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REVIEW ARTICLE

The Genetics of Papillary Microcarcinomas of the Thyroid: Diagnostic and Prognostic Implications

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Abstract: Papillary microcarcinoma of the thyroid (mPTC) is defined by the WHO as a papillary thyroid cancer measuring 10mm or less in diameter and it is nowadays a topic of intense debate among the members of the medical community due to its apparent "epidemic" rise. Although these tumors follow almost always an indolent clinical course and carry an excellent prognosis, it is known that a small subset may display a potentially aggressive behavior. Nevertheless, we still lack an accurate way of predicting those which will cause significant disease. In an attempt to address this problem, a number of clinico-pathologic features have been studied as poor prognostic markers in mPTC, and their association with known genetic alterations in thyroid cancer has been evaluated. Herein we review the present knowledge concerning mPTC's genetic profile, namely the prevalence of BRAF (V600E), RAS and TERT promoter mutations and RET/PTC and PAX8-PPARG rearrangements and report the results of the evaluation in the putative prognostic value of these genetic alterations in mPTC.

Keywords: Papillary microcarcinoma of the thyroid, mPTC, BRAF, RAS, TERT, RET/PTC, Genetics, Prognosis.

1. INTRODUCTION

Papillary carcinoma of the thyroid (PTC) is the most common malignancy of the thyroid gland, and its size range varies widely. Papillary microcarcinoma (mPTC) is defined by the World Health Organization (WHO) as a papillary thyroid cancer measuring 10mm or less in diameter, and constitutes a topic of intense debate, not only because of its increasing incidence, but also because its clinical management still raises some concerns. Recently, it has been shown that the increased incidence of thyroid cancer between 2000 and 2010 may be mainly due to the increased incidence in the diagnosis of mPTC, that has raised from 32% of all thyroidectomies in the first half to 40% in the second half of the decade, in a Brazilian study [1]. Whether this observation is due to a true increase in mPTC incidence or just a higher number of early diagnoses as a result of better diagnostic tools still remain controversial.

mPTCs are usually indolent on their clinical course. Disease specific mortality is <1% [2-4], loco regional recurrence rates varies between 2-6% [4], extrathyroidal extension can be observed in about 2–21% [2] of the patients and lymph node (LNM) and distant metastases may occur in about 9-51% [2, 3, 5] and <1-2% [4] of the cases, respectively. In agreement with these observations, a large study that included 900 patients treated in Mayo Clinic with a mean follow up period of 13.5 years detected 30% of LNM, 2% of extrathyroidal extension, 0.3% of distant metastases at presentation and a 20 year and 40 year recurrence rates of 5.7% and 8%, respectively [6]. The overall expected survival did not differ from that of the general population [6]. Despite its overall excellent prognosis, data on record indicate that mPTC may include at least two biologically distinct subpopulations, the major group comprising indolent tumors with minimal or no potential for progression and a minor group including tumors with a potentially aggressive course.

Therapeutic options in mPTC range from pure observational follow-up to total thyroidectomy and iodine remnant ablation (RAI). The observational approach is an option offered to very few mPTC patients outside of two centers in Japan [4, 7, 8] and can be chosen in alternative to immediate surgery in some cases, specifically: (i) patients classified as very low risk (*e.g.* mPTC without clinically evident local invasion or metastases, or cytological signs and/or molecular alterations of aggressive tumor) and (ii) patients with a very low life-expectancy due to serious comorbidities [4]. Another option is surgery, either by lobectomy or total thyroi-

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dectomy, and the 2015 American Thyroid Association (ATA) management guidelines recommend that "thyroid lobectomy alone is sufficient treatment for small, unifocal, intrathyroidal carcinomas in the absence of prior head and neck irradiation, familial thyroid carcinoma, or clinically detectable cervical nodal metastases (Strong Recommendation, Moderate-quality evidence)" [4]. Although most of the patients are included in the low risk group and would therefore benefit from lobectomy, there are few centers following this approach, with the majority preforming total thyroidectomy. Regarding this aspect, one should notice that the risk of complications in total thyroidectomy is significantly greater than in lobectomy, with the former leading to higher rates of recurrent laryngeal nerve injury, hypocalcemia and hemorrhage/hematoma [9]. Finally, although RAI therapy is another therapeutic option, the best available observational evidence suggests that it is unlikely to improve disease-specific or disease-free survival in mPTC (uni- or multi-focal) without other high risk features [6, 10-13].

Distinguishing the mPTCs which will cause significant disease from those that will not cause harm to the patient would therefore be of great clinical value, as it would help to select the patients who should be treated more aggressively and to separate them from the vast majority of mPTC patients who should be spared from overtreatment. Unfortunately, there is no consensual way of accurately predict the outcome of mPTC so far. Research has been conducted in the last two decades with the aim of finding clinico-pathologic features that could help predicting tumor behavior. Currently, the line of investigation is also focused on finding genetic and molecular markers of tumor aggressiveness. Despite the large amount of data on the genetic characterization of thyroid tumors, the genetic profile of mPTC remains far from a detailed characterization.

The genetic alterations most frequently detected in PTC belong to the MAPK and PI3KCA/AKT pathways and include BRAF (V600E), RAS mutations (all the three members of the RAS family) and also RET/PTC and PAX8-PPARG rearrangements [14]. Recently, TERT promoter mutations were reported as a newly discovered genetic alteration with prognostic significance in PTC [15]. Although mPTCs are considered to be histologically and biologically similar to PTC [16] and, by some authors, as early lesions [16] there is a general agreement that mPTCs are not mere "newborn" PTCs, meaning that most mPTC can be "old" lesions that will not ever evolve towards PTC, remaining quiescent for many years [17].

In the present study we review the genetics of mPTC, namely the prevalence of BRAF (V600E), RAS and TERT promoter mutations and also of RET/PTC and PAX8-PPARG rearrangements. We aim to complement such information with an overview of the recent results concerning the prognostic value of genetic markers in mPTC.

2. GENETIC ALTERATIONS IN mPTC

It is surprising to realize that only few studies on mPTC analyzed genes other than BRAF, RAS and RET/PTC. The first publications investigating this issue analyzed RET/PTC rearrangements [18-20], whereas more recently the focus shifted towards BRAF V600E mutation, the most studied genetic alteration in these neoplasms so far (Table **1**). Less often, one can also find some publications analyzing RAS mutation [21-24] and preliminary studies addressing the presence of TERT promoter mutation [15, 25] in mPTC (Table **1**). Despite its relevance in PTC biological behavior, predominantly in follicular patterned lesions, we did not find any article investigating PAX8-PPARG rearrangements specifically in mPTCs. Moreover, only 6 articles analyzed more than one genetic alteration at the same time, namely BRAF V600E, RAS and RET/PTC [21, 24], BRAF and RAS [22, 26] or BRAF and TERT mutation [25]. None of these studies discovered any overlap among MAPK genetic alterations, fitting with the observations reported from PTCs [27].

The overall prevalence of BRAF V600E mutation in mPTCs from the series reviewed in the present study is 57% (5.741/10.004) (Table **1**). Considering each study separately, it becomes evident the huge variation in the BRAF mutational status in mPTC, ranging from 0% in a study of 3 cases of mPTC with fatal outcome [26] to 90.7% in a study conducted in korean patients [28]. A number of hypotheses have been proposed to explain these differences, namely differences in the criteria of selection of patients and in the mutation detection procedures, as well as differences respecting geographic variation and the lack of integration between mutation analyses and tumor histologic subtype.

The separate analysis of the different studies from each country, discloses an overall BRAF mutation rate of about 69% in Korea, 64% in USA, 44% in Italy and 40% in China, the most representative countries herein reviewed (Table **2**). Although globally USA presents with high mutation detection, it should be stressed the wide range of prevalence of the mutation (21-85%) in the different series, in contrast with Korea or China whose ranges are shorter (Table **2**). Thus, the high overall prevalence of BRAF V600E mutation in USA may result from an overestimated value obtained in particular studies. Similarly, the 69% of BRAF mutation found in mPTC from a polish series may be overestimating the global prevalence of BRAF mutation in Poland, as this is the only study in this population [29]. Nevertheless, there seems to exist a higher prevalence of BRAF mutation in mPTC in korean series in comparison with other countries, namely Italy and China. The precise reason behind this observation is unclear and demands further investigation.

There is few data analyzing specifically the prevalence of BRAF mutations in the various histological types of mPTC. The considerable difficulty of categorization results from their small size together with the fact that WHO classification does not specifically subtype mPTC neoplasias. Preliminary studies indicate that BRAF mutation is more prevalent in conventional [29-32] and tall cell types [30, 33] than in the follicular patterned mPTC [30, 33-35]. In these studies, BRAF mutation in conventional mPTC ranged between 43-81%, between 93%-100% in tall cell variant and between 0–67% in the follicular variant of mPTC. The differences in BRAF mutation rate may thus also result in part from the different histological composition of the various series.

Evidence suggests that the prevalence of BRAF mutation in occult mPTC is higher in tumors diagnosed by ultrasonography before surgery than in those incidentally found after

Table 1. Summary of the prevalence of the major genetic alterations detected in mPTC.

Study	Country	No. Cases	Mutation, n (%)			Rearrangement, n (%)	Sample Type
			BRAF (V600E)	NRAS, KRAS, HRAS	TERT Promoter	RET/PTC	
Sugg *et al*. 1998 [18]	Canada	39	---	---	---	30 (77%)	FFPE
Tallini *et al*. 1998 [19]	USA	55	---	---	---	25 (45.6%)	FFPE
Corvi *et al*. 2001[20]	UK, Italy	21	---	---	---	11 (52%)	FFPE
Nikiforova *et al*. 2003 [39]	USA/Italy	10	2 (20%)	---	---	---	FT, FFPE
Kim *et al*. 2005 [31]	Korea	60	31 (52%)	---	---	---	FFPE
Trovisco al 2005 [32]	Portugal, Spain, Brazil, Russia	20	6 (30%)	---	---	---	FFPE
Adeniran *et al*. 2006 [21]	USA	4	2 (50%)	0 (0%)	---	1 (25%)	FT
Lupi *et al*. 2007 [40]	Italy	230	90 (39.1%)	---	---	---	FFPE
Rodolico *et al.* 2007 [41]	Italy	214	88 (41.1%)				FFPE
Ugolini *et al*. 2007 [36]	Italy	132	33 (25%)	---	---	---	FFPE
Frasca *et al*. 2008 [42]	Italy	103	25 (24.3%)	---	---	---	FFPE
Lin *et al*. 2008 [43]	USA	19	4 (21.1%)	---	---	---	FFPE
Min *et al*. 2008 [44]	Korea	60	32 (53.3%)	---	---	---	FFPE
Ito *et al*. 2009 [45]	Japan	110	31 (28.2%)	---	---	---	FFPE
Kwak *et al.* 2009 [46]	Korea	339	213 (62.8%)	---	---	---	FNAB
Lee *et al*. 2009 [47]	China	64	24 (37.5%)	---	---	---	FFPE
Nasr *et al*. 2009 [48]	USA	5	2 (40%)	---	---	---	FFPE
Park *et al*. 2010 [16]	Korea	279	92 (67.4%)	---	---	---	FFPE
Basolo *et al*. 2010 [49]	Italy	581	229 (39.4%)	---	---	---	FFPE
Lin *et al*. 2010 [50]	China	61	21 (34%)	---	---	---	FNAB
Kurtulmus *et al.* 2012 [51]	Turkey	64	19 (29.70%)	---	---	---	FFPE
Lee *et al*. 2012 [37]	Korea	275	223 (81%)	---	---	---	FNAB
Niemeier *et al*. 2012 [22]	USA	59	28 (47.4%)	2 (N; H) (5.1%)	---	---	FFPE
Zhou *et al*. 2012 [52]	China	100	31 (31%)	---	---	---	FNAB
Jung t al 2012 [34]	Korea	721	573 (79.5%)	---	---	---	FFPE
Schulten *et al*. 2012 [53]	Saudi Arabia	56	10 (17.9%)	---	---	---	FFPE
Ahn *et al*. 2012 [54]	Korea	78	61 (78.2%)	---	---	---	FFPE
Joo *et al*. 2012 [38]	Korea	115	57 (49.6%)				FNAB
Kim *et al*. 2012 [55]	Korea	404	267 (66.1%)				FFPE

(Table 1) contd….

Study	Country	No. Cases	Mutation, n (%)			Rearrangement, n (%)	Sample Type
			BRAF (V600E)	NRAS, KRAS, HRAS	TERT Promoter	RET/PTC	
Xing *et al*. 2013 [56]	USA, Italy, Japan, Poland, Australia, Spain, Czech Republic	435	168 (38.62%)	---	---	---	NS
Lim *et al*. 2013 [57]	Korea	2124	1532 (72%)	---	---	---	FFPE
Choi *et al*. 2013 [58]	Korea	101	72 (71.3%)	---	---	---	FFPE
Mussazhanova *et al*. 2013 [35]	Japan	13	6 (46.2%)	---	---	---	FFPE
Piana *et al*. 2013 [26]	Italy	3 [a]	0 (0%)	0 (0%)	---	---	FFPE
Rossi *et al*. 2013 [59]	Italy	73	45 (61.6%)	---	---	---	FNAB
Vinagre *et al*. 2013 [15]	Portugal	5	1 (20%)		0 (0%)		FFPE
Virk *et al*. 2013 [33]	USA	129	90 (69.8%)	---	---	---	FNAB, FFPE
Zheng *et al*. 2013 [60]	China	977	392 (40.1%)	---	---	---	FFPE
Chung YJ *et al*. 2013 [28]	Korea	86	78 (90.7%)	---	---	---	FFPE
Chung SY *et al*. 2013 [61]	Korea	111	86 (77.5%)	---	---	---	FNAB, FFPE
Bernstein *et al*. 2013 [62]	USA	53	25/27 tall cell (92.6%) 20/26 classic (76.9%)	---	---	---	FFPE
Valcavi *et al*. 2013 [63]	Italy	3	2 (66.7%)	---	---	---	FNAB
Yang *et al*. 2014 [64]	China	291	124 (42.6%)	---	---	---	FNAB
Park *et al*. 2014 [65]	Korea	514	335 (65.2%)	---	---	---	FFPE
Walczyk *et al*. 2014 [29]	Poland	113	78 (69%)	---	---	---	FFPE
Tallini *et al*. 2015 [30]	Italy	298	145 (49%)	---	---	---	FFPE
Yu *et al*. 2015 [66]	China	44	22 (50%)	---	---	---	FT
Bastos *et al*. 2015 [24]	Brazil	40	11 (27.5%)	2 (5%) Nras	---	3 (7.5%)	FFPE
de Biase *et al*. 2015 [25]	Italy	428	261(61%)	---	19/404 (4.7%)	---	FFPE
TOTAL			**5741/10004 (57.4 %)**	**4/106 (3.8%)**	**19/409 (4.6 %)**	**70/159 (44%)**	

ND - not determined; FFPE – formalin fixed, paraffin-embedded; FNAB- fine-needle aspiration biopsy; FT- frozen tissue; NS- not specified

[a] all cases had a fatal outcome.

thyroidectomy secondary to benign disease [36]. In light of this information, one may also hypothesize that different criteria for selection of patients (selection based in different outcomes or selection of patients after surgery *vs* incidental diagnosis) could also impact BRAF mutation rate in different studies.

Table 2. BRAF V600E prevalence by country.

Country	BRAF V600E Prevalence % (Range)
Korea [16, 28, 31, 34, 37, 38, 44, 46, 54, 55, 57, 58, 61, 65]	69.3% (49.6 – 90.7)
Poland [29]	69%
USA [21, 22, 33, 43, 48, 62]	63.6% (21.1 – 84.9)
Italy [25, 26, 30, 36, 39-42, 49, 59, 63]	44.3% (0 - 66.7)
China [47, 50, 52, 60, 64, 66]	39.9% (31 - 50)
Multicentric [32, 56]	38.2%
Japan [35, 45]	30.1% (28.2 – 46.2)
Turkey [51]	29.7%
Brazil [24]	27.5%
Portugal [15]	20%
Saudi Arabia [53]	17.9%

The comparison of BRAF mutation prevalence in mPTC and PTC yields conflicting results. Some authors did not find significant differences in the prevalence of BRAF mutational status between mPTC and PTC [16, 34, 35, 37, 38], whereas others observed a significantly higher rate of mutation in PTC [24, 42, 43, 45, 49, 51, 57, 65, 66]. Out of 10.000 mPTCs collected in this review, 5741 were positive for BRAF mutation, and the global prevalence of BRAF in mPTC (57.4%) is similar to the 51% (27.3% - 87.1%) reported in a recent meta-analysis comprising 30 studies on PTC [67].

Although V600E is by far the most frequently observed alteration in BRAF gene, some other mutations were reported in mPTC, namely BRAF V600-K601E [29, 40, 49], BRAF E611K [29] and BRAF T599-V600T 598-599I [29]. Similarly to what was reported in PTC, some authors have correlated the morphology of the mPTC and the prevalence of the different BRAF mutations [32].

RET/PTC rearrangements were the second most commonly studied genetic alteration in mPTC and those rearrangements were found to be present overall in 44% (70/159) of the mPTC (Table **1**). It has been advanced by some authors that RET/PTC rearrangements are significantly more frequent in mPTC than in PTC [18, 20, 80], but these observations were not confirmed by others, who found similar rates of RET/PTC rearrangements in both types of tumors [24, 30]. The knowledge about the prevalence of the three more frequent RET/PTC rearrangements, RET/PTC-1, -2 and -3, in mPTC is still scarce and incomplete; data published in the literature indicate a higher prevalence of RET/PTC-1 [18]. Another still largely unexplored association is the one between RET/PTC and mPTC histological subtypes, with only one paper addressing this issue and failing to demonstrate a significant correlation with any of the histological types of mPTC [20].

Finally, RAS mutation was found to be less prevalent than BRAF mutation or RET/PTC rearrangements in mPTC, being present in only 4% of the tumors included in this review (4/106). Nevertheless, it must be taken into account that this genetic alteration was far less studied than the former two.

Only two published studies address the prevalence of TERT promoter mutation in mPTC and present a range of percentages of 0% [15] and 4.7% [25]. Due to the scarcity of data, it is premature to advance if RAS or TERT mutations are associated with any particular feature of mPTC (*e.g.* histologic type).

Besides the aforementioned genetic alterations, a small number of studies have addressed other genetic alterations in mPTC being the most recurrently found in the literature cyclin D1 [16, 44, 45, 68-71], p53 [72, 73] and Bcl2 [74]. As referred above, in the whole, the full genetic profile of mPTC still remains incomplete and unexplored.

3. GENETIC ALTERATIONS AS PROGNOSTIC MARKERS IN mPTC

Given the guarded prognosis of a small subset of mPTC, there have been several authors investigating the association of clinico-pathologic features of mPTC with poor prognosis. Age [75], gender [76], presence of LNM [6, 76], multifocality [6], extrathyroidal extension [75], tumor size [75] and concomitant autoimmune thyroid diseases [75], have been proposed to carry prognostic information. In a meta-analysis conducted by Roti and colleges [3], it was found a statistically significant association between younger age, multifocallity and LNM at diagnosis and recurrence in mPTC, although the same was not true for gender, tumor size and presence of extrathyroidal extension.

A number of publications have addressed the association of these clinico-pathological features with known genetic alterations in mPTC. Articles published so far evaluating the association between RET/PTC rearrangements [20, 24] or RAS mutation [24] and guarded clinico-pathologic features failed to establish a significant correlation (Table **3**).

Table 3. Summary of the associations between genetic alterations in mPTC and clinico-pathologic features.

Study	Age	Gender	LNM [c]	Multifocality	Extrathyroidal Extension	Advanced Disease Stage [d]	Size
RET/PTC rearrangements							
Corvi *et al*. 2001[20]	No	---	---	---	---	---	No
Bastos *et al*. 2015 [24]	---	---	No	---	No	No	---
Ras mutation							
Bastos *et al*. 2015 (NRAS) [24]	---	---	No	---	No	No	---
TERT promoter mutation							
de Biase *et al*. 2015 [25]	No [b]	No	No	No	---	No	No [e]
BRAF V600E mutation							
Kim *et al*. 2005 [31]	No [a]	No	No	No	No	No	---
Lupi *et al*. 2007 [40]	No [a]	No	No	No	**Yes**	**Yes**	---
Rodolico *et al*. 2007 [41]	**Yes** [b]	No	**Yes**	No	No	---	No [g]
Ugolini *et al*. 2007 [36] [h]	No	No	---	---	---	---	No
Frasca *et al*. 2008 [42]	---	---	No	No	**Yes**	**Yes**	---
Min *et al*. 2008 [44]	---	---	No	---	---	---	---
Kwak *et al*. 2009 [46]	No [b]	No	No	No	---	No	#
Lee *et al*. 2009 [47]	No [b]	No	**Yes**	---	**Yes**	**Yes**	---
Basolo *et al*. 2010 [49]	**Yes** [a]	No	**Yes**	No	**Yes**	**Yes**	---
Lin *et al*. 2010 [50]	No [b]	No	**Yes**	**Yes**	**Yes**	**Yes**	---
Kurtulmus al 2012 [51]	---	---	**Yes**	No	No	---	---
Lee *et al*. 2012 [37]	No [b]	No	No	No	No	---	No [f]
Zhou *et al*. 2012 [52]	---	---	---	---	---	---	---
Lim *et al*. 2013 [57]	---	---	**Yes**	---	**Yes**	**Yes**	**Yes** [e]
Choi *et al*. 2013 [58]	No [a]	No	No	No	No	No	---
Mussazhanova *et al*. 2013 [35]	No [b]	No	No	---	No	---	No [e]
Rossi *et al*. 2013 [59]	---	---	**Yes**	---	No	---	---
Virk *et al*. 2013 [33]	No [a]	No	**Yes**	No	**Yes**	---	No [f]
Zheng *et al*. 2013 [60]	No [a]	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes** [f]
Chung YJ *et al*. 2013 [28]	---	---	No	---	---	---	---
Chung SY *et al*. 2013 [61]	---	---	No	---	---	---	---
Yang *et al*. 2014 [64]	---	---	**Yes**	---	---	---	---
Walczyk *et al*. 2014 [29]	No [a]	No	No	---	---	---	No [e]
Tallini *et al*. 2015 [30]	**Yes** [b]	No	**Yes**	No	**Yes**	**Yes**	**Yes** [f]
Yu *et al*. 2015 [66]	---	---	No	---	---	---	---
Bastos *et al*. 2015 [24]	---	---	No	---	No	No	---
de Biase *et al*. 2015 [25]	No [b]	No	**Yes**	No	---	**Yes**	**Yes** [e]
Total BRAF studies	**3/16**	**1/16**	**12/25**	**1/14**	**9/17**	**9/13**	**4/10**

Only univariate analysis are shown. **No** – without statistically significant association; **Yes** – with statistically significant association; # data not clear; [a] cut off < or > 45 years old; [b] mean age; [c] either for central or lateral LNM; [d] classified by TNM/AJCC staging system; [e] cut off mean size; [f] cut off < or > 5mm; [g] maximal diameter; [h] Only occult mPTC.

Nevertheless, it should be taken into account that such studies are based on small series, with few mPTC harboring the aforementioned genetic alterations. That may help to explain the difficulty in reach significance. The absence of a clear association with indicators of poor outcome together with the higher prevalence of RET/PTC rearrangements in mPTC comparing with PTC lead some authors to suggest that this genetic alteration may be indicator of an indolent behavior [19].

The only article that focused on TERT promoter mutation as a prognostic indicator in mPTC showed the absence of any association between TERT mutation and unfavorable clinical features or presence of persistent/recurrent disease in mPTC (Table **3**), at variance with the association with guarded prognosis observed in TERT mutated PTC [15, 25, 77].

BRAF V600E, in contrast with the aforementioned genetic alterations, was largely evaluated as a prognostic marker in mPTC. Eleven out of 27 papers on BRAF mutation did not establish a significant correlation between BRAF V600E and any of the clinico-pathologic features previously associated with poor outcome [24, 28, 29, 31, 35-37, 44, 58, 61, 66] (Table **3**). Nevertheless, the majority of them (16/27) observed a significant association between BRAF and at least one of such characteristics (Table **3**). LNM (either analyzing central lymph nodes, lateral lymph nodes or both) was the most frequently studied clinico-pathologic feature and 12 out of 25 studies observed a significant association between BRAF mutation and the presence of LNM [25, 30, 33, 41, 47, 49-51, 57, 59, 60, 64]. The second most commonly investigated association was the one between BRAF mutation and extrathyroidal extension, with 9 out of 17 papers describing a positive correlation with BRAF status [30, 33, 40, 42, 47, 49, 50, 57, 60]. Age at diagnosis provided more conflicting results. Three out of 16 groups established a significant association between age and BRAF status in mPTC [30, 41, 49], but the target of comparison varied from study to study, with some investigating the mean age [35, 41, 47, 50] and others studying it as a categorical variable (cut-off of 45 years old) [49, 58, 60] rendering the results difficult to compare. Out of the three groups who demonstrated a significant correlation with age, two observed an association of BRAF mutation with older age [30, 41] and the remaining one with younger age of the patient [49]. These discrepancies must be seen in light of the natural history of mPTC that, as stated before, are known to be frequently a quiescent, silent tumor, only revealed in autopsy studies, but comprises also early lesions that can evolve to clinical evident tumors.

Tumor size was analyzed by some groups as the mean tumor size [25, 29, 35, 41, 57], and by others as a categorical variable (cut-off of 5mm) [30, 33, 37, 46, 60]. Four out of 10 groups have observed a significant association between BRAF and larger tumor size [25, 30, 57, 60].

Another commonly evaluated association was the one between BRAF mutation and gender but only one out of the 16 groups that evaluated this parameter observed a significant association with male gender [60].

With regard to clinical stage at diagnosis, classified by TNM/AJCC staging system, it was analyzed in 13 studies and 9 of them found a positive association between higher clinical stage and the presence of BRAF mutation [25, 30, 40, 42, 47, 49, 50, 57, 60]. Multifocality of the primary tumor was another clinico-pathologic feature reviewed here and we found it was associated with BRAF mutational status in only one of the 14 studies that evaluated it.

Other less explored variables include bilaterality (with two [52, 59] out of three groups [41, 52, 59] finding a positive association with BRAF mutation), capsular invasion (positive association established in one [46] out of two studies [30, 46]) and concomitant Hashimoto thyroiditis (with a significant correlation with lower prevalence of BRAF mutation in one [57] out of four papers [35, 47, 57, 58]) (data not shown).

Complementing the results described here, a very recently published meta-analysis evaluated the association between BRAF V600E mutation and clinico-pathologic features of mPTC. The authors concluded that multifocality, extrathyroidal extension, LNM, advanced clinical stage (III or IV), larger tumor size (>5mm) and tall cell variant were significantly associated with presence of BRAF V600E mutation [78]. The same was not true for age (either continuous or 45 years or older), gender, concomitant Hashimoto thyroiditis and nodular goiter [78].

Despite the increasing interest in BRAF mutation and its association with indicators of poor prognosis, very few groups have performed a direct prognostic evaluation using tumor recurrence or survival rate as outcome variables. The association between BRAF status and mortality rate in mPTC was investigated in only one study [56], where a positive correlation with mortality was detected in the univariate analysis, but was not confirmed in the adjusted hazard ratio. Furthermore, evidence suggests that there is no association between BRAF mutation and distant metastases [58, 60]. In contrast with what has been described in the literature [29, 30, 79], Zheng and colleagues found a significantly higher recurrence rate in mPTC with BRAF mutation [60].

Although the association between genetic alterations, namely BRAF V600E, and some clinico-pathologic features predictive of tumor aggressiveness has been found in many studies [25, 30, 33, 40-42, 46, 47, 49-52, 57, 59, 60, 64] and in a recent meta-analysis [78], it should be stressed that most of such studies show also an high prevalence of BRAF mutations, usually around 40% but in some series reaching values as high as 70%. This observation indicates that this mutation alone has a low positive predictive value for detecting those mPTC with more aggressive behavior and thus, its clinical utility, in case it exists, may be dependent from the evaluation of other genetic alterations or histopatological features of the tumor.

In one study, a “risk score” including a certain number of features was assayed. A formula comprising BRAF mutation status together with 3 histopatological features, namely superficial tumor location, intraglandular tumor spread/multifocality (IGS/MF) and tumor fibrosis have been advanced, in order to predict mPTC aggressiveness [22]. The proposed simplified algorithm is MPu score = superficial tumor location + BRAF(+) + IGS/MF(+) + fibrosis(2+). This score separates the tumors in three categories of aggressiveness: low risk, intermediate risk and high risk groups, each having a probability of extrathyroid spread or recurrence of 0, 20 and 60%, respectively. The authors suggest that this score may have clinical implications and propose that low risk tumors should be followed conservatively and high risk patients may benefit from total thyroidectomy and possibly central compartment or lateral neck lymph node sampling together with more intensive postoperative follow-up surveillance [22]. Further studies testing other score systems including other criteria may be useful in the clinical practice. The same is true for studies addressing the coexistence of BRAF mutation and other genetic alterations as predictors of less favorable outcomes in mPTC. In this regard, the presence of BRAF V600E together with TERT promoter mutation, evaluated in one study, was not associated with higher rates of recurrent/persistent disease [25].

In summary, we believe that available evidence indicates that nor BRAF V600E mutation alone, nor any of the other genetic alterations are an accurate way of predicting mPTC aggressive behavior. The 2015 ATA guidelines for management of thyroid nodules address this issue and do not routinely recommend BRAF mutational evaluation for initial pre or post-operative risk stratification in differentiated thyroid carcinomas. Additionally, there appears to be little role for BRAF mutational testing as an aid to risk stratification in mPTC tumors that do not demonstrate other worrisome clinico-pathologic features (such as extrathyroidal extension, aggressive histology, vascular invasion, or lymph node metastases), as those tumors are classified as ATA low risk tumors independent of its BRAF mutational status. Thus, clinical usefulness of testing genetic alterations in mPTCs may be more relevant if investigated as a panel of different genetic alterations, and used as negative predictive indicators of an aggressive behavior when there is no genetic alteration detected. To our knowledge, investigation in this setting is currently lacking.

4. GENOMIC STUDIES IN mPTC

Allowing to a better understanding of complex biological systems, genomic studies may be useful for further characterization of mPTC's genetics and behavior.

To date, very few studies performed genomic analysis in mPTC. The few available papers address specific points such as the genomic differences between mPTC and PTC, comparison of mPTC with and without cervical lymph node metastasis and the genetic heterogeneity in multifocal mPTC [80].

Lin and colleagues investigated 19 molecular markers (including BRAF mutation and loss of heterozygosity profile) on 12 multifocal mPTCs in order to identify clonality and thus differentiate intrathyroidal metastasis (ITM) from independent primary (IP) mPTC. Although half of the cases represented ITM, the clinical relevance of such information is still unclear [81].

Kim *et al.*, compared the genomic profile between mPTC and PTC and did not found significant differences in gene expression between the two groups [81]. Others found similar results [82].

Recently, one group used oligonucleotide array analysis in order to investigate differences between six mPTC with and without cervical lymph node metastasis. They found 12 differentially expressed genes and endoplasmic reticulum aminopeptidase 2 (ERAP2) was the one presenting the biggest difference between mPTC and PTC [83].

Although the present data is very preliminary a few studies start to incorporate genomic data in diagnostic and prognostic scoring systems in order to develop more accurate stratification of the patients.

CONCLUDING REMARKS

The major limitation of this work is that a considerable number of publications do not analyze separately mPTC and PTC, particularly with regard to the genetic alterations analysis and its association with clinico-pathologic features. Therefore, we used mainly studies in which mPTC were the main target of investigation and thus we may possibly have missed some relevant articles. Nevertheless, from the literature reviewed, we concluded that the most intensely studied genetic alteration in mPTC is BRAF V600E mutation, with RET/PTC rearrangements and RAS mutation being surprisingly less examined despite their well-known importance in PTC biology.

Regarding BRAF mutation status, significant differences between series were observed. Although histological composition of the series and inclusion criteria of the patients, together with geographic variation, may help to explain these differences, more studies are needed in order to clarify the reason behind these observations. Data available suggests a higher prevalence of the mutation in Korea [16, 28, 31, 34, 37, 38, 44, 46, 54, 55, 57, 58, 61, 65] in comparison with Italy [25, 26, 30, 36, 39-42, 49, 59, 63] and China [47, 50, 52, 60, 64, 66] and in series with higher proportion of tall cell variant mPTC [62].

With respect to the usefulness of these genetic alterations as prognostic markers in mPTC, the studies on record on RET/PTC rearrangement, RAS mutation and TERT promoter mutation are too preliminary to be taken into consideration when deciding the therapeutic strategy. Regarding BRAF mutation, there is a general agreement that this genetic alteration alone is not enough to guide clinical management in mPTC. Our belief is that future investigation should address this issue analyzing different combinations of these genetic alterations and maybe evaluate the value of their absence as negative predictors of an aggressive outcome.

It is obvious the relevance of being able to select those patients with an aggressive mPTC to more intensive treatment. However, the balance between treat aggressively those who need and spare the others from the dangers of overtreatment should be managed carefully. Unfortunately, we have not found yet an accurate way of predicting mPTC aggressiveness. The search for markers of prognosis, genetic or not, that may help us deciding appropriate clinical management of these patients is therefore of huge clinical relevance and efforts must be made in order to find such markers.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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