

SHORT COMMUNICATION:

Chromosome alterations in two cases of thyroid papillary adenocarcinoma

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ABSTRACT

Papillary carcinomas are the most frequent thyroid tumors. We report a cytogenetic study of two cases of epithelial thyroid carcinoma of the papillary type using G/TG banding techniques. The modal number was 46 chromosomes in both cases. No structural alteration was detected. Numerical alterations were present in a random manner, probably due to the instability of the neoplastic tissue.

INTRODUCTION

Cancer of the thyroid in the general population accounts for approximately 1% of the total cancer incidence. It is one of the least frequent causes of death from cancer. The understanding of the clinical course of thyroid cancer and its treatment have been hampered by the failure of pathologists and thyroidologists to agree on a reasonably uniform pathologic classification, and the lack of a significant number of patients with the various types of the disease, under observation for a sufficient length of time. Because of these problems in classification, and inadequate knowledge concerning the long-term natural history of the disease, there are differences of opinion concerning the surgical and medical management of thyroid cancer (Hill, 1982).

Thyroid carcinomas may be of the follicular, papillary, medullary or anaplastic type. Papillary carcinomas are typically multifocal and non-encapsulated

and metastasize almost exclusively through the lymphatic system. They represent the most frequent thyroid tumors and occur in patients of all ages. When they occur in young patients they are of small size and of low histopathological grade, with a good prognosis (Williams, 1980). Thyroid papillary carcinomas, contrary to the follicular type, present a high frequency of familial occurrence (Ozak *et al.*, 1988).

The most frequent and consistent cytogenetic alterations reported in the literature for this type of tumor are rearrangements on the long arm of chromosome 10 (10q), though few cases are described thus far. These rearrangements may affect the proto-oncogene *ret* located on the 10q11.2 band, which is activated in some thyroid papillary carcinomas (Herrmann *et al.*, 1991a,c).

MATERIAL AND METHODS

The cytogenetics and histopathology of two cases of epithelial thyroid adenocarcinoma of the papillary type were analyzed. Tumor material was extracted from two female patients, respectively aged 30

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and 35 years, who had been admitted to the University Hospital, Faculty of Medicine of Ribeirão Preto.

Cytogenetics

Surgical tumor fragments were removed under sterile conditions, cut into small pieces and placed in sterile flasks containing HAM-F10 medium (Sigma), supplemented with 10% fetal calf serum and antibiotics. The flasks were incubated at 37°C for a few days. For cytogenetic analysis, cells in the exponential growth phase were treated with 0.0016% colchicine for at least three hours. The cells were then removed with 0.05% trypsin, treated with hypotonic 0.075 M KCl for approximately 20 minutes at 37°C and fixed in methanol:acetic acid (3:1). Metaphases were submitted to trypsin-Giemsa banding (GTG bands). The karyotypes and cytogenetic clones are described according to the ISCN (1985, 1991).

Histopathology

The material was fixed in 10% formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin-eosin.

RESULTS

Cytogenetics

We analyzed approximately 100 cells (standard staining) of two thyroid tumors with a histopathological diagnosis of papillary adenocarcinoma, to determine chromosome distribution and modal number (Tables I and II). In both cases the modal number was 46: 40% in case 1 and 34% in case 2.

Table III lists the eight karyotypes analyzed by G banding for case 1, which showed three normal cells with 46,XX and five cells with nonclonal chromosome

Table I - Chromosome distribution and modal number for case 1.

														Near-haploidy (≤ 34 chromosomes)										
No. of chromosomes	25	26	28	30	31	32	33	34						Total										
No. of cells	1	1	1	1	3	1	1	1						10										
														Near-diploidy (35-57 chromosomes)										
No. of chromosomes	36	38	39	40	41	42	43	44	45	46	47	48	Total											
No. of cells	1	3	4	1	1	8	5	4	18	41	1	4	91											
														Near-tetraploidy (81-103 chromosomes)										
No. of chromosomes	89	92											Total											
No. of cells	1	1											2											

Table II - Chromosomes distribution and modal number for case 2.

																Near-haploidy (≤ 34 chromosomes)										
No. of chromosomes	22	23	25	29	33											Total										
No. of cells	1	2	1	2	1											7										
																Near-diploidy (35-57 chromosomes)										
No. of chromosomes	35	36	38	39	40	41	42	43	44	45	46	47	48	52	Total											
No. of cells	1	1	2	2	8	1	8	4	6	10	35	2	1	1	82											
																Near-tetraploidy (81-103 chromosomes)										
No. of chromosomes	82	86	88	89	90	91	92	93	96							Total										
No. of cells	1	2	1	1	1	1	3	1	1							12										

losses and/or gains. No structural alteration was observed. Figure 1 shows karyotype 45,XX, -9, detected in case 1 by G banding.

Table IV describes the karyotypes analyzed by G banding for case 2, showing four normal 46,XX cells and four cells with nonclonal numerical chromosome alterations. Again, no structural chromosome alteration was detected in this case.

Table III - Karyotypes (G-banding) detected in case 1.

Karyotypes	No. of cells
39,XX, -3, -4, -5, -8, -13, -18, -18	1
42,XO, -X, -10, -17, -18	1
43,XX, -16, -20, -21, -22, +mar1	1
45,XX, -9	1
46,XX, -12, +mar1	1
46,XX	3

Table IV - Karyotypes (G-banding) detected in case 2.

Karyotype	No. of cells
43,XX, -1, -4, -7, -18, +mar1	1
45,XX, -3	1
46,XX	4
48,XO, -X, -21, +mar1, +mar2, +mar3, +mar4	1
91,XXXO, -X, -1, -13, -22, +mar1, +mar2, +mar3	1

Histopathology

Case 1 - Neoplastic glandular epithelial tissue had a predominantly papillary pattern with areas of a follicular pattern, consisting of cells that exhibit slight cytonuclear polymorphism and a low mitotic index. These cells are cylindrical or cubic in shape, have acidophilic cytoplasm and have an ovoid or rounded nucleus of weak chromatin arranged at the periphery, with a single and barely visible nucleolus. The papillae, formed by a delicate vascular connective axis, present a discrete lymphoid infiltrate and are interspersed with frequent multinucleate giant cells. The described neoplastic proliferation infiltrates the pseudocapsule that surrounds it and embolizes the lymphatic vessels. Diagnosis - Papillary thyroid adenocarcinoma.

Case 2 - Neoplastic glandular epithelial tissue of a predominantly papillary pattern with areas of a follicular pattern, at times consisting of cylindrical cells lining the delicate vascular connective axis, and at others of cubic cells lining acini that contain colloidal material. These cells have a rounded or ovoid nucleus with weak chromatin shifted to the periphery conferring the appearance of ground glass. The cytoplasm is acidophilic and there is a single, barely visible nucleolus. The described neoplastic tissue exhibits multiple focal points of calcification and invades the pseudocapsule and underlying skeletal muscle tissue, in addition to frequently embolizing the lymphatic vessels. There are frequent karyokinesis figures and

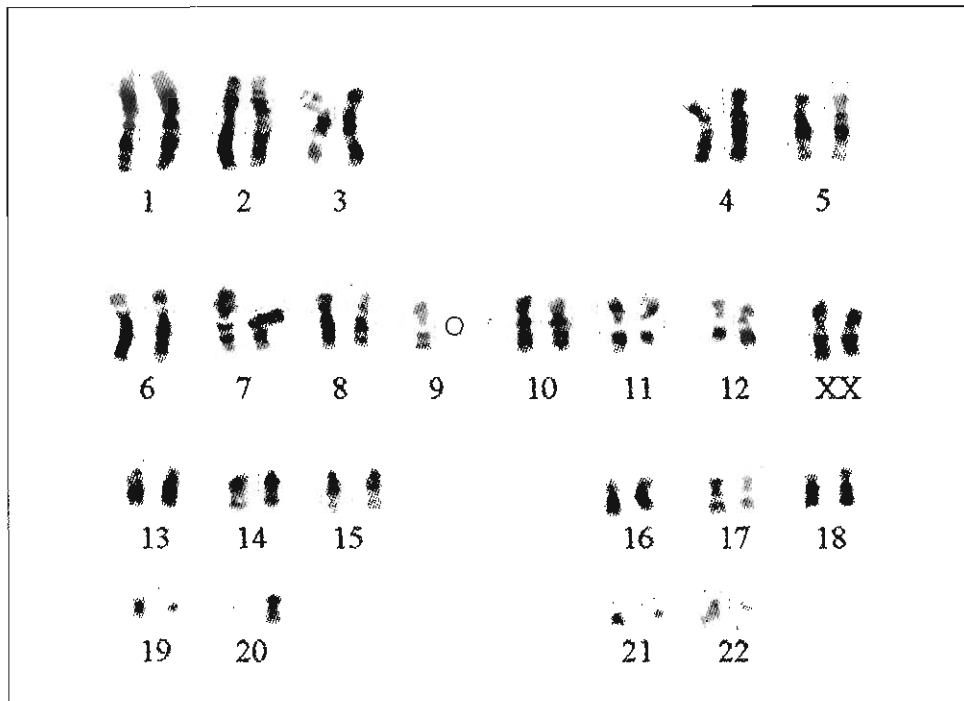


Figure 1 - Karyotype 45,XX, -9, detected in case 1 by G banding.

discrete cytonuclear polymorphism. Diagnosis - Papillary thyroid adenocarcinoma infiltrating the underlying muscle tissue.

DISCUSSION

On average, the chromosome number obtained in the present study was diploid or hypodiploid, as also described by others (Teyssier *et al.*, 1990; Olah *et al.*, 1990). However, Hermann *et al.* (1991d) described two cases of papillary carcinoma with tetraploidy, whereas in our cases tetraploidy appeared only sporadically.

Chromosome alterations in papillary thyroid carcinomas have been demonstrated in some reports. Hermann *et al.* (1991d) described clonal aberrations (trisomy 7 and a rearrangement of chromosome 10) in one of four successfully harvested papillary cancers. Jenkins *et al.* (1990), in an analysis of seven cases, detected four with a single clonal alteration (10q) and three with no apparent abnormality. Bondeson *et al.* (1989) analyzed five cases, four of them being 46,XX and one having a clonal-ins(2;15) alteration. In the two cases of papillary carcinoma reported here, no clonal alterations were detected. This demonstrates that, contrary to other types of thyroid tumors (Bondeson *et al.*, 1989; Antonini *et al.*, 1991; Hermann *et al.*, 1991b), papillary carcinomas generally present close to normal karyotypes.

The chromosome deviations (gain and/or loss) detected in our two cases of papillary carcinoma may be related to the chromosome instability of this neoplastic tissue, thus also being related to the process of tumor development, and may not characterize, of themselves, the establishment (origin) of the neoplastic process. These alterations, therefore, may be of proliferative significance in this neoplasia.

Rearrangements on the long arm of chromosome 10 have been described as the major markers for this type of carcinoma by several investigators (Jenkins *et al.*, 1990; Herrmann *et al.*, 1991a,c,d). In addition to 10q rearrangements, alterations in chromosome 7 have also been implicated as an aggressiveness factor (Antonini *et al.*, 1989; Teyssier *et al.*, 1990; Herrmann *et al.*, 1991a,c). Olah *et al.* (1990) suggested that the chromosome alteration in 11q detected in their sample (three cases) may be a deletion specific for papillary carcinoma of the thyroid. This demonstrates that, as the number of cases analyzed increases, other chromosome alterations may arise that may be involved in the process of development of these neoplasias.

Since the development of thyroid cancer has been considered to be a sequential process of

hyperplasia → adenoma → well differentiated carcinoma (Schauer *et al.*, 1984), it would be important to use this molecular and biochemical approach in all types of thyroid tumors, since chromosome 10, which is involved in papillary carcinoma, may also be altered in other thyroid neoplasias (Antonini *et al.*, 1991).

It is probable that the neoplastic process of papillary carcinoma is related to small alterations at the molecular level that cannot be detected by conventional cytogenetic analysis, with the consequent need for the application of more modern molecular techniques such as *in situ* hybridization for the detection of these changes. Another approach could be the biochemical detection of the expression of oncogene PTC (papillary thyroid carcinoma) located in the 10q11 region (Fusco *et al.*, 1987).

Since the alterations on the long arm of chromosome 10 have been defined as being exclusively detected in papillary carcinomas when compared to the other types of thyroid tumors (Herrmann *et al.*, 1991b), it would be useful to perform a molecular evaluation of small alterations in this chromosome region in cases of thyroid tumors in which the established karyotypes do not present this alteration. Papillary carcinomas without this alteration could represent a subtype of tumor; perhaps less malignant. This may explain apparently normal karyotypes in malignant neoplastic thyroid tissue.

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RESUMO

Os carcinomas papilíferos são os mais frequentes tumores de tiróide. Dois casos foram analisados cariotipicamente. O número modal em ambos os casos foi de 46 cromossomos. Nenhuma alteração estrutural foi encontrada. Alterações numéricas estiveram presentes de maneira aleatória, provavelmente em decorrência da instabilidade do tecido neoplásico.

REFERENCES

- Antonini, P., Venuat, A.M., Linares, G., Caillou, B., Berger, R. and Parmentier, C. (1989). A translocation (7,10)(q35;q21) in a differentiated papillary carcinoma of the thyroid. *Cancer Genet. Cytogenet.* 41: 139-144.

- Antonini, P., Venuat, A.M., Linares, G., Caillou, B., Schlumberger, M., Travagli, J.P., Berger, R. and Parmentier, C.** (1991). Cytogenetic abnormalities in thyroid adenomas. *Cancer Genet. Cytogenet.* 52: 157-164.
- Bondeson, L., Bengtsson, A., Bondeson, A.-G., Dahlenfors, R., Grimelius, L., Wedell, B. and Mark, J.** (1989). Chromosome studies in thyroid neoplasia. *Cancer* 64: 680-685.
- Fusco, A., Grieco, M., Santoro, M., Berlingieri, M.T., Pilloti, S., Pierotti, P., Della Porta, G. and Vecchio, G.** (1987). A new oncogene in human thyroid papillary carcinomas and their lymphodal metastases. *Nature* 320: 170-172.
- Herrmann, M.A., Hay, I.D., Bartelt, D.H.Jr., Ritland, S.R., Dahl, R.J., Grant, C.S. and Jenkins, R.S.** (1991a). Cytogenetic and molecular genetic studies of follicular and papillary thyroid cancers. *J. Clin. Invest.* 88: 1596-1604.
- Herrmann, M.A., Hay, I.D., Bartelt, D.H.Jr., Spurbeck, J.L., Dahl, R.J., Grant, C.S. and Jenkins, R.B.** (1991b). Cytogenetics of six follicular thyroid adenomas including a case report of an oxyphil variant with t(8,14)(q13;q24.1). *Cancer Genet. Cytogenet.* 56: 231-235.
- Herrmann, M.E., Mohamed, A., Talpos, G. and Wolman, S.R.** (1991c). Cytogenetic study of papillary thyroid carcinoma with a rearranged chromosome 10. *Cancer Genet. Cytogenet.* 57: 209-217.
- Herrmann, M.E., Talpos, G.B., Mohamed, A.N., Saxe, A., Ratner, S., Lalley, P.A. and Wolman, S.R.** (1991d). Genetic markers in thyroid tumors. *Surgery* 110: 941-948.
- Hill, C.S.Jr.** (1982). Thyroid gland. In: *Cancer Medicine*. (Holland, J.F. and Frei III, E., eds.). Lea & Febiger, Philadelphia, pp. 1685-1692.
- ISCN** (1985). *An International System for Human Cytogenetic Nomenclature*. (Harnden, D.G. and Klinger, H.P., eds.). Published in collaboration with Cytogenetic Cell Genet. Karger, Basel.
- ISCN** (1991). *Guidelines for Cancer Cytogenetics, Supplement to An International System for Human Cytogenetic Nomenclature*. (Mitelman, F., ed.). S. Karger, Basel.
- Jenkins, R.B., Hay, I.D., Herath, I.F., Schultz, C.G., Spurbeck, J.L., Grant, C.S., Goellner, J.R. and Dewald, G.W.** (1990). Frequent occurrence of cytogenetic abnormalities in sporadic nonmedullary thyroid carcinoma. *Cancer* 66: 1213-1220.
- Olah, E., Balogh, E., Bojan, F., Juhasz, F., Stenszky, V. and Farid, N.R.** (1990). Cytogenetic analyse of three papillary carcinomas and a follicular adenoma of the thyroid. *Cancer Genet. Cytogenet.* 44: 119-129.
- Ozak, O., Ito, K., Kobayashi, K., Suzuki, A., Manabe, Y. and Hosoda, Y.** (1988). Familial occurrence of differentiated, nonmedullary thyroid carcinoma. *World J. Surg.* 12: 565-571.
- Schauer, A.** (1984). Pathogenese and pathologische anatomie. In: *Maligne Schilddrusentumoren* (Becker, H.D. and Heize, H.G., eds.). Springer Verlag, Berlin, pp. 2-61.
- Teyssier, J.R., Liautaud-Roger, F., Ferre, D., Patey, M. and Dufer, J.** (1990). Chromosomal changes in thyroid tumors. Relation with DNA content, karyotypic features, and clinical data. *Cancer Genet. Cytogenet.* 50: 249-263.
- Williams, E.D.** (1980). Pathologic and natural history. In: *Recent Results in Cancer Research: Thyroid Cancer* (Duncan, W., ed.). Springer Verlag, Berlin, pp. 47-55.

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