The Molecular Biological Characteristics of Childhood Thyroid Carcinoma

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Abstract:

We have used molecular biology to study mutation and expression of key oncogenes in childhood thyroid carcinomas from Belarus and Ukraine. All cases were histologically verified by two or more pathologists including at least one from the CIS and one from the EU. We chose to study six genes which have been shown to be involved in thyroid carcinogenesis in adults: ret, Ha, Ki and N ras genes, p53 and the TSH receptor. Expression of the ret oncogene, which has been shown to be activated by translocation in a proportion of papillary carcinomas has been studied by two independent methods. The first, used by the Cambridge group uses RT-PCR to identify the expression of the tyrosine kinase domain of the gene; as the gene is normally silent in follicular cells, this approach allows demonstration of activation of ret, but does not identify the particular translocation involved. The second approach, used by the Naples group, also uses RT-PCR, but amplifies across the breakpoint of each of the three translocations already identified to provide information on the proportion of tumours which express the individual translocations of this gene. Mutations in the TSH receptor, a key modulator of thyroid follicular growth have been sought by the Brussels group using SSCP and direct sequencing. The Munich group have analysed the samples for presence of mutation in p53, which is believed to play a role in genetic instability which is a features of carcinomas derived from may different tissues. Mutations in the common sites of the ras oncogenes have been studied by the Cambridge group.

Analysis of 26 papillary carcinomas so far studied has shown that mutations in the TSH receptor and in p53 do not play a significant role in the genesis of the tumours studied. The proportion of tumours showing ret expression does not differ significantly from that found in a control non exposed population from the UK. However, the pathological study shows that nearly all the increased number of thyroid carcinomas found in children exposed to fallout from Chernobyl are of the papillary type, and although the proportion showing ret activation does not increase, the numbers of tumours in which ret activation plays a role in carcinogenesis has very greatly increased. We therefore consider that radiation leading to ret translocation is a major feature in the increased number of childhood

thyroid carcinomas in the population exposed to fallout from Chernobyl.

1: Introduction

The thyroid is an organ which is extremely sensitive to the carcinogenic effect of radiation. It has been known for many years that radiation causes an increase in thyroid cancer in animals (1) and that exposure to external radiation in childhood, usually as a result of clinical treatment increases the risk of developing thyroid cancer in later life (2). More recently, there has been a dramatic increase in juvenile thyroid cancer reported in the areas of Belarus and Ukraine exposed to fallout from the Chernobyl nuclear disaster (3-6). This may be related to the fact that the thyroid is the only organ in the body that actively takes up and then binds iodine. Large amounts of radioiodine were released during the Chernobyl accident. However, radioiodine is widely used in man for the treatment of Graves Disease, and it has not been shown to be carcinogenic to man in the doses used in adults.

There are three types of differentiated thyroid cancer, medullary carcinoma, derived from the minority C cell component of the thyroid, follicular and papillary carcinomas which both derive from the thyroid follicular cell. Thyroid follicular cells require iodide in order to synthesise the iodide containing hormones T3 and T4, which are essential for metabolic regulation in vertebrates. Although both follicular and papillary carcinomas increase in frequency after radiation exposure , it is papillary carcinoma which shows a greater relative increase (7). There is little evidence to suggest that medullary carcinoma is associated with exposure to radiation.

Molecular biological studies have recently suggested that papillary and follicular carcinomas in humans show different oncogene involvement; ras genes being more frequently mutated in follicular carcinoma (8) and translocations of the ret and trk oncogenes being more frequent in papillary carcinomas (9). So far three different translocations of the ret oncogene have been identified, two intrachrosomal and one interchromosomal. Interestingly the ret oncogene has been shown to be frequently mutated, rather than translocated, in medullary carcinoma (10).

2: Material studied

Sections of paraffin embedded material were obtained from 26 cases of thyroid cancer from children under 15 at the time of operation from Ukraine and 50 cases from Belarus. All cases were histologically verified and all except one (a medullary carcinoma) were papillary carcinoma. Sections were circulated to each of the collaborating centres for analysis.

3: Results

3.1: Ras gene mutation

The three ras genes have been studied in 14 papillary carcinomas by PCR and direct sequencing. No mutations have been observed in the commonly mutated codons (12,13 and 61). Similar studies carried out on a control group of childhood thyroid papillary carcinomas from England and Wales also showed no mutation at these sites. However, mutations of these genes have been identified in 3 of 10 adult follicular carcinomas using the same approach. This suggests that ras gene mutation is probably not normally involved in the genesis of thyroid papillary carcinomas, whether induced by radiation or not.

3.2: Ret gene expression

Expression of the ret gene has been studied using RT-nPCR for a 90 base pair sequence within the tyrosine kinase domain and direct sequencing. Ret expression was identified in 6 of 18 (33%) Chernobyl associated childhood papillary carcinomas so far studied. This was a significantly lower frequency than that found in a study which used the same technique on adult papillary carcinomas (11), and was also lower than the frequency observed in 20 childhood papillary carcinomas from

England and Wales. However, due to the smaller number of carcinomas from children so far studied, we are not yet able to say whether the irradiated series shows a significant reduction in the frequency of ret expression. A small study carried out on frozen material from the Ukraine showed ret expression in a similar proportion (3/11) of childhood thyroid papillary carcinomas, suggesting that the low frequency found in the paraffin embedded material was not due to a decreased sensitivity of the system. There does not appear to be a correlation between the expression of the ret oncogene as observed by RT-nPCR analysis and morphological subtype of papillary carcinoma. Positivity for actin amplification was used as a control for quality of the RNA extracted from the sections.

3.3: Ret gene translocation

Twenty five cases of childhood thyroid carcinomas have so far been studied; only 11 yielded sufficient RNA for further analysis. Two controls for RNA quality were used: amplification of actin mRNA by RT-PCR and Northern blot with an 18S RNA probe. Using primers which allow detection of the three individual translocations of the ret oncogene so far identified, three papillary carcinomas with ret translocation have so far been identified by PCR and Southern blotting. All 11 cases which provided sufficient RNA have been analysed for the PTC1 translocation; only one case was positive. Two of the 7 cases so far analysed have been found to be positive for the PTC3 translocation. Analysis of the presence of PTC1 in a small series of adenomas from the Ukraine has also been carried out and shown to be absent. Interestingly one of the carcinomas found to be positive for ret expression by RT-PCR was not found to possess one of the 3 known translocations. Further studies on the remaining papillary carcinomas for PTC2 and PTC3 expression and to identify other translocations involving the ret oncogene are underway.

3.4: Mutations in the TSH receptor

DNA extraction has been performed on material from 41 cases and regions of interest in exon 10 of the TSH receptor have been amplified using PCR. The rgions studied include the third intracellular loop abd the third transmembrane segment of the TSH receptor gene. Single stranded conformational polymorphism (SSCP) in all cases followed by sequencing (in 15 papillary carcinomas) has been used to identify TSH receptor mutations in exon 10. So far no mutations have been observed in 41 cases of papillary thyroid carcinoma or in 18 follicular adenomas and 3 follicular carcinomas from children and adolescents from the radiation exposed population. Cases known to be positive for mutations in exon 10 have been used as control for the technique used. The absence of mutations in the TSH receptor correlates with the morphological observations that the childhood thyroid tumours identified in the Ukraine after Chernobyl are of the papillary subtype.

3.5: p53 mutation

Exons 5 and 7 and 8 have been successfully amplified from 23 papillary carcinomas using nested PCR. SSCP analysis under four different running conditions has so far been applied to all 23 samples, but no aberrant cases have been found. p53 mutation involvement in thyroid carcinoma is usually a late phenomenon, at the interface between differentiated and undifferentiated carcinoma. All of the tumours so far received have been well differentiated and do not show widespread positivity for p53 on immunocytochemistry. However, exposure to radiation has been reported to increase the frequency of mutation in the p53 gene in other tissues. From the results presented here that p53 mutation does not appear to play a major role in papillary thyroid carcinogenesis post Chernobyl.

4: Conclusions

The results obtained from this study so far suggest that contrary to a previous report (10) there is no increase in the frequency of ret translocation in thyroid carcinomas post Chernobyl. However, we cannot yet exclude the possibility that there may be a difference in the frequency of the three known translocations of ret

when compared to the data from an adult population. Whether any such difference is due to radiation or is due to a differing frequency in children requires study of the proportion of PTC 1, 2 and 3 positive tumours in a non radiation exposed population of children. Previous reports had also suggested that there was an increased frequency of mutation in Ki ras in human follicular thyroid carcinomas following radiation (13). However, we have been unable to demonstrate any Ki ras mutations in papillary carcinomas whether from children exposed to radiation or not. Other work has failed to find any ras mutation in adult papillary carcinomas (8). The papillary carcinomas we have studied showed evidence of aggressivity, but we were also unable to demonstrate mutations in N ras. N ras mutations have recently been shown to be more common in aggressive thyroid tumours (14). The lack of mutations in the TSH receptor is related to the fact that the tumours examined so far have all been papillary carcinomas, and the lack of p53 mutations is not surprising as all tumours analysed so far have been well differentiated. p53 immunopositivity has been noted only in occasional nuclei in sections from the same tumours suggesting that alterations in p53 gene expression have not played a major role in the development of these tumours.

The molecular biological results so far obtained provide additional evidence to support the view that ret translocations are one of the key events in papillary carcinogenesis, while ras mutations are one of the key events in follicular carcinogenesis. The post Chernobyl tumours do not appear to differ significantly in their pattern of mutations from a non radiation exposed comparison group of children, although the frequency of different types of ret translocation have not yet been adequately studied. Overall there has been a very great increase in the frequency of childhood thyroid cancer in the children exposed to fallout from Chernobyl, this increase has been very specifically in the papillary subgroup of thyroid tumours, and the oncogene changes have been those found in papillary tumours from unirradiated patients, not those found in follicular carcinoma whether associated with radiation exposure or not. They suggest therefore that exposure to radiation has led to a great increased in tumours showing a ret translocation, and also a great increase in the ret negative papillary carcinomas, where the molecular biological changes have yet to be characterised.

5: References

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