Radiation signatures in childhood thyroid cancers after the Chernobyl accident: Possible roles of radiation in carcinogenesis

Suzuki, Keiji; Mitsutake, Norisato; Saenko, Vladimir; Yamashita, Shunichi

Cancer Science, 106(2), pp.127-133; 2015

© 2014 The Authors. Cancer Science published by Wiley Publishing Asia Pty Ltd on behalf of Japanese Cancer Association. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
Radiation signatures in childhood thyroid cancers after the Chernobyl accident: Possible roles of radiation in carcinogenesis

Keiji Suzuki, Norisato Mitsutake, Vladimir Saenko and Shunichi Yamashita

Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

_key words_
Chernobyl, chromosome rearrangement, radiation, signature, thyroid cancer

Correspondence
Keiji Suzuki, Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
Tel: +81-95-819-7116; Fax: +81-95-819-7117;
E-mail: kzsuzuki@nagasaki-u.ac.jp

Funding information
Ministry of Education, Culture, Sports, Science and Technology, Japan.

Received September 27, 2014; Revised November 26, 2014; Accepted November 30, 2014

Cancer Sci 106 (2015) 127–133

doi: 10.1111/cas.12583

It is generally recognized that radiation exposure takes part in cancer development in the human body. For example, increased risks in cancer mortality/incidence have been well described among atomic bomb survivors in Hiroshima and Nagasaki.\(^1\) After the accident in the Chernobyl nuclear power plant (CNPP) in 1986, large amounts of radioactive materials were released into the environment, which caused excessive numbers of thyroid cancers among children living in contaminated areas neighboring the CNPP.\(^2,3\) Clear dose-dependent induction of childhood thyroid cancers has proven that radiation exposure is the primary cause of thyroid cancer induction.\(^4–6\) Thus, the Chernobyl childhood thyroid cancers have provided unequalled examples to unveil the molecular mechanisms of radiation-induced carcinogenesis.

After the Tokyo Electric Power Company Fukushima Daiichi nuclear power plant accident in 2011, people in Fukushima prefecture and across Japan expressed widespread concerns about health effects due to the release of radioactive materials.\(^7,8\) Although the radiation doses to the public were not appreciably high, the worry is about the late effects of radiation, such as cancer induction.\(^9–11\) A part of this anxiety, so-called radiation phobia, is ascribable to numerous uncertainties in our knowledge of the health effects from low-dose radiation exposure. As we have insufficient scientific evidence to depict the effects of low-dose exposure to radiation, current radiation protection policy has adopted the hypothesis called the linear no-threshold (LNT) model. It assumes that even a very low dose of radiation brings about non-zero risk of cancer induction. Although the LNT model has been evaluated for many years, there is still uncertainty about the linear relationship of low-dose exposure, such as to doses below 100 mSv.\(^11\) One of the reasons for this uncertainty is insufficient mechanistic evidence available from epidemiological studies, so that the applicability of the LNT model to low-dose radiation exposure has not been fully evaluated. Moreover, the LNT model has been challenged by recent experimental observations, including non-targeted effects, which cast some doubts on the linearity of the dose–effect relationship, especially in the low-dose range.

An even more complicated issue is the applicability of the LNT model to life-long exposure to low-dose radiation at a low-dose rate. Although the dose and dose-rate effectiveness factor is used in current radiation protection guidelines, the linear concept is based on the assumption that stochastic radiation-induced oncogenic mutations persist in the target stem cells in tissues/organs. However, recent advances in stem cell biology have suggested that the integrity of stem cells is protected by multiple mechanisms, such as efficient DNA repair, stem cell competition, and tissue turnover. Thus, there is an urgent need to reconcile the recent observations that challenge...
the persistence of stochastic oncogenic events in tissues and organs.\(^\text{12}\) Moreover, through these findings, we have recognized the immediate need of extensive reconsideration of the theoretical basis of radiation-induced carcinogenesis in order to ascertain whether recent scientific observations sufficiently support the current carcinogenesis model, in which radiation-induced oncogenic mutations are involved in cancer development.

**Childhood Thyroid Cancer after the Chernobyl Accident**

After the accident at the CNPP on April 26, 1986, large amounts of radioactive materials were released, which lead to radiation exposure in the residents of affected areas.\(^{2,3}\) Particularly, the fallout of radioactive iodine caused noticeable internal exposures in children through ingestion of contaminated milk and foodstuffs, which resulted in significant numbers of childhood thyroid cancer – one of the main health effects of the accident.\(^{13,14}\) Four to 5 years after the accident, excessive cases of childhood thyroid cancers started to be reported. The increases in thyroid cancer were particularly profound among children aged between 0 and 4 years, whereas no such increase was observed in adults. Between 1991 and 2005, 5127 cases of thyroid cancers were reported among children under the age of 14 years in Belarus, while 6848 cases were diagnosed in individuals exposed at when aged under 18 years.\(^{5}\) Amongst children born after 1986, the incidence rate of thyroid cancer significantly declined almost to the background level, indicating that the considerable increase in thyroid cancers in children born after 1986, the incidence rate of thyroid cancer was observed among children exposed at when aged under 18 years.\(^{3}\) Amongst children born after 1986, the incidence rate of thyroid cancer significantly declined almost to the background level, indicating that the considerable increase in thyroid cancers in children born after 1986, the incidence rate of thyroid cancer was observed among children exposed at when aged under 18 years.\(^{3}\) Amongst children born after 1986, the incidence rate of thyroid cancer significantly declined almost to the background level, indicating that the considerable increase in thyroid cancers in children born after 1986.

**Oncogenic Rearrangements in Childhood Thyroid Cancer after the Chernobyl Accident**

After the Chernobyl accident, the highest risk for radiation-induced thyroid cancer was observed among children exposed at the age of 0–4 years. Early childhood thyroid cancer cases showed significantly higher prevalence of rearrangements between the rearranged during transfection (RET) gene and the PTC3 gene (RET/PTC3 rearrangement).\(^{25–27}\) The RET/PTC1 as well as RET/PTC2 rearrangements were also reported.\(^{27}\) It has been established that RET/PTC1 gene rearrangement is the most prevailing genetic alteration in childhood PTCS after the Chernobyl accident overall.\(^{19,20,28,29}\)

Fusions of the RET proto-oncogene with several partner genes, which have been collectively designated the PTC genes, have been described (Table 1).\(^{30}\) The RET gene encodes a transmembrane receptor tyrosine kinase. The binding of the ligands stimulates receptor dimerization, the critical step for activation of tyrosine kinase activity.\(^{31,32}\) The fusion partner genes are commonly expressed in thyroid follicular cells and possess coiled-coil domains that enable homodimerization of the fusion RET/PTC proteins (Fig. 1). As a result, RET/PTC proteins constitutively activate the MAPK pathway without any ligand binding (Fig. 2).\(^{28,33–36}\)

Other types of rearrangements identified in childhood thyroid cancer related to the Chernobyl accident include juxtaposition of the A kinase anchor protein 9 (AKAP9) gene and \(\text{v}-\text{raf viral oncogene homolog B1 (BRAF)}\), designated AKAP9–BRAF\(^{37,38}\) rearrangement between translocated protein region (TPR) and the neurotrophic tyrosine kinase receptor type 1 (NTRK1) gene (TPR–NTRK1)\(^{38}\) rearrangement between the ETS variant 6 (ETV6) gene and the NTRK3 gene (ETV6–NTRK3)\(^{38,39}\) rearrangement between the acylglycerol kinase (AGK) gene and the BRAF gene (AGK–BRAF)\(^{38}\) rearrangement between the cAMP-responsive element binding protein 3-like 2 (CREB3L2) gene and the peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) gene (CREB3L2–PPAR\(\gamma\))\(^{38}\) and rearrangement between the paired box 8 (PAX8) gene and the PPAR\(\gamma\) gene (Pax8–PPAR\(\gamma\)) (Table 1).\(^{38,40}\)

The RET/PTC1 and RET/PTC3 rearrangements are created through the paracentric (intrachromosomal) inversion within chromosome 10, where the RET, CCDC6, and NCOA4 genes are assigned (Fig. 1).\(^{34–36}\) Other RET/PTC rearrangements arise from interchromosomal translocations. Theoretically, at least two independent DNA double-strand breaks are necessary to produce a rearrangement. Therefore, these observations have logically brought about the hypothesis that radiation exposure from internal \(\text{I}^{131}\) causes DNA double-strand breaks, resulting in oncogenic genome rearrangements after illegitimate recombination.\(^{28}\) Notable association between radiation...
exposure and the induction of oncogenic rearrangement was demonstrated in experimental studies, in which radiation-induced RET/PTC rearrangements were confirmed in X-irradiated primary thyroid tissues transplanted into SCID mice.\(^{[41]}\) However, one should be cautious about the conclusion, because the experiments used high-dose radiation exposure over 50 Gy. More recently, the generation of RET/PTC rearrangements have been identified in thyroid epithelial cells receiving much lower doses, although the frequency was quite low and dose-dependent induction was not clear.\(^{[42]}\)

Although in vitro experiments seem to substantiate the hypothesis, in vivo studies have drawn a different picture. After the earlier studies, several independent groups have evaluated the prevalence of RET/PTC rearrangements in childhood thyroid cancer after the Chernobyl accident and compared the results with the frequency of RET/PTC rearrangements in sporadic childhood PTCs. The compiled data indicated that RET/PTC rearrangements were detectable to a comparable extent in both childhood thyroid cancers after the Chernobyl accident and sporadic childhood thyroid cancers.\(^{[43–45]}\)

Extensive studies showed that the frequency of thyroid cancer with RET/PTC rearrangements decreases with age in sporadic cases, whereas those with the BRAF mutation becomes greater.\(^{[19]}\) It is well established that these two genetic changes are mutually exclusive. Individuals born before the accident are now aged 28 years or older, and a recent report has suggested that the frequency of thyroid cancer harboring the BRAF mutation has tended to grow in the affected group, while RET/PTC rearrangements are still detectable.\(^{[46]}\) This is another epidemiological observation indicating that molecular changes in thyroid cancer after the Chernobyl accident mirror those occurring spontaneously.

### Table 1. Oncogenic rearrangements in childhood thyroid cancers related to the Chernobyl accident

<table>
<thead>
<tr>
<th>Oncogenes Rearrangements</th>
<th>Rearrangement partners</th>
<th>Chromosome locations</th>
<th>Type of rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RET/PTC1</td>
<td>CCDC6 (also H4)</td>
<td>10q11.2</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>RET/PTC2</td>
<td>PRKAR1A</td>
<td>17q24.2</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>RET/PTC3</td>
<td>NCOA4 (also Eie1)</td>
<td>10q11.2</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>RET/PTC4</td>
<td>NCOA4 (also Eie1)</td>
<td>10q11.2</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>RET/PTC5</td>
<td>GOLGA5 (also RFG5)</td>
<td>14q32.12</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>RET/PTC6</td>
<td>TRIM24</td>
<td>7q32-q34</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>RET/PTC7</td>
<td>TRIM33 (also RFG7)</td>
<td>1p13.1</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>RET/PTC8</td>
<td>KTN1</td>
<td>14q22.1</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>RET/PTC9</td>
<td>RFG9 (also MBD1)</td>
<td>18q21</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>BRAF rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td></td>
<td>7q34</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>AKAP9/BRAF</td>
<td>AKAP9</td>
<td>7q21-q22</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>AGK/BRAF</td>
<td>AGK</td>
<td>7q34</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>NTRK rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTRK1</td>
<td></td>
<td>1q21-q22</td>
<td></td>
</tr>
<tr>
<td>NTRK3</td>
<td></td>
<td>15q25</td>
<td></td>
</tr>
<tr>
<td>TPR/NTRK1</td>
<td>TPR</td>
<td>1q25</td>
<td>Paracentric inversion</td>
</tr>
<tr>
<td>ETV6/NTRK3</td>
<td>ETV6</td>
<td>12p13</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>PPARγ rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td></td>
<td>3q25</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>PAX8/PPARγ</td>
<td>PAX8</td>
<td>2q13</td>
<td>Interchromosomal translocation</td>
</tr>
<tr>
<td>CREB3L2/PPARγ</td>
<td>CREB3L2</td>
<td>7q34</td>
<td>Interchromosomal translocation</td>
</tr>
</tbody>
</table>

![Fig. 1. Schematic representation of RET/PTC1 rearrangements. A paracentric inversion of chromosome 10 gives rise to a fusion gene between the tyrosine kinase domain of the RET gene and the amino terminal region of the CCDC6 gene. The fusion protein is constitutively activated through the dimer formation mediated by the coiled-coil domain of the CCDC6 protein.](#)

![Fig. 2. Activation of the MAPK pathway in thyroid cancer. Most of the rearrangements identified in post-Chernobyl childhood thyroid cancers impair the physiological function of receptor tyrosine kinase activity, which results in constitutive activation of the MAPK pathway.](#)
Thus, accumulating in vivo observations suggest that RET/PTC rearrangements observed in childhood thyroid cancer after the Chernobyl accident might not be the result of internal exposure to radiation from $^{131}\text{I}$, but rather radiation exposure might play a non-targeted role in providing a tissue microenvironment, which eventually selects thyroid follicular cells with spontaneous RET/PTC rearrangement.

**Copy Number Alteration as a Radiation Signature in Childhood Thyroid Cancer after the Chernobyl Accident**

Radiation exposure is an efficient inducer of DNA double-strand breaks, therefore it is highly expected to cause gains or losses of DNA; however, this notion was challenged by array comparative genomic hybridization analysis. A variety of copy number alterations (CNAs) have been identified in childhood thyroid cancers after the Chernobyl accident, mostly gains of DNA, and these were compared with CNAs in sporadic cases, in which losses were more frequent than gains. Consequently, it turned out to be clear that most studies have failed to demonstrate specific CNAs associated with radiation exposure, while one study, using an age- and ethnicity-matched cohort, described a unique gain of chromosome 7q11, which was absent in all unexposed cases. A few gains are assigned to this chromosome band, although the overexpression of such gene products seems not to be the driver in childhood thyroid cancers. Thus, some copy number signatures might be associated with radiation-induced childhood thyroid cancers, however, their involvement in childhood thyroid carcinogenesis remains to be determined.

**Gene Expression Signature**

Previous studies have shown the differences in gene expression profiles between PTCs and normal thyroid tissues. The strategy has been used to identify gene expression signatures that distinguish radiation-induced childhood thyroid cancers from sporadic cases. Several studies have been carried out and some of them reported gene expression changes unique to radiation-induced childhood PTCs, whereas others have failed to identify the signatures. Importantly, the identified genes were very different between the studies, with few recurrent genes. More recently, gene expression profiles were compared in normal contralateral thyroid tissues obtained from exposed and unexposed children after the Chernobyl accident. The study identified a gene expression signature, whose gene products are related to overall cell proliferation.

It should be taken into account that gene expression profiles could be affected by possible confounding factors such as age, ethnicity, and pathological features of the tumors, and these might have caused large discrepancies between the studies. At present, it seems unlikely that common gene expression signatures could be associated with radiation-induced childhood thyroid cancers. As suggested by previous reports, the signatures might be dispensable for childhood thyroid carcinogenesis but rather they might reflect the results of radiation exposure.

**Radiation Signatures and Possible Mechanisms of Radiation Carcinogenesis**

It is generally accepted that cancer has arisen as a result of accumulation of oncogenic mutations. Mathematical considerations show that cancers, especially the solid cancers, show age-depen-
anomalous tissue development could be targeted by radiation exposure.

Possible involvement of tissue disturbance in thyroid carcinogenesis has been discussed in the observations, in which chronic autoimmune thyroiditis, such as Hashimoto’s thyroiditis, is sometimes accompanied by cancer.\(^{72}\) Although the link is still debated, it seems likely that PTCs may develop if the cells with oncogenic mutations preexisted in the region with Hashimoto’s thyroiditis. It should be noted that proliferative response was observed in Hashimoto’s thyroiditis,\(^{73}\) therefore, the disturbance of tissue homeostasis by chronic inflammation could create a condition for the cells harboring spontaneous RET/PTC rearrangement to undergo cell proliferation.\(^{74}\)

In fact, some adverse effects of the Chernobyl accident on thyroid function have been reported in several studies, although the results are not always consistent due to the limited sample sizes and a lack of individual dose estimations. Earlier studies have shown the increased prevalence of thyroid autoimmune disorders among children exposed to the Chernobyl radioactive fallout 6–8 years after the accident, which was no more evident 12–14 years after the accident.\(^{75,76}\) More recent studies have indicated that subclinical hypothyroidism still persisted among the individuals who were younger than 18 years of age on the day of the accident.\(^{77}\) These observations imply that internal exposure to radioactive iodine may result in not a detrimental but notable disturbance in the thyroid gland of the affected children.

Recently, it has been recognized that ionizing radiation induces senescence-like cell death in thyroid follicular cells.\(^{78}\) Moreover, senescence-like cell death promotes secretion of inflammatory cytokines,\(^{79}\) so that it is tempting to speculate that radiation-induced tissue disruption could result in inflammatory circumstances that promote the initial stage of thyroid carcinogenesis (Fig. 3). Thus, taking all of this information into consideration, it is plausible to propose that a role of radiation in childhood thyroid cancers after the Chernobyl accident could be an introduction of tissue disturbance by inducing thyroid follicular cell death as well as introducing the secretory phenotype of dead cells (Fig. 3).

One should be cautious about this scenario, because many of the above speculations have to be experimentally proven. Also, the idea suggests that the stochastic induction of oncogenic mutations by radiation might not be the primary role of radiation exposure in childhood cancer development, rather, deterministic cell death could be involved. The risk of thyroid cancer incidence was estimated to increase linearly with radiation dose; however, these findings may cast doubt on the use of the LNT model, on which current risk estimation relies, especially at low doses. Thus, with further scientific investigations, we should reconsider the scientific significance of the LNT model especially for low-dose and low-dose-rate exposure. As such a condition currently exists in Fukushima prefecture, thorough studies will undoubtedly provide invaluable insights into this complication.

Conclusions

Internal exposure to radioactive iodine caused childhood papillary thyroid cancer after the Chernobyl accident. Molecular analyses have shown that RET/PTC rearrangements are the most prevailing oncogenic alteration in both radiation-induced and sporadic childhood thyroid cancer. Thyroid follicular cells might display selective growth, if the cells harbor spontaneous oncogenic rearrangements and if the tissue and tissue microenvironment are perturbed by cell death caused by ionizing radiation. The hypothetical model may cast some doubt on the current model of stochastic radiation carcinogenesis. Future studies will define the non-targeted role of radiation exposure, which should improve our understanding of multistep carcinogenesis induced by radiation exposure.

Acknowledgments

We thank Dr. Michiko Matsuse for critical reading of the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Disclosure Statement

The authors have no conflict of interest.

References

Role of radiation in thyroid cancers

Leeman-Neill RJ, Howe GW, Bogdanova TI et al. A cohort study of thyroid cancer and other thyroid diseases after the chernobyl accident: thyroid cancer in Uk-


Gembicki M, Stozharov AN, Arinchin AN et al. Iodine deficiency in Belaru-


Gembicki M, Stozharov AN, Arinchin AN et al. Iodine deficiency in Belarussian children as a possible factor stimulating the irradiation of the thyroid gland during the Chernobyl catastrophe. Environ Health Perspect 1997; 91: 1487–90.


