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ETV6-NTRK3 is a common chromosomal rearrangement in radiation-associated thyroid cancer

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Abstract

Background—Our previous analysis of papillary thyroid carcinomas (PTC) from the Ukrainian-American cohort exposed to ^{131}I from the Chernobyl accident found *RET/PTC* rearrangements and other driver mutations in 60% of tumors.

Methods—In this study, we analyzed the remaining, mutation-negative tumors using RNA-Seq and RT-PCR to identify novel chromosomal rearrangements and characterize their relationship with radiation dose.

Results—The *ETV6-NTRK3* rearrangement was identified by RNA-Seq in a tumor from a patient who received a high ^{131}I dose. Overall, it was detected in 9/62 (14.5%) of post-Chernobyl and in 3/151 (2%) of sporadic PTCs (p=0.019). The most common fusion type was between exon 4 of *ETV6* and exon 14 of *NTRK3*. The *ETV6-NTRK3* prevalence in post-Chernobyl PTCs was associated with increasing ^{131}I dose, albeit at borderline significance (p=0.126). The group of rearrangement-positive PTCs (*ETV6-NTRK3*, *RET/PTC*, *PAX8-PPARγ*) was associated with significantly higher dose response compared to the group of PTCs with point mutations (*BRAF*, *RAS*) (p<0.001). In vitro exposure of human thyroid cells to 1 Gy of ^{131}I and γ-radiation resulted in the formation of *ETV6-NTRK3* with a rate of 7.9×10^{-6} and 3.0×10^{-6} cells, respectively.

Conclusions—We report here the occurrence of *ETV6-NTRK3* rearrangements in thyroid cancer and show that this rearrangement is significantly more common in tumors associated with exposure to ^{131}I and has a borderline significant dose response. Moreover, *ETV6-NTRK3* can be

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directly induced in thyroid cells by ionizing radiation *in vitro* and therefore may represent a novel mechanism of radiation-induced carcinogenesis.

Keywords

thyroid cancer; radiation; chromosomal rearrangements; NTRK3; Chernobyl

INTRODUCTION

Thyroid cancer is the most common type of endocrine malignancy, and its incidence has been steadily growing in the U.S. and many other countries during the last four decades.[1] Exposure to ionizing radiation during childhood is a well-established risk factor for thyroid cancer. The increased risk of thyroid cancer after radiation exposure was first suggested in 1950 in infants who received external beam radiation for enlarged thymus.[2] This has been later confirmed in multiple studies of patients exposed to environmental or medical radiation, including X-ray or γ-radiation as wells as radioiodines, mainly iodine-131 (131I).[3] In the decades following the 1986 Chernobyl accident, the surrounding geographic area experienced a marked increase in incidence of thyroid cancer among those who were children or young adults at the time of the accident.[4] The post-Chernobyl case-control and cohort studies confirmed previous observations that papillary thyroid carcinoma (PTC) is the predominant type of thyroid cancer associated with radiation exposure,[4–6] and that the risk for PTC following ^{131}I exposure increases with dose, with the magnitude of the increase comparable to that following external radiation.[7–10]

Activating mutations in the mitogen activated protein kinase (MAPK) signaling cascade are common in thyroid cancer and believed to be essential for tumorigenesis.[11] The most common events include point mutations in the *BRAF* and *RAS* genes as well as chromosomal rearrangements involving the *RET* gene, known as *RET/PTC*. However, the mutational mechanisms leading to MAPK activation in sporadic and radiation-related PTCs appear to be different. Whereas in sporadic tumors, point mutations in *BRAF* and *RAS* genes are by far most common (∼60% of all PTCs), in post-Chernobyl or post-radiotherapy PTCs, 50–80% of tumors typically harbor chromosomal rearrangements of the *RET* gene known as *RET/PTC* whereas point mutations are rare.[12–14] Other chromosomal rearrangements, such as *AKAP9-BRAF* and those involving the *NTRK1* and *PPARγ* genes, are also more frequently found in radiation-associated PTCs.[15,16] However, a significant proportion of radiation-associated tumors harbor none of the known mutations, suggesting that other, unknown genetic events may occur in these tumors.

In our recent study, we performed genotypic analysis of 62 PTCs from a well-characterized cohort of Ukrainian individuals (UkrAm) who received 0.008–8.6 Gy of ^{131}I to the thyroid after the Chernobyl accident.[15] The study confirmed the *RET/PTC* rearrangements as the most common genetic event in these tumors and found different trends with dose in PTCs harboring chromosomal rearrangements and point mutations. However, 40% of these tumors had none of the known genetic events and were further analyzed in this study using the RNA-Seq analysis to discover novel genetic events that might occur in radiation-related thyroid cancer. This analysis revealed the *ETV6-NTRK3* chromosomal rearrangement, previously unknown to occur in thyroid cancer, is a common genetic event in radiation-related but not in sporadic thyroid cancer, and showed that *ETV6-NTRK3* can be directly induced in human thyroid cells by ionizing radiation *in vitro*.

MATERIALS AND METHODS

Study cases and samples

The study was approved by the University of Pittsburgh, National Cancer Institute, and Institute of Endocrinology and Metabolism (IEM) (Kyiv, Ukraine) Institutional Review Boards. The cases of radiation-associated PTCs were diagnosed among individuals from the UkrAm study who were younger than 18 years old at the time of the Chernobyl accident.[17] Individual radioactivity measurements in thyroid gland were performed within the two months following the accident and individual ^{131}I thyroid doses were estimated based on these measurements, interview data concerning dietary and lifestyle habits, and environmental transfer models.[18,19] PTC was diagnosed in 104 individuals (age at surgery: range, 14–32 years; mean, 22.7±5.1 years) between 1998 and 2008 at the Laboratory of Morphology of Endocrine System of the IEM, after four sequential screenings.[20] Pathologic diagnoses were reviewed by the International Pathology Panel of the Chernobyl Tissue Bank (CTB). Frozen tissue samples were available for 75 cases of PTCs. For 74 PTCs DNA and/or RNA were extracted at IEM or Imperial College (London, UK) and received through the CTB. Four cases exposed to ^{131}I *in utero* and 8 cases that lacked either DNA (n=3) or RNA (n=5) were excluded. In addition, a series of 151 consecutive sporadic PTC cases (age at surgery: range, 15–97 years; mean, 45.6±17.7 years) and additional 92 PTC cases (age at surgery: range, 4–77 years; mean, 44.2±16.7 years) previously found to be negative for known genetic alterations,[21] were available through the University of Pittsburgh Health Sciences Tissue Bank (HSTB).

RNA-Seq and data analysis

Tumor RNA samples were processed to remove ribosomal RNA using the Ribozero Magnetic Gold kit (Illumina), followed by library preparation for RNA sequencing using the IlluminaTruSeq RNA Sample Preparation Kit v2. Briefly, polyadenylated RNA was fragmented, reverse transcribed, indexed, amplified and purified to produce individual barcoded libraries, according to the manufacturer's instructions. The prepared libraries were assessed using a Bioanalyzer and the High Sensitivity DNA kit (Agilent). Paired-end sequencing was performed on Illumina HiSeq2000 at the High Throughput Genome Center at the Department of Pathology, University of Pittsburgh. Sequence reads obta were analyzed for gene fusion events using the ChimeraScan[22,23] and deFuse[24] programs. The predicted fusion events from the two programs were integrated and combined with genomic annotation to generate a list of candidate gene fusions. Before the analysis, sequences with low quality (base quality < 13) at both ends of reads were trimmed and trimmed reads with less than 25 bp were removed. The reference human genome (NCBI build 37.1, hg19) and gene annotation database (Ensembl v69 and UCSC hg19) were used for the analysis. To reduce false positive findings, the fusion events detected by both programs were further narrowed down by excluding (i) fusion events between adjacent genes (called as read-through), (ii) fusion events with no reads spanning the predicted breakpoints, and (iii) fusion events predicted to have five or more fusion partners and lacking specificity of target regions.

Detection of *ETV6-NTRK3* fusions by RT-PCR

Tumor RNA was reverse transcribed and amplified using the following primers: 5'-CATTCTTCCACCCTGGAAAC-3' (forward *ETV6* exon 4), 5'-AAGCCCATCAACCTCTCTCA-3' (forward *ETV6* exon 5), 5'-TCCTCACCACTGATGACAGC-3' (common reverse *NTRK3*). PCR product was analyzed by agarose gel electrophoresis. The presence of the fusion was confirmed by Sanger sequencing.

Cell irradiation and *in vitro* induction of *ETV6-NTRK3* fusions

HTori-3 human thyroid cells[25] were grown in RPMI1640 supplemented with 10% FBS. Cells were authenticated using the STRS analysis.[26] For γ-radiation, 1×10^6 HTori-3 cells were exposed to 1Gy from ^{137}Cs source (Gamma Cell 40 irradiator) at a dose-rate of 0.58 Gy per minute. For ^{131}I irradiation, 1×10^6 HTori-3 cells in a T25 flask were incubated for 24 hours in 2 mL of culture media in the presence of NaI^{131} to deliver 1 Gy of radiation. Calculation of dose received by a monolayer of cells growing in the T25 flask and exposed to 1 Gy of ^{131}Iwas performed based on Kernel integrations as previously described[27] and was found to be 1.02 Gy per hour per 1 mCi. Two replicates of each experiment were performed. Delivery of radiation was monitored by formation of γH2AX nuclear foci using nnti-phosphorylated histone H2AX primary antibody (Upstate Biotechnology). Following γ or ^{131}I irradiation, HTori-3 cells were split into thirty T25 flasks, transferred to T75 flasks for continuous growth, and harvested 9 days after irradiation. RNA was extracted using Trizol (Invitrogen), mRNA was purified using Oligotex mRNA kit (Qiagen). Following the reverse transcription step, multiplex PCR was performed to detect *ETV6*e4-*NTRK3*e14 and *ETV6*e5-*NTRK3*e14 rearrangements using the sequence specific primers described above. PCR products were resolved in the agarose gel and detected by Southern blot hybridization with ^{32}P-labeled oligonucleotide probes specific for *ETV6*e4-*NTRK3*e14 (5'-ACCATGAAGAAGGTCCCGT-3') and *ETV6*e5-*NTRK3*e14 (5'-AGAATAGCAGGTCCCGTGG-3').

Statistical analysis

Univariate analyses were performed using the two-sample T-test. Mutation prevalence data was analyzed using standard logistic regression models as previously described. (18) Briefly, the following model was used to examine the probability of *ETV6-NTRK3*rearrangement with ^{131}I dose *D* (in Gy) controlling for effect of age at surgery *a*, gender *s*, oblast (province) of residence *O*:

$$P[\,mutation|D,a,s]=\frac{\exp[\alpha_0+\alpha_1(a-25)+\alpha_2 1_{gender=female}+\alpha_3 1_{O=Zhytomir}+\alpha_4 1_{O=Kyiv}+\alpha_5 D+\alpha_6 D^2]}{1+\exp[\alpha_0+\alpha_1(a-25)+\alpha_2 1_{gender=female}+\alpha_3 1_{O=Zhytomir}+\alpha_4 1_{O=Kyiv}+\alpha_5 D+\alpha_6 D^2]}$$

For some analyses quartic polynomials or categorical functions of age replaced the α_1(a–25) term. Most analyses used log-linear functions of dose, D, but a few involved log-quadratic functions of dose. Twenty five years were subtracted from age (at surgery) to aid convergence of fitted models. All tests were two-sided and based on the likelihood-ratio test, and confidence intervals for the logistic regression analyses were derived from the profile likelihood.[28] Likelihood-ratio tests were also used to assess heterogeneity by endpoint, using an extension of methods previously described.[29] Linear regression analyses were performed using Stata and log-linear logistic regression analyses using Epicure.[30]

RESULTS

Identification and prevalence of *ETV6-NTRK3* rearrangements in radiation-related and sporadic PTCs

The previous genotyping analysis of 62 post-Chernobyl PTCs from the UkrAm cohort identified driver mutations in 37 tumors (60%), whereas 25 (40%) of the tumors did not harbor any other mutations previously reported to occur in thyroid cancer.[15] From this group, two tumors had a sufficient amount of RNA and were selected for whole-transcriptome (RNA-Seq) analysis to search for novel chromosomal rearrangements. One of these tumors was from a patient who was 1 year old at the time of the Chernobyl accident

and received an estimated ^{131}I dose of 7.5 Gy to the thyroid, among the highest doses in this series. Another tumor was from a patient who was 10 years old at the time of exposure and received an estimated ^{131}I dose of 0.34 Gy. The analysis of PTC from the first patient revealed an in-frame fusion event between exon 4 of the ETS variant gene 6 (*ETV6*) and exon 14 of the neurotrophin receptor 3 (*NTRK3*) gene, which was detected by both programs, ChimeraScan and deFuse. The RNA-seq analysis of PTC sample from the second patient did not yield any promising gene fusions involving potential oncogenes. The presence of the *ETV6-NTRK3* rearrangement was validated by RT-PCR and confirmed by Sanger sequencing (Fig. 1).

Upon screening the rest of post-Chernobyl tumors using RT-PCR, 8 additional cases positive for *ETV6-NTRK3* fusion were found, all involving exon 4 of *ETV6* and exon 14 of *NTRK3* genes. Overall, 9 out of 62 (14.5%) of post-Chernobyl PTCs harbored this rearrangement. One of these tumors also harbored *BRAF* V600E mutation and another tumor also had the *RET/PTC1* rearrangement, whereas the remaining seven *ETV6-NTRK3* positive tumors lacked known common driver mutations (*BRAF*, *RAS*, *RET/PTC*, or *PAX8-PPARγ*).

Screening of 151 consecutive sporadic PTCs from the general U.S. population revealed three positive cases, resulting in a prevalence of 2%. None of the three *ETV6-NTRK3* positive sporadic tumors harbored other common driver mutations known to occur in thyroid cancer. The prevalence of *ETV6-NTRK3* in post-Chernobyl PTCs was significantly higher than in sporadic PTCs, including both crude prevalence (p=0.01) and prevalence after adjustment for age and gender (p=0.019).

Analysis of additional 92 sporadic PTCs selected based on the lack of other known driver mutations identified 4 tumors positive for *ETV6-NTRK3* (4.3%). Of the seven *ETV6-NTRK3* rearrangements totally identified in sporadic PTCs, 6 involved the fusion of exon 4 of *ETV6* to exon 14 of *NTRK3* (*ETV6*e4-*NTRK3*e14) and one revealed a larger PCR product, which on Sanger sequencing was found to result from the fusion of exon 5 of *ETV6* to exon 14 of *NTRK3* (*ETV6*e5-*NTRK3*e14) (Fig. 1). None of the 7 patients with sporadic PTCs carrying *ETV6-NTRK3* rearrangement was found to have a documented history of radiation exposure. Re-screening of post-Chernobyl tumors for *ETV6*e5-*NTRK3*e14 rearrangement revealed no additional positive cases. No *ETV6-NTRK3* was found in TPC1 cell line established from PTC.

Exposure-related features of *ETV6-NTRK3*-positive tumors

In 62 post-Chernobyl PTCs, an average thyroid dose received from ^{131}I was 1.27 Gy. Among the *ETV6-NTRK3*-positive tumors (n=9), the average ^{131}I dose was 2.27 Gy (Fig. 2A). The age of these individuals at the time of exposure ranged from 0.5 to 17.2 years (mean, 8.1 years) and the age at surgery ranged from 14.2 to 35.1 years (mean, 23.9 years) with the average time between exposure and surgery of 15.8 years (Table 1). In multivariate analysis (adjusting for age at surgery, gender, and place (oblast) of residence), tumors harboring the *ETV6-NTRK3* rearrangement were found to be associated with increasing ^{131}I dose, albeit at borderline significance (p=0.126) (Table 2). The dose response adjusted for these variables is shown in Fig. 2B.

When the *ETV6-NTRK3*-positive tumors were grouped with tumors that harbored other types of chromosomal rearrangements, i.e. *RET/PTC* or*PAX8/PPARγ*, rearrangement-positive tumors were associated with a significantly higher dose response compared to the tumors with point mutations (*BRAF*, *NRAS*, *HRAS*) adjusting for age, gender, and oblast (p<0.0001) (Table 2). Specifically, the adjusted excess odds ratio (EOR) per Gy for all chromosomal rearrangements was 0.09 (95% CI: −0.24, 0.46) while that for point mutations was −3.29 (95% CI: −6.06, −1.38) (Table 2). There was a similar degree of heterogeneity

($p<0.0001$) if adjustment was also made for a possible quadratic term in the dose response for rearrangements.

Histopathologic features of *ETV6-NTRK3*-positive PTCs

The majority (6/9, 67%) of post-Chernobyl PTCs found to have the *ETV6-NTRK3* rearrangement demonstrated a follicular growth pattern and were classified as the follicular variant of PTC (Fig. 3A). The remaining tumors (3/9, 33%) had a significant papillary component and were classified as classic papillary type of PTC (Fig. 3B). All nine tumors showed some component of a solid growth pattern, comprising approximately 10% of the examined tumor in seven cases and 20–30% of the examined tumor in two cases (Fig. 3B). Among sporadic PTCs positive for *ETV6-NTRK3*rearrangement, four were classic papillary type (57%) and three were follicular variant PTC (43%). All four classic papillary PTCs had a significant component of follicular growth, but no well-defined solid component was observed in any of the sporadic tumors. Among radiation-associated tumors, all were AJCC/UICC stage I at presentation, whereas among sporadic tumors, 5 were stage I and two were stage III based on the presence of minimal extrathyroidal extension in patients over the age of 45.

In vitro induction of *ETV6-NTRK3* rearrangements by ionizing radiation

High prevalence of *ETV6-NTRK3* rearrangement in radiation-associated PTCs and its association with high thyroid dose suggested that this rearrangement could be induced by ionizing radiation. To test this possibility, we studied the induction of *ETV6-NTRK3* rearrangement in HTori-3 human thyroid cells after exposure to 1 Gy of ^{131}I or γ-radiation. The ^{131}I dose distribution in the monolayer of cells within a T25 flask generated by Kernel integration is shown in Fig. 4A. Induction of double-stranded DNA breaks by radiation was monitored by formation of γH2AX nuclear foci (Fig. 4B).Whereas no rearrangements were observed in the unexposed cells, both radiation types resulted in the generation of *ETV6-NTRK3* rearrangements (Fig. 4C).The average rate of *ETV6-NTRK3* induction was 7.9×10^{-6} cells per 1 Gy of ^{131}I and 3.0×10^{-6} per 1 Gy of γ-radiation. Both *ETV6*e4-*NTRK3*e14 and *ETV6*e5-*NTRK3*e14 fusions were induced by ionizing radiation, with the predominance of *ETV6*e4-*NTRK3*e14 after both ^{131}I and γ-radiation (Fig. 4D).

DISCUSSION

In this study, we report for the first time the occurrence of the *ETV6-NTRK3* chromosomal rearrangement in thyroid cancer, which was found to be common in PTCs associated with ^{131}I exposure from the Chernobyl accident. In fact, in this well-characterized series of radiation-related thyroid cancer, *ETV6-NTRK3* was the second most common rearrangement type after *RET/PTC*. Moreover, this study demonstrates that this rearrangement can be directly induced in human thyroid cells *in vitro* by exposure to both ^{131}I and γ-radiation.

The fusion between the *ETV6* gene on chromosome 12 and the *NTRK3* gene on chromosome 15 was first described in congenital fibrosarcoma in 1998.[31] Since then, *ETV6-NTRK3* rearrangements have been found in several other tumor types including acute myeloid leukemia (AML),[32,33] chronic eosinophilic leukemia (CEL),[34] congenital mesoblastic nephroma, [35] secretory breast carcinoma,[36] and mammary analogue secretory carcinoma of the salivary gland.[37] *ETV6*, also known as *TEL*, is a transcription factor from the ETS transcription factor family, which is involved in various oncogenic gene fusions resulting from chromosomal translocations, mostly reported in subtypes of AML. *NTRK3* is a transmembrane receptor tyrosine kinase, for which ligand is neurotrophin-3, which is primarily involved in neuronal cellular processes.[38] The rearrangement results in fusion of the SAM domain of *ETV6*, which is required for dimerization, with the tyrosine kinase

domain of *NTRK3*, such that the transcribed product is a constitutively active tyrosine kinase.[38]

Most of *ETV6-NTRK3* fusions that occur in various tumor types involve the fusion point initially identified in congenital fibrosarcoma, i.e. between exon 5 of *ETV6* and exon 13 of *NTRK3* genes[31] A shorter variant, in which exon 4 of *ETV6* is fused to *NTRK3*, has been found in cases of AML and CEL.[32,34] Here, we report the occurrence of *ETV6-NTRK3* rearrangements in radiation-related and sporadic PTCs, with fusion points that differ from those previously identified in other tumor types as they lack exon 13 of *NTRK3*.

Our results provide several lines of evidence that link *ETV6-NTRK3* rearrangements in papillary thyroid cancer with radiation exposure. First, this rearrangement is found with a significantly higher prevalence in PTC's of patients exposed to ^{131}I from the Chernobyl accident than in PTCs arising in the general U.S. population. Second, among post-Chernobyl PTCs we observed a borderline statistically significant association between ^{131}I dose and prevalence of *ETV6-NTRK3* rearrangement. Finally, our *in vitro* experiments demonstrated the induction of both types of *ETV6-NTRK3* fusions in human thyroid cells by radiation. Both ^{131}I and γ-radiation were efficient at inducing this rearrangement in cultured human cells. This suggests that, in addition to patients exposed to ^{131}I after the Chernobyl accident, thyroid cancers developing after external beam radiation therapy may also harbor these rearrangements.

Among post-Chernobyl tumors in this study, *ETV6-NTRK3*-positive PTCs arose in individuals exposed to an average ^{131}I dose of 2.3 Gy, higher than the average dose received by patients who developed PTCs driven by other oncogenes. The average age of patients with PTCs positive for *ETV6-NTRK3* was about 8 years old at the time of the accident and 24 years old at the time of surgery, resulting in the average time between exposure and thyroid surgery of 16 years. The UkrAm study includes individuals followed since 1998, and therefore it remains unknown whether *ETV6-NTRK3* may also be common in tumors that developed less than 12 years after the accident. Of note, a sharp increase in thyroid cancer incidence in the area surrounding the Chernobyl nuclear power plant was observed as early as 4–6 years following the accident.[39]

Phenotypically, both radiation-associated and sporadic tumors harboring *ETV6-NTRK3*rearrangement were either follicular variant of PTC or classic papillary cancer. Interestingly, the presence of solid growth pattern was a microscopic feature found only in radiation-associated PTCs carrying this rearrangement. We and others have previously demonstrated a common presence of solid growth pattern in post-Chernobyl tumors as compared to sporadic PTCs, and the association of this growth pattern with *RET/PTC3* rearrangement.[40,41] The results of this study suggest that *ETV6-NTRK3* may represent another type of chromosomal rearrangement associated with the solid growth pattern of PTC in patients exposed to radiation.

Findings in this study provide additional evidence for association between specific types of mutations and etiologic factors implicated in the development of thyroid cancer. Previous studies have shown that thyroid cancers developing after exposure to ionizing radiation have a high prevalence of chromosomal rearrangements such as *RET/PTC* and *AKAP9-BRAF*, and low prevalence of point mutations.[12–14] The results of this study extend evidence supporting such as association, adding *ETV6-NTRK3* to the list of fusions that preferentially occur in patients exposed to radiation and that can be induced by radiation *in vitro*.

The association between *ETV6-NTRK3* and radiation exposure of thyroid gland found in this study raises a possibility that these fusion observed in other cancer types may also be related

to radiation exposure. This is particularly plausible for AML, which has a strong dose-dependent relationship with environmental or medical irradiation.[42]

In summary, we report here the occurrence of *ETV6-NTRK3* rearrangement in papillary thyroid cancer and show that this is common event in thyroid tumors associated with ^{131}I radiation exposure. Moreover, we demonstrate that this rearrangement can be directly induced by ^{131}I or γ-radiation *in vitro* and therefore may represent a novel molecular mechanism contributing to the development of radiation-induced thyroid cancer.

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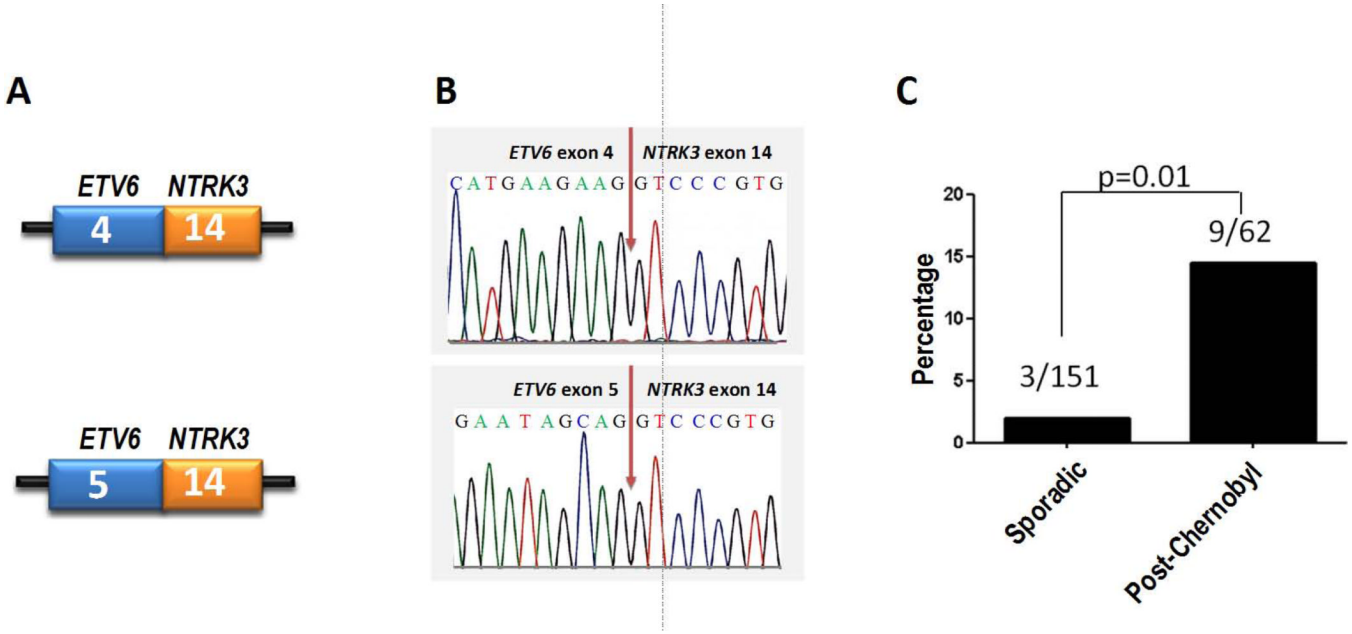

Figure 1.

ETV6-NTRK3 fusions identified in post-Chernobyl and sporadic thyroid tumors. (**A**) Schematic representation of the fusion point between exons of the two gene in mRNA. (**B**) Confirmation of *ETV6-NTRK3* fusions by Sanger sequencing. (**C**) Frequency of *ETV6-NTRK3* fusions in sporadic and post-Chernobyl PTCs.

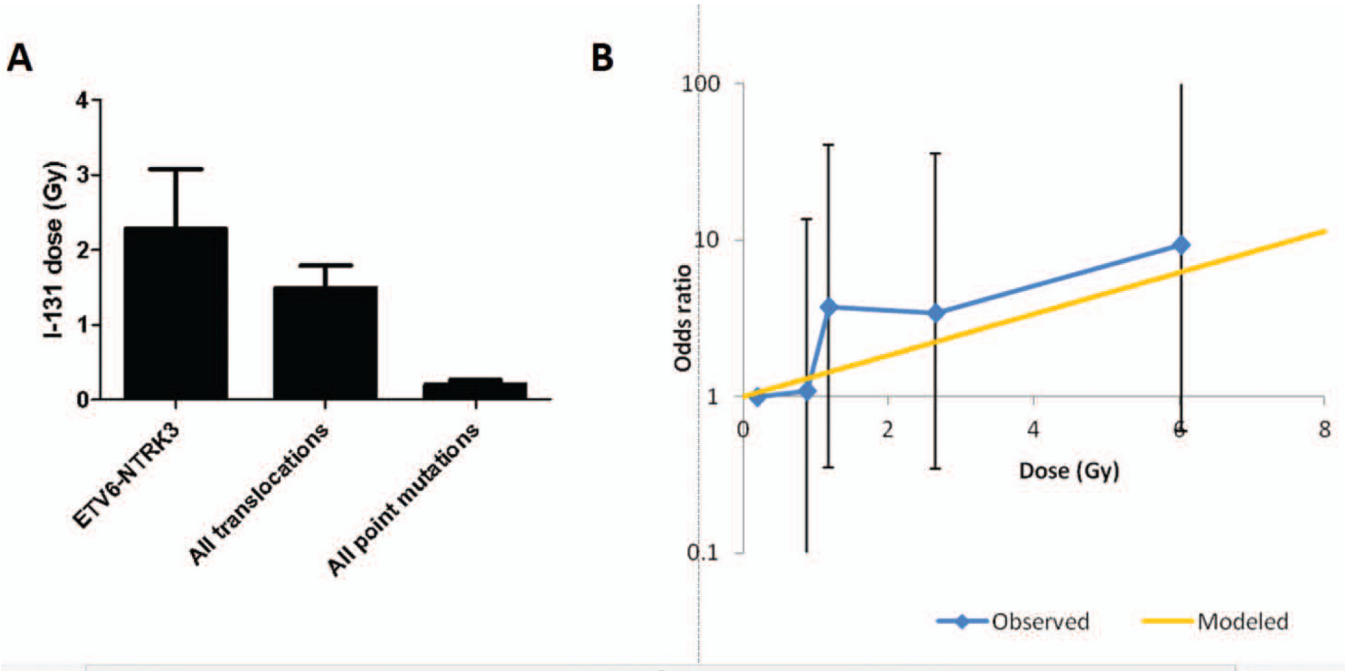

Figure 2.

Exposure-related characteristics of *ETV6-NTRK3*-positive tumors. (**A**) Mean ^{131}I thyroid dose received after the Chernobyl accident by individuals who subsequently developed PTC carrying specific molecular alterations. (**B**) Observed and modeled dose response for post-Chernobyl PTCs adjusted for age at surgery, gender and place of residence.

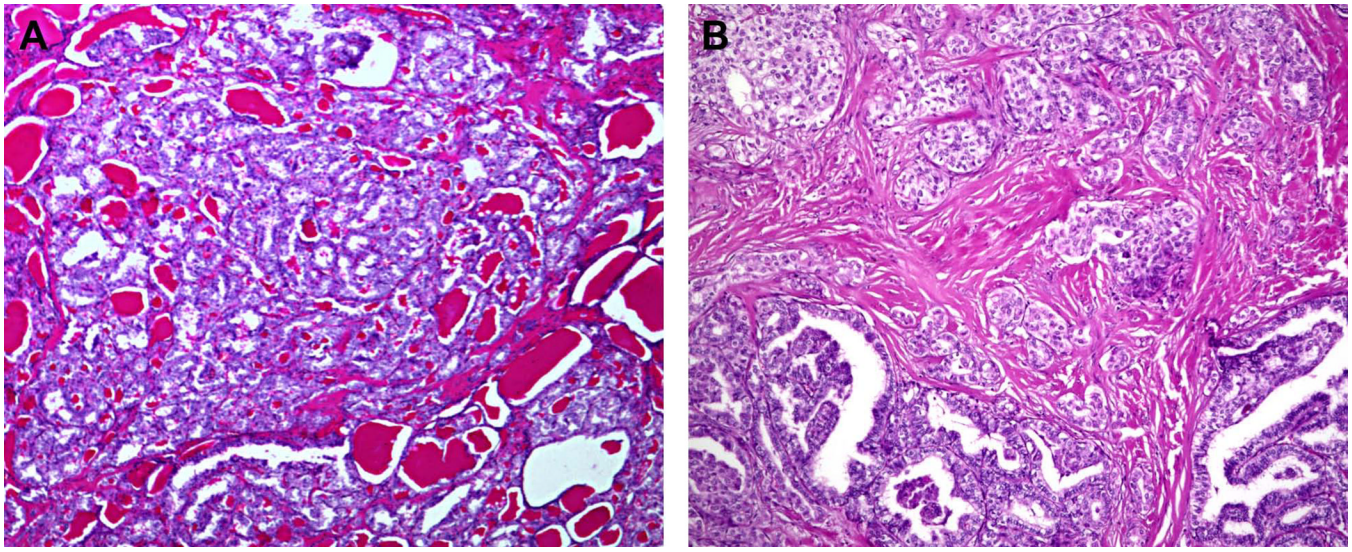

Figure 3.
Histopathologic features of *ETV6-NTRK3*-positive tumors. (**A**) Follicular variant of PTC showing follicular growth pattern and no well-formed papillary structures. (**B**) Classic papillary type of PTC showing well-formed papillae (bottom) and focal areas of solid growth (top).

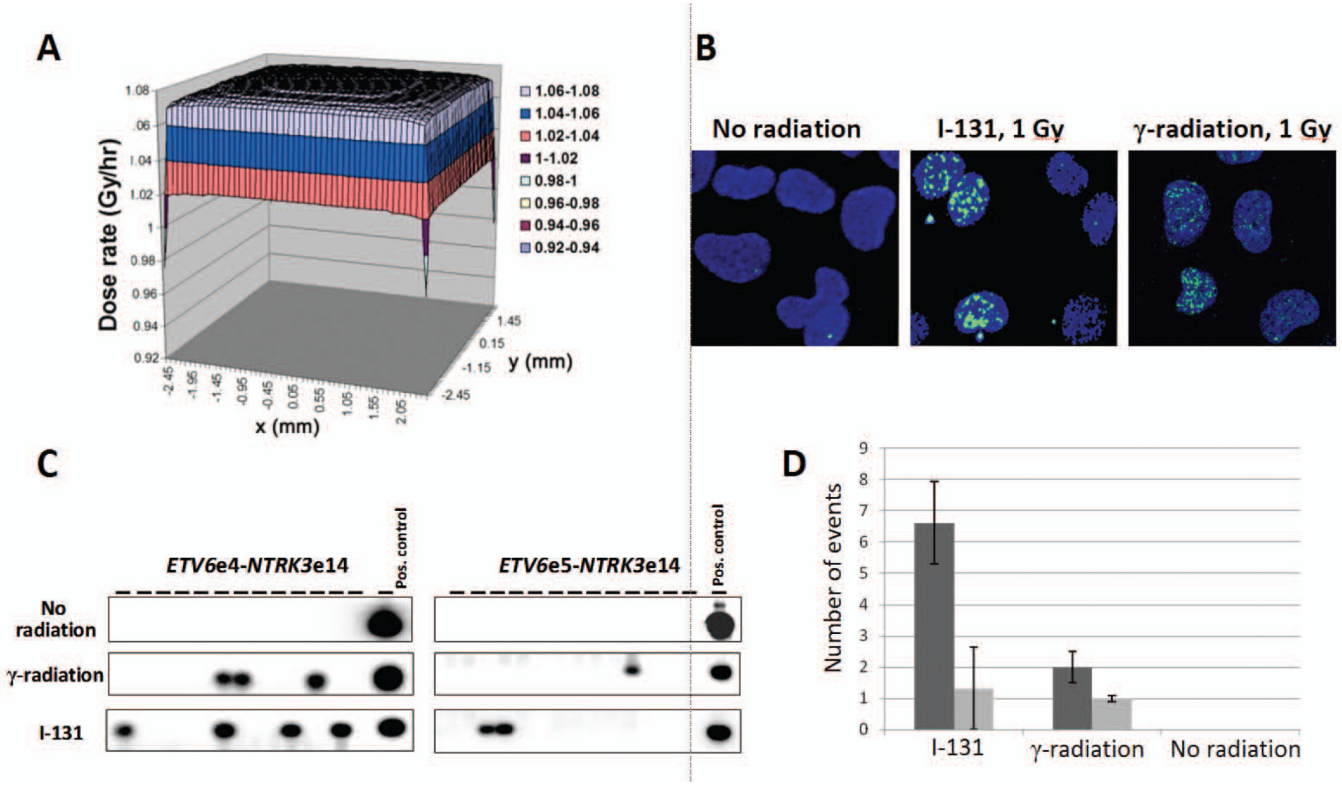

Figure 4.

In vitro induction of *ETV6-NTRK3* rearrangements by radiation exposure. (**A**) Distribution of the ^{131}I dose in the monolayer of cells within a T25 flask generated by Kernel integration. (**B**) Induction of DSBs by ionizing radiation as evident by formation of gH2AX nuclear foci. (**C**) Identification of *ETV6-NTRK3* rearrangements in human thyroid cells after exposure to 1 Gy of ^{131}I or γ-radiation. (**D**) Frequency of specific types of *ETV6-NTRK3* rearrangements induced *in vitro* by ^{131}I and g-radiation.

Table 1

^{131}I dose and other exposure-related characteristics by mutation type in post-Chernobyl papillary thyroid cancer

Genetic Alteration	N (%)*	^{131}I dose, mean (Gy)	Age at exposure, mean (yrs)	Age at surgery, mean (yrs)	Latency, mean (yrs)
BRAF	9 (14.5%)	0.27	10.2	27.0	16.8
RAS	6 (9.7%)	0.21	10.9	29.5	18.6
PAX8-PPARγ	2 (3%)	0.62	12.2	25.8	13.5
RET/PTC	22 (36%)	1.22	6.4	22.3	15.9
ETV6-NTRK3	**9 (14.5%)**	**2.27**	**8.1**	**23.9**	**15.8**
None	18 (29%)	1.71	7.9	24.6	16.7
Total	62	1.27	8.1	24.5	16.5

*Four tumors had two mutations each: *BRAF* and *NRAS, NRAS* and *PAX8-PPARγ, BRAF* and *ETV6-NTRK3, RET/PTC1* and *ETV6-NTRK3*.

Table 2

Dose response for different groups of mutations in post-Chernobyl papillary thyroid cancer*

Genetic Alteration	Risk*** Gy^{-1} (95% CI)	*p*
Assessment of trend		
ETV6-NTRK3	0.30 (−0.09, 0.74)	0.1263**
Assessment of heterogeneity (log-linear dose response)		
All translocations	0.09 (−0.24,0.46)	<0.0001^
All point mutations (*BRAF, NRAS, HRAS*)	−3.29 (−6.06, −1.38)	
Assessment of heterogeneity (log linear-quadratic dose response for translocations)		
All translocations (linear term)	0.77 (−0.07,1.69)	<0.0001^
All point mutations (*BRAF, NRAS, HRAS*)	−3.20 (−5.94, −1.32)	

* All analyses adjusted for age at surgery, gender, and oblast.

** *p*-value for linear trend.

^ *p*-value for heterogeneity in linear terms.

*** Based on log-linear and log-linear quadratic models.