Protein Expression of Carcinoembryonic Antigen Cell Adhesion Molecules in Benign and Malignant Melanocytic Skin Lesions

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Upon completion of this activity you will be able to:
• define cell adhesion molecules such as members of the carcinoembryonic antigen (CEA) family.
• describe the relationship between cell adhesion molecules such as CEA and CEA adhesion molecule-1 (CEACAM1) and the development and progression of malignant melanoma.

Abstract

Dysregulation of cell adhesion molecules is associated with progression of malignant melanoma. Immunohistologic study of benign nevi (BN), dysplastic nevi (DN), and primary superficial spreading melanoma (SSM) was performed for carcinoembryonic antigen (CEA) and CEA cell adhesion molecule-1 (CEACAM1) using monoclonal antibodies. We investigated BN (n = 42), DN (n = 22), thin SSM (n = 21), and thick SSM (n = 21). CEA expression in melanomas and DN was significantly increased compared with BN. CEA expression in thick SSM was significantly higher than in DN. Compared with BN, expression of CEACAM1 in melanomas was significantly increased. CEACAM1 expression in thick SSM was significantly increased compared with DN and thin SSM. Our data support the findings of previous studies indicating that cell adhesion molecules of the CEA family may have a role in the development and progression of cutaneous melanoma and potentially serve as prognostic markers.

Cutaneous malignant melanoma is a tumor developing by transformation of melanocytes. The worldwide incidence and mortality rate in fair-skinned populations are on the increase. The presence of metastases carries a severe prognosis because efficacious systemic treatments are still lacking. An earlier differentiation and detection of the primary melanoma would help improve diagnosis and prognosis. Toward this aim, markers identifying malignant lesions are needed. Moreover, understanding the molecular bases of oncogenicity in melanocytic proliferations may contribute to the development of efficacious therapies.

Dysregulation of cell adhesion molecules is associated with tumor progression in melanoma. The expression of cell adhesion molecules may be up-regulated or down-regulated when melanomas progress from the radial to the vertical growth phase. Among the most prominent cell adhesion molecules down-regulated during tumor progression is E-cadherin. On the other hand, integrins and the melanoma cell adhesion molecule are expressed preferentially in the vertical growth phase.

In this context, emphasis has recently been placed on the cell adhesion molecule CEACAM1, which is also known as biliary glycoprotein I or CD66a. CEACAM1 is a member of the carcinoembryonic antigen (CEA) family also belonging to the immunoglobulin superfamily. There is a lack of studies systematically investigating CEA and CEACAM1 expression in malignant melanoma and its precursor lesions. The present study was designed to investigate the protein expression pattern of CEA and CEACAM1 in superficial spreading melanoma (SSM), dysplastic nevi (DN), and benign nevi (BN).
Materials and Methods

Cases

We studied paraffin-embedded sections of BN, DN, and SSM of patients who underwent surgery between 2002 and 2008 in the Department of Dermatology, Ruhr-University Bochum, Bochum, Germany. Diagnosis and staging of nevi and melanomas had been performed on the basis of clinical, histopathologic, ultrasonic, and computed tomographic findings. Clinical data, including the Breslow vertical tumor thickness and stage of disease according to the 2002 American Joint Committee on Cancer system, were recorded from the original reports of patients with melanoma. We stratified SSM by the Breslow vertical tumor thickness as thin (<1 mm) and thick (≥1 mm). This study adhered to the Declaration of Helsinki, and ethics approval for research was obtained from the local review board of the Ruhr-University Bochum.

Immunohistologic Studies

Immunohistochemical staining was performed for CEA and CEACAM1 as follows: 4-µm paraffin-embedded sections were mounted on silanized slides and stored for 1 hour in a humid chamber at 60°C. Sections were deparaffinized in xylene and washed with 100%, 96%, 70%, and 50% ethanol for 5 minutes each and rinsed with demineralized water. After washing with Target Retrieval Solution (DAKO, Hamburg, Germany) for 20 minutes, sections were stored for 20 minutes in a water bath at 96°C. Sections were covered with 200 µL of monoclonal antimouse CEACAM1 antibody (29H2, catalog No. ab49510; Abcam, Cambridge, MA) at a dilution of 1:50 and monoclonal antimouse CEA (II-7 clone) antibody (DAKO) at a dilution of 1:100 for 60 and 30 minutes at 25°C in an autostainer (DAKO), respectively. As a secondary antibody, ChemMate Link, biotinylated secondary antibody (DAKO) containing antimouse, antirabbit, and antigoat immunoglobulins, was used. After washing with 10× Wash Buffer (DAKO) for 2 minutes, streptavidin-alkaline phosphatase (DAKO) was used as the enzyme for 30 minutes. Chromogen red (Red permanent, DAKO) was used for visualization before counterstaining with hematoxylin and mounting in Mowiol (Roche Molecular Biochemicals, Mannheim, Germany). Specificity testing was performed by blocking of the primary antibody, and negative control staining was performed by omitting the primary antibody.

Microscopic Evaluation

All immunohistochemical slides were separately evaluated by the same observer (S.G.) for patterns of immunohistochemical labeling. Microscopic evaluation (magnification ×100) was by blinded analysis in a coincident order of the tumor types assessed. Three randomly chosen fields of view were assessed in the tumor tissue. The percentage of positively stained tumor cells per field on total tumor cell count per field was determined. Quantitative results were expressed as the averaged percentages of positively stained tumor cells in the fields selected.

Statistical Analysis

Data analysis was performed by using the statistical package MedCalc Software (Mariakerke, Belgium). Distribution of data was assessed by using the D’Agostino-Pearson test. Normally distributed data were expressed as mean ± SD and nonnormally distributed data as median (range). The 1-way analysis of variance (ANOVA) and the Kruskal-Wallis ANOVA, including a Mann-Whitney post hoc test for independent data, were used for analysis of normally and nonnormally distributed data, respectively. Categorical data were assessed by using the χ² test. The Spearman coefficient of correlation (r) was also analyzed. A P value of less than .05 was regarded as statistically significant.

Results

In total, we studied 106 cases of histopathologically proven BN (n = 42), DN (n = 22), thin SSM with Breslow vertical tumor thickness less than 1 mm (mean ± SD, 0.46 ± 0.24 mm; n = 21), and thick SSM with Breslow thickness 1 mm or more (mean ± SD, 1.7 ± 0.8 mm; n = 21). Of the patients with thin SSM, 16 had stage IA disease, 1 had stage IIA, 2 had IIIA, 1 had IIIB, and 1 had stage IV disease. In the group with thick SSM, 1 patient had stage IA disease, 6 had stage IB, 5 had stage IIA, 1 had stage IIB, 2 had stage IIIA, 4 had stage IIIB, and 2 had stage IV disease. With regard to the tumor localization, there was no significant difference between groups (P = .23). Most lesions were on the lower extremities (28/106 [26.4%]) and the upper (40/106 [37.7%]) areas of the trunk; the remaining lesions were on the upper extremities (10/106 [9.4%]) and the head (3/106 [2.8%]). Sex distribution between groups did not statistically significantly differ (P = .07). However, age differed significantly between the groups, indicated by increased age in patients with SSM (P < .0001) (Table 1).

In all lesions studied, melanocytes in normal skin adjacent to the tumor did not show CEA or CEACAM1 immunoreactivity. Both antibodies exhibited slight to moderate membranous and cytoplasmic melanocytic staining intensity (Image 1). Staining intensity of CEACAM1 was more pronounced at the invasive front of SSM. Median and range values for CEA and CEACAM1 protein expression are detailed in Table 1. Kruskal-Wallis ANOVA revealed significant differences of CEA expression between the groups studied (Figure 1, P < .0001). Median CEA expression in thick SSM (P < .0001),
Table 1
Clinical Characteristics and Lesional Protein Expression of CEA and CEACAM1 in Patients With Benign and Malignant Melanocytic Skin Lesions

<table>
<thead>
<tr>
<th></th>
<th>Benign Nevus (n = 42)</th>
<th>Dysplastic Nevus (n = 22)</th>
<th>&lt;1 mm (n = 21)</th>
<th>≥1 mm (n = 21)</th>
<th>Differences Between Groups (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>16/26</td>
<td>9/13</td>
<td>11/10</td>
<td>13/8</td>
<td>.07</td>
</tr>
<tr>
<td>Mean (SD) age (y)</td>
<td>39 ± 12</td>
<td>47 ± 16</td>
<td>58 ± 13</td>
<td>61 ± 12</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Median (range) CEA expression (%)</td>
<td>0 (0-4.9)</td>
<td>1.8 (0-12.9)</td>
<td>5 (0-76.8)</td>
<td>7.7 (0-74.8)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Median (range) CEACAM1 expression (%)</td>
<td>1 (0-78)</td>
<td>9.6 (0-62.7)</td>
<td>18 (0-82)</td>
<td>74 (7.2-100)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

CAM, cell adhesion molecule; CEA, carcinoembryonic antigen.

Discussion

The human CEA protein family encompasses several forms of proteins with different biochemical features. CEA is an oncofetal glycoprotein, containing 50% carbohydrate with a molecular weight of approximately 200 kDa. CEA is overexpressed in several tumor types of epithelial origin and is known as an important and extensively used clinical tumor marker for colorectal and other carcinomas. Hence, CEA is an attractive target for immunotherapeutic purposes because

Thin SSM (P < .0001), and DN (P = .007) was significantly increased compared with expression in BN. Compared with DN, median CEA expression of thin SSM was not significantly increased (P = .052). However, median CEA expression in thick SSM was significantly higher than in DN (P = .013). CEA expression in SSM did not significantly correlate with Breslow tumor thickness and Clark level (r = 0.16, P = .3; and r = 0.22, P = .16, respectively).

Kruskal-Wallis ANOVA revealed significant differences of CEACAM1 expression between the groups studied (P < .0001). Compared with BN, median expression of CEACAM1 in thin SSM (P = .022) and thick SSM (P < .0001) was significantly increased. Median CEACAM1 expression of BN did not significantly differ from expression in DN (P = .39). Median CEACAM1 expression of thick SSM was significantly increased compared with DN (P < .0001) and thin SSM (P = .0009). There was a significant positive correlation between CEACAM1 expression and Breslow tumor thickness (r = 0.57, P = .0002) and Clark level (r = 0.52, P = .0009).
of its expression profile, its role in tumor progression, and its immunogenicity. The CEA proteins are encoded by 29 genes tandemly arranged on chromosome 19q13.2. All CEA family genes have been classified into 2 major subfamilies, namely CEACAM and the pregnancy-specific glycoprotein subgroups. In nonpathologic conditions, CEACAM is expressed on the surface of epithelial, endothelial, and hematopoietic cells. Alternative splicing events generate 2 major isoforms, CEACAM1-L and CEACAM1-S, which differ in the length of the cytoplasmic tail. Both CEACAM1 isoforms mediate homophilic intercellular adhesion and are used as a receptor by pathogens. CEACAM1 triggers various cellular functions, such as proliferation, differentiation, morphogenesis, and apoptosis.\(^{23}\)

Melanocytic nevi are both markers of an increased risk of cutaneous melanoma and direct precursor lesions. Benign and atypical moles have been shown to exist in clinical and histologic contiguity with cutaneous melanoma, in particular SSM and lentigo maligna melanoma, suggesting that these melanocytic nevi are also susceptible to malignant transformation.\(^{25,26}\) Therefore, we stratified our analysis by
different types of pigmented skin lesions comprising a spectrum of benign and malignant melanocytic proliferations.

In the present study using a monoclonal antibody against CEA, we have shown that there is apparently a stepwise increase of CEA protein expression in melanocytic tumors when comparing BN, DN, and SSM. Indeed, we observed significantly increased CEA expression in DN and SSM compared with expression in BN. CEA expression in thick SSM was also increased compared with expression in DN.

All in all, the data in the literature on CEA expression in benign and malignant melanocytic lesions are conflicting. A previous report indicated that polyclonal CEA staining is not rare in malignant melanoma. However, monoclonal CEA staining was negative in all malignant melanomas. Similar findings were also reported by Sanders et al and Ravindranath et al. By contrast, Selby et al found CEA immunoreactivity (polyclonal) in a significant number of malignant melanoma cases. The authors hypothesized that this may be due to CEA expression by tumor cells or to cross-reactivity of the polyclonal antibody with substances such as nonspecific cross-reacting antigens that share antigenic sites with CEA. Pavoni et al used a completely human single-chain CEA antibody of high affinity. Interestingly, in 7 of 34 malignant melanomas, they found that CEA positively stained tumor cells. Furthermore, Egawa et al demonstrated that members of the CEA family (monoclonal and polyclonal) were strongly expressed in all subtypes of melanocytic nevi studied, except blue nevi.

Our data indicate that CEACAM1 protein is significantly overexpressed in thick SSM, thin SSM, and DN compared with BN. Moreover, CEACAM1 expression was significantly increased in thick SSM compared with DN. Similar to the CEA expression observed in this study, CEACAM1 expression showed stepwise overexpression in melanocytic skin lesions at more advanced stages of neoplastic progression.

As previously observed by Thies et al, we observed the staining intensity of CEACAM1 to be more pronounced at the invasive front of melanomas. Moreover, we found a significant positive correlation between CEACAM1 protein expression and Clark level and Breslow tumor thickness of SSM. Preclinical malignant melanoma studies have shown that CEACAM1 is an independent factor of the metastasis risk, with a predictive value superior to that of vertical tumor thickness. Moreover, specific CEACAM1 gene mutation inhibits the invasive growth of malignant melanoma, and treatment with anti-CEACAM monoclonal antibodies blocks CEACAM1-enhanced cell invasion and cell migration in a dose-dependent manner. By interaction with integrins, particularly integrin β3, CEACAM1-L was shown to enhance the migratory and metastatic potential of melanoma cells. In fact, clinical studies in a 10-year follow-up indicate that expression of CEACAM1 in primary tumors in patients with malignant melanoma is associated with the subsequent development of metastatic disease.

Thies et al suggested that 4D1/C2 against CEACAM1 antibodies is highly sensitive and may be a worthy complement to standard antibody panels for diagnosis of melanoma cells. They found that for detection of CEACAM1, the monoclonal 4D1/C2 antibody was by far superior to the commercial CEACAM1 antibody for detecting malignant melanoma cells. In particular, the sentinel lymph node sensitivity (95% vs 40%) of 4D1/C2 was significantly higher than that of the commercial antibody. Thies et al also showed that the sensitivity of the antibody 4D1/C2 against CEACAM1 is superior to the classic trio—S-100, Melan-A, and HMB45—for detecting melanoma cells. Markel et al for example, provided mechanistic evidence that CEACAM1 is actively up-regulated in response to interferon-γ on melanoma cells surviving specific lymphocyte-mediated attack to emerge with transiently enhanced resistance. This dynamic evasion mechanism reveals a potential sophisticated tumor strategy that uses an offensive agent as a sensor for the presence of nearby immune attack to actively increase the protection of surviving cells. The aforementioned findings point to the important role of CEACAM1 in the development of aggressive melanoma and mark it as an attractive target for novel immunotherapy.

We performed a systematic immunohistochemical study on CEA and CEACAM1 protein expression in melanoma and different precursor lesions. Limitations of our study include the absence of survival data and functional studies. Nevertheless, our data give support to previous studies indicating that cell adhesion molecules of the CEA family may have a role in the development and progression of cutaneous melanoma and potentially serve as prognostic markers.

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References


