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Screening of selected genomic areas potentially involved in thyroid neoplasms

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Abstract

Loss of heterozygosity (LOH) studies have been used to identify sites harbouring tumour suppressor genes (TSGs) involved in tumour initiation or progression. To further elucidate the genetic mechanisms for follicular and papillary thyroid tumours development, we studied the frequency of LOH in 36 thyroid tumours (21 follicular thyroid adenomas (FAs) and 15 papillary thyroid carcinomas (PTCs)) on 10 specific genomic areas: 3p22, 3p25, 7q21, 7q31, 10q23, 10q25-26, 11q13, 11q23, 13q13 and 17p13.3-13.2 using 20 polymorphic markers. We have selected these areas for two reasons: (a) Even though LOH in thyroid neoplasms has been described in some of these areas, results are controversial, and (b) we have also studied areas described as involved in other epithelial or endocrine tumour types, but not studied up to now in thyroid neoplasms. Two areas showed a high percentage of LOH: 7q31 and 11q23. A 62% LOH was found at 7q31 in the FAs, suggesting, as other authors have proposed, that at least one TSG must be present in the vicinity of the *c-met* locus. The second area in frequency was at the 11q23 locus, with a 45% LOH in the FAs. This area was studied because it has been described as being involved in the development of epithelial and endocrine cancers. This locus had not been studied before in thyroid neoplasms. This result is interesting because the *LOH11CR2A* gene is localised at this locus. We suggest that this gene and/or an other TSG nearby may be involved in the progression to FA. In our study, a low percentage of LOH was found in the PTC samples, indicating that TSGs present in the areas we have studied are not significantly involved in their progression. Our data also suggest that TSGs located in areas where no LOH was detected (*PTEN, MEN1, Cyclin D1, BRCA2 and RFC3*) are not involved or do not have an important role in tumour progression.

Keywords: Follicular thyroid adenoma; Papillary thyroid carcinoma; Loss of Heterozygosity; Tumour supressor gene

1. Introduction

Thyroid neoplasms are an attractive model to study the molecular genetics of tumorigenesis. They comprise a broad spectrum of phenotypes probably because evolution of these neoplasms, despite having a clonal origin, is a multistep phenomenon [1,2]. Multiple genetic lesions have been reported in different subsets of thyroid cancer. Briefly, mutations in the oncogene *ras* in follicular and papillary thyroid carcinomas [3,4], mutations in the thyrotropin receptor or gsp genes in toxic thyroid nodules [2,5,6], *ret/PTC* rearrangements in papillary thyroid carcinoma (PTC) [7–9] and *TP53* gene mutations in anaplastic carcinomas [10,11] have been reported, but with different mutational frequencies and different prevalences among geographical regions.

However, several genomic areas have been described as possibly being involved in the neoplastic transformation of thyrocytes due to the detection of loss of heterozygosity (LOH), suggesting that tumour suppressor genes (TSGs) can also participate in such progression. Two approaches are generally used to find TSGs: a 'general at random genomic scanning' or the study of a chromosome segment in order to localise a smallest common deleted region (SCDR). All of these studies are performed using the polymerase chain reaction (PCR) microsatellite analysis.

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In general, percentages of LOH obtained by both approaches in thyroid neoplasms are not high and the results are variable. However, some candidate areas have been proposed. LOH has been found at 3p22-12 in follicular thyroid adenomas (FA) (20%) and carcinomas (FTC) (71%), but not in PTC [12]. LOH at the same locus is also present in prostatic adenocarcinomas [13]. Near this area, in 3p25-26, LOH has been found in FTC (86%) [12] and again in prostatic adenocarcinomas [13]. Moreover, Kroll and colleagues [14] have found a t(2;3)(q13;p25) translocation in 5 of 8 follicular carcinomas, but in 0% of follicular adenomas, papillary carcinomas or multinodular hyperplasias. At 7q31, LOH has been described in FTC (78%), but not in PTC [15]. Recently, Trovato and colleagues [16] obtained at 7q21 and 7q31 100% of LOH in FTC and 10–29% in FA, but 0% in PTC.

10q is an interesting area because deletions along the long arm of chromosome 10 have been described in thyroid tumours as well as in carcinomas of the endometrium [17], prostate and brain. Using a general at random genomic scanning, Zedenius [18] found low LOH frequencies in follicular thyroid tumours, except in 10q. Later, using the SCDR approach, he found LOH in the 10q23-26 region in follicular tumours [19].

The *PTEN* gene is a TSG present in this area. It has been suggested that mutations in this gene could be involved in thyroid neoplasias, but when the *PTEN* gene was sequenced, a low percentage of mutations was found [20]. No LOH was present in 10q in PTC [21]. With this information in mind, some similar hypothetical models for the initiation and progression of thyroid neoplasms have been proposed [22–24].

TSGs in other epithelial cancers can also participate in the development of thyroid neoplasms. A way to prove or deny the role of these proposed TSGs in thyroid neoplasms is to study the presence of LOH with markers close to these candidate genes. A candidate area is 11q13, where the *MEN1* gene is present. In this area, the presence of a TSG has been proposed in epithelial endocrine neoplasms such as breast cancer [25]. Deletions at chromosome 11q23 are also frequent in breast, lung and ovarian carcinomas. The *LOH11-CR2A* gene has been found at this locus [26]. 13q23 is another interesting locus because of the presence of the *BRCA2 gene*. 17p13.3-13.2 is an additional area where a 'cluster' of TSGs like *OVCA1* and *2*, *ABR*, *TP53*, etc., is found. Some reports have described LOH at this point in medulloblastomas [27] and in breast cancer [28].

Because no specific genes have been found to be linked to FA or PTC in a significant number of cases (except *ret*/*PTC* rearrangements in some geographical areas in PTC), our aim was to study the prevalence of LOH in thyroid neoplasms at selected genomic points where TSGs are localised. It is suggested that these TSGs may be involved in the development of thyroid cancer or other epithelial cancers.

2. Materials and methods

Normal and tumour tissues were obtained from 36 patients with thyroid lesions: 21 FAs and 15 PTCs. Upon surgical removal, the normal and tumour tissues were immediately snap frozen in liquid nitrogen and stored at −70 °C pending analysis. Tumour tissue specimens were confirmed histologically to contain neoplastic cells, with a proportion of tumour cells above 50% in all specimens.

For DNA extraction, tissue samples were homogenenised. DNA was extracted and purified using standard procedures based on ionic detergent lysis and proteinase K digestion, followed by repeated phenol-chloroform extraction, precipitation in absolute ethanol and washing twice consecutively with 70% (v/v) ethanol. Extracted DNA samples were redissolved in 10 mM Tris–HCl, 0.1 mM ethylene diamine tetraacetic acid (EDTA) (pH 7.5) and stored at 4 °C until use.

Each of the tumour and matched normal DNA samples were subjected to PCR-based analysis using the markers described in Table 1. Primers were obtained from http://www.ncbi. nlm. nih. gov/genome/guide and selected for regions where higher rates of LOH had been previously described for either thyroid neoplasms or other epithelial cancers. Standard PCR reactions were carried out in a final volume of 25 μl, containing 50 ng high-molecular-weight DNA. Aliquots of the PCR product were denatured with formamide, heated to 80 °C and electrophoresed on standard denaturing 8% (w/v) polyacrylamide DNA sequencing gels. Gels were then fixed, dried and subjected to autoradiography for 24 h.

Definition of allelic loss was limited to the informative cases. In the LOH technique, a given case is considered informative for a given microsatellite marker when the corresponding non-tumoral DNA is heterozygous. Homozygous non-tumoral DNA are, therefore non-informative. The signal intensity of the polymorphic alleles was evaluated visually by two reviewers in an independent and blind fashion. A sample had LOH when a loss of intensity of one allele by more than 50% was seen in a tumour sample with respect to the matched allele from normal tissue.

3. Results

In several tumour types, detection of genetic loss by studying LOH has led to the localisation of TSGs. TSGs play a critical role in the progression of most human epithelial cancers. To define the chromosomal areas in which these critical TSG loci may reside, we

Table 1
Locations of the chromosome markers used in this study and summary of LOH versus informative cases in each locus studied

Locus	Band location	LOH/informative marker (%)	
		Follicular adenoma (FA)	Papillary carcinoma (PTC)
D3S1298	3p22	1/14 (7)	1/12 (8)
D3S1079	3p25	2/14 (14)	0/11 (0)
D3S1110	3p25	4/11 (36)	1/8 (13)
D7S660	7q21.1	0/12 (0)	0/9 (0)
D7S492	7q21.1	0/17 (0)	0/11 (0)
D7S486	7q31.1	8/13 (62)	1/8 (13)
D7S655	7q31.3	3/10 (30)	1/10 (10)
D10S1765	10q23	2/14 (14)	1/10 (10)
D10S541	10q23	1/12 (8)	1/10 (10)
D10S216	10q25-26	0/14 (0)	2/13 (15)
D10S587	10q25-26	0/13 (0)	0/10 (0)
PYGM	11q13	0/13 (0)	0/11 (0)
D11S1889	11q13	0/13 (0)	0/8 (0)
D11S1328	11q23	5/11 (45)	0/7 (0)
D11S1345	11q23	3/12 (25)	0/10 (0)
D13S260	13q13	0/14 (0)	1/10 (10)
D13S171	13q13	0/12 (0)	0/9 (0)
D13S267	13q13	0/13 (0)	2/7 (29)
D17S675	17p13.3–13.2	0/12 (0)	0/10 (0)
D17S731	17p13.3–13.2	1/13 (8)	0/10 (0)

LOH, loss of heterozygosity.

performed an allelotype for FA and PTC by rapid PCR-based microsatellite analysis. We studied the prevalences of LOH in the thyroid neoplasms of our population by amplifying 20 microsatellite markers (10 genomic areas on six chromosomal arms). A comprehensive list of all the markers used and the results are shown in Table 1 and a representative example of LOH is shown in Fig. 1.

The percentage of loci that were informative varied from 47–78% of the cases analysed, with an average of 62.2%. Usually, LOH in 15% or below does not significantly exceed that expected as 'background noise'. Overall, we found few areas of chromosomal loss. 18 of the 36 samples analysed (50%) displayed LOH with one or more of the markers tested. 13 FAs (62%) and 5 PTCs (33%) displayed LOH at one of more loci. The highest incidence (62%) of LOH was at locus D7S486 (7q31) followed by 45% at D11S1328 (11q23) and 36% in D3S1110 (3p25), all in the FA. A moderate percentage of LOH was found in the PTCs at D13S267 (29%), but not at the other loci indicating LOH is a rare event in these areas in PTCs. 10/13 FAs and 2/5 PTCs with LOH showed two or more chromosome loci affected by deletions.

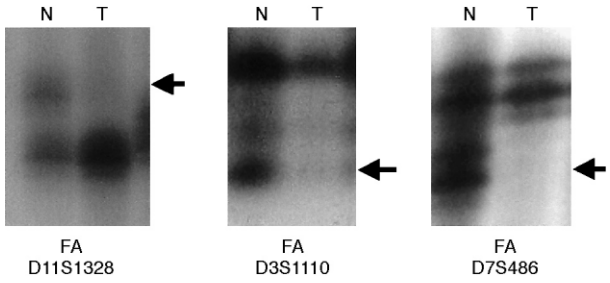

Fig. 1. Microsatellite analysis on the D11S1328, D3S1110 and D7S486 loci are shown in some follicular adenomas (FA). Paired samples of normal thyroid (N) and tumour DNA (T). The arrow indicates loss of heterozygosity (LOH).

4. Discussion

Areas like 3p22–25, 7q21–31, 10q23, 10q25–26, 11q13 and 17p13.3–13.2 have been suggested as involved in the development of thyroid cancer, but the findings are still controversial. As the results in the literature are inconclusive, and as some of these areas have also shown LOH in epithelial tumours, we analysed all of these points. Moreover, we studied other loci like 11q23 and 13q13 as they present LOH in epithelial and/or endocrine tumours, but have not been previously tested in thyroid neoplasms.

Our results corroborate that LOH at 7q31 is a frequent event in FAs and not in PTCs, as has been previously reported [15,16]. However, the low percentage of LOH (13%) present in the PTCs (probably background), does not allow a 100% discrimination between FAs and PTCs. LOH percentage is higher at D7S486 (near the *c-met* locus) than D7S655. As suggested by other authors [16], we also believe there must be at least one TSG in the vicinity of the *c-met* locus.

We have also found LOH at 11q23 using the marker D11S1328 in the FAs (45%), but not in the PTCs (0%).

This area was studied because of the presence of the *LOH11CR2A* gene, that has been associated with the development of epithelial and endocrine cancers. We suggest this gene (and/or perhaps other nearby TSGs) may participate in the progression to FA, but not in the PTCs. 3p25 also showed a significant percentage of LOH (36% at D3S1110) in the FAs but only a 13% in the PTCs. In this area, the *VHL* gene is found, but no mutations have been observed in thyroid neoplasms [12]. Recently, Kroll TG and colleagues have reported a t(2;3)(q13;p25) translocation [14], and as result, the fusion of the *PAX8* and *PPARγ* genes, demonstrating an oncogenic role. This translocation is found in follicular carcinomas, but not in FAs or papillary carcinomas.

Another area where a moderate LOH was found was at 13q13 (29% at D13S267) in the PTCs, but not in the FAs (0%). This is the only area where LOH was present in the PTCs. Genes described near this locus are *BRCA2* and *Replication factor C (RFC3)*. In our opinion, if a TSG is implied in the progression of PTC in this area its contribution is not very important.

In some areas we have not detected LOH or found only low percentages indicative of background levels. Therefore, our results exclude a major role of some TSGs located in these areas in the development of FAs or PTCs. We suggest the exclusion of TSGs located at 3p22 in both FAs and PTCs, at 7q21 in both FAs and PTCs, at 7q31 in PTCs, at 10q23 (where the *PTEN* gene is present) in both FAs and PTCs, at 10q25-26 in both, at 11q13 (where the *MEN1* and *Cyclin D1* genes are located) in both FAs and PTCs, at 11q23 in PTC, at 13q13 (where the *BRCA2* and *RFC3* genes are located) in both FAs and PTCs, and at 17p13.3.13.2 also in both. However, we cannot exclude a role of these genes in the development of FTCs from FAs because we have not studied these kind of samples.

The contrast in the prevalence of the allelic deletions between the FAs and PTCs we have found in this study has been previously reported. It suggests that there is an intrinsic difference in the factors controlling chromosomal integrity in the two main phenotypes of thyroid neoplasms. Many of the mechanisms of thyroid tumour initiation and progression as yet remain unknown. Some of the defects described above are not highly prevalent; however, as more genetic abnormalities are found which differ among the various types of thyroid cancer, a more accurate approach for the differential molecular diagnosis of tumour material may become available, especially when the cytology is ambiguous.

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