

Anaplastic Thyroid Cancer Evolved From Papillary Carcinoma

Demonstration of Anaplastic Transformation by Means of the Inter-Simple Sequence Repeat Polymerase Chain Reaction

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Background: In thyroid tumors, the coexistence of well- and poorly differentiated tumor types has led to the hypothesis that poorly differentiated thyroid tumors develop from well-differentiated thyroid tumors. By evaluating the genomic instability of histologically distinct but coexisting tumor foci, this study aimed to develop an improved understanding of thyroid tumorigenesis and tumor evolution.

Design: Laser capture microdissection (LCM) was carried out on archival formalin-fixed, paraffin-embedded sections from a tumor containing foci of classic papillary thyroid cancer and anaplastic thyroid cancer. DNA was extracted from each microdissected tumor focus. In addition, cryopreserved bulk normal and neoplastic thyroid tissue underwent DNA extraction. All DNA samples were subsequently evaluated for genomic instability by means of inter-simple sequence repeat polymerase chain reaction.

Results: The LCM DNA from each archival paraffin-

embedded tumor focus demonstrated unique patterns of banding as compared with the cryopreserved tumor and normal tissue DNA. Thus, intratumoral variability in genomic instability was observed. Comparison of inter-simple sequence repeat polymerase chain reaction patterns of LCM DNA from adjacent foci of papillary and anaplastic tumors showed conserved genome alterations.

Conclusions: At the genome level, thyroid tumors may be highly heterogeneous. The intratumoral histologic heterogeneity observed in thyroid neoplasms reflects genetically heterogeneous underlying tumor cell populations that are demonstrated by the observed differences in their rates and extents of genomic instability. The conserved genomic alterations in the microdissected papillary and anaplastic foci suggest intratumoral evolution, with transformation of a preexisting papillary tumor to anaplastic carcinoma.

Arch Otolaryngol Head Neck Surg. 2003;129:96-100

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THE RAPIDLY lethal course of disease that occurs in individuals with a diagnosis of anaplastic thyroid cancer is in stark contrast to the usually indolent clinical behavior of differentiated thyroid cancer (DTC). It seems paradoxical that anaplastic tumors are believed to evolve from DTC. This intratumoral evolution has been termed *anaplastic transformation*. Despite multiple clinical, pathological, and experimental studies, evidence demonstrating this evolutionary process has been limited. We used inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) as a technique for evaluating histologically distinct but co-occurring anaplastic and papillary tumor foci, to provide experimental evidence demonstrating this intratumoral evolutionary process.

REPORT OF A CASE

A 79-year-old white woman presented to our clinic with a 3-month history of progressive dysphagia and hoarseness. Her medical history was unremarkable, and she reported no previous exposure to radiation. A 7-cm mass involving the thyroid gland was noted on clinical examination. Indirect mirror examination showed paralysis of the right vocal cord. A computed tomographic scan demonstrated an extensive tumor of the thyroid gland, located primarily on the right side, that was encasing the esophagus and displacing the trachea (**Figure 1**). There was also evidence of neck nodal and lateral pharyngeal nodal metastases on the scan. Results of examination of a preoperative needle biopsy specimen were suggestive of papillary carcinoma. The patient un-

derwent a total thyroidectomy, a right extended neck dissection, and a partial esophagectomy because of local tumor extension. She received radioiodine and external beam radiation treatments postoperatively. She refused chemotherapy treatment. She died 7 months postoperatively of brain metastases.

METHODS

TUMOR SPECIMEN

Fresh frozen specimens from histologically normal and tumor tissue were procured immediately after the patient underwent thyroidectomy. This tissue was obtained under the supervision of our institutional review board, and informed consent for participation in this study was obtained from the patient preoperatively. The tumor and normal tissue were stored at -70°C . Sections for routine morphologic examination were fixed in neutral buffered formalin (10% vol in water; pH 7.4) and embedded in paraffin. Hematoxylin-eosin-stained sections were reviewed to select appropriate areas for laser capture microdissection (LCM). The matching paraffin block was sectioned at 4-mm thickness, mounted on uncharged glass slides, and stained with hematoxylin-eosin.

LASER CAPTURE MICRODISSECTION

A laser capture apparatus (PixCell II; Arcturus Engineering, Mountainview, Calif) was used for the LCM, which was carried out on foci of anaplastic thyroid carcinoma and papillary thyroid carcinoma. Two thousand pulses with a laser diameter of $30\ \mu\text{m}$ at 50 mW were carried out on each of these 2 tumor foci (**Figure 2**). The LCM specimens were then digested for 12 hours at 42°C in 1-mg/mL proteinase K, 0.1% sodium dodecyl sulfate, and 100mM Tris hydrochloride, pH 8.0. The digested material was diluted to 500 μL with water and extracted with an equal volume of phenol-chloroform-isoamyl alcohol. The DNA was ethanol precipitated and diluted to 25 ng/ μL for further analysis. DNA was extracted from the fresh frozen tumor and normal thyroid specimen as previously described.¹

THE ISSR-PCR

The ISSR-PCR is a technique for scanning the genome and amplifying sequences that are located between (but not including) microsatellites.^{1,2} The ISSR-PCR 3' end anchored primer used was $(\text{CG})_4\text{RY}$. This primer was synthesized by the Roswell Park Cancer Institute Biopolymer Facility, Buffalo, NY, and end-labeled with γ -phosphorus 32 (^{32}P)-adenosine triphosphate by T4 polynucleotide kinase. The amplification reaction was carried out in a 20- μL mixture containing 1 μM primer, 50 ng of genomic DNA, and 0.3 U of *Taq* polymerase (Life Technologies, Inc, Bethesda, Md) in polymerase chain reaction (PCR) buffer (10mM Tris hydrochloride, pH 9.0, 2% formamide, 50mM potassium chloride, 0.2mM deoxynucleotide triphosphates, 1.5mM magnesium chloride, 0.01% gelatin, 0.01% Triton X-100). After initial denaturation at 94°C for 3 minutes, 30 PCR cycles were performed at 94°C , then 45 seconds at 52°C , then 2 minutes at 72°C , followed by a final 7-minute extension at 72°C . Five microliters of each PCR was then analyzed on a nondenaturing 8% polyacrylamide gel, buffered with 1X TBE (0.89mM Tris borate, pH 8.3, 2mM EDTA). Electrophoresis was then performed at 70 W for 10 minutes followed by 50 W for 4300 volt-hours. The gels were then dried and placed on film (Kodak XAR; Eastman Kodak Co, Rochester, NY) for overnight exposure. The assay was repeated 4 times to ensure reproducibility. The area of the gel examined represents PCR products below 500 base pairs. The ISSR-PCR using template DNA



Figure 1. Computed tomographic scan of study patient's neck demonstrating encasement of esophagus by anaplastic thyroid tumor.

from fixed tissues fails to amplify products above that molecular weight.

RESULTS

The histopathological evaluation of the thyroid tumor showed extensive spindle features with focal squamoid differentiation consistent with an anaplastic (undifferentiated) carcinoma. Intermingled with the spindle cell areas were foci of papillary carcinoma (**Figure 3**). The tumor showed extensive extrathyroidal extension with invasion of the surrounding soft tissue and direct extension to the wall of the esophagus and the retropharyngeal space. Metastatic tumor was present in the retropharyngeal, paratracheal, and right cervical lymph nodes.

A representative electrophoretic analysis of ISSR-PCR products is illustrated in **Figure 4**. In this analysis, the gross frozen normal thyroid tissue specimen and the gross frozen thyroid tumor specimen displayed identical banding patterns when compared with each other by means of a $(\text{CG})_4\text{RY}$ primer. The LCM papillary tumor specimen showed an altered banding pattern that displayed 2 new bands relative to the gross specimens (bands B and C). The LCM anaplastic tumor specimen retained both of the additional bands that were present in the banding pattern of the LCM papillary specimen. In the LCM anaplastic specimen, band C exhibited decreased intensity when compared with the LCM papillary specimen. A third band, found in the gross tumor specimen, the gross normal tissue specimen, and the LCM papillary specimen, was lost in the LCM anaplastic specimen (band A).

COMMENT

It is not surprising that both patients and physicians fear the possibility that anaplastic transformation may occur in untreated DTC. Despite accounting for only a few thyroid tumors (1.6%),³ anaplastic carcinoma is responsible for more than half of the 1200 deaths annually attributed to thyroid cancer in the United States.⁴ With a mean survival ranging from 4 to 12 months and a 5-year survival rate ranging from 1.0% to 7.1%, anaplastic thyroid carcinoma ranks among the most lethal of all human neoplasms.⁵⁻⁸ These tumors are characterized by rapid growth and dissemina-

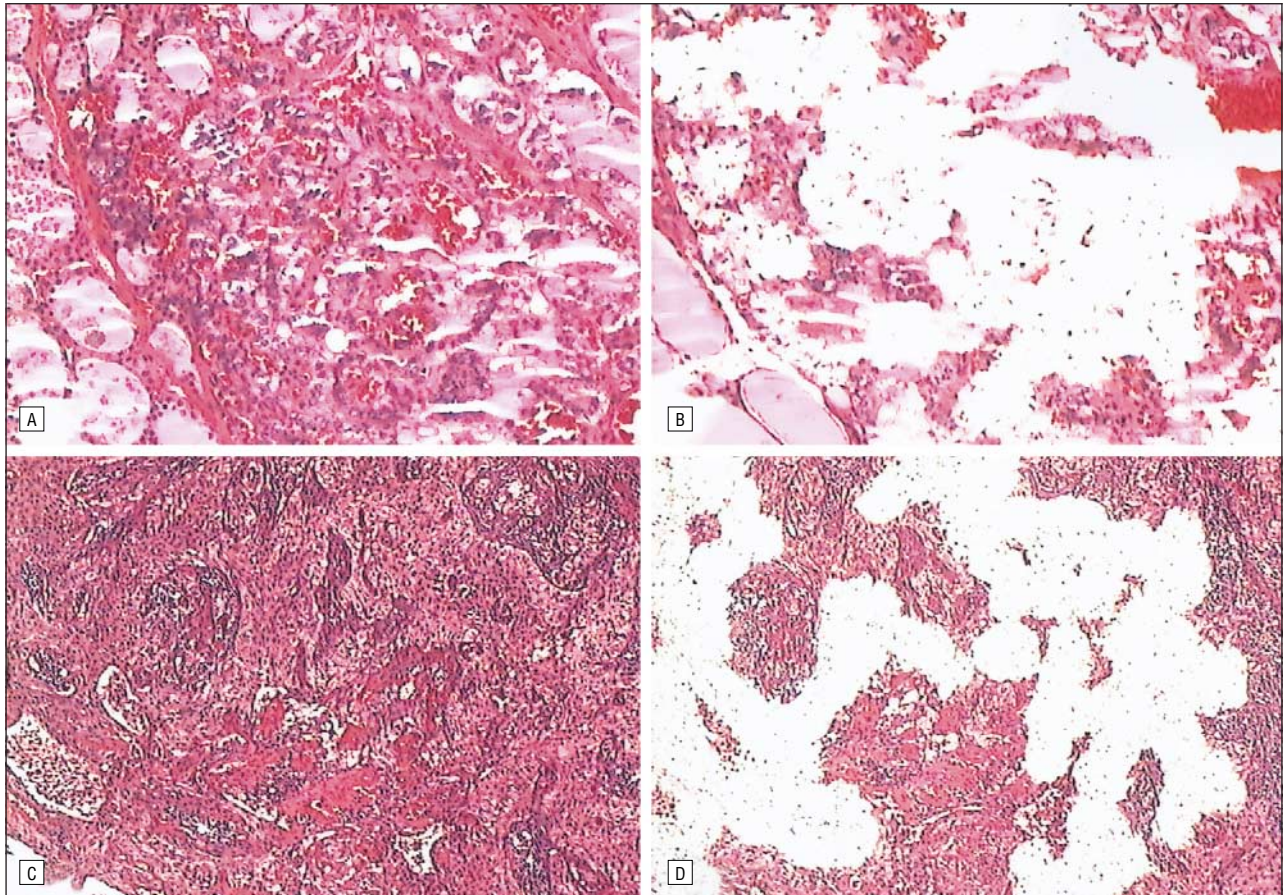


Figure 2. Photomicrographs demonstrating removal of the tumor foci for analysis before and after laser capture microdissection (LCM). A, Pre-LCM papillary carcinoma. B, Post-LCM view showing removal of the papillary carcinoma. C, Pre-LCM anaplastic carcinoma. D, Post-LCM view showing removal of the anaplastic carcinoma (hematoxylin-eosin, original magnification $\times 100$).

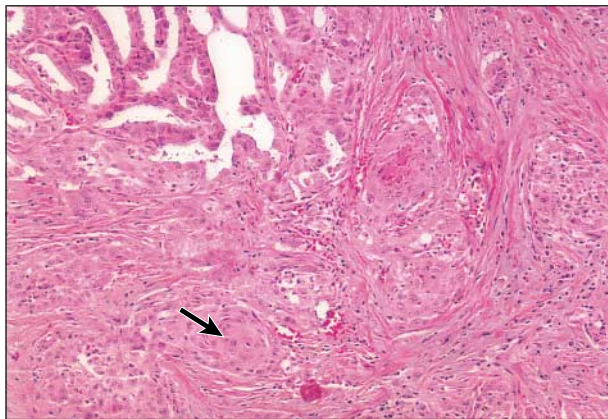


Figure 3. Anaplastic carcinoma with spindle cell features and focal squamous differentiation (arrow). Focal papillary carcinoma is present in the upper left corner (hematoxylin-eosin, original magnification $\times 200$).

tion. It is not unusual for tumor volume to double during a week of observation.⁹ Approximately one half of affected individuals will have distant metastases present at the time of their diagnosis,^{4,6} and, despite tracheostomy, the most common cause of death in this population is suffocation caused by tumor obstructing the thoracic inlet.¹⁰ Unfortunately, there has been little progress in the management and control of this thyroid malignancy. Current treatment strategies are multimodal and include surgery,

radiation therapy, and chemotherapy.^{4,6-8,10-20} Regardless of treatment, few patients with anaplastic cancer survive longer than 1 year. Reports of survival of these individuals beyond a year raises doubt of the validity of their diagnosis.¹¹ As well, in most survivors the anaplastic component of their tumor tends to represent an incidental finding on histopathological review of a specimen from surgery for other thyroid disease.¹¹ The relative rarity and rapidly fatal nature of anaplastic carcinoma have made it difficult to study both its underlying biology and its response to treatment. Currently, there are no good methods to predict which thyroid neoplasms will progress to anaplastic cancer. Through the development of an understanding of anaplastic tumor evolution and progression, an effective treatment for this aggressive disease may be developed.

The pathological association of anaplastic tumors with DTCs has led to the emergence of the concept of anaplastic transformation.²¹⁻²⁵ It has generally become accepted that the development of anaplastic thyroid cancer can be considered part of the natural course of untreated DTC. Approximately 2% of DTCs are believed to develop into anaplastic tumors.^{10,26}

Clinical evidence suggesting the occurrence of anaplastic transformation of DTC can be appreciated in case series where the presence of a DTC component in an anaplastic tumor was found to represent a good clinical prognosticator.^{20,24} An associated DTC component may sug-

gest that the tumor is at an earlier stage of disease progression. In a review of the 24-year experience in managing anaplastic thyroid cancer at our institution, Tan et al¹⁹ showed that the presence of a DTC component did not have any value as a clinical prognosticator. While some investigators have described similar findings,⁸ other groups have found a beneficial prognostic value of a coexisting DTC focus.^{20,24} Other authors have provided further clinical support for anaplastic transformation of DTC by suggesting that the fall in the reported incidence of anaplastic cancer is a consequence of increased aggressiveness in the surgical treatment of thyroid malignancies.⁹

The cause of anaplastic transformation, or tumorigenesis, currently remains unclear. A number of oncogenes and tumor suppressor genes have been investigated and are believed to play a role in anaplastic tumor development. These genes have included *TP53* (tumor suppressor gene),¹⁵ *bcl-2* (proto-oncogene),²⁷ cyclin D1 (oncogene),²⁸ β -catenin (gene is a key component of the Wnt signaling pathway),²⁹ *c-myc* (proto-oncogene),³⁰ *Nm23* (metastasis suppressor gene),³¹ and *ras* (oncogene).³² Mutation of the *TP53* gene, a tumor suppressor gene that encodes the P53 protein, has been best studied and is believed to play an important role in anaplastic transformation.¹⁵ Although some genes involved in anaplastic tumor evolution have been identified, the underlying molecular basis for this process remains largely unknown.

The ISSR-PCR is a novel experimental tool that was developed to study molecular evolution in eukaryotic organisms.³³ It has also been used to quantify intrachromosomal genomic instability in thyroid cancer.³⁴ Stoler et al³⁴ were able to differentiate benign from malignant thyroid neoplasms by means of ISSR-PCR measures of genomic instability. By coupling this technique with LCM, ISSR-PCR can be used to study intratumoral genetic evolution. The results of ISSR-PCR in this case have demonstrated that the LCM focus of papillary carcinoma displays a similar but unique banding pattern, or genetic fingerprint, when compared with the gross tumor specimens. The LCM focus of anaplastic carcinoma displayed a fingerprint different from that of the gross tumor that shared 2 alterations observed in the papillary carcinoma focus. An additional band is uniquely lost in the anaplastic tumor. The simplest model for explaining these results suggests that intratumoral genetic heterogeneity is present in thyroid cancer. The LCM dissection has allowed for the very directed evaluation of intratumoral cellular subpopulations. The unique genetic makeup of these cellular subpopulations is not obvious in the grossly dissected tumor, where the banding pattern represents an overall average of the genetic changes present in the entire specimen. The presence of an overall similar banding pattern, with discrete differences, when the papillary tumor was compared with the anaplastic tumor (Figure 4), is consistent with anaplastic thyroid cancer evolving from a preexistent papillary tumor.

CONCLUSIONS

Our experimental findings have demonstrated that, with the use of an ISSR-PCR technique, the similarities between the genetic fingerprint of co-occurring anaplastic

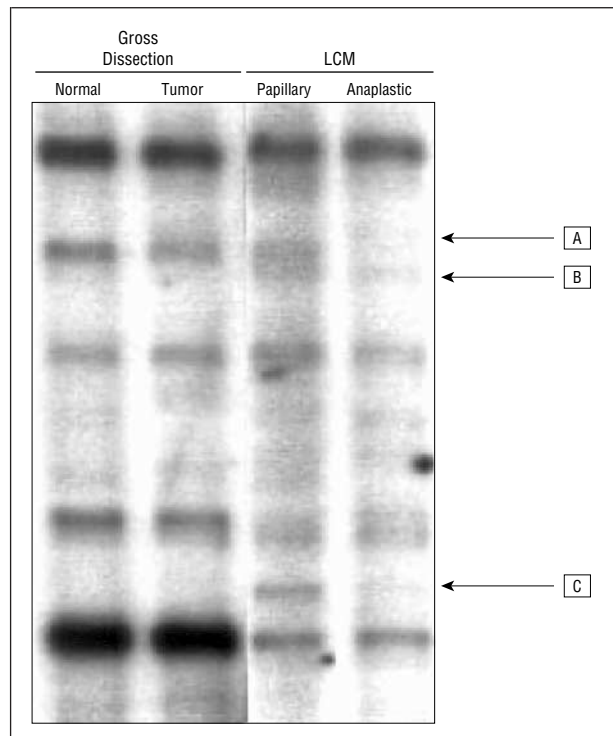


Figure 4. Inter-simple sequence repeat polymerase chain reaction products from normal and tumor tissue (gross) and foci of papillary and anaplastic carcinoma (laser capture microdissection [LCM]). The LCM anaplastic specimen shows retention of the 2 new bands exhibited by the LCM papillary specimen (bands B and C), although band C exhibits decreased intensity. Band A, which is present in all other specimens, is absent in the LCM anaplastic specimen.

carcinoma and papillary carcinoma indicate an intratumoral evolution of anaplastic carcinoma from the papillary tumor. These findings are consistent with clinical, pathological, and experimental evidence from the current literature that also strongly suggests that anaplastic transformation from DTC does take place. This is the first report, to our knowledge, to describe use of ISSR-PCR to evaluate LCM DNA from archival paraffin-embedded tissue. This technique appears promising and warrants further evaluation.

Currently, regardless of treatment, a rapid death is often imminent in individuals with a diagnosis of anaplastic thyroid cancer. Only through further study of the mechanisms that underlie anaplastic transformation will insights fundamental to the development of new and effective treatments emerge for this uniformly fatal thyroid cancer.

Accepted for publication June 17, 2002.

This study was presented as a poster at the annual meeting of the American Head and Neck Society, Boca Raton, Fla, May 10-14, 2002.

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REFERENCES

- Basik M, Stoler DL, Kontzoglou KC, Rodriguez-Bigas MA, Petrelli NJ, Anderson GR. Genomic instability in sporadic colorectal cancer quantitated by inter-simple sequence repeat PCR analysis. *Genes Chromosomes Cancer*. 1997;18:19-29.

2. Stoler DL, Chen N, Basik M, et al. The onset and extent of genomic instability in sporadic colorectal tumor progression. *Proc Natl Acad Sci U S A*. 1999;96:15121-15126.
3. Gilliland FD, Hunt WC, Morris DM, Key CR. Prognostic factors for thyroid carcinoma: a population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973-1991. *Cancer*. 1997;79:564-573.
4. McIver B, Hay ID, Giuffrida DF, et al. Anaplastic thyroid carcinoma: a 50-year experience at a single institution. *Surgery*. 2001;130:1028-1034.
5. Venkatesh YS, Ordonez NG, Schultz PN, Hickey RC, Goepfert H, Samaan NA. Anaplastic carcinoma of the thyroid: a clinicopathologic study of 121 cases. *Cancer*. 1990;66:321-330.
6. Demeter JG, De Jong SA, Lawrence AM, Paloyan E. Anaplastic thyroid carcinoma: risk factors and outcome. *Surgery*. 1991;110:956-961; discussion 961-963.
7. Carcangiu ML, Steeper T, Zampi G, Rosai J. Anaplastic thyroid carcinoma: a study of 70 cases. *Am J Clin Pathol*. 1985;83:135-158.
8. Passler C, Scheuba C, Prager G, et al. Anaplastic (undifferentiated) thyroid carcinoma (ATC): a retrospective analysis. *Langenbecks Arch Surg*. 1999;384:284-293.
9. Ain KB. Anaplastic thyroid carcinoma: a therapeutic challenge. *Semin Surg Oncol*. 1999;16:64-69.
10. Junor EJ, Paul J, Reed NS. Anaplastic thyroid carcinoma: 91 patients treated by surgery and radiotherapy. *Eur J Surg Oncol*. 1992;18:83-88.
11. Nilsson O, Lindeberg J, Zedenius J, et al. Anaplastic giant cell carcinoma of the thyroid gland: treatment and survival over a 25-year period. *World J Surg*. 1998;22:725-730.
12. Hadar T, Mor C, Shvero J, Levy R, Segal K. Anaplastic carcinoma of the thyroid. *Eur J Surg Oncol*. 1993;19:511-516.
13. Sugitani I, Kasai N, Fujimoto Y, Yanagisawa A. Prognostic factors and therapeutic strategy for anaplastic carcinoma of the thyroid. *World J Surg*. 2001;25:617-622.
14. Lu WT, Lin JD, Huang HS, Chao TC. Does surgery improve the survival of patients with advanced anaplastic thyroid carcinoma? *Otolaryngol Head Neck Surg*. 1998;118:728-731.
15. Lam KL, Lo C, Chan K, Wan K. Insular and anaplastic carcinoma of the thyroid: a 45-year comparative study at a single institution and a review of the significance of p53 and p21. *Ann Surg*. 2000;231:329-338.
16. Kobayashi T, Asakawa H, Umeshita K, et al. Treatment of 37 patients with anaplastic carcinoma of the thyroid. *Head Neck*. 1996;18:36-41.
17. Voutilainen PE, Multanen M, Haapiainen RK, Leppaniemi AK, Sivula AH. Anaplastic thyroid carcinoma survival. *World J Surg*. 1999;123:975-978; discussion 978-979.
18. Lo C, Lam K, Wan K. Anaplastic carcinoma of the thyroid. *Am J Surg*. 1999;177:337-339.
19. Tan RK, Finley RK III, Driscoll D, Bakamjian V, Hicks WL Jr, Shedd DP. Anaplastic carcinoma of the thyroid: a 24-year experience. *Head Neck*. 1995;17:41-47; discussion 47-48.
20. Haigh PI, Ituarte PH, Wu HS. Completely resected anaplastic thyroid carcinoma combined with adjuvant chemotherapy and irradiation is associated with prolonged survival. *Cancer*. 2001;91:2335-2342.
21. Rodriguez JM, Pinero A, Ortiz S, et al. Clinical and histological differences in anaplastic thyroid carcinoma. *Eur J Surg*. 2000;166:34-38.
22. Spires JR, Schwartz MR, Miller RH. Anaplastic thyroid carcinoma: association with differentiated thyroid cancer. *Arch Otolaryngol Head Neck Surg*. 1988;114:40-44.
23. Ibanez ML, Russell WO, Albores-Saavedra J, Lampertico P, White EC, Clark RL. Thyroid carcinoma—biologic behavior and mortality: postmortem findings in 42 cases, including 27 in which the disease was fatal. *Cancer*. 1966;19:1039-1052.
24. Nishiyama RH, Dunn EL, Thompson NW. Anaplastic spindle-cell and giant-cell tumors of the thyroid gland. *Cancer*. 1972;30:113-127.
25. Aldinger KA, Samaan NA, Ibanez M, Hill CS Jr. Anaplastic carcinoma of the thyroid: a review of 84 cases of spindle and giant cell carcinoma of the thyroid. *Cancer*. 1978;141:2267-2275.
26. Saunders CA, Nayar R. Anaplastic spindle-cell squamous carcinoma arising in association with tall-cell papillary cancer of the thyroid: a potential pitfall. *Diagn Cytopathol*. 1999;21:413-418.
27. Pilotti S, Collini P, Rilke F, Cattoretto G, Del Bo R, Pierotti MA. Bcl-2 protein expression in carcinomas originating from the follicular epithelium of the thyroid gland. *J Pathol*. 1994;172:337-342.
28. Wang S, Lloyd RV, Hutzler MJ, Safran MS, Patwardhan NA, Khan A. The role of cell cycle regulatory protein, cyclin D1, in the progression of thyroid cancer. *Mod Pathol*. 2000;13:882-887.
29. Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL. Frequent mutation and nuclear localization of β -catenin in anaplastic thyroid carcinoma. *Cancer Res*. 1999;59:1811-1815.
30. Hoang-Vu C, Dralle H, Scheumann G, et al. Gene expression of differentiation and dedifferentiation markers in normal and malignant human thyroid tissues. *Exp Clin Endocrinol*. 1992;100:51-56.
31. Zou M, Shi Y, al-Sedairy S, Farid NR. High levels of Nm23 gene expression in advanced stage of thyroid carcinomas. *Br J Cancer*. 1993;68:385-388.
32. Stringer BM, Rowson JM, Parkar MH, et al. Detection of the H-RAS oncogene in human thyroid anaplastic carcinomas. *Experientia*. 1989;45:372-376.
33. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*. 1994;20:176-183.
34. Stoler DL, Datta RV, Charles MA, et al. Genomic instability measurement in the diagnosis of thyroid neoplasms. *Head Neck*. 2002;24:290-295.