the present review, we highlight that the molecular diagnosis of thyroid cancer is already possible (Eszlinger et al., 2008; Giordano, 2008). We therefore examine whether, to improve prognostic/predictive ability, molecular diagnosis should now supplant or at least supplement the clinicohistopathological evaluation of DTC. This question is increasingly pertinent, as in recent years, both research on single, cancer-specific mutations and genome-wide DNA microarray-based approaches have added greatly to our understanding of DTC biology and clinical behavior. In our discussion of the prognostic relevance of molecular studies, we focus on data regarding gene mutations, essential for neoplastic transformation of follicular cells and, then, for gene expression changes, judged both by RNA and protein levels. We present details of microarray-based comparative genome hybridisation (CGH) studies on gene amplifications/deletions and of studies on epigenomic changes insofar as this information is germane to the current role and future investigation of molecular prognostic factors in DTC. Since the B/Raf oncogene (v-ras murine sarcoma viral oncogene homolog B1) is the major genetic factor in PTC (Puxeddu and Moretti, 2007; Xing, 2007), an important focus of the present review is to weigh the current arguments for and against applying B/Raf mutation status in clinical practice. As we discuss in more detail below, the data on the correlation of B/Raf status with DTC clinicopathological factors are substantial and even show an impact on survival. However, from the clinical point of view, a factor present in 50% of cases is of limited use in managing a disease which has a poor outcome in no more than 10–15% patients, thus, a more detailed stratification is necessary and we look for this possibility.
Our review begins by summarizing the current understanding of histopathological and clinical prognostic factors in DTC. Next, we describe \( \text{BRAF} \) mutations and other molecular events occurring early in the thyroid neoplastic transformation in the context of their relationship to thyroid cancer prognosis. Subsequently, we discuss the expression changes in other single genes other than \( \text{BRAF} \) that are known to have prognostic utility in DTC. Lastly, we present the status of relevant genome-wide analyses and provide some closing remarks where we consider not only the prognostic value of genetic markers but also comment briefly on their predictive contribution.

2. Pathological and clinical basis for evaluating prognosis in papillary and follicular thyroid cancers

2.1. Histopathological and clinical characteristics of DTCs and their influence on disease prognosis

The follicular cells of the thyroid gland give rise to a variety of tumors that differ markedly in their morphology and biological and clinical behavior. Based on a number of diagnostic criteria and morphological hallmarks, these tumors may be divided into five major types: papillary, follicular, oncocytic, poorly differentiated, and undifferentiated (anaplastic), the first two of which in turn may be subdivided into a number of variants (LiVolsi and Asa, 1994; LiVolsi and Baloch, 2004). By far the most common is PTC, representing up to 80% of all thyroid malignancies. This prevalence is distant from that of FTC, ranging from less than 10% or 15% to not more than 25%, depending on the series, while oncocytic histotype (either variant of PTC or FTC) constitutes not more than 2% of thyroid cancers (Kushchayeva et al., 2008). Several histopathological features of DTC have been claimed to influence its prognosis. In PTC, the tall cell variant has been shown to have a worse prognosis than does the classical type (Ito et al., 2008 ). Concomitant logical features of DTC have been claimed to influence its prognosis.

In PTC, the tall cell variant has been shown to have a worse prognosis than does the classical type (Ito et al., 2008). Concomitant thyroiditis, diffuse lymphocytic infiltration, or both may favorably influence the course of PTC as well as that of FTC (Kashima et al., 1998; Loh et al., 1999; Modi et al., 2003).

FTC, especially if widely invasive or oncocytic, was claimed to have a worse prognosis than does PTC (Gulcelik et al., 2007; Passler et al., 2004). However, a recent study of more than 1000 patients found no difference in cancer-specific survival between PTC and FTC when one accounted for clinical prognostic factors such as age at diagnosis, primary tumor size and the presence of extrathyroidal invasion or of metastases (Verburg et al., 2009). In FTC, the prognosis for the minimally invasive subtype is excellent (Thompson et al., 2001). Fig. 1 compares a variety of commonly used clinical prognostic factors with respect to the associated significant increases in the risks of death or DTC recurrence that were found in a recent large, long-term study of DTC patients conducted at our center (Czarniecka, 2004). As seen in that figure, the presence of distant metastases is the strongest poor prognostic factor for overall survival in DTC (Elisei et al., 2008; Mazzaferri and Jhiang, 1994; Mazzaferri and Kloos, 2001). However, there are exceptions to this rule, as distant metastases in young patients are not regarded as a poor prognostic factor. In fact, younger patients with distant metastases have a better prognosis than do older patients diagnosed with locally invasive tumors, lymph node metastases, or both (Jarzab et al., 2005a).

The prognostic impact of lymph-node involvement in DTC has long been a matter of controversy, especially regarding survival. Several studies failed to demonstrate an influence on mortality rates (DeGroot et al., 1990; Mazzaferri and Young, 1981) while others have identified an association between lymph node metastases and an increased risk of cancer-related death, especially when the involved lymph nodes are large, bilateral or located in the mediastinum (Czarniecka, 2004; Mazzaferri and Kloos, 2001; Podnos et al., 2005; Tubiana et al., 1985; Zaydfudim et al., 2008). In fact, in the past, lymph-node involvement was even considered to be a favorable prognostic sign until the effect of age was recognized: children and young adults with PTC have both far more frequent lymph-node involvement and a far better outcome than do older patients. However, almost all studies unequivocally agree that lymph-node involvement is associated with a higher risk of locoregional recurrences (Beasley et al., 2002; Handkiewicz-Junak et al., 2007; Harwood et al., 1978; Sakorafas et al., 2009).

2.2. Clinicopathological staging of DTC and its limitations

At least 18 staging systems for PTC, FTC (Akslen, 1993; Cadry and Rossi, 1988; DeGroot et al., 1990; Greene et al., 2002; Hay et al., 1987, 1993; Mazzaferri and Jhiang, 1994; Pasi et al., 1992; Shahaa et al., 1994; Sherman et al., 1998) or both, have been formulated based on retrospective patient outcome evaluations (Lang et al., 2007a,b). In Europe, the UICC/TNM system is the most widely adopted. It considers three main factors:

- T status—primary tumor size and extrathyroidal extension,
- N status—presence or absence of lymph node metastases,
- M status—presence or absence of distant metastases.

The UICC/TNM system also considers patient age at diagnosis, a factor not commonly used in other non-TNM-based DTC staging systems. All patients below 45 years of age who are diagnosed without distant metastases, are considered to have stage I disease, i.e., belong to the “low-risk” group.

The clinicopathological factors upon which the majority of staging systems are based are summarized in Table 1. These variables frequently overlap, e.g., older or very young patients tend to more often have lymph node or distant metastases or both; young patients with lymph node metastases more often suffer from distant metastases (Jarzab et al., 2005a). Such overlap is usually addressed with multivariate analysis, which can help select independent variables while rejecting factors that are only surrogates for those variables. Nonetheless, even with that statistical approach, results are sometimes contradictory, as in the evaluation of tumor histopathology (Gulcelik et al., 2007; Verburg et al., 2009) or of lymph node metastases (Bardet et al., 2008; McConahey et al., 1986). In the case of histopathology, the contradictory results may be attributable to the somewhat subjective, not fully standardized method of specimen evaluation. The rate of disagreement during DTC evaluation can be high, especially in FTC where it can reach up to 50% of cases (Franc et al., 2003; Lange et al., 2006).

Table 2 provides the survival by disease stage or risk group according to the most popular staging systems. As seen in Table 2, the systems show marked survival differences, especially for “high-risk” groups/advanced stages, in which, according to most systems, more than 50% of patients eventually succumb to disease (Durante et al., 2006; Sampson et al., 2007; Schlumberger et al., 1996). It is however not established which patients in less advanced subgroups do or do not require more intensive treatment to cure their cancer. This results in a very wide “gray zone” of DTC prognosis, which leads to a significant risk of over- or undertreatment in a substantial number of patients (Gulcelik et al., 2007; Tuttle et al., 2008). The MACIS and TNM staging systems have been demonstrated to be the most accurate prognostic systems in some (Lang et al., 2007a,b; Passler et al., 2003) but not all studies (Brierley et al., 1997). Nevertheless, even if evaluated with the best score, they explained barely about 20% of the observed variation in survival time (Lang et al., 2007a,b) and only a few studies yielded better results (D’Avanzo et al., 2004).

Exacerbating this problem, the extent of treatment (e.g., radicalness of surgery or use or activity of radioiodine) that is
recommended in guidelines is not consistent between “high-risk” or “low-risk” groups according to different staging systems. It is, of course, well known that the extent of treatment can influence disease outcome (Czarniecka, 2004; Gulcelik et al., 2007; Lang et al., 2007a,b; Passler et al., 2003). In addition, since almost all current clinicohistopathological prognostic factors are based on postsurgical tumor and patient evaluation, none of these staging systems can be applied pre-operatively, and hence help tailor the extent of surgery.

In summary, then, the utility of the current clinicohistopathological staging systems for DTC is limited by the abundance of systems, their frequent overlap and sometimes contradictory results, their far from perfect match with short- or long-term prognosis, and their inapplicability pre-operatively.

2.3. Molecular mechanisms of neoplastic transformation in papillary and follicular thyroid cancer: prognosis-related aspects

From the molecular point of view, papillary and follicular thyroid cancers are regarded as different diseases. This opinion is supported by the disparate molecular initiating events leading to neoplastic transformation. Also supporting this perspective are the differences in DNA ploidy level (PTCs are generally diploid, FTCs aneuploid) and the differences in localization and in the number of gains and losses seen in comparative genomic hybridisation (CGH)-based analysis. In PTC, aneuploidy, although observed in minority of tumors (Rodrigues et al., 2007), is significantly associated with cancer-specific death (Sturgis et al., 1999). The profound differences in gene expression will be described below. On the other hand, many subsequent down-stream molecular mechanisms are very similar between PTC and FTC, which explains the commonalities in the clinical course of both cancers and creates a rationale for similar therapeutic approaches to both of them.

2.3.1. Activation of the RAS–RAF–MEK–ERK1/2 pathway and the PI3K/AKT pathway in thyroid cancers

DTC harbors several highly prevalent genetic alterations, some of which are seen only in this cancer or are characteristic of one of its histotypes. In PTC, the most important oncogenic mechanism, seen in about 80% of tumors, is the activation of the mitogen-activated protein kinase (MAPK) pathway (Fagin, 2004; Fagin and Mitsiades, 2008), which once constitutively activated, leads to tumorigenesis (Hilger et al., 2002; Peyssonnaux and Eychene, 2001). Three most important initiating events, RET/PTC (rearranged during transfection/papillary thyroid cancer), RAS (resistance to audiogenic seizures) and BRAF mutation, are regarded as mutually exclusive, alternative triggers for the activation of the pathway (Fagin, 2004). The mutual exclusivity between BRAF and the RAS mutation is characteristic not only of thyroid cancer but also of several other human cancers (Xing, 2005). The activating BRAF mutation has only been recognized relatively recently (Davies et al., 2002), but in fact is the most frequent trigger in PTC.

A single oncogenic alteration along the receptor tyrosine kinase–RAS–RAF–MEK–ERK1/2 pathway is likely sufficient to drive thyroid cell neoplastic transformation, although further supportive molecular events are necessary. BRAF mutation and RET/PTC (and NTRK1—neurotrophic tyrosine kinase, receptor, type 1) rearrangements differ to some extent in their effects on the shared oncogenic pathway, respectively, resulting more frequently in the classic variant or the solid variant of PTC, while RAS mutations and RASSF1A (Ras association (RalGDS/AF-6) domain family member 1) methy-
Table 1
Clinical prognostic factors used in different staging systems for DTC.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Positive prognostic indicator</th>
<th>Negative prognostic indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Younger (usually below 40–45 years of age)</td>
<td>Older (usually above 40–45 years of age)</td>
</tr>
<tr>
<td>Primary tumor—diameter</td>
<td>Small (the best prognosis for tumors ≤ 1 cm)</td>
<td>Large (the worst prognosis for tumors &gt; 1 cm)</td>
</tr>
<tr>
<td>Primary tumor—extension beyond thyroid capsule</td>
<td>No</td>
<td>Present</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Surgical resection</td>
<td>Total thyroidectomy and appropriate extent of lymphadenectomy ensuring complete removal of cancer tissues</td>
<td>Incomplete removal of cancer tissues and/or lack of total thyroidectomy and of appropriate extent of lymphadenectomy</td>
</tr>
</tbody>
</table>

Table 2
Survival by disease stage or risk group in selected DTC staging systems.

<table>
<thead>
<tr>
<th>Staging system</th>
<th>DTC type (number of patients)</th>
<th>Primary outcome end-point</th>
<th>Disease stage or risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNM stage (Greene et al., 2002)</td>
<td>PTC (6590)</td>
<td>5-Year survival</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>FTC (1129)</td>
<td>5-Year survival</td>
<td>95%</td>
</tr>
<tr>
<td>MACIS (Hay et al., 1993)</td>
<td>PTC (1779)</td>
<td>20-Cause-specific survival rates</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99%</td>
</tr>
<tr>
<td>Ohio State University Staging system (Mazzaferrri and Jhiang, 1994)</td>
<td>PTC and FTC (1355)</td>
<td>Cancer-related deaths</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>AMES (Cady and Rossi, 1988)</td>
<td>PTC and FTC (821)</td>
<td>Cancer-related deaths</td>
<td>Low</td>
</tr>
<tr>
<td>NTCTCS system (Sherman et al., 1998)</td>
<td>PTC and FTC (1607)</td>
<td>5-Year CSS</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99.8%</td>
</tr>
<tr>
<td>University of Chicago (DeGroot et al., 1996)</td>
<td>PTC (269)</td>
<td>10-Year CSS</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>GAMES Risk Groups (Shaha et al., 1994)</td>
<td>PTC and FTC (1038)</td>
<td>10-Year survival</td>
<td>Low</td>
</tr>
<tr>
<td>AGES (Hay et al., 1987)</td>
<td>PTC (860)</td>
<td>25-Year CSS</td>
<td>Low</td>
</tr>
<tr>
<td>DAMES (Pasieka et al., 1992)</td>
<td>PTC (74)</td>
<td>Cancer-related death or recurrence</td>
<td>Low</td>
</tr>
<tr>
<td>SAG (Akslen, 1993)</td>
<td>PTC (173)</td>
<td>10-Year CSS</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>98.3%</td>
</tr>
</tbody>
</table>

CSS, cancer-specific survival; DTC, differentiated thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer.

3. Prognostic impact of the initiating molecular events in papillary and follicular thyroid cancers

3.1. The role of BRAF proto-oncogene in PTC

BRAF is a serine–threonine kinase, expressed in a tissue-specific manner and abundant in thyroid follicular cells, which among the three known RAF kinases, most potently activates the MAPK pathway (Peyssonnaux and Eychene, 2001) (for reviews see Puxeddu and Moretti, 2007; Xing, 2005). Activating BRAF mutations are not thyroid cancer-specific. To the contrary, BRAF belongs to the commonest oncogenes, mutated in 7–9% of all malignant solid tumors, with the highest prevalence (about 60%) in melanomas (DeLuca et al., 2008), and a frequent presence in ovarian, colorectal, and lung cancers and even some low-grade astrocytomas (Pfister et al., 2008). Interestingly, although the activation of the MAPK signaling pathway is regarded as frequent in human cancer, the evidence for this is most compelling in thyroid cancer and melanoma (Johansson et al., 2007; Knauf and Fagin, 2009).

3.1.1. BRAF mutation is the most frequent initiating molecular event in PTC

After melanoma, PTC is the second human malignancy where BRAF mutations are especially frequent, found in 29–87% (with a mean frequency of about 45%) of cases (Kim et al., 2009; Kimura et al., 2003; Puxeddu et al., 2004; Xing, 2007). BRAF mutations are not found exclusively in PTC but also, albeit less often (not more than 20–26% of cases, mainly those originating from PTC) in ATC (Nikiforova et al., 2003a; Smallridge et al., 2009; Takano et al., 2007).

Although more than 40 mutations have been identified in the BRAF gene, the most significant hot spot mutation, accounting for over 90% of all BRAF mutations, is a thymidine to adenine transversion at nucleotide 1799 (T1799A) in exon 15. This substitution leads to a valine-to-glutamate transversion at residue 600 near the catalytic center of the protein (BRAFV600E) and is believed to produce a constitutively active kinase by disrupting hydrophobic interactions between residues in the activation loop and residues in the adenosine triphosphate (ATP) binding site (Wan et al., 2004).
BRAFV600E potentiates the catalytic activity of the enzyme by more than 500 times (Kim et al., 2005).

**BRAF** mutation is an early event in PTC tumorigenesis, which can be found even in minute PTCs, less than 4 mm in diameter (Ugolini et al., 2007). The constitutively active kinase subsequently leads to tumorigenesis through aberrant activation of the MAPK pathway (Davies et al., 2002; Garnett and Marais, 2004). Such activation involves hyperphosphorylation of retinoblastoma protein (RB), which releases inhibition of E2F-dependent transcription factors, allowing the cell to pass from G1 into S phase, increasing growth and promoting survival (for a review of this process see Knauf and Fagin, 2009). At the same time, apoptosis is inhibited, probably due to the action of NFκB transcription factor (Palona et al., 2006). Transgenic mouse studies clearly demonstrated the driving force of the **BRAF** mutation in promoting malignant transformation of thyrocytes and extrathyroidal invasion of PTC (Knauf et al., 2005). One important molecular mechanism involved in this process is **BRAF** mutation-promoted methylation and hence silencing of the tissue inhibitor of metalloproteinase-3 (TIMP-3; Hu et al., 2004) and resultant overexpression of metalloproteinases (Melillo et al., 2005; Mesa et al., 2006; Palona et al., 2006).

The **BRAF**-oncogene induces chromosomal instability (Mitsutake et al., 2005), and is it not only an initiator of PTC but is necessary for the maintenance of PTC proliferation and tumorigenicity (Liu et al., 2007).

### 3.1.2. BRAF mutations are associated with poorer prognosis of PTC

Based on molecular results, numerous studies have investigated the clinical significance of **BRAF** mutation in PTC. The general tendency of the studies to show the association of this mutation with factors related to poor prognosis is rather convincing (Table 3). However, a careful overview of the literature indicates that the clinical data on the link between **BRAF** mutation and DTC outcome are not very consistent. The first data were summed up in a large meta-analysis of 1168 patients and showed that **BRAF** mutation correlated with histologic subtype, presence of extrathyroidal extension, and advanced clinical stages, but not with age, sex, race, or tumor size of PTC patients (Lee et al., 2007). More recent studies addressed even more carefully the correlation between **BRAF** status and PTC clinicopathological factors: two such studies (Frasca et al., 2008; Wang et al., 2008a), which altogether included more than 400 patients, found in multivariate analysis a positive correlation between **BRAF** mutation and extrathyroidal extension, while two other such studies totaling about twice as many patients did not (Kebebew et al., 2007; Lupi et al., 2007). In the analysis of Lupi et al., encompassing 500 consecutive PTC patients at the University of Pisa, the one-way univariate correlations of **BRAF** mutation positivity with extrathyroidal extension and tumor stage-related data were very distinct. However, the multivariate analysis discarded all the factors except lack of a tumor capsule (whether the tumor was single or multifocal) (P = 0.0005 in multivariate analysis). In the Lupi study, lack of encapsulation remained statistically significant in separate multivariate analyses for micro-PTC, larger tumors and follicular variant PTC but not for classical variant PTC. Presence of tumor capsule, although relatively rare in PTC (present in 21.7% of the Lupi series), is a strong predictor of an especially good disease-free prognosis, seen very distinctly in follicular variant PTC (Kakudo et al., 2004).

Summing up the analysis of the relationship of **BRAF** mutations with clinicopathological factors, there is only one factor shown by the majority of studies to correlate with **BRAF** mutations—extrathyroidal tumor extension, which is however questioned if tumor encapsulation is also included in the analysis. For one perusing these studies, questions arise on the origins of the discrepancies between the study results. Many studies on the correlation of **BRAF** with clinicopathological data (Costa et al., 2008; Elisei et al., 2008; Guan et al., 2009; Kebebew et al., 2007; Lee et al., 2009; Rosenbaum et al., 2005) were based on univariate analyses that could not distinguish between overlapping significant factors. In that case what seems to be **BRAF** related in univariate analysis can result from other unknown factors affecting studied outcome (e.g., disease stage or recurrences) and be correlated with **BRAF** status. For example, **BRAF** mutations are relatively rare in PTC cases with concomitant autoimmune thyroiditis (Nikiforova et al., 2002), and here the immune response may influence the clinical course more than do the molecular consequences of initiating mutation itself. Although the multivariate analysis may be somewhat helpful in making the distinction between diverse confounding factors, it may only solve some but not all the problems, as only a limited number of factors can be included and other, theoretically decisive factors in the outcome of the disease can be unintentionally omitted. Nevertheless at present, multivariate analysis seems to be the best way to obtain the least biased results. A good example is the already discussed study by Lupi et al., where **BRAF** mutation positively correlated with extrathyroidal extension in univariate analysis, but was abolished by the status of thyroid capsule in multivariate approach.

A second issue in interpreting these studies is how precisely clinicopathological factors are defined. For example, both minimal invasion beyond the thyroid capsule and gross invasion of muscles and other neck tissue can be regarded as extrathyroidal disease extension, but their clinical significance differs substantially: tumors with gross invasion into surrounding tissues have a worse prognosis and a higher risk for recurrence. Differences in the methodology of **BRAF** detection can also cause conflicting results among studies.

Surprisingly, distant metastases, the most significant factor of poor PTC outcome, were not associated with **BRAFV600E** mutation as a single factor. However, when that mutation was associated with other genetic events, the conjunction of events appeared as an independent predictor of distant metastasis (Costa et al., 2008).

A more definitive assessment of the impact of **BRAF** status on PTC prognosis can be derived solely from studies evaluating the long-term outcome of the disease. Only recently have reports on disease-free survival or overall survival in **BRAF**-positive versus **BRAF**-negative PTC patients been published (Table 4). Ito et al. (2009) evaluated disease-free survival and distant metastases-free survival with respect to **BRAF** mutation status in more than 600 patients followed for a mean 83 ± 35 months. The **BRAFV600E** mutation was detected in 38% of their series, but these investigators did not search for other far less frequent **BRAF** point mutations or rearrangements. The only significant difference among patient subgroups was the frequency of **BRAF** mutations in micro-PTC (28%) versus tumors larger than 1 cm (41%) (P = 0.0175), however, **BRAF** mutation prevalence did not successively increase with size in patients having tumors larger than 1 cm. In multivariate analysis, **BRAF** mutation was not linked to patient gender, disease stage, massive extrathyroidal extension, or lymph node/distant metastases at diagnosis. Importantly, the authors also did not find any difference in 5- or 10-year disease-free survival or metastases-free survival, which were about 90% in both **BRAF**-positive or **BRAF**-negative cases. This observation confirmed the findings of a previous multicenter Italian report involving PTC of all types (Fugazzola et al., 2004) and a Korean study (Kim et al., 2006b) involving the classic PTC variant. Contrary conclusions, supporting the correlation of **BRAF** status with disease-free survival, were reported by Kebebew et al. (2007) (mean follow-up of 6 years) who in multivariate analysis, noted a significant association of **BRAF** mutation with recurrence risk.

The recent study by Elisei et al. (2008), who analyzed 102 patients with a median follow-up of >10 years, is the only published study until now to address the relationship of **BRAF** mutation status...
Table 3
Correlation of BRAF mutation and clinicopathological prognostic factors in papillary thyroid carcinoma: data from recent studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Type of statistical analysis</th>
<th>Number of BRAF-positive cases (%)</th>
<th>Tumor size</th>
<th>Gender</th>
<th>Age</th>
<th>Extrathyroidal extension</th>
<th>Tumor capsule</th>
<th>Lymph node metastases</th>
<th>Distant metastases</th>
<th>Cancer stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosenbaum et al. (2005)</td>
<td>85</td>
<td>Univariate</td>
<td>54 (64%)</td>
<td>–</td>
<td>–</td>
<td>0.0001</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kim et al. (2006a)</td>
<td>103</td>
<td>Univariate</td>
<td>34 (33%)</td>
<td>0.0001</td>
<td>ns</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
<td>0.0001</td>
<td>–</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kim et al. (2006b)</td>
<td>203</td>
<td>Univariate</td>
<td>140 (73%)</td>
<td>0.006</td>
<td>0.006</td>
<td>ns</td>
<td>0.06</td>
<td>–</td>
<td>0.005 (gross invasion)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Kebebew et al. (2007)</td>
<td>274</td>
<td>Univariate</td>
<td>133 (49%)</td>
<td>ns</td>
<td>ns</td>
<td>0.03</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.04</td>
</tr>
<tr>
<td>Lupi et al. (2007)</td>
<td>500</td>
<td>Univariate</td>
<td>217 (44%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.00001</td>
</tr>
<tr>
<td>Lee et al. (2007)</td>
<td>1168</td>
<td>Univariate</td>
<td>570 (49%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.000</td>
<td>ns</td>
<td>–</td>
<td>ns</td>
<td>0.000</td>
</tr>
<tr>
<td>Costa et al. (2008)</td>
<td>49</td>
<td>Univariate</td>
<td>27 (55%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Elisei et al. (2008)</td>
<td>102</td>
<td>Univariate</td>
<td>38 (37%)</td>
<td>ns</td>
<td>ns</td>
<td>0.02</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.03</td>
</tr>
<tr>
<td>Wang et al. (2008a,b)</td>
<td>108</td>
<td>Univariate</td>
<td>54 (50%)</td>
<td>ns</td>
<td>–</td>
<td>0.2</td>
<td>0.02</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.04</td>
</tr>
<tr>
<td>Frasca et al. (2008)</td>
<td>323</td>
<td>Univariate</td>
<td>125 (39%)</td>
<td>0.005</td>
<td>–</td>
<td>–</td>
<td>0.0001</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.05</td>
</tr>
<tr>
<td>Lee et al. (2009)</td>
<td>64</td>
<td>Univariate</td>
<td>24 (38%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.001</td>
<td>–</td>
<td>0.003</td>
<td>–</td>
<td>0.001 (only T status)</td>
</tr>
<tr>
<td>Ito et al. (2009)</td>
<td>631</td>
<td>Univariate</td>
<td>242 (38%)</td>
<td>ns</td>
<td>ns</td>
<td>0.05</td>
<td>ns</td>
<td>ns</td>
<td>0.005</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Guan et al. (2009)</td>
<td>1032</td>
<td>Univariate</td>
<td>639 (62%)</td>
<td>ns</td>
<td>ns</td>
<td>0.003</td>
<td>ns</td>
<td>ns</td>
<td>0.005</td>
<td>–</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ns, not significant.
Table 4
Association of BRAF with disease-free and overall survival.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Disease-free survival (decrease)</th>
<th>Overall survival (decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al. (2006a,b)</td>
<td>203</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Fugazzola et al. (2004)</td>
<td>260</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Kebebew et al. (2007)</td>
<td>274</td>
<td>0.03 by multivariate logistic regression analysis</td>
<td>–</td>
</tr>
<tr>
<td>Abubaker et al. (2008)</td>
<td>536</td>
<td>0.01 by Kaplan-Meier analysis</td>
<td>–</td>
</tr>
<tr>
<td>Elisei et al. (2008)</td>
<td>102</td>
<td>0.03 by multivariate logistic regression analysis</td>
<td>0.015 by log-rank analysis</td>
</tr>
<tr>
<td>Ito et al. (2009)</td>
<td>631</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Costa et al. (2008)</td>
<td>202</td>
<td>ns</td>
<td>–</td>
</tr>
</tbody>
</table>

With overall survival. A higher rate of persistent disease, mortality, or both in was reported in BRAF-positive PTC patients ($P=0.005$). Multivariate logistic regression, that also included well-accepted clinicopathological factors, found significant correlation with negative outcomes only for BRAF status. Log-rank analysis confirmed the negative prognostic impact of BRAF mutation on overall survival, with deaths occurring practically exclusively in patients harboring BRAF-positive tumors. It must be stressed that patients in the Elisei study were followed substantially longer than were patients in studies negative for prognostic significance of BRAF mutation (Fugazzola et al., 2004; Ito et al., 2009; Kim et al., 2006b). However, genetic background differences also may explain the discrepant results of the studies.

A recent study on the molecular biology of recurrent PTC (Henderson et al., 2009) is far less convincing than the Elisei et al. study (Elisei et al., 2008). The authors report that the BRAF mutation was found in 77.8% of recurrences, which far exceeds the frequency seen historically in primary PTCs. However, in the majority of patients, these investigators did not compare the BRAF status in the recurrent tumors versus in the primary tumors. Additionally, they analyzed only lymph node recurrences, and the lower limit of their range of times-to-recurrence started at 4 months, an interval short enough to suggest that the disease might be attributable to primary treatment failure rather than true recurrence.

3.2. Is it the time to apply BRAF as clinically relevant prognostic/predictive factor in PTC?

The question, whether we are ready to implement the BRAF-oncogene as a molecular prognostic factor in PTC is answered positively by the majority of recent publications. However, the authors of this review would like to adopt a more cautious position and indicate what we believe is still necessary before BRAF might be used as a clinically relevant factor of poor prognosis in DTC. Let us list our major doubts:

1. As stated earlier, the frequency of BRAF mutation in PTC is high—45% on average, with values over 70—80% in some populations. This high prevalence will mean that implementing BRAF mutation as a factor of poor prognosis will shift many DTC patients, considered up to now as low risk patients, in the group of patients who require more extensive therapy (Table 5). Our evaluation indicates that as many as 31% of all PTC patients and 39% of those diagnosed with stage I–II disease, will face the risk of overtreatment if the decision will be based on the BRAF-positivity of their tumors, among them at least of (1/4) of patients with micro-PTC, who constitute up to 44% of patients with tumor ≤2 cm (Bonnet et al., 2009) and who were considered until now as having an excellent prognosis. We think that, as of now, the evidence-based support for such consequences is very weak and the risk of overtreatment is significant. As an example, a 32-year-old pregnant woman (10th week of pregnancy) with an incidentally detected hypoechoic nodule 8 mm in diameter was referred to our department because of cytologic diagnosis of PTC. No enlarged lymph nodes were palpated or seen by sonography. The recommendation based on current guidelines (Cooper et al., 2009) would be to monitor carefully and, if no signs of distinct progression are observed, to perform thyroidectomy after delivery. However, for better evaluation, BRAF mutation was sought and confirmed to be present in the cytologic material obtained by fine needle aspiration biopsy. Due to the presence of this molecular factor, the consulting surgeon decided to perform surgery in the second trimester. In our opinion, the risk of overtreatment based on molecular diagnosis in this case was significant, since at least 20% of women of this age presenting with PTC would bear BRAF-positive tumors, while the clinical data clearly show that the prognosis is excellent in these cases.

On the other hand, the risk of undertreatment in the young BRAF-negative patients should be considered.

2. The relevance of BRAF status to the indications for radioiodine treatment seems still not well explained. On the one side, there are plenty of data showing that BRAF mutation correlates with a poor response to radioiodine due to a lack of proper membrane sodium–iodine symporter expression (see further discussion below). On the other side, there are proposals to apply more radioiodine in BRAF-positive patients due to the poorer prognosis. What we lack is evidence that more radioiodine will be helpful in these patients. Approaches to increase tumor radiosensitivity or to apply complementary pharmacological therapy in these patients would appear to be more reasonable.

For the evaluation of the prognostic significance of BRAF in thyroid cancer, a comparison with one of the most aggressive cancers, melanoma, may be of value. As already mentioned, BRAF mutations are frequent in both cancers and are found at early disease stage—in thyroid in micro-PTC and in skin, in the majority of benign nevi, the precursors of melanomas. In benign nevi, the clonal activation of MAPK by BRAF is followed by induction of senescence which provides a barrier against tumor progression (Wajapeeyee et al., 2008). Similarly, the expression of mutated BRAF in lung epithelium results in development of benign tumors that express senescence markers and only rarely progress to malignant tumors unless functional p53 or p16 are absent (Dankort et al., 2007). BRAF-induced senescence has not been analyzed in thyroid tissues and can difficult due to lack of benign counterpart to PTC. However, this hypothesis may explain why BRAF is surprisingly frequent in indolent micro-PTCs—only the next molecular event(s) is/are necessary to accelerate its growth to clinically evident disease. In this case, we should focus on identifying events, secondary to BRAF mutation, which may be of better prognostic/predictive value then BRAF itself. According to Knauf and Fagin (2009), in PTC, mechanisms for overcoming senescence probably do not require loss of functional p53, which occurs late in progression to poorly differentiated thyroid carcinoma, nor rise in p16.
3.3. Other molecular initiating events specific for PTC—do they have prognostic potential?

3.3.1. RET/PTC rearrangements

RET is a transmembrane tyrosine kinase receptor, the constitutive activation of which by point mutation in parafollicular C cells leads to medullary thyroid cancer. RET/PTC rearrangements, where the 3′ or tyrosine kinase domain of the RET gene is fused with the 5′ domain of one of several constitutively expressed genes, cause the constitutive activation of the RET gene, which otherwise is normally silent in follicular cells (Santoro et al., 2004). The fusion leaves intact the tyrosine domain (TK) of the RET receptor and enables the RET/PTC oncoprotein to activate the MAPK cascade (Knauf et al., 2003b). Several studies suggest that the oncogenic effects of RET/PTC rearrangements require signaling along the MAPK pathway in the presence of functional BRAF kinase (Knauf et al., 2003a; Mitsutake et al., 2006).

RET/PTC rearrangements may arise from fusion with a wide range of house-keeping genes (until now, 12 affected genes and circa 15 rearrangements have been described), and constitute an event specific both for organ (thyroid) and histotype (PTC) (Fagin and Mitsiades, 2008; Santoro et al., 2004). However, some data indicate the possibility of RET/PTC rearrangements also in lymphocytic thyroiditis (Rhoden et al., 2006).

Transfection of the RET/PTC1 gene in normal rat thyroid cells resulted in loss of differentiation and of TSH growth dependency. However, the cells were totally transformed only after transfection with RET/PTC and mutated RAS genes, suggesting that simultaneous activation of several genes was necessary for tumor progression. This observation, together with the frequent occurrence of RET/PTC rearrangements in papillary microcarcinoma, suggests that such rearrangements are an early event in lymphocytic thyroiditis (Rhoden et al., 2006).

Although molecular changes observed in other cancer cannot be directly translated to PTC, we should also take into account that in other types of cancer (e.g., colorectal, ovarian, lung) BRAF mutation is not related to poorer prognosis, on the contrary, it is observed in cases with better outcome. In fact, the generally more favorable outcome of BRAF-induced cancers may be the cause of the generally good prognosis in PTC. This stresses once more the need to search for molecular markers which shall stratify BRAF-positive PTC cases and help to specify patients with truly poor prognosis.

### 3.3. Other molecular initiating events specific for PTC—do they have prognostic potential?

<table>
<thead>
<tr>
<th>Stage</th>
<th>% of patients with RET/PTC</th>
<th>Risk of over-treatment</th>
<th>Risk of undertreatment</th>
<th>General evaluation of the risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13%</td>
<td>(A) &lt;1 (1-B) x (1-C)</td>
<td>1%</td>
<td>Acceptable</td>
</tr>
<tr>
<td>II</td>
<td>10%</td>
<td>(B) 26%</td>
<td>3%</td>
<td>Considerable</td>
</tr>
<tr>
<td>III</td>
<td>5%</td>
<td>(C) 5%</td>
<td>5%</td>
<td>Acceptable</td>
</tr>
<tr>
<td>IV</td>
<td>5%</td>
<td>(D) 2%</td>
<td>9%</td>
<td>Acceptable</td>
</tr>
<tr>
<td>All ages</td>
<td>10%</td>
<td>A x B x C</td>
<td>3%</td>
<td>Considerable</td>
</tr>
</tbody>
</table>

---

CSS: cancer-specific survival; DFS, disease-free survival; PTC, papillary thyroid cancer.

---

Data extracted from Jonklaas et al. (2006).

---

Data extracted from the meta-analysis of Lee et al. (2007).

---

Because of poor prognosis in this subgroup.
higher occurrence of lymph node metastases was correlated with younger age rather than with RET/PTC rearrangements (Collins et al., 2002).

3.3.2. NTRK rearrangements

NTRK1 is another membrane tyrosine kinase receptor which regulates growth, differentiation and apoptosis in the peripheral and central nervous system, with nerve growth factor acting as its ligand. Similarly to RET, NTRK-3 is activated by rearrangements with genes constitutively expressed in thyroid follicular cells. Activation of the oncoproteins TRK1-3 is ensured by autophosphorylation of the tyrosine kinase domain (Pierotti et al., 1996). NTRK rearrangements are rare and are found in <10% of PTCs (Brzezianska et al., 2008; Roque et al., 2001). Their rarity precludes the studies on their prognostic roles.

3.3.3. Gene rearrangements are frequent in thyroid cancers

Until recently, gene rearrangements were regarded as characteristic of hematological malignancies. Among solid tumors, they were found only in sarcomas and thyroid carcinomas. This picture is now being changed by the detection of very small chromosomal deletions/amplifications in other solid tumors, e.g., prostate cancer (Carver et al., 2009; Tomlins et al., 2009). Nevertheless, predisposition of thyroid cells to undergo gene rearrangement is not completely understood (Teixeira, 2006; Wang et al., 2008b). The juxtaposing of the loci participating in RET/PTC and NTRK1 rearrangements in interphase nuclei of thyroid follicular cells has been reported (Gandhi et al., 2006; Nikiforova et al., 2000; Roccato et al., 2005). Interestingly, these rearrangements are more frequently found in young patients (Collins et al., 2002; Fenton et al., 2000; Wang et al., 2008a). Of note, though the decisive factor(s) explaining the good prognosis of PTC in young patients, remains unclear: is it the biology of the tumor (which is caused by a gene rearrangement, not BRAF mutation) or of the host (differing immunity in young patients) or of both (Jarzab et al., 2005a).

3.4. Is there some multiclonality in papillary thyroid cancer?

Many data indicate that, despite the general rule of non-overlap between the main PTC-inducing mutations, which are regarded as mutually exclusive (Fagin, 2004), there is a substantial degree of multiclonality in this type of thyroid cancer. Both BRAF mutations and RET/PTC rearrangements can be found as non-clonal changes and different types of RET/PTC rearrangement may be found in different tumor foci from the same patient (Aherne et al., 2006; Wang et al., 2008b). The juxta-positioning of the loci participating in RET/PTC and NTRK1 rearrangements in interphase nuclei of thyroid follicular cells has been reported (Gandhi et al., 2006; Nikiforova et al., 2000; Roccato et al., 2005). Interestingly, these rearrangements are more frequently found in young patients (Collins et al., 2002; Fenton et al., 2000; Wang et al., 2008a). Of note, though the decisive factor(s) explaining the good prognosis of PTC in young patients, remains unclear: is it the biology of the tumor (which is caused by a gene rearrangement, not BRAF mutation) or of the host (differing immunity in young patients) or of both (Jarzab et al., 2005a).

3.5. Gene mutations important for development of both papillary and follicular thyroid cancer

3.5.1. RAS mutations

The RAS–RAF–MEK–ERK1/2 pathway is hyperactivated in 30% of human cancers (Balmanno and Cook, 2009). RAS is a membrane-associated, small protein with guanosine triphosphate (GTP) binding ability, involved in proliferation, differentiation and cell survival. Translocation of RAS to the cytoplasmatic membrane is an important step in the protein’s activation. Farnesylation of RAS is the first obligatory step in a series of post-translational modifications leading to membrane association, which, in turn, determines the switch from an inactive to an active form. RAS is activated by a variety of membrane receptors, mainly belonging to the tyrosine kinase receptor family, rarely G proteins. Non-membrane coupled tyrosine kinases can also to activate RAS, with the down-stream activation of different effector pathways.

The RAS protein is encoded by three genes, H-, N- and K-RAS and each of them may underlie activating point mutations, occurring most frequently at codon 12 or 61. Such mutations lead to the change in conformation of the protein so that it remains bound to GTP, i.e., constitutively active (while in normal conditions GTP-ase activity of RAS leads to RAS deactivation). In some cancers, like colon cancer, RAS mutation is regarded as the first oncogenic event necessary but not sufficient for the cell to cross over the benign adenoma/cancer barrier (Takayama et al., 2006) as RAS mutations are found with the same frequency in colonic adenomas as in colonic cancers. In some other types of cancer RAS mutations occur at later stages of carcinogenesis.

In thyroid gland, RAS mutations are found already in follicular thyroid adenomas (FAs) and the transition from the follicular adenoma to follicular cancer requires the next molecular step (although, unlike in colon cancer, adenomas are not regarded as precancerous stage for FTC). RAS mutations were described in some 19% of FAs and in up to about 50% of FTC (Esapa et al., 1999; Shi et al., 1991; Suarez et al., 1990), however, in more recent studies RAS mutations are evaluated with an overall frequency of about 20% (Garcia-Rostand et al., 2003; Vasko et al., 2003).

Importantly, RAS mutations are not thyroid cancer histotype-specific and are also found in FTC, with the prevalence ranging from 0% to 15% (Abrosimov et al., 2007b; Adeniran et al., 2006; Hou et al., 2007; Vasko et al., 2003). These mutations are rarest in the classical variant of PTC, and more frequent in the follicular variant, where their prevalence may reach even 20–50% (Goutas et al., 2008; Zhu et al., 2003). Some studies indicate that N2-RAS mutations are particularly frequent (Abubaker et al., 2008; Hou et al., 2007; Wang et al., 2007), other focus on K-RAS (Goutas et al., 2008) which are seen in FTC with lower frequency (Fagin and Mitsiades, 2008). Their frequency in anaplastic thyroid cancer is estimated as to be about 20–25% (Smallridge et al., 2009).

RAS mutation can promote thyroid tumorigenesis through the RAF–MEK–ERK1/2 pathway or through its interaction with PI3K/AKT pathway (Xing, 2005). Garcia-Rostand et al. (2003) reported the association of RAS mutations with aggressive thyroid cancer phenotypes and poor prognosis: 55% (11/20) of patients with RAS-positive well-differentiated thyroid cancers died in comparison to 15% (9/58) RAS-negative ones (P = 0.016). There are no other reports on this issue, however, the observation that RAS mutations are rather frequent in poorly differentiated thyroid cancers (40–55%) supports this notion (Fagin and Mitsiades, 2008).

3.6. Follicular thyroid cancer-specific gene mutations

3.6.1. PAX8/PPARγ rearrangements

This type of rearrangement results from the translocation between the PAX8 (paired box gene 8) gene, which encodes a paired domain transcriptional factor, and the peroxisome proliferator-activated receptor (PPARγ) gene. The resulting fusion protein (PPF; PAX8/PPARγ fusion protein) encodes a nearly full-length PPARγ, the expression of which is under the transcriptional regulation of the PAX8 promoter (Kroll et al., 2000). The functional consequences of this PAX8/PPARγ rearrangement are yet not fully understood. Some studies have shown negative effect of PAX8/PPARγ rearrangement on the function of wild-type PPARγ (Gregory et al., 2004), however other have questioned this negative effect and showed the up-regulation of many genes which are transcriptional targets of PPARγ (Giordano et al., 2005). Another possible oncogenic mechanism of PAX8/PPARγ rearrangement is deregulation of PAX8 function, which is critical for thyroid cell differentiation (Reddi...
3.7.3. the non-clonal event in 14/18 PTCs investigated (Puxeddu et al., 2007). In FTC, 18
D. Handkiewicz-Junak et al. / Molecular and Cellular Endocrinology
naling pathway and is in fact a Wnt nuclear effector (Fagin and
of growth-promoting genes. It constitutes an element of Wnt sig-
and non-degraded, plays a role in gene transcription regulation
2008; Sahin et al., 2005).
3.7.2. PTEN mutations
3.6.2. RAS homolog 1 (ARH1) silencing
As already mentioned, the mechanisms of transition from a
benign to a malignant tumor, from FA to FTC, are not well known.
High frequency of loss of heterozygosity in imprinted genomic
regions has been suggested to contribute to this transition (Sarquis
et al., 2006). One of the proposed mechanisms is the reduction of the
number of ARH1 copies. Weber et al. (2005a) showed that relatively
few FAs but the majority of FTCs, including minimally invasive FTCs,
show marked ARH1 mRNA underexpression, due to deletion of the
non-imprinted second allele in conjunction with hypermethylation of
the genomically imprinted allele.
3.7. Mutations of other genes and their importance for thyroid
cancer and its outcome
3.7.1. PIK3CA mutations and amplification
PIK3CA mutation is not a common mechanism in the activation of
PI3K/AKT in thyroid carcinoma while amplification of this gene
is more frequent. In PTC, rare mutations and much more frequent
amplifications, comprise altogether 15–53% of tumors (Abubaker
et al., 2008; Hou et al., 2007). PIK3CA amplification is observed also in
FTC and even in FAs (Hou et al., 2007; Wu et al., 2005); however, it
is the most frequent in anaplastic thyroid cancer. Its down-stream
target, AKT, is phosphorylated in at least half of PTC, but independ-
ently of PIK3CA mutation or amplification (Abubaker et al., 2008).
3.7.2. PTEN mutations
PTEN (phosphatase and tensin homolog) is a dual-specific
phosphatase, a negative regulator of the AKT/PI3K pathway
by dephosphorylation of phosphatidylinositol-3,4,5-triphosphate
(PIP3), which can also influence the MAPK pathway. PTEN is a tumor
suppressor gene and its germline loss of function mutations pre-
dispose to multiple tumors including FTC (Yeh et al., 1999). Single
cases of loss of function somatic mutation of PTEN were described in
FTC (7%), somewhat more frequently in FTC (12–50%) and rarely
in PTC (2%) (Friisk et al., 2002; Garcia-Rostan et al., 2005; Gimm et
al., 2000; Hou et al., 2007; Smallridge et al., 2009). A novel rear-
drangement involving PTEN and the H4 gene, has been described as
the non-clonal event in 14/18 PTCs investigated (Puxeddu et al.,
2005) and as a feature present in 4.8% of a larger series of PTCs in
Chinese patients (Wang et al., 2008b). All these rearrangements are
paracentric inversions of chromosome 10q, the same chromosome
which is responsible for RET rearrangements.
3.7.3. β-Catenin mutations
The β-catenin protein, when bound to E-cadherin, mediates
cell skeleton/adhesion interactions, and when non-sequestered and
non-degraded, plays a role in gene transcription regulation of
growth-promoting genes. It constitutes an element of Wnt sig-
naling pathway and is in fact a Wnt nuclear effector (Fagin and
Mitsiades, 2008). The Wingless (Wnt) family of secreted glyco-
proteins controls early developmental processes including cellular
migration, proliferation and differentiation. Non-canonical Wnt
signaling is associated with tumorigenesis and Wnt5a has been
reported to be increased in the majority of FTCs but in only some
FTCs (Kremenevskaja et al., 2005). Mutations of β-catenin gene
(CTNNB1) are most frequent in poorly differentiated and anaplastic
cancer (Garcia-Rostan et al., 1999; Garcia-Rostan et al., 2001).
3.7.4. TP53 mutations
TP53 encodes a multifunctional nuclear protein which is impor-
tant for cell cycle arrest at DNA damage, senescence or apoptosis
and is one of the best known tumor suppressor genes. Contrary
to what is seen in many other cancers, TP53 loss of function
mutations occur late in thyroid tumorigenesis and are practically
absent in DTC (0–9%), while the prevalence of these mutations
rises as the tumor grade worsens, reaching 17–38% in poorly dif-
ferentiated and 55–88% (Fagin and Mitsiades, 2008; Smallridge
et al., 2009) in anaplastic cancers. At this moment, the features
of poor differentiation are easily detected histologically, thus, the
immunohistochemical or molecular investigation of TP53, docu-
mented widely in other tumors, has not gained clinical significance
in thyroid cancer.
4. Prognostic aspects of other gene expression changes in
papillary and follicular thyroid cancer
Over the last two decades many gene expression changes have
been described in papillary and follicular thyroid cancers and
related to the stage of the disease or its outcome. The clear distinc-
tion between the changes characteristic for papillary and follicular
cancers is however not possible for many reasons: First, due to
the much higher frequency of PTC, most of the genes described
below were either not examined in FTC or investigated in small
groups of FTC cases, not allowing for specific distinction. Second,
many of those genes in which such comparison was performed,
showed only minor differences between histotypes because they
were late consequences of the neoplastic transformation of the fol-
icular thyroid cell. Thus, it is rationale to look for their prognostic
significance independently from the histotype or variant of thyroid
cancer. In the given below brief list of genes, expression changes of
which has putative or proved prognostic relevance, we inform on
histotype-specific changes only then, when they were investigated
with sufficient power.
4.1. Single genes expression changes
4.1.1. MET proto-oncogene
The MET proto-oncogene encodes a membrane tyrosine kinase
receptor for hepatocyte growth factor (HGF). HGF is a potent mito-
gen for epithelial cells and promotes cell motility and invasion.
About 50% of cases of PTC are characterized by MET overexpression
(Di Renzo et al., 1992), which is believed to be a sign of more aggres-
sive disease (Mineo et al., 2004; Ramirez et al., 2000). BRAF-positive
tumors were associated wit MET overexpression in aneuploid PTCs
(Rodrigues et al., 2007).
4.1.2. Epithelial growth factor receptor (EGFR)
Among receptor tyrosine kinases EGFR belongs to the best
known. EGFR (HER1) protein levels are increased in PTCs in com-
parison to normal thyroid tissue and the high expression of EGFR
has been suggested to be associated with worse outcome of PTC
after thyroidectomy (Akslen and Varhaug, 1995), an observation
not repeated in other studies (Ruan et al., 2008). Another early
study indicated on HER2 as the predictor of metastasis in PTC
(Kremser et al., 2003). The immunohistochemical evaluation of
EGFRVIII showed its expression in the majority of PTCs (Omidfar et al., 2009). In the study of Wiseman et al. (2008a), which investigated expression of HER1–4 genes, overexpression of at least one of them was observed in 76% of DTC and only HER3 was positively correlated with DTC stage, while HER4 was inversely correlated with T stage. In this context it is important that HER3 (ERBB3) is characteristic for BRAF-induced PTCs (Giordano et al., 2005).

Until recently, it was assumed that activating mutations of EGFR do not play any role in the molecular biology of DTC (Omidfar et al., 2009). However, the recent finding of Masago et al. (Masago et al., 2009) indicated that EGFR mutations commonly seen in pulmonary carcinoma may be observed in a subset of papillary or poorly differentiated thyroid cancers which may make tumor cells oncogene-addicted.

4.1.3. Mitogen-inducible gene-6 (MIG-6)
MIG-6 is an immediate early response gene, expression of which is induced by cellular stress, hormones or growth factors in many cells. It exerts a negative effect on EGFR signaling that is mediated by the MAPK cascade (Ferby et al., 2006). Because MIG–6 expression is induced by MEK–ERK activation, such expression may be regarded as a negative feedback loop signal in normal cells, lost in cancer. In PTC, MIG-6 directly correlated with EGFR expression and its higher mRNA level was associated with better overall survival in a population of 106 PTC patients followed for six years. Interestingly, MIG-6 expression was independently predictive of disease-free survival in BRAF-positive patients (Ferby et al., 2006).

4.1.4. Vascular epithelial growth factor (VEGF) and VEGF receptor
VEGF overexpression is a characteristic feature of malignant tumors, seen in thyroid cancer as in other cancers (Wiseman et al., 2008b) and may be interpreted as marker of tumor hypoxia. However, not in all cancers it is correlated with HIF-1alpha expression. Thyroid cancer belongs to those tumors in which hypoxia-inducible factor-1alpha is up-regulated at the same time that VEGF is overexpressed (Jubb et al., 2004). The correlation of VEGF expression with the expression of other angiogenic factors is strong (Tanaka et al., 2002). Quantitative evaluation of VEGF expression in PTC showed the association of this phenomenon with metastatic PTC (Klein et al., 2001). VEGF expression was closely correlated with tumor size, extrathyroidal invasion and BRAF presence (Jo et al., 2006), while Tian et al. (2008) observed the correlation with lymph node metastases both for VEGF and metalloproteinase MMP2. In the study of Jo et al. (Jo et al., 2006), VEGF was the only molecular marker associated with tumor size, extrathyroidal invasion and cancer stage by univariate analysis and the association with extrathyroidal invasion was confirmed in multivariate analysis, while VEGF expression was only weakly associated with PTC recurrence in young patients (Fenton et al., 2000).

4.1.5. E-cadherin and catenins
E-cadherin (CDH1) is a calcium-dependent transmembrane glycoprotein that functions as a cell-cell adhesion molecule. It has an intracellular domain that complexes with catenin proteins for regulation of further Wnt-mediated transcriptional signaling to the nucleus. CDH1 expression is reduced in thyroid carcinomas and has been associated with poor prognosis, not only in PTC (von Rhein et al., 1997) but also in FTC (Brecelj et al., 2005) as well as with PTC-to-ATC transition (Wiseman et al., 2007). Dislocalization of catenins in thyroid cancer may be secondary to loss of E-cadherin (Bohm et al., 2000; Rocha et al., 2003). However, the study of Kapran et al. (2002) did not show any association of E-cadherin or catenins expression with PTC recurrence.

4.1.6. Cyclin D1 and other cell cycle regulators
Association of overexpression of cyclin D1, one of the target genes of Wnt signaling pathway, with more advanced PTC, has been addressed for a long time (Ferenc et al., 2005; Ito et al., 2005). Although cyclin D1 and other cell cycle regulators, such as cyclin E, p16, p21 were overexpressed in thyroid cancer, only p16 expression correlated significantly with extrathyroidal tumor extension and the presence of lymph node metastases in the study of Melck et al. (2007). Also, in the study of Pesutic-Pisac et al. (2008) loss of p27 was more essential than cyclin D1 overexpression, while Khoo et al. (2002) suggested the contribution of both markers. The study of Hoos et al. (2002) was negative for correlation of cyclin D1 expression with indices of prognostic relevance in oncocytic thyroid cancers.

4.1.7. Mucin 1
Mucin 1 (MUC1) is a glycoprotein important for cell adhesion, overexpressed in about 25% of PTC (Wreesmann et al., 2004). Preclinical studies support the role of MUC1 in promoting an aggressive phenotype of PTC (Patel et al., 2005). MUC1 overexpression is especially frequent in the tall-cell variant of PTC, known for its poor prognosis (Ghossein and LiVolsi, 2008). Identified as an independent prognostic marker by genome-wide gene expression profiling of PTC, MUC1 overexpression was described to be significantly associated with the treatment outcome (Wreesmann et al., 2004), independent of extrathyroidal extension or of the PTC variant. However, other reports question the correlation of MUC1 overexpression with PTC prognosis (Abrosimov et al., 2007a; Min et al., 2008). The significance of MUC1 overexpression for cancer outcome may be more essential if we consider MUC1 contribution to the survival network, causing resistance to genotoxic agents in thyroid cancer (Siraugsu et al., 2007).

4.1.8. S100A4 calcium-binding protein
This small calcium-binding protein also called calvasculin has been reported to promote metastasis and was indicated as a molecular factor of poor prognosis in PTC (Zou et al., 2004, 2005). In papillary microcarcinomas, the expression of S100A4 correlated more strongly with lymph node metastases than did any other molecular factor investigated and was more significant for association with stage than BRAF status (Min et al., 2008).

4.1.9. Osteopontin and CD44v6
Osteopontin (SPP1) is a cytokine regulating cell trafficking within the immune system, binding a splice variant of CD44, that is overexpressed in PTC and contributes to the motigenesis (Figge et al., 1994), survival and motility of PTC thyrocytes and their invasion potential (Guriano et al., 2005). In PTC, the gene expression level of SPP1 was associated with metastasis but not with the BRAF status of the tumor (Oler et al., 2008).

4.1.10. Immunity-related genes
Activation of the MAPK pathway induces up-regulation of chemokines and their receptors on tumor cells, relevant for their sustained proliferation and cell motility. Tumors use molecules of the innate immune system for their growth, survival and metastasis (Melillo et al., 2005). Expression of toll-like receptor 3 (TLR3) is characteristic for PTC but not FTC (McCall et al., 2007). The basally expressed receptors are functional and able to activate the NF-kB pathway and to increase the expression of interferon IFNβ and CXCL10 chemokine. Interestingly, TLK5 overexpression is coordinated with WNT5a expression.

Chemokine receptors CXCR2–CXCR4 and CCR7 are expressed on PTC cells (Melillo et al., 2005; Sancho et al., 2006; Wagner et al., 2008) and CCR7 was reported to be associated with pathologic factors of PTC aggressiveness such as extrathyroidal tumor extension,
The same procedure done for extrathyroidal extension was even more sensitive. Unfortunately, this gene signature has not yet been verified by another group.

4.2.2. Metastasis-associated genes in thyroid cancer

Although the survival rate in well-differentiated thyroid cancer is excellent, that is not the case in the 5–10% of patients with distant metastases. Thus, identification of a metastasis-related gene signature is desirable. One of the first approaches was carried out by Zou et al. (2004), who established a thyroid cancer cell line with high metastatic capacity and specified genes characteristic of this line, among them MET, ezrin, integrin, motility-related protein-1, cadherin P, NEDD5 and S100A4. In their quantitative real-time PCR (qPCR) validation study, mentioned already above, they confirmed S100A4 to be associated with poor thyroid cancer prognosis. Two other genes were listed by Oler et al. (2008) as metastasis-associated; CXCL14 and cystatin M. However, the definition of metastatic PTC was very poor in this study. Some comparisons were also performed by Rodrigues et al. (2007) who reported low expression of many genes in M1 aneuploid PTCs.

4.2.3. Epithelial-to-mesenchymal transition in PTC

In the search for key molecular prognostic factors in thyroid cancer, the mechanisms of tumor invasion must be considered. The process of epithelial-to-mesenchymal transition (EMT) has been implicated in the conversion of early-stage tumors to invasive malignancy and has been extensively studied in invasive PTC (Vasko et al., 2007). After loss of cell-to-cell contacts and remodeling of the cytoskeleton, tumor cells are more prone to migration and this process is marked by loss of cadherin-E expression. Integrin pathway, Notch, MET, TGF-beta, NFkB, PI3K and p21-activated kinase belong to EMT-regulating proteins, also fibronectin 1 is considered as EMT related. In the study of Vasko et al. (2007), microarray analysis of the central part and of the invasive front of selected PTC tumors has been performed, showing in the latter the overexpression of TGF-beta, NFkB and integrin pathway as well as small G protein regulators and CDC42. Overexpression of vimentin, the hallmark of EMT, was associated with tumor invasion and nodal metastasis and was correlated with RUNX2 expression, as judged by immunohistochemistry. Interestingly, there was no correlation of expression of these invasion-related proteins with tumor genotype, e.g., BRAF status. However, in the study of Watanabe et al. (2009) silencing of BRAF reduced the increased expression of vimentin. For PTC cell invasion, enhanced AKT activity is especially important, but there was no correlation of AKT with BRAF presence in PTC (Vasko et al., 2007). According to Wang et al. (2007), BRAF mutation and AKT pathway activation are mutually exclusive in PTC.

4.3. Genetic germline background and the prognosis of papillary and follicular thyroid cancer

Some 5–10% of all cancers developing in follicular cells occur on the background of familial predisposition, referred as familial non-medullary thyroid carcinoma (FNMC), with autosomal dominant mode of inheritance and penetrance increasing with age (Cavaco et al., 2008; Handkiewicz-Junak et al., 2006; Malchoff and Malchoff, 2006). This clinical entity develops most often (in over 85% of cases) as familial PTC and differs from sporadic cancer by earlier age of onset, more frequent multifocal disease, higher degree of aggressiveness and higher recurrence rate. Not all involved family members suffer from thyroid cancer, some develop multinodular goiter or follicular adenomas. The diseases are genetically heterogeneous, with some genes identified in single families but with only a few general susceptibility genes described (Canzian et al., 1998; Gudmundsson et al., 2009; Jadzieszewski et al., 2008; McKay et al., 2001; Ngan et al., 2009). Somatic BRAF or RAS mutations are found in...
familial PTCs with similar frequency as in sporadic tumors (Cavaco et al., 2008). In this aspect it is important to add that there are no data suggesting that any particular genetic background facilitates the \textit{BRAF} mutation what could be suspected considering the presence of a unique \textit{BRAF} mutation present in a large proportion of PTC cases. However, environmental influences are also conceivable, as suggested by recent Chinese data showing that \textit{BRAF}-positive PTCs are significantly more frequent in areas of extremely high iodine intake (Guan et al., 2009) and by comparison of different Sicilian areas (Frasca et al., 2008).

5. Final considerations on prognostic impact of genetic factors in papillary and follicular thyroid cancer

5.1. Relation between \textit{BRAF} status and gene expression profile

5.1.1. Is \textit{BRAF}-positive DTC a distinct molecular subtype?

The mutational status of PTC correlates well with its gene expression profile, allowing bioinformatical analysis of genome-wide data to clearly differentiate \textit{BRAF}, \textit{RAS} or \textit{RET}/PTC mutants, even if an unsupervised approach, Principal Component Analysis, is applied. The most important paper on this issue, published by Giordano et al. (2005), showed distinctly that PTC mutational status was even more strongly correlated with gene expression profile than with morphology as evaluated by subdivision into PTC variants. When comparing \textit{RET}/PTC mutants versus \textit{BRAF} mutants, 3891 probesets with $P < 0.01$ were specified, with a similar distance of both of these mutant types from \textit{RAS} mutants, the number of different genes being on average approximately 20 times as many as obtained for permuted data sets. By further selection of genes for those that yielded $P < 0.01$ as well as a fold-change <0.5 or >2 for any group compared to any other, the \textit{BRAF} mutants were characterized by 82 probesets (31 up-, 51 down-expressed). These probesets had a very strong significance, verified, as required in genomic studies, by the False Discovery Ratio (FDR), which was 0.5 (it is well accepted in genomic studies that significant genes should show FDR under 5–10%). Many genes were down-regulated in \textit{RAS} mutants, while many increases were common between \textit{BRAF} and \textit{RET}/PTC mutant tumors. The most differentially expressed gene in the \textit{BRAF} mutants was \textit{TM7SF4}. Its product is a dendritic cell protein, likely related to the immune response in these tumors.

Although the other studies published were less powerful (Frattini et al., 2004; Musholt et al., 2006), they did not contradict the basic message of the Giordano study: the gene expression profile of \textit{BRAF}-positive PTC is very distinct and easily recognizable. Oler et al. (2008) described that the expression of a newly discovered chemokine, CXCL14, and of cystatin M, a secreted inhibitor of lysosomal cysteine proteases, was not only markedly elevated in PTC, but also significantly higher in \textit{BRAF}-positive versus \textit{BRAF}-negative tumors. Both genes were simultaneously associated with metastatic PTC. Also, expression of such PTC-characteristic genes as fibronectin 1, CITED1 and vimentin were positively correlated
with the expression of BRAFV600E (Watanabe et al., 2009). The conditional expression of BRAFV600E and RET/PTC showed that the more invasive potential of BRAF-mutated tumors may be at least partially explained by more distinct overexpression of metalloproteinases MMP3, MMP9 and MMP13 (Mesa et al., 2006).

All these data, especially regarding genome-wide differences, substantiate the hypothesis that BRAF-induced PTC is in fact a distinct molecular subtype of PTC, with a different, more aggressive disease course and a poorer outcome, which demands a subtype-specific treatment. However, prospective data still are necessary to prove this theory.

5.1.2. BRAF-mutated PTCs exhibit fewer thyroid-specific gene expression features

Many authors have confirmed the lower expression of thyroid-specific genes in BRAF-positive PTCs: the gene expression profiling study by Giordano et al. (2005) noted a down-expression of TPO and the study of Durante et al. (2007) confirmed the earlier observations of the more intensive loss of gene expression of NIS (−82%), AIF-B (−86%), TPO (−90%), and TG (−46%) in BRAF-mutated PTCs. When BRAF-positive and BRAF-negative PTCs are compared, the level of NIS gene expression is at least 5 times lower in the former (Durante et al., 2007). Not only is there a BRAF-dependent down-regulation of NIS expression on the transcriptional level, but also, the trafficking of the NIS protein to the cell membrane is impaired in BRAF-positive PTCs (Riesco-Eizaguirre et al., 2006). Moreover, suppression of the BRAF/MEK/MAPK pathway restores expression of iodoide-metabolizing genes (Liu et al., 2007a). Unlike the case with NIS or TPO genes, the expression of TSH-R and PAX8 seems not to be different in BRAF-positive tumors in comparison to other PTCs (Durante et al., 2007).

To interpret these data, some more general remarks seem necessary: the down-expression of thyroid-specific genes is not specific to BRAF-induced tumors, but is seen on the RNA level, the protein level or both in every case of thyroid cancer (Ambroziak et al., 2005; Huang et al., 2001; Krause et al., 1999; Skubis-Zegadlo et al., 2005). Especially, the direct attribution of the poorer outcome of BRAF-positive PTC to the down-regulation of thyroid differentiation-related genes seems precarious. These two facts are only associated, the causative relation is not proven. It appears to be more aggressive tumor biology rather than poorer response to radioiodine therapy which causes poorer outcome in BRAF-positive tumors. The impact of the lower expression of NIS protein in BRAF-mutated tumors is diminished by the fact that both BRAF-positive and BRAF-negative tumors exhibit mainly cytoplasmic localization of NIS, which precludes that protein's functionality. The down-expression of NIS in BRAF-positive PTCs is seen also in microcarcinomas (Oler and Cerutti, 2009) and even in these low-risk tumors, BRAF mutation has been related to development of more advanced disease, while the prognosis is excellent.

5.1.3. BRAF mutation is associated with the more aggressive tumor phenotype in PTC

The exact molecular mechanisms of the invasive potential of BRAF-induced tumors are still not known. As mentioned above, the poorer outcome in patients bearing BRAF-positive tumors is with certainty not merely the result of poorer efficacy of radioiodine treatment, due to impaired radioiodine uptake and retention. The prominent activation of the MEK–ERK1/2 pathway in BRAF-mutated thyrocytes is well known, but the cascade of subsequent events is not well defined. Interestingly, MET overexpression, an event related to poorer PTC prognosis, is seen mainly in BRAF-positive tumors (Giordano et al., 2005). Also, the mean Ki-67 index, an indicator of tumor cell proliferation, has been reported to be significantly higher in BRAF-positive than in BRAF-negative patients (1.01% versus 0.1%) (Nakayama et al., 2007); importantly, both these Ki-67 values are rather low, which is a known feature of PTC (Saltman et al., 2006). The increased GLUT-1 expression in BRAF-positive tumors may be interpreted as the sign of such tumors’ higher aggressivity. A multivariate analysis concluded that BRAF-positive tumors have higher VEGF expression (Jo et al., 2006). Recent reports indicate that prohibitin, a multifunctional protein, is overexpressed only in PTCs bearing a BRAF mutation. The most plausible mechanism for prohibitin overexpression is the increased gene promoter activity by the BRAF oncoprotein (Franzoni et al., 2009).

Nevertheless, recent publications indicate that some molecular markers correlate more closely with clinicopathological factors of poor prognosis than does BRAF-oncogene presence itself. In contrast to BRAF-positivity, S100A4 and cyclin D1 overexpression predicted lymph node metastasis in a series of nearly 200 PTC microcarcinomas (Min et al., 2008). The S100A4 gene was also investigated in a more detailed study and shown to be significantly associated with metastases (Zou et al., 2005).

5.2. Major questions in the therapeutic strategy in DTC which can be solved by molecular diagnosis

In fact, currently, the major clinically relevant problems we face in DTC are:

1. How to differentiate between indolent and progressing micro-PTC? BRAF estimation may help to find the progression-potent tumors, but obviously at the present status of knowledge is not powerful enough to predict the need for more aggressive treatment than is presently recommended.

2. How to specify the more precise indications for lymphadenectomy in DTC?

Until now, the decision regarding lymphadenectomy is based on the following rules: routine central lymphadenectomy and selective lateral lymphadenectomy meaning that the latter procedure is performed only when evidence or at least strong suspicion exists for the presence of lymph node metastases (Cooper et al., 2006; Pacini et al., 2006). Knowing the BRAF status in the primary tumor may prompt omission of central lymphadenectomy in BRAF-negative cases, however, this may lead to undertreatment of RET/PTC-positive young patients. On the other side, detection of a BRAF mutation would prompt a decision to perform central lymphadenectomy in small tumors, a strategy that is not obligatory today, and hence could lead to overtreatment.

3. How to optimize the decision for adjuvant radioiodine after surgery, especially in the “gray zone” of T1-T2N0-N1a patients?

Basing the decision on BRAF status would mean that about in 2/3 of the patients in this stage adjuvant treatment after surgery would be necessary. However, the indications for a standard adjuvant treatment with radioiodine are doubtful in this group in view of decreased expression of genes responsible for iodine metabolism in BRAF-positive cancers.

4. How to treat patients with advanced DTC?

We do not feel that the information on BRAF status changes anyhow the present treatment rules—even the success of sorafenib, a tyrosine kinase inhibitor which is able to target BRAF as well, has not been shown dependent on BRAF mutation status (Kloos et al., 2009). On the other hand, data from other cancers suggest that tumors bearing a BRAF mutation may be particularly prone to respond to MEK inhibitors (Ball et al., 2007), an observation that deserves confirmation in the context of advanced thyroid cancer.
5.3. Conclusions from the transcriptome-wide analyses of DTCs

1. Among the genetic factors analyzed for their prognostic significance, the presence of activating BRAF mutation in papillary thyroid cancer seems the only one to be considered in clinical practice. However, the putative significance of BRAF-positivity is limited by the fact of its frequent occurrence. Further studies are necessary to stratify BRAF-positive papillary thyroid cancers and to indentify molecular factors related to the subset with poor prognosis.

2. By gene signature, the difference between BRAF-positive and BRAF-negative PTC is so distinct that BRAF-positive cancer should be regarded as a molecular subtype of PTC. There is an urgent need to investigate, which gene signature within the BRAF-related gene expression profile is specifically correlated with poor prognosis.

3. Differences between papillary and follicular cancers shown until now by gene expression profiling are profound, but they are still not sufficiently well characterized, thus, their relation to the putative differences in prognosis between these histotypes is premature.

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