

Xeroderma Pigmentosum Complementation Group C Protein (XPC) Expression in Basal Cell Carcinoma

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Abstract. *Background: The Xeroderma pigmentosum complementation group C protein (XPC) is a general sensor of damaged DNA. Individuals carrying a mutation in XPC genes exhibit marked photosensitivity and increased occurrence of skin cancers. Little is known about the distribution of XPC protein in basal cell carcinoma (BCC). Aim: To determine whether the XPC protein is associated with basal cell carcinoma. Materials and Methods: In the present study, we investigated the protein expression of XPC by immunohistochemistry in 86 cases of BCC and paired-adjacent normal epidermis. Results: The intensity of nuclear XPC expression was significantly higher in BCC compared to adjacent normal epidermis ($p < 0.001$). Attenuated XPC expression was associated with high-risk BCC ($p = 0.045$) but was not significantly associated with age, gender and body area. Conclusion: Our results indicate that XPC is associated with BCC and further studies are warranted to determine if the XPC-BCC interaction is specific to just one cancer cell type and to investigate potential mechanisms.*

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Key Words: Xeroderma pigmentosum complementation group C protein (XPC), Basal cell carcinoma (BCC), skin cancer.

Basal cell carcinoma (BCC) is the most common human cancer and its incidence is increasing (1, 2). BCC is primarily caused by exposure to ultraviolet radiation but there are additional risk factors, such as ionizing radiation, chemical toxins, arsenic, immunosuppression and human papilloma virus (3, 4). Most BCCs are non-metastatic but there are incidents where BCC has metastasized (3).

Xeroderma pigmentosa (XP) patients have an autosomal recessive inherited *XP* gene mutation responsible for susceptibility to UV-induced skin cancers (5). Seven XP complementation groups (*XPA*, *XPB*, *XPC*, *XPD*, *XPE*, *XPF*, *XPG*) and a variant group (*XPV*) have been identified (corresponding to mutations in distinct genes involved in nucleotide excision repair) (6). The XPC group is the most prevalent complementation group in Europe, United States, and North Africa (6). Xeroderma pigmentosum group C (XPC) is a DNA damage recognition protein that functions in the early recognition of nucleotide excision repair (NER) and binds to damaged DNA at a very early stage during DNA repair (7). Recent reports suggest that XPC also stimulates repair of oxidative lesions by base excision repair (7), and several investigations demonstrated that a *XPC* mutation may be involved in certain types of cancer (8-11). The purpose of the present study was to determine if the XPC protein is associated with basal cell carcinoma.

Materials and Methods

Study samples. This study was approved by the Chung Shan Medical University Hospital (IRB No CS11077) Institutional Review Board. The tissue specimens from the Chung Shan Medical University Hospital pathology laboratory files from 2004 to 2012 were examined and diagnoses were confirmed by two pathologists. A total of 86 tissue samples were used for this study: BCC (n=86)

and paired-adjacent normal epidermis (n=86). Based on the clinical presentation and histological examination, the BCC group was divided into two groups (high-risk and low-risk). High-risk BCC was defined as recurrent aggressive histological subtypes of BCC, such as a prior history of ≥ 3 non-melanoma skin lesions, infiltrative type, basosquamous type, tumor size > 2 cm and tumor at the periorbital area. There were no nodal-involved BCC or metastatic BCC in this study. Clinical information was obtained from the medical records. All patient data were de-identified.

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, Copenhagen, Denmark). Appropriate positive and negative controls were included. Positive XPC staining was noted by ascertaining nuclear expression, whereas any cytoplasmic staining was considered background artifact. XPC-positive tumor cell nuclei were evaluated by light microscopy. Patients were classified into either a positive group, with $> 10\%$ positive cancer cells in the tissue, or a negative group, with $< 10\%$ positive cancer cells.

Statistical analysis. Statistical analysis was performed using the SPSS statistical software program (Version 11.0 SPSS Inc., Chicago, IL, USA). Statistical analysis was carried out using analysis of variance (ANOVA) and the Student's *t*-test. A 1-way ANOVA was performed to assess if there was any difference among the groups and, also using a 1-way nonparametric ANOVA, in case the data might not be considered normally or symmetrically skewed. Regression was also assessed to determine if there was a linear trend across the groups with significant values of $p < 0.05$.

Results

Sample demographics. Out of the 86 BCC patients, 49 (57%) were men. Patients had a mean age of 67.3 ± 12.7 . BCC subtypes were: noduloulcerative (72%), pigmented (9%), superficial (11%) and infiltrative (8%). In the BCC group, 78 (91%) of the lesions were located at the sun exposure site and 26 (30%) were at high-risk, as shown in Table I.

Increased XPC protein levels in basal cell carcinoma compared to adjacent normal skin. XPC protein was observed in almost all BCC specimens and the expression of XPC in normal epidermis was mostly located in the basal layers (Figure 1). Seventy three (84.9%) BCC and 30 (34.9%) adjacent normal epidermis were XPC-positive. The intensity of XPC expression was significantly higher in BCC compared to adjacent normal epidermis ($p < 0.001$).

XPC protein levels are reduced in high-risk basal cell carcinoma. In the BCC group, 19 (73.1%) of 26 high-risk BCC were positive for XPC and 54 (90%) of low-risk BCC were positive for XPC expression. XPC protein levels were also lower in the infiltrative type of BCC compared with the noduloulcerative type. Overall XPC protein expression was

Table I. Clinical and pathological characteristics of basal cell carcinoma patients.

BCC (n=86)	n	%
Age		
Elderly (≥ 65 y)	55	64
Younger (< 65 y)	31	36
Gender		
Men	49	57
Women	37	43
Site		
Sun exposure	78	91
Non-sun exposure	8	9
Histopathologic type		
Noduloulcerative	62	72
Pigmented	8	9
Superficial	9	11
Infiltrative	7	8
High-risk	26	30
Low-risk	60	70

significantly lower in the high-risk BCC to compared the low-risk BCC ($p = 0.045$). Age and gender were not significantly different between the groups (Table II).

Discussion

UV radiation causes DNA damage, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) and pyrimidone photoproducts (6-4PPs) that may contribute to carcinogenesis. Nucleotide excision repair (NER) removes cyclobutane pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs) and other helix-distorting DNA lesions (12-14). Xeroderma pigmentosum (XP) patients have a more than 10,000-fold increased risk of skin cancer, which is associated with defective nucleotide excision repair (NER) of UV radiation-induced CPDs and 6-4 photoproducts (6-4PPs) (15). XPC is considered to be a damage-recognition protein responsible for nucleotide excision repair of UVB damage to DNA (7, 16). NER-deficient *Xpc*^{-/-} mice exhibit a divergent spontaneous tumor spectrum and accumulate mutations slowly (17, 18). We found that XPC was present in 84.9% of BCC specimens, supporting the hypothesis that there was DNA damage in BCC that could provide molecular evidence of UV radiation in BCC.

Our results demonstrated that XPC was expressed predominantly in the nucleus of basal cell carcinoma. XPC was also expressed in the nuclei of basal keratinocytes of adjacent normal epidermis, which is in agreement with de Feraudy *et al.* (19) who also observed that XPC protein expression was reduced as cells progressed to the upper layers of keratinocytes. Attenuated expression of XPC protein was noticed in high-risk BCC in our study (Table II). These high-

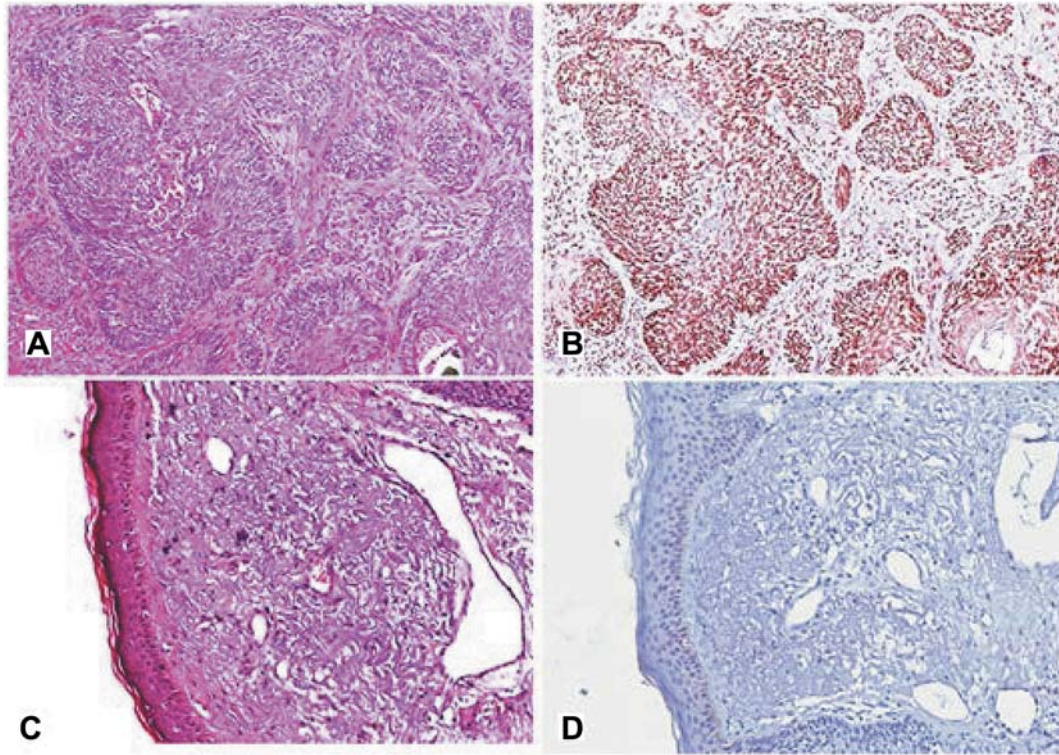


Figure 1. XPC protein expressions in basal cell carcinoma and adjacent normal epidermis. Nuclear expression of XPC in BCC (A, B) and adjacent normal epidermis (C, D). A, C=Hematoxylin–eosin ($\times 200$) in BCC and normal epidermis, respectively. B, D=XPC expression in BCC and normal epidermis, respectively.

risk BCC patients with a prior history of ≥ 3 non-melanoma skin lesions, infiltrative type, tumor size > 2 cm and tumor at the periorbital area are prone to relapse exhibiting local invasiveness and difficulty to completely excise.

De Feraudy *et al.* found that XPC is a DNA damage-binding protein frequently inactivated in squamous cell carcinomas (20), while the loss of XPC provides a selective advantage for initiation of UV-induced SCCs. Several hypotheses explained the loss of XPC protein expression, such as genomic instability, loss of the XPC locus at chromosome 3 p25 (20), p53 mutations which regulated XPC in UV-induced non-melanoma skin cancers (21, 22) and inactivation of both alleles of XPC by either mutagenesis or promoter methylation (20, 23). Phosphatase and tensin homolog (PTEN) expression and AKT activation are also required for XPC expression and thus better DNA repair. PTEN loss also suppressed expression of XPC through the AKT/p38 signaling axis (24). Further investigation is ongoing to clarify the regulatory pathway for diminishing XPC expression in high-risk BCC.

In conclusion, we demonstrated the probable tumorigenic role of XPC protein in cutaneous basal cell carcinoma and decreased XPC expression in the high-risk BCC.

Table II. XPC protein expressions in basal cell carcinoma patients.

Clinical characteristic	XPC protein (by immunohistochemistry)			
	Number	Negative n (%)	Positive n (%)	p-Value
BCC	86	13 (15.1)	73 (84.9%)	<0.001
Adjacent normal epidermis	86	56 (65.1)	30 (34.9%)	
BCC				
Elderly (≥ 65 y)	55	9 (16.3)	46 (83.7)	0.672
Younger (< 65 y)	31	4 (12.9)	27 (87.1)	
Men	49	7 (14.3)	42 (85.7)	0.807
Women	37	6 (16.2)	31 (83.8)	
Sun exposure	78	13 (16.7)	65 (83.3)	0.215
Non-sun exposure	8	0	8 (100)	
High-risk	26	7 (26.9)	19 (73.1)	0.045
Low-risk	60	6 (10)	54 (90)	

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Conflicts of Interest

The Authors declare no conflict of interest.

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