P53 Expression and Polysomies of Chromosome 9, 17 in Head and Neck Cancer Prognosis

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Abstract

Sixty-nine cases of head and neck squamous cell carcinoma were examined by immunohistochemistry for p53 and chromosome *in situ* hybridization for chromosome 9 and 17 to determine the relationship between p53 expression and polysomies of chromosome 9 and 17 with the development of a second primary tumor as well as recurrence of primary tumor of head and neck squamous cell carcinoma. We found early expression of p53 in the normal and premaligant lesions adjacent to tumor which was associated with a gradual increase in the fraction of positive nuclei as well as numbers of cancer. We also found statistically significant increments of polysomies of chromosome 9 and 17 in terms of the polysomy index seen through the histologic changes occuring during multistep tumorigenesis. Our results could not demonstrate statistically significant correlation between p53 expression and PI 9 and 17 in tumorigenesis. Interestingly, however, there was a strong correlation between p53 expression and second primary tumor as well as recurrence of primary tumor. The p53 expressed group had a seven fold increased incidence in developing second primary tumor and a two and a half times increased incidence for recurrence of primary tumor, compared to the non-expressed group.

We conclude that p53 expression and polysomies of chromosome 9 and 17 have an important role in multistep tumorigenesis in HNSCC. There was no significant correlation between p53 expression and polysomies of chromosome 9 and 17. However, the expression of p53 was statistically significant for association with second primary tumor and recurrence of primary tumor of head and neck squamous cell carcinoma.

Key word : P53 Expression, Polysomies of Chromosome 9 and 17, Poor Prognosis, Head and Neck Cancer

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Head and neck squamous cell carcinoma (HNSCC) is one of the major worldwide health problems. Despite advances in therapeutic interventions, two major problems of treament failure are recurrence of primary tumor (RPT) and development of second primary tumors (SPT)[1,2]. Malignant transformation and tumor progression are currently thought to be the result of the accumulation of somatic mutations in critical protooncogenes and the tumor suppressor genes. Current evidence suggests that abrogation of normal p53 pathway is a common feature in human cancers, and it appears to be a critical step in the pathogenesis and progression of tumors[3-5]. Furthermore, the frequency of p53 expression or mutation and concurrent genetic instability occured very early from normal cells adjacent to tumor lesions (ANL) and progress to hyperplasia (HYP), dysplasia (DYP), and ultimately to carcinoma of the head and neck. This suggested the association of genetic instability with the loss of normal p53 function and of its potential role in multistep tumorigenesis in H&N cancer[6]. However, association between p53 expression, genetic instability, advanced tumor stages, and poor prognosis remains unclear.

The product of wild-type p53 (wt-p53) gene is a nuclear phosphoprotein that is constitutively expressed in most normal tissues and characterized by a short half-life. Conversely, several mutant p53 proteins that are found in adenomas and carcinomas, are metabolically more stable with a longer half-life, and consequently are present at high concentrations. Such evidence along with availability of monoclonal antibodies (MAbs) that are potentially able to detect wt-p53 and mutant forms made it possible to evaluate the prognostic relevance of p53 levels in a large series of human tumors[7-9]. The correlation between immunohistochemical detection of p53 protein and the presence of mutations in the p53 gene has been investigated in many tumors[10,11]. Currently, chromosome *in situ* hybridization (CISH) has been adapted for use on formalin-fixed, paraffin embedded tissues using non-isotopic chromosome specific DNA probes and enzyme-mediated (e.g. peroxidase) immunochemical procedures[12-15]. This technique allows direct visualization of chromosome changes in normal, premalignant, and tumor tissues without loss of architecture.

In this report, we evaluated p53 expression in the role of genetic instability and of its important role in development of RPT and SPT in H&N cancer.

MATERIAL AND METHOD

Patients. We designed to detect the expression of the p53 protein by IHC, and demonstrate the genetic instability in terms of polysomy index (PI) using CISH. The sixty-nine patients who were selected for this study had presented with primary tumors (excluding recurrence or multiple primary tumors). They received definitive therapy and long-term follow-up. The RPT or SPT were recorded according to the criteria of Warren and Gates[16]. By definition, a SPT of the same histologic type as the first had to be separated from it by more than 2 cm of normal epithelium or had to occur at least 3 years after the diagnosis of the first primary tumor. When the disease had occurred within 3 years or after 3 years from the first diagnosis and was within 2 cm of the normal epithelium, it was considered a recurrence. Any new tumor of a different histologic type was recorded as a SPT without the requirement of separation of more than 2 cm. Seven biopsy specimens of oral mucosa obtained from normal volunteers (cancer-free nonsmokers) were used as normal controls.

Tissue Preparation. Formalin fixed, paraffin embedded tissues of primary tumor specimens were obtained from patients with HNSCC who were treated surgically between 1980 and 1991. Some specimens contained not only carcinoma but also adjacent tissue lesions, and some specimens contained only adjacent tissue without carcinoma. Of the 69 cases, 56 exhibited ANL; 63 HYP; 33 mild DYP (MD), 18 moderate DYP (ModD), 28 severe DYP/carcinoma *in situ* (SD/CIS); and 68 squamous cell carcinomas (SCC). Four μm thick sections were mounted on aminoalkylsilance-coated slides (Histology Control Systems, Glen Head, NY). A cell block section of paraffin-embedded A431 cells, known to express a mutated p53 gene (CGT to CAT at codon 273), was attached to each slide that was prepared for IHC as positive controls. Lymphocytes on each the sections were used as internal negative controls. For CISH, lymphocytes on the same sections served as internal controls. The slides were stored at room temperature.

Probe. A biotinylated classical satellite chromosome 9 (D9Z1) specific for the pericentric heterochromatin of human chromosome 9 and a biotinylated alpha satellite chromosome 17 (D17Z1) specific for centromere of human chromosome 17 (Oncor, Inc., Gaitherburg, MD) were used for ISH. A monoclonal anti-p53 antibody (clone D07; Biogenex Inc., San Romon, CA) was chosen for IHC.

IHC. The IHC procedure was performed as described in detail elsewhere[17,18]. In brief, after deparaffinization and blocking of endogenous peroxidase, 4 μm paraffin sections of primary cancer of HNSCC were reacted with anti-p53 mouse monoclonal antibody-D07 and incubated at 37°C for 2 hours. The slides were then incubated with a biotinylated antimouse secondary antibody (Vector Labs.,Burlingame, CA) and reactivity was visualized with avidin-biotin immunoperoxidases system (Vector) using 0.1 per cent diaminobezidine as the chromogen and 4 per cent methyl green as counterstain. Areas for analysis were selected by the pathologist by comparing the hybridized slides to a corresponding hematoxylin-eosin-stained adjacent section. A minimum of 200 cells from the most positively stained area on each slide was selected and scored; p53 expression was quantitated as per cent positive cells.

CISH. The CISH procedure was performed as described earlier[19] with slight modifications. The specimens were deparaffinized after 65°C overnight incubation and were treated with 1 mg/ml RNase in 2XSSC. Each specimen was digested with 0.4 per cent pepsin (Sigma, St. Louis, MO) in 0.2N HCI. The optimal digestion for each was carefully determined under microsopic examinaton. Endogenous peroxidase activity was blocked with 3 per cent H_2O_2 in methanol for 5 min. The hybridization solution contained 60 per cent formamide in 2XSSC, 5 per cent dextran sulfate, 1 mg/ml salmon sperm DNA, and 0.8 ng/nL biotinylated DNA probes. Twenty to thirty μL of the hybridization solution was applied to each section pending upon the size of section. The probe and target DNA were denatured together at 95°C for 4min for chromosome 17 and at 96°C for 6 min for chromosome 9, with further incubation overnight at 37°C in a sealed wet chamber. The slides were washed in 50 per cent formamide (pH7), and 0.1XSSC (pH7.0). Histochemical detection of probe was performed by immunoperoxidase staining. The slides were incubated for 30 min at 37°C with avidin and biotinylated anti-avidin biotin-peroxidase complex solution (Vectastain ABC kit; Vector Labs., Inc.) Visualization was achieved with 50ml PBS solution containing 50 mg diaminobezidine tetrahydrochloride (Sigma), 35mg of $NiCl_2$, and 10 μL of 30 per cent H_2O_2. The slides were then counterstained with Giemsa stain (0.02%) and mounted in Eukitt (Calibrated Instruments, Inc., Hawthorne, NY), and examined under a light microscope. Areas for analysis were selected by the pathologist by comparing the hybridized slides to a corresponding adjacent hematoxylin-eosin-stained section. At least 200 nuclei were scored in each defined histological area according to the previous described criteria[20]. The total number of signal spots was analyzed under light microscope. A polysomy index (PI) was calculated from the percentage of cells which expressed three or more signal of a chromosome in each nucleus.

Statistical Analysis. Statistical analyses were performed using the unpaired student's *t* test for testing significance. $P<0.05$ was considered statistically significant.

RESULTS

Due to technical problems and limited number of tissue samples, after ISH and IHC procedures, we had 49 ANL, 55 HYP, 30 MD, 16 ModD, 28 SD/CIS, and 60 SCC for chromosome 9; 48 ANL, 51 HYP, 28 MD, 15 ModD, 25 SD/CIS, and 60 SCC for chromosome 17; and 56 ANL, 63 HYP, 33 MD, 18 ModD, 27 SD/CIS, and 68 SCC for p53 protein staining.

EXPRESSION OF P53 PROTEIN

In normal control epithelium, no tissue samples expressed p53 protein.

Evidence for Accumulation of p53 Expression during Multistep Tumorigenesis. Performing IHC, we found not only a gradual accumulation in the fraction of positively stained nuclei (percentage of p53 expressed cells) as tissue abnormalities progressed but also the number of cases. (Fig. 1) As tissue progressed to hyperplasia, dysplasia, and squamous cell carcinoma, the number of cases of p53 expressed cells increased. Overall, 20 of 56 (35.71%) samples of ANL, 25 of 63 (39.68%) HYP, 50 of 78 (64.10%) DYP, and 47 of 68 (69.12%) tumors expressed p53 protein.

Association of p53 Nuclear Reactivity with SPT. Our data demonstrated the higher trend of accumulation of p53 expression in terms of the fraction of positively stained nuclei (%) in the group with SPT. A strong trend was seen in SD/CIS (24.05% in the group without SPT *vs* 35.00 per cent in the group with SPT) and in SCC (31.88% *vs* 39.69%), but no statistical significance was seen. When we assessed the p53 expression by number of cases, we found increment in cases (% case) in the group with SPT seen through histologic changes

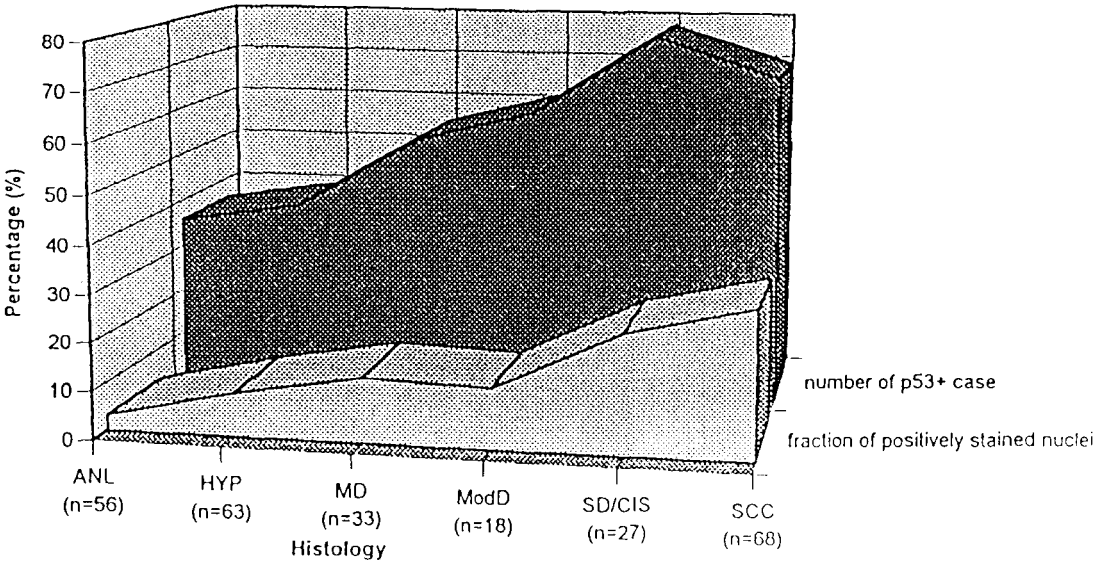

Fig. 1. Demonstration of the fraction of positively stained nuclei (% of p53 expressing cell) and incidence of case (%) during head and neck tumorigenesis.

Table 1. The correlation between p53 expression and second primary tumors during head and neck tumorigenesis.

Histology	No. cases of second primary tumors (% total of each histology)		P value
	p53-	p53+	
Normal adjacent to tumor (n=56)	5 (8.93)	2 (3.57)	<0.100
Hyperplasia (n=63)	7 (11.11)	4 (6.35)	<0.100
Mild dysplasia (n=33)	6 (18.18)	3 (9.09)	<0.100
Moderate dysplasia (n=18)	3 (16.67)	4 (22.22)	<0.400
Severe dysplasia / carcinoma *in situ* (n=27)	1 (3.27)	5 (18.52)	<0.050*
Squamous cell carcinoma (n=68)	2 (2.94)	14 (20.59)	<0.001*

* statistical significance

from 3.75 per cent in ANL to 20.59 per cent in SCC. The inverse was observed in the group without SPT. In SD/CIS and SCC, the p53 expression demonstrated five (18.52% *vs* 3.70%, p<0.50) and seven (20.59% *vs* 2.94%, p<0.001) folds of SPT development respectively over the p53 non-expressed group. (Table 1).

Association of p53 Immunoreactivity with Recurrence. A nonsignificant trend of p53 immunoreactivity was observed in the SCC group.

Table 2. The correlation between p53 expression and recurrence of primary tumor during head and neck tumorigenesis.

Histology	No. cases of recurrence of primary tumor (% total of each histology)		P value
	p53-	p53+	
Normal adjacent to tumor (n=56)	18 (32.14)	10 (17.86)	<0.005
Hyperplasia (n=63)	18 (11.11)	11 (6.35)	<0. 010
Mild dysplasia (n=33)	5 (15.15)	5 (15.15)	1.000
Moderate dysplasia (n=18)	6 (33.33)	8 (44.44)	<0.050
Severe dysplasia / carcinoma *in situ* (n=27)	3 (14.29)	8 (26.63)	<0.025
Squamous cell carcinoma (n=68)	10 (14.71)	24 (35.29)	<0.001

* statistical significance

Table 3. The polysomy index of chromosome 9 and 17 during head and neck tumorigenesis.

Histology	Polysomy Index (± SD)			
	chromosome 9	P value	chromosome 17	P value
Normal adjacent to tumor (n=56)	1.15 (±1.07) (n=49)	<0.001*	1.35 (±1.03) (n=48)	<0.001*
Hyperplasia (n=63)	3.26 (±2.92) (n=55)	>0.100	3.52 (±2.49) (n=51)	>0.100
Mild dysplasia (n=33)	3.77 (±2.98) (n=30)	>0.100	3.90 (±2.47) (n=28)	<0.050*
Moderate dysplasia (n=18)	5.27 (±4.33) (n=16)	<0.001*	9.68 (±8.45) (n=15)	>0.100
Severe dysplasia / carcinoma *in situ* (n=28)	11.38 (±6.47) (n=28)	<0.001*	15.00 (±9.11) (n=25)	<0.050*
Squamous cell carcinoma (n=68)	17.09 (±9.23) (n=60)		19.83 (±11.28) (n=60)	

* statistical significance

There was two (29.63% *vs* 14.29% , p<0.025) and two and a half (35.29% and 14.71%, p>0.001) fold increase in SD/CIS and SCC recurrence fates repectively over the p53 non-expressed group. (Table 2)

GENETIC INSTABILITY

Positive chromosome signals appear as dark dots on interphase nuclei in tissue section. Normal control epithelium from cancer-free, non-smoking individuals showed 0, 1, or 2 signal/nuclei.

Evidence for Increased PI During Multistep Tumorigensis. In contrast to that found in normal control epithelium, using CISH for chromosome 9 and 17, we demonstrated a statistically significant increment of PI from ANL to HYP to DYP to SCC. (Table. 3)

Association of PI and Development of SPT. We divided the tissue specimens to the group that developed SPT and the group that did not. The results showed a higher trend in the group with SPT

but did not reach the level of statistical significance in this sample size. (Fig. 2. A and B)

Association of PI and Development of RPT. We also divided the tissue specimens according to the development of RPT. Our results demonstrated a general trend toward increased PI as the tissue progressed from ANL to HYP to DYP to SCC in the group with RPT. However, they were not statistically significant in our sample size. (Fig. 3. A and B)

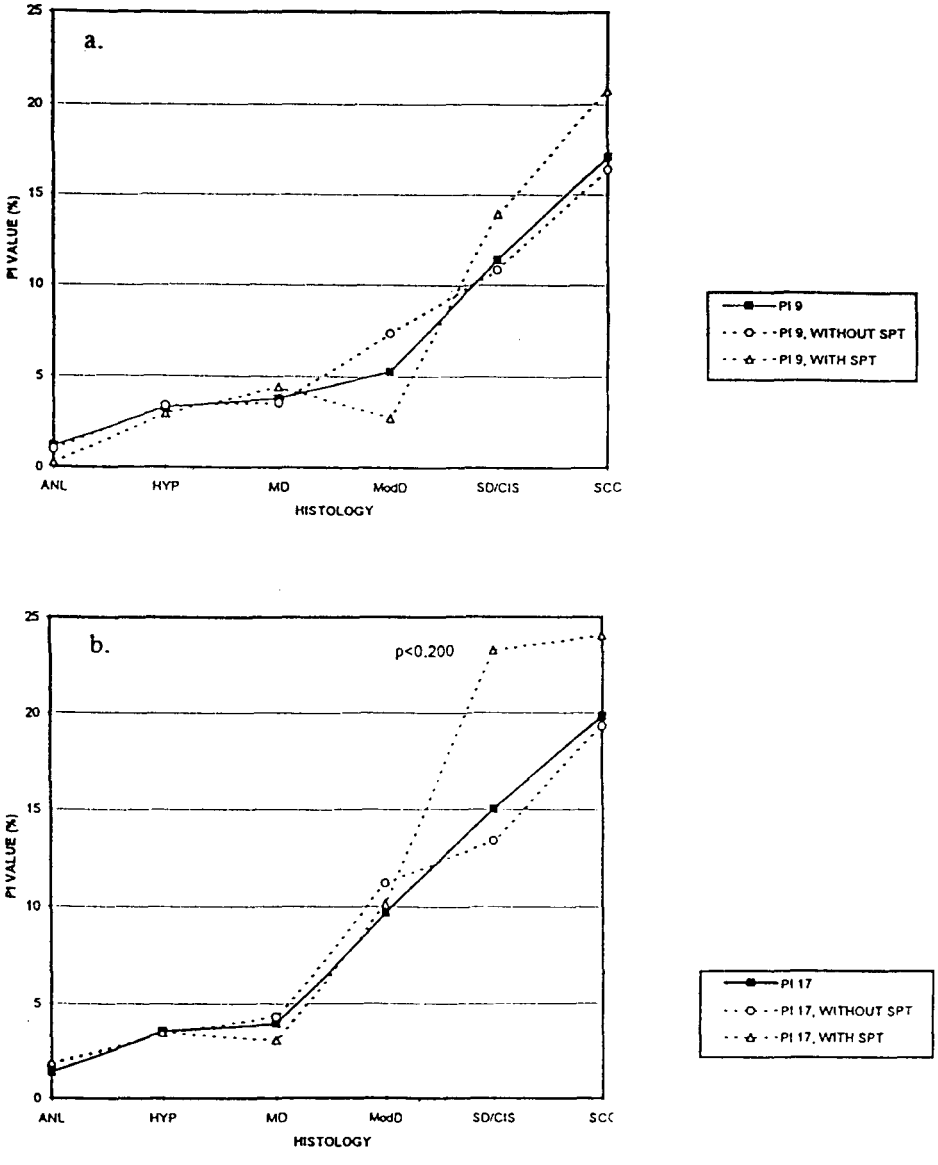

Fig. 2. The correlation between polysomy index (PI) and second primary tumors (SPT) (data is presented by mean).
A. The correlation between PI of chromosome 9 and SPT, there is no statistical significance.
B. The correlation between PI of chromosome 17 and SPT, there is no statistical significance.

CORRELATION OF P53 EXPRESSION AND GENETIC INSTABILITY

We determined the PI in the p53 expressed group and the p53 non-expressed group during multistep tumorigenesis. The results showed a higher trend toward the histologic progression of tissue specimens in the p53 expressed group. However, they did not reach the level of statistical significance in our sample size. (Fig. 4. A and B)

DISCUSSION

We demonstrated the early expression of p53 in the normal and premalignant lesion adjacent to tumors which not only showed a gradual increase

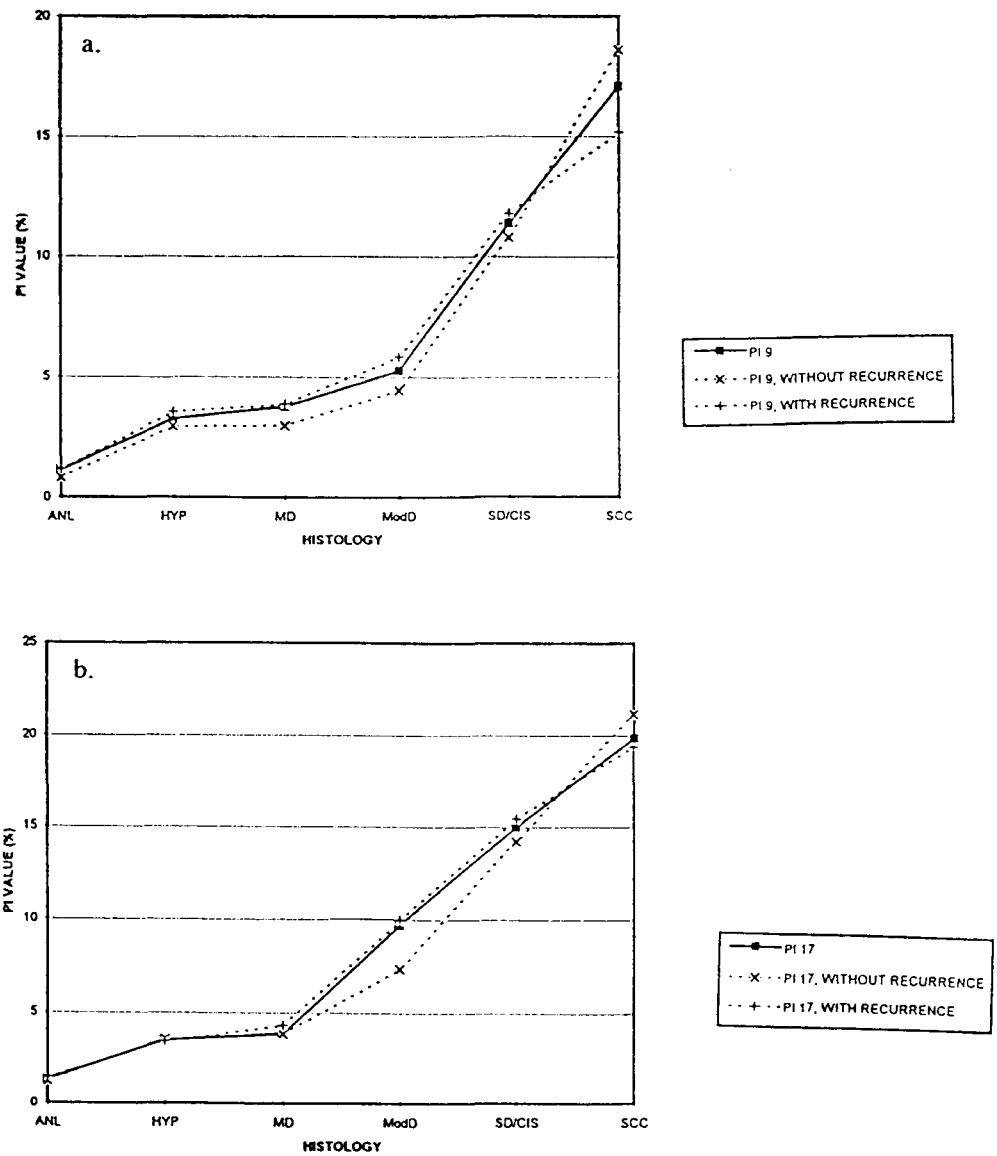

Fig. 3. The correlation between polysomy index (PI) and recurrence of primary tumors (RPT) (data is presented by mean).
A. The correlation between PI of chromosome 9 and RPT, there is no statistical significance.
B. The correlation between PI of chromosome 17 and RPT, there is no statistical significance.

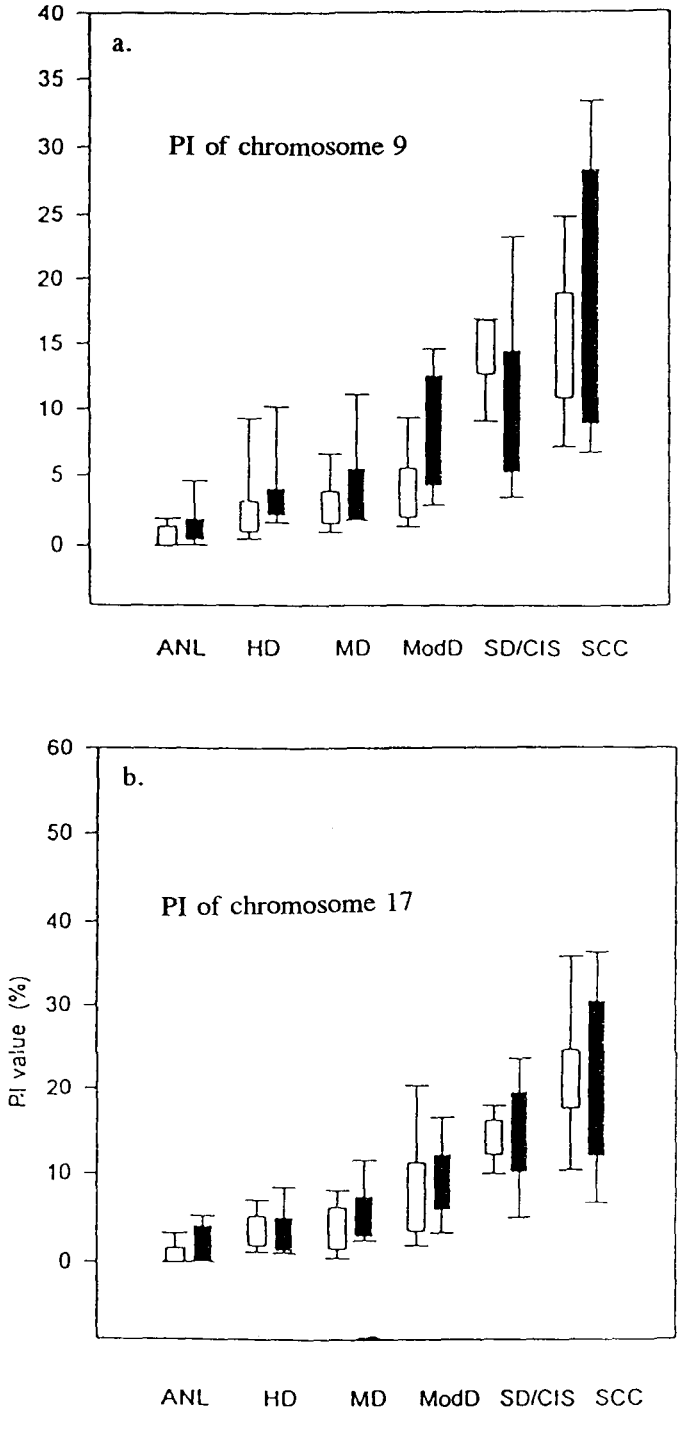

Fig. 4. **The correlation between p53 expression and polysomy index (PI). The opened bars represent the p53 non-expressed group and the closed bars represent the p53 expressed group.**
A. The correlation between p53 and PI of chromosome 9, there is no statistical significance.
B. The correlation between p53 and PI of chromosome 17, there is no statistical significance.

in the fraction of positive nuclei but also the number of cases (Fig. 1). This supports the notion that expression of p53 in the head and neck cancer is an important event[6]. The implication for carcinogenesis is that when p53 function is lost, the cell lacking p53 function becomes genetically unstable and thus predisposed to gross genomic alterations such as gene amplification, aneuploidy, translocations, deletions, and the like. The p53 tumor-suppressor gene fits this observation, being altered in a vast majority of human cancers. The p53 protein acts as a molecular switch that activates a cell-cycle checkpoint in the G1 phase of the cell cycle[21]. When p53 is activated, it transactivates a whole battery of downstream effector genes, whose products are themselves involved in negative growth control. The likely purpose of this cell-cycle checkpoint is to provide the cell with a time window for repair of DNA damage prior to S-phase entry. The loss of p53 function thus creates conditions favorable for genetic instability. However, our results could not demonstrate the statistical significance of correlation between p53 expression and genetic instability during tumor progression (Fig. 4. A and B). One of the reasons for this may be the use of IHC for p53 screening. Some tumors with mutations in p53 gene as detected by more specific techniques (such as, single stand conformational polymorplism analysis (SSCP) or DNA sequencing analysis) had no detectable p53 nuclear accumulation, and substantial proportion of tumors that showed evidence of p53 nuclear reactivity demonstrated no mutations by SSCP and DNA sequencing analysis[10,11]. The mechanisms that induced the loss of p53 function were not just only mutations[22]. The second reason may be that CISH can not demonstrate all varieties of genetic instabilities. It was just used to detect aneuploidy[12-15]. Third, the frequency of aneuploidy may not be the majority of genetic instability, that is observed during tumorigenesis[23,24]. Fourth, our limited number of tissue specimens limited statistical calculations. Fifth, the loss of p53 function is an early event in carcinogenesis of head and neck cancer which created genetic instability and followed by gross genomic alterations. Finally, 50 per cent of head and neck cancer have no p53 expression[6,25], but still demonstrate genomic instability, suggesting that there is/are other pathway/pathways to control genetic instability and tumorigenesis. From our data, we demonstrated the strong correlation between p53 expression and SPT and RPT (Table 1 and 2). The P53 expressed group had a strong tendency for a higher incidence of SPT (20.59% *vs* 2.94% $p<0.001$) and RPT (35.29%; and 14.71% $p<0.001$) than the p53 non-expressed group.

The evidence of genetic instability was observed not only in the malignant regions but also in the normal and premalignant regions adjacent to the tumors. A statistically significant difference was observed throughout the histologic changes. (Table 3) This not only supports the notion of multistep tumorigenesis[26] and field cancerization[27], but also the idea of genetic instability as the driving force behind multistep carcinogenesis[28]. The stepwise accumulation of genetic instability that progressed significantly through ANL to HYP to DYP to carcinoma, suggested its potential role during these steps. Cancer is a multistep process which is initiated by at least two hits to the genome, usually in the form of mutation in growth controls. Cells in which proliferative controls are relaxed or uncoupled from appropriate regulatory cues, as a result of one or more primary hits, have apparently a higher probability for the acquisition of secondary genomic alteration. The predominant view of initiation is that the concomitant gain of growth-promoting oncogene functions along with the loss of negative regulators. (i.e., tumor suppressors) acts to propel the cell toward increasingly aberrant cell-cycle control at the molecular level and an increasingly malignant state at the anatomic level. Secondary genomic alterations include a wide array of chromosomal aberrations including aneuploidy, gene amplification, translocation, and mutation that may in turn lead to the activation of additional cellular oncogenes and the loss of additional tumor suppressors[27]. The attainment of primary plus secondary alterations results in tumor promotion and progression. By CISH, the accumulation of polysomy was observed from our results to have a higher trend in the specimens that developed SPT (Fig. 2. A and B) and RPT (Fig. 3. A and B). This suggested its association with more aggressive disease with poorer prognosis. However, we could not demonstrate a statistical significance from our results. The reasons for this have already been mentioned.

These results suggest that p53 expression and genetic instability have an important role in multistep tumorigenesis of head and neck cancer. There was no correlation between p53 expression and polysomies of chromosome 9 and 17. The p53 expression had a statistically significant correlation

with SPT and RPT. The p53 expressed group had a seven fold increased incidence to develop SPT as well as a two and a half fold increase for RPT, both compared to p53 non-expressed groups. Further studies are needed to demonstrate the correlation between p53 function and genetic instability and to understand their specific roles in head and neck tumorigenesis.

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โปรตีนพี 53 และโพลีโซมีของโครโมโซมที่ 9 และ 17 ในการพยากรณ์โรคมะเร็งของศีรษะและคอ

นวพรรณ จารุรักษ์, พ.บ., ดอง เอ็ม ชิน, พ.บ.**, นรินทร์ วรวุฒิ, พ.บ.***, เจ วาย โร, พ.บ., ปร.ด.****, วอง คิ ฮอง, พ.บ.**, วอลเตอร์ เอ็น ฮิทเทิลแมน, ปร.ด.******

คณะผู้วิจัยศึกษาผู้ป่วยโรคมะเร็งศีรษะและคอจำนวน 69 ราย โดยใช้วิธีอิมมูโนฮิสโตเคมีสทรี (immunohistochemistry) ตรวจย้อมโปรตีนพี 53 และวิธีโครโมโซมอินไซตูไฮบริไดเซชั่น (chromosome in situ hybridization) ตรวจหาโพลีโซมีของโครโมโซมที่ 9 และ 17 ในชิ้นเนื้อมะเร็งปฐมภูมิ (primary tumor) เพื่อตรวจหาความสัมพันธ์ระหว่างโปรตีนพี 53 และโพลีโซมีของโครโมโซมที่ 9 และ 17 ต่อการเกิดมะเร็งปฐมภูมิขึ้นใหม่ (second primary tumor) และการกลับเป็นใหม่ของมะเร็งปฐมภูมิ (recurrence of primary tumor) ในโรคมะเร็งศีรษะและคอ คณะผู้วิจัยพบว่าการย้อมพบโปรตีนพี 53 นี้ สามารถพบได้ตั้งแต่เนื้อเยื่อที่มีเซลล์รูปร่างปกติ เนื้อเยื่อผิดปกติก่อนเป็นเซลล์มะเร็งจนกระทั่งเนื้อเยื่อมะเร็ง โดยมีลักษณะเพิ่มขึ้นทั้งจำนวนสัดส่วนของการย้อมพบในเนื้อเยื่อนั้น ๆ และจำนวนรายผู้ป่วย นอกจากนี้คณะผู้วิจัยพบว่ามีการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติของดัชนีโพลีโซมี (polysomy index) ตามการเปลี่ยนแปลงของเนื้อเยื่อจากบริเวณที่เซลล์มีรูปร่างปกติจนผิดปกติและกลายเป็นเซลล์มะเร็งอย่างเป็นขั้นตอน (multistep tumorigenesis) คณะผู้วิจัยไม่สามารถแสดงนัยสำคัญทางสถิติของความสัมพันธ์ระหว่างการพบโปรตีนพี 53 และการเกิดโพลีโซมีของโครโมโซมที่ 9 และ 17 แต่พบว่ากลุ่มผู้ป่วยที่ตรวจพบโปรตีนพี 53 เกิดมะเร็งปฐมภูมิขึ้นใหม่สูงกว่ากลุ่มผู้ป่วยที่ตรวจไม่พบโปรตีนพี 53 เจ็ดเท่า และมีความเสี่ยงต่อการกลับเป็นใหม่ของมะเร็งปฐมภูมิสูงกว่ากลุ่มผู้ป่วยที่ตรวจไม่พบโปรตีนพี 53 สูงถึงสองเท่าครึ่ง

คณะผู้วิจัยสรุปว่าการพบโปรตีนพี 53 และการเกิดโพลีโซมีของโครโมโซมที่ 9 และ 17 มีความสำคัญต่อการเกิดเป็นโรคมะเร็งศีรษะและคออย่างเป็นขั้นตอน แต่คณะผู้วิจัยไม่สามารถแสดงนัยสำคัญทางสถิติของความสัมพันธ์ระหว่างการพบโปรตีนพี 53 และการเกิดโพลีโซมีของโครโมโซมที่ 9 และ 17 อย่างไรก็ตามการตรวจพบโปรตีนพี 53 นี้ มีความสัมพันธ์กับการเกิดมะเร็งปฐมภูมิขึ้นใหม่และการกลับเป็นใหม่ของมะเร็งปฐมภูมิของโรคมะเร็งของศีรษะและคออย่างมีนัยสำคัญทางสถิติ

คำสำคัญ : การย้อมพบโปรตีน พี 53, โพลีโซมีของโครโมโซมที่ 9 และ 17, การพยากรณ์โรคที่ไม่ดี, โรคมะเร็งของศีรษะและคอ

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