**Introduction**

Insulin-like growth factor-II (IGF-II) messenger RNA (mRNA)-binding protein-3 (IMP-3) is known as a homology domain-containing protein overexpressed in cancer (KOC) and L232S, a member of the IGF-II mRNA-binding protein (IMP) family, which also includes IMP-1 and IMP-2. IMP-3 is a 270 amino-acid protein encoded by a 14350-bp mRNA transcript produced by a gene located on chromosome 7p11.5. It is associated with cell proliferation and considered an oncogenic protein due to its expression during embryogenesis and in some malignancies including pancreatic carcinoma, renal cell carcinoma, endometrial carcinoma, germ cell neoplasms, ovarian carcinoma, extrapulmonary small-cell carcinoma, as well as high-grade neuroendocrine carcinoma, squamous cell carcinoma, and adenocarcinoma of the lung. Its exact role in carcinogenesis is still unclear.

IMP-3 has been shown to be a prognostic marker in renal cell carcinoma, colorectal carcinoma and gastric adenocarcinoma, and has been proposed as a potential therapeutic target for lung cancer. IMP-3 expression increases with the degree of dysplasia in the pancreatic ductal epithelium; it is related to tumor stage in pancreatic carcinomas and to aggressive behavior of urothelial carcinomas. Moreover, IMP-3 has been claimed as a diagnostic clue in cutaneous melanocytic neoplasms as it is expressed in malignant melanomas but not in benign melanocytic nevi, even when dysplastic features are present. Squamous cell carcinoma of head and neck, tongue and uterine cervix have been shown to express IMP-3 but, to the best of our knowledge, cutaneous squamous cell tumors have never been investigated.

Keratoacanthoma is an intriguing tumor, most often considered a benign neoplasm intended to involute with complete resolution within a few months. Other authors classify it as a subtype of squamous cell carcinoma. In routine practice, histologic and cytopathic features of keratoacanthoma and squamous cell carcinoma are often difficult to distinguish and a reliable marker to differentiate these lesions has not been found. The question has been raised as to whether keratoacanthoma is an unreliable histological diagnosis or these tumors have a latent, although rare, malignant potential. The understanding of the nature of keratoacanthoma has been controversial since its original description between 1950 and 1980. The consensus of the dermatology and dermatopathology community has been to classify this lesion as a benign condition, although papers describing malignant behavior in keratoacanthoma have been published.

The objective of this study was to analyze immunohistochemical IMP-3 expression in keratoacanthomas and cutaneous squamous cell carcinomas to determine whether IMP-3 can aid in the differential diagnosis of these lesions.

**Materials and Methods**

**Samples**

A retrospective study was initiated to review the medical records of all patients with a diagnosis of keratoacanthoma and squamous cell carcinoma between 2010 and 2011 at the Pathology Division, University of Cagliari, Italy. Through a careful clinicopathological correlation, we identified 67 squamous cell skin lesions grouped into 34 cases of keratoacanthoma and 33 of squamous cell carcinoma, 17 in situ and 16 invasive. Clinicopathological variables such as patients’ age and sex, maximum diameter of lesions, growth phase of KA, ulceration, Clark level and depth of invasion of SCC were recorded.

**Immunohistochemistry**

Five micron paraffin sections were immunostained for IMP-3 (code M3626 monoclonal mouse anti-Human IMP-3; Dako Cytomation, Carpenteria, CA, USA). We used the Dako Cytomation LSAB2 system-HRP with a Dako Autostainer (Dako Cytomation). This system is based on a technique that employs a modified labeled avidin-biotin (LAB) where
a biotinylated secondary antibody forms a complex with peroxidase-conjugated streptavidin molecules. Endogenous peroxidase activity was quenched by incubating (5 min) specimens with 3% hydrogen peroxide. Heat-induced antigen retrieval was adopted (20 min at 98° in Tris/EDTA pH 9). Tissue sections were incubated (30 min at room temperature) with the IMP-3 antibody. Staining was completed after incubation (10 min) with AEC (3-amino-9-ethyl carbazole, substrate chromogen) and it resulted in a red-colored precipitate at the antigen site. Slides were reviewed by two pathologists (SS and LP) who were not aware of the clinical data, and evaluated for both tumor cell percentage and intensity of immunoreactivity. Cytoplasmic staining was considered positive for IMP-3 expression. The percentage of positive cells was recorded as 0 (negative); 1+ (<5% of cells stained); 2+ (5-9.9% of cells stained); 3+ (10-49.9% of cells stained); and 4+ (50-90%); and >90% of cells stained.\textsuperscript{26} Intensity was scored as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong),\textsuperscript{27} and evaluated by comparison with contiguous sebaceous glands (considered moderately positive).

### Statistical analysis

The response variables involved in the analysis, such as the percentage of positive cells or their intensity, are of the semiquantitative type, more precisely, they are ordered polytomous categorical values. Therefore, of interest is not their value (\textit{i.e.,} 1, 2, etc.), but instead their frequency distribution and how it changes across different values of the predictor variables. To regress such variables over a specified set of predictors it is more appropriate to use the proportional odds model;\textsuperscript{26} basically, this is a generalization of the regression model for polytomous response variables. The P-values obtained from the proportional odds model for the regression coefficients resemble the evidence for the association between the specific regressor variable and the polytomous response one. The preselected significance is 5% (P<0.05). The proportional odds model is a rather standard model implemented in software as, for instance, \textit{R}.\textsuperscript{27}

### Results

#### Clinicopathological features of squamous skin lesions

Clinical features of squamous skin lesions reviewed in our study are presented in Table 1. The keratoacanthomas were from 34 subjects (22 males and 12 females) ranging from 39 to 90 years of age (mean age 69.5). The lesions consisted clinically of a firm, dome-shaped nodule ranging from 6 to 30 mm in maximum diameter with a horn-filled crater. They reached their full size with rapid growth in a period ranging from a few weeks to some months. Histologically, keratoacanthomas were symmetric exo-endophytic lesions with central horn-filled crater and overhanging epithelial lips, composed of glassy keratinocytes with intracytoplasmatic glycogen and intraepithelial elastic fibers, characterized by a sharp outline between tumor and stroma, not extending to a depth below the eccrine glands. A rather pronounced mixed inflammatory infiltrate was present in the surrounding dermis, sometimes with development of eosinophilic or neutrophilic epithelial microabscesses. Keratinocytes showed variable degrees of nuclear atypia and mitotic figures, usually confined to the basal layers and more pronounced in keratoacanthomas in the early proliferative stage. Perineural or vascular invasion was not observed in any of our cases. Sixteen cases had at least partial features of regressing lesions showing flattening of the central horn-filled crater.

The 33 squamous cell carcinomas included 17 intraepithelial \textit{(in situ)} carcinomas (Clark I) from 9 males and 8 females ranging from 54 to 84 years of age (mean age 74.4) and 16 invasive carcinomas (3: Clark II, 1: Clark III, 9: Clark IV, 3: Clark V) from 11 males and 5 females ranging from 63 to 94 years of age (mean age 78) (Tables 1 and 2). Clinically, SCCs \textit{in situ} appeared as slowly enlarging erythematos patches showing little or no infiltration, with areas of scaling and crusting. Twelve of the selected invasive SCCs presented as

### Table 1. Clinical data.

<table>
<thead>
<tr>
<th>N.</th>
<th>Max diam. (mm)</th>
<th>Sex</th>
<th>Age</th>
<th>Mean age</th>
<th>Median age</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>KA</td>
<td>34</td>
<td>M (22)</td>
<td>39-90 y</td>
<td>69.5</td>
<td>71.5</td>
<td>Face (11), arm (6), hand (6), trunk (3), thigh (2), scalp (2), leg (2), neck (1), shoulder (1)</td>
</tr>
<tr>
<td>SCC invasive</td>
<td>16</td>
<td>M (11)</td>
<td>63-94 y</td>
<td>78.0</td>
<td>77</td>
<td>Face (9), scalp (4), arm (1), ear (1), hand (1)</td>
</tr>
<tr>
<td>SCC \textit{in situ}</td>
<td>17</td>
<td>M (9)</td>
<td>50-84 y</td>
<td>74.4</td>
<td>77</td>
<td>Face (6), leg (5), scalp (2), ear (2), hand (1), trunk (1)</td>
</tr>
</tbody>
</table>

\textit{KA}, keratoacanthoma; SCC, squamous cell carcinoma; M, male; F, female.
ulcerative skin lesions with a keratinous crust and elevated, indurated surrounding, while 5 of them presented as nodular tumors often misdiagnosed as basal cell carcinomas. Histologically, irrespective of the presence or absence of ulceration, SCCs were characterized by nests of atypical squamous cells with abundant eosinophilic cytoplasm and a large, often vesicular nucleus arising from the epidermis and invading the dermis to variable extent.

**IMP-3 expression by immunohistochemistry**

Data on IMP-3 immunohistochemical staining are presented in Table 3. Of 34 KAs, 25 (74%) were negative for IMP-3 staining (Figure 1a,b). Nine positive KAs (6 proliferative and 3 regressing) showed IMP-3 cytoplasmic expression in <50% of tumor cells. IMP-3 staining intensity was weak (1+) in six out of nine and moderate (2+) in three out of nine. The positivity was usually confined to basal layers of atypical keratinocytes (Figure 2a,b). The growth phase (proliferative or regressing) was not related to IMP-3 expression (P=0.02569). On the contrary, 19 of 33 SCCs (57%) were IMP-3 positive (8/16 invasive, 11/17 in situ) (Figure 3a,b; Figure 4a,b). The pattern of IMP-3 expression in these cases was variable, ranging from focal and weak to intense and diffuse positivity. Fourteen of thirty-three SCCs (43%) (8/16 invasive, 6/17 in situ) were completely negative for IMP-3 (Figure 5a,b). The percentage of IMP3-positive cells in invasive SCCs was not related to the three clinicopathological features considered: ulceration (P=0.7152), Clark level (P=0.6924) and depth of invasion (P=0.8695). Age, sex, and lesion diameter were not related to IMP-3 in any of the groups. To compare immunohistochemical data on KA to SCC, we found statistical evidence for the percentage of IMP3-positive cells to increase significantly in the invasive SCC group (P=0.0111), and particularly in the SCC in situ group (P=0.0021) with respect to the KA group. IMP-3 intensity staining increased significantly in invasive SCCs (P=0.0213), and particularly in SCCs in situ (P=0.008) with respect to KA. The intensity was not significantly related to the percentage of IMP-3 positive cells.

**Discussion**

Keratoacanthoma is a controversial lesion considered either benign or a subtype of squamous cell carcinoma.19 In routine histopathological examination there are tumors that are difficult to classify as either KA or SCC. Helpful criteria for the diagnosis of a KA include epithelial lips, a sharp outline between tumor and stroma and absence of ulceration.28-33 Criteria more commonly seen in SCCs include a
Table 3. Immunohistochemical data.

<table>
<thead>
<tr>
<th>N</th>
<th>Negative</th>
<th>&lt;5%</th>
<th>5-9.9%</th>
<th>10-49.9%</th>
<th>50-90%</th>
<th>&gt;90%</th>
<th>weak 1+</th>
<th>moderate 2+</th>
<th>strong 3+</th>
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<tbody>
<tr>
<td>KA</td>
<td>34</td>
<td>25</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>SCC invasive</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SCC in situ</td>
<td>17</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

KA, keratoacanthoma; SCC, squamous cell carcinoma.

References
12. Ozdemir NO, Türk NS, Düzcan E. IMP3 Expression in urethelial carcinomas of the


