P16^{INK4A} Expression in Bowen's Disease and Bowenoid Papulosis

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Background: *Bowen's disease (BD) is a skin carcinoma in situ occurring over the entire body surface. It shares similar histopathological features with Bowenoid papulosis (BP) of the genitalia, but differs in etiology and clinical course. Increased p16^{INK4A} (p16) tumor suppressor protein expression has been demonstrated in relation to the progression of cutaneous squamous neoplasms.*
Objective: *To evaluate the difference in p16 expression between Bowen's disease and Bowenoid papulosis.*
Material and Method: *Biopsies of 46 cases of BD in the period 1994 - 2003 and 14 cases of BP during 1987 - 2003 in the Anatomical Pathology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Thailand were studied by immunohistochemical methods using the P16 kit (CINTec^{TM} Histology Kit, clone E6H4, Code-Nr. K5334, DakoCytomation, Denmark). Nuclear/cytoplasmic immunoreactivity in more than 10% of neoplastic cells was considered positive.*
Results: *P16 expression was positive in 37 of 46 BD cases (80.4%) which was higher than that of BP (6 of 14 cases or 42.9%) (p value < 0.05, Chi-square test). The expression among the three groups of BD: extragenital (28 of 35), chronic arsenical-related (7 of 8) and genital lesions (2 of 3) was not significantly different (p value = 0.734, Chi-square test).*
Conclusion: *P16 expression was more frequent in BD than BP. This suggests a possible association between p16 expression and tumorigenesis of these lesions.*

Keywords: *P16^{INK4A}, Bowen's disease, Bowenoid papulosis*

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Bowen's disease (BD) is a form of Carcinoma in situ of the epidermis. It can progress to invasive squamous cell carcinoma (SCC), which is one of the most common cutaneous cancers[1-3]. Normally, BD presents as a discrete, slowly enlarging erythematous, well-demarcated plaque with scale and crust varying in size from a few millimeters to several centimeters[4]. It can be found on all parts of the body including the genitalia, particularly of an older age group. Sun exposure, arsenic exposure, ionizing radiation, immunosuppression [and] certain types of human papillomavirus (HPV) have been implicated in the etiology of BD. It has been reported that anogenital Bowen's disease has a strong association with HPV type16[5]. The histopathology of BD shows full thickness dysplasia. Atypical pleomorphism and hyperchromatic keratinocytes have been found to distribute throughout the epidermis with disordered maturation, atypical mitosis and complete disorganization of the epidermal architecture.

Bowenoid papulosis (BP) is characterized clinically by the presence of reddish brown pigmented verrucous papules and plaque on genitalia[3,6,7]. Histopathology reveals a Bowen's disease-like pattern. Its important cause is HPV infection, especially types 16, 18, 31-34, 42 and 51-54[5,6,8-10]. It is usually found in sexually active young males and females[1,5]. Spontaneous regression of this lesion is considered common[11],

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although in rare cases it may progress to invasive SCC[12].

$P16^{INK4A}$ (p16), a tumor suppressor protein, can inhibit cyclin-dependent kinase (CDK) 4 and 6 which regulate the G1 checkpoint of the cell cycle and then suppress cellular proliferation[13-15]. Functional or structural loss of p16 could therefore, lead to all cell cycle propagation of potential genetically damaged cells and subsequent risk of tumor development[16-18]. Increased p16 expression has been demonstrated in cervical squamous neoplasms as lesions progress from premalignant to malignant phases of proliferation and is correlated with high risk type of HPV[19,20].

In skin, increased expression of p16 during the progression from actinic keratosis to in situ SCC to invasive SCC has been demonstrated[21-23]. Furthermore, this expression corresponds to the presence of atypical keratinocytes. In the present study the authors aimed to evaluate the difference between p16 immunohistochemical staining of BD and BP which share similar histopathological features, but differ in etiology and clinical course.

Material and Method

Tissue samples coded as BD, SCC in situ were collected from January 1994 to December 2003 and BP samples were collected from January 1987 to December 2003 from the files of the Anatomical Pathology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Thailand. The authors used a longer collection period for BP than BD because there were fewer BP cases.

Clinical and demographic data were retrieved from patient files. Second excisions of the residual tumors were excluded. Clinical features and all hematoxylin-eosin stained sections were reviewed and categorized into 2 groups as BD and BP. BD cases were selected from the skin lesion on any part of the body having a full thickness histological pattern of dysplasia with disordered maturation, loss of polarity and disorientation, accompanied by parakeratosis and hyperkeratosis. BP cases consisted of lesions clinically characterized by hyperpigmented papules on the genitalia and having a histological feature of full thickness dysplasia. Cases in which the differential diagnosis between BD and BP was doubtful were excluded.

Immunohistochemistry

P16 protein expression was immunohistochemically examined by a ready-to-use P16 kit (CINTec™ Histology Kit, clone E6H4, Code-Nr. K5334, DakoCytomation, Denmark). Briefly, five-micron sections of formalin-fixed, paraffin-embedded tissue were deparaffinized and rehydrated. The sections were subjected to high temperature epitope retrieval by microwaving in epitope retrieval solution. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The sections were then incubated with a 1:150 primary mouse anti-human p16 antibody overnight. The visualization was made by incubation with dextran polymer conjugated with horseradish peroxidase and was further developed with diaminobenzidine (DAB) then were counterstained with hematoxylin. Appropriate positive and negative controls were run with each staining batch.

Evaluation of p16 staining

Either nuclear or cytoplasmic brownish staining indicated a positive result. By low power filed, the well stained area was selected and then the reactivity was assessed in 1000 consecutive tumor cells. The percentage of positive cells was recorded. Intensity was scored from 1-3 with 3 indicating the most intense dark brown stain. The normal skin next to the tumor was regarded as a control. For statistical analysis, cases with more than 10% staining were considered positive. The Chi-square test was used to assess the association between variables.

Results

The present study included 46 cases of BD and 14 cases of BP. Three cases were excluded due to doubtful diagnosis. Clinical characteristics of cases are shown in Table 1. It was found that both groups of patients had wide age ranges. Among the 46 cases of BD, 35 cases were obtained from extragenital sites and were not associated with arsenic exposure. Eight cases were chronic arsenical-related and 3 cases were genital BD.

Immunoreactivity to p16 of BD and BP was found both in the nucleus and in the cytoplasm (Fig. 1). The intensity was related to the percentage of positive staining. The high percentage, positive cases (more than 25% stained cells) showed both nucleus and cytoplasmic staining while the lower ones showed predominantly cytoplasmic staining. More than half of the cases showed heterogeneity of staining.

P16 expression was positive in 37 of 46 BD cases (80.4%), and 6 of 14 BP cases (42.9%). This difference was statistically significant (p value = 0.006, Chi-square test) (Table 2). Among the three subgroups of BD, p16 expression was positive in 28 of 35 extra-

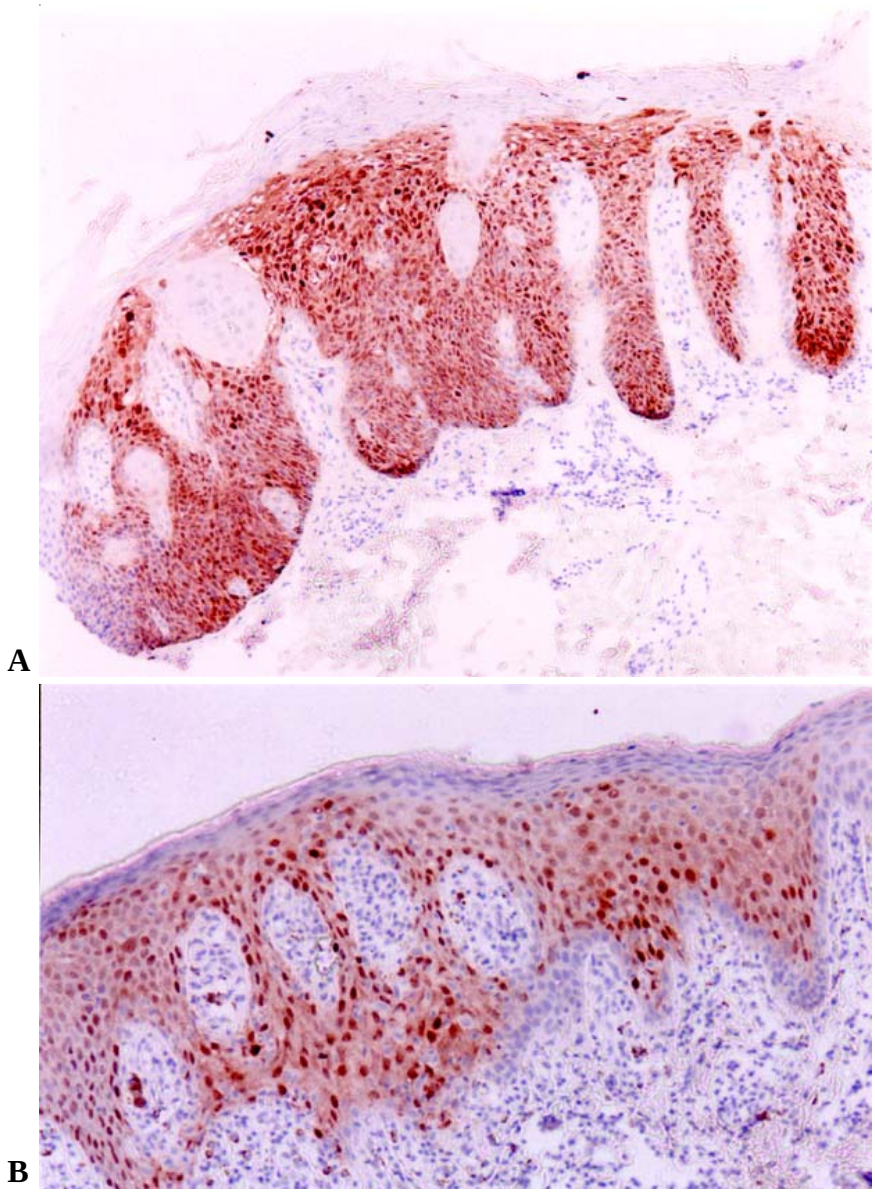

Fig. 1 P16 expression in Bowen's disease (A) and Bowenoid papulosis (B). The mmunoreactivity is found on both nucleus and cytoplasm

Table 1. The characteristics of Bowen's disease and Bowenoid papulosis

Variables	Bowen's disease (n = 46)	Bowenoid papulosis (n = 14)
Age : median (range) years	47.5 (31-97)	30.5 (19-75)
Sex, male: female	23:23	5:9
Sites		
Extragenitalia: no arsenic exposure	35	-
Extragenitalia: arsenical-related	8	-
Genitalia	3	14

Table 2. P16 expression in Bowen's disease and Bowenoid papulosis

Types of lesions	P16-	P16+	p value
Bowenoid papulosis (BP)	8 (57.1)	6 (42.9)	0.006*
Bowen's disease (BD)	9 (19.6)	37 (80.4)	
BD extragenital: no arsenic exposure	7 (20.0)	28 (80.0)	0.734**
BD extragenital: arsenical-related	1 (12.5)	7 (87.5)	
BD genital	1 (33.3)	2 (66.7)	

p value of Chi-square test
* Testing between BD and BP, ** Testing among subgroups of BD.
Parenthesis indicates percentage of total cases

genital lesions (80.0%), 7 of 8 chronic arsenic lesions (87.5%) and 2 of 3 genital lesions (66.7%). The expression among these 3 groups was not significantly different (p value = 0.734, Chi-square test). In addition, among the 35 cases of extragenital BD, the expression in sun-exposed and sun-protected sites (10/13 versus 18/22) showed no significant difference (p value = 0.726).

P16 expression on normal squamous epithelium next to the tumor was negative in all cases.

Discussion

BD and BP sometimes share similar histopathological features, but carry different etiologies and prognosis. Immunohistochemical staining for p16 protein, which has been demonstrated to correlate with progression of the tumor was used as a marker to distinguish between these two entities. The authors hypothesized that BD would have higher frequency of p16 expression compared to BP. The present results indicated that BD had 2 times higher frequency of p16 expression than BP and this confirmed the authors' hypothesis. To date, there has been no specific factor to predict the tendency of malignant transformation of both BD and BP. From the present data, it could be postulated that the p16 positive cases may predict subsequent cancer development. Therefore, evaluation of the outcome of BD and BP patients correlated with p16 expression should be further studied.

In the present study, a high proportion of BD demonstrated p16 expression. This result was consistent with other studies. Salama et al demonstrated p16 immunostaining in 90 of 107 (84.1%) BD cases[22]. Hodges and Smoller demonstrated p16 expression in approximately 90% of in situ SCC and 100% of invasive SCC[21]. It has been reported that the etiology of BD and invasive SCC of the skin is multifactorial, including UV light, chemical agents such as arsenic and certain types of HPV in periungual and anogenital lesions. The development of a sun-induced squamous neoplasm is the result of a complex sequence of events initiated by exposure to UV radiation. The high frequency of p16 expression in cutaneous BD and invasive SCC may therefore indicate the contribution of defective p16 tumor suppressor gene activity in the UV carcinogenesis pathway. The demonstration of multiple mutations including in-frame deletion, and frame-shift and nonsense mutations of the INK4a locus support this hypothesis[24]. Defects of the p16 gene may also be involved in arsenic-related carcinogenesis. Inorganic arsenicals alter the methylation patterns and the expression of p16 gene in BEP2D cells, suggesting that the hypermethylation of p16 gene CpG islands may be one of the mechanisms of carcinogenesis caused by inorganic arsenicals[25]. HPV 16 and 18, the high-risk type, are also proposed to be etiologies of defective p16 activity[17]. It has been reported that expression of p16 correlated with the presence of high-risk HPV in preinvasive and invasive cervical neoplasm[19]. This evidence might explain the high frequency of p16 expression in BD over the entire body and of chronic arsenical-related cases in the present study. This may imply that alteration of p16 function in cutaneous squamous premalignant and malignant lesions results from a variety of etiologic-induced tumorigenesis pathways.

It has been shown that BP can be induced by either high-risk or low-risk HPV infection[5,6,8-10]. An increase in cervical intraepithelial neoplasm p16 expression has also been demonstrated in relation to the presence of high-risk HPV[19,20]. However, a low proportion of p16 expression was found in BP group. This suggests that the presented BP cases are likely to be associated with low-risk rather than high-risk HPV. In order to clarify this situation, the types of HPV in Thai BP need further investigation.

In conclusion, p16 expression was more frequent in BD than in BP. The high expression among BD subgroups may result from various etiological pathways. The low expression in BP suggests that the presented BP may be associated with low-risk rather than high-risk HPV. These suggest a possible association between p16 expression and tumorigenesis of these lesions.

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การแสดงออกของโปรตีน P16^{INK4A} ในมะเร็งผิวหนังระยะเริ่มต้น Bowen's disease และรอยโรค Bowenoid papulosis

เสาวรัตน์ เอื้อเพิ่มเกียรติ, ปารมี ทองสุกใส, ปลื้มจิต บุณยพิพัฒน์

วัตถุประสงค์: *Bowen's disease (BD) เป็นระยะเริ่มต้นของมะเร็งผิวหนังที่เกิดขึ้นทั่วร่างกาย มีลักษณะทางพยาธิวิทยาคล้ายรอยโรค Bowenoid papulosis (BP) ที่อวัยวะเพศ แต่สาเหตุและลักษณะทางคลินิกต่างกัน พบว่าการแสดงออกของโปรตีน P16^{INK4A} (p16) เพิ่มขึ้นสัมพันธ์กับความรุนแรงของมะเร็งผิวหนัง การศึกษานี้ เพื่อหาความแตกต่างของการแสดงออกของโปรตีน p16 ในรอยโรคทั้งสอง*

วัสดุและวิธีการ: *นำชิ้นเนื้อมะเร็งผิวหนังระยะเริ่มต้น BD จำนวน 46 ราย ตั้งแต่มกราคม พ.ศ. 2537 ถึงธันวาคม พ.ศ. 2546 และรอยโรค BP จำนวน 14 ราย ตั้งแต่มกราคม พ.ศ. 2530 ถึงธันวาคม พ.ศ. 2546 จากภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ มาตรวจย้อมโดยวิธีทางอิมมูโนเคมี โดยใช้น้ำยาสำเร็จรูป P16 (CINTec^{TM} Histology Kit, clone E6H4, Code-Nr. K5334, DakoCytomation, Denmark) การแสดงออกของ p16 ในนิวเคลียสและ/หรือไซโตพลาสมที่มากกว่าร้อยละ 10 ถือว่าเป็นผลบวก*

ผลการศึกษา: *พบการแสดงออกของ p16 ใน BD 37 ใน 46 ราย (80.43%) และใน BP 6 ใน 14 ราย (42.86%) (p value < 0.05, Chi-square test) การแสดงออกของ p16 ใน BD ที่นอกอวัยวะเพศ (28 ใน 35), ที่สัมพันธ์กับการได้รับสารหนู (7 ใน 8)และที่อวัยวะเพศ (2 ใน 3) ไม่แตกต่างกัน (p value = 0.734, Chi-square test)*

สรุป: *การแสดงออกของ p16 ใน BD มากกว่าใน BP ทำให้สันนิษฐานได้ว่า การแสดงออกของ p16 น่าจะสัมพันธ์กับกลไกการเกิดโรค ที่ต่างกันของรอยโรคเหล่านี้*