Symbiosis

ISSN Online: 2378-1726
www.symbiosisonlinepublishing.com

Research Article | Clinical Research in Dermatology: Open Access | Open Access

Expression of FOXE1 in Cutaneous Squamous Cell Carcinoma

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Received: May 24, 2019; Accepted: May 31, 2019; Published: June 3, 2019

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Abstract

Background: Cutaneous squamous cell carcinoma (cSCC) is one of the common non-melanoma skin malignant tumors with sophisticated pathogenesis. FOXE1 is considered to be a tumor suppressor involved in the development of various tumors; however, the expression and significance of FOXE1 in cSCC remains to be investigated.

Methods: Skin samples were collected from patients with cSCC and healthy controls (HCs). Immunohistochemistry was used to detect FOXE1 protein expression.

Results: In HCs, FOXE1 is mainly expressed in the basal layer of epidermis and the positive rate was 33.33%. In cSCC, the expression of FOXE1 protein was significantly elevated, and the positive rate was 74.07%.

Keywords: FOXE1; cutaneous squamous cell carcinoma;

Abbreviations

SCC: cutaneous squamous cell carcinoma;

FOXE1: Forkhead Box E1;

HCs: Healthy Controls;

DBD: DNA-Binding Domain;

TTF-2: Thyroid Transcription Factor;

Dvl2: Dishevelled Segment Polarity Protein 2;

HCC: Hepatocellular Carcinoma;

GLI2: GLI Family Zinc Finger 2;

Duox2: Dual Oxidase 2;

Introduction

Cutaneous squamous cell carcinoma (cSCC) is a malignant tumor originating from the epidermal or accessory keratinocytes. The incidence of cSCC is second in non-melanocyte-derived skin malignancies, which increases year by year from 3% to 10%. Most cSCC can be cured by surgical resection, but a small part of cSCC can be life-threatening [1]. The occurrence of cSCC may be related to physical and chemical factors, viral infection, long-term inflammatory stimuli and immune status, while the specific mechanism is still unclear.

Forkhead-box (FOX) family members are DNA-binding proteins involved in transcriptional regulation and DNA repair, cell growth, differentiation and embryonic development [2]. FOXE1 is functionally equivalent to a pioneer factor, helping to open a dense chromosomal structure, thus facilitating the binding of other transcription factors. The function of FOXE1 has been studied in several kinds of tumors. Some studies have shown that the hypermethylation status of FOXE1 is related to the development of cancer [3-5]. This study explores the role of FOXE1 in the development of squamous cell carcinoma.

Methods

a. Clinical samples

27 patients (13famale and 14male, age range = 47-85 years) who were diagnosed with cSCC in the dermatology department of the Second Affiliated Hospital of Xi'an Jiaotong University were enrolled in this study. Another 21 cases (11 female and 10 male, age range = 13-54 years) of healthy control were obtained from cosmetic surgery. There was no significant difference in gender and age between the two groups, and there were no other skin or systemic diseases. Institutional Ethics Committee of Xi'an Jiaotong University approved protocol and procedure of the study. Written informed consent was obtained from all patients before samples collection and analysis.

b. Immunohistochemistry

Paraffin-embeded tissues from 27 patients with cSCC and 21 HCs were obtained from the Department of Dermatology, Second Hospital of Xi'an Jiaotong University. The samples were incubated overnight at 4 °C with the primary antibody (anti- FOXE1; cat. no ab5080; Abcam, Cambridge, UK) at a final dilution of 1:100, then incubated with an appropriate biotinylated streptavidin-horseradish oxidase-conjugated secondary antibody, (ZSGB-BIO, Beijing, China), followed by haematoxylin counterstaining and finally visualization with 3′-diaminobenzidine (ZSGB-Bio, Beijing, China). Stained sections were examined under a microscope. Two experienced pathologists evaluated the staining results under microscope independently and the results were quantified according to the following systems. The staining results were evaluated according to the staining intensity and the percentage of stained cells in a semi quantitative manner. The staining

intensity in both cytoplasm and nucleus was graded as follows: 0 (negative), 1 (weak positive), 2 (moderate positive) and 3 (strong positive). The percentage of positively stained cells was scored as follows: 0 (≤5%), 1 (6–25%), 2 (26–50%), score 3 (51–75%) and 4 (>76%). The score was obtained for each microscopic by multiplying the two scores. The final immunoreactivity score was defined as the average score of five fields.

c. Statistical analysis

SPSS statistical software (v21.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The Pearson chi-square test was used for immunohistochemistry analysis.

Results

Immunohistochemistry results indicated expression of FOXE1 is increased in cSCC lesions

The expression level of FOXE1 in cSCC was significantly higher than that in HCs. ($\chi^2 = 10.309$, $P < 0.05$) the quantification of FOXE1 expression level in specimens is listed in Table 1. Staining of FOXE1 can be observed in both cytoplasm and nucleus. FOXE1 was mainly expressed in the basal layer of the epidermis in the normal skin (Figure 1); however, in cSCC skin, FOXE1 was mainly expressed in cancerous tissue and keratin pearl (Figure 2 and Figure 3). Semi-quantitative analysis of the immunohistochemistry results showed that the expression of FOXE1 is significantly elevated in cSCC skins compared to the healthy controls (Table 1 and Figure 4).

Table 1: Expression of FOXE1 in normal skin and cutaneous squamous cell carcinoma

Group		Expression grade(n)				Positive rate (%)
	n	-	+	++	+++	
cSCC	27	7	7	11	2	74.07
Healthy controls	21	14	5	2	0	33.33

Note : * $P < 0.05$, compared with healthy controls

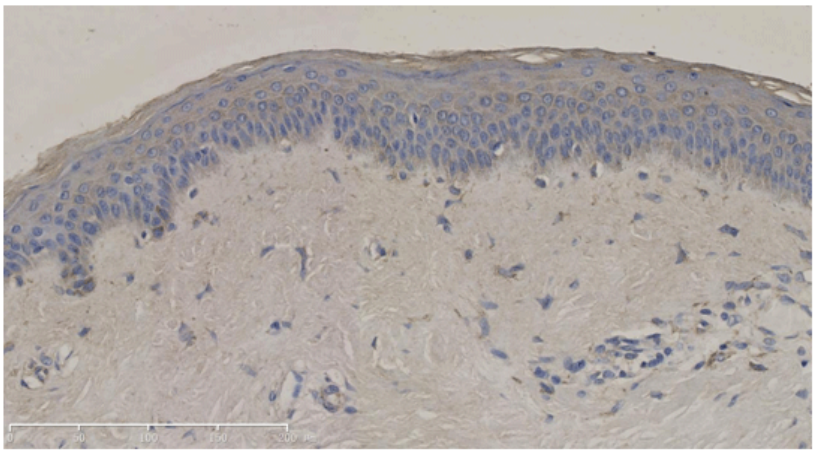

Figure 1: Expression of FOXE1 in healthy control (bar length = 500 μm)

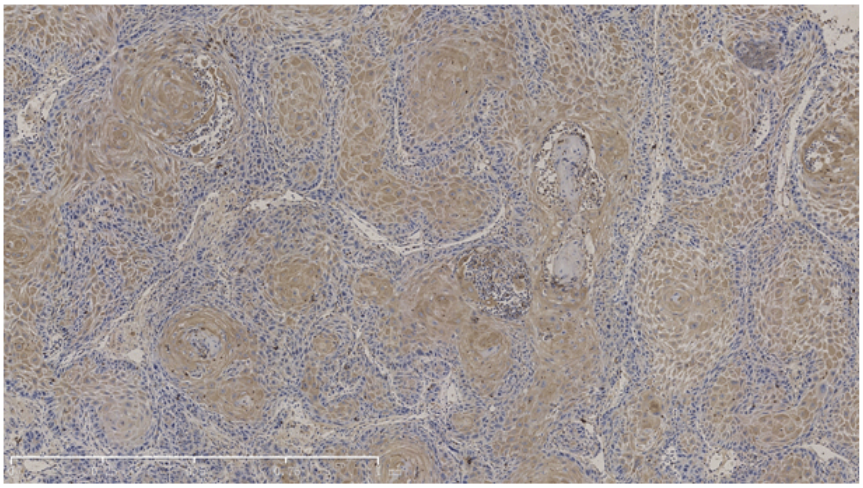

Figure 2: Expression of FOXE1 in cSCC (bar length = 1 mm)

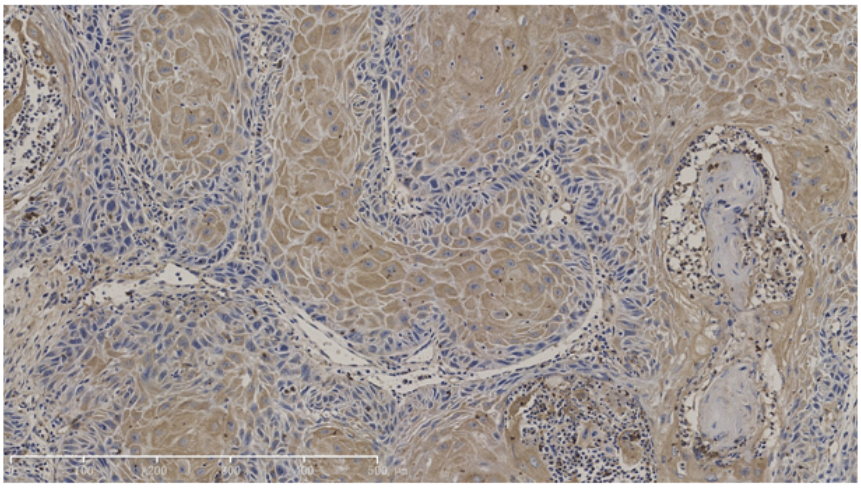

Figure 3: Expression of FOXE1 in cSCC (bar length = 500 μm)

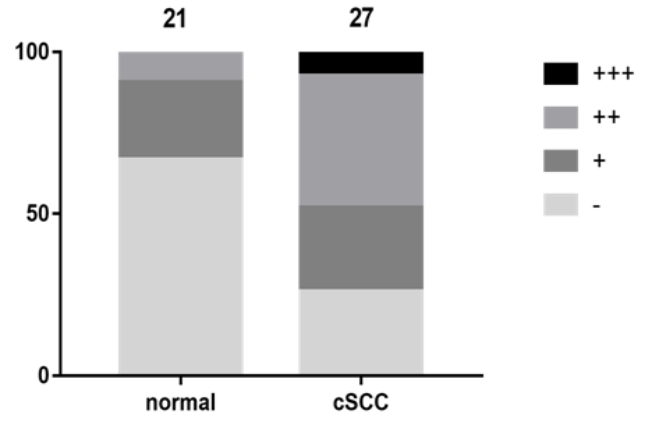

Figure 4: Semi quantitative analysis of FOXE1 staining

Discussion

Cutaneous squamous cell carcinoma is a malignant proliferation of cutaneous epithelium and represents 20% to 50% of skin cancers. It often occurs in exposed parts such as the head, face, neck and back of the hand. The incidence of the disease is rising rapidly in recent years [6-7]. As previously reported, loss-of-function mutation of notch receptor 1 is early event in the progression of cSCC [8], but the knowledge on the molecular events of cSCC progression is incomplete.

Forkhead box (Fox) proteins contain a common DNA-binding domain (DBD) termed the forkhead box or winged helix domain and involve in a series of biological processes. Forkhead proteins bind DNA as monomers and promote gene activation directly by regulation of chromatin structure. [2,9]. FOXE1, also called thyroid transcription factor 2 (TTF-2), regulates the expression of

thyroid-specific genes and is also necessary for the maintenance of the thyroid differentiated state [10]. Mutations of the FOXE1 gene cause human syndromes that are associated with thyroid agenesis [11]. In addition, FOXE1 variations have been associated with susceptibility to several types of cancer. In pancreatic cancer, loss of afadin leads to increased formation of the Dvl2-FOXE1 complex in the snail gene promoter region, which activates snail gene transcription, thereby promoting pancreatic cancer metastasis [12]. In another study, FOXE1 was identified as the direct functional targets of miR-422a and involved in HCC tumor genesis and metastasis [13]. FOXE1 and GLI2 both expressed in normal epidermis and basal cell carcinoma, and FOXE1 maybe participate in executing the transcriptional program triggered by GLI2[14]. Moreover, FOXE1 was identified to be upregulated in the tissues of PTC patients and over expression of FOXE1 reversed the effects of Gli2 siRNA on levels of Wnt/β-catenin. Therefore, these maybe exist a crosstalk among Gli2, FOXE1 and Wnt pathway [15]. Of note, through genomic and transcriptomic analyses, Wnt signaling network was identified being potentially aberrantly regulated in cSCC [16], and a study showed that tumor volume was reduced and tumor-free survival was increased as a result of knockdown of β-catenin in human cSCC cells in xenotransplantation models [17]. Interestingly, Duox2 was upregulated when FOXE1 was silenced and Duox2 may play an important role in the mechanisms of cutaneous anti-inflammation [18,19].

In conclusion, our study indicated that FOXE1 may play an important role in the pathogenesis of cSCC. The potential mechanism may involve the network between FOXE1 and Wnt/β-catenin. To some extent, FOXE1 may be a new therapeutic target for cSCC. To clarify the pathogenesis, further studies are needed to elucidate the exact role of the FOXE1 in cSCC pathogenesis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (81573055)

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