p53 Protein Accumulation and Genomic Instability in Head and Neck Multistep Tumorigenesis[1]

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Abstract

Head and neck cancer develops in a multistep process and is associated with increasing frequencies of p53 alterations and with increasing genomic instability. To study the relationship of *p53* alterations and genomic instability during head and neck tumorigenesis, we analyzed p53 protein expression and chromosome 9 and 17 polysomy in 48 squamous cell carcinomas of the head and neck and their adjacent normal epithelium (31 sites), hyperplastic (24 sites), and dysplastic lesions (26 sites). Normal oral epithelium obtained from seven nonsmoking, cancer-free individuals served as negative controls. Six (19%) of 31 lesions in adjacent normal epithelium, 7 (29%) of 24 hyperplastic lesions, 12 (46%) of 26 dysplastic lesions, and 28 (58%) of 48 squamous cell carcinomas expressed p53. In contrast, no normal control epithelium had detectable p53 expression. To determine the relationship between dysregulated p53 expression and genomic instability during tumorigenesis, we compared p53 immunohistochemistry distributions and chromosome polysomy levels (by chromosome *in situ* hybridization) in different histological groups associated with tissue progression. Although the degree of chromosome polysomy increased for all of the groups during histological progression, lesions with dysregulated p53 expression showed nearly 2–4-fold increased levels of chromosome polysomy. This trend was significant for dysplastic lesions ($P = 0.005$ and $P = 0.002$ for chromosomes 9 and 17, respectively) and for squamous cell carcinoma ($P = 0.005$ and $P = 0.002$ for chromosomes 9 and 17, respectively). Image analysis studies for 28 p53-expressing tumors and their adjacent premalignant lesions demonstrated a strong spatial correlation between stepwise transitions from low to high p53 expression and increased chromosome polysomy frequencies in 13 (46%) of 28 cases. These findings suggest that altered p53 expression is associated with increased genetic instability in preneoplastic epithelium and may play a driving force for increasing the rate of accumulation of genetic events during head and neck tumorigenesis.

Introduction

Clarifying the biological processes driving human tumorigenesis may help researchers to identify and develop future preventive and therapeutic strategies. Head and neck squamous cell carcinoma has been suggested as an example of a multistep tumorigenesis process in a carcinogen-exposed tissue field (1–3), wherein premalignant lesions are frequently observed in areas adjacent to frank malignancy (4). One driving force of the multistep process is the accumulation of genetic damage, the rate of which may be influenced by the degree of carcinogen exposure, the inherent sensitivity of the individual, and the tissue reactivity to carcinogens (5–7). Although the whole carcinogen-exposed field presumably has accumulated genetic damage, only a few premalignant foci become clinically evident and may eventually develop into carcinoma. It is hypothesized that those cells having the right, physiologically relevant "genetic hits" or alterations progress toward malignancy (8). Several potentially important genetic events in head and neck tumorigenesis have been described recently (9–12), although the timing of different genetic events and their functional physiological consequences require further exploration.

Genetic instability has been proposed to be a major driving force determining the rate of accumulation of specific genetic hits in several human cancers (13–15). To determine the rate of accumulation of genetic alterations during head and neck tumorigenesis and to assess the risk of tumor formation in the genetically altered tissue field, we previously probed (11) squamous cell carcinomas of the head and neck and their adjacent normal epithelia and premalignant lesions for numerical chromosomal aberrations by nonisotopic *in situ* hybridization using chromosome-specific DNA probes for chromosomes 7 and 17. Normal oral epithelium from nonsmoking individuals free of cancer showed no evidence of chromosome polysomy (*i.e.*, cells with three or more chromosome copies), whereas histologically normal epithelia adjacent to the tumors and their premalignant lesions exhibited chromosome polysomy, suggesting that a process of genomic instability is ongoing in the preneoplastic tissue from which the tumor originates (16). Furthermore, the frequency of cells with polysomy increased with histological progression (11). Nevertheless, within each histological grade, there was a significant intersubject variation in the levels of chromosome polysomy present, suggesting that biological factors might influence the rate of accumulation of genetic hits.

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Table 1 Patient characteristics

Patient number	48
Median age (range)	57 (25 –77)
Sex	
Male	30
Female	18
Smoking (pack-yr.) median (range)	48 (15–160)
Tumor site	
Oral cavity	14
Oropharynx	10
Hypopharynx	8
Larynx	16
Stage	
I	5
II	10
III	16
IV	17
Histology	
Normal (adjacent to tumor)	31
Hyperplasia	24
Dysplasia	26
Squamous cell carcinoma	48

Alteration of the *p53* gene is one of the most common genetic events in human tumors, including head and neck cancer (17–20). Although *p53* has been shown to participate in many cellular physiological response mechanisms, its functional role in cancer development is still not well understood. We and others have reported previously (9, 10, 12) in a similar study setting of tumors and their contiguous adjacent premalignant lesions that dysregulated p53 protein abundance can be detected in 21% of cases of histologically normal epithelium, 29% of hyperplastic lesions, and 45% of dysplastic lesions adjacent to tumors. This finding suggests that altered p53 expression plays a functional role in head and neck tumor development.

Because *in vitro* studies have indicated that *p53* gene product is critically involved in the up-regulation of cell cycle checkpoints after a variety of cellular injuries, perhaps participating in a surveillance mechanism to detect DNA damage and elicit cellular protection, it has been proposed that cells with altered p53 function might show increased genomic instability *in vivo* after cellular insult (*e.g.,* Li-Fraumeni syndrome; Ref. 21). Thus, in carcinogen-altered tissues (*e.g.,* the tobacco-exposed upper aerodigestive tract), alterations of p53 function might be expected to increase the levels of genomic instability and accelerate the rate of accumulation of genetic alterations. To explore this possibility, we determined the relationship between p53 expression status and chromosome polysomy in head and neck tissue specimens containing squamous cell carcinomas and their adjacent premalignant lesions. Altered p53 expression was shown to be highly correlated with increased rates of genomic instability and thus might be a therapeutic target for the prevention of head and neck cancer.

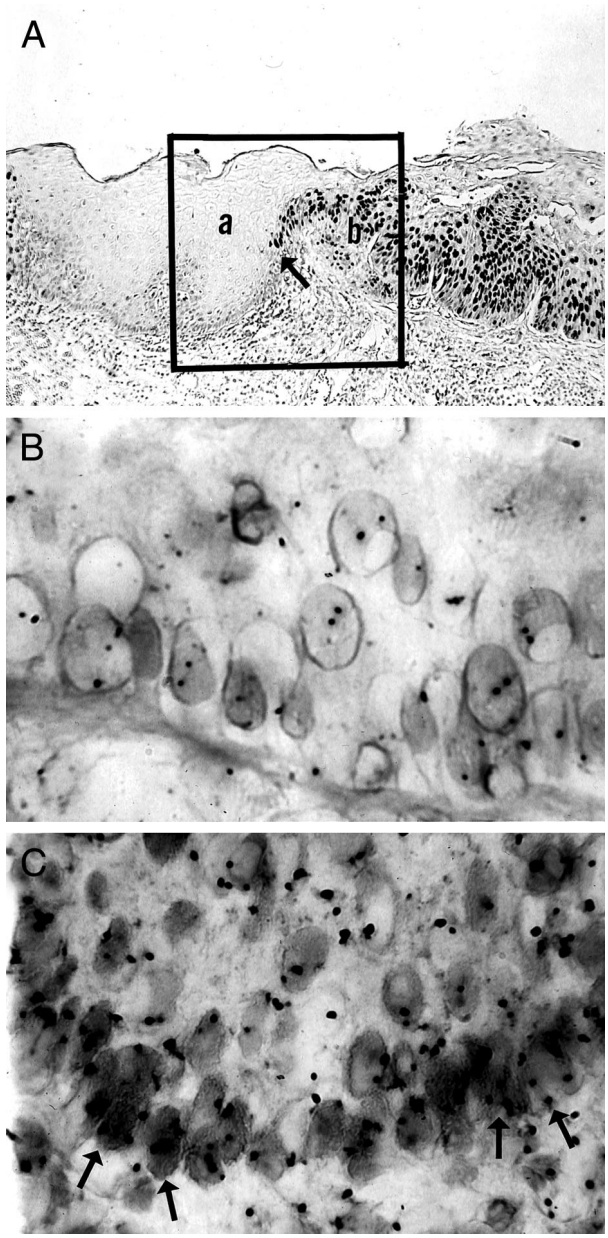


Fig. 1. Immunohistochemical analysis of p53 expression and chromosome 17 changes in a transitional area of normal epithelium adjacent to a tumor and in a mild dysplastic area. *A,* a few cells expressed p53 protein in the epithelium on *left side of arrow* (*area a*). Note the dramatic increase in p53 expression with histological changes on *right side of arrow* (*area b*; ×10, original magnification). *B,* in the same area (*A, area a*), note chromosome 17 changes in *area a* when most of the cells display two or fewer chromosome copy numbers (×100, original magnification). *C,* chromosome 17 copies increased dramatically in the cells shown in *area b* (*A*). Most of the cells display three or more copies (*arrows*; × 100, original magnification).

Materials and Methods

Formalin-fixed, paraffin-embedded tumor specimens were obtained from patients with head and neck squamous cell carcinoma whose tumors were surgically resected at The University of Texas M. D. Anderson Cancer Center. Forty-eight specimens containing carcinomas with adjacent normal epithelium and premalignant lesions (*i.e.,* hyperplasia or dysplasia) were selected for this study. Seven biopsy specimens of normal oral mucosa obtained from healthy volunteers (*i.e.,* cancer-free nonsmokers) were used as controls. Slides stained with H&E were reviewed by a pathologist to identify areas of normal, hyperplastic, dysplastic, and cancerous histological type according to criteria described previously (22). Sections (4-μm thick) were mounted on aminoalkylsilane-coated slides (Histology Control Systems, Glen Head, NY) for immunohistochemical staining and chromosome *in situ* hybridization.

Table 2 Polysomy of chromosome 17 in relation to p53 protein expression

Histology	Chromosome polysomy (mean ± SD, percentage)						
	No.	Overall	No.	p53 negative	No.	p53 positive	P^a
Control	7	0 ± 0	7	0 ± 0	NA[b]	NA	NA
Adjacent normal	31	1.8 ± 2.9	25	1.3 ± 1.9	6	4.1 ± 5.1	0.14
Hyperplasia	24	4.3 ± 3.9	17	3.5 ± 2.4	7	6.4 ± 6.3	0.38
Dysplasia	26	10.4 ± 12.5	14	4.6 ± 5.1	12	17.6 ± 15.3	0.005
Tumors	48	15.7 ± 12.8	20	8.6 ± 6.8	28	21.0 ± 13.8	0.0006

[a] Wilcoxon's rank-sum test.
[b] NA, not applicable.

Table 3 Polysomy of chromosome 9 in relation to p53 protein expression

Histology	Chromosome polysomy (mean ± SD, percentage)						
	No.	Overall	No.	p53 negative	No.	p53 positive	P^a
Control	7	0 ± 0	7	0 ± 0	NA[b]	NA	NA
Adjacent normal	31	1.42 ± 1.7	25	1.14 ± 1.5	6	2.6 ± 2.2	0.14
Hyperplasia	24	4.1 ± 3.4	17	3.2 ± 2.6	7	6.3 ± 4.1	0.07
Dysplasia	26	6.5 ± 8.5	14	2.4 ± 1.9	12	11.3 ± 10.7	0.002
Tumors	48	13.8 ± 11.9	20	6.6 ± 6.5	28	18.9 ± 12.2	0.0001

[a] Wilcoxon's rank-sum test.
[b] NA, not applicable.

After identification of premalignant and carcinomatous areas, tissue sections were immunohistochemically stained for p53 using a monoclonal anti-p53 antibody (clone D07; Biogenex, Inc., San Ramon, CA). This antibody has been shown previously (23) to react to both wild-type and mutant forms of the p53 protein. Immunohistochemical analysis involved a modification of the avidin-biotin-immunoperoxidase method as described previously (24). To overcome variations in the degree of staining from slide to slide, cell-block sections of paraffin-embedded A431 cells were attached to each slide. A431 cells express a *p53* gene mutation (CGT to CAT at codon 273) and thus served as an internal positive control for each tissue section. This allowed quantitative comparisons between different samples.

To detect genomic instability in the premalignant lesions and tumors, the sections adjacent to those immunostained for p53 expression were processed for chromosome *in situ* hybridization as described previously (11). Tissue sections were hybridized with a classical satellite DNA probe specific for the pericentromeric region of chromosome 9 [D9Z1] as well as with an α satellite probe specific for the centromeric region of chromosome 17 [D17Z1]. These probes were obtained from Oncor, Inc. (Gaithersburg, MD). At least 200 nuclei were scored in each defined histological area, and each nucleus was assessed for the chromosome copy number. Chromosome polysomy was defined as the fraction of cells demonstrating three or more signals in each nucleus.

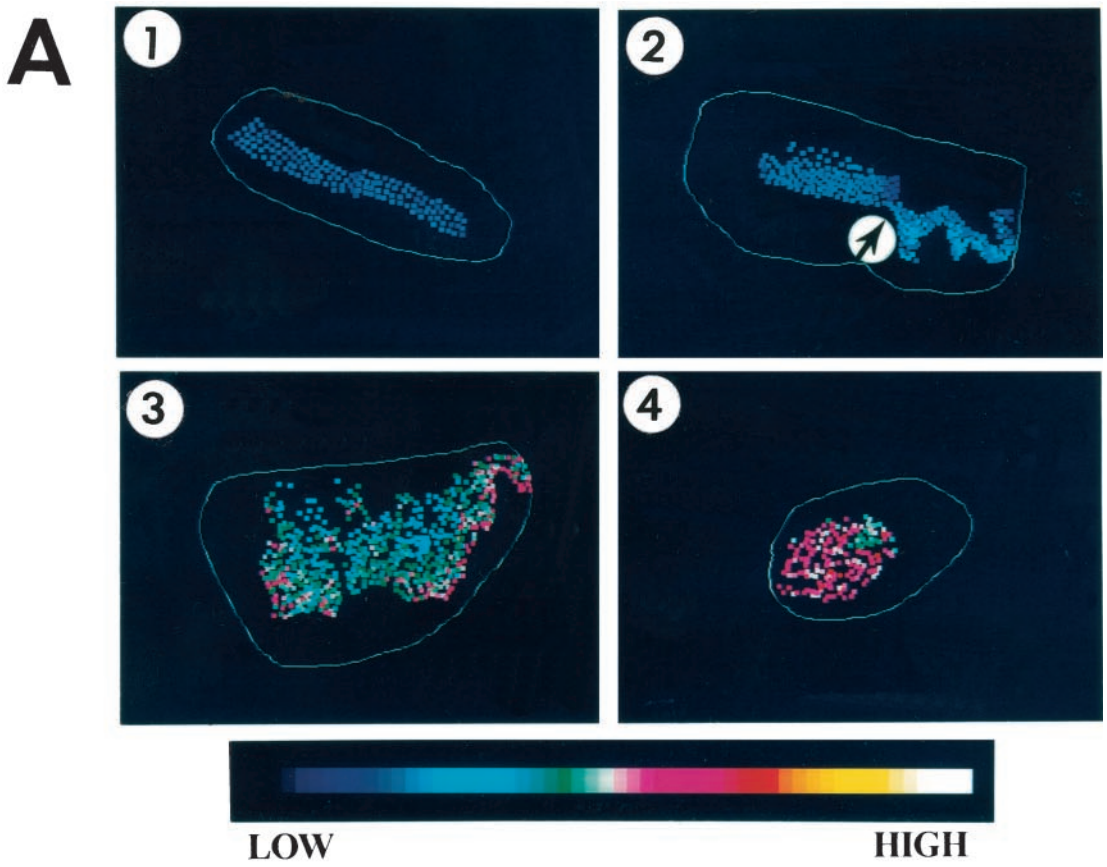
To assess the amount and distribution of p53 protein expression and its relationship to chromosome polysomy on the tissue sections, we mapped the topographical distribution of p53 expression and chromosomal changes using an image analysis system as described previously (12). In brief, using the Magiscan Image Analysis System (Joyce-Loebl, Ltd., Dukesway, England) attached to a light microscope with a controller-driven stage, the mapping involved visual identification of premalignant lesions of the epithelial layer and tumors and manual circling of each nucleus with a light pen on particular p53-expressed areas. Each circled region was characterized by a measurement of total integrated density over the nucleus, the area of the measured nuclear region, and its relative coordinates. The specific intensity of each region was calculated as the integrated intensity over each nucleus divided by the area of that nucleus and was normalized to that measured in the control A431 cells. Because the location of each object measured was recorded in relative coordinates, the pattern of p53 expression could be displayed as a two-dimensional array. This technique allowed the visualization of the topographical distribution of p53 expression in each tissue section. The chromosomal distribution was mapped in a similar way according to the number of signals in each nucleus after chromosome *in situ* hybridization was performed on the adjacent tissue sections of p53 immunostaining.

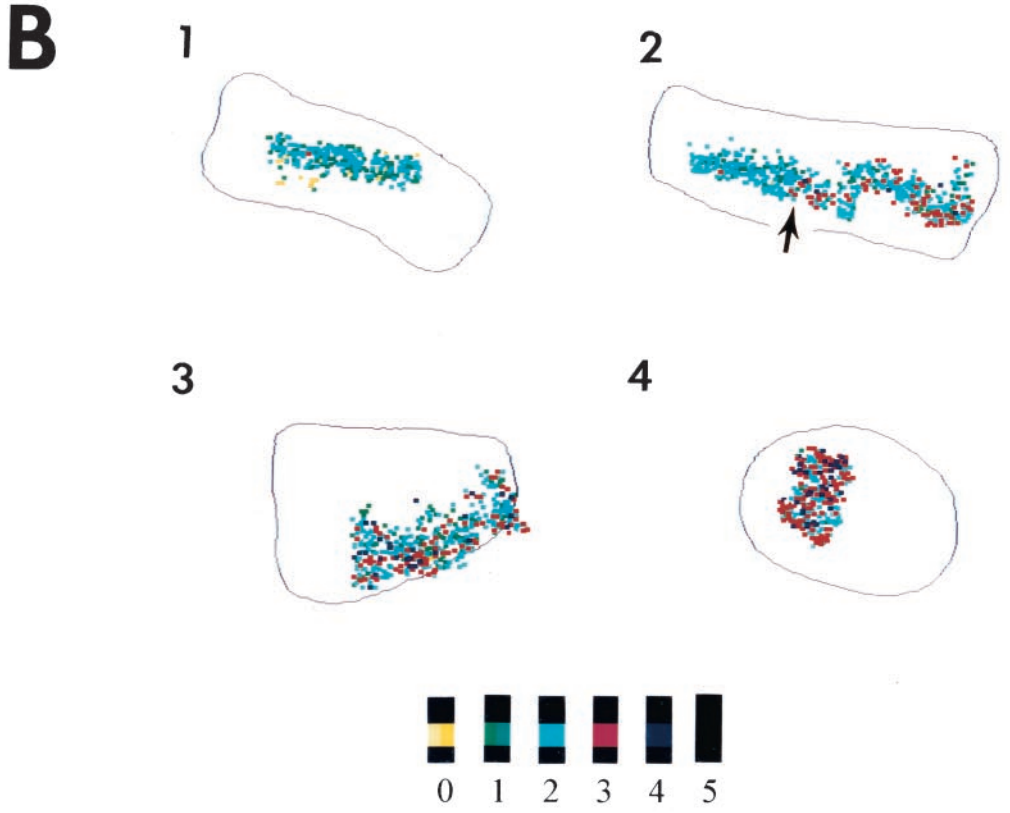
Frequency tabulation and descriptive statistics were given to summarize the distribution of discrete and continuous variables. Statistical analyses were performed using nonparametric Wilcoxon's rank-sum test to test for equal distribution of chromosome polysomy between the two groups. Two-sided *P*s were compared, and *P*s of <0.05 were considered statistically significant.

Results

Surgically resected tumor specimens were obtained from 48 patients with head and neck squamous cell carcinoma. These 48 specimens were derived from four head and neck tumor sites: oral cavity (14 specimens), oropharynx (10 specimens), hypopharynx (8 specimens), and larynx (16 specimens). The 48 tumor specimens were chosen because they contained contiguous regions of adjacent normal (31 sites), hyperplastic (24 sites), and dysplastic epithelium (26 sites). All of the 48 specimens examined contained invasive carcinoma. Table 1 shows the characteristics of the patients from whom the tumors were resected. All but three of the cases had a documented smoking history, with a median of a 48 pack-year exposure. None of the patients had received previous treatment.

The p53-positive expression group was defined by $\geq$10% of tumor cells showing nuclear reactivity, and the p53-negative group was defined by $<$10% of tumor cells showing nuclear





reactivity. p53 protein levels were detected in 6 (19%) of 31 samples of normal epithelium adjacent to tumors (Fig. 1*A*, *area b*), in 7 (29%) of 24 samples of hyperplasia, in 12 (46%) of 26 samples of dysplasia, and in 28 (58%) of 48 carcinomas. In contrast, none of the normal control epithelia expressed detectable levels of p53. As the tissues progressed from normal to hyperplasia, dysplasia, and squamous cell carcinoma, the frequency of p53-expressing cells continuously increased.

To determine the relationship between dysregulated p53 expression levels and the degree of genomic instability during multistage head and neck tumorigenesis, nonisotopic chromosome *in situ* hybridization was performed using chromosome 17- and chromosome 9-specific DNA probes on the tissue sections adjacent to those used for the p53-immunostaining studies. In this setting, chromosome-specific signals appeared as dark dots on the interphase nuclei in the tissue section (Fig. 1, *B* and *C*). Normal diploid cells would be expected to exhibit two chromosome copies/nucleus if whole nuclei were being examined. However, because nuclei are frequently truncated in 4-μm tissue sections, chromosome copy numbers are underrepresented. Thus, a finding of cells with three or more signals would be considered a very infrequent event in unperturbed populations. On the other hand, genomic instability would lead to chromosome nondisjunction and the generation of cells with zero, one, two, and three or more chromosome copies. Therefore, the presence and frequency of cells exhibiting three or more chromosome copies (defined here as chromosome polysomy) might be considered a quantitative marker of ongoing or accumulated genomic instability in tissues.

As shown in Table 2 and Table 3, normal control epithelia from cancer-free, nonsmoking individuals showed no evidence of chromosome polysomy. In contrast, when chromosome 17 polysomy levels were determined as a function of histological progression and compared with p53 immunostaining status, chromosome polysomy increased overall with histological progression. p53-positive normal and hyperplastic epithelia adjacent to the tumor showed mean chromosome polysomy frequencies that were 2–3-fold higher than those of p53-negative lesions (percentages ± SE of cells with polysomy, 4.1 ± 5.1% *versus* 1.3 ± 1.9%, respectively, $P = 0.14$, in normal epithelium; 6.4 ± 6.3% *versus* 3.5 ± 2.4%, respectively, $P = 0.38$, in hyperplastic epithelium). In the dysplastic tissue and squamous cell carcinoma cases, chromosome 17 polysomy rates were significantly higher in the p53-positive sites than in the p53-negative sites (17.6 ± 15.3% *versus* 4.6 ± 5.1%, respectively, $P = 0.005$, in dysplasia; 21.0 ± 13.8% *versus* 8.6 ± 6.8%, respectively, $P = 0.0006$, in squamous cell carcinoma; Table 2).

Increased chromosome polysomy in p53-positive sites was also observed using a chromosome 9 probe, suggesting that generalized chromosome polysomy can be detected with any chromosome probe. Adjacent normal epithelium and hyperplastic tissue showed 2-fold higher polysomy 9 in the p53-positive lesions than that observed in the p53-negative lesions (2.6 ± 2.2% *versus* 1.1 ± 1.5%, respectively, $P = 0.14$, in adjacent normal epithelium; 6.3 ± 4.1% *versus* 3.2 ± 2.6%, respectively, $P = 0.07$, in hyperplasia). The more advanced lesions of dysplasia and squamous cell carcinoma showed significantly higher chromosome 9 polysomy frequencies in the p53-positive lesions than that seen in the p53-negative lesions (11.3 ± 10.7% *versus* 2.4 ± 1.9%, respectively, $P = 0.002$, in dysplasia; 18.9 ± 12.2% *versus* 6.6 ± 6.5%, respectively, $P = 0.0001$, in squamous cell carcinoma; Table 3). These data clearly indicate that dysregulated p53 expression is associated with increased genomic instability during head and neck tumorigenesis.

In some cases, the level of p53 expression was found to increase suddenly in the epithelium during multistep histological progression. This provided the opportunity to determine whether the transition from low to high p53 expression levels was spatially associated with a transition in the degree of chromosome polysomy. To address this issue, we spatially mapped p53 expression levels and chromosome copy numbers in adjacent tissue sections of the same specimens by image analysis using a computer-controlled microscope. An example of this type of analysis is illustrated in Fig. 1. The histologically normal adjacent epithelium of this patient showed a dramatic shift in the level of p53 expression, from a low (but abnormal) level in *area a* to a higher level in *area b* (Fig. 1*A*). To represent this staining pattern as a spatial map, the location of each nucleus scored for p53 expression was indicated on a two-dimensional display as a dot, the color of which indicates its normalized specific staining intensity. The quantitation of this transition in p53 expression levels is illustrated in Fig. 2*A*, where a pseudocolor scale ranging from deep blue at the lowest intensity to white at the highest intensity level of p53 expression (as indicated in the *color bar* at the *bottom*) was chosen. An *arrow* marks the transition from low-p53 staining to high-p53 intensity in the normal adjacent epithelium. As the tissue progressed to dysplasia and to squamous cell carcinoma, p53 expression continually increased (Fig. 2*A*).

At the chromosome level, although an occasional nucleus within *area a* of the histologically normal adjacent epithelia exhibited three or more copies of chromosome 17, the dramatic shift in p53 expression was associated with increased chromosome 17 polysomy levels. To better examine the spatial pattern of chromosome polysomy, the location of each nucleus scored was recorded along with the number of chromosome copies detected, and the data were displayed as a two-dimensional array where each dot represents a nucleus, and the color of the dot represents the chromosome copy number (yellow, green, cyan, red, blue, and black representing 0, 1, 2, 3, 4, and 5 chromosome copies, respectively). A comparison of Fig. 2*A* and Fig. 2*B* demonstrates a direct spatial correlation between increased p53 expression levels and increased chromosome 17 polysomy. Chromosome 17 polysomy was found to increase in a stepwise fashion from normal epithelium adjacent to the tumor to dysplastic tissue (Fig. 2*B*). By the time squamous cell carcinoma developed, a majority of the cells showed increased chromosome 17 copy numbers, suggesting the outgrowth of an aneuploid clone (Fig. 2*B*).

Chromosome 9 polysomy frequencies also correlated with p53 accumulation levels in the same tissue. Similar topological correlations of p53 expression transitions and chromosome 17 and

Fig. 2. Topographical mapping of p53 expression and chromosome 17 changes during head and neck tumorigenesis. *A*, *pseudocolor bar at the bottom* indicates the range of p53 expression from *deep blue* at the lowest intensity to *white* at the highest. *1*, normal epithelium adjacent to a tumor showing very few cells expressing p53 protein; *2*, dramatic increase in expression in the transitional zone (*arrow*); *3*, higher, expanded p53 expression in the dysplastic area; *4*, markedly increased p53 expression in squamous cell carcinoma. The mapping of p53 expression in each panel was performed under ×40, original magnification. *B*, the chromosome 17 copy numbers are illustrated by a color-coding system whereby *yellow*, *green*, *cyan*, *red*, *blue*, and *black* represent chromosome copy numbers 0, 1, 2, 3, 4, and 5, respectively. Chromosome 17 changes in areas *1*, *2*, *3*, and *4* indicate the exact same area as shown in *A*. Note that p53 expression and chromosome 17 changes are well matched in each histological type. Note *panel 2*, wherein chromosome 17 copies greatly increase from *right side of arrow*, marking the dramatic accumulation of p53 protein. The mapping of each panel for chromosome 17 copy numbers were performed under ×100, original magnification.

chromosome 9 polysomy transitions occurred in 13 (46%) of 28 of the p53-positive samples examined. These findings suggest that dysregulated p53 expression is strongly associated with an increase in genomic instability during head and neck tumorigenesis.

Discussion

We reported previously (12) that abnormal p53 protein accumulation can occur early in head and neck tumorigenesis. The degree of p53 expression was found to increase continually, both in frequency and quantity, as the tissue changed from adjacent normal epithelium to hyperplasia to dysplasia to squamous cell carcinoma (12). We also reported previously (11) that chromosome polysomy occurred not only in cancer cells but also in normal epithelium adjacent to tumor and in premalignant cells during the multistep process of head and neck tumorigenesis. Although cells must undergo numerous genetic changes before they can progress to the malignant phenotype (25–27), the forces that control the rate of chromosome change or genomic instability are still largely unknown. The results reported here provide support for the notion that p53 status might play one of the determining roles for the ongoing degree of genomic instability during head and neck tumorigenesis.

Preclinical and clinical data support the notion that *p53* plays a key role in regulating genomic stability (14, 28–30). In fact, the association between *p53* abnormality and genomic instability was also reported in breast (31), ovarian (32), renal cell (33), and head and neck squamous cell carcinomas (34). Three categories of cell regulatory defects are presumed to lead to genomic instability, especially in the setting of cell stress: defects in cell proliferation, defects in repair processes, and defects in the machinery of DNA replication and chromosome segregation. Importantly, *p53* function has been reported to influence each of these pathways; *e.g.*, carcinogen-exposed normal cells generally arrest or delay in G_1 phase of the cell cycle before entering S phase. This delay has been proposed to be an important cell cycle checkpoint allowing necessary DNA repair to take place before DNA replication past damaged regions (35). Cells lacking normal *p53* function do not show G_1 arrest in response to DNA damage, and this may lead to an accumulation of unrepaired lesions and increased chromosomal abnormalities, including numerical aberrations, deletions, rearrangements, and gene amplification (36–38). An alternative explanation is that disrupted *p53* function alters the capacity of damaged cells to undergo apoptosis and cell turnover (*i.e.*, "cellular proof-reading"; Ref. 39). This would allow more damaged cells to survive and would result in an increased apparent genomic instability pattern in the face of carcinogen exposure. Although the results shown here do not distinguish between these alternative explanations, this head and neck tumor model system provides a unique model system for exploring these possibilities *in situ.*

An important observation gained in this study was that lesions that overexpress p53 had relatively increased levels of genomic instability not only in the tumor fraction but also in the premalignant lesions. Not only was this association detected on a population level at each stage of histological progression, it was also detected by topological mapping of p53 expression and chromosomal polysomy on adjacent tissue sections where strong spatial correlations were observed. On the other hand, the correlation between transitions of p53 staining and chromosome polysomy was not always perfect; *e.g.*, in some cases, a transition from low to high p53 immunostaining was not spatially correlated with an increase in the frequency of chromosome polysomy. This might suggest that p53-mediated genomic instability itself is a multistep process and requires the cooperation of other events; *e.g.*, if the function of *p53* were to control cell cycle progression in the face of cellular stress, then an effect on genomic instability would require both cellular injury and cell proliferation in addition to disrupted *p53* function to elicit increased genomic instability. Alternatively, whereas overabundance of p53 protein levels in tissues is frequently associated with disrupted *p53* function or *p53* mutation, not all of the *p53* alterations may necessarily have genomic instability as a downstream consequence. Recent studies (40–42) into the consequences of specific *p53* mutations and/or regulatory control mechanisms should provide insight into this possibility.

Although dysregulated p53 protein levels were generally associated with increased genomic instability in the tissues studied, in some cases we also observed them in lesions without apparent increases in p53 protein levels. One possible explanation for this observation is that a functional *p53* alteration occurred in a gene site that did not lead to tissue accumulation of protein (*e.g.*, truncating mutation or deletion). Alternatively, genomic instability during tumorigenesis may also occur through *p53*-independent pathways that also influence cell-cycle regulatory pathways; *e.g.*, Glick *et al.* (43) demonstrated that transfection of transforming growth factor β can suppress genomic instability independent of G_1 arrest, *p53*, or retinoblastoma gene function. Similarly, alterations that disrupt cell-cycle regulatory controls, such as cyclin-dependent kinase activities (44) or the transition through mitosis (45), also induce genomic instability. Along these lines, our own group has shown in the same head and neck tumorigenesis model that cyclin D1 overexpression and loss of p16 function can spatially cooperate to promote increased chromosome polysomy frequencies (46).

The development of efficient clinical trials for the prevention of aerodigestive tract cancers (*i.e.*, lung cancer and head and neck cancer) is dependent upon the identification of individuals at high risk for cancer development who might best benefit from such intervention (47). It is also dependent on the identification of the processes that drive the tumorigenesis process, which may then be targeted for intervention. We demonstrated recently (48, 49) that increased p53 expression in premalignant lesions was associated with a decreased clinical response to therapy with 13-*cis*-retinoic acid and/or IFN, and we demonstrated previously (50, 51) that individuals whose oral premalignant lesions showed high degrees of chromosome polysomy were at increased risk for subsequently developing aerodigestive tract cancer. These previous results together with those reported here suggest that restoring normal *p53* function in head and neck tissues at risk for malignancy have a potential role in future chemoprevention approaches (52).

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