Variable Genetic Alterations and Survival in Head and Neck Cancer

Lyon L. Gleich, MD; Ya Qin Li, MD; Xin Wang, MD; Peter J. Stambrook, PhD; Jack L. Gluckman, MD

Objectives: To evaluate multiple genetic loci in patients with head and neck cancer to determine if, as in colorectal carcinoma, there is an orderly occurrence of genetic alterations, and if an accumulation of alterations affects patient survival.

Design: Cohort study of patients with head and neck cancer in which fresh tissue was retrieved.

Setting: Academic medical center.


Main Outcome Measures: The DNA from tumor and healthy tissue was evaluated for loss of heterozygosity at p53, retinoblastoma, and chromosome 16q and for amplification of cyclin D1. The respective RNA was probed for levels of p53, p16, p21, and p27 messenger RNA. These findings were compared with tumor stage and patient survival.

Results: DNA analysis showed that loss of heterozygosity occurred at p53 in 21% of tumors, at retinoblastoma in 35%, and at 16q in 21%, and that cyclin D1 was amplified in 42%. Messenger RNA levels of the assessed proteins were variably increased and decreased compared with healthy tissues obtained from the same patients with no discernable pattern. There was no correlation between any one of these genetic alterations and overall survival. When patients were analyzed for loss of heterozygosity at p53, retinoblastoma, 16q, or altered cyclin D1 in combination, 19 patients had no detectable alterations, 13 had 1, 6 had 2, and 5 had 3. Single genetic alterations did not affect survival; however, there was a trend toward decreased survival with multiple alterations. The 2-year Kaplan-Meier survival in patients with less than 1 genetic loss was 78% vs 58% in patients with 2 or more losses.

Conclusions: The lack of a pattern of genetic alterations in head and neck cancer demonstrates that its progression can be mediated by a multitude of pathways, complicating its genetic evaluation. Single genetic alterations do not appear to affect survival; however, when multiple alterations are detected—regardless of combination—survival is affected. This observation lends credence to the theory that multiple genetic alterations contribute to cancer progression; however, the lack of a pattern of this genetic change is a significant obstacle to applying genetic findings to routine cancer therapy.


©1999 American Medical Association. All rights reserved.
MATERIALS AND METHODS

In accordance with the University of Cincinnati Medical Center Institutional Review Board, fresh malignant and healthy tissue specimens were obtained from 43 patients at the time of surgical treatment for a primary squamous cell carcinoma of the head and neck from October 1991 through June 1994. There were 35 men and 8 women, aged 31 to 81 years (median, 59 years). Primary cancer sites were the oropharynx (15), larynx (12), oral cavity (11), and hypopharynx (5). Advanced stage tumors predominated (stage I, 2; stage II, 4; stage III, 12; and stage IV, 25). All tumors were treated by surgical excision followed by radiation therapy, except for 3 early stage tumors that received surgery alone. Healthy tissue was obtained during the procedure distant to the cancer (ie, sternocleidomastoid muscle or distant mucosa).

The specimens were frozen immediately after excision in liquid nitrogen and stored at −70°C. High-molecular-weight DNA and messenger RNA (mRNA) were extracted from the tissues and transferred to nylon membranes as previously described.3,4 To assess for LOH at p53, the probe p68RS2.0 was applied to RBRB. To detect LOH at 16q24, the following microsatellite repeats were analyzed: D16S 285, D16S 408, D16S 398, D16S 402, D16S 449, D16S 305, and D16S 303. If the tumor demonstrated greater than 50% reduction in intensity of one of the informative alleles compared with the normal tissue, LOH was scored as positive. To assess for amplification of DNA and increased levels of mRNA at cyclin D1, Southern and Northern blot analyses were performed as previously described using a cyclin D1 probe.4

Malignant and healthy tissue mRNA were analyzed for levels of p53, p16, p21, and p27 mRNA by Northern blot analysis using B-actin as an internal control.7 The p53 probe was provided by Harvey Preiser, MD; the p16 probe was provided by Alexander Kamb, PhD, and Priya Dayanant, PhD; the p21 probe was provided by Bert Vogelstein, MD; and the p27 probe was provided by Joan Massague, PhD. The level of mRNA found in each tumor was compared with that in healthy tissue from the same patient.

pared with patient survival to determine if specific patterns of genetic change can predict patient outcome.

RESULTS

The LOH analyses for p53 and RB were performed for all 43 specimens. There was LOH at p53 in 9 patients (21%) and at RB in 15 patients (33%). The LOH at 16q was analyzed in 29 patients with sufficient DNA and was observed in 6 patients (21%). Cyclin D1 was analyzed in 24 patients with sufficient DNA and showed amplification in 10 patients (42%), in 9 of whom increased cyclin D1 mRNA was detected.

In the 35 patients with sufficient mRNA for analysis, the level of p53 mRNA in tumor compared with healthy tissue was increased in 11 patients, equal in 3, decreased in 11, and undetectable in 8. Observed mRNA levels of p16, p21, and p27 followed no pattern and appeared random. The results of the DNA and mRNA analyses and data on patient outcome are shown in the Table.

Of the 43 patients, 26 have died: 15 died from the cancer, 10 from unrelated causes, and 1 from an unknown cause. Seventeen patients remain alive and free of disease for 26 to 66 months (mean, 46.1 months). The patients who died from other causes in less than 24 months (2 patients) or from an unknown cause in less than 24 months (1 patient) were excluded from the statistical analysis of survival. Review of the pathology reports found pathologically positive nodes in 28 of the 43 specimens.

Statistical analysis for a relationship between each individual genetic marker found no such correlation. Statistical analysis by \( \chi^2 \) testing found no relationship between the number of loci affected and any of the following: patient age, sex, T stage, N stage, staging group, site, or pathologic nodal status. In assessing the data to determine if any 2 genes together were strong predictors of patient survival, no such combination was found.

Trends were present for each DNA alteration studied, suggesting that loss of genetic material was associated with decreased survival. The DNA results were therefore studied to determine if LOH at multiple loci or in combination with amplification at cyclin D1 was associated with poorer survival. There were 16 patients with no DNA alterations in the sites studied, only 4 of whom died from cancer (25%). There were 13 patients with 1 DNA alteration, 6 of whom died from cancer (46%). There were 6 patients with 2 DNA alterations and 5 patients with 3 DNA alterations. Six of these 11 patients with multiple alterations died from cancer (55%). Kaplan-Meier survival analysis demonstrated that the patients with multiple alterations had poorer survival, with only 58% of patients surviving 2 years compared with 78% of those surviving 2 years in the group with less than 1 alteration (log rank \( \chi^2 \) analysis \( P = .28 \) (Figure).

COMMENT

Knowledge of the genetic alterations that lead to cancer has increased greatly in the past decade. In some cancers, genes have been found that predict genetic predisposition to cancer, such as the APC gene in colorectal cancer and BRCA-1 and BRCA-2 in breast cancer. Researchers investigating head and neck squamous cell carcinoma have searched for similar genes that predict cancer development or prognosis. Although p53 and other genes initially appeared promising, this study shows that alterations of these genes individually is not a sufficient predictor of patient outcome to be clinically useful at this time.

Current research in colorectal carcinoma has continued to support a pattern of genetic losses that lead to
carcinogenesis and then to more aggressive disease. In head and neck cancer, LOH at multiple sites is associated with poorer prognosis, but an orderly pattern of genetic loss has not been found. This current study examined interrelated genes to determine if a pattern of loss is present in head and neck cancer.

The genes studied included the tumor suppressor genes p53 and RB. The p53 gene is located on chromosome 17 and is mutant in most cancer types. In response to cell stressors, such as hypoxia, the level of p53 protein is increased. The RB gene is located on chromosome 13 and produces the retinoblastoma protein. The retinoblastoma protein and p53 interact and exert regulatory control on the cell cycle by directly or indirectly affecting the cyclin-dependent kinases whose activities depend on cyclins, such as cyclin D1. The proteins p16, p21, and p27 are involved in the interactions between p53, the retinoblastoma protein, and the cyclin-dependent kinases.

Under normal circumstances, these genes can cause a cell undergoing severe stress to die by apoptosis. Mutations in some or all of these genes can abrogate this effect. Chromosome 16q, which demonstrates partial loss in several tumor types, encodes many of the cadherins, which are related to cell adhesion. Loss of this chromosomal region is therefore theorized to promote metastases.

Despite the described interactions of these genes, no orderly derangement of the genetic material was found. In fact, there was no discernible pattern of genetic loss.
nosis. However, the statistical power of both studies is insufficient at this time to alter current therapy based on these complex genetic analyses. In this study, patients survived despite many genetic losses, and no pattern of genetic changes was seen when evaluating many of the genetic loci that have been proposed to be meaningful. Although comparative genomic hybridization has suggested other loci, such as 10q, that may be significant, evaluations at these loci for LOH failed to show a survival impact.  

As technology continues to improve, with genetic innovations such as chip technology for genetic analysis, evaluations of patient tissue for multiple genetic alterations may become more practical. Until that time, the search for genetic markers and patterns that relate to patient outcome should continue. However, this study suggests that there are many obstacles to overcome before genetic data can be applied to routine care of patients with head and neck cancer.

Accepted for publication September 11, 1998.

This work was supported by the Ruth Lyons Cancer Fund, Cincinnati, Ohio.


Reprints: Lyon L. Gleich, MD, Department of Otolaryngology–Head and Neck Surgery, University of Cincinnati Medical Center, PO Box 670528, 213 Bethesda Ave, Cincinnati, OH 45267-0528 (e-mail: lyon.gleich@uc.edu).

REFERENCES


or alteration of expression found in either the DNA or mRNA analyses, respectively. This suggests that head and neck cancers are not like many other cancers but, rather, are associated with a variable and apparently unpredictable pattern of genetic losses. It is possible that a pattern of losses may be seen early in carcinogenesis, but this study failed to show any pattern associated with developed head and neck cancer.

This study used tissues obtained from sites throughout the head and neck, and its statistical power has limitations due to the number of patients examined at every genetic locus. This study evaluated many different genetic loci, but regions of reported genetic abnormalities, such as on chromosomes 3p, 8p, and 9p were not analyzed. Additionally, we analyzed tumor suppressor genes by LOH. Whereas LOH is one common mechanism by which genetic activity is lost, tumor suppressor function may be lost by other mechanisms, including homozygous deletion, point mutations, posttranslational events, and epigenetic inactivation. However, despite these limitations, our results failed to show any discernible pattern of genetic loss.

Our analysis showed that multiple genetic alterations are associated with poorer prognosis. However, there was no single pattern of genetic loss that emerged as the most meaningful. Rather, the number of losses appeared to be the most meaningful, and even this finding was not strongly supported by statistical analysis. Additionally, there were patients with no DNA losses who died from their cancers, and other patients with 3 losses and high-stage disease who survived.

This study and that of Li et al support the theory that multiple genetic losses are indicative of a poorer prognosis. However, the statistical power of both studies is insufficient at this time to alter current therapy based on these complex genetic analyses. In this study, patients survived despite many genetic losses, and no pattern of genetic changes was seen when evaluating many of the genetic loci that have been proposed to be meaningful. Although comparative genomic hybridization has suggested other loci, such as 10q, that may be significant, evaluations at these loci for LOH failed to show a survival impact.  

As technology continues to improve, with genetic innovations such as chip technology for genetic analysis, evaluations of patient tissue for multiple genetic alterations may become more practical. Until that time, the search for genetic markers and patterns that relate to patient outcome should continue. However, this study suggests that there are many obstacles to overcome before genetic data can be applied to routine care of patients with head and neck cancer.

Accepted for publication September 11, 1998.

This work was supported by the Ruth Lyons Cancer Fund, Cincinnati, Ohio.


Reprints: Lyon L. Gleich, MD, Department of Otolaryngology–Head and Neck Surgery, University of Cincinnati Medical Center, PO Box 670528, 213 Bethesda Ave, Cincinnati, OH 45267-0528 (e-mail: lyon.gleich@uc.edu).

REFERENCES