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Original Article

Expression of DKK1 and β-catenin in epidermal neoplasms and their correlation

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Abstract: Objective: To investigate the expression of Dickkopf-1 (DKK1) and β-catenin and their correlation in epidermal neoplasms. Methods: Immunohistochemical staining was applied to detect the expression of DKK1 and β-catenin in tissue samples of 19 cases of seborrheic keratosis (SK), 16 cases of actinic keratosis (AK), 24 cases of Bowen's disease (BD), 25 cases of cutaneous squamous cell carcinoma (SCC), and 22 cases of normal epidermal tissue (NET). Results: DKK1 was expressed in cytoplasm in normal epidermis. The positive expression rates of DKK1 in SK, AK, BD and SCC were 63.16%, 50.00%, 12.50% and 8.00%, respectively. β-catenin was expressed in cell membrane in normal epidermis. The abnormal expression rates of β-catenin in SK, AK, BD and SCC were 15.79%, 56.25%, 91.67% and 96.00%, respectively. Additionally, significant negative correlation was observable between the expression of β-catenin and DKK1 (r=-0.692, P=0.000). Conclusion: The Wnt signaling pathway may play an important role in the process of epidermal neoplasms formation. The loss of DKK1 promotes the abnormal expression of β-catenin through Wnt signaling pathway.

Keywords: Epidermis neoplasms, DKK1, β-catenin, immunohistochemistry

Introduction

Squamous Cell Carcinoma (SCC) is a cancer of a kind of epithelial cells, originating from precancer diseases. However, the deteriorated process and mechanisms involved in SCC are largely unknown. For example, seborrheic keratosis (SK), nominated as noncancerous benign skin growth of the delayed maturation of keratinocytes has the possibility to change into SCC. Actinic keratosis (AK) and Bowen's disease (BD) are the prophase lesions of the cancer and epidermis in situ squamous cell carcinoma, respectively. Both of these two diseases have the ability to transfer into invasive SCC.

The Wnt signaling pathway is a network of proteins best known for their roles in embryogenesis and maintaining the inner environmental stability of the human body. Abnormal of this signal cascade will lead to diseases, even formation of the tumors [1]. Dickkopf-1 (DKK1) is nominated as the typical antagonist of the Wnt/β-catenin signal pathway and performs critical roles in the development of tumors via inhibiting β-catenin-dependent transcription of a series of genes [2]. Down-regulated or loss of DKK1 expression was distinct in a variety of cancers, including gastrointestinal cancer [3], cervical cancer [4], breast cancer [5] and melanoma [6]. However, the roles of DKK1 in the cancers originated from keratinocytes aggressiveness are still elusive. This present study described here examined DKK1 and β-catenin expression in varied epidermal tissues to explore the mechanisms underlying the onset of epidermal neoplasms.

Materials and methods

Materials

Patients' information: All the benign tumors and cancer specimens (106) were collected from the patients in the first hospital of Chongqing Medical University from 2006 to 2011. Wax specimens have been deposited in the pathology room of Dermatology Department. There were 19 cases of SK, 16 cases of AK, 24 cases of BD, 25 cases of SCC at an earlier stage

Table 1. Information of the people

Diagnosis	Numbers	Age	Sex (male:female)
NET	22	65±8	10:12
SK	19	63±12	11:8
AK	16	67±10	9:7
BD	24	24	16:8
SCC	25	68±14	15:10

involved in this study. The specimens were pathologically indentified and the patients were free of medication before diagnosis. Additionally, normal epidermal tissues (NET) obtained from circumcision and eye pouch removal operations with the healthy people were applied as positive controls. The basic information of the people was listed in **Table 1**.

Reagents: The primary antibodies against DKK1 (polyclonal rabbit antibody against human) and β-catenin (monoclonal mouse antibody against human) were obtained from Beijing Zhongshan Bio-Tek company (China) were diluted by 1:100. The second antibody and DAB assay kit were obtained from Wuhan Boster Bio-engineering Limited Company (China).

Methods

Immunohistochemistry: SABC assay was used in immunohistochemical staining of DKK1 and β-catenin. 3-4 μm slides were prepared from the wax specimens and roasted overnight, and then the slides were incubated 2-3 times in xylene and dehydrated by placing in a concentration gradient ethanol. Peroxidase was inactivated by incubation with 3% hydrogen peroxide for 10 minutes. Microwave heat was used to repair antigen. After placing slides into buffer containing goat serum at 37°C for 20 minutes, the slides were incubated in a humidified chamber overnight with primary antibody at 4°C. After washing, the slides were kept with the second antibody for 20 min at 37°C. SABC was applied at 37°C for 20 min and DAB was used to display the staining (monitoring under the microscope). Hematoxylin-staining was applied to double-stain the slides. Then, the slides went through protocol steps, differentiating with hydrochloric acid in ethanol, dehydrating with gradient ethanol ion, xylene, and blocking the slides with neutral resin. As the negative control, PBS was used instead of primary antibody. The slides from esophageal squamous epithelial tissue were used as the positive control to normalize DKK1 and liver cancer tissues were applied as the positive control to indicate β-catenin expression.

Results analysis

Brown particles colored by DAB indicated the positive reactive product. DKK1 was normally expressed in cytoplasm, sometimes in nuclear. At least 10 high powder fields were chosen for each group and 100 cells were counted with each field. Semi-quantitative analysis was conducted by analyzing the counts of positive cells, as well as grey value of the positive staining. According to the color of the staining, results were divided into no staining (with the same color as background), low staining (with a color a little higher than background), middle staining (with a color obviously higher than background) and high staining and each rate got a score of 0, 1, 2 and 3, respectively. According to the percentage of the positive cells in the field, the values "<10%", between 10%-25%, between 25%-50% and ">50%" would get a score of 0, 1, 2 and 3, respectively. Then, these two values were combined to decide the expression of proteins in each group (the score 0-2 represents negative "-", 3-4 represents positive "+", 5-6 represents strong positive "++"). β-catenin was normally expressed on the cell membrane and more than 70% expression on the membrane was defined as the normal expression. By contrast, less than 70% expression was defined as low expression or expression loss. While β-catenin expression in the cytoplasm or nuclear was more than 10%, it was defined as ectopic expression. Both of low expression or expression loss, as well as ectopic expression were proposed as abnormal expression.

Data analysis

The data were analyzed by SPSS 20.0. Sample rates were compared using χ test and Fisher method. The correlation of different index was analyzed by Spearman software. The desired significance level was chosen to be $\alpha=0.05$, thus an estimated $P<0.05$ was considered to indicate a significant difference between two experimental conditions.

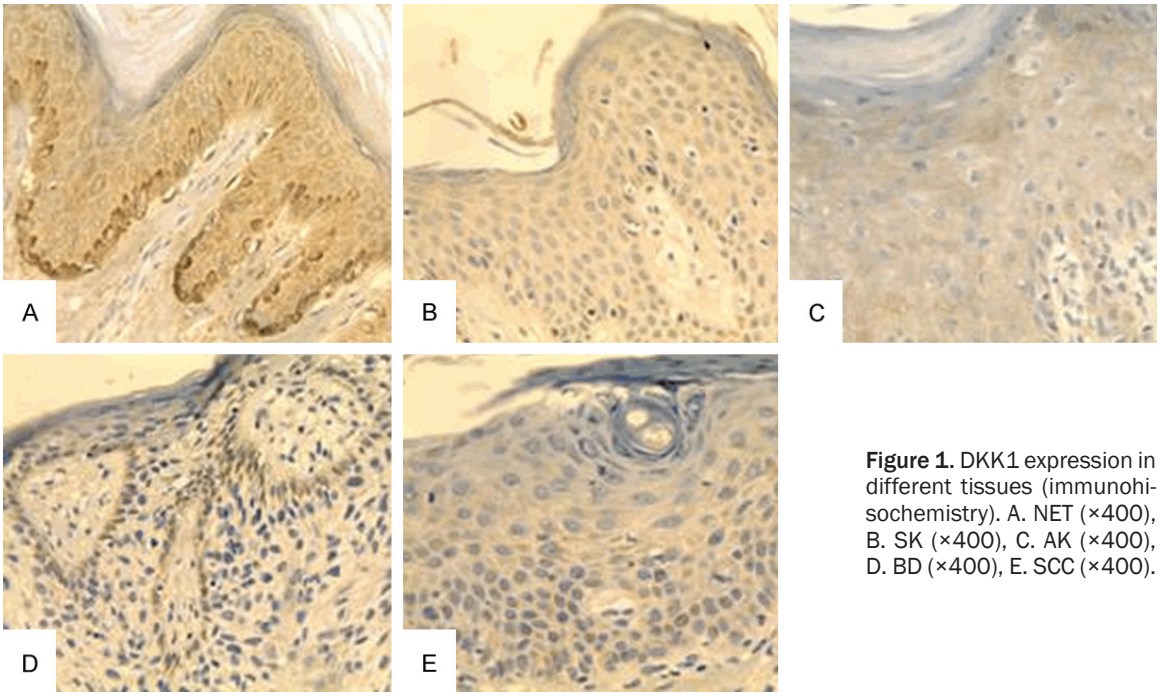

Figure 1. DKK1 expression in different tissues (immunohisochemistry). A. NET (×400), B. SK (×400), C. AK (×400), D. BD (×400), E. SCC (×400).

Table 2. The expression of DKK1 and β-catenin in NET, SK, AK, BD and SCC

Groups	Numbers (N)	DKK1 positive (%)	Abnormal β-catenin expression (%)
NET	22	21 (95.45)	0 (0)
SK	19	12 (63.16)	3 (15.79)
AK	16	8(50.00)	9 (56.25)
BD	24	3 (12.50)	22 (91.67)
SCC	25	2 (8.00)	24 (96.00)

Results

DKK1 expression in NET, SK, AK, BD and SCC

DKK1 expression was located in the cytoplasm of the normal epidermal tissue, while the expression would be down-regulated in the pathological epidermal tissues. As shown in our results, the expression rate of DKK1 in SK, AK, BD and SCC were 63.16%, 50.00%, 12.50% and 8.00%, respectively and positive expression rates were significantly lower than NET group ($P<0.05$) (**Figure 1**). The difference among SK, AK, BD and SCC was statistically significant ($P=0.000$, $P<0.05$). Meanwhile, SCC, SK or AK also showed a significant expression difference when compared with BD. However, there was no obvious difference between SK and AK, as well as BD and SCC (**Table 2**).

β-catenin expression in SK, AK, BD and SCC

The normal expression of β-catenin in the epidermal tissues locates at cell membrane. It was showed that cell membrane of basal layer and spinous layer was continually positively stained with β-catenin antibody. Also, ectopic expression was not observable in the normal tissues. However, under pathological alteration of the epidermal tissue, the expression was abnormal, including low expression or expression loss on the cell membrane or ectopic expression from cell membrane to cytoplasm or nuclear. For example, cell membrane β-catenin expression was down-regulated or depleted in SK tissues. By contrast, AK, BD and SCC showed more ectopic expression (**Figure 2**). The abnormal expression rate in SK, AK, BD and SCC were 15.79%, 56.25%, 91.67%, 96.00%, respectively. Through statistical analysis, the values in AK, BD and SCC were significant higher than NET group ($P<0.05$), while no statistical difference was observed between SK and NET ($P>0.05$). AK, BD and SCC also showed significant different in β-catenin expression with each other. When compared with AK, BD or SCC also showed significantly different ($P<0.05$). There was no obvious difference of β-catenin expression between BD and SCC ($P>0.05$, **Table 3**).

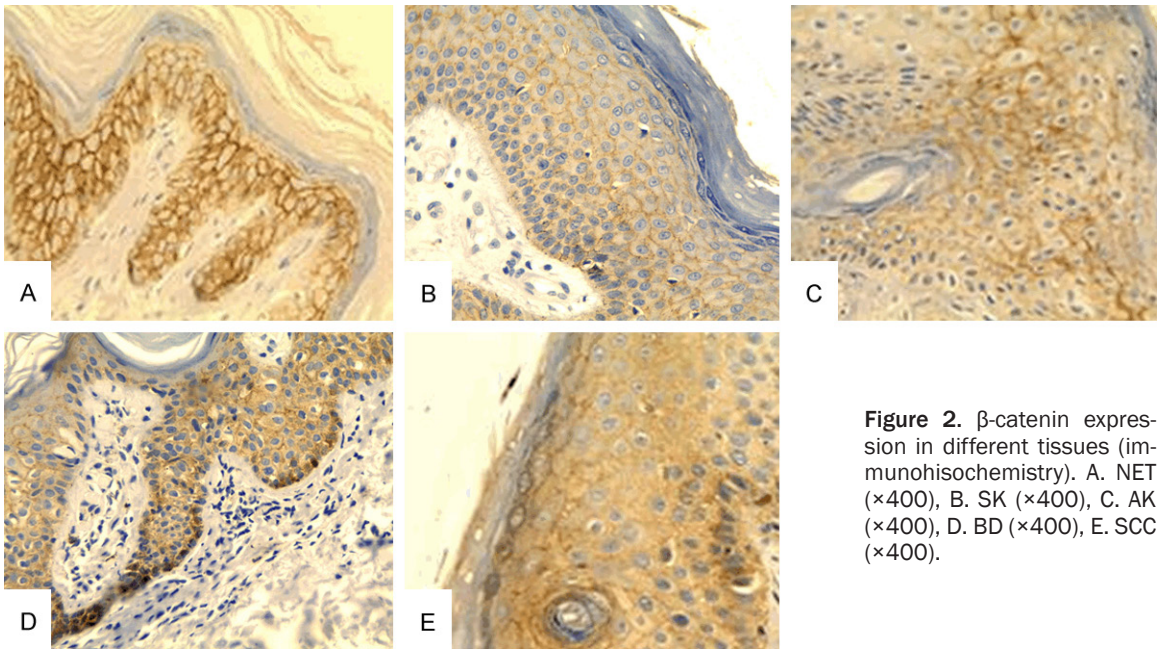

Figure 2. β-catenin expression in different tissues (immunohisochemistry). A. NET (×400), B. SK (×400), C. AK (×400), D. BD (×400), E. SCC (×400).

Table 3. Analysis of the correlation between DKK1 and β-catenin in SSC

β-catenin	DKK1		Total
	Positive	Negative	
Normal expression	1	23	24
Normal expression	1	0	1
Total	2	23	25

r=-0.692, P=0.000.

Correlation of DKK1 with β-catenin in SCC

Based on expression values of DKK1 and β-catenin, we analyzed the correlation of DKK1 with β-catenin in SCC. The data showed that the spearman gradient value r was -0.692 and *p* value was 0.000. These data might implicate the negative correlation of these two proteins expression in SCC (**Table 2**).

Discussions

SSC is one of the common cancers observed in Dermatology. The typical characteristics of SSC are its invasion and metastasis, which seriously affect patients' health, as well as life quality. Hence, it is of great significance to find ways to prevent epidermal lesion-induced tissue malignancy or to diagnose malignance as early as possible. Wnt signal transduction pathway consists of 3 major branches, including typical Wnt/β-catenin signal transduction pathway [7], cell polarity [8] and Wnt/Ca^{2+} pathway [9]. Recently, the abnormal activation of Wnt signal transduction pathway was found to be related to onset and development of several cancers. Hence, to deeply understand the property of this signal pathway and its roles in malignancy of epidermal tissues will help to elucidate the mechanisms of SCC progression. DKK1 is one tumor suppressor gene, which antagonizes the Wnt signal pathway. The inhibition of this pathway will increase phosphorylation of β-catenin, leading to inhibition of β-catenin-dependent transcription.

In this present study, DKK1 was showed a cytoplasm expression in the normal epidermal tissues. By contrast, DKK1 expression took on a gradually down-regulated trend in SK, AK, BD and SSC. These results implicate that with deterioration of SCC, DKK1 will be gradually down-regulated. That further explains that DKK1 performs a key role in suppressing the epidermal cancer development. Meanwhile, activation of Wnt/β-catenin pathway entails an accumulation of β-catenin in cytoplasm, which subsequently leads to its nuclear translocation, afterward, the increase of β-catenin-dependent transcription, especially c-myc and cyclin D1. Uncontrollable cell growth is supposed to be the key step in mediating cell malignancy. β-catenin encompass some additional functions, such as maintaining the normal morphol-

ogy and mediating intercellular adhesion when combined with E-cadherin to form a complex [10]. The loss of cell membrane expression of this protein will damage the intercellular adhesion system, facilitating the metastasis of the tumor cells. This study showed that β-catenin in normal epidermal tissues expressed on cell membrane and the continual staining of cells at basal layer and spinous layer to some extent reflect its adhesion property and function. By applying immunohisochemical staining, Fukumaru and workers examined β-catenin expression in normal epidermis and 140 cases of epidermal neoplasm. They demonstrated that there was no significant difference between cells in normal keratinocytes and benign tumors (SK, common warts or keratoacanthoma) about β-catenin expression on cell membrane. By contrast, malignant cancers, including BD, basal cell carcinoma and squamous cell carcinoma show a decreased membrane expression and increased cytoplasm or nuclear expression. Hence, membrane expression of β-catenin may correlate with differentiation of keratinocytes [11].

Some other reports suggested a decrease of membrane β-catenin expression is preferable observed in SSC, then AK and BD [12]. However, our investigation indicates that β-catenin expression on the cell membrane is gradually decreased in SK, AK, BD and SCC, while ectopic expression displays gradually increase. This increase of abnormal expression rate is consistent with other report. Based on these data, the abnormal expression of β-catenin probably increases cell atypia, hence, playing critical roles in the formation of tumor cells from epidermal keratinocytes. Dual roles in cell adhesion and cellular signal transduction performed by β-catenin further demonstrate the molecular mechanisms of tumor malignancy. Additionally, our study also demonstrates a negative correlation between DKK1 and β-catenin in SSC. These results in SSC are consistent with the measurement in other cancers, such as colorectal cancer [13], etc. That implicates the existence of activation of Wnt/β-catenin pathway in SSC formation. Expression loss of DKK1 would increase β-catenin abnormal expression. According to this study, the measurements of DKK1 and β-catenin expression might help to screen the epidermal cell malignancy and Wnt/β-catenin pathway could be one therapeutic target for cancer prevention.

In this study, we present the similarity expression trend of β-catenin as the other reports widely. Since the rare report on DKK1 expression, selective bias still exists. The abundant samples are still desired to further understand the roles of DKK1 played in the development of epidermal neoplasms. Additionally, our study just used a single immunohistochemistry method to semi-quantify DKKI and β-catenin expression. DNA and RNA level, as well as protein quantification of the two proteins and their related factors are still needed.

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Disclosure of conflict of interest

None.

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