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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Keywords: squamous cell carcinoma, Xeroderma pigmentosum group C (XPC)

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 > 2 cm, 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

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Gender</th>
<th>Age*</th>
<th>Tumor grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC</td>
<td>92</td>
<td>47 male;</td>
<td>72.8±12.9</td>
<td>Broders’1:5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 female</td>
<td></td>
<td>Broders’2:47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Broders’3:40</td>
</tr>
<tr>
<td>AK</td>
<td>37</td>
<td>15 male;</td>
<td>75.2±10.3</td>
<td>KIN 1:13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 female</td>
<td></td>
<td>KIN 2:13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KIN 3:11</td>
</tr>
<tr>
<td>NE</td>
<td>91</td>
<td>48 male;</td>
<td>39.9±14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>43 female</td>
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</tr>
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</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Positive (n)</th>
<th>Negative (n)</th>
<th>p-value</th>
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<tbody>
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<td><strong>Age</strong></td>
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<tr>
<td>&lt;65</td>
<td>16</td>
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<tr>
<td>≧65</td>
<td>44</td>
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<tr>
<td><strong>Sex</strong></td>
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<tr>
<td>Sun exposure</td>
<td>21</td>
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<td>0.928</td>
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<tr>
<td>Non-sun-exposure</td>
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<td><strong>Differentiation</strong></td>
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<td>Poor</td>
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<td>25</td>
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<tr>
<td>Moderate</td>
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<td>Well</td>
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<td><strong>Recurrence</strong></td>
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<tr>
<td>No</td>
<td>31</td>
<td>43</td>
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<tr>
<td>Yes</td>
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<td>17</td>
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<td><strong>Metastasis</strong></td>
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<tr>
<td>No</td>
<td>32</td>
<td>58</td>
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<tr>
<td>Yes</td>
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<td>2</td>
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<td><strong>Risk</strong></td>
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<td>Low</td>
<td>26</td>
<td>21</td>
<td>0.033*</td>
</tr>
<tr>
<td>High</td>
<td>11</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

*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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