Decreased Expression of Xeroderma Pigmentosum Group C (XPC) Protein in Cutaneous Squamous Cell Carcinoma

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Abstract: Cutaneous squamous cell carcinoma (SCC) is the second most common form of malignant tumors in humans and the incidence rate is still increasing. Actinic keratosis (AK) is a pre-malignant neoplasm within the continuum of SCC. However, the actual etiology and carcinogenesis of an individual AK progressing to invasive SCC is still remains unclear. Xeroderma pigmentosum group C (XPC) is the first DNA repair protein to recognize DNA damage site during nucleotide excision repair process, but the evidence of XPC in carcinogenesis from AK to SCC is sparse. In this study, we investigated the protein expression of XPC by immunohistochemistry in 349 cases of SCC, AK, and normal epidermis. The intensity of nuclear XPC expression was significantly lower in SCC compared with adjacent normal epidermis, pre-cancerous AK, and normal epidermis. Decreased XPC expression was also associated with recurrent rate and high-risk SCC. We suggest that attenuated XPC protein expression might be a prognostic marker for tumor recurrence in SCC.
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1. Introduction

Cutaneous squamous cell carcinoma (SCC) is the second most common human cancer [1,2], and the incidence is around 22.65/100,000 to 60.2/100,000 [3]. Ultraviolet (UV) irradiation, radiation, immunosuppressants, virus, scars, burns, toxins such as arsenic are the risk factors [4]. SCC is generally more aggressive, and potentially lifethreatening, than basal cell carcinoma [5]. A study from Australia estimated the fatality rate at 4%–5%, whereas U.S. studies suggest a 1% rate [6,7]. Actinic keratoses (AK) are pre-malignant neoplasm and are the most common neoplasms within the continuum of squamous cell carcinoma. An estimation of individual AK progressing to invasive SCC varies from as low as 0.025% to as high as 16% [1], but the actual risk of an individual AK progressing to invasive SCC still remains unclear.

Xeroderma pigmentosa is an autosomal recessive photosensitivity syndrome and strong evidence of the role of DNA repair in cancer susceptibility is derived from studies of xeroderma pigmentosum patients who have an incidence of skin cancers approximately 1000 times that of the general population. Eight XP complementation groups (A~G, and a variant) have been distinguished (corresponding to mutations in distinct genes involved in nucleotide excision repair) [8]. Xeroderma pigmentosum group C (XPC) is the main damage-recognition protein responsible for nucleotide excision repair of UVB damage to DNA, and the high incidence of skin malignancies in XP-C patients suggests that loss of expression of XPC protein might also provide a selective advantage for initiation and progression of similar cancers in non XP-C patients in the general population [9]. There is a paucity of knowledge of XPC protein expression in carcinogenesis of AK progressed to SCC. This study is to investigate the role of XPC protein in SCC, AK, and normal epidermis.

2. Methodology

2.1 Sample collection

Formalin-fixed paraffin embedded (FFPE) tissues cutaneous squamous cell carcinoma (SCC), pre-cancers: actinic keratosis (AK) and normal epidermis (NE) were obtained from department of dermatology and pathology, Chung Shan Medical University Hospital. Histological sections of all were reviewed and the diagnoses were confirmed by two pathologists. Clinical information was extracted from the medical records. All patient data were de-identified. Normal epidermis specimens were obtained from the facial benign subcutaneous tumors: neurofibroma, epidermal cyst, lipoma, and we excluded those patients with malignancy. The study was approved by Chung Shan Medical University Hospital (IRB No: CS11077) institutional review board. A total of 349 tissue samples were used for this study: SCC (n=92), SCC paired-adjacent

epidermis (SCC-N)(n=92), AK (n=37), AK paired-adjacent epidermis (AK-N)(n=37), and normal epidermis (n=91) were collected. Based on the clinical presentation and histological examination, the SCC group was divided into two groups (high risk and low risk). High risk SCC was defined as recurrent aggressive histological subtypes of SCC, such as a prior history of ⩾ 3 non-melanoma skin lesions, tumor size > 2cm, perineural infiltration, metastasis, and tumor at periorbital area and lip area.

2.2. Immunohistochemical Analysis

Immunohistochemical studies were performed on 5-mm thick sections of formalin-fixed paraffin-embedded tissue. Antigen retrieval was carried out with heat-induced epitope retrieval buffer. The slides were stained on a DAKO Autostainer using primary antibodies against XPC (LSAB Kit K675, Dako, USA). Positive XPC staining was noted by ascertaining nuclear expression and any cytoplasmic staining was considered background artifact. The immunohistochemical stains were reviewed and the staining was evaluated by intensity (SI) and percentage of positive cells (PC) using the following scale per previously established protocols.(Gonzalez LO 2010; Muller M 2010). Briefly, in terms of SI, the scales are 0 (no staining), 1+ (minimal staining), 2+ (moderate staining), and 3+ (strong staining) and in terms of PC, 0 (no staining), 1+ (less than 25% of cells), 2+ (26–50% of cells), and 3+ (more than 51% of cells). A total score (TS) was calculated by adding the SI and PC scores, and the mean of the TS was used for statistical analysis Proportion scoring was performed only if the intensity of tumor cells staining was more than that of the internal controls limiting errors in semiquantitation as a consequence of nonspecific background staining.

2.3 Statistical analysis

All experiments were performed in triplicate and presented as means ± SD. Statistical analyses were performed using oneway analysis of variance (ANOVA) followed by Tukey post hoc test (SPSS 17.0 software) to determine significant differences among the groups. The difference was considered significant when $p<0.05$.

3. Results

3.1 Demographic data

In this study, the mean age was 72.8, 75.2, 39.9 for SCC, AK, and normal epidermis group, respectively. SCC samples were graded using Broders' classifications [10,11]. For AK, a three-tiered keratinocytic intraepithelial neoplasia (KIN) grading was used [12]. The clinicopathological characteristics are summarized in Table 1.

Table 1 Clinic-pathological characteristics of squamous cell carcinoma, actinic keratosis and normal epidermis

Diagnosis	n	Gender	Age*	Tumor grade
SCC	92	47male; 45female	72.8±12.9	Broders'1:5 Broders'2:47 Broders'3:40
AK	37	15male; 22female	75.2±10.3	KIN 1:13 KIN 2:13 KIN 3:11
NE	91	48male; 43female	39.9±14.2	

SCC: squamous cell carcinoma; AK: actinic keratosis; NE: normal epidermis; *mean±SD

3.2 Immunohistochemistry data

In tumor cells, mean total score of XPC was as follows: SCC= 2.11 (SD, 1.3), SCC-N = 5.14 (SD, 0.90), AK = 5.08 (SD, 0.76), AK-N= 5.86 (SD, 0.35), and NE = 5.60 (SD, 0.61). Statistically significant differences were observed between the following groups: SCC versus SCC-N ($P < 0.0001$) and SCC versus AK and NE ($P< 0.0001$ and $P < 0.0001$, respectively) (Fig. 1). There were no statistically significant differences between the other groups: SCC-N versus AK, AK-N, NE (P= 0.097, P=0.061, P=0.071, respectively); AK versus AK-N, NE (P= 0.054, P=0.083, respectively); AK-N versus NE (p=0.587).

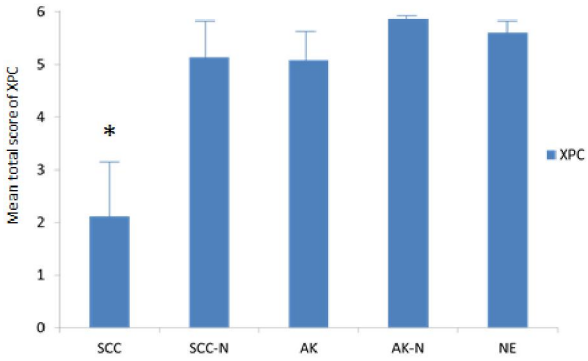

Fig. 1 Nuclear expression of XPC in SCC, SCC paired-adjacent epidermis (SCC-N), AK, AK paired-adjacent epidermis (AK-N), and normal epidermis (NE). *$p<0.05$.

XPC protein was observed in almost all of normal epidermis specimens, and the expression of XPC was mostly located in the low third of epidermis, especially in the basal layers (Fig. 2E).

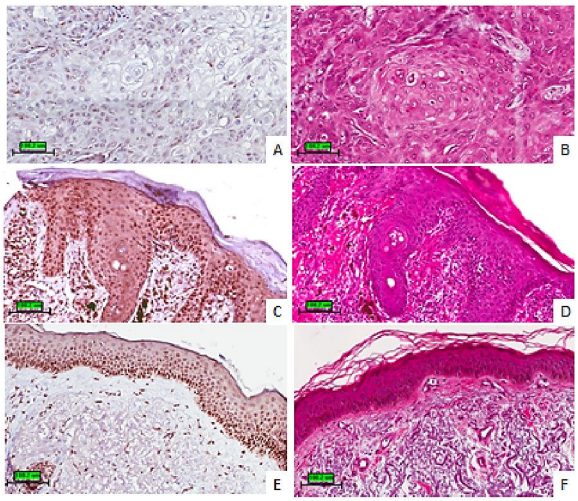

Fig. 2 Decreased XPC expression in cutaneous squamous cell carcinoma were detected by immunohistochemical studies. A, C, E = XPC expressions in SCC, AK, and normal epidermis, respectively. B, D, F = hematoxylin–eosin staining in SCC, AK, and normal epidermis, respectively.

Table 2 Xeroderma pigmentosum group C (XPC) protein in cutaneous squamous cell carcinoma

	Positive (n)	Negative (n)	p-value
Age			
＜65	16	5	0.775
≧65	44	27	
Sex			
Male	13	34	0.282
Female	19	26	
Location			
Sun exposure	21	40	0.928
Non-sun-exposure	11	20	
Differentiation			
Poor	15	25	0.757
Moderate	16	31	
Well	1	4	
Recurrence			
No	31	43	0.046
Yes	1	17	
Metastasis			
No	32	58	0.391
Yes	0	2	
Risk			
Low	26	21	0.033*
High	11	34	

*Compared mean total score of XPC protein between high risk group vs. low risk group, $p<0.05$

The correlation of XPC expression with clinicopathologic features was analyzed. Decreased expression of XPC was significantly associated with recurrence (p=0.046) and high risk (p=0.033) in SCC (Table 2). However, XPC expression was not associated with age, gender, differentiation, and metastasis.

4. Discussion

DNA repair plays a fundamental role in the maintenance of genomic integrity. The ability to repair DNA is quite variable within human populations [13], and decreased DNA repair has been associated with increased risk of a variety of human neoplasms, including skin cancer [14]. Xeroderma pigmentosum group C (XPC) is a general sensor of damaged DNA and inactivating XPC mutations are associated with xeroderma pigmentosa and an extremely high risk of skin cancer [15,16]. Our study detected that the intensity of nuclear XPC expression was significantly lower in SCC compared with adjacent normal epidermis, pre-cancerous AK, and normal epidermis. Attenuated XPC expression was associated with recurrence and high-risk SCC. Similar results were reported by de Feraudy *et al*. that XPC inactivation is a frequent target in squamous cell carcinomas [9,17], and several hypotheses were proposed [9]. Our sample size is larger than de Feraudy *et al*. and we found that XPC is significantly lower in recurrent SCC and high risk SCC. We proposed that attenuated XPC protein might be a prognostic factor in SCC, and be worth further investigation.

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