Proliferative Activity of Human Thyroid Cells in Various Age Groups and Its Correlation with the Risk of Thyroid Cancer after Radiation Exposure

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Context: The thyroid gland is vulnerable to the carcinogenic effects of ionizing radiation, and there is a well-documented inverse correlation between thyroid cancer and age at exposure, particularly for ages less than 20 yr. One of the factors responsible for this phenomenon may be more rapid cell proliferation in children.

Objective: The objective of this study was to determine the proliferative rate of normal human thyroid cells in different age groups.

Design: We used immunohistochemical analysis to determine the Ki-67 proliferative index in 117 thyroid glands obtained at autopsy, including 25 fetal thyroids (11–40 wk gestation), 55 childhood thyroids (0–19 yr), and 37 adult thyroids (20–60 yr).

Results: The rate of Ki-67 labeling in the three groups was 7.4 ± 6.10, 0.23 ± 0.15, and 0.08 ± 0.04% respectively, demonstrating an overall trend for diminishing proliferative activity of thyroid cells with increasing age. However, a lack of correlation was noted between the slopes of cancer risk calculated from previous studies of irradiated populations and proliferative rate in the pediatric age intervals of 0–4 and 5–9 yr, suggesting that other factors are likely to be responsible for the particularly high sensitivity to radiation-induced thyroid cancer among the youngest children.

Conclusions: Our findings of a general decrease in proliferative activity of thyroid cells with age may explain, at least in part, the higher risks of radiation-related thyroid cancer in children compared with adults. However, the variation in the rate of cell proliferation is unlikely to be responsible entirely for this phenomenon and other factors may also be involved. (J Clin Endocrinol Metab 91: 2672–2677, 2006)

Ionizing radiation during childhood is a well-established risk factor for thyroid cancer (1, 2). The strong association between radiation exposure and thyroid cancer was first reported more than 50 yr ago (3) and was confirmed by studies of patients exposed to external medical radiation for treatment of childhood diseases and conditions, such as enlarged thymus (4), tinea capitis (5), childhood cancer (6), and other conditions of the head and neck (7), and survivors of the atomic bomb explosions in Hiroshima and Nagasaki (8, 9). More recently, children in Belarus, Ukraine, and Russia who were exposed primarily to internally deposited radio- active iodines, mostly I-131, as a result of the Chernobyl accident exhibited a significantly elevated risk of thyroid cancer (10–12).

The increased risk of thyroid cancer in these cohorts was documented for a wide range of doses, and a linear dose-response relation for doses from 0.1 Gy to up to 1–2 Gy described the data well (1, 9, 13–15). At higher doses, a flattening of the dose-response curve has been found in some studies (15), but in a recent evaluation of young cancer survivors, a linear dose-response relation was seen up to 10 Gy (6). Most studies have found that age at exposure was a strong modifier of thyroid cancer risk. This was observed after various doses of external, mostly therapeutic radiation (1, 13, 14). In a pooled analysis of several studies, the excess relative risk (ERR) per gray was highest among those exposed at 0–4 yr of age, and it progressively decreased with increasing age of children (13). Among atomic bomb survivors, one of the few populations in which people were exposed to radiation at all ages, the risk decreased with increasing age at exposure, so that by about 20 yr of age the risk of thyroid cancer was no longer significantly elevated and after the age of 40 yr no increased risk could be observed (9). In populations exposed after the Chernobyl accident, the incidence of thyroid cancer also was inversely correlated with age at exposure in many (11, 16, 17) but not all studies (18, 19). Although, in part, this can be explained by a significantly higher radiation dose to the thyroid in young children, it is likely that age at exposure may also serve as a risk modifier in these populations (11).

The factors responsible for a higher risk of thyroid cancer after childhood radiation exposure remain unknown. It has been suggested that this is likely due to the growth pattern of thyroid cells, which are generally believed to proliferate actively in childhood, but have a very limited potential for proliferation during adult life (11, 13). This has been studied in detail in animal thyroid, and it was assumed that the same
growth pattern would be present in humans (11). Surprisingly limited empirical data are available on the proliferative activity of normal human thyroid cells at different ages. Some information comes from studies of proliferative activity of thyroid tumors, where normal thyroid tissues were used as a control. Using MIB-1 antibody that reacts with the Ki-67 nuclear antigen found throughout the cell cycle but absent in resting (G0) cells, it was shown that overall proliferative rate of normal adult thyroid cells is as low as 0.2% (20). Comparable findings (0.6%) were observed when another proliferative marker, proliferating cell nuclear antigen/cyclin, was studied in several normal thyroid glands (21).

Recently, Faggiano et al. (22) reported their analysis of a series of 31 thyroid glands from patients with families affected by medullary thyroid carcinomas and ages ranging from 3–39 yr. They found that, in individuals under 12 yr of age, thyroid follicles are of a significantly smaller size as compared with older individuals, and there was a higher expression of proteins involved in the iodine trafficking and metabolism in this age group (22). As for the proliferative activity, no significant Ki-67 labeling was observed in this study, possibly due to a relatively limited number of cases in each age group and of microscopic fields examined in each case. The finding of age-related size of thyroid follicles and variation in the expression of sodium/ioidide symporter and other proteins involved in iodine metabolism provides a possible mechanism for a much higher radiation dose in young children after exposure to radioiodines, but cannot explain the age-related risk of radiation-related cancer after external irradiation. An age-related difference in the proliferative activity of thyroid cells would provide the basis for this paradigm for both radiation types.

In this study, we determined the proliferative activity of normal thyroid cells in a large series of cases including the entire spectrum of ages from the 11th wk of gestation throughout childhood and adulthood. This was compared with the reported data on the age-related risks of thyroid cancer from various populations exposed to radiation. The prenatal group was included because three cases of thyroid cancer were diagnosed among children living in Gomez, Belarus who were exposed in utero (23). The Ki-67 labeling by immunohistochemistry was chosen as a tool to study the proliferative activity in formalin-fixed tissues because it has been widely accepted and validated in nonneoplastic and neoplastic tissues as a reliable marker of cell proliferation, and none died of neoplastic diseases. The spectrum of unmet unmet and infection, which were distributed approximately evenly across the spectrum of specific age intervals, except for the 11–15 wk gestation group, where spontaneous abortion was the only anatomical diagnosis. Within the pediatric group, the most common causes of death within the fetal group were acute asphyxia, congenital malformations, and infection, which were distributed approximately evenly across the spectrum of specific age intervals, except for the 11–15 wk gestation group, where spontaneous abortion was the only anatomical diagnosis. Within the pediatric group, the most common causes of death within the fetal group were acute asphyxia, congenital malformations, and infection, which were distributed approximately evenly across the spectrum of specific age intervals, except for the 11–15 wk gestation group, where spontaneous abortion was the only anatomical diagnosis. Within the pediatric group, the most common causes of death were congenital malformations and infections. Seven patients in this group (13%) died of nonthyroid neoplasms. Within the adult group, most patients died of infection, heart disease, or acute bleeding, and none died of neoplastic diseases. The spectrum of underlying diseases was not expected to affect differentially the proportion of proliferating thyroid cells in specific age intervals.

Proliferative rate at various ages

Proliferative rate of thyroid cells in different age intervals was determined based on the expression of the Ki-67 nuclear
antigen (Fig. 1). The results are summarized in Table 2. The highest levels of cell proliferation were in early fetal life, particularly at 11–15 and 16–20 wk gestation, when 16 and 12% of cells were labeled, respectively. However, the rate of cell proliferation continuously decreased, with a particularly sharp drop at 31–35 wk gestation, when less than 2% of cells remained cycling. The rate decreased, further approaching the age of fetal maturity, with a proliferative index of 0.4% found at 35–40 wk gestational age. After birth, the proliferative index remained low, with 0.2% proliferating cells in infants under 1 yr of age. Some fluctuation in the rate of cell proliferation was observed within the pediatric group, with most age intervals demonstrating the rate of cell proliferation within the range of 0.2–0.3% (Table 2). In the adult population, the proliferative activity was close to 0.1% throughout the spectrum of age intervals. Overall, the mean rate of Ki-67 labeling was 7.4 ± 6.1% in the fetal group, 0.23 ± 0.15% in the pediatric group, and 0.08 ± 0.04% in the adult group. To ensure that no single cause of death unduly influenced our findings, we also analyzed the data removing the individuals who died from the two largest causes of death (infections and congenital malformations). There was no appreciable change in the results when either cause of death was excluded. The difference in the mean proliferative rate between the groups was statistically significant (P < 0.001). Figure 2 shows the smoothed proliferative index by weeks from conception. Whereas there is evidence of an overall trend for decreasing proliferative index with increasing age (P < 0.01), patterns of variation differed within the different age groups. There was a steep decline during the fetal period (P < 0.001) and the first few postnatal months, almost no difference between 1–10 yr (P > 0.5), a significant drop between 10–19 yr (P = 0.04), and a small drop after 20 yr (P = 0.26).

### Correlation with radiation-associated cancer risks

Although the trend for decreasing risk of radiation-associated thyroid cancer with increased age at exposure has been observed in many studies, only a few have provided quantitative analysis of the ERR per Gy in specific age groups. Among the atomic bomb survivors in Japan, the calculated ERR per Gy was 6.4 for those exposed at age 0–4 yr, 3.7 for those 5–9 yr old, 2.1 for 10–19 yr old, 0.7 for 20–29 yr old, 0.9 for 30–39 yr old, and 0.0 for older than 40 yr (1, 27). In a pooled analysis of seven studies of populations exposed to external radiation, including those exposed to

![Fig. 1. Representative microscopic images of Ki-67 immunostaining of thyroid glands at different ages. A, Sixteen weeks gestation, proliferative rate 14.15%; B, 1 yr old, proliferative rate 0.35%; C, 20 yr old, proliferative rate 0.05%. The arrows indicate the only two thyroid follicular cell nuclei positive for immunostain in this field. Magnification, ×200.](image-url)
therapeutic irradiation as children and adults and atomic bomb survivors of all ages, the modifying effect of age was 1.0, 0.5, 0.2 in those exposed at the ages of 0–4, 5–9, and 10–14 yr, respectively (13). The results from these studies were used to compare with the trend of the proliferative rate in the corresponding age groups. We observed mean proliferative rates of 0.22 for 0–4 yr old, 0.28 for 5–9 yr old, 0.20 for 10–14 yr old, 0.18 for 15–19 yr old, 0.09 for 20–29 and 30–39 yr old, and 0.08 for individuals over 40 yr old.

Comparison between the risks of radiation-related thyroid cancer and cell proliferation in the same age intervals revealed a generally similar tendency for a decrease in both parameters with age (Fig. 3). However, a lack of correlation was noted between the slopes of risk and proliferative rate in the early pediatric age intervals of 0–4 and 5–9 yr and in those over 40 yr old.

Discussion

In this study, we report the proliferative rates of histologically normal human thyroid cells at various ages. These data reveal that thyroid cells proliferate at significantly different rates during the fetal period, in children, and in adults, suggesting that the rate of cell proliferation may be a contributing factor to the known variability in the risks of radiation-related thyroid cancer in pediatric and adult populations. On the other hand, correlation between proliferative rate and cancer risk was lacking in several age intervals, most noticeably among young children, suggesting that other factors are likely to be responsible for the particularly high sensitivity to radiation-induced thyroid carcinogenesis in the youngest pediatric populations.

The highest levels of growth activity of thyroid cells were observed during the early fetal period, particularly at 11–20 wk gestation. Given the high rate of proliferation at this time, there is surprisingly little evidence of a large increase in thyroid cancer after in utero radiation exposure. This partly may be because most studies of in utero radiation exposure have only followed subjects through childhood and, therefore, could not evaluate the relationship with adult cancers. Another possible explanation can be drawn from the hypothesis proposed by Ohtaki et al. (28). They have determined that fetal lymphocytes did not show a high frequency of chromosome translocations after radiation exposure of more than 0.1 Sv, and suggested that fetal cells may eliminate damaged cells more efficiently than adult cells through apoptosis or possess enhanced ability to repair genotoxic damage.

The pace of cell proliferation declines sharply during the late fetal period. Beyond 26–30 wk gestation, less than 2% of cells participate in cell cycling at any given period of time. After birth, less than 0.4% of cells are dividing at the same time, and less than 0.2% of cells are proliferating after the age.
of 19 yr. The low rate of cell cycling during late fetal life and within the first years after birth is a somewhat unexpected finding. Indeed, the weight of the thyroid gland further increases in postnatal life from approximately 2–4 g at birth to approximately 15–20 g in adulthood (29–31). However, it is likely that, in significant part, this is due to the accumulation of colloid within the follicular spaces, rather than cell multiplication. Formation of colloid is known to begin at 12 wk postconception, and by wk 14, the gland reveals first small colloid-containing follicles, which progressively increase in size during fetal life and after birth. This process apparently continues during childhood, as evident from the steady increase in the follicle size and proportion of follicles with abundant colloid in older children (22, 32). The low proliferative rate of thyroid cells in adults observed in this study confirms the previous observation that Ki-67 labeling ranges from 0.00–0.34% in adult normal thyroid glands (20).

The risk of radiation-related thyroid cancer has a well-documented relationship with age at exposure after external irradiation (1, 9, 13). In this study, we document an overall decrease in the proliferative rate as a function of age, which correlates well with the overall decrease in risk of radiation-related thyroid cancer with increasing age at exposure. However, we also observed significant inconsistency between the rate of cell proliferation and the pattern of radiation-related cancer risk in several specific age intervals. The major discrepancy was observed within the first 10 yr of life, where the proliferative rate was fairly stable but the risk of radiation-related thyroid cancer showed a strong decline with a large drop between 0–4 and 5–9 yr of age (13). Another possible inconsistency is that in the late fetal period, e.g. 31–40 wk gestation, the proliferative rate was severalfold higher than in newborns, whereas the current literature does not provide evidence of a high risk of thyroid cancer after late in utero exposure. These discrepancies suggest that additional and still unknown factors may modify further the risk of thyroid cancer in the youngest children. One possibility would be that it is due to a higher functional state of thyroid glands in early pediatric ages. Indeed, immediately after birth, thyroid glands have almost no stored colloid, so that follicular cells are required to produce thyroid hormone not only for immediate use but also for storage within the follicles. This correlates with the progressive decrease in the height of follicular cells, which reflects directly the functional status of these cells. Columnar cells (most functionally active) are seen in normal thyroids only during the first 3 months of life, and the proportion of cuboidal cells continually decreases during childhood, so that between 6 and 15 yr of age the majority of follicular cells change their shape from cuboidal to flattened (32). However, if the functional status plays a role, the exact mechanisms of such an effect are not clear. It would not be directly related to the expression levels of sodium/iodide symporter or other proteins involved in iodine transport and organization, because the age-related risk of thyroid cancer has been documented primarily for external irradiation.

In summary, our findings of a general decrease in proliferative activity of thyroid cells with age may explain, at least in part, the overall higher radiosensitivity of children compared with adults. However, the variation in the rate of cell proliferation is unlikely to be responsible entirely for this phenomenon, and other mechanisms predisposing to a significantly higher risk of thyroid cancer among the youngest children are likely to exist. Better understanding of these mechanisms should allow more effective approaches for prevention of radiation-induced thyroid carcinogenesis in humans.

Acknowledgments

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