p53 Protein Expression in Cutaneous Squamous Cell Carcinoma

SAUVARAT AUEPEMKIATE, M.D.*,
PLEUMJIT BOONYAPHIPHAT, M.Sc.*,
HUTCHA SRIPLUNG, M.D.*

Abstract

Background: p53 is a nucleoprotein encoded by a tumor suppressor gene. It's mutations are implicated in the genesis of a wide variety of malignant neoplasia including skin cancers.

Objective: To study the expression of the p53 protein in cutaneous squamous cell carcinoma (SCCs) and evaluate the relationships between this expression and sites, varying degrees of differentiation and amounts of apoptotic cells.

Method: Sixty-seven tissue samples of SCCs from Songklanagarind Hospital obtained from January 1991 to December 1996 were examined by immunohistochemistry using polyclonal anti p53-CM1. (Novocastra Laboratories, Newcastle, England, dilution 1:700)

Result: p53 Immunoreactivity was demonstrated in 26.87 per cent of SCCs. This was observed in 15/51 of sun-exposed cases and 3/16 of sun-protected cases ($p = 0.401$). The more differentiated the tumor, the less p53 staining was observed ($p = 0.043$). There was no association between p53 positivity and the amounts of apoptotic cells.

Conclusion: The p53 expression is not related to the sun exposure. It does not represent a commitment to apoptosis. However, it may indicate the differentiation and/or proliferative status of the tumor cells.

Key word : p53, Cutaneous Squamous Cell Carcinoma

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p53 Protein is a 53000-dalton phosphoprotein located in all nuclei. This protein is composed of a sequence of 393 amino acids(1). The gene coding for p53 is a tumor suppressor gene which has been assigned to the short arm of chromosome 17 in the 17p13 region(2). The natural form

* Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

or wild-type of p53 can bind to DNA. This process, in turn, prevents the cells from entering the S phase of the cell cycle[3,4]. In other words, p53 acts as a tumor suppressor by inhibiting DNA replication, regulating transcription, and eventually exerting control over the cell cycle[5,6]. In addition, wild-type p53 is found to be unstable with a short half-life of 20-30 minutes[7,8] and a small quantity below the detection of routine immunohistochemistry is produced[9]. In contrast, a mutant p53 protein has been reported to lose these tumor suppression properties. Comparatively, this mutant is degraded much more slowly and has a longer half-life of 6 to 7 hours. As a consequence, its level which accumulated within the nuclei is high enough to be detectable with immunohistochemistry staining technique[7,8].

Mutation of p53 gene has been found to be involved in the genesis of a wide variety of malignant neoplasm[10,11]. This mutation has been detected in malignant tumors of various organs including the lungs, breast, colon, esophagus, liver, bladder, ovary and brain[12-17]. Furthermore, the mutation of this gene has been observed in many skin lesions such as actinic keratosis, basal cell carcinoma, squamous cell carcinoma, and a small proportion of malignant melanoma[3,18-20]. Several reports have shown the p53 over-expression in invasive squamous cell carcinoma of the skin within a range of 15 per cent to 72 per cent[21-26]. Squamous cell carcinoma is one of the most common skin cancers for which UV radiation is the major epidemiologic risk[27]. In addition, UV-like mutations have been found in the p53 gene[28].

The p53 gene has also functioned as both a repair gene and as a regulator of apoptosis. These two activities appear interrelated. Cells lacking functional p53 are thus unable to repair their DNA damage and/or initiate apoptosis[29]. Even though quantitative correlations between UV-induced p53 mutation, inhibition of apoptosis and the development of squamous cell carcinoma of the skin have been reported, the relationships among these remain unclear[30,31].

In this study, we aimed to detect the expression of p53 protein in cutaneous squamous cell carcinoma (SCCs) using an immunohistochemistry staining technique. In addition, the relationships between this expression and sites, varying degrees of the differentiation of the tumors and amounts of apoptotic cells were evaluated.

MATERIAL AND METHOD

Tissue samples coded as SCCs were collected from the file of Anatomical Pathology Unit, Department of Pathology, Songklanagarind Hospital from January 1991 to December 1996. Sixty-seven specimens of SCCs from the entire body skin with the exception of the genitalia and lips were selected. Metastatic squamous cell carcinoma from other organs were then excluded. Clinical information including sex, age and sites were recorded.

Demonstration of p53 protein expression was performed by immunohistochemical technique as previously described[32]. In brief, the paraffin-embedded tissues were cut, and these sections were deparaffinized, rehydrated and then treated sequentially with 3 per cent H_2O_2, normal goat serum, rabbit polyclonal anti p53-CM1 (Novocastra Laboratories, Newcastle, England, dilution 1:700), biotinylated goat anti rabbit Ig G (1:400) and avidin-biotin peroxidase complex. Antigen-antibody reactions were illustrated by diaminobenzidine (DAB). The slides were then lightly stained with hematoxylin for histology visualization.

A positive result of staining was defined when the nucleus was stained. The staining was quantitated according to the number of positive nuclei per 1000 tumor cells and to intensity. The intensity was graded as follows: 1+ if nuclear staining was present but barely visible, 2+ if a light to medium brown, staining was clearly visible and 3+ if staining was dark brown. The normal skin at the periphery of the tumor was regarded as a control. Apoptotic cells have been referred to dyskeratotic cells which consist of central basophilic pyknotic nuclei and eosinophilic homogeneous cytoplasm[29]. They were counted per 20 high power fields.

Association between variables was initially analyzed by Chi square test and *t*-test, and then multivariate using logistic regression was applied.

RESULTS

The incidence of males was about 2 times higher than females (male = 47, female = 20 cases). Their ages ranged from 14 to 90 years (mean = 62.05 years). The distributions of sites and differentiation of SCCs are shown in Table 1. The lesions were common on the head and face and more common in a sun-exposed area than a sun-protected area. These tumors were divided into three grades based on the degree of nuclear pleo-

morphism and the extent of cytoplasm keratinization. Most of the tumors were well differentiated.

p53 Immunoreactivity was varying in percentage of positivity from 1-99 per cent. Using the cut point of 10 per cent referred by the previous studies,[18,33] p53 staining was positive in18 of 67 cases (26.87%). In poorly differentiated tumors, p53 staining was diffusely distributed in all layers of the epidermis. Within the well and moderately differentiated tumors the staining was concentrated toward the basal cell layer and less observed in the keratinized zones. Intensity of the overall staining was faint (1+) in 8 cases, moderate (2+) in 9 cases and strong (3+) in 7 cases. There was no significant difference between the grading of the tumor with regard to intensity of p53 staining. All normal skin peripheral to the tumors was negative.

The correlations of p53 expression and sites, differentiation and amounts of apoptotic cells are shown in Table 2. In univariate and multivariate analyses, only differentiation of the tumor was significantly associated with p53 expression. The more differentiation of the tumor the less p53 immunostaining was noted. The odd ratios were 2.93, 95 per cent CI 0.57-15.07 and 7.82, 95 per cent CI 1.26-48.35 for moderately and poorly differentiated tumors compared to well differentiated tumor respectively. There was no association of p53 immunoreactivity with sun-exposed/protected skin or with amounts of apoptotic cells.

Table 1. The distributions of site and differentiation of SCCs.

	No.	%
Site		
Sun-exposed	*51*	*76.12*
Head & Face	27	40.30
Foot	20	29.85
Hand	4	5.97
Sun-protected	*16*	*23.88*
Trunk	8	11.94
Arm	3	4.48
Thigh	5	7.46
Differentiation		
Poor	6	8.96
Moderate	8	11.94
Well	53	79.10

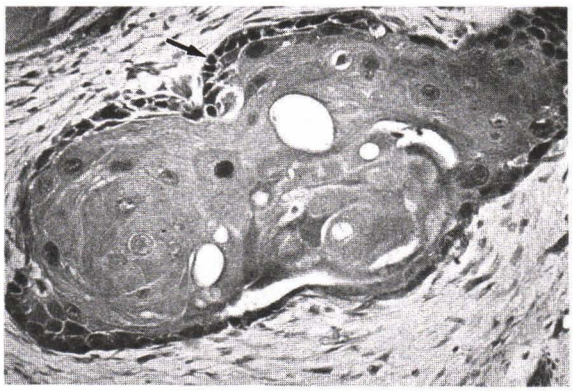

Fig. 1. p53 Expression, brown-colored nuclei, are confined mainly toward the basal layer of well differentiated SCCs.

Table 2. Factors associated with p53.

Characteristics of cases	p53 negative No. of cases	p53 positive No. of cases	p-value
Site			
Sun-exposed	36(70.59%)	15(29.41%)	0.401
Sun-protected	13(81.25%)	3(18.75%)	
Differentiation			
Poor	2	4	0.043
Moderate	5	3	
Well	42	11	
No. of apoptotic cells			
Mean ± S.D.	13.35±20.8	13.5±16.75	0.977

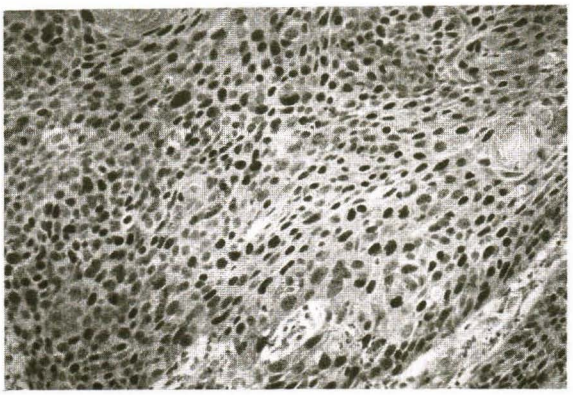

Fig. 2. p53 Expression is present throughout all tumor nuclei of poorly differentiated SCCs.

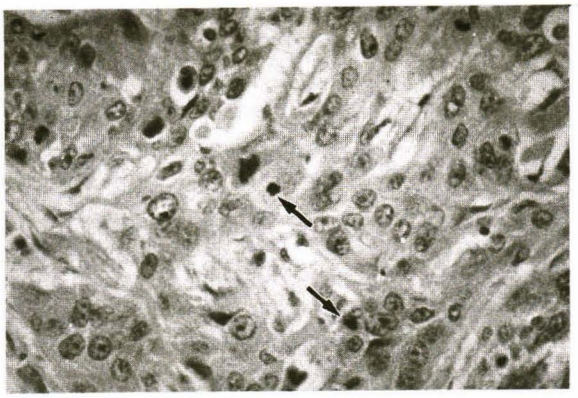

Fig. 3. Apoptotic cells are demonstrated by pyknotic nuclei and homogeneous cytoplasm. (arrows).

DISCUSSION

In this study, SCCs were more common in sun-exposed areas than sun-protected areas. This confirmed the role of UV light as a major carcinogen of SCCs[27]. p53 Staining in our series was expressed in a low percentage (26.87%), however, it was still within the range of other reports (15-72%)[21-26]. This diversity may be due to the different geographical origins, etiology of the tumors studied, sample size, immunohistochemical methods, antibody used or degree of differentiation of SCCs [19,23,25,34]. No difference of p53 over-expression was found in tumors arising from either sun-exposed or sun-protected skin. This finding was similar to the results of other studies[25,33]. Thus, it suggested that UV light may not be the sole factor causing p53 protein over expression. Other carcinogens might also be participants[25]. It was noted that a single large dose of UV radiation[35] obtained in the initiation step, followed by exposure to other tumor-promoting agents might contribute. In addition, the accumulation of wild-type p53 protein might be another possible factor[36].

Our results strongly showed a significant association between greater differentiation of the tumors and less expression of p53. Similar findings have also been reported by previous studies[20,33]. Since mutant p53 can not prevent the cell which has damaged DNA from entering the cell cycle, this will then cause no check point of cell division, thereby resulting in more poorly differentiated cells. Therefore, the presence of p53 expression in a cell may indicate the differentiation and/or proliferative status of that cell.

In this study the positivity of p53 was not correlated with the amounts of apoptotic cells. It may imply that p53 immunoreactivity represents only a part of the cellular repair period. It does not even further represent a commitment to programmed cell death. Alternatively, this supports that apoptosis is not triggered only by p53-dependent pathway but appears to involve several mechanisms[37]. More importantly it is likely that inhibited apoptosis as a consequence of either p53 mutation or p53 independence may be a significant mechanism of tumorigenesis. Although a few studies have shown a strong correlation between positive p53 immunoperoxidases staining and the presence of a mutation in sequencing data,[38] the relation between such over-expression and an actual mutation of the p53 gene is still controversial. Additional molecular analysis of p53 is needed. In this study, the apoptotic cells were counted from H&E stain by recognizing them as dyskeratotic cells. This may not be as accurate as when sophisticated techniques such as electron microscopy or immunohistochemistry are used. However, the agreement of dyskeratotic cells and apoptotic cells demonstrated by TUNEL method has been found[39]. Furthermore, counting the dyskeratotic cells from H&E stain is easy and economical to perform.

In conclusion, our study does not support the crucial role of UV on the expression of p53 pro-

tein. In addition, the negative association between this expression and apoptosis implies that either p53-dependent or independent pathways may account for tumorigenesis and programmed cell death. With regard to the fact that the immunohistochemistry may not represent the actual mutation, further study in molecular analysis is needed.

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โปรตีน p53 ในมะเร็งผิวหนังชนิดสแควมัส

เสาวรัตน์ เอื้อเพิ่มเกียรติ, พ.บ.,*
ปลื้มจิต บุญยพิพัฒน์, วท.ม., หัชชา ศรีปลั่ง, พ.บ.**

วัตถุประสงค์ : เพื่อหาอัตราการตรวจพบโปรตีน p53 ในมะเร็งผิวหนังชนิดสแควมัส และหาความสัมพันธ์ของโปรตีน p53 กับตำแหน่งของโรค พัฒนาการของมะเร็งและจำนวนเซลล์ที่มีลักษณะ apoptotic

วิธีการ : นำชิ้นเนื้อมะเร็งผิวหนังชนิดสแควมัสในโรงพยาบาลสงขลานครินทร์ ตั้งแต่มกราคม พ.ศ.2534 ถึง ธันวาคม พ.ศ.2539 จำนวน 67 ราย มาตรวจย้อมโดยวิธีทางอิมมูโนเคมี โดยใช้ polyclonal anti p53–CM1. (Novocastra Laboratories, Newcastle, England, dilution 1:700)

ผลการศึกษา : พบโปรตีน p53 ในมะเร็งผิวหนังชนิดสแควมัส ร้อยละ 26.87 มะเร็งในบริเวณที่ถูกแสงแดดย้อมติด 15 ใน 51 ราย และบริเวณที่ไม่ถูกแสงแดดย้อมติด 3 ใน 16 ราย ($p = 0.401$) ในมะเร็งที่มีพัฒนาการมาก พบโปรตีน p53 น้อยกว่ามะเร็งที่พัฒนาการน้อย ($p = 0.043$) ไม่พบความสัมพันธ์ระหว่างโปรตีน p53 กับจำนวนเซลล์ที่มีลักษณะ apoptotic

สรุป : โปรตีน p53 ที่ตรวจพบไม่สัมพันธ์กับตำแหน่งของมะเร็งไม่ว่าจะเป็นที่ถูกแสงแดดหรือไม่ และไม่ได้บ่งถึงการเกิด apoptosis แต่อาจเป็นตัวบ่งชี้พัฒนาการหรือการเจริญของเซลล์มะเร็งได้

คำสำคัญ : โปรตีน p53, มะเร็งผิวหนังชนิดสแควมัส

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* ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์, อ.หาดใหญ่, จ.สงขลา 90110