Investigation of biomarker properties of DARS2 to distinguish squamous cell carcinoma from keratoacanthoma

Ozlem Ucer[a], Nevin Kocaman[b,*]

[a]Firat University, Faculty of Medicine, Department of Pathology, Elazig, Türkiye
[b]Firat University, Faculty of Medicine, Department of Histology and Embryology, Elazig, Türkiye

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Abstract

Aim: This study was aimed at investigating the effectiveness of DARS2 protein expression in the differential diagnosis of squamous cell carcinoma (SCC) and keratoacanthoma (KA).

Materials and Methods: A total of 60 cases, 30 SCC and 30 KA, diagnosed in the Fırat University, Faculty of Medicine, Department of Pathology, were analysed immunohistochemically by studying the DARS2 protein.

Results: DARS2 expression values were found to be statistically significant in the differential diagnosis of SCC and KA ($p < 0.05$).

Conclusion: The marker used in this study was found useful in the differential diagnosis of SCC and KA, and it is recommended that, further molecular and genetic studies be carried out, this will facilitate the accurate diagnosis of SCC from KA.

Introduction

The second most common of the non-melanoma skin cancer cells has been identified to be the Squamous cell carcinoma (SCC) worldwide. Current studies have shown that, there has been an increase in the occurrence of SCC in all age groups, with USA alone having more than 400,000 people diagnosed of invasive SCC each year [1].

The etiology of this condition has been attributed to extreme exposure to sunlight and UV radiation B, which are some of the causes of DNA damage. It is possible for SCC to arise from any part of the skin and squamous epithelium. Histological diagnosis in SCC is made by findings such as stromal desmoplasia, the presence of atypical squamous cells, and absence of a sharp border between tumour clusters and stroma [2,3].

Keratoacanthomas (KA) on the other hand are tumors that develop from follicular infundibular/istmatic (isthmic) keratinocytes and are usually seen on sun-exposed parts of the skin [4]. Distinguishing keratoacanthomas from squamous cell carcinomas have until now beena diagnostic challenge in dermatopathology. Various immunohis-tochemical and cytogenetic markers have been employed to demystify this challenge.

However, up till date, there has not been any conclusive evidence to support the practical application of any of those markers [5].

DARS2 is a mitochondrial protein, which has recently been discovered to have an effect on tumour formation and progression. This has resulted in several studies to bring to conclusion the mitochondrial mechanisms involved in tumorigenesis [6].

In this study, the value of DARS2 protein was investigated to provide new opportunities for the diagnosis of SCC patients and to differentiate them from KA.

Materials and Methods

The study was approved by the local ethics committee of the Firat University (Approval No:21.04 2022/06-12). Thirty (30) SCC and thirty (30) KA cases were included in this study. Patients were identified retrospectively by reviewing a pathological database. Pathological data were obtained from the hospital medical archives and pathology reports.

*Corresponding author:
Email address: drnkocaman@gmail.com (Nevin Kocaman)

Immunohistochemistry

Immunohistochemical procedures as described by Kocaman and Artas were used in this study [7]. Histological tissue microarray slides which were 3 µm thick were used in the Immunohistochemistry (IHC) experiment.

The antibodies used in the experiment include: Anti AspRS antibody (Sc-166535; Santa Cruz Biotechnology, Oregon, USA). Immunohistochemical staining was used in the calculation of the histoscore which was subsequently employed in the measurement of tissue levels of DARS2.

Microscopic evaluation of staining intensity

The distribution of staining was scored as 0.1, < 25%; 0.4, 26-50%; 0.6, 51-75%; 0.9, 76-100%, whilst the intensity of staining was scored as 0 for no staining; 0.5 as very little staining; 1 for little staining; 2 for moderate staining; and 3 as very strong staining. The formula for the calculation of the histoscore was as follows: histoscore = distribution × intensity [7].

Statistical analysis

IBM SPSS 22 statistical package was used in the statistical analysis of the data derived from this study. Determination of the level of distribution of data was done employing the Shapiro-Wilk test. The descriptive statistics of the data are expressed as Mean ± Standard Deviation for normally distributed variables in continuous data, as [Median (Minimum-Maximum)] for non-normally distributed variables, and as percentage [n(%)] for categorical variables. For normally distributed continuous data, the Independent Sample t-test was used to compare two independent groups, and for non-normally distributed continuous data, the Mann-Whitney U test was used to compare two independent groups. Pearson Chi-Square test was used to compare categorical variables. Significance level was determined at $p < 0.05$

Results

Demographic findings

A total of 36 patients from the SCC and KA groups included in the study were evaluated, no gender or age difference was found between the patients in the study (Table 1).

Immunohistochemical findings
DARS2 immunoreactivity

As a result of examining the immunohistochemical staining for DARS2 immunoreactivity under light microscopy;

Table 1. Summary of patients' clinical data.

Characteristic	SCC	KA
Sex n (%)		
Female	63.3%	50.0%
Male	36.7%	50.0%
Age median (min-max)	60(min 54- max 78)	63(min 54- max 74)

SCC(Squamous Cell Carcinoma), KA (Keratoakantom). Comparison of SCC and KA groups (p < .05).Descriptives are expressed as median (min-max).

Table 2. Histoscore of DARS2.

Histoscore of DARS2	SCC	KA	P value
DARS2 median (min max)	2.7 (min1.8- max 2.7)[a]	1.2 (min 0.9- max 1.2)	[a]0.001

SCC (Squamous Cell Carcinoma), KA (Keratoakantom).
[a] : Comparison of SCC and KA groups. (p < .05) Descriptives are expressed as median (min-max).
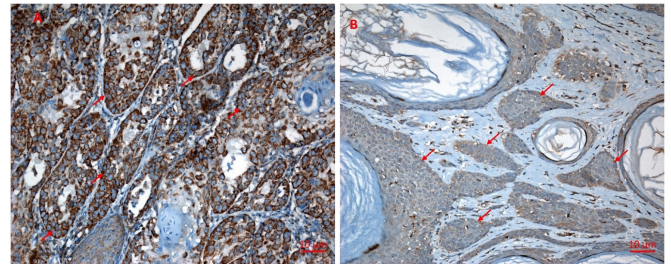

Figure 1. Immunohistochemical analysis of DARS2 protein at lesion sites with SCC and Keratoacanthoma. (A) SCC skin tissue DARS2 immunoreactivity, (B) Keratoacanthoma skin tissue DARS2 immunoreactivity.

DARS2 immunoreactivity was found to be statistically significantly increased in the SCC group when compared to the KA group (Figure 1) (Table 2).

Discussion

Differentiating keratoacanthoma (KA) from SCC has been a subject of discussion for some time now. [8]. According to some researchers, KA is a variant of SCC, whereas others describe it as a primary stage which progresses into SCC through time [9,10]. Notwithstanding the fact that cytological features display great similarities between KA and SCC cases, a distinction is made according to the structural features of their tumors [11]. However, even in the most accurate biopsy specimen, KA cases may sometimes be histologically indistinguishable from SCC cases due to the lack of histological features showing sufficient sensitivity or specificity. As a result of this observation, various immunohistochemical markers have been tested over the years for the accurate differential diagnosis of KAs from SCCs [12].

SCCs are immunohistochemically stained positively with cutaneous epithelial markers, high molecular weight cytokeratin, involucrin, vimentin and epithelial membrane antigens (EMA) [13]. However, in a comparative study of keratoacanthoma and squamous cell carcinoma, Cribier et al.(date) reported that the immunohistochemical criteria were not sufficiently specific enough to distinguish the two [14].

This study was carried out to test a new molecule to differentiate keratoacanthoma from squamous cell carcinoma. Data from this study demonstrated that DARS2 protein was expressed in keratoacanthoma and squamous cell carcinoma samples, but staining was most severe in SCC. DARS2 is a mitochondrial protein being studied in re-

cent times. Mitochondrion is a vital organelle for physiological mechanisms. Furthermore, mitochondrial dysfunctions have been found to be associated with tumorigenesis and disease progression [15]. Mitochondria are complex organelles of bioenergetics, biosynthetics, and signaling that are associated with various disease conditions, including cardiovascular diseases, neurological disorders, and metabolic disorders [16,17]. The mechanisms of mitochondria involved in tumorigenesis have been extensively studied, and some specific nuclear mitochondrial genes have been recognized as potential targets for the development of next-generation cancer therapeutics [18].

Mitochondrial aminoacyl-tRNA synthetases are a group of catalytic enzymes which play an important role in protein translation by supplying amino acids to the newly formed polypeptide chain. They also contribute to the functions of protein synthesis and oxidative phosphorylation enzymes. There are 19 mitochondrial aminoacyl-tRNA synthetase genes. One of them being DARS2 [19,20].

The fact that DARS2 expression was significantly higher in this study, especially in tissues with SCC, suggests that DARS2 is effective in SCC diagnosis and thus, can be used to differentiate it from KA.

Although keratoacanthomas are diffuse, self-limiting squamous proliferations, there are sporadic reports of "metastasizing keratoacanthomas" [21]. It is necessary to distinguish KA from SCC as precisely and quickly as possible. There exists the tendency of accepting KA as a subtype of SCC or malignant rather than seeing it as a subtype of SCC researchers have reported that, up to 25% of undiagnosed keratoacanthomas transition to squamous cell carcinoma. Some reports indicated that, keratoacanthoma is indeed a benign and self-healing proliferation, but may become malignant in up to a quarter of cases, particularly in elderly patients predisposed to skin cancer [22].

Due to the spontaneous regression tendency, suspicion has developed whether KAs are tumors or not. For this reason, the pathogenesis and natural course of KAs have been a matter of debate for many years [23]. Conversely, there are studies showing that keratoacanthoma is distinctly different from squamous cell carcinoma on molecular bases [24]. In recent times, one of the most important debates in dermatopathology is on the relationship of keratoacanthoma with squamous cell carcinoma. In practice, there are keratoacanthomas which are indistinguishable from squamous cell carcinoma characterized by cords or micronodules and composed of pleomorphic keratinocytes, especially at the base of the tumor [25]. Most keratoacanthomas are histologically characterized by infiltrative lobules and nests of tumor cells with low-grade nuclear features and abundant, glassy eosinophilic cytoplasm that mature towards the centre. Keratoacanthoma usually shows a sharp border between tumor nests and stroma [26].

SCC, on the other hand, consists of clusters and cords of polygonal shaped squamous epithelial cells originating from the epidermis. These cells have extensive eosinophilic cytoplasm, often large, vesicular nuclei, and prominent eosinophilic nucleoli. Depending on the differentiation of the tumour, varying amounts of central keratinization and keratin pearls are present. Single cell keratinization is frequently observed and the degree of anaplasia is used to determine the grade of the tumor [27].

In this study, 6 (36.7%) of SCC cases were female, 15 (63.3%) were male, and the mean age was 60. SCC was mostly observed on the face, hands and forearms of male, and on the face and legs in female patients. 7 (50.0%) of KA cases were female, 8 (50.0%) were male, and the mean age was 63 years. Normally, SCC is observed more frequently in the elderly than in the young. In this study, the mean age of patients with SCC was 60, which was consistent with the results reported in literature [28]. KA is a rapidly growing, solitary, self-limiting and regressed squamoproliferative lesion that develops in fair-skinned individuals, elderly individuals, and sun-exposed areas. KA was observed more commonly in male patients than female patients and is usually diagnosed after the age of 50, with the peak period in the sixth decade [29]. In this study, the mean age for KA was 63 years. However, there was no significant difference in terms of gender and age between the patients in the SCC and KA groups included in the study.

Conclusion

In conclusion, this study postulates that, DARS2 may be a potential determinant for the differentiation of keratoacanthoma and well-differentiated SCC from KA.

Ethics approval

The study was approved by the local ethics committee of the Firat University (Approval No:21.04 2022/06-12).

Funding

Declaration of competing interest

The authors declare no conflicts of interest.

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