***TERT* promoter mutational screening as a tool to predict malignant behaviour in follicular thyroid tumours—three examples from the clinical routine**

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Abstract

Follicular thyroid adenomas (FTAs) and carcinomas (FTCs), collectively the most common thyroid neoplasms, constitute a significant clinical challenge since histological evidence of invasive behaviour is required for a malignant diagnosis. Small subsets of FTAs relapse as manifest malignant FTCs, indicating that histology is not always adequate to predict malignant potential. Lately, recurrent mutations in the promoter of the *Telomerase reverse transcriptase* (*TERT*) gene have been coupled to FTCs, whereas FTAs usually lack this aberrancy. We describe three patients with follicular thyroid tumours in which *TERT* promoter mutational screening was employed as part of the clinical work-up to pinpoint malignant potential. In two retrospective analyses of seemingly benign lesions, the detected mutations predicted future skeletal metastases, and in one prospective case, the mutational screening led to a different clinical management of the afflicted patient. We therefore consider *TERT* promoter mutational screening an adjunct tool of value in equivocal cases.

Keywords *TERT* · Thyroid cancer · Mutation · Clinical practice

Introduction

Two point mutations of the *Telomerase reverse transcriptase* (*TERT*) promoter (C228T and C250T, corresponding to positions –124 and –146 base pairs from the ATG site) were recently shown to confer increased *TERT* expression, and these events have been demonstrated for various malignancies, including thyroid cancer [1–6]. More specifically, these mutations have been intimately associated with follicular thyroid cancer (FTC) and follicular tumours of uncertain malignant potential (FT-UMP) as opposed to a near-total absence in

follicular thyroid adenoma (FTA) [7]. *TERT* encodes the catalytic subunit of telomerase, an enzyme capable of extending telomere length—a pivotal mechanism for cancer cells to evade telomere shortening and subsequent programmed cell death [8]. Therefore, *TERT* promoter mutations and the ensuing *TERT* overexpression are expected to provide malignant tumours with a selective advantage. In this study, three cases from the clinical practice are highlighted for which *TERT* promoter mutational screening conveyed an adjunct tool for pinpointing malignant potential in follicular thyroid tumours with an equivocal histology.

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Case presentations

The first case is a 62-year-old male of Swedish ethnicity with a history of ankylosing spondylitis. In 2012, he presented with a 30-mm nodule in his right thyroid lobe, and a preoperative cytology fine-needle aspiration biopsy (FNAB) suggested a follicular tumour (Bethesda IV). A diagnostic hemithyroidectomy was performed, and the histopathological examination revealed a 40-mm, well-circumscribed FTA without signs of vascular or

capsular invasion, but with slightly elevated Ki-67 proliferation index (4.6%) (Fig. 1(A)). In 2018, 6 years after the operation, the patient was diagnosed with a pathologic fracture of the humerus, and radiology suggested a metastatic lesion. An intra-operative biopsy from the fractured area revealed metastatic thyroglobulin-positive adenocarcinoma with a follicular growth pattern, indicative of metastatic FTC (Fig. 1(B)). Careful histopathological re-examination of the original thyroid tumour revealed small foci of intra-capsular tumour cell deposits, but no evident malignant phenotype, suggesting a diagnosis of “follicular tumour of uncertain malignant potential” as outlined by the novel 2017 WHO criteria (Fig. 1(C)) [9]. We then retrospectively performed *TERT* promoter mutational analyses from the patient’s original thyroid

tumour and the FTC metastatic lesion using extraction of genomic DNA from the formalin-fixed paraffin-embedded (FFPE) material and bi-directional Sanger sequencing of the *TERT* promoter region covering upstream positions C228 and C250 using the Genetic Analyser 3500 (Applied Biosystems, Foster City, USA). These analyses revealed a C228T *TERT* promoter mutation in both the original FT-UMP and the subsequent metastatic lesion, providing molecular evidence that the original thyroid tumour exhibited malignant potential (Table 1). If the pathologist reviewing the original follicular tumour would have had access to *TERT* promoter mutational screening, the final histopathological diagnosis would in theory remain unaltered—but the finding of such a mutation would certainly have intensified the follow-up

Fig. 1 Photomicrographs illustrating the cases detailed in the study. All cases are magnified $\times 100$ unless otherwise specified. (A) Case 1: the primary thyroid lesion diagnosed as FTA. Upon re-investigation 6 years later, this tumour was found to harbour a C228T *TERT* promoter mutation. (B) Case 1: scrapings from a pathological fracture of the humerus demonstrating metastatic FTC with strong immunoreactivity for thyroglobulin, magnified $\times 400$. (C) Case 1: the originally diagnosed FTA was re-classified as an FT-UMP. Arrow indicates area with questionable capsular invasion. Insert ($\times 400$) highlighting the capsular engagement. Note the focal nuclear atypia within this part of the tumour. (D) Case 2: the primary thyroid lesion originally classified as multinodular goitre, and later re-classified as a follicular tumour, in which the distinction between FTA and FTC could not be ascertained based on the limited original sampling. This sample carried a C228T

TERT promoter mutation. (E) Case 2: neck scar recurrence, initially interpreted as a tumoural implant. An asterisk marks the epidermal layer of the skin. Magnification $\times 40$. (F) Case 2: neck scar recurrence. Upon re-investigation, foci suggestive for vascular invasion were detected (arrows). A C228T *TERT* promoter mutation was demonstrated for this lesion. (G) Case 3: pulmonary lesion demonstrating thyroid tissue arranged in a macrofollicular growth pattern (left) encapsulated from the lung tissue (right). This lesion carried a C228T *TERT* promoter mutation, thereby favouring metastatic FTC over ectopic thyroid tissue as the final diagnosis. Magnification $\times 40$. (H) Case 3: widely invasive FTC in the right thyroid lobe positive for the C228T *TERT* promoter mutation, macroscopic image. Arrow indicates a focus of capsular invasion. (J) Case 3: histological representation of the capsular invasion. An asterisk marks the capsular layer

Table 1 Clinico-pathological characteristics of the three cases

Case no.	Gender	Age at diagnosis	Tissue or site	Original diagnosis	Year diagnosed	<i>TERT</i> promoter genotype	Revised diagnosis	Status
1	M	62	Thyroid	FTA	2012	C228T mutation	FT-UMP	–
1	–	68	Humerus	Metastatic FTC	2018	C228T mutation	–	Alive
2	F	64	Thyroid	Multinodular goitre	2001	C228T mutation	Follicular tumour NOS	–
2	–	71	Neck scar	“Implanted FTA”	2008	C228T mutation	Metastatic FTC	–
2	–	80	Pelvis	Metastatic FTC	2017	No material available	–	Alive
3	F	74	Lung	Metastatic FTC	2018	C228T mutation	–	–
3	–	74	Thyroid	wiFTC	2018	C228T mutation	–	Alive

FTA, follicular thyroid adenoma; FT-UMP, follicular tumour of uncertain malignant potential; FTC, follicular thyroid cancer; NOS, not otherwise specified; wiFTC, widely invasive FTC

scheme of the patient. The patient underwent a completion hemithyroidectomy in April 2018 and is currently awaiting radioiodine treatment. Thyroglobulin 1 week post-operatively was elevated (2122 microgram/L). He is clinically well.

The second case is a 64-year-old female of Swedish ethnicity who was diagnosed with multinodular goitre following a right-sided hemithyroidectomy (Fig. 1(D)) at another hospital. Seven years later, the patient noted a subcutaneous, nodular expansion underneath the neck scar. FNAB suggested a follicular thyroid tumour (Bethesda IV), and the lesion was surgically removed. The histopathological report was consistent with a subcutaneously located 24-mm encapsulated follicular thyroid tumour without overt signs of malignancy (Fig. 1(E)). The Ki-67 index was 2%. The lesion was therefore believed to constitute an implantation remnant from a previous hypothetical thyroid tissue seeding at the initial surgery. Nine years later, the patient complained of pain from the right pelvic area, and an MRI scan visualised a large destruction from which an FNAB suggested metastatic FTC. A completion left-sided hemithyroidectomy was performed, in which two small (3 and 0.4 mm respectively) conventional papillary thyroid cancer lesions were demonstrated. No follicular tumour was visualised, and hence the question arose whether the original diagnoses of multinodular goitre as well as subcutaneous implantation tumour were correct. Histopathological re-examination of the right thyroid lobe revealed a probable follicular thyroid tumour, in which the amount of tissue submitted for examination was too diminutive to characterise the relation to the surrounding capsule. Moreover, the implantation tumour in the neck was also re-investigated—in which small foci of lymphovascular invasion were noted, and hence the nomenclature for this lesion was changed to metastatic FTC (Fig. 1(F)). Interestingly, tissues from both the original right thyroid lobectomy and the subsequent subcutaneous excision were positive for the C228T *TERT* promoter mutation (Table 1). This indicates that the original lesion in

the right thyroid lobe was indeed an FTC, and that the subcutaneous manifestation was a scar recurrence of the same lesion. Thus, had the latter lesion been screened for this genomic alteration, the diagnostic outcome might have been different, and the patient would have been considered for adjuvant treatment. The patient received radioiodine ablation with 7.3 gigabecquerel in February 2018. Further radiological examinations have revealed additional metastases in the shoulder, ribs and chest. Latest thyroglobulin count was elevated (14,830 micrograms/L).

The third case is a 74-year-old female of Swedish ethnicity. In 2015, a small mass in her left superior lobe of the lung was detected by conventional chest X-ray as a part of an investigation for intermittent chest pain. An ensuing FDG-PET scan was negative, and she was followed clinically. After 2 years, the slow growth rate raised suspicion that the lesion represented a hamartoma or a neuroendocrine tumour, and since the lesion was also positive for a DOTATOC-positron emission tomography (DOTATOC-PET) CT scan, a diagnostic wedge excision of the lesion was performed. The histopathological diagnosis was surprisingly consistent with a 20-mm large macrofollicular lesion indicative of thyroid tissue (Fig. 1(G)). The cells demonstrated positivity for thyroglobulin and TTF1, were without cellular atypia and endowed with a low Ki-67 proliferation index (<1%). The preliminary pathology report listed two differential diagnoses: metastatic FTC or an inclusion of entrapped benign thyroid tissue. A subsequent mutational screening revealed a C228T mutation of the *TERT* promoter, strongly suggestive for metastatic FTC (Fig. 2a). The patient was then referred to the endocrine surgery unit at our institution, and a 21-mm nodule was visualised in the right thyroid lobe by ultrasonography. An FNA biopsy was consistent with a follicular tumour (Bethesda IV), and an ensuing analysis of the cytology material could pinpoint the C228T *TERT* promoter mutation (Fig. 2b). With this information at hand, the patient underwent total thyroidectomy upfront, and the histopathological examination was consistent with a macrofollicular variant of FTC (Fig. 1(H–J)). This case

Fig. 2 Chromatograms illustrating the *TERT* promoter mutation C228T in case 3. **a** Chromatogram from the *TERT* promoter sequencing using genomic DNA from the excised pulmonary FTC metastasis. A heterozygous C>T mutation at position 228 corresponding to –124 bp from the ATG site of the *TERT* gene was demonstrated (highlighted in light blue). **b** Chromatogram from the *TERT* promoter sequencing from the subsequent cytological examination of the primary tumour in the right thyroid lobe, in which the heterozygous C228T mutation was visualised (highlighted in light blue). The mutations were verified using reverse complement sequencing (data not shown)

demonstrates the advantage of *TERT* promoter mutational screening when determining whether extrathyroidal lesions of thyroid origin represent metastatic tissue or trapped embryological tissue [10]. The patient is currently awaiting radioiodine treatment, and she is clinically well.

Discussion

The introduction of *TERT* promoter mutational screening in the clinical setting has given physicians an instrument to better estimate the true malignant potential of follicular thyroid tumours. Although the histopathological diagnosis requires unequivocal signs of malignancy, such as capsular and/or vascular invasion [9], there is a disturbing gap between the current histopathological classification algorithm and the true clinical outcome—as subsets of cases with worrisome histological features (denoted FT-UMP) do recur as full-blown malignant FTCs [7]. This is not least exemplified in this study, in which two of the cases were initially interpreted as benign, but later recurred. Moreover, *TERT* promoter mutational screening is cheap, fast, easy to perform and interpret, and the two mutational sites are easily amplified by the same primer pair. Therefore, the test is also well suited for low-volume pathology centres without experience and/or economics to accommodate more elaborate next-generation sequencing platforms, such as Thyroseq [11]. In our institution, a post-operative *TERT* promoter mutational screening is routinely

employed for all cases of FT-UMP. If a mutation is detected, the patient is offered a contralateral lobectomy to allow follow-up using thyroglobulin to detect eventual relapses. However, it should be noted that *TERT* promoter mutations are coupled to older patient age, and as our three cases were all > 60 years at initial presentation, the true value of *TERT* promoter mutational analyses in younger patients is not established [12]. Moreover, a general pitfall when analysing thyroid tumours with a follicular growth pattern is the distinction between a follicular tumour and a follicular variant of PTC [13, 14]. In our case series, no sample displayed PTC-like nuclear changes. Moreover, nuclear atypia is sometimes associated to an underlying malignant potential of follicular thyroid tumours [14]. Among our cases, mild atypia was seen in one case only, the FT-UMP of case 1 (Fig. 1(C)).

We conclude that *TERT* promoter mutational screening entails several clinical benefits and constitutes a useful tool for a proper detection of follicular thyroid tumours with potential for aggressive behaviour in histologically equivocal cases. We further advocate that the finding of such a mutation should lead to an intensified follow-up scheme, as molecular attributes of malignancy might precede the required histological signs.

Contributions MH and CCJ conceived and designed the study, researched and analysed data and wrote, edited and reviewed the manuscript. JOP and NW analysed data and edited and reviewed the manuscript. KJ, CL, NFM, IS and JZ provided clinical details and edited and reviewed the manuscript. All authors gave final approval for publication.

CCJ takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript.

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Compliance with ethical standards

Informed consent Informed consent was collected from all patients enlisted in the study, and ethical permission has been granted by a local ethics committee (Regionala etikprövningsnämnden Stockholm).

Conflict of interest The authors declare that they have no conflict of interest.

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