ENDOCRINE TUMOURS

Genetic predictors of thyroid cancer outcome

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Abstract

Genetic predictors of outcome are reviewed in the context of a disease – cancer – that can be (too) simplistically described as a ‘successful, invasive clone of our own tissues’. Context has many faces that determine a thyroid cancer patient’s outcome beyond the influence of genetic markers. There is also plenty of evidence on the prognostic meaning of the interplay between genetics and context/microenvironment factors (encapsulation, degree of invasion, staging, etc.). This review addresses only genetic alterations detected by molecular methods in surgically resected specimens, thus ruling out immunohistochemistry and (F)ISH, despite their crucial relevance as topographically oriented methods. For the sake of the discussion, well-differentiated carcinomas were divided into two main morphologic types: papillary carcinoma (classic and most variants) displaying BRAFV600E mutations and RET/papillary thyroid carcinoma rearrangements and the group of follicular patterned carcinomas that encompasses follicular carcinoma and the encapsulated form of follicular variant of papillary carcinoma, displaying RAS mutations and PAX8/PPAR γ rearrangement. TERT promoter mutations have been recently described (and associated with distant metastases and reduced survival) in papillary and follicular carcinomas, as well as in poorly differentiated and undifferentiated carcinoma. TP53 mutations, previously thought to be restricted to less differentiated carcinomas, were also detected in papillary and follicular carcinoma and found to carry a guarded prognosis. Besides their putative importance for targeted therapies, the prognostic meaning of such mutations is discussed *per se* and in the setting of concurrent BRAF mutation.

European Journal of
Endocrinology
(2016) 174, R117–R126

Invited Author’s profile

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Introduction

Assuming that cancer can be defined, in an oversimplified way, as a 'highly regulated, successful invasive clone of our own tissues' or, in a less simplified but still too simplistic way, as a 'highly regulated, successful, invasive clone of our own tissues, involving a multistep accumulation of mutations in genes regulating major signalling pathways that are frequently heterogeneous genetically, epigenetically and phenotypically, as well as the cross talk of such mutations with cellular and extracellular alterations at the surrounding tissues', it does not make sense to discuss genetic predictors of thyroid cancer (or any other cancer type) outside host and surgical pathology context.

The aforementioned context has many faces that determine patients' outcomes beyond the influence of genetic markers. This applies to the age and/or gender of the patients, and the site, size and macroscopic characteristics of the cancer – namely, its pushing or infiltrative borders. The degree of invasiveness, both locally and at a distance, is measured by the TNM staging, which is the most powerful predictor of outcome of almost all cancer patients. The histological characteristics of the cancer are also a major factor of prognosis: morphological subtype, degree of differentiation, extension of necrosis, mitotic index and signs of invasion (parenchymatous, lymphovascular and to adjacent organs). The histological context can be, and frequently is, enriched by immunohistochemical data that allow to evaluate more precisely cell proliferation, overexpression (or misplacement) of oncogene products and underexpression (or, again, misplacement) of tumour-suppressor gene products and the number and the type of cells involved in the immunomodulation of cancer development.

The sort of molecular approach that immunohistochemistry provides is also achieved, and frequently reinforced, by *in situ* demonstration of gene rearrangement and gene amplification (FISH is frequently the best method to detect such genetic alterations). Both immunohistochemistry and *in situ* methods provide, furthermore, topographic information that complements the molecular data and are often crucial for understanding carcinogenesis. This has been demonstrated, for instance, by Eloy *et al.* (1) who showed that the interaction between transforming growth factor beta/Smad pathway activation and BRAF mutation plays different roles in circumscribed and infiltrative papillary thyroid carcinoma (PTC); in the latter, the interaction is associated with epithelial-to-mesenchymal transition and local invasion, as well as to nodal metastization of infiltrative PTCs (1).

Thyroid carcinomas are classified according to the cell type they derive from, their degree of differentiation and their cytoarchitecture. Follicular cell-derived tumours comprise well-differentiated thyroid carcinoma (WDTC), poorly differentiated thyroid carcinoma (PDTC) and undifferentiated thyroid carcinoma (UTC). The well-differentiated group encompasses, according to cytoarchitecture and nuclear features of the neoplastic cells, follicular thyroid carcinoma (FTC) and PTC, with the latter having two main variants: classic PTC (cPTC) and follicular variant PTC (FVPTC). The minority of carcinomas that derive from parafollicular C cells are named medullary thyroid carcinoma (2).

In this review, we will just focus on genetic alterations detected by molecular methods in surgically resected specimens, thus skipping their usefulness in cytopathology. To keep the paper within an adequate size, we will only address the importance of the genetic predictors of outcome of patients with follicular cell-derived carcinomas displaying good or moderate differentiation, thus avoiding medullary carcinoma and UTC. PDTC will be discussed together with the respective better differentiated counterparts PTC and its variants, namely, FVPTC and FTC.

Clinico-pathological factors vs genetic predictors of outcome

In a recent article on the usefulness of molecular biomarkers in thyroid cancer, we concluded that, for the moment, clinical and histopathological prognostic factors remain much more important than genetic factors for diagnostic and prognostic purposes (3). This conclusion is, however, challenged almost every day by the publication of new molecular data in the different types of thyroid cancer. The most important of such publications was the 'Integrated genomic characterization of papillary thyroid carcinoma' that provided a detailed description of the genomic landscape of 496 cases of PTC under the auspices of The Cancer Genome Atlas (TCGA) Research Network Initiative (4).

Besides a huge amount of genetic and epigenetic information that will take time to fully understand, it is interesting to realize that the aforementioned study (4) confirmed the existence of two main genetic types of differentiated thyroid carcinoma (DTC) that correspond to cPTC (and some variants of PTC such as the tall cell and Warthin-like variant) and to the group of follicular

patterned carcinomas that encompass FVPTC, as our group and others have suggested years ago (5, 6, 7). The absence of solid prospective studies on thyroid cancer and the close relationship between clinical, pathological, immunohistochemical and genetic factors turn very difficult to discuss out of the global context the prognostic role played by the latter (8).

Of the numerous genetic alterations detected in WDTC and PDTC, we included in the present review those that are more prevalent and/or seem to play a more important prognostic role. It is the case of BRAF, RAS, TERT promoter and TP53 mutations and of RET/PTC and PAX8/PPAR γ rearrangements.

RET/PTC and PAX8/PPAR γ rearrangements

RET/PTC rearrangements are quite frequent in PTC, whereas PAX8/PPAR γ rearrangement is often detected in follicular patterned lesions (FVPTC and FTC) (3, 5, 6, 7, 9); the overall evidence indicates that tumours with either of these rearrangements rarely evolve to less differentiated forms (i.e. their prevalence is very low in PDTC and UTC). RET/PTC is a chimeric gene generated by the fusion of the RET tyrosine kinase (TK) domain with the 5' terminal region of genes that are constitutively expressed in thyroid follicular cells (10) allowing dimerization of the RET TK domain and its constitutive activation. The most frequent forms of this oncogene in PTC are RET/PTC1 and RET/PTC3, both arising from chromosome 10 inversions (11). RET/PTC1 rearrangement appears to be associated with small, classic type PTC displaying low proliferation and occurring in young patients (12, 13, 14, 15). At variance with this, RET/PTC3 rearrangement is prevalent in the solid variant of PTC that is frequent in children and was often found in PTCs occurring in the setting of the Chernobyl accident (16), being more prone to a more aggressive behaviour (13, 14, 15, 17). Despite being associated with signs of clinical aggressiveness (namely nodal and lung metastases), cases of solid variant of PTC arising in young patients, with or without RET/PTC3 rearrangement, respond well to radioactive iodine (RAI) treatment and are not significantly associated with a worse survival of the patients.

Taking the data on record in the literature as well as our own experience into account, it may be concluded that the prognostic value of RET/PTC rearrangement in thyroid cancer has not been fully clarified yet.

PAX8/PPAR γ rearrangement has been associated with some adverse prognostic features (e.g. multifocality and

vascular invasion) in some series, but the gathered evidence is not strong enough to identify this rearrangement as a genetic predictor of outcome in thyroid cancer (9, 18). Furthermore, PAX8/PPAR γ rearrangements have been also detected in 14% of the cases of follicular thyroid adenoma (FTA) (19).

RAS mutations and prognosis

RAS are small GTPase-proteins that act as a molecular switch propagating signals from TK and non-TK receptors and activating the MAPK and other signalling pathways. RAS mutations are more prevalent and seem to be more relevant as a prognostic indicator in follicular patterned lesions (FVPTC, FTC and, namely, PDTC) than in cPTC (18). All of the three RAS genes (H, K and N-RAS) were shown to be mutated in both benign and malignant thyroid tumours but the frequency of the mutations is higher in FTC (36%), PDTC (55%) and UTC (52%) and more frequently affects the N-RAS gene (20).

RAS mutations are less prevalent in benign and malignant Hürthle cell tumours (5 and 11% respectively) than in their non-Hürthle cell counterparts and less prevalent in PTC (10%) than in FTC (25–30%) (7, 20). Within PTC, RAS mutations are rare in its classic form, whereas in FVPTC, its prevalence falls within the range of other follicular patterned tumours (~25%) (6).

The controversy on the prognostic value of RAS mutations in thyroid cancer results partially, at least, from the fact that RAS mutations are present along all of the whole spectrum of thyroid lesions, from FTA to the deadly UTC. Garcia Rostan *et al.* (21) have shown that patients with RAS mutated carcinomas, namely PDTC, harbour distant metastases more frequently and have higher mortality, being RAS mutations an independent predictor of poor survival (21). Other studies disclosed a similar association between (N) RAS mutation and distant metastases and/or lower survival in FTC (22, 23).

The assumption that RAS mutations can predispose to differentiation loss in thyroid cancer derives from their presence in DTC with areas of dedifferentiation and from their greater prevalence in PDTC and UTC than in DTC (24).

It has been difficult to demonstrate the prognostic value of RAS mutations due to the relatively small size of the majority of the series (in particular concerning FTC, PDTC and UTC that are less frequent than PTC) and the too short follow-up in most situations. Large, multicentric studies will be necessary to establish definitely the prognostic value of RAS mutations.

BRAF and NIS expression

BRAF gene encodes a serine/threonine kinase that belongs to the RAS–RAF–MEK–ERK–MAP kinase pathway, whose biological role is to mediate cellular responses to growth factors. There are several BRAF mutations, the BRAFT1796A (in exon 15) is largely the more prevalent, leading to a substitution of a valine by a glutamic acid at position 600. Such a mutation causes increased BRAF kinase activity and the subsequent phosphorylation of MEK1/2 and ERK1/2, turning the activation of the MAP kinase pathway independent from upstream factors activation (25).

BRAFV600E mutation is the most prevalent point mutation in PTC, being present in 36–83% of cases. It rarely co-exists with other prevalent genetic events such as RET/PTC rearrangement or RAS mutation (18). BRAFV600E mutation exhibits a strong genotype–phenotype association; it is (almost) exclusively detected in PTC exhibiting a papillary or mixed follicular/papillary growth pattern, regardless of being a cPTC or any of the PTC variants (other than the encapsulated FVPTC) (5).

Besides the frequent BRAFV600E mutation, other alterations were detected in the BRAF gene in PTCs: the BRAFK601E mutation, which occurs mainly in FVPTC (<10% of the cases) (5), and the in-frame deletion VK600-1E that has been detected in rare cases of solid variant of PTC. BRAF rearrangements, namely the AKAP9–BRAF fusion, were also described as rare events preferentially found in radiation-induced PTC (18). At present, there is not enough evidence to evaluate the putative prognostic role of the aforementioned rare BRAF alterations.

Although functional studies, using thyroid-targeted BRAFV600E transgenic mice (26) and BRAFV600E transfected thyroid cell lines (27), indicate that BRAF mutations lead to a more ‘aggressive type’ of PTC, several other studies, addressing the correlation between BRAFV600E and the clinical features of PTC, provided discrepant results (see below).

Some studies reported significant associations between BRAF mutation and poor prognostic indicators like older age (28, 29), male gender (30, 31), extrathyroid extension (28, 32), regional metastases (29, 32), distant metastases (33), higher tumour staging (28, 32, 33), tumour size (31, 34, 35) and tumour recurrence (32, 36). Other studies have not observed the aforementioned associations (37, 38, 39). Furthermore, Elisei *et al.* (40) have demonstrated that the search for BRAFV600E mutation may prove useful to modulate the treatment among low-risk PTC patients, those who require less or

more aggressive treatment. Recently, a multicenter retrospective study showed that BRAFV600E was significantly associated with increased cancer-related mortality among patients with PTC, but the association was not independent of several clinico-pathological features of aggressiveness (41).

We observed that BRAFV600E PTCs tended to occur in older patients and did not exhibit a significant association with signs of clinico-pathological aggressiveness – namely larger size, extrathyroidal extension, vascular invasion and lymph node metastases (5, 8) – or poor circumscription (8). This does not mean, however, that BRAF mutation cannot contribute for progression of PTC toward less differentiated carcinomas in the appropriate context, because our group and others (28, 33, 42) detected BRAFV600E mutation in 10–35% of UTC.

Despite the BRAF mutation controversial association with guarded prognostic features, its association with a decrease in expression of several ‘thyroid specific genes’ or ‘iodine handling genes’ (36, 43, 44) is widely acknowledged. The association of BRAF mutation with the loss of RAI avidity in recurrent PTC has been confirmed *in vitro* and *in vivo* (36, 45). It was recently shown that MEK inhibition may restore RAI incorporation, turning BRAF and/or MEK inhibitors into promising targets to treat RAI-refractory thyroid cancers (45, 46).

TERT promoter mutations

About two-thirds of thyroid carcinomas display telomerase activation that is more frequent in UTC than in DTC (42). Capezzone *et al.* (47) observed telomerase activity in most sporadic and familial malignant thyroid tumours, as well as in some adenomas. Recently, mutations in the promoter region of the telomerase (*TERT*) gene were reported in follicular cell-derived thyroid carcinomas (FCDTC) (48, 49, 50). These mutations occur in two hotspot positions, located at –124 and –146 bp upstream from the ATG start site (–124G>A and –146G>A, C>T on opposite strand) and confer enhanced *TERT* promoter activity, putatively by generating a consensus-binding site (GGAA) for ETS transcription factors within the *TERT* promoter region (51).

In a large series of 469 carcinomas, we found *TERT* promoter mutations in 7.5% of PTC, 17.1% of FTC, 29.0% of PDTC and 33.0% of UTC (52). This stepwise increase in the frequency of *TERT* promoter mutations from well to poorly differentiated and undifferentiated carcinomas was also reported in other studies (49, 50). No *TERT* promoter mutations were found in normal tissues, benign lesions or

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medullary thyroid carcinomas. Like RAS mutations, the frequency of *TERT* promoter mutations seems to be lower in tumours with oncocytic features than in their non-oncocytic counterparts; these observations reinforce the assumption that oncocytic tumours have a different set of molecular alterations and probably also alternative mechanisms for cell survival (53, 54, 55). The majority (about 80%) of mutated cases present the $-124G>A$ mutation. In PTC, *TERT* promoter mutations were significantly more frequent in *BRAF* mutated tumours (50, 52). *TERT* promoter mutations were associated with increased mRNA expression, and this increase was more pronounced in tumours harbouring both *BRAF* and *TERT* promoter mutations (48).

Several studies analysed the relationship between *TERT* promoter mutations and clinico-pathological features (49, 50, 52, 56, 57), and four studies also analysed the implications of the presence of these mutations on patients' clinical outcomes (52, 56, 58, 59). *TERT* promoter mutations were associated with older age of the patients at diagnosis, larger tumour size, distant metastases and a higher stage in several studies (50, 52, 57). The association with distant metastases seems to be particularly consistent and has been reported in most of the studies, strongly suggesting that there is a link between *TERT* promoter mutations and the metastatic potential of FCDTC. From the clinical standpoint, this association is extremely relevant because distant metastases are major determinants of prognosis, especially in older patients (60).

In our study (52), patients with DTC harbouring *TERT* promoter mutations were less prone to be disease free at the end of follow-up, and similar results were found in three other studies (56, 58, 59). Our study also showed that patients with *TERT*-mutated tumours were submitted to more treatments with radioiodine with higher cumulative doses, as well as to other treatment modalities like surgery for recurrent disease, external beam irradiation or treatment with TK inhibitors (52). Furthermore, patients with tumours harbouring *TERT* promoter mutations had increased disease-specific mortality, and this finding was independent of age and gender (52).

As previously mentioned, *TERT*-mutated PTC harbours more frequently *BRAF* mutations than *TERTwt* tumours. Horn *et al.* (51) advanced that the mutation creates newly consensus binding sites for *TCF* subfamily transcription factors (Elk1 and Elk4) that can be activated by *BRAF*. Our results in *TERT* mRNA expression corroborated this assumption, showing an increased *TERT* expression in tumours harbouring *BRAF* and *TERT* mutation (48). Because *BRAF* has also been associated

with worse prognosis in some studies, several authors hypothesized that both mutations could cooperate toward a worse prognosis (50, 61). One still ignores the mechanism behind the putative cooperation between *BRAF* and *TERT* promoter mutation. It is nevertheless tempting to speculate, considering the pro-senescent effect of *BRAF* mutation alone (62), that *TERT* promoter mutations may contribute to abrogate such effect through their role leading to evasion from senescence (63, 64, 65). Taking into account that the prognostic value of *BRAF* is currently under debate and that *TERT* promoter mutations were independently associated with aggressive clinico-pathological features and worse outcome in all of the large series published to date (66), we think that, at present, the most important question is to clarify, whether or not, after controlling the clinical importance of *TERT* mutations, *BRAF* goes on adding a significant prognostic value (66). Multicentric studies with large series of patients will be necessary to clarify if the 'addition' of *BRAF* mutational status to a *TERT*-mutated tumour has indeed value for prognostic stratification (66).

TP53 mutations

Most *TP53* mutations lead to the expression of a mutant protein or, less commonly, to its absence (67, 68). In thyroid carcinomas, *TP53* mutations are not different from those of cancers at other sites and have been described in exons 5–9, with 273 being the codon most often altered (42, 67, 69, 70, 71, 72, 73). No p53 expression or mutation has been found in normal thyroid or in benign lesions, including follicular adenoma, adenomatous goitre and chronic thyroiditis (72, 73, 74, 75, 76). For years it was repeated that more than 98% of DTC (PTC and FTC) had a normal *TP53* gene (18, 69, 70, 71, 72, 73, 75, 77), even when cases secondary to radiation exposure were included (78). This scenario may be changing due to the utilization of next-generation sequencing; using this methodology, Nikiforova *et al.* (79) reported the presence of *TP53* mutations in 3.5% of PTC (2/57) and in four of 36 FTC (11.1%); the four FTC cases were oncocytic carcinomas and three were widely invasive (75). In the recent TCGA study (4), *TP53* mutations were detected in 0.7% of PTC thus confirming their scarcity in PTC, but no clinico-histopathological data were provided on the mutated cases. The results of the study by Nikiforova *et al.* (75) study regarding the high clinical aggressiveness of *TP53* mutated DTC fit with previously reported results. A small proportion of aggressive PTC are associated with *TP53* mutations and/or p53 expression; the tall cell variant of

PTC is associated with a significantly higher rate of p53 than common PTC (80). Positivity for p53 protein has been detected in rare aggressive thyroid tumours such as a mixed columnar and tall cell variant of PTC (81) and a squamous cell carcinoma associated with the tall cell variant of PTC (82). Positivity for p53 protein has also been reported in some aggressive cases of the cribriform-morular variant of PTC (83, 84). Immunohistochemical evaluation of the columnar cell variant of PTC showed a predominantly weak nuclear p53 staining in both indolent and aggressive tumours (81).

Loss of cellular polarity/cohesiveness, hobnail features and micropapillary structures, either alone or in combination, are independent predictive factors for lymph node metastasis both in common PTC and in papillary microcarcinoma (82, 83, 84, 85). All of these peculiar morphological features are characteristic of the so-called micropapillary/hobnail variant of PTC (85, 86, 87, 88, 89), an aggressive type of PTC carrying poor outcome, which is consistently positive for p53 (85, 86, 87, 89) at the immunohistochemical level. Hobnail features were most commonly observed in association with PDTC and UTC (90). These features have also been associated with other histologic variants that are known to be more clinically aggressive, such as increased mitotic activity and/or necrosis and lymph node metastases at presentation. It has therefore been suggested that hobnail features may be a manifestation of 'higher-grade transformation' (90). The recent observation by our group (91) of two fatal cases of the micropapillary/hobnail variant of PTC positive for p53 by immunohistochemistry (Fig. 1) and TP53 mutated at the molecular level with progression to UTC supports the involvement of p53 in such transformation (90, 92). Inactivating TP53 mutations have been reported in about 26% of PDTC (71, 73, 85) and in more than 60% of UTC (42, 69, 70, 71, 73, 85). The results of the studies based on the detection of nuclear accumulation of p53 protein (73, 74, 75, 76, 86, 87) fit with molecular studies. p53 expression is more obvious in areas showing active infiltrative growth and/or at the periphery in PDTC, and widespread positivity for p53 is characteristic of UTC. The analysis of TP53 mutations and/or p53 expression in PTC co-existing with UTC has shown that p53 expression/mutation is limited to the undifferentiated components (70, 71, 86, 88). Moreover, re-expression of WT p53 in human UTC cell lines with a mutated p53 has been associated with re-expression of the paired box domain transcription factor Pax-8, thyroglobulin, thyroperoxidase and TSH receptor (72, 89). All of these findings strongly support that TP53 inactivation plays an important role in

Figure 1

Micropapillary/hobnail variant of PTC. (A) The papillary structures are lined by cells with dense eosinophilic cytoplasm and the nuclei placed in the apex of the cytoplasm producing a surface bulge (hobnail appearance). There are also areas of cellular discohesiveness and micropapillary pattern (H&E, 400×). (B) The nuclei of the tumour cells show strong positivity for p53 (clone DO-7, Dako, Denmark, 400×).

the progression from differentiated to undifferentiated carcinoma, as a final event in the tumourigenic process, contributing to the highly aggressive phenotype of these tumours (90).

miRNA and lncRNA in thyroid cancer outcome

Of the numerous molecules and mechanisms described in recent years in the oncology field, miRNA and lncRNA arise as major players due to their action on the modulation of known cancer genes and/or their products (oncogenes, tumour suppressor genes and apoptotic proteins).

It has been hypothesized that some of the miRNA and/or lncRNA (or a set of) can help in the differential diagnosis of benign and malignant tumours, however scarce information is available regarding their putative role on prognosis. Nevertheless, some miRNA have been repeatedly found dysregulated in thyroid cancer, in particular in PTC (miR-146b, miR-181b, miR-187, miR-221 and miR-222) and the same set of molecules has been associated with tumour aggressiveness in some studies (92). Unfortunately, the relevant set of miRNAs varies from one report to the other, turning difficult or even impossible to draw, at present, any meaningful conclusions.

The same holds true concerning the available data on lncRNAs. The complexity of the available evidence is huge because these long (longer than 200 nt) RNAs can play a role at both the transcriptional and the post-transcriptional gene regulation level. lncRNAs NAMA, AK023948 and PTCSC3AA (PTC susceptibility candidate 3) are among the (yet) reduced number of lncRNAs that have been associated with PTC (93, 94). Until now it has not been possible to ascertain any role to lncRNA in the prognosis of thyroid cancer patients.

Final remarks

The importance of genetic markers for predicting thyroid cancer outcome is limited by the pre-eminence of clinical, histopathological, immunological and other context-driven features. Despite this, there is enough evidence to claim that TERT promoter mutations and TP53 mutations are major molecular biomarkers of prognosis and to suggest that BRAF and RAS mutations may also play a prognostic role in some conditions. Besides prognosis, the aforementioned mutations and the respective molecular pathways, as well as other genetic and epigenetic alterations recently identified by the Cancer Genome Atlas (4), will probably serve as targets for the so-called personalized therapy.

Declaration of interest

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

Funding

This study was supported by FCT, the Portuguese Foundation for Science and Technology through a PhD grant to C Tavares. Further funding was obtained from the project 'Microenvironment, metabolism and cancer' that was partially supported by Programa Operacional Regional do Norte (ON.2 – O Novo Norte) under the Quadro de Referência Estratégico Nacional (QREN) and the Fundo Europeu de Desenvolvimento Regional (FEDER). IPATIMUP integrates the i3S Research Unit, which is partially supported by FCT. This study was funded by FEDER funds through the Operational Programme for Competitiveness Factors – COMPETE and National Funds through FCT, under the project PEst-C/SAU/LA0003/2013. The work of J M Cameselle-Teijeiro was supported by grant PI12/00749-FEDER from Instituto de Salud Carlos III, Ministry of Economy and Competitiveness, Madrid, Spain.

References

- Eloy C, Santos J, Cameselle-Teijeiro J, Soares P & Sobrinho-Simoes M. TGF- β /Smad pathway and BRAF mutation play different roles in circumscribed and infiltrative papillary thyroid carcinoma. *Virchows Archiv* 2012 **460** 587–600. (doi:10.1007/s00428-012-1234-y)
- Rosai JD, Frable WJ & Tallini G. In *Tumors of the Thyroid & Parathyroid Glands*. Chapter 9, pp 500–530. American Registry of Pathology: Washington DC, 2014.
- Soares P, Celestino R, Melo M, Fonseca E & Sobrinho-Simoes M. Prognostic biomarkers in thyroid cancer. *Virchows Archiv* 2014 **464** 333–346. (doi:10.1007/s00428-013-1521-2)
- Cancer Genome Atlas Research N. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* 2014 **159** 676–690. (doi:10.1016/j.cell.2014.09.050)
- Trovisco V, Soares P, Preto A, de Castro IV, Lima J, Castro P, Maximo V, Botelho T, Moreira S, Meireles AM *et al*. Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. *Virchows Archiv* 2005 **446** 589–595. (doi:10.1007/s00428-005-1236-0)
- Castro P, Reboredo AP, Soares RJ, Magalhaes J, Roque L, Trovisco V, Vieira de Castro I, Cardoso-de-Oliveira M, Fonseca E, Soares P *et al*. PAX8–PPAR γ rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 213–220. (doi:10.1210/jc.2005-1336)
- Nikiforov YE & Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nature Reviews. Endocrinology* 2011 **7** 569–580. (doi:10.1038/nrendo.2011.142)
- Eloy C, Santos J, Soares P & Sobrinho-Simoes M. The preeminence of growth pattern and invasiveness and the limited influence of BRAF and RAS mutations in the occurrence of papillary thyroid carcinoma lymph node metastases. *Virchows Archiv* 2011 **459** 265–276. (doi:10.1007/s00428-011-1133-7)
- Armstrong MJ, Yang H, Yip L, Ohori NP, McCoy KL, Stang MT, Hodak SP, Nikiforova MN, Carty SE & Nikiforov YE. PAX8/PPAR γ rearrangement in thyroid nodules predicts follicular-pattern carcinomas, in particular the encapsulated follicular variant of papillary carcinoma. *Thyroid* 2014 **24** 1369–1374. (doi:10.1089/thy.2014.0067)
- Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, Della Porta G & Vecchio G. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. *Nature* 1987 **328** 170–172. (doi:10.1038/328170a0)
- Romei C & Elisei R. RET/PTC translocations and clinico-pathological features in human papillary thyroid carcinoma. *Frontiers in Endocrinology* 2012 **3** 54. (doi:10.3389/fendo.2012.00054)
- Soares P, Fonseca E, Wynford-Thomas D & Sobrinho-Simoes M. Sporadic ret-rearranged papillary carcinoma of the thyroid: a subset of slow growing, less aggressive thyroid neoplasms? *Journal of Pathology* 1998 **185** 71–78. (doi:10.1002/(SICI)1096-9896(199805)185:1<71::AID-PATH42>3.0.CO;2-S)
- Thomas GABH, Cook HA, Williams ED, Nerovnya A, Cherstvoy ED, Tronko ND, Bogdanova TI, Chiappetta G, Viglietto G, Pentimalli F *et al*. High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4232–4238. (doi:10.1210/jcem.84.11.6129)
- Nikiforov YE. RET/PTC rearrangement in thyroid tumors. *Endocrine Pathology* 2002 **13** 3–16. (doi:10.1385/EP:13:1:03)
- Mochizuki KKT, Nakazawa T, Iwashina M, Kawasaki T, Nakamura N, Yamane T, Murata S, Ito K, Kameyama K, Kobayashi M *et al*. RET rearrangements and BRAF mutation in undifferentiated thyroid carcinomas having papillary carcinoma components. *Histopathology* 2010 **57** 444–450. (doi:10.1111/j.1365-2559.2010.03646.x)
- Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H & Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Research* 1997 **57** 1690–1694.
- Sugg SL, Ezzat S, Zheng L, Freeman JL, Rosen IB & Asa SL. Oncogene profile of papillary thyroid carcinoma. *Surgery* 1999 **125** 46–52. (doi:10.1016/S0039-6060(99)70287-4)

Review	C Tavares, M Melo and others	Genetic predictors of cancer outcome	174:4	R124
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- 18 Sobrinho-Simoes M, Maximo V, Rocha AS, Trovisco V, Castro P, Preto A, Lima J & Soares P. Intragenic mutations in thyroid cancer. *Endocrinology and Metabolism Clinics of North America* 2008 **37** 333–362, viii. (doi:10.1016/j.ecl.2008.02.004)
- 19 Marques AR, Espadinha C, Catarino AL, Moniz S, Pereira T, Sobrinho LG & Leite V. Expression of PAX8–PPAR γ 1 rearrangements in both follicular thyroid carcinomas and adenomas. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3947–3952. (doi:10.1210/jcem.87.8.8756)
- 20 Howell GM, Hodak SP & Yip L. RAS mutations in thyroid cancer. *Oncologist* 2013 **18** 926–932. (doi:10.1634/theoncologist.2013-0072)
- 21 Garcia-Rostan G, Zhao H, Camp RL, Pollan M, Herrero A, Pardo J, Wu R, Carcangiu ML, Costa J & Tallini G. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. *Journal of Clinical Oncology* 2003 **21** 3226–3235. (doi:10.1200/JCO.2003.10.130)
- 22 Jang EK, Song DE, Sim SY, Kwon H, Choi YM, Jeon MJ, Han JM, Kim WG, Kim TY, Shong YK *et al.* NRAS codon 61 mutation is associated with distant metastasis in patients with follicular thyroid carcinoma. *Thyroid* 2014 **24** 1275–1281. (doi:10.1089/thy.2014.0053)
- 23 Fukahori M, Yoshida A, Hayashi H, Yoshihara M, Matsukuma S, Sakuma Y, Koizume S, Okamoto N, Kondo T, Masuda M *et al.* The associations between RAS mutations and clinical characteristics in follicular thyroid tumors: new insights from a single center and a large patient cohort. *Thyroid* 2012 **22** 683–689. (doi:10.1089/thy.2011.0261)
- 24 Zhu Z, Gandhi M, Nikiforova MN, Fischer AH & Nikiforov YE. Molecular profile and clinical–pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. *American Journal of Clinical Pathology* 2003 **120** 71–77. (doi:10.1309/ND8D9LAJTRCTG6QD)
- 25 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W *et al.* Mutations of the BRAF gene in human cancer. *Nature* 2002 **417** 949–954. (doi:10.1038/nature00766)
- 26 Knauf JA, Ma X, Smith EP, Zhang L, Mitsutake N, Liao XH, Refetoff S, Nikiforov YE & Fagin JA. Targeted expression of BRAFV600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. *Cancer Research* 2005 **65** 4238–4245. (doi:10.1158/0008-5472.CAN-05-0047)
- 27 Melillo RM, Castellone MD, Guarino V, De Falco V, Cirafici AM, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhföffer M *et al.* The RET/PTC–RAS–BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. *Journal of Clinical Investigation* 2005 **115** 1068–1081. (doi:10.1172/JCI200522758)
- 28 Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A *et al.* BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5399–5404. (doi:10.1210/jc.2003-030838)
- 29 Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, Shihru D, Bastian B & Griffin A. The prevalence and prognostic value of BRAF mutation in thyroid cancer. *Annals of Surgery* 2007 **246** 466–470 (discussion 470–471). (doi:10.1097/SLA.0b013e318148563d)
- 30 Xu X, Quiros RM, Gattuso P, Ain KB & Prinz RA. High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. *Cancer Research* 2003 **63** 4561–4567. (doi:10.1111/j.1365-2265.2006.02605.x)
- 31 Kim TY, Kim WB, Rhee YS, Song JY, Kim JM, Gong G, Lee S, Kim SY, Kim SC, Hong SJ *et al.* The BRAF mutation is useful for prediction of clinical recurrence in low-risk patients with conventional papillary thyroid carcinoma. *Clinical Endocrinology* 2006 **65** 364–368. (doi:10.1111/j.1365-2265.2006.02605.x)
- 32 Xing M. BRAF mutation in thyroid cancer. *Endocrine-Related Cancer* 2005 **12** 245–262. (doi:10.1677/erc.1.0978)
- 33 Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, Ohtsuru A, Saenko VA, Kanematsu T & Yamashita S. Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4393–4397. (doi:10.1210/jc.2003-030305)
- 34 Oler G & Cerutti JM. High prevalence of BRAF mutation in a Brazilian cohort of patients with sporadic papillary thyroid carcinomas: correlation with more aggressive phenotype and decreased expression of iodide-metabolizing genes. *Cancer* 2009 **115** 972–980. (doi:10.1002/cncr.24118)
- 35 Elisei R, Ugolini C, Viola D, Lupi C, Biagini A, Giannini R, Romei C, Miccoli P, Pinchera A & Basolo F. BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3943–3949. (doi:10.1210/jc.2008-0607)
- 36 Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M & Santisteban P. The oncogene BRAF V600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of Na⁺/I[−] targeting to the membrane. *Endocrine-Related Cancer* 2006 **13** 257–269. (doi:10.1677/erc.1.01119)
- 37 Fugazzola L, Puxeddu E, Avenia N, Romei C, Cirello V, Cavaliere A, Faviana P, Mannavola D, Moretti S, Rossi S *et al.* Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature. *Endocrine-Related Cancer* 2006 **13** 455–464. (doi:10.1677/erc.1.01086)
- 38 Fugazzola L, Mannavola D, Cirello V, Vannucchi G, Muzza M, Vicentini L & Beck-Peccoz P. BRAF mutations in an Italian cohort of thyroid cancers. *Clinical Endocrinology* 2004 **61** 239–243. (doi:10.1111/j.1365-2265.2004.02089.x)
- 39 Abrosimov A, Saenko V, Rogounovitch T, Namba H, Lushnikov E, Mitsutake N & Yamashita S. Different structural components of conventional papillary thyroid carcinoma display mostly identical BRAF status. *International Journal of Cancer* 2007 **120** 196–200. (doi:10.1002/ijc.22290)
- 40 Elisei R, Viola D, Torregrossa L, Giannini R, Romei C, Ugolini C, Molinaro E, Agate L, Biagini A, Lupi C *et al.* The BRAF(V600E) mutation is an independent, poor prognostic factor for the outcome of patients with low-risk intrathyroid papillary thyroid carcinoma: single-institution results from a large cohort study. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 4390–4398. (doi:10.1210/jc.2012-1775)
- 41 Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, Yip L, Mian C, Vianello F, Tuttle RM *et al.* Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *Journal of the American Medical Association* 2013 **309** 1493–1501. (doi:10.1001/jama.2013.3190)
- 42 Soares P, Lima J, Preto A, Castro P, Vinagre J, Celestino R, Couto JP, Prazeres H, Eloy C, Maximo V *et al.* Genetic alterations in poorly differentiated and undifferentiated thyroid carcinomas. *Current Genomics* 2011 **12** 609–617. (doi:10.2174/138920211798120853)
- 43 Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, Barbi F, Avenia N, Scipioni A, Verrienti A *et al.* BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2840–2843. (doi:10.1210/jc.2006-2707)
- 44 Romei C, Ciampi R, Faviana P, Agate L, Molinaro E, Bottici V, Basolo F, Miccoli P, Pacini F, Pinchera A *et al.* BRAFV600E mutation, but not RET/PTC rearrangements, is correlated with a lower expression of both thyroperoxidase and sodium iodide symporter genes in papillary thyroid cancer. *Endocrine-Related Cancer* 2008 **15** 511–520. (doi:10.1677/ERC-07-0130)
- 45 Chakravarty D, Santos E, Ryder M, Knauf JA, Liao XH, West BL, Bollag G, Kolesnick R, Thin TH, Rosen N *et al.* Small-molecule MAPK inhibitors restore radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. *Journal of Clinical Investigation* 2011 **121** 4700–4711. (doi:10.1172/JCI46382)

Review	C Tavares, M Melo and others	Genetic predictors of cancer outcome	174:4	R125
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- 46 Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, Pentlow KS, Zanzonico PB, Haque S, Gavane S *et al.* Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. *New England Journal of Medicine* 2013 **368** 623–632. (doi:10.1056/NEJMoa1209288)
- 47 Capezzone M, Cantara S, Marchisotta S, Busonero G, Formichi C, Benigni M, Capuano S, Toti P, Pazaitou-Panayiotou K, Caruso G *et al.* Telomere length in neoplastic and nonneoplastic tissues of patients with familial and sporadic papillary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E1852–E1856. (doi:10.1210/jc.2011-1003)
- 48 Vinagre J, Almeida A, Populo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, Lima L *et al.* Frequency of TERT promoter mutations in human cancers. *Nature Communications* 2013 **4** 2185. (doi:10.1038/ncomms3185)
- 49 Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimipasic T, Ghossein RA & Fagin JA. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1562–E1566. (doi:10.1210/jc.2013-2383)
- 50 Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar AK & Xing M. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. *Endocrine-Related Cancer* 2013 **20** 603–610. (doi:10.1530/ERC-13-0210)
- 51 Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K *et al.* TERT promoter mutations in familial and sporadic melanoma. *Science* 2013 **339** 959–961. (doi:10.1126/science.1230062)
- 52 Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, Celestino R, Almeida A, Salgado C, Eloy C *et al.* TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E754–E765. (doi:10.1210/jc.2013-3734)
- 53 de Vries MM, Celestino R, Castro P, Eloy C, Maximo V, van der Wal JE, Plukker JT, Links TP, Hofstra RM, Sobrinho-Simoes M *et al.* RET/PTC rearrangement is prevalent in follicular Hurthle cell carcinomas. *Histopathology* 2012 **61** 833–843. (doi:10.1111/j.1365-2559.2012.04276.x)
- 54 Maximo V, Botelho T, Capela J, Soares P, Lima J, Taveira A, Amaro T, Barbosa AP, Preto A, Harach HR *et al.* Somatic and germline mutation in GRIM-19, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hurthle cell) tumours of the thyroid. *British Journal of Cancer* 2005 **92** 1892–1898. (doi:10.1038/sj.bjc.6602547)
- 55 Maximo V, Lima J, Prazeres H, Soares P & Sobrinho-Simoes M. The biology and the genetics of Hurthle cell tumors of the thyroid. *Endocrine-Related Cancer* 2012 **19** R131–R147. (doi:10.1530/ERC-11-0354)
- 56 Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, Larsson C & Xu D. The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. *Oncogene* 2014 **33** 4978–4984. (doi:10.1038/onc.2013.446)
- 57 Gandolfi G, Ragazzi M, Frasoldati A, Piana S, Ciarrocchi A & Sancisi V. TERT promoter mutations are associated with distant metastases in papillary thyroid carcinoma. *European Journal of Endocrinology* 2015 **172** 403–413. (doi:10.1530/EJE-14-0837)
- 58 Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, Pai S & Bishop J. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *Journal of Clinical Oncology* 2014 **32** 2718–2726. (doi:10.1200/JCO.2014.55.5094)
- 59 Muzza M, Colombo C, Rossi S, Tosi D, Cirello V, Perrino M, De Leo S, Magnani E, Pignatti E, Vigo B *et al.* Telomerase in differentiated thyroid cancer: promoter mutations, expression and localization. *Molecular and Cellular Endocrinology* 2015 **399** 288–295. (doi:10.1016/j.mce.2014.10.019)
- 60 Sampson E, Brierley JD, Le LW, Rotstein L & Tsang RW. Clinical management and outcome of papillary and follicular (differentiated) thyroid cancer presenting with distant metastasis at diagnosis. *Cancer* 2007 **110** 1451–1456. (doi:10.1002/cncr.22956)
- 61 Liu X, Qu S, Liu R, Sheng C, Shi X, Zhu G, Murugan AK, Guan H, Yu H, Wang Y *et al.* TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E1130–E1136. (doi:10.1210/jc.2013-4048)
- 62 Michaloglou C, Vredevelde LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ & Peeper DS. BRAF600-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005 **436** 720–724. (doi:10.1038/nature03890)
- 63 Bell RJ, Rube HT, Kreig A, Mancini A, Fouse SD, Nagarajan RP, Choi S, Hong C, He D, Pekmezci M *et al.* Cancer. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. *Science* 2015 **348** 1036–1039. (doi:10.1126/science.aab0015)
- 64 Preto A, Singhrao SK, Haughton MF, Kipling D, Wynford-Thomas D & Jones CJ. Telomere erosion triggers growth arrest but not cell death in human cancer cells retaining wild-type p53: implications for anti-telomerase therapy. *Oncogene* 2004 **23** 4136–4145. (doi:10.1038/sj.onc.1207564)
- 65 Chiba K, Johnson JZ, Vogan JM, Wagner T, Boyle JM & Hockemeyer D. Cancer-associated TERT promoter mutations abrogate telomerase silencing. *eLife* 2015 **4** e07918. (doi:10.7554/eLife.07918)
- 66 Melo M, da Rocha AG, Vinagre J, Sobrinho-Simoes M & Soares P. Coexistence of TERT promoter and BRAF mutations in papillary thyroid carcinoma: added value in patient prognosis? *Journal of Clinical Oncology* 2015 **33** 667–668. (doi:10.1200/JCO.2014.59.4614)
- 67 Levine AJ & Oren M. The first 30 years of p53: growing ever more complex. *Nature Reviews. Cancer* 2009 **9** 749–758. (doi:10.1038/nrc2723)
- 68 Lane D & Levine A. p53 Research: the past thirty years and the next thirty years. *Cold Spring Harbor Perspectives in Biology* 2010 **2** a000893. (doi:10.1101/cshperspect.a000893)
- 69 Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi T, Hayashi Y, Dohi K, Nakamura N & Akiyama M. Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. *Cancer Research* 1992 **52** 1369–1371.
- 70 Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi Y, Dohi K, Nakamura N & Akiyama M. Genetic alterations in thyroid tumor progression: association with p53 gene mutations. *Japanese Journal of Cancer Research* 1993 **84** 526–531. (doi:10.1111/j.1349-7006.1993.tb00171.x)
- 71 Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G & Pierotti MA. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. *Journal of Clinical Investigation* 1993 **91** 1753–1760. (doi:10.1172/JCI116385)
- 72 Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH & Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *Journal of Clinical Investigation* 1993 **91** 179–184. (doi:10.1172/JCI116168)
- 73 Dobashi Y, Sakamoto A, Sugimura H, Mernyei M, Mori M, Oyama T & Machinami R. Overexpression of p53 as a possible prognostic factor in human thyroid carcinoma. *American Journal of Surgical Pathology* 1993 **17** 375–381. (doi:10.1097/00000478-199304000-00008)
- 74 Soares P, Cameselle-Teijeiro J & Sobrinho-Simoes M. Immunohistochemical detection of p53 in differentiated, poorly differentiated and undifferentiated carcinomas of the thyroid. *Histopathology* 1994 **24** 205–210. (doi:10.1111/j.1365-2559.1994.tb00511.x)
- 75 Park KY, Koh JM, Kim YI, Park HJ, Gong G, Hong SJ & Ahn IM. Prevalences of Gsz, ras, p53 mutations and ret/PTC rearrangement in differentiated thyroid tumours in a Korean population. *Clinical Endocrinology* 1998 **49** 317–323. (doi:10.1046/j.1365-2265.1998.00515.x)
- 76 Farid P, Gomb SZ, Peter I & Szende B. bcl2, p53 and bax in thyroid tumors and their relation to apoptosis. *Neoplasma* 2001 **48** 299–301.

Review	C Tavares, M Melo and others	Genetic predictors of cancer outcome	174:4	R126
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- 77 Wright PA, Lemoine NR, Goretzki PE, Wyllie FS, Bond J, Hughes C, Roher HD, Williams ED & Wynford-Thomas D. Mutation of the p53 gene in a differentiated human thyroid carcinoma cell line, but not in primary thyroid tumours. *Oncogene* 1991 **6** 1693–1697.
- 78 Nikiforov YE, Nikiforova MN, Gnepp DR & Fagin JA. Prevalence of mutations of ras and p53 in benign and malignant thyroid tumors from children exposed to radiation after the Chernobyl nuclear accident. *Oncogene* 1996 **13** 687–693.
- 79 Nikiforova MN, Wald AI, Roy S, Durso MB & Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1852–E1860. (doi:10.1210/jc.2013-2292)
- 80 Ruter A, Dreifus J, Jones M, Nishiyama R & Lennquist S. Overexpression of p53 in tall cell variants of papillary thyroid carcinoma. *Surgery* 1996 **120** 1046–1050. (doi:10.1016/S0039-6060(96)80053-5)
- 81 Putti TC & Bhuiya TA. Mixed columnar cell and tall cell variant of papillary carcinoma of thyroid: a case report and review of the literature. *Pathology* 2000 **32** 286–289.
- 82 Kleer CG, Giordano TJ & Merino MJ. Squamous cell carcinoma of the thyroid: an aggressive tumor associated with tall cell variant of papillary thyroid carcinoma. *Modern Pathology* 2000 **13** 742–746. (doi:10.1038/modpathol.3880129)
- 83 Cameselle-Teijeiro J, Menasce LP, Yap BK, Colaco RJ, Castro P, Celestino R, Ruiz-Ponte C, Soares P & Sobrinho-Simoes M. Cribriform-morular variant of papillary thyroid carcinoma: molecular characterization of a case with neuroendocrine differentiation and aggressive behavior. *American Journal of Clinical Pathology* 2009 **131** 134–142. (doi:10.1309/AJCP7ULS0VSISBEB)
- 84 Nakazawa T, Celestino R, Machado JC, Cameselle-Teijeiro JM, Vinagre J, Eloy C, Benserai F, Lameche S, Soares P & Sobrinho-Simoes M. Cribriform-morular variant of papillary thyroid carcinoma displaying poorly differentiated features. *International Journal of Surgical Pathology* 2013 **21** 379–389. (doi:10.1177/1066896912473355)
- 85 Pita JM, Figueiredo IF, Moura MM, Leite V & Cavaco BM. Cell cycle deregulation and TP53 and RAS mutations are major events in poorly differentiated and undifferentiated thyroid carcinomas. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E497–E507. (doi:10.1210/jc.2013-1512)
- 86 Quiros RM, Ding HG, Gattuso P, Prinz RA & Xu X. Evidence that one subset of anaplastic thyroid carcinomas are derived from papillary carcinomas due to BRAF and p53 mutations. *Cancer* 2005 **103** 2261–2268. (doi:10.1002/cncr.21073)
- 87 Evans JJ, Crist HS, Durvesh S, Bruggeman RD & Goldenberg D. A comparative study of cell cycle mediator protein expression patterns in anaplastic and papillary thyroid carcinoma. *Cancer Biology & Therapy* 2012 **13** 776–781. (doi:10.4161/cbt.20560)
- 88 Pilotti S, Collini P, Del Bo R, Cattoretto G, Pierotti MA & Rilke F. A novel panel of antibodies that segregates immunocytochemically poorly differentiated carcinoma from undifferentiated carcinoma of the thyroid gland. *American Journal of Surgical Pathology* 1994 **18** 1054–1064. (doi:10.1097/00000478-199410000-00009)
- 89 Moretti F, Farsetti A, Soddu S, Misiti S, Crescenzi M, Filetti S, Andreoli M, Sacchi A & Pontecorvi A. p53 re-expression inhibits proliferation and restores differentiation of human thyroid anaplastic carcinoma cells. *Oncogene* 1997 **14** 729–740. (doi:10.1038/sj.onc.1200887)
- 90 DeLellis RA, Lloyd RV, Heitz PU & Eng C. In *WHO Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs*. Lyon: IARC Press, 2004.
- 91 Cameselle-Teijeiro J, Rodríguez-Pérez I, Celestino R, Eloy C, Piso Neira I, Abdulkader Nallib I, Soares P & Sobrinho-Simões M. Hobnail/micropapillary variant of papillary thyroid carcinoma: Evidence of progression to undifferentiated carcinoma with molecular analysis. *Virchows Archiv* 2015 **467** (Supplement 1) S69.
- 92 Maze H. MicroRNA as a diagnostic tool in fine-needle aspiration biopsy of thyroid nodules. *Oncologist* 2012 **17** 1032–1038. (doi:10.1634/theoncologist.2012-0013)
- 93 Jendrzewski J, He H, Radomska HS, Li W, Tomsic J, Liyanarachchi S, Davuluri RV, Nagy R & de la Chapelle A. The polymorphism rs944289 predisposes to papillary thyroid carcinoma through a large intergenic noncoding RNA gene of tumor suppressor type. *PNAS* 2012 **109** 8646–8651. (doi:10.1073/pnas.1205654109)
- 94 Kentwell J, Gundara JS & Sidhu SB. Noncoding RNAs in endocrine malignancy. *Oncologist* 2014 **19** 483–491. (doi:10.1634/theoncologist.2013-0458)

Received 18 June 2015

Revised version received 7 October 2015

Accepted 28 October 2015