Role of immunohistochemistry in the diagnosis and staging of cutaneous squamous-cell carcinomas (Review)

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Abstract. Non-melanoma skin cancer (NMSC) is the most common type of neoplasm affecting Caucasian individuals, with squamous-cell carcinoma (cSCC) being the second most common type of NMSC after basal-cell carcinoma. The immunohistochemical study of cSCC is of particular importance, especially for the diagnosis of its rare forms, for which accurate and early diagnosis is crucial for survival. In the present review of the literature, the potentially significant value of immunohistochemical markers were highlighted to more accurately assess the biological behaviour, the prognosis of cSCC and to optimize case management. The immunohistochemical markers were classified from a pathophysiological point of view in order to present the mechanism by which carcinogenesis occurs with its subsequent evolution and therefore, to develop a more accurate novel risk staging criteria for this type of neoplasm.

Contents

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1. Introduction

In the last 10 years, as a result of the collaboration between various European experts in Dermatology and Dermato-Oncology, it has been concluded that non-melanoma skin cancer (NMSC) is the most common type of neoplasm affecting Caucasian individuals and squamous-cell carcinoma (cSCC) is the second most common type of NMSC after basal-cell carcinoma. cSCC is a form of carcinoma characterised by the proliferation of keratinocytes that occurs in a long process of intraepidermal dysplasia (1). Previous data indicated that there has been a significant increase in the incidence of cSCC, especially in the fair-skinned elderly population exposed to more chronic levels of ultraviolet radiation (UV) (2). The chronic, cumulative exposure of UV in areas such as the upper extremities, as well as advanced age, fair skin and immunosuppression are the most important causal risk factors for cSCC. Chronic exposure to UV radiation can cause genetic mutations responsible for the degree of tumour differentiation and tumour aggression.

The incidence of cSCC is constantly increasing worldwide, but the precise causes are not known, and the primary reasons underlying this shortcoming are the lack of registration of diagnosed cases, heterogeneity of treatments and low mortality rates (3). Although the vast majority of cSCC cases are curable via surgical excision, a small percentage of patients with locally advanced or metastatic cSCC may relapse and have a high risk of mortality (4).

There are several staging systems for cSCC [the American Joint Committee on Cancer, 7th and 8th edition (AJCC 7, AJCC 8) staging system, the Breuninger staging system (5), the Brigham and Women's Hospital (BWH) staging system] (5-7), with a number being difficult to use in clinical practices due to the lack of uniformity of the evaluated criteria (5). For instance, the AJCC 7 staging system comprises a number of elements considered to be risk factors such as thickness, poor degree of differentiation, perineural infiltration, involvement of the ear or lower lip and level of invasion, while the AJCC 8 includes tumour size, infiltration, deep invasion, perineural invasion and bone erosion in cutaneous tumours of the head and neck excluding those located in the eyelid. However, AJCC 8 excludes the degree of histological differentiation, tumour stage and associated risk factors in contrast to the Breuninger staging system (5) and the Brigham and Women's Hospital staging systems, which use clinical and pathological classification and also associated risk factors (6,7). An easy-to-use system for assessing tumour and prognosis based on the evaluation of tumour parameters and the elements of disease aggression would have a double benefit both in diagnosis and staging but also in the application of personalised therapies. Considering the importance of staging due to the risk of metastasis of cSCC, in the present review, the immunohistochemical aspects of cSCC were highlighted. The immunohistochemical study of cSCC is of particular importance, especially for the diagnosis of its rarer forms, for which accurate and early diagnosis are important for survival (5,6).

The present systematic review aimed to summarize the literature on the immunohistochemical markers currently used and/or studied for the accurate diagnosis and staging of cSCC. As well as their incidence and treatment, the stratification of the risk of cSCC is uncertain, and the aim of this review was to highlight immunohistochemical markers that could be incorporated into diagnostic criteria to more accurately assess the risk, the prognosis of these tumours, the need for further investigation and to guide adjustments to treatment plans.

The immunohistochemical markers from the reviewed articles have been classified from the pathophysiological point of view in order to represent the mechanism by which carcinogenesis occurs with its subsequent evolution and, therefore, to develop a more comprehensive and representative novel risk staging criteria for this type of neoplasm.

2. Search strategy and inclusion/exclusion criteria

Studies addressing both non-melanoma skin cancers and other non-skin sites of cSCC were excluded. Studies addressing cSCC exclusively with immunohistochemical studies relevant to the diagnosis and prognosis of this type of carcinoma were included. The references in the articles obtained in the initial search were evaluated to see if they were relevant or not for inclusion.

A search strategy was developed using PubMed (https://pubmed.ncbi.nlm.nih.gov/), ScienceDirect (https://www.sciencedirect.com/), SpringerLink (https://link.springer.com/), Wiley Online (https://onlinelibrary.wiley.com/) and Elsevier (https://www.elsevier.com/), with the following search terms to identify the scientifically relevant papers for this systematic review: 'Squamous skin cell carcinoma', 'cutaneous squamous cell carcinoma', 'cSCC', 'immunohistochemistry', 'angiogenesis', 'cellular invasion', 'cellular infiltrate' and 'inflammation infiltrate', with suitable use of Boolean modifiers as appropriate.

The search strategy identified 63 articles of which 37 were deemed to be eligible for inclusion in this systematic review. Of these, five studies addressed markers of epithelial-mesenchymal transition (EMT), seven studies addressed invasion markers, three studies addressed vascular proliferation markers, 17 studies focused on cell proliferation markers and five studies addressed inflammation markers.

3. Macroprocesses involved in the development and/or progression of cSCC

EMT. EMT is a biological process that consists of multiple biochemical changes of the polarised epithelial cell, which typically interacts with the basement membrane. The epithelial cell takes on the phenotype of a mesenchymal cell, which includes increased migratory capacity, invasiveness, increased resistance to apoptosis and increased production of extracellular matrix components (8). EMT is a physiological and important process involved in embryogenesis and healing. In cancer, elements found in normal development have been identified, and this observation supports the hypothesis that cells 'reactivate their developmental properties out of context in adults', thus contributing to carcinogenesis and in EMT facilitating tumour progression (9). The specific co-localization of markers associated with epithelial and mesenchymal phenotypes suggests that the cells have partially undergone the process of EMT (8).

A total of three types of EMT have been proposed: Type 1, which is associated with implantation, embryo formation and organ development; type 2, associated with healing, tissue regeneration and fibrosis, in addition to inflammation; finally, type 3, which occurs in neoplastic cells (8,10).

Invasion. Invasion is the first step in the metastasis of tumour cells. Morphologically, invasion is the process by which neoplastic cells detach from the tumour mass, and acquire the ability to actively move and invade neighbouring tissues through the basement membrane. EMT plays a key role in tumour dissemination, but is not always necessary for invasion and metastasis (10,11). Therefore, the occurrence of EMT cannot always predict whether the tumour cell will migrate at some point (11,12). Although the mechanisms of invasiveness have been well studied and described, to the best of our knowledge, there is currently no validated panel of effective markers for the identification of migratory cells in cSCC or to assess their invasive potential (11).

The perineural region is the space between the nerve fascicles and the perineurium. Cancer cells migrate around the nerve or even invade the nerve. Perineural invasion (PNI) is associated with a particularly poor prognosis, and patients with PNI are more likely to possess distant metastases and local recurrence (13).

Vascular proliferation. Tumour growth and the process of metastasis is dependent on the formation of new blood vessels. Tumours cannot grow >2 mm in diameter if they

are not vascularised (14). Malignant cells can stimulate angiogenesis or vasculogenesis, but tumour vascularization is relatively less common (15). The vessels are permeable and dilated, and their organization appears random. Angiogenesis is necessary not only for the continuation of tumour growth, but also for neoplastic cells to become vascularised, as well as for metastasis (14). Tumour angiogenesis is a multifactorial process in which, in addition to the factors that promote it [vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), pan endothelial marker CD34, endoglin (CD105) or mammary serine protease inhibitor (maspin)], other growth factors, extracellular matrix proteins and cellular adhesion molecules are also involved. It takes place in several stages, which are regulated by a complex interaction of stimulatory and inhibitory factors. The angiogenetic stages include: Increase in vascular permeability, destabilization of endothelial-peri endothelial cell contact, enzymatic dissolution of the vascular basement membrane, endothelial cell migration, endothelial cell proliferation, lumen formation and, finally, formation of a stable blood vessel (16).

The spread of malignant tumour cells through the lymphatic system is of great clinical importance, and the state of the lymph nodes influences the stage of the tumour, and is often the basis for selection of an appropriate therapeutic regimen. The markers of lymphatic proliferation include podoplanin and VEGF receptor-3 (16).

Cellular proliferation. Cell proliferation plays a key role in cell injury, including injury leading to neoplasia. Increased proliferation and changes in the cell cycle are essential processes required for successful carcinogenesis (17).

Inflammation markers. Tumour-infiltrating-lymphocytes and other immune infiltrates in the peritumoral tissue have prognostic relevance in several types of cancer (18,19). The mechanisms by which local inflammation modulates the tumour behaviour of invasive cSCC are not fully established; however, the inflammatory tumour microenvironment is a major determinant for the regression of *in situ* lesions and the control of cSCC development in immunocompetent patients (20,21).

4. EMT markers

The first study reviewed (22) addressing markers of EMT, conducted in Korea, included the markers axis inhibition protein-2 (AXIN2) and Snail family of zinc-finger transcription factors (SNAIL). AXIN2 enhances SNAIL activity via the wingless-related integration site (Wnt) signalling pathway. This study included 111 tissue biopsies from 93 patients without recurrence of the carcinoma and 18 patients with recurrence after Moh's micrographic surgery at 156 months. No patients exhibited distant metastasis. A significant association was observed between SNAIL expression in the expressing tissues and AXIN2, and the clinicopathological significance was demonstrated by the fact that the expression of both markers were detected more frequently in patients with recurrence. Additionally, the expression of AXIN2 and SNAIL was also correlated with the size of the tumour, with their expression being more prominent in patients with larger tumours. Kaplan-Meier survival curve analysis demonstrated that survival without recurrence of cSCC was associated with tumour size, degree of differentiation and expression levels of AXIN2 and SNAIL. It was therefore concluded that these two markers should be considered in predicting the risk of recurrence. Unfortunately, this study had some limitations, such as the lack of all information on >50% of patients (for example, history of organ transplantation, diabetes and other types of cancer) (22).

The next large study by Toll *et al* (23) in Spain, with patients who developed SCC between 2001 and 2011. EMT markers were analysed in primary cSCC cases without metastases, in those with metastases and in lymphatic metastases. This retrospective study included 146 biopsies, of which 51 were non-metastatic, 56 were metastatic and 39 were lymphatic metastases. Immunohistochemistry (IHC) was performed for the following markers: E-cadherin, vimentin, SNAIL1, β-catenin, Twist, Zinc finger E-box-binding homeobox 1 (Zeb1), podoplanin and the AE1-AE3 cytokeratin panel. Loss of membrane E-cadherin expression was observed in metastatic and non-metastatic SCCs, with no significant differences between the two. Conversely, the nuclear localization of E-cadherin was predominantly observed in metastatic SCC compared with non-metastatic SCC. E-cadherin loss is associated with the release of β-catenin from the adherens junctions, the adherens area and its translocation to the nucleus (23,24). Loss of cellular β-catenin is frequently observed in both metastatic and non-metastatic SCC groups, but its nuclear localization has been detected only in patients with metastases (23-25). Thus, β-catenin is a very specific marker for risk assessment of metastases, but its sensitivity is relatively low (33%). Additionally, the acquisition of mesenchymal markers was studied and thus, the presence of vimentin was observed immunohistochemically, and was revealed to be present in 67.9% of metastatic and 31.4% of non-metastatic SCC tissues. The presence of the EMT-associated transcription factors SNAIL, Twist and Zeb1 were also assessed. Unlike the previous study, in the cases reported by Toll *et al* (23), ≤1% cells were positive for SNAIL and were thus considered negative for this marker. Twist expression was revealed in 40% of metastatic SCCs, whereas non-metastatic SCCs were all negative for Twist expression. Finally, Zeb1 was observed in 48.1% of metastatic and 19.6% of non-metastatic SCCs. Finally, increased expression of podoplanin was observed in metastatic SCCs. The study by Toll *et al* (23) concluded that vimentin was a more accurate marker for predicting metastatic risk compared with loss of E-cadherin expression at the membrane, which was later confirmed in 2014 by Barrette *et al* (26), and Twist was an even more specific marker for the development of lymphatic metastases (23,26).

Toll *et al* (23) in 2013 also demonstrated what was concluded in the study conducted by Vinicius *et al* (27) in 2011: E-cadherin expression is not correlated with the risk of metastasis or survival, and that podoplanin is a promising prognostic marker, the expression of which is correlated with disease progression and indicative of the presence of an aggressive tumour (23,27). Podoplanin has also stood out in

Table I. Epithelial-mesenchymal transition markers.

First author (year)	Markers	Role/signalling pathway	Involved in disease progression/ invasiveness	Prediction of risk of recurrence	Prediction of risk of metastasis	Potential therapeutic target	(Refs.)
Zhao (2020)	AXIN2, SNAIL	AXIN2 enhances SNAIL activity via the Wnt signalling pathway (wingless-related integration site)		+			(22)
Toll (2013)	Nuclear β-catenin				+		(23)
Hesse (2016)	E-cadherin	Marker of cancer invasion in various types of cancer			+		(28)
Toll (2013), Barrette (2014)	Loss of membrane E-cadherin	Expression of the members of the AKT signalling pathway is decreased in metastatic tissue compared with the corresponding primary tumour			+		(23,26)
Vinicius (2011)	Cytoplasmic accumulation E-cadherin		+				(27)
Toll (2013)	Zeb1				+		(23)
Toll (2013), Vinicius (2011), Hesse (2016)	Podoplanin	Marker of EMT, single-cell invasion	+		+	+	(23,27,28)
Toll (2013)	SNAIL1						(23)
Toll (2013), Barrette (2014)	Twist		+		+ lymphatic metastases		(23,26)
Vinicius (2011)	HER-4	EGFR, HER-2, HER-3 and HER-4. Involved in cell proliferation, differentiation, and apoptosis			+ lymph node metastasis		(27)

AXIN2, axis inhibition protein-2; SNAIL, Snail family of zinc-finger transcription factors; Zeb1, Zinc finger E-box-binding homeobox 1; HER, human epidermal growth factor receptor; EGFR, epidermal growth factor receptor.

subsequent studies as an independent marker of prognosis and metastatic risk (28) (Table I).

5. Invasion markers

A 2006 study (13) addressed the nerve growth factor receptor ($p75^{NGFR}$) marker with the aim of determining its value in detecting PNI compared with the non-specific serum S-100 protein, which results in staining of a wide variety of cells. The 2006 study (13) revealed that S-100 was useful and easier to perform with regard to nerve detection. $P75^{NGFR}$ was not a more specific marker for the detection of PNI; however, unlike S-100, which identifies axons, $P75^{NGFR}$ analysis tended to stain the endoneurium and perineurium. This allowed the assessment of the space between the two structures, a key location for detecting PNI. Therefore, this study demonstrated that $p75^{NGFR}$, although non-specific, may exhibit potential as a diagnostic marker due to its superior haematoxylin and eosin staining sensitivity and may thus be used as an alternative or adjunct to S-100 (13).

A study by Nissinen *et al* (29), which included 65 SCC cases, 65 *in situ* SCC cases, 31 actinic keratoses cases, seven seborrheic keratoses cases and 16 normal skin samples, examined the expression of claudin-11 in all these samples. The conclusion of this study was that claudin-11 was not expressed in the samples of *in situ* SCC, actinic keratosis or in benign skin tumours (seborrheic keratosis). Additionally, the expression of claudin-11 was negative in metastatic and poorly-differentiated SCC cases. Its expression was positive in well- and moderately-differentiated SCC samples. Therefore,

claudin-11 may serve as a potential biomarker for analysis of tumour progression to the invasive stage (29).

Focal adhesion kinase (FAK) and cortactin are two proteins known for their role in cell migration and invasion in other types of cancer (30,31). A study conducted by Munguía-Calzada *et al* (32) in Spain, published in 2019, investigated the involvement of FAK and cortactin in metastatic SCC compared with a control group of patients who possessed tumours that had not metastasised. FAK expression in at least 1% of tumour cells was observed in 69 of the cases, and that of cortactin in 54 of the 100 cases included in the study. Regarding the relationship between the two proteins, no connection was observed. The data from the study indicated that cortactin expression was not correlated with lymph node metastases and had no impact on the prognosis. Conversely, the positive expression of FAK highlighted its value as a biomarker for the stratification of metastatic risk and moreover, its value as a therapeutic target has also been studied (32,33).

The study conducted by Rose *et al* (34) addressed the expression of phosphorylated (p-)SMAD2 and p-SMAD3 as potential markers. Of the 230 lesions examined, 225 were SCC and five were keratoacanthomas. This study demonstrated the link between invasive SCC and low TGF-β pathway activity (the pathway in which SMAD2 and SMAD3 are activated) (34); however, given the limitations of the study this was not a definitive conclusion and further investigation is required (34).

A recent retrospective cohort study at the Mayo Clinic, published in 2020, demonstrated that low immunohistochemical expression of inositol polyphosphate-5-phosphatase (INPP5A) was associated with a poor prognosis of cSCC (28). INPP5A plays a role in the progression of actinic keratosis to SCC, as well as from localised to metastatic tumour (29). Loss of INPP5A expression in cSCC is correlated with aggressive tumour behaviour and a more severe clinical presentation, as demonstrated by Cumsky *et al* (35) in 2019, in which it was demonstrated that tumours with low expression levels of INPP5A were likely to exhibit high-risk characteristics (a Brigham and Women's Hospital stage ≥T2a, tumours with large diameter, moderate-to-poor differentiation, perineural invasion), thus it became a potential immunohistochemical marker for stratifying the risk of cSCC. The Mayo Clinic study complemented the study by Cumsky *et al* (35), and it further demonstrated the potential of evaluating INPP5A expression as an adjuvant tumour marker for clinical management and risk stratification of recurrent and metastatic carcinoma. However, given the limitations of the study, including the small number of patients, further research with larger cohorts are required to determine whether INPP5A can be used as a biomarker for the prognosis of patients with metastatic disease (35,36) (Table II).

6. Vascular proliferation markers

Angiogenesis is an important phenomenon in tumour evolution; however, the mechanisms underlying its occurrence and its role in cSCC have not yet been identified. In 2011 Florence *et al* (37) published a study, which included both cSCC of varying degrees of invasiveness and actinic keratosis. It examined the following membrane markers: CD34, which is a pan endothelial marker, and CD105, also known as endoglin, a marker for neo-angiogenesis (37). CD34 can be used to assess the tumour vascular bed, but not the status of angiogenesis. It also does not differentiate between the different stages of carcinoma (37). CD105 is associated with proliferation and can be induced by hypoxia, thus it has an important role in vascular development and remodelling. It has been observed that CD105 expression is lower in samples from patients with chronic exposure of the skin to the sun compared with those with squamous cell carcinoma, thus it may be used to indicate progression. The results of the study by Florence *et al* (37) have assisted in understanding the evolution of SCC in relation to angiogenesis and in the identification of novel therapeutic targets, in particular CD105 (37).

The following study, conducted in Romania at the University of Medicine and Pharmacy Târgu Mureș and published in 2015 (38), evaluated 38 cases of SCC, using VEGF-A, cyclooxygenase-2 (COX-2), CD31, mammary serine protease inhibitor (maspin), p16 and discovered-on-gastrointestinal stromal tumour (DOG-1). The conclusion of this study was that DOG-1/COX-2 interactions were responsible for the sun exposure-independent carcinogenesis of cSCC, which could be influenced by androgens. In addition, in terms of the potential therapeutic targets, DOG-1 positive cases responded to anti-COX-2 therapy. Inhibition of angiogenesis outside of these cases could be achieved with anti-maspin medication (38).

The following two studies refer to lymphovascular proliferation. In 2012 Toll *et al* (39) used anti-podoplanin antibodies (also known as D2-40) to evaluate metastatic squamous-cell carcinoma with lymphovascular infiltrate. It aimed to identify lymphovascular invasion in skin tumours. Podoplanin is a non-specific vascular proliferation marker and is also involved in EMT, but Toll *et al* (39) concluded that podoplanin could be used to differentiate the lymphatic endothelium from that of blood vessels, and was associated with the risk of metastasis (39). The most recent study on this topic, published in 2020, supported previous research on the presence of podoplanin in cSCC, but further studies are required to outline in detail how podoplanin can be used as a prognostic marker in patients with cSCC (40).

7. Cellular proliferation markers

In 2009, Takahara *et al* (41) published a study based on the hypothesis that the marker CD10 (also known as neprilysin) in peritumoral stromal cells contributed to cell proliferation and the progression of cSCC. The results of the study on CD10 were as follows: Samples of normal skin, seborrheic keratosis and actinic keratosis demonstrated no marker expression; two of the 15 cases of Bowen's disease and keratoacanthoma presented weak expression; and all cases of SCC clearly presented strong CD10 expression in peritumoral stromal cells. It was concluded that CD10-positive stromal cells induced the migration of tumour-associated macrophages (TAMs) and the decrease in the number of Langerhans cells in the skin. These phenomena were strongly correlated with cell proliferation, highlighted by analysis of Ki-67 expression (41). In 2013 Yun *et al* (42) continued the study of the CD10 marker, assessing its expression within tumour cells, not in the peritumoral stromal cells. They compared the presence of CD10 in

Table II. Invasion markers.

First author (year)	Markers	Role/signalling pathway	Involved in disease progression/ invasiveness	Prediction of risk of recurrence	Prediction of risk of metastasis	Potential therapeutic target	(Refs.)
Lewis (2006)	$p75^{NGFR}$	$p75^{NGFR}$ marker (nerve growth factor receptor), increased detection of Perineural invasion (PNI)	+	+	+		(13)
Lewis (2006)	S-100	S-100 identifies axons, stains the endoneurium and perineurium, and reveals the space between the two structures, a key location for detecting perineural invasion	+	+	+		(13)
Nissinen (2017)	Claudin-11	Tight junction transmembrane protein involved in cell-cell adhesion, apoptosis and tumour invasion via the p38 MAPK signalling pathway	+				(29)
Munguia-Calzada (2019)	FAK	Involved in cell migration and invasion			+ lymph node metastases	+	(32)
Rose (2018)	Low expression of INPP5A PO_4-SMAD2 and PO_4-SMAD3	TGF-β signalling pathway activity (the pathway in which SMAD2 and SMAD3 are activated)	+				(34)
Cumsky (2019); Maly (2020)	Low expression of INPP5A	A membrane-associated type I inositol phosphatase	+	+	+		(35,36)

FAK, focal adhesion kinase; INPP5A, inositol polyphosphate-5-phosphatase.

selected cases of actinic keratosis, Bowenoid actinic keratosis, Bowen's disease and cSCC. All cases of precursor lesions were CD10-negative, and eight of the 25 squamous cell carcinomas (32%) were positive, suggesting that the presence of the marker in epithelial tumour cells was possibly associated with its proliferation and progression (42).

Regarding the Ki-67 marker mentioned above, in 2009 Kreuter *et al* (43) addressed its expression in invasive and *in situ* SCC due to α- and β-HPV infections of the hands, compared with an HPV-negative control group. Cells in the HPV-positive tumours demonstrated considerably higher Ki-67 expression compared with the HPV-negative cells, particularly periungual cells. Therefore, high proliferative activity, assessed by immunohistochemical staining of Ki-67, was associated with the aggressiveness and risk of recurrence of periungual cSCC (43). A study published in Romania, conducted in Craiova by Marinescu *et al* (44) in 2016, also assessed Ki-67 expression in all samples included as follows: 20 of the 28 actinic keratoses (71.4%), two cases of Bowen's disease (100%) and 54 of 61 squamous cell carcinomas (88.5%) were positive. The involvement of the p16 marker was also demonstrated in these two studies. In the study of Kreuter *et al* (43), p16 expression was shown to be present in a considerably higher percentage of HPV-positive lesions compared with HPV-negative lesions, which may have explained their increased aggressiveness (43,44).

In the study by Marinescu *et al* (44), p53 was examined, which is physiologically a tumour suppressor; however, when the encoding gene gathers certain mutations, it functions as an oncogene, and may thus serve as a tumour marker. Therefore, immunohistochemistry using Ki-67, p16 and p53 as markers may assist in differentiation between precancerous lesions and invasive SCC, and their expression supports the idea of continuous evolution of these lesions, whereas their absence suggests that other mechanisms are involved (44).

The next proliferation marker is insulin growth like factor 1 receptor (IGF-1R), which has been revealed to be predominantly associated with poorly differentiated SCC, and numerous anti-IGF-1R drugs are under study for management of inoperable tumours (45). Epidermal growth factor receptor

(EGFR), whose activation promotes cell proliferation and increases epidermal thickness and cellularity, is associated with tumour progression and a poor prognosis. Anti-EGFR therapies may be useful in select cases of metastatic carcinoma, but further studies are required to establish this (46).

The clinico-pathological and prognostic implications of the p300 marker in cutaneous squamous cell epithelium were not studied until 2014. In a study by Chen *et al* (47), all 165 samples demonstrated p300 expression, of which 79 47.9%) had low expression and 86 (52.1%) exhibited increased marker expression. Thus, the increased presence of the p300 marker was associated with aggressive traits, such as lymphatic metastases, which have also been shown to be an independent marker for a poor prognosis of the disease (47).

Another potential marker of proliferation, CD133, is strongly expressed in cancerous stem cells, which possess the ability to initiate and maintain tumour growth (48). In a study by Xu R *et al* (49), 81 of the 165 carcinoma samples (49.1%) had low CD133 expression and 84 had high expression (50.3%). Therefore, CD133 was a useful biomarker that can be used to assess the poor prognosis of the patients and can also serve as a novel therapeutic target.

With regard to absent in melanoma 2 (AIM2), a component of the inflammasome, there is evidence of its involvement in the progression of carcinoma. In normal skin samples, AIM2 expression was either absent or minimal, and in samples of actinic keratosis, Bowen's disease and invasive SCC, its expression was low, moderate and increased, respectively. These results suggest that AIM2 is a biomarker for the progression of premalignant lesions to invasive carcinoma (50).

One of the most widely studied markers in this category, especially for differential diagnosis, is p63, a member of the p53 family. Overexpression of p63 in SCC has led to the conclusion that it plays an oncogenic role. In a 2007 article by Ko *et al* (51), which included eight cases of SCC, p63 expression was evaluated, although it was a limited evaluation due to a lack of tissues for immunohistochemical staining in their study. Of the eight cases, six exhibited p63 staining and were all considered positive for it. However, four cases presented difficulties for a conclusive evaluation due to the atypical presentation of SCC; a spindle cell subtype. p63 together with cytokeratin MNF116 has been shown to assist in the diagnosis of cases of cSCC with atypical presentations of various subtypes (51).

The study by Alomari *et al* (52) addressed the diagnosis of poorly differentiated cutaneous SCC, which in most cases is a challenge for pathologists. Control cases include atypical fibroxanthoma, cutaneous leiomyoma and giant cell tumours of the soft parts. The aim of their study was to compare an isoform of p63, the p40 protein, with the other markers often used for diagnosis (p63, CKMNF116) in terms of specificity, and p40 proved to be superior (52).

The differential diagnosis is particularly important for the rare types of cSCC, such as spindle cell type, which is difficult to distinguish from atypical fibroxanthoma, desmoplastic melanoma, leiomyosarcoma and dermatofibrosarcoma protuberans (53,54). Studies show that p63 and its more specific isoform, p40, are the most important markers of differentiating spindle cell squamous-cell carcinoma from other control samples. Cytokeratin CK34βE12 (CK903) has also been shown to be promising for this purpose (54-58) (Table III).

8. Inflammation markers

In a study by Tahakara *et al* (41) from 2009, CD68 expression in dermal macrophages was predominantly positive in cases of SCC compared with the precancerous lesions. By contrast, Langerhans epidermal CD1a cells were rarer in SCC compared with the precancerous lesions (41)

Additionally, the leukocyte tumour microenvironment was analysed in 2018 by Strobel *et al* (21), and anti-CD68 (macrophages), CD20 (mature B lymphocytes), CD8 (T-lymphocytes), CD4 (helper T-lymphocytes, macrophages, monocytes and dendritic cells) were used. This comparative study included 20 renal transplant patients and 18 immunocompetent patients, and a low density of peritumoral inflammatory infiltrate in renal transplant and immunocompromised patients was observed (16).

CD4, CD8 together with CD3 (T-lymphocytes), CD123 (plasmacytoid dendritic cells) and forkhead-box-protein 3 (FOXP3; regulatory T-lymphocytes) were analysed by Mühleisen *et al* (59) in 2009, in which SCC samples from 43 immunocompetent patients and 42 transplanted and immunocompromised patients were used. Low peritumoral inflammatory infiltrate in immunocompromised patients indicates a negative prognosis, with more aggressive neoplastic growth and metastasis (59).

In a study by Pettersen *et al* (60) from 2011, it has been shown that the marker CD163 is much more specific compared with CD68 for identifying macrophages. It was noted that in the same sample, cells that simultaneously expressed CD163 and CD68 were highlighted, as well as $CD163^+$ cells that did not express CD68. This translates into the fact that CD163 has higher specificity and sensitivity compared with CD68 for macrophages. Moreover, $CD68^+$ cells exhibit double immunohistochemical staining with CD11c (dermal myeloid dendritic cells), which supports the advantage of CD163 (60).

In 2015, Sandvik *et al* (61) studied the markers CD11c (for dermal myeloid dendritic cells), CD303 (CLEC4C; for dermal plasmacytoid dendritic cells), CD163 (macrophages) and FOXP3 (T-lymphocytes) in immunocompromised patients and a control group of immunocompetent patients. Peritumoral $CD11c^+$ labelled myeloid dendritic cells were revealed to be fewer in immunocompromised patients. The proportion of $FOXP3^+$ T-lymphocytes was also decreased in this group. The number of macrophages was revealed to be similar in both groups. Contrary to the previous article published by Mühleisen *et al* (59), the number of plasmacytoid dendritic cells, this time labelled with CD303, was similar in both groups (61) (Table IV).

9. Conclusions

In conclusion, the present review summarized the literature on IHC markers used for the diagnosis and staging of cSCC, which may be of relevance as appropriate staging can provide important insights into the extent of the disease and allow for suitable treatment. In addition, characteristics such as the

Table III. Vascular, lymphatic and cell proliferation markers.

First author (year)	Marker	Role/signalling pathway	Involved in disease progression/ invasiveness	Prediction of risk of recurrence	Prediction of risk of metastasis	Potential therapeutic target	(Refs.)
Florence (2011)	CD105	A marker for neo-angiogenesis. associated with proliferation and can be induced by hypoxia. It has an important role in vascular development and remodelling as well	+			+	(37)
Ciortea (2015)	Maspin	Maspin cytoplasmic to nuclear translocation	+			+	(38)
Ciortea (2015)	DOG-1, COX-2	DOG-1/COX-2 interaction is responsible for the sun-independent carcinogenesis of cutaneous squamous-cell carcinoma that can be influenced by androgens				+	(38)
Toll (2012)	D2-40 (anti-podoplanin) antibodies		+ tumour lymph vessel invasion		+		(39)
Gulseren (2020)	Podoplanin	Involved in the EMT	+ tumour lymph vessel invasion				(40)
Takahara (2009); Yun (2013)	CD10 (neprilysin)	Marker of stromal fibroblasts	+				(41,42)
Kreuter (2009); Marinescu (2016)	Ki67	Marker of proliferation	+	+	+		(43,44)
Oh (2014)	IGF-1R	Detected in the cell surface membrane of well-differentiated cSCC. It is predominantly present in the cytoplasm in moderately differentiated cSCC, and is poorly expressed in the nuclei of tumour cells of poorly differentiated cSCC	+			+	(45)
Canueto (2017)	EGFR	Promotes cell proliferation and increases epidermal thickness and cellularity	+		+ lymph node metastasis	+	(46)
Chen (2015)	p300 marker	Transcriptional coactivator participates in the regulation of a wide range of cell biological processes	+		+ lymph node metastasis		(47)
Xu (2016)	CD133	Initiates and maintains tumour growth	+			+	(49)

DOG-1, discovered-on-gastrointestinal stromal tumour; COX-2, cyclooxygenase-2; EMT, epithelial-mesenchymal transition; cSCC, squamous-cell carcinoma.

Table IV. Markers of inflammation.

First author (year)	Marker	Role/signalling pathway	Involved in disease progression/ invasiveness	Prediction of risk of recurrence	Prediction of risk of metastasis	Potential therapeutic target	(Refs.)
Farshchian (2014)	AIM2	Progression of premalignant lesions to invasive carcinoma		+		+	(50)
Ko (2008), Alomari (2014), Morgan (2008), Dotto (2006), Hall (2008), Gleason (2009), Ha (2014)	p63, p40, CKMNF116, CK34βE12 (CK903)	p63, a member of the p53 family, is expressed as two distinct isoforms [ΔNp63 (p40) and TAp63]. p63, p40 and CK903 are important markers used for differentiating spindle cell squamous-cell carcinoma from other lesions	+	+			(51,52,54-58)
Takahara, (2009)	CD10, CD68	CD10 marker for stromal fibroblasts, CD68 marker for dermal macrophages		+			(41)
Strobel (2018)	A decrease in immune cell infiltrates	Anti-CD68 markers (macrophages), CD20 (mature B lymphocytes), CD8 (T lymphocytes), CD4 (helper T lymphocytes, macrophages, monocytes, dendritic cells)		+			(21)
Mühleisen, (2009)	A decrease in perineoplastic inflammatory infiltrate	CD3, CD4, CD8, FOXP3, CD123 and STAT1 in immunocompromised individuals		+	+		(59)
Pettersen (2011)	$CD163^+$ and $CD68^+$ TAMs	$CD163^+$ TAMs produced pro-tumoral factors MMP9 and MMP11, at the gene and protein levels		+			(60)
Sandvik (2015)	A decrease in CD11c in dermal myeloid dendritic cells			+			(61)

AIM2, absent in melanoma 2; FOXP3, forkhead-box-protein 3; TAMs, tumour-associated macrophages.

size of tumour, thickness, tumour depth, margins, histological grade, histological subtype, perineural and lymphovascular invasion are valuable predictors of disease progression and disease-specific death.

In the studies reviewed, some of the IHC markers have proven useful in diagnosing cutaneous poorly differentiated SCC, for example: p63, p40, CKMNF116 and CK34βE12 (CK903) (51,52,54-58).

IHC markers that allow for prediction of the aggressiveness, invasiveness and risk of tumour progression included markers of mesenchymal epithelial transition [E-cadherin (27), podoplanin (23,26-28) and Twist (5,23)], markers of invasion [$p75^{NGFR}$, S-100 (13), Claudin-11 (29), INPP5A (34-36)], markers of vascular/lymphatic cell proliferation [CD105 (37), DOG-1, maspin (38), anti-podoplanin antibodies (23), CD10 (41,42), IGF-1R (45), EGFR (46), p300 marker (47) and CD133 (49)] and inflammatory markers [AIM2 (50), CD10, CD68 (41), a decrease in immune cell infiltrates (21), reduced peri neoplastic inflammatory infiltrate (59), TAMs, CD163, CD68 (60) and CD11c (61)].

In prediction of the risk of recurrence, EMT markers [AXIN2 and SNAIL (22)], invasion markers [S-100, $p75^{NGFR}$ (13), INPP5A (35,36)] and Ki-67, a proliferation marker (43,44) hold value.

To predict the risk of metastasis, EMT markers [nuclear β-catenin (23), E-cadherin (23,26,28), Zeb1 (23), podoplanin (23,27,28), Twist (23,26) and human epidermal growth factor receptor 4 (27)], invasion markers [$p75^{NGFR}$, S-100 (13), FAK (32) and INPP5A (35,36)], proliferation markers [anti-podoplanin antibodies (23), Ki-67 (43,44) EGFR (46), p300 marker (47), and a lower density of peri-neoplastic inflammatory infiltrate in immunocompromised individuals (59) may have predictive value.

The therapeutic targets identified included podoplanin (a marker of EMT) (23,27,28), FAK (a marker of invasion) (32), CD105 (37), cytoplasm to nuclear translocation of maspin, DOG-1 (38), IGF-1R (45), EGFR (46), CD133 (all markers of proliferation) (49) and AIM2 (a marker of inflammation) (50).

Molecular mechanisms, as determined by the expression of certain immunohistochemical markers, may be useful in diagnosing atypical forms of cSCC and highlighting the specific features of aggressive tumours. Therefore, the present study placed an emphasis on the importance of understanding the pathophysiological mechanisms at the different stages of evolution and progression of SCC.

The primary limitation of the present systematic review arises from the design of the included studies (observational but also experimental studies), the heterogeneity of the studies (human tissue probes and also cell line studies), and the large differences between the number of cases included in each of these studies. Due to the limitations of most of the analysed studies, no final conclusions can be postulated. Further studies are required to better understand the value of these immunohistochemical markers, and also for the discovery of novel biomarkers and more adequate therapeutic strategies. Newer therapies, such as targeted therapy and immunotherapy used for SCC cases with limited treatment options are evidence of the value of deciphering the biological behaviours of cSCCs. Understanding the molecular mechanisms underlying the development and progression of cSCC, as well as highlighting the value of IHC markers with a role in disease progression, may be the basis for the discovery of novel biomarkers and development of improved therapeutic strategies.

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ACG, RIN, EB, AB, AM, GT, MA, AH, LM, MB, RA, IH, CGP, LN, MDC and SAZ conceived of the presented idea, performed data acquisition and drafted the manuscript. EB contributed to documentation on the topic of interest, work conception and revised the manuscript. RIN, EB, DAI performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. RIN and DAI gave final approval. All authors read and approved the final version of the manuscript. Data sharing is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Stanganelli I, Spagnolo F, Argenziano G, Ascierto PA, Bassetto F, Bossi P, Donato V, Massi D, Massone C, Patuzzo R, *et al*: The multidisciplinary management of cutaneous squamous cell carcinoma: A comprehensive review and clinical recommendations by a panel of experts. Cancers (Basel) 14: 377, 2022.
2. Parekh V and Seykora JT: Cutaneous squamous cell carcinoma. Clin Lab Med 37: 503-525, 2017.
3. Lomas A, Leonardi-Bee J and Bath-Hextall F: A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol 166: 1069-1080, 2012.
4. Claveau J, Archambault J, Ernst DS, Giacomantonio C, Limacher JJ, Murray C, Parent F and Zloty D: Multidisciplinary management of locally advanced and metastatic cutaneous squamous cell carcinoma. Curr Oncol 27: e399-e407, 2020.
5. Roscher I, Falk RS, Vos L, Clausen OPF, Helsing P, Gjersvik P and Robsahm TE: Validating 4 staging systems for cutaneous squamous cell carcinoma using population-based data: A nested case-control study. JAMA Dermatol 154: 428-434, 2018.
6. Morgado-Carrasco D, Bosch-Amate X, Fusta-Novell X and Garcia-Herrera A: RF-staging cutaneous squamous cell carcinoma: Challenges and questions. Actas Dermosifiliogr (Engl Ed) 111: 261-262, 2020 (In English, Spanish).
7. Ruiz ES, Karia PS, Besaw R and Schmults CD: Performance of the American joint committee on cancer staging manual, 8th Edition vs. the Brigham and Women's hospital tumor classification system for cutaneous squamous cell carcinoma. JAMA Dermatol 155: 819-825, 2019.
8. Kalluri R and Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest 119: 1420-1428, 2009.
9. Micalizzi DS, Farabaugh SM and Ford HL: Epithelial-mesenchymal transition in cancer: Parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia 15: 117-134, 2010.

10. Ribatti D, Tamma R and Annese T: Epithelial-mesenchymal transition in cancer: A historical overview. Transl Oncol 13: 100773, 2020.
11. Gerashchenko TS, Novikov NM, Krakhmal NV, Zolotaryova SY, Zavyalova MV, Cherdyntseva NV, Denisov EV and Perelmuter VM: Markers of cancer cell invasion: Are they good enough? J Clin Med 8: 1092, 2019.
12. Tsai JH and Yang J: Epithelial-mesenchymal plasticity in carcinoma metastasis. Genes Dev 27: 2192-2206, 2013.
13. Lewis Kelso R, Colome-Grimmer MI, Uchida T, Wang HQ and Wagner RF Jr: p75(NGFR) immunostaining for the detection of perineural invasion by cutaneous squamous cell carcinoma. Dermatol Surg 32: 177-183, 2006.
14. Kumar V, Abbas A, Aster JC and Robbins SL: Robbins Basic Pathology, 9th Edition. Elsevier Inc., Philadelphia, PA, 2013.
15. Lugano R, Ramachandran M and Dimberg A: Tumor angiogenesis: Causes, consequences, challenges and opportunities. Cell Mol Life Sci 77: 1745-1770, 2020.
16. Hawighorst T: Angiogenesis, lymphangiogenesis, and tumor progression. Zentralbl Gynakol 124: 497-505, 2002 (In German).
17. Iatropoulos MJ and Williams GM: Proliferation markers. Exp Toxicol Pathol 48: 175-181, 1996.
18. Zitvogel L, Tesniere A and Kroemer G: Cancer despite immunosurveillance: Immunoselection and immunosubversion. Nat Rev Immunol 6: 715-727, 2006.
19. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C and Fridman WH: Immune infiltration in human tumors: A prognostic factor that should not be ignored. Oncogene 29: 1093-1102, 2010.
20. Bui JD and Schreiber RD: Cancer immunosurveillance, immunoediting and inflammation: Independent or interdependent processes? Curr Opin Immunol 19: 203-208, 2007.
21. Strobel SB, Safferling K, Lahrmann B, Hoffmann JH, Enk AH, Hadaschik EN, Grabe N and Lonsdorf AS: Altered density, composition and microanatomical distribution of infiltrating immune cells in cutaneous squamous cell carcinoma of organ transplant recipients. Br J Dermatol 179: 405-412, 2018.
22. Zhao G, Kim KY, Zheng Z, Oh Y, Yoo DS, Lee ME, Chung KY, Roh MR and Jin Z: AXIN2 and SNAIL expression predict the risk of recurrence in cutaneous squamous cell carcinoma after Mohs micrographic surgery. Oncol Lett 19: 2133-2140, 2020.
23. Toll A, Masferrer E, Hernandez-Ruiz ME, Ferrandiz-Pulido C, Yébenes M, Jaka A, Tuneu A, Jucglà A, Gimeno J, Baró T, *et al*: Epithelial to mesenchymal transition markers are associated with an increased metastatic risk in primary cutaneous squamous cell carcinomas but are attenuated in lymph node metastases. J Dermatol Sci 72: 93-102, 2013.
24. Kourtidis A, Lu R, Pence LJ and Anastasiadis PZ: A central role for cadherin signaling in cancer. Exp Cell Res 358: 78-85, 2017.
25. Zhang Y, Liu B, Zhao Q, Hou T and Huang X: Nuclear localizaiton of β-catenin is associated with poor survival and chemo-/radioresistance in human cervical squamous cell cancer. Int J Clin Exp Pathol 7: 3908-3917, 2014.
26. Barrette K, Van Kelst S, Wouters J, Marasigan V, Fieuws S, Agostinis P, van den Oord J and Garmyn M: Epithelial-mesenchymal transition during invasion of cutaneous squamous cell carcinoma is paralleled by AKT activation. Br J Dermatol 171: 1014-1021, 2014.
27. Vinicius de LV, Scapulatempo C, Perpetuo NM, Mohamed F, de Carvalho TS, de Oliveira AT, Segalla JG and Carvalho AL: Prognostic and risk factors in patients with locally advanced cutaneous squamous cell carcinoma of the trunk and extremities. J Skin Cancer 2011: 420796, 2011.
28. Hesse K, Satzger I, Schacht V, Köther B, Hillen U, Klode J, Schaper K and Gutzmer R: Characterisation of prognosis and invasion of cutaneous squamous cell carcinoma by podoplanin and E-Cadherin expression. Dermatology 232: 558-565, 2016.
29. Nissinen L, Siljamaki E, Riihila P, Piipponen M, Farshchian M, Kivisaari A, Kallajoki M, Raiko L, Peltonen J, Peltonen S and Kähäri VM: Expression of claudin-11 by tumor cells in cutaneous squamous cell carcinoma is dependent on the activity of p38δ. Exp Dermatol 26: 771-777, 2017.
30. Clark ES, Whigham AS, Yarbrough WG and Weaver AM: Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia. Cancer Res 67: 4227-4235, 2007.
31. Canel M, Secades P, Garzon-Arango M, Allonca E, Suarez C, Serrels A, Frame M, Brunton V and Chiara MD: Involvement of focal adhesion kinase in cellular invasion of head and neck squamous cell carcinomas via regulation of MMP-2 expression. Br J Cancer 98: 1274-1284, 2008.
32. Munguia-Calzada P, Fernandez-Vega I, Martinez-Camblor P, Díaz-Coto S, García-Pedrero JM, Vivanco B, Osuna CG, Vazquez-Lopez F, Rodrigo JP and Santos-Juanes J: Correlation of focal adhesion kinase expression with nodal metastasis in patients with head and neck cutaneous squamous cell carcinoma. Head Neck 41: 1290-1296, 2019.
33. Serrels A and Frame MC: FAK goes nuclear to control anti-tumor immunity-a new target in cancer immuno-therapy. Oncoimmunology 5: e1119356, 2016.
34. Rose AM, Spender LC, Stephen C, Mitchell A, Rickaby W, Bray S, Evans AT, Dayal J, Purdie KJ, Harwood CA, *et al*: Reduced SMAD2/3 activation independently predicts increased depth of human cutaneous squamous cell carcinoma. Oncotarget 9: 14552-14566, 2018.
35. Cumsky HJL, Costello CM, Zhang N, Butterfield R, Buras MR, Schmidt JE, Drenner K, Nelson SA, Ochoa SA, Baum CL, *et al*: The prognostic value of inositol polyphosphate 5-phosphatase in cutaneous squamous cell carcinoma. J Am Acad Dermatol 80: 626-632 e1, 2019.
36. Maly CJ, Cumsky HJL, Costello CM, Schmidt JE, Butterfield RJ, Zhang N, DiCaudo DJ, Nelson SA, Smith ML, Ochoa SA, *et al*: Prognostic value of inositol polyphosphate-5-phosphatase expression in recurrent and metastatic cutaneous squamous cell carcinoma. J Am Acad Dermatol 82: 846-853, 2020.
37. Florence ME, Massuda JY, Brocker EB, Metze K, Cintra ML and Souza EM: Angiogenesis in the progression of cutaneous squamous cell carcinoma: An immunohistochemical study of endothelial markers. Clinics (Sao Paulo) 66: 465-468, 2011.
38. Ciortea CD, Jung I, Gurzu S, Kovecsi A, Turdean SG and Bara T: Correlation of angiogenesis with other immunohistochemical markers in cutaneous basal and squamous cell carcinomas. Rom J Morphol Embryol 56 (2 Suppl): S665-S670, 2015.
39. Toll A, Gimeno-Beltran J, Ferrandiz-Pulido C, Masferrer E, Yébenes M, Jucglà A, Abal L, Martí RM, Sanmartín O, Baró T, *et al*: D2-40 immunohistochemical overexpression in cutaneous squamous cell carcinomas: A marker of metastatic risk. J Am Acad Dermatol 67: 1310-1318, 2012.
40. Gulseren D, Gokoz O, Karahan S and Karaduman A: Podoplanin expression in cutaneous squamous cell carcinomas and its relationship to histopathological prognostic factors. J Histotechnol 43: 147-152, 2020.
41. Takahara M, Chen S, Kido M, Takeuchi S, Uchi H, Tu Y, Moroi Y and Furue M: Stromal CD10 expression, as well as increased dermal macrophages and decreased Langerhans cells, are associated with malignant transformation of keratinocytes. J Cutan Pathol 36: 668-674, 2009.
42. Yun JH, Roh JY, Park SH and Lee JR: CD10 expression in cutaneous squamous cell carcinoma and its precursor lesions: Evaluation using tissue microarray. Ann Dermatol 25: 515-517, 2013.
43. Kreuter A, Gambichler T, Pfister H and Wieland U: Diversity of human papillomavirus types in periungual squamous cell carcinoma. Br J Dermatol 161: 1262-1269, 2009.
44. Marinescu A, Stepan AE, Margaritescu C, Marinescu AM, Zăvoi RE, Simionescu CE and Niculescu M: P53, p16 and Ki67 immunoexpression in cutaneous squamous cell carcinoma and its precursor lesions. Rom J Morphol Embryol 57 (2 Suppl): S691-S696, 2016.
45. Oh ST, Eun YS, Yoo DS, Park HJ, Kim TY, Cho BK, Stark A and Reichrath J: Expression of insulin-like growth factor-1 receptor in conventional cutaneous squamous cell carcinoma with different histological grades of differentiation. Am J Dermatopathol 36: 807-811, 2014.
46. Canueto J, Cardenoso E, Garcia JL, Santos-Briz Á, Castellanos-Martín A, Fernández-López E, Blanco Gómez A, Pérez-Losada J and Román-Curto C: Epidermal growth factor receptor expression is associated with poor outcome in cutaneous squamous cell carcinoma. Br J Dermatol 176: 1279-1287, 2017.
47. Chen MK, Cai MY, Luo RZ, Tian X, Liao QM, Zhang XY and Han JD: Overexpression of p300 correlates with poor prognosis in patients with cutaneous squamous cell carcinoma. Br J Dermatol 172: 111-119, 2015.
48. Sellheyer K: Basal cell carcinoma: Cell of origin, cancer stem cell hypothesis and stem cell markers. Br J Dermatol 164: 696-711, 2011.

49. Xu R, Cai MY, Luo RZ, Tian X, Han JD and Chen MK: The expression status and prognostic value of cancer stem cell biomarker CD133 in cutaneous squamous cell carcinoma. JAMA Dermatol 152: 305-311, 2016.
50. Farshchian M, Nissinen L, Siljamaki E, Riihilä P, Piipponen M, Kivisaari A, Kallajoki M, Grénman R, Peltonen J, Peltonen S, *et al*: Tumor cell-specific AIM2 regulates growth and invasion of cutaneous squamous cell carcinoma. Oncotarget 8: 45825-45836, 2017.
51. Ko CJ, McNiff JM and Glusac EJ: Squamous cell carcinomas with single cell infiltration: A potential diagnostic pitfall and the utility of MNF116 and p63. J Cutan Pathol 35: 353-357, 2008.
52. Alomari AK, Glusac EJ and McNiff JM: p40 is a more specific marker than p63 for cutaneous poorly differentiated squamous cell carcinoma. J Cutan Pathol 41: 839-845, 2014.
53. Yanofsky VR, Mercer SE and Phelps RG: Histopathological variants of cutaneous squamous cell carcinoma: A review. J Skin Cancer 2011: 210813, 2011.
54. Morgan MB, Purohit C and Anglin TR: Immunohistochemical distinction of cutaneous spindle cell carcinoma. Am J Dermatopathol 30: 228-232, 2008.
55. Dotto JE and Glusac EJ: p63 is a useful marker for cutaneous spindle cell squamous cell carcinoma. J Cutan Pathol 33: 413-417, 2006.
56. Hall JM, Saenger JS and Fadare O: Diagnostic utility of P63 and CD10 in distinguishing cutaneous spindle cell/sarcomatoid squamous cell carcinomas and atypical fibroxanthomas. Int J Clin Exp Pathol 1: 524-530, 2008.
57. Gleason BC, Calder KB, Cibull TL, Thomas AB, Billings SD, Morgan MB, Hiatt KM and Smoller BR: Utility of p63 in the differential diagnosis of atypical fibroxanthoma and spindle cell squamous cell carcinoma. J Cutan Pathol 36: 543-547, 2009.
58. Ha Lan TT, Chen SJ, Arps DP, Fullen DR, Patel RM, Siddiqui J, Carskadon S, Palanisamy N and Harms PW: Expression of the p40 isoform of p63 has high specificity for cutaneous sarcomatoid squamous cell carcinoma. J Cutan Pathol 41: 831-838, 2014.
59. Mühleisen B, Petrov I, Gachter T, Kurrer M, Schärer L, Dummer R, French LE and Hofbauer GF: Progression of cutaneous squamous cell carcinoma in immunosuppressed patients is associated with reduced CD123+ and FOXP3+ cells in the perineoplastic inflammatory infiltrate. Histopathology 55: 67-76, 2009.
60. Pettersen JS, Fuentes-Duculan J, Suarez-Farinas M, Pierson KC, Pitts-Kiefer A, Fan L, Belkin DA, Wang CQ, Bhuvanendran S, Johnson-Huang LM, *et al*: Tumor-associated macrophages in the cutaneous SCC microenvironment are heterogeneously activated. J Invest Dermatol 131: 1322-1330, 2011.
61. Sandvik LF, Skarstein K, Krynitz B, Volchenkov R, Sviland L, Leivestad T, Jonsson R and Appel S: Peritumoral dermis of squamous cell carcinomas in renal transplant recipients contains less CD11c+ myeloid dendritic cells and FoxP3+ T cells compared to immunocompetent controls. J Eur Acad Dermatol Venereol 29: 2128-2135, 2015.