Follicular variant of papillary thyroid carcinoma: a diagnostic challenge for clinicians and pathologists

A Salajegheh, E B Petcu, R A Smith and A K-Y Lam

Postgrad. Med. J. 2008;84;78-82
doi:10.1136/pgmj.2007.064881

Follicular variant of papillary thyroid carcinoma: a diagnostic challenge for clinicians and pathologists

A Salajegheh, E B Petcu, R A Smith, A K-Y Lam

Discipline of Pathology (School of Medicine), Griffith University, Gold Coast, Queensland, Australia

Correspondence to: Professor A Lam, Department of Pathology, Griffith Medical School, Gold Coast Campus, Gold Coast, Queensland, 4222, Australia; a.lam@griffith.edu.au

Received 27 September 2007
Accepted 15 January 2008

ABSTRACT

The follicular variant of papillary thyroid carcinoma (FVPTC) presents a type of papillary thyroid cancer that has created continuous diagnosis and treatment controversies among clinicians and pathologists. In this review, we describe the nomenclature, the clinical features, diagnostic problems and the molecular biology of FVPTC. It is important for clinicians to understand this entity as the diagnosis and management of this group of patient may be different from other patients with conventional PTC. The literature suggests that FVPTC behaves in a way similar, clinically, to conventional papillary thyroid carcinoma. However, there are some genotypic differences which may characterise this neoplasm. These parameters may account for the phenotypic variation described by some scientists in this type of cancer. Further understanding can only be achieved by defining strict pathological criteria, in-depth study of the molecular biology and long term follow-up of the optional patients with FVPTC.

Papillary thyroid carcinoma (PTC) is the most common histological type of thyroid cancer and accounts for more than 70% of primary thyroid malignancies.[1–5] Recently, its incidence has been increasing, which is believed to be partly related to the detection of small early lesions by ultrasonography and fine needle aspiration biopsy.[6 7] The diagnosis of the follicular variant of papillary thyroid carcinoma (FVPTC) also adds to the numbers of PTC cases recorded worldwide. Although FVPTC has been recognised for more than 50 years, its diagnostic features, molecular biology and prognosis are still debatable.[2 8] It is important for clinicians to understand this entity as the diagnosis and management of this group of patients may be different from other patients with conventional PTC. In this review, the current knowledge of FVPTC will be evaluated.

THE MILESTONES OF FVPTC

FVPTC was first described in 1953 by Crile and Hazard who named this lesion alveolar variant of PTC.[9] The identification was confirmed by Lindsay in 1960.[10] The author observed that although the neoplasm had a follicular architectural pattern, the nuclear features were that of the conventional PTC. Therefore, the tumour should be designated as a FVPTC. Also, the author hypothesised that the biological behaviour of this condition should be similar to that of conventional PTC.[10] In 1976, Hawk and Hazard performed a study of 300 consecutive cases of PTC and noted significant diagnostic problems.[11] In 1977, Chen and Rosai stressed the importance of nuclear rather than architectural pattern in making a diagnosis of PTC. They showed that FVPTC behaves similarly to the conventional variant of PTC.[12] Thereafter, the FVPTC was diagnosed with greater frequency.

DIAGNOSTIC CHALLENGE

Most frequently, FVPTC presents as a thyroid nodule that is discovered incidentally or on routine examination. Sometimes, patients present with metastasis in a neck lymph node or with hoarseness of voice caused by involvement of the recurrent laryngeal nerve. Rarely, FVPTC gives rise to lung metastases in the absence of lymph node secondaries.[13]

Ultrasound imaging and fine needle aspiration biopsy are common tools necessary for investigation of patients suspected of having thyroid cancer. The ultrasound makes a clear differentiation between solid and cystic lesions and also identifies calcifications.[14] Fine needle aspiration biopsy is also useful in the diagnosis of papillary carcinoma.[1] However, some studies have reported a very low sensitivity with fine needle aspiration for the identification of FVPTC. It is accepted that the only reliable way to diagnose this tumour is histological examination of the thyroidectomy specimen.

The main differential diagnoses of this tumour are follicular adenoma or follicular carcinoma. Morphologically, FVPTC may appear partially or completely encapsulated.[15 16] This is very important to know since FVPTC can be misdiagnosed as follicular adenoma or as follicular thyroid carcinoma if capsular or vascular invasion are present.[15] Also, conventional PTC with follicular pattern and papillary microcarcinoma may be misdiagnosed as FVPTC.

LiVolsi and Baloch have suggested that the challenge posed by an FVPTC diagnosis can be explained by the following: (1) large numbers of this malignancy arise in a background of nodular goitre, resembling adenoma or adenomatoid nodules which mostly lack of capsular and vascular invasion; (2) some tumours show multifocal rather than diffuse distribution of typical nuclear features of papillary thyroid carcinoma; (3) the majority of the encapsulated FVPTCs are solitary, lack any invasive characteristics, are confined to the thyroid and behave in an indolent fashion.[17] In a multicentric study, experienced thyroid pathologists were asked to make a diagnosis of this papillary variant. Surprisingly, the concordance rate was only 40%. Nevertheless, the authors concluded that cytoplasmic invagination into the nucleus, abundant nuclear grooves, ground glass nuclei, psammoma bodies, enlarged overlapping nuclei

and irregularly shaped nuclei were the most important criteria for diagnosis of FVPTC. Dark staining colloid, irregular contours of follicles, scalloping of colloid, elongated follicles and multinucleated macrophages in the lumen of follicles were viewed as less important diagnostic parameters.[8]

CLINICAL BEHAVIOUR

It has been always difficult to document the clinical behaviour and long term outcome of FVPTC. Many published series evaluating clinicopathologic features and outcome of this tumour lack a clear definition of this condition. Some studies also evaluate relatively few cases, and no long term follow-up was included in the assessment.[3 5 18 19] Many authorities believe that FVPTC has the same prognosis as conventional PTC.[3 18 19]

FVPTC patients present with larger tumour size and younger age groups.[3 18 20 21] However, in some reports, FVPTC was found to mimic the pathologic features and clinical behaviour of follicular neoplasms.[2 20] Some encapsulated FVPTC metastasise to distant sites in the absence of lymph node metastases, mimicking the behaviour of follicular carcinoma.[13] Also, some FVPTC cases demonstrated a significantly higher prevalence of angiovascular and capsular invasion, distant metastases and poorly differentiated areas.[19 22 23] However, it was noted on histological examination that FVPTC has significantly lower rates of lymph node metastases, is more often encapsulated and shows extra-thyroidal invasion less often than conventional PTC.[2 5 18 19 22] Furthermore, FVPTC showed less calcification, psammoma bodies and bone formation in comparison with PTC.[21]

In a recent study that involved more than 500 thyroid cancer patients and with more than 15 years of follow-up, FVPTC was concluded to have more favourable clinicopathological features and a better tumour risk group profile. However, long term outcome was similar to conventional PTC patients.[5]

MOLECULAR BIOLOGY

In recent years, advances in molecular biology have shed some light on the diagnosis and prognosis of thyroid cancer. The controversial issues related to the follicular variant of PTC could be explained by molecular biology. In papillary thyroid cancer, the three most commonly reported genetic alterations in the carcinogenesis are the BRAF, RAS and RET/PTC oncogenes.[24] All these genes are involved in the control of cellular growth and differentiation, occupying various positions in the major cell growth signalling pathway, and the most common genetic abnormalities in PTC are located at this level.

RET is a proto-oncogene located on chromosome 10q11.2 that encodes a protein tyrosine kinase receptor with an extracellular domain, a transmembrane domain, and an intracytoplasmic kinase domain.[25] There are a number of RET/PTC gene rearrangements, all produced by the fusion of the tyrosine kinase portion of RET with 5′-portions of different genes. RET/PTC1 and RET/PTC3 are the most common types, making up more than 90%, whereas RET/PTC2 and several other types are uncommon.[26–32] The rearrangement is considered to be an early event in thyroid tumourogenesis. While in some studies it has been reported that the prevalence of RET/PTC rearrangements was similar in CPTC and FVPTC (40–45%), it has been shown that FVPTC has lower frequencies of RET/PTC rearrangements than conventional PTC.[29 33–35]

BRAF is a protein kinase that has an important role in cell proliferation, differentiation, and programmed cell death. Initially, activating mutations of BRAF were found in malignant melanomas, and colorectal and ovarian carcinomas.[36] Now, it is recognised that BRAF is also mutated in PTC with high frequency, between 29–69% of cases.[37 38] The most common mutation is characterised by a change of a valine amino acid to glutamate in codon 600 (BRAF mutation V600E, previously labelled V599E) which causes increased kinase activity.[37–41] The prevalence of BRAF mutations has been shown to correlate positively with age and it has been shown that BRAF mutation may be related to prognosis.[38 42 43]

A distinct BRAF mutation (K601E) has been detected in FVPTC.[38] Trovisco *et al* claimed that this mutation is typical of FVPTC. However, it is less frequent than BRAF V600E in CPTC. The substitution of a lysine by a glutamate in codon 601 (BRAF K601E) in the FVPTC causes increased kinase activity and resembles the BRAF V600E mutation found in CPTC.[44] It is worth noting that BRAF V600E has about 2.5× the kinase activity of BRAF K601E.[36 45]

Ras proto-oncogenes, known individually as H-ras, K-ras and N-ras are members of the large family of guanosine triphosphate (GTP) binding proteins. The proteins are associated with the cellular membrane and are bound to guanosine diphosphate

Scientific advances that have allowed development of understanding of the clinical aspects of FVPTC

- The concordance rate of diagnosing FVPTC in the hands of experts was only 40%.
- FVPTC patients present with larger tumour size and younger age groups than conventional PTC.
- FVPTC shows less calcification, psammoma bodies and bone formation in comparison with PTC.
- FVPTC has more favourable clinicopathological features (lower rates of lymph node metastases, more often encapsulated and shows extra-thyroidal invasion less often than conventional PTC).
- FVPTC has a better tumour risk group profile than conventional PTC.
- The long term outcome of patients with FVPTC was similar to patients with conventional PTC.

Outstanding research questions or things we need to know

- The three most commonly reported genetic alterations in PTC are the BRAF, RAS and RET/PTC oncogenes.
- FVPTC has lower frequencies of RET/PTC rearrangements than conventional PTC.
- FVPTC can have a different mutation (K601E) from conventional PTC.
- Ras mutation pattern in FVPTC is similar to that of follicular carcinoma.
- PAX8-PPARγ gene fusion is mostly seen in follicular carcinomas but can be seen in FVPTC.
- Cylcooxygenase-2 was often positive in conventional PTC but negative in FVPTC.
- There was a frequent lack of p16 protein expression and promoter methylation in FVPTC when compared with conventional PTC.
- Caution is needed in interpreting the literature of molecular biology in thyroid carcinoma.

Learning points

- Papillary thyroid carcinoma (PTC) is the most common histological type of thyroid cancer.
- Increasing incidence of PTC is related to the detection of small early lesion and increased diagnosis of the follicular variant of papillary thyroid carcinoma (FVPTC).
- Nuclear features rather than architectural pattern are important in making a diagnosis of PTC.
- The only reliable way to diagnose FVPTC is histological examination of the thyroidectomy specimen.
- The main differential diagnoses of FVPTC are follicular adenoma, follicular carcinoma, conventional papillary carcinoma and papillary microcarcinoma.
- The decision to perform a total thyroidectomy mostly depends on the patient's risk factors, tumour characteristics, the presence or absence of nodal metastases, and the patient's choice.

Key references

- Lloyd RV, Erickson LA, Casey MB, *et al*. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am J Surg Pathol* 2004;**28**:1336–40.
- Lang BH, Lo CY, Chan WF, *et al*. Classical and follicular variant of papillary thyroid carcinoma: a comparative study on clinicopathologic features and long-term outcome. *World J Surg* 2006;**30**:752–8.
- LiVolsi VA, Baloch ZW. Follicular neoplasms of the thyroid view, biases, and experiences. *Adv Anat Pathol* 2004;**11**:279–87.
- Lam AKY, Lo CY, Lam KSL. Papillary carcinoma of thyroid: a 30-yr clinicopathological review of the histological variants. *Endocr Pathol* 2005;**16**:323-30.
- Xing M. BRAF mutation in thyroid cancer. *Endocr Relat Cancer* 2005;**12**:245–62.

(GDP) when inactive. They have been shown to play a key role in signal transduction as molecular switches modulating proliferation and malignant transformation. Activating point mutations of the ras genes arise in codons 12, 13, or 61. They are usually found in follicular carcinomas, at frequencies of between 18–52%.[23 46 47] Their existence has been reported with a significantly lower frequency in PTC.[23 48] Interestingly, PTC and follicular carcinoma have different patterns of ras oncogene activating mutations. However, it has been reported that the ras mutation pattern in FVPTC is similar to that of follicular carcinoma.[23 49] There is a possibility of a strong correlation between ras mutation and follicular differentiation in thyroid carcinomas.

Changes to these three prototype genes above have been found in approximately 70% of PTC.[24] In other words, approximately 30% of thyroid cancers may have other genetic alterations. Some of the observed candidates for these genetic changes are detailed below.

PAX8-PPARγ gene fusion is mostly seen in follicular carcinomas (53–63%) and in some follicular adenomas (8–13%).[50–55] It has been cytogenetically identified as translocation t (2;3) (q13;p25).[55] Its prevalence in follicular carcinomas may be higher in tumours associated with radiation exposure as it has been seen in Belarusian citizens exposed to ionising radiation after the Chernobyl nuclear accident in 1986. It is also believed that the existence of this rearrangement may be involved in the invasive behaviour of the follicular neoplasm.[50 54 56–58] Roque *et al* found PAX8-PPARγ gene fusion in a case of FVPTC by using conventional cytogenetics.[59] Furthermore, there is an association between PAX8-PPARγ and the presence of multifocality and vascular invasive characteristics in FVPTC.[60] Thus, this rearrangement may have a possible role in promoting metastases.[54 58]

Cylcooxygenases are known to play a role in the formation of prostaglandins and consist of two identified isoforms, COX-1 and COX-2. Cyclooxygenase-2 (COX-2) has a role in the carcinogenesis of PTC. Expression of COX-2 has been mostly observed in PTC but not in benign thyroid nodules. Previous studies have revealed that COX-2 expression was significantly lower in anaplastic and follicular carcinomas than in PTC.[61–63] It was also shown that COX-2 expression in PTC was noticeably reduced in elderly patients, large sized tumours and in cases associated with advanced stage and satellite tumours. Therefore, there was a suggestion that COX-2 may be important in the early phase of pathogenesis of thyroid carcinoma.[61 62] The gene may therefore represent a useful target for the identification of thyroid tissue transitioning from a benign to a malignant state. Lo and colleagues demonstrated a significant difference in the level of COX-2 mRNA in PTC compared with adjacent non-tumorous and benign thyroid tissues. This study has also investigated the expression of COX-2 in the FVPTC. It was shown that most FVPTC cases are negative for COX-2.[63]

The p16 gene encodes the p16 protein, which competes with cyclin D for binding to CDK4. This inhibits the ability of the cyclin D–CDK4 complex to phosphorylate RB (retinoblastoma), thus causing cell cycle arrest in the late G1 phase. The roles of this gene in PTC have been investigated in a few studies.[64–68] Studies indicated that different types of p16 alterations are present in approximately 80% of patients with PTC. It is worth noting that there was a frequent lack of p16 protein expression and promoter methylation in FVPTC when compared with conventional PTC.[68]

The limited data available in the literature suggest that FVPTC has different molecular alterations compared to conventional papillary thyroid carcinoma. However, we should be cautious in interpreting the literature. Firstly, the number of follicular variant tumours described in some studies was small. Secondly, due to the difficult morphological assessment, some cases of follicular neoplasm, papillary microcarcinoma or conventional PTC may have been misdiagnosed as FVPTC. Nevertheless, we believe that FVPTC has some genotypic differences compared with conventional PTC which lead to the phenotypic difference between these two entities. More studies are necessary to address those issues.

MANAGEMENT

As the long term outcome of FVPTC is similar to conventional PTC patients, FVPTC may be managed similarly to conventional PTC.[5] Surgery as a standard preferred procedure of choice has been performed for many years. Patients would undergo total or near total thyroidectomy. The decision to perform a total thyroidectomy mostly depends on the patient's risk factors, tumour characteristics, the presence or absence of nodal metastases and the patient's choice.[5 18 69] Decisions on the subsequent management after surgery are often made by oncologists and surgeons. Postoperative radioactive iodine

(I^{131}) ablation (dose 80–100 mCi), followed by whole body scintigraphy and thyroxin suppression therapy, should be given to patients with high risk factors. Follow-up examinations may include clinical investigations, chest *x* ray, cervical ultrasonography or magnetic resonance image (MRI) scan and measurement of thyroglobulin levels without thyrotropin stimulation.[5] Furthermore, radioactive iodine (I^{131}) whole body scintigraphy should be repeatedly performed for the detection of suspicious recurrences and for patients with an elevated thyroglobulin level.[3–5 18 19 69] In addition, pathological consultation may be needed on follow-up if the patient develops features suggestive of follicular neoplasm rather than papillary carcinoma (for example, vascular metastases). In this circumstance the initial diagnosis needs to be reviewed as follicular neoplasms may be misdiagnosed as FVPTC.

CONCLUSIONS

FVPTC has distinct clinicopathological characteristics. The correct histopathological diagnosis of this entity is difficult. Moreover, some of the molecular features of FVPTC may differ from those of conventional PTC. Nevertheless, it is likely that some specimens of FVPTC may mimic the pathologic features and clinical behaviour of follicular adenoma or carcinoma. Further understanding of this malignancy can only be achieved by attention to pathological criteria, in-depth study of the carcinoma's molecular biology and long term follow-up of patients.

MULTIPLE CHOICE QUESTIONS (TRUE (T)/FALSE (F); ANSWERS AFTER THE REFERENCES)

1. Epidemiology of papillary thyroid carcinoma and its follicular variant (FVPTC):

(A) Papillary thyroid carcinoma (PTC) is the most common histological type of thyroid cancer

(B) PTC's increasing incidence is partly related to the detection of small early lesions by ultrasonography and fine needle aspiration biopsy

(C) FVPTC accounts for >70% of primary thyroid malignancies

(D) FVPTC accounts partly for the increasing number of PTC cases recorded worldwide in the recent years

(E) FVPTC is mostly discovered as a thyroid nodule on routine examination

2. Pathological characteristics of FVPTC:

(A) FVPTC is often smaller than conventional PTC

(B) FVPTC often occurs in the elderly groups

(C) FVPTC has nuclear grooves and intranuclear inclusions

(D) FVPTC does not metastasise to lymph node

(E) FVPTC has the tumour cells arranged in follicles

3. Diagnosis of FVPTC:

(A) Ultrasound imaging and fine needle aspiration biopsy are necessary tools for the investigation of patients suspected of having FVPTC

(B) In pathological diagnosis of FVPTC, the cytoplasmic features are more critical than nuclear features of the neoplasm

(C) The most reliable way to diagnose FVPTC is histological examination of the thyroidectomy specimen

(D) FVPTC is the neoplasm that has a follicular architectural pattern but the nuclear features of the conventional papillary thyroid carcinoma

(E) Most physicians now believe that FVPTC behaves similarly to the classic variant of papillary thyroid carcinoma

4. Molecular biology of papillary thyroid carcinoma:

(A) The BRAF, RAS and RET/PTC are the most common genetic alterations in papillary thyroid cancer

(B) RET/PTC rearrangement is an early event in thyroid tumourogenesis

(C) The BRAF mutation pattern of FVPTC is different from that in conventional papillary thyroid carcinoma

(D) RAS mutation pattern in FVPTC is similar to that of follicular carcinoma of thyroid

(E) The expression of COX-2, p16 protein expression and promoter methylation are more frequent in FVPTC when compared with conventional PTC

5. Management of FVPTC:

(A) Total or near total thyroidectomy is the standard preferred procedure of choice in the management of FVPTC

(B) The management of the patient depends on the patient's risk factors, tumour characteristics, the presence or absence of nodal metastases and the patient's choice

(C) Postoperative radioactive iodine ablation, followed by whole body scintigraphy and thyroxin suppression therapy, should be given to patients with low risk factors

(D) Clinical investigations, chest *x* ray, cervical ultrasonography or MRI scan and measurement of thyroglobulin levels are performed as the follow-up examinations of patients

(E) Pathological consultation on follow-up is necessary if the patient develops features suggestive of follicular neoplasm such as vascular metastases

Funding: None

Competing interests: None.

REFERENCES

1. **Al-Brahim N,** Asa SL. Papillary thyroid carcinoma: an overview. *Arch Pathol Lab Med* 2006;**130**:1057–62.
2. **Liu J,** Singh B, Tallini G, *et al*. Follicular variant of papillary thyroid carcinoma: a clinicopathologic study of a problematic entity. *Cancer* 2006;**107**:1255–64.
3. **Burningham AR,** Krishnan J, Davidson BJ, *et al*. Papillary and follicular variant of papillary carcinoma of the thyroid: initial presentation and response to therapy. *Otolaryngol Head Neck Surg* 2005;**132**:840–4.
4. **Liska J,** Altanerova V, Galbavy Š, *et al*. Thyroid tumours: histological classification and genetic factors involved in the development of thyroid cancer. *Endocr Regul* 2005;**39**:73–83.
5. **Lang BH,** Lo CY, Chan WF, *et al*. Classical and follicular variant of papillary thyroid carcinoma: a comparative study on clinicopathologic features and long-term outcome. *World J Surg* 2006;**30**:752–8.
6. **Burgess JR.** Temporal trends for thyroid carcinoma in Australia: an increasing incidence of papillary thyroid carcinoma (1982–1997).*Thyroid* 2002;**12**:141–9.
7. **Mackenzie EJ,** Mortimer RH. Thyroid nodules and thyroid cancer. *Med J Aust* 2004;**180**:242–7.
8. **Lloyd RV,** Erickson LA, Casey MB, *et al*. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am J Surg Pathol* 2004;**28**:1336–40.
9. **Crile G,** Hazard JB. Relationship of the age of the patient to the natural history and prognosis of carcinoma of the thyroid. *Ann Surg* 1953;**138**:33–8.
10. **Lindsay S.** *Carcinoma of the thyroid gland: a clinical and pathologic study of 293 patients at the University of California Hospital*. Springfield, Illinois: Charles C Thomas, 1960;42–52
11. **Hawk WA,** Hazard JB. The many appearances of papillary carcinoma of the thyroid. *Cleve Clin Q* 1976;**43**:207–15.
12. **Chen KT,** Rosai J. Follicular variant of thyroid papillary carcinoma: a clinicopathologic study of six cases. *Am J Surg Pathol* 1977;**1**:123–30.

13. **Baloch ZW,** LiVolsi VA. Follicular-patterned lesions of the thyroid: the bane of the pathologist. *Am J Clin Pathol* 2002;**117**:143–50.
14. **Reading CC,** Charboneau JW, Hay ID, *et al*. Sonography of thyroid nodules: a "classic pattern" diagnostic approach. *Ultrasound Q* 2005;**21**:157–65.
15. **Castro P,** Fonseca E, Magalhães J, *et al*. Follicular, papillary, and hybrid carcinomas of the thyroid. *Endocr Pathol* 2002;**13**:313–20.
16. **Rosai J,** Zampi G, Carcangiu ML. Papillary carcinoma of the thyroid: a discussion of its several morphologic expressions, with particular emphasis on the follicular variant. *Am J Surg Pathol* 1983;**7**:809–17.
17. **LiVolsi VA,** Baloch ZW. Follicular neoplasms of the thyroid view, biases, and experiences. *Adv Anat Pathol* 2004;**11**:279–87.
18. **Passler C,** Prager G, Scheuba C, *et al*. Follicular variant of papillary thyroid carcinoma: a long-term follow-up. *Arch Surg* 2003;**138**:1362–6.
19. **Zidan J,** Karen D, Stein M, *et al*. Pure versus follicular variant of papillary thyroid carcinoma: clinical features, prognostic factors, treatment, and survival. *Cancer* 2003;**97**:1181–5.
20. **Wreesmann VB,** Ghossein RA, Hezel M, *et al*. Follicular variant of papillary thyroid carcinoma: genome-wide appraisal of a controversial entity. *Genes Chromosomes Cancer* 2004;**40**:355–64.
21. **Lam AKY,** Lo CY, Lam KSL. Papillary carcinoma of thyroid: a 30-yr clinicopathological review of the histological variants. *Endocr Pathol* 2005;**16**:323–30.
22. **Chang HY,** Lin JD, Chou SC, *et al*. Clinical presentations and outcomes of surgical treatment of follicular variant of the papillary thyroid carcinomas. *Jpn J Clin Oncol* 2006;**36**:688–93.
23. **Zhu Z,** Gandhi M, Nikiforova MN, *et al*. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma: an unusually high prevalence of ras mutations. *Am J Clin Pathol* 2003;**120**:71–7.
24. **Adeniran AJ,** Zhu Z, Gandhi M, *et al*. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. *Am J Surg Pathol*. 2006;**30**:216–22.
25. **Takahashi M,** Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cells* 1985;**42**:581–8.
26. **Elisei R,** Romei C, Vorontsova T, *et al*. RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated malignant and benign thyroid lesions in children and adults. *J Clin Endocrinol Metab* 2001;**86**:3211–16.
27. **Thomas GA,** Bunnell H, Cook HA, *et al*. High prevalence of RET/PTC rearrangements in Ukrainian and Belarusian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. *J Clin Endocrinol Metab* 1999;**84**:4232–8.
28. **Di Cristofaro J,** Vasko V, Savchenko V, *et al*. Ret/PTC1 and ret/PTC3 in thyroid tumours from Chernobyl liquidators: comparison with sporadic tumours from Ukrainian and French patients. *Endocr Relat Cancer* 2005;**12**:173–83.
29. **Rabes HM,** Demidchik EP, Sidorow JD, *et al*. Pattern of radiation induced RET and NTRK1 rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clin Cancer Res* 2000;**6**:1093–103.
30. **Tallini G,** Santoro M, Helie M, *et al*. RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumour phenotypes. *Clin Cancer Res* 1998;**4**:287–94.
31. **Bongarzone I,** Vigneri P, Mariani L, *et al*. RET/NTRK1rearrangements in thyroid gland tumours of the papillary carcinoma family: correlation with clinicopathological features. *Clin Cancer Res* 1998;**4**:223–8.
32. **Nikiforova MN,** Caudill CM, Biddinger P, *et al*. Prevalence of RET/PTC rearrangements in Hashimoto's thyroiditis and papillary thyroid carcinomas. *Int J Surg Pathol* 2002;**10**:15–22.
33. **Puxeddu E,** Moretti S, Elisei R, *et al*. BRAF (V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. *J Clin Endocrinol Metab* 2004;**89**:2414–20.
34. **Lima J,** Trovisco V, Soares P, *et al*. BRAF mutations are not a major event in post-Chernobyl childhood thyroid carcinomas. *J Clin Endocrinol Metab* 2004;**89**:4267–71.
35. **Lam AK,** Montone KT, Nolan KA, *et al*. Ret oncogene activation in papillary thyroid carcinoma: prevalence and implication on the histological parameters. *Hum Pathol* 1998;**29**:565–8.
36. **Davies H,** Bignell GR, Cox C, *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54.
37. **Nikiforova MN,** Kimura ET, Gandhi M, *et al*. BRAF mutations in thyroid tumours are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *J Clin Endocrinol Metab* 2003;**88**:5399–404.
38. **Trovisco V,** Vieira De Castro I, Soares P, *et al*. BRAF mutations are associated with some histological types of papillary thyroid carcinoma. *J Pathol* 2004;**202**:247–51.
39. **Kimura ET,** Nikiforova MN, Zhu Z, *et al*. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RASBRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res* 2003;**63**:1454–7.
40. **Cohen Y,** Xing M, Mambo E, *et al*. BRAF mutation in papillary thyroid carcinoma. *J Natl Cancer Inst* 2003;**95**:625–27.
41. **Soares P,** Trovisco V, Rocha AS, *et al*. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. *Oncogene* 2003;**22**:4578–80.
42. **Xing M.** BRAF mutation in thyroid cancer. *Endocr Relat Cancer* 2005;**12**:245–62.
43. **Xing M,** Westra WH, Tufano RP, *et al*. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab* 2005;**90**:6373–9.
44. **Trovisco V,** Soares P, Preto A, *et al*. Type and prevalence of BRAF mutations are closely associated to papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. *Virchows Arch* 2005;**446**:589–95.
45. **Wan PT,** Garnett MJ, Roe SM, *et al*. Mechanism of activation of the RAF–ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004;**116**:855–67.
46. **Esapa CT,** Johnson SJ, Kendall-Taylor P, *et al*. Prevalence of Ras mutations in thyroid neoplasia. *Clin Endocrinol* 1999;**50**:529–35.
47. **Shi YF,** Zou MJ, Schmidt H, *et al*. High rates of ras codon 61 mutation in thyroid tumours in an iodide-deficient area. *Cancer Res* 1991;**51**:2690–3.
48. **Lemoine NR,** Mayall ES, Wyllie FS, *et al*. Activated ras oncogenes in human thyroid cancers. *Cancer Res* 1988;**48**:4459–63.
49. **Vasko V,** Ferrand M, Di Cristofaro J, *et al*. Specific pattern of RAS oncogene mutations in follicular thyroid tumours. *J Clin Endocrinol Metab* 2003;**88**:2745–52.
50. **Nikiforova MN,** Biddinger PW, Caudill CM, *et al*. PAX8- PPARgamma rearrangement in thyroid tumours: RT-PCR and immunohistochemical analyses. *Am J Surg Pathol* 2002;**26**:1016–23.
51. **Marques AR,** Espadinha C, Catarino AL, *et al*. Expression of PAX8-PPARγ1 rearrangements in both follicular thyroid carcinomas and adenomas. *J Clin Endocrinol Metab* 2002;**87**:3947–52.
52. **Cheung L,** Messina M, Gill A, *et al*. Detection of the PAX8-PPARγ fusion oncogene in both follicular thyroid carcinomas and adenomas. *J Clin Endocrinol Metab* 2003;**88**:354–7.
53. **Lacroix L,** Mian C, Barrier T, *et al*. PAX8 and peroxisome proliferator-activated receptor γ 1 gene expression status in benign and malignant thyroid tissues. *Eur J Endocrinol* 2004;**151**:367–74.
54. **Castro P,** Rebocho AP, Soares RJ, *et al*. PAX8-PPARγ Rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2006;**91**:213–20.
55. **Kroll TG,** Sarraf P, Pecciarini L, *et al*. PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma. *Science* 2000;**289**:1357–60.
56. **Dwight T,** Thoppe SR, Foukakis T, *et al*. Involvement of the PAX8/peroxisome proliferator- activated receptor γ rearrangement in follicular thyroid tumours. *J Clin Endocrinol Metab* 2003;**88**:4440–5.
57. **Au AY,** McBride C, Wilhelm KG Jr, *et al*. PAX8-Peroxisome proliferator-activated receptor γ (PPARγ) disrupts normal PAX8 or PPARγ transcriptional function and stimulates follicular thyroid cell growth. *Endocrinology* 2006;**147**:367–76.
58. **Nikiforova MN,** Lynch RA, Biddinger PW, *et al*. RAS point mutations and PAX8-PPARγ rearrangement in thyroid tumours: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab* 2003;**88**:2318–26.
59. **Roque L,** Nunes VM, Ribeiro C, *et al*. Karyotypic characterization of papillary thyroid carcinomas. *Cancer* 2001;**92**:2529–38.
60. **Castro P,** Roque L, Magalhães J, *et al*. A subset of follicular variant of papillary carcinoma harbours PAX8–PPARγ translocation. *Int J Surg Pathol* 2005;**13**:235–238.
61. **Kim SJ,** Lee J, Yoon JS, *et al*. Immunohistochemical expression of COX-2 in thyroid nodules. *Korean J Intern Med* 2003;**18**:225–9.
62. **Ito Y,** Yoshida H, Nakano K, *et al*. Cyclooxygenase-2 expression in thyroid neoplasms. *Histopathology* 2003;**42**:492–7.
63. **Lo CY,** Lam KY, Leung PP, *et al*. High prevalence of cyclooxygenase 2 expression in papillary thyroid carcinoma. *Eur J Endocrinol* 2005;**152**:545–50.
64. **Boltze C,** Zack S, Quednow C, *et al*. Hypermethylation of the CDKN2/p16INK4A promotor in thyroid carcinogenesis. *Pathol Res Pract* 2003;**199**:399–404.
65. **Ferenc T,** Lewinski A, Lange D, *et al*. Analysis of p161NK4A protein expression in follicular thyroid tumours. *Pol J Pathol* 2004;**55**:143–8.
66. **Schagdarsurengin U,** Gimm O, Hoang-Vu C, *et al*. Frequent epigenetic silencing of the CpG island promoter of RASSF1A in thyroid carcinoma. *Cancer Res* 2002;**62**:3698–701.
67. **Elisei R,** Shiohara M, Koeffler HP, *et al*. Genetic and epigenetic alterations of the cyclin-dependent kinase inhibitors p16INK4b and p16INK4a in human thyroid carcinoma cell lines and primary thyroid carcinomas. *Cancer* 1998;**83**:2185–93.
68. **Lam AKY,** Lo CY, Leung P et al. Clinicopathological roles of alterations of tumor suppressor gene p16 in papillary thyroid carcinoma. *Ann Surg Oncol* 2007;**14**:1772–9.
69. **Lo CY,** Chan WF, Lam KY, *et al*. Optimizing the treatment of AMES high-risk papillary thyroid carcinoma. *World J Surg* 2004;**28**:1103–9.

Answers

1. (A) T (B) T (C) F (D) T (E) T
2. (A) F (B) F (C) T (D) F (E) T
3. (A) T (B) F (C) T (D) T (E) T
4. (A) T (B) T (C) T (D) T (E) F
5. (A) T (B) T (C) F (D) T (E) T