TERT promoter mutations: a genetic signature of benign and malignant thyroid tumours occurring in the context of tinea capitis irradiation

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Abstract

Objective: The aim of this study is to evaluate the frequency and molecular characteristics of TERTp mutations in thyroid adenomas and carcinomas occurring in the low-dose radiation exposure tinea capitis setting.

Design and methods: Twenty-seven patients with 34 well-differentiated thyroid carcinomas and 28 patients with 29 follicular adenomas diagnosed in a Portuguese tinea capitis cohort were studied. Blood samples were obtained from all the patients. Screening for TERTp mutations was performed by PCR amplification followed by Sanger sequencing. A series of 33 sporadic thyroid adenomas was used as control.

Results: TERTp mutations were detected in six of the 28 patients with adenoma (21.4%) and in four of the 27 patients with carcinoma (14.8%). Three tumours (two carcinomas and one adenoma) had the tandem mutation –124/–125 GG>AA (30.0%), whereas the remaining seven had the –124G>A. The 20.7% frequency of TERTp mutations in adenomas contrasts with the absence of mutations in the adenomas from the control group and from most series on record, whereas the one found in carcinomas (11.8%) is similar to those reported in the literature for sporadic carcinomas.

Conclusion: TERTp mutations, including the tandem mutation –124/–125 GG>AA not described previously in thyroid tumours, appear to represent a genetic signature for thyroid tumours in patients submitted to low-dose X-ray irradiation. The high frequency of TERTp mutations in the adenomas of our cohort contrasts with their absence in sporadically occurring, as well as in adenomas of the Chernobyl series.

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Introduction

Tinea capitis is a fungal disease of the scalp that attained epidemic proportions in the 5th–6th decades of last century due to the lack of anti-fungal oral treatment (1). Before the appearance of griseofulvin, the best way to eradicate the disease was to induce scalp epilation with X-ray irradiation using the Adamson–Kienbock technique (2, 3). The children received 7 Gy in the scalp (4), 1.5 Gy in the brain (5) and 0.045–0.5 Gy in the thyroid (6).

It is well recognised that X-ray radiation exposure is associated with cancer development (7). Increased frequencies of basal cell carcinoma (8, 9), thyroid carcinoma (1, 6) and meningioma (5) have been shown in the tinea capitis cohorts.

We have been following such a cohort since 2006 encompassing individuals who were submitted to scalp X-ray epilation in Portugal at the former Dispensário

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Central de Higiene Social do Porto (DCHSP) between 1950 and 1963 (1, 10). In thyroid carcinomas from these patients (with a long latency period), we found that the genetic alterations profile was more similar to that of the sporadic context, regarding for example, a higher frequency of *BRAF* mutation than that of the Chernobyl radiation-induced context (11, 12). A study from our group had previously shown that *BRAF* mutations were not a major event in early onset post-Chernobyl childhood thyroid carcinomas (13).

Regarding the presence of *RET/PTC* rearrangements in radiation context, conflicting results have been presented regarding radiation exposure, age at exposure and histology of the tumours (14, 15, 16). Elisei *et al.* stated that the presence of *RET/PTC* rearrangements is not higher in radiation-induced thyroid tumours than in sporadic, is not different after exposure to radioiodine or external radiation and is not associated with the young age of the patients also (17).

In Chernobyl studies, it was shown that the frequency of *BRAF* increases (18), whereas the frequency of *RET/PTC* rearrangements decreases with an increased latency period between exposure and clinical presentation (7, 19, 20). These data fit with the hypothesis advanced by Williams *et al.* that Chernobyl-related carcinomas will appear in 'waves', a first 'wave' with very short latency, in very young patients and showing a distinct genetic profile (frequent *RET/PTC* rearrangements and low *BRAF* mutations), and a late-onset 'wave' affecting older patients (20–40 years) and displaying a genetic profile similar to that found in adult sporadic thyroid carcinoma (low *RET/PTC* rearrangements and high *BRAF* mutation) (19, 20). In fact, Dinets *et al.* (21) found frequent *BRAF* mutations in a series of 70 adult patients exposed in childhood or as teenagers to the Chernobyl radioactive fallout and surgically treated between 2004 and 2008 (late-onset lesions) (21). In summary, these observations suggest that molecular changes in thyroid cancer after the Chernobyl accident mirror those occurring spontaneously, age (or time) of onset being more relevant than the etiopathogenic factor.

Telomerase, a ribonucleoprotein that maintains telomere length at the end of the chromosomes, is expressed at functionally significant levels in several human cancer cells, promoting their immortality (22). The *TERT* gene encodes the reverse transcriptase component of telomerase. Mutations in its promoter have been recently described in several cancers (23) and are particularly important as they provide the first evidence of driver mutations in the so-called dark matter of the

human genome (24). They have been described in thyroid carcinomas where they are an indicator of clinically aggressive tumours (25, 26, 27). There are limited data on the prevalence of *TERT*p mutations in radiation-induced thyroid carcinomas. To explore the influence of the external radiation context (X-rays), we decided to search for such mutations in thyroid tumours from the tinea capitis-irradiated cohort. As far as we know, *TERT* promoter mutations have never been studied in cancers associated with X-ray external radiation, namely in the tinea capitis cohort submitted to low-dose radiation in the thyroid.

Subjects and methods

Patients

We have previously observed from the clinical standpoint 1375 individuals from an original cohort of 5356 individuals who had undergone scalp irradiation for tinea capitis treatment during their childhood. Thirty-eight of those 1375 individuals were found to present thyroid carcinoma (2.8%) (1). The molecular study was conducted using the DNA from the tinea capitis patients from whom we had retrieved thyroid carcinomas and/or adenomas, whenever material was available (11). We also used DNA from blood collected from all the patients to search for mutations in blood cells.

In total, we studied 34 carcinomas from 27 patients (five patients had two carcinomas and one patient had three carcinomas), and 29 adenomas from 28 patients, as one patient had two adenomas. Three patients presenting carcinomas and adenomas were included in both groups – two with one adenoma and one carcinoma, and the third with two adenomas and two carcinomas. Thirty-two carcinomas were classified as papillary thyroid carcinoma (PTC): 40.6% classical variant and 59.4% follicular variant. The remaining two carcinomas were a follicular carcinoma (FTC) and a well-differentiated carcinoma not otherwise specified (WDT-NOS).

Additionally, we have studied a series of sporadically occurring follicular adenomas, retrieved from the files of Centro Hospitalar de S. João, comprising 33 adenomas from 33 non-irradiated individuals.

All the procedures were performed under strict ethical and confidentiality procedures according to the Portuguese ethical rules. The study has been approved by the Ethics Committee of the Hospital Pedro Hispano, and all the patients signed up an informed consent.

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Table 1 Clinicopathological characteristics and *TERT*p mutation frequency in thyroid carcinomas and thyroid adenomas of X-ray-irradiated patients.

Clinicopathological characteristics	Thyroid carcinoma			Thyroid adenoma		
	Total	<i>TERT</i> p wt	<i>TERT</i> p mut	Total	<i>TERT</i> p wt	<i>TERT</i> p mut
Number of patients	27	23	4	28	22	6
Age at diagnosis (mean $\pm$ s.d.)	56.7 $\pm$ 6.7	55.7 $\pm$ 6.0	62.0 $\pm$ 8.7	54.3 $\pm$ 7.7	54.6 $\pm$ 7.6	54.6 $\pm$ 10.2
Latency (mean $\pm$ s.d.)	50.2 $\pm$ 6.3	49.4 $\pm$ 6.2	54.5 $\pm$ 5.7	47.9 $\pm$ 7.2	48.0 $\pm$ 6.8	48.0 $\pm$ 10.9
Age at irradiation (mean $\pm$ s.d.)	6.4 $\pm$ 2.9	6.2 $\pm$ 2.7	7.3 $\pm$ 4.3	6.5 $\pm$ 2.4	6.7 $\pm$ 2.4	6.6 $\pm$ 2.7
Gender (n (%))						
Female	19 (70.4)	16 (69.6)	3 (75.0)	22 (78.6)	19 (86.4)	4 (66.7)
Male	8 (29.6)	7 (30.4)	1 (25.0)	6 (21.4)	3 (13.6)	2 (33.3)
Irradiation dose (n (%))*						
325–400 R	24 (92.3)	20 (90.9)	4 (100.0)	28 (100.0)	22 (100.0)	6 (100.0)
$\geq$ 630 R	2 (7.7)	2 (9.1)	0 (0.0)	0 (0)	0 (0)	0 (0)

*One case of carcinoma without information on the dose received.

Identification of *TERT*p mutations

To screen for *TERT* promoter mutations, we analysed the hot spots previously identified by PCR followed by Sanger sequencing. *TERT* promoter mutation analysis was performed with the pair of primers forward *TERT*F, CAGCGCTGCCTGAACTC; and reverse *TERT*R, GTCCTGCCCTTCACCTT. Amplification of genomic DNA (25–100 ng) was performed by PCR using the QIAGEN multiplex PCR kit, using the following cycling conditions: 30 s at 95°C, 90 s at 62°C and 20 s at 72°C for 35 cycles. All PCR products were purified and directly sequenced on an ABI Prism 3130 xl Automatic sequencer (Perkin-Elmer) using the ABI Prism Dye Terminator Cycle sequencing kit (Perkin-Elmer). The sequencing reaction was performed in the forward direction, and an independent PCR amplification, both in the forward and reverse directions, was performed in the samples that were suspected to carry the mutations.

Statistical analysis

The PASW Statistics 23 program was used. Proportions were compared using χ^2 test; the significance of differences between means was assessed by Student's unpaired *t*-test. A *P* value <0.05 was considered statistically significant with a 95% confidence interval.

Results

In the non-irradiated setting, composed by 33 patients with thyroid adenomas, 27 women (81.8%) and 6 men (18.2%), with a mean age at diagnosis of 53.8 ± 7.8 , no mutations were detected in *TERT* promoter. In the radiation-exposed setting, *TERT*p mutations were detected in four patients

with thyroid carcinoma (14.8%) and in six patients with thyroid adenoma (21.4%). No mutations were found in the corresponding blood samples. The cases had been previously analysed for *BRAF* and *NRAS* mutations, *RET/PTC* and *PAX8/PPARG* rearrangements (11); only one *BRAF*-mutated carcinoma had *TERT*p mutation (Table 1). We detected a case with *NRAS* mutation, which was wild type for *TERT*p mutation.

Table 1 summarises the clinicopathological characteristics of the irradiated patients according to the mutation status. One patient presented one mutated adenoma, one wild-type adenoma and one wild-type carcinoma and another patient presented one mutated adenoma and two wild-type carcinomas (Table 2). The other eight patients with mutated tumours had only one tumour. In the carcinoma group, age at diagnosis (*TERT*pmut 62.0 ± 8.7 vs *TERT*pwt 55.7 ± 6.0), age at irradiation (*TERT*pmut 7.3 ± 4.3 vs *TERT*pwt 6.2 ± 2.7), and latency, defined as time between irradiation and

Table 2 Type of *TERT*p mutations found in the mutated tumours.

Case	Adenomas	Carcinomas
2 ^a	–124:G>A	Wt
4 ^b	–124:G>A	wt/wt
5	–	–124:G>A*
7	–	–124:G>A*
16	–124/–125:GG>AA	–
17	–	–124/–125:GG>AA*
20	–124:G>A	–
23	–124:G>A	–
41	–	–124/–125:GG>AA**
45	–124:G>A	–

^aPatient with two independent lesions; ^bpatient with three independent lesions; *papillary thyroid carcinoma; **well-differentiated thyroid carcinoma not otherwise specified.

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Table 3 *TERT*p mutation frequency according to the histological type and *BRAF* mutation in the 34 carcinomas.

	Total	<i>TERT</i> p wt	<i>TERT</i> p mut
Number of carcinomas	34	30	4
Histological type			
PTC	32 (94.1)	29	3 (75.0)
FTC	1 (2.9)	1	0 (0)
WDT-NOS	1 (2.9)	0	1 (25.0)
<i>BRAF</i>			
<i>BRAF</i> wt	25 (73.5)	22 (73.3)	3 (75.0)
<i>BRAF</i> mut	9 (26.5)	8 (26.7)	1 (25.0)*

FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma; WDT-NOS, well-differentiated thyroid carcinoma not otherwise specified. *PTC.

surgery (*TERT*p mut 54.5 ± 5.7 vs *TERT*p wt 49.4 ± 6.2), were higher in *TERT* patients with mutated tumours, although the differences were not statistically significant. Regarding the adenoma group, age at diagnosis (*TERT*p mut 54.0 ± 9.3 vs *TERT*p wt 54.6 ± 7.6), age at irradiation (*TERT*p mut 6.3 ± 2.5 vs *TERT*p wt 6.7 ± 2.4), and latency (*TERT*p mut 47.7 ± 9.8 vs *TERT*p wt 48.0 ± 6.8), were similar in patients with *TERT*p-mutated and -non-mutated tumours. No significant differences were found, in both groups, for gender and irradiation dose. Also, comparing together all the mutated lesions with the wild-type lesions did not reveal any significant differences in the aforementioned clinicopathological characteristics (data not shown). Regarding the size of the tumours, there was also no significant correlation with *TERT*p mutations, either considering all the mutated lesions (*TERT*p mut 1.6 ± 1.2 cm vs *TERT*p wt 1.7 ± 1.8 cm) or comparing separately adenomas (*TERT*p mut 1.5 ± 1.0 cm vs *TERT*p wt 2.7 ± 2.3 cm) and carcinomas (*TERT*p mut 1.8 ± 1.5 cm vs *TERT*p wt 0.9 ± 0.6 cm).

The mutations comprised the -124G>A mutation that was present in six cases (five FTA and one PTC), and the tandem GG>AA mutation at position -124/-125 bp in three cases (one FTA, one PTC and one WDC-NOS); the tandem mutation was observed in 50% of the mutated

thyroid carcinomas (2/4) and in 16.7% of the mutated adenomas (1/6) (Table 2).

The histological subtype and the *BRAF*^{V600E} mutation status of the carcinomas are presented in Table 3. One of the *TERT*p-mutated tumours was a classic PTC, two were follicular variant of PTC and the fourth was a WDC-NOS. There were no significant differences between wild-type and *TERT*p-mutated carcinomas concerning the PTC histological subtype or the *BRAF*^{V600E} mutation status, but the series is too small to allow a meaningful comparison.

We observed *TERT*p mutations in 6 of the 29 adenomas (20.7%) at variance with none in the 33 sporadically occurring adenomas and in the cases we have previously observed in other selected series (25, 28). *TERT*p mutations were detected in 3 of 32 PTCs (9.2%). This percentage fits with those we had previously observed in PTC (7.5% and 7.7% respectively) (25, 28). The same holds true regarding the group of well-differentiated carcinomas (encompassing PTC, FTC and WDC-NOS) (11.8% in the present study, and 9.2% and 9.4% respectively) (25, 28). These data are summarised in Table 4.

Discussion

To better clarify the genetic profile of low-dose external radiation-induced thyroid tumours, we decided to search for *TERT*p mutations in thyroid tumours from the Portuguese tinea capitis cohort. For comparison, we analysed a similar series of non-radiation-exposed follicular adenomas, and also used, for comparative purposes, the results we have previously obtained in sporadically occurring thyroid tumours (25, 28).

In the radiation-exposed setting, we detected *TERT*p mutations in 14.8% of the patients with carcinoma (11.8% of the carcinoma lesions) and in 21.4% of the patients with adenoma (20.7% of the adenoma lesions). Regarding the carcinoma, the frequencies we detected in the present study are in the range of those found

Table 4 *TERT*p mutations in thyroid tumours series studied by our group.

Thyroid tumours	<i>TERT</i> p mutations (n/total (%))			
	Present series (X-irradiated)	Present series (non-irradiated control group)	Vinagre et al. (2013) (28)	Melo et al. (2014) (25)
Follicular adenomas and adenomatous nodules (FTA) (n (%))	6/29 (20.7)	0/33 (0)	0/60 (0)	–
Papillary carcinoma (PTC) (n (%))	3/32 (9.2)	–	13/169 (7.7)	25/332 (7.5)*
Well-differentiated carcinoma (FTC+PTC+WDC-NOS) (n (%))	4/34 (11.8)	–	22/233 (9.4)	37/402 (9.2)*

*The cases presented in Melo et al. (25) study include part of the cases published in Vinagre et al. (28).

for the sporadic cases (9.2–22.5%) (25, 26, 27, 29). This observation fits with those previously obtained on *BRAF*, *NRAS* mutations and *RET/PTC* rearrangements (11), as the frequency of such genetic alterations in this irradiated, late-onset cohort was also similar to what is found in the sporadic context. At variance with this, in early-onset radiation-associated thyroid cancers, such as the Chernobyl cohorts, no *TERTp* mutations were detected in the carcinomas analysed (25, 30). The biggest series, with 51 cases, is from Liu (30) who concludes that this genetic event is not related to radiation in thyroid cancer. In the present study, besides exploring the effect of a different type of radiation (X-rays), we evaluated a series of late-onset carcinomas, as the latency period between exposure and diagnosis was more than 40 years.

Regarding the adenomas, we found that *TERTp* mutations were a frequent event (20.7% of the patients) in the X-ray-exposed setting. Previous studies have shown a 0–2% prevalence in the non-irradiated context (25, 28, 31, 32); the 2% prevalence (1/58 cases) has been reported by Wang *et al.* (31) who considered the mutation as an early genetic event in thyroid follicular tumour malignancy. In the present study, we confirmed the absence of *TERTp* mutations in non-exposed follicular adenomas by screening a new series of 33 sporadic adenomas. In the Chernobyl radiation context, similar to what was reported for carcinomas, the mutation was not found in adenomas also (25), in accordance with our previous results (25, 28). Therefore, at the best of our knowledge, this is the first report of *TERTp* mutations in thyroid tumours (either carcinomas or adenomas) associated with X-ray external radiation exposure.

Furthermore, in our ten mutated cases, it is interesting to emphasise that 30% of the mutations were tandem mutations. This was confirmed by the absence of *TERTp* polymorphisms or mutations in the patient's blood. In thyroid tissues, these mutations have been reported only in BCPAP cell line (26, 28) and are infrequent in other cancer models (26, 33, 34, 35). Interestingly, in basal cell carcinomas from the patients of this tinea capitis cohort, we have found a different pattern, with a lower frequency of the –124G>A and a higher frequency of the –146G>A mutation, comparing with the sporadic BCC cases (34). In the present study, all the mutated cases were in the same –124/–125 hotspot; no cases presented the –146G>A mutation. This is also in agreement with what has been described by others in sporadic thyroid cancer, as the –146G>A is the less frequent one (10–17.2%) (25, 27) or absent (26). This difference may be due to a different field effect, namely due to differences in the organs involved

(skin vs thyroid) and differences in the dose received (5–6 Gy on the skin – an intermediate dose – compared to 0.5 Gy, at maximum, on the thyroid – a low dose).

Comparing the clinicopathological characteristics of the mutated and non-mutated tumours (adenomas and carcinomas), we did not find any significant differences regarding age at irradiation, age at diagnosis and latency period. These negative results should be interpreted with caution due to the restricted number of mutated cases.

TERTp mutations were found to be frequent in adenomas from X-ray-irradiated patients, comparing to the 0–2% detected in the sporadic context (28, 31) and to the 0% in the Chernobyl setting (25), and showing a frequency similar to that one found in carcinomas. These results raise the possibility that the *TERTp* mutation may be an early genetic event in this context as advanced by Wang *et al.* (31). Curiously, such event does not appear to drive carcinogenesis, at least *per se*, as *TERTp* mutations are not more prevalent in the carcinomas arising in this setting than in sporadically occurring well-differentiated (papillary and follicular) carcinomas. This apparent dissociation between *TERTp* mutation and carcinogenesis in the X-irradiation context is reinforced by the finding of two cases in which *TERTp* mutation was detected in adenomas of the patients and not in the concurrent PTCs. Moreover, this also raises a very interesting point, which is the need to find the partner or partners that, together with *TERTp* mutation, may induce tumourigenesis.

The data herein presented suggest that *TERTp* mutations may represent a genetic signature for thyroid tumours submitted to low-dose X-ray external radiation as they were detected both in adenomas and in different histotypes of carcinoma. Moreover, in 30% of the mutated cases, we observed the tandem mutation –124/–125GG>AA, which is to the best of our knowledge, never described before in thyroid tumours. It will be interesting to explore other thyroid low-dose radiation settings to see if the tandem mutation –124/–125GG>AA is specific for this radiation model.

Declaration of interest

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

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