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Expression of connexin 32 and connexin 43 gap junction proteins in basal and squamous cell carcinomas

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Abstract

Gap junctions play a role in intercellular communication in the epithelium, muscle and nerve tissues, and many more tissues and organs. These linking regions and the proteins forming them are called connexins (Cx). Recent studies show that cellular homeostatic equilibrium can be influenced by connexins. In this study, the expression of Cx43 and Cx32 in basal cell carcinoma (BCC) and squamous cell carcinomas (SCC) is tried studied. A total of 45 cases, 23 male and 22 female, were included in the study. Twenty of the cases were SCC and twenty-five were BCC. Retrospective data from records were examined and specimens were investigated by immunohistochemistry staining. It was determined that the results of Cx 43 and Cx 32 staining changed according to the tumor type of cases (P = 0,020). It was determined that the results of Cx43 and Cx32 staining did not change according to the sex and age, BCC and SCC subtype, and location. Losses of Cx43 and Cx32 have been shown to be associated with tumor types. We believe that it can be used as a support marker of SCC and BCC differential diagnosis.

Keywords: Basal cell carcinoma, Squamous cell carcinoma, Connexin 32, Connexin 43

Introduction

Gap junctions (GJ) between two neighboring cells have great importance. GJ are intercellular membrane channels that allow direct cell-to-cell communication, ion, small molecules, and cellular metabolites to enter and exit between neighboring cells. These linking regions and the proteins that constituent them are called connexins and show a widespread in all mammalian cells [1].

Recent studies have shown that cellular homeostatic balance can independently affect intercellular communication (GJIC), the structural premises of gap junctions [2]. These highly diverse proteins make their specific responsibilities as vital functions, such as facilitating the intracellular passage of small regulatory molecules by spreading electrical signals between cells. In many cell types, changes in the connexin family can be seen and adaptive mechanisms may develop for the continuation of function. However, these changes can lead to significant pathologies. In many cases, single-point mutations cause dramatic consequences due to insufficient quantification of the junctional regions of the connexions and non-uniform internalization [3].

The sizes of the connections are their classification criteria. Membrane proteins form extracellular and intracellular structures in the form of thread loops in the membrane due to amino acid

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sequences. These layouts form the topology of the connections. This formation has been confirmed by several investigators for Cx43, Cx26, and Cx32 [4-6].

Despite numerous linkages, evidence has been shown that these proteins are superimposed on cell and tissue proliferation [3].

First, in many tissues and cell types, two or more members of the connective family are explicitly exposed. Cx43 was observed in keratinocytes and Cx32 in hepatocytes. There are studies of the expression of connexin in mammals and in particular of Cx43 expression. It is known that Cx43 is endogenously present and contains 35 different tissues, including keratinocytes, astrocytes, endothelial cells, smooth muscle cells [3-7].

When the literature is investigated, researches are suggesting that Cx43 can stimulate invasion and metastasis by regulating the interaction between stromal cells and tumors [7]. There are also studies evaluating the expression of Cx 32 and 43 between normal tissue and neoplastic tissue [8].

In this study, the expression of basal cell carcinoma (BCC) and squamous cell carcinomas (SCC) Cx43 and Cx 32 was investigated and the relationship with clinical parameters was investigated.

Material and Method

Design of study

The study was planned retrospectively. BCC and SCC cases sent to the pathology department between 2014 and 2015 were included in the study. The location, diameter, differentiation grades and age and gender of cases were reported. A total of 45 cases were included in the study. Twenty of the cases were SCC, twenty-five of the cases BCC. 23 of the cases were male and 22 of the cases were female. Paraffin blocks of cases were selected. 3 micrometer thick sections were taken from these paraffin blocks. Sections were taken on a polylysine-coated slides.

Immunohistochemistry

Sections of 3 µm thickness were prepared from paraffin blocks consisting of skin tissues. Immunohistochemical staining was performed using a Leica Bond automatic tissue staining device . The antibody and dilution ratios are as follows; Cx32 (recombinant anti-connexin 32/GJB1) (dilution ratio 1:200) and Cx43 (recombinant anti-connexin 43/GJA1) (dilution ratio 1:200). Slides were evaluated under the Nikon Eclipse Ni U microscope for grading. The stained slides were examined and photos were taken. Cx32 and Cx43 expression were evaluated semi-quantitatively according to staining intensity as none, mild, moderate and strong (0,1,2,3). Mild, moderate and strong staining grades were combined (as positive). Data were classified as negative and positive. It was observed that positivity was found in the basal cells (Figures 1-4).

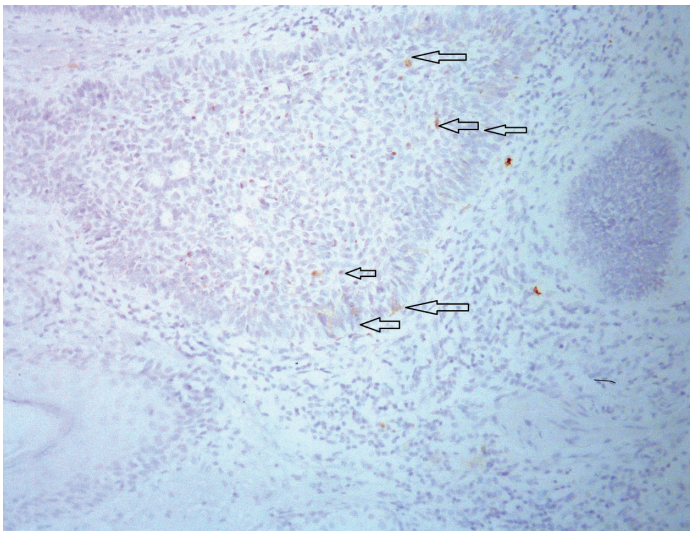

Figure 1. Connexin 43 positivity of Basal Cell Carcinomas (x200)

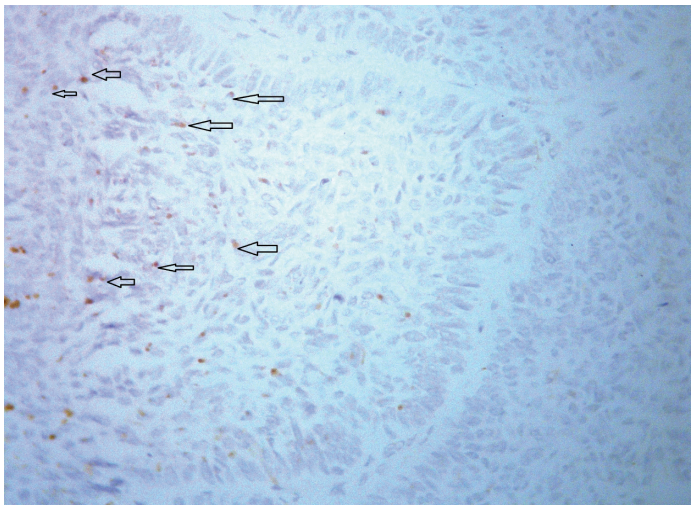

Figure 2. Connexin 32 positivity of Basal Cell Carcinomas (x400)

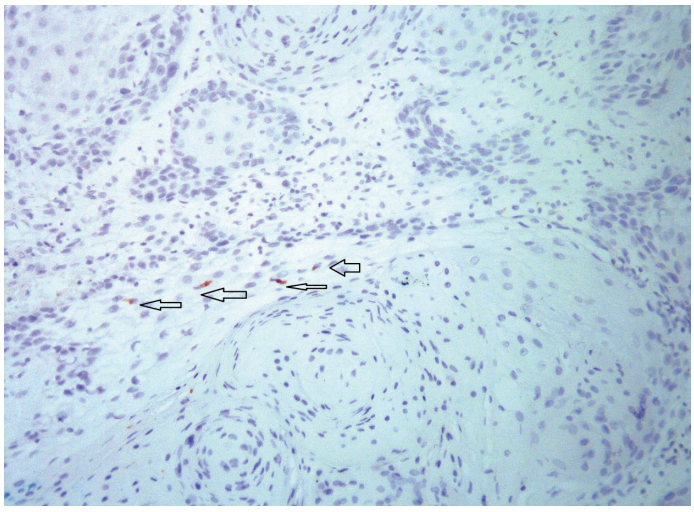

Figure 3. Connexin 43 positivity of Squamous Cell Carcinomas (x200)

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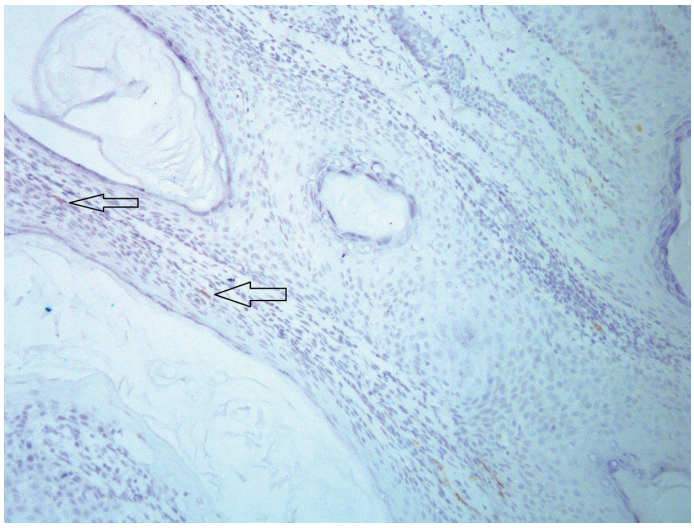

Figure 4. Connexin 32 positivity of Squamous Cell Carcinomas (x200)

Statistical analysis of data

Chi-square analysis was used to investigate the relation between sex, age, type, differentiation grade, location, and the results of the Cx43 and Cx32 staining of the cases. In this study, evaluation; below 50 years of age and 51-70 age group values were combined and evaluated. Diameter less than 5 mm and from 6 to 10 mm were combined and evaluated. SCC distribution according to the differentiation is as follows; 12 well-differentiated, 8 moderate and poorly differentiated tumors. The subtype distribution of BCC was as follows; 14 nodular, 3 infiltrative, 5 basosquamous, 1 micronodular, 1 keratotic, 1 pigmented. In both tumors, location , diameter, and age were evaluated together. The location of the nose and the upper region was 29 cases and under the nose were 26 cases. The number of cases between 6-10 mm and over 11mm is 22 and 23, respectively.

Chi-square analysis was used to investigate the relationship between gender, age, type, degree of differentiation and location of cases with Cx43 and Cx32 staining results (negative, positive).

In addition, for significant results, the effect of staining intensity was demonstrated by bivariate logistic regression analysis. In the study, multivariate logistic regression analysis was not performed because the effect of species was significant only in the presence of staining ($P < 0.25$).

All statistical calculations were made in the SPSS 19.0 V statistical package program and were expressed in terms of findings and percentages.

Results

Statistically, it was determined that the results of Cx 43 and Cx 32 staining changed according to the tumor type of the cases ($P = 0,020$) (Table-1). As a result of the bivariate logistic regression analysis performed, the intensity of staining in BCC type according to SCC type is 8,175 times more (Tables 2,3). As a result of the bivariate logistic regression analysis performed, the intensity of staining in BCC type according to SCC type is 7,071 times more.

Table 1. Results of the Cx 43 and Cx 32 according to the type of tumor

Type of tumor	**n (%)**		Total
Staining	Negative	Positive	
BCC	14 (56.0)	11 (44.0)	25 (100.0)
SCC	18 (90.0)	2 (10.0)	20 (100.0)
Total	32 (71.1)	13 (28.9)	45 (100.0)
x^2 :6.252	P=0.020		

Table 2. Potential risk factors associated with Cx43 staining in the univariate logistic regression equation

Variable	No	Total No	Prevalence (%)	B	S.E.	Wald	Sig.	Exp(B)	95% CIfor Exp(B)	
									Lower	Upper
Constant				-2.20	0.745	8.690	0.003	0.111		
TYPE										
SCC	2	20	10.0							
BCC	11	25	44.0	1.96	0.847	5.330	0.021	7.071	1.344	37.215

Table 3. Potential risk factors associated with Cx32 staining in the univariate logistic regression equation

Variable	No	Total No	Prevalence (%)	B	S.E.	Wald	Sig.	Exp(B)	95% CIfor Exp(B)	
									Lower	Upper
Constant				-2.20	0.745	8.690	0.003	0.111		
TYPE										
SCC	2	20	10.0							
BCC	11	25	44.0	1.96	0.847	5.330	0.021	7.071	1.344	37.215

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Besides, the results of Cx 43 and Cx 32 staining did not change according to the subtype of BCC and SCC (respectively; P = 0,188, P = 0.514). Moreover, the results of Cx 43 and Cx 32 staining in the cases did not change according to the location (P = 0,322).

Cx 43 and 32 staining results were evaluated according to the diameter of the tumor. It was determined that the staining results did not change (P=0.372).

Distribution of Cx43 and Cx32 staining results according to the sex of tumor types are evaluated. It was determined that Cx32 staining results were the same as Cx43 staining results and did not change (P = 0,815).

Statistically, Cx 43 and Cx 32 staining results were evaluated according to the age of cases. It was determined that the staining results did not change (respectively; P=0.095, P=0.441).

Discussion

Some Cx's are very specific and are excreted in many tissues. One of these cases, the Cx43, has been reported to be extended in more than twenty-five tissues [9,10].

Hieber et al. have studies showing increased expression of Cx43 [11]. In the aforementioned study, they observed that carotenoids increased protein levels and Cx43 expression in the suprabasal layers of human keratinocytes in human and mouse fibroblasts and organotypic cultures [11]. As a result of this study, they concluded that it may be important in the growth of human tumor cells and claimed that Cx43 expression can potently inhibit the in vitro marker of malignancy [12].

Cx43 expression in human carcinoma cells has been shown to decrease both in vivo and in vitro [13,14].

In Bertram's study, increased Cx43 expression and decreased normal and neoplastic tissue proliferation were observed. It has been observed to decrease in displaced tissue and tumor progression [15].

In a study by Puzzo et al., they reported negative or poor expression of Cx43 expression in poorly differentiated carcinoma [16].

In this study, staining in SCC was lesser than BCC. Since SCCs are more aggressive tumors than BCC, loss of Cx staining showed results consistent with the literature with tumor type. Since the prognosis will worsen with loss of differentiation, the expected result in this study is loss of Cx staining with loss of differentiation. However, although this interpretation was made clinically in this study, it could not be proved statistically because the number of cases was small.

The study of Wilgenbus et al. investigated the expression of Cx 26, 32, and 43 gap-junction proteins in malignant and non-malignant human tissues. In their study, Cx32, the major gap-junction protein in rat and mouse liver, was also detected in the human liver and kidneys [17]. Cx26 was detected in different epithelium, whereas Cx43 was expressed in epithelial and mesenchymal tissues. All benign tumors and some malignant tumors in the study showed stable expression in gap-junction proteins. In addition, a decrease in gap-junction proteins was observed in many cancers compared to normal tissues. To give an example of these cancers; breast cancer, renal cell carcinoma and sarcoma. They observed in their study in basal cell carcinoma tissue samples [17].

Tada et al. showed that Cx expression was weak in BCC and SCC, and expression was absent in eccrine and apocrine glands [18].

In a study by Schneider et al. they report 43 cases of basal, parabasal, and middle-layer Cx [8]. In this study, it was noticed that the staining is prominent in the basal cells.

Danos et al. reported a positive correlation between the expression of Cx 43 and the patients's survival and head and neck carcinomas [19].

Limitations of the study

The sample number in this study was a few. Therefore, SCC and BCC subtype could not be analyzed separately in terms of demographic data. In this study, it was observed that the expression of Cx43 and Cx32 in BCC and SCC cases did not change with age, sex, location, and diameter. Since the study was included between 2014 and 2015, the relationship between Cx32 and Cx43 was not assessed with the survival of the cases. This is one of the limitations of this study.

Conclusion

Expression of CX32 and Cx 43 may be useful in the differential diagnosis of BCC and SCC. We believe that analysis with larger case series can yield more reliable results in order to reveal the differences between the subtypes and demographic variables of both tumors.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

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Ethical approval
Local Ethics Board of Ordu University approved this study with decision number 2015/6.

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