Abstract. Background: Melanoma is one of the most aggressive types of skin cancer. The purpose of this study was to evaluate the use of two biomarkers, ProEx C and glucose transporter isoform 1 (GLUT1), in the diagnosis and prognostication of melanoma. Materials and Methods: We analyzed 129 melanomas and 59 benign nevi in a tissue microarray using immunohistochemical method with antibodies to topoisomerase IIα (TOP2A) and minichromosome maintenance complex component 2 (MCM2) using ProEx C and to GLUT1. Results: The average proliferative index by ProEx C immunostain was significantly higher in melanomas (37.5%) compared to benign nevi (1.9%) as was the expression of GLUT1 (p<0.0001 respectively). Dermal mitosis was found to correlate positively with both ProEx C and GLUT1 (p=0.003 and p<0.001, respectively). Ulceration and tumor thickness positively correlated with GLUT1 expression (p=0.013 and p=0.033, respectively), but not with ProEx C staining. There was a significant association between increasing ProEx C index and stronger expression of GLUT1 (p<0.001). Kaplan–Meier disease-specific survival analyses indicated that patients whose melanoma exhibited expression of GLUT1 had a significantly lower rate of disease-specific survival than patients whose melanoma did not (p=0.039). However, staining by ProEx C did not show a prognostic significance in disease-specific survival. Conclusion: ProEx C and GLUT1 are potentially useful markers in differentiation of melanoma from nevi. Absence of GLUT1 expression in patients with primary melanoma predicts better survival.

Malignant melanoma is one of the most aggressive and deadly types of skin cancer and is the fifth most commonly diagnosed cancer in the United States (1). Among melanocytic lesions in the skin, distinction between malignant melanoma and benign nevi requires evaluation of both cytological and architectural features of the lesions. Histopathological criteria sometimes may be inadequate in differentiating difficult melanocytic lesions, even for experienced dermatopathologists. Studies have shown poor consistency in the diagnosis of difficult melanocytic lesions, even by expert dermatopathologists (2, 3). Dermatopathologists are faced with almost daily examples of melanocytic lesions that cannot easily be categorized based on histological criteria.

Biomarkers as measurable molecules are valuable in assisting diagnosis, prognostic evaluation and treatment of malignant melanoma. Recently, molecular and chromosomal aberration detected by fluorescence in situ hybridization and single nucleotide polymorphism array/comparative genomic hybridization have been shown to be promising in improving differentiation of malignant melanoma from benign melanocytic proliferation. Immunohistochemical studies still remain rapid and convenient tools for assisting in diagnosis (4-6). Yet there are no reliable immunohistochemical markers that are both sensitive and specific for diagnosing malignant melanoma. Among the commonly available biomarkers, Ki67 is one of the most well-studied and widely used proliferation markers for differentiating melanoma from benign nevi. Studies have shown significant differences in proliferative index by Ki67 between benign nevi and malignant melanoma (7-10), and Ki67 staining index in melanoma has been shown to correlate positively with tumor thickness, mitotic figures, tumorigenic vertical growth phase, vascular invasion and metastatic potential. However, Ki67 as an independent prognostic indicator in overall survival has shown variable significance (11-14). Immunohistochemical staining by ProEx C antibody has recently been studied as another
proliferative marker. The nature of ProEx C has been discussed in depth previously (15). It is a cocktail of immunohistochemical antibodies directed against two proteins intimately involved in the cell cycle, namely topoisomerase IIα (TOP2A) and minichromosome maintenance complex component 2 (MCM2). The former is involved in deconstruction and separation of DNA strands prior to replication, while the latter is a member of the DNA-licensing factor family involved in forming the pre-replication complex. These two proteins have been both demonstrated as useful markers of aberrant S-phase induction (15) and identified as cancer markers in cervical squamous intraepithelial lesions (16, 17), pre-malignant and malignant lesions of the lung, and also in non-cervical neoplasia (15, 18), including perineal melanoma (19).

In addition to proliferation markers, other diagnostic markers indicating biological behavior may be helpful in the differentiation of melanoma from benign nevi. Malignant neoplasms not only have increased proliferation, but also enhanced glycolytic metabolism (20), which is characterized by an increased rate of glucose uptake mediated by glucose transporter proteins. During malignant transformation, up-regulation of specific glucose transporters and their translocation from the cytoplasm to the cell membrane leads to increased glucose transport (21). Among the family of seven glucose transporters, glucose transporter isoform 1 (GLUT1) is a key rate-limiting factor in glucose metabolism and is widely expressed in a broad range of human cancer types, including those of the lung (22), thyroid (23), ovary (24), breast (25), colon (26), prostate (27), stomach (28) and pancreas (29). The expression of GLUT1 in melanoma appears to be variable in different tumor biopsies (30) and overexpression of GLUT1 has been shown to be closely related to 2-deoxy-2-[18F]-fluoro-D-glucose uptake in malignant melanoma (31). However, another study showed that the expression of GLUT1 was down-regulated in 55% of malignant melanomas, while GLUT1 was present in the majority of benign nevi (32). Overall, the status of GLUT1 expression in melanocytic lesions, especially its correlation with patient survival and prognosis, has not been well studied.

The purpose of this study was to investigate the use of potential immunohistochemical staining by ProEx C and for GLUT1 in melanoma and nevi through use of a tissue microarray. In addition, we sought to correlate staining by ProEx C and for GLUT1 in primary melanomas with a variety of important clinicopathological parameters, the status of developing metastatic disease, and disease-specific survival in follow-up.

Materials and Methods

Tissue microarray construction. A tissue microarray (TMA) was constructed, as previously described (33). The cohort of tissues was selected from formalin-fixed, paraffin-embedded tissue blocks obtained from the archives of the Department of Pathology and Laboratory Medicine at Dartmouth-Hitchcock Medical Center. The cohort characteristics are presented in Table I. Primary melanomas were staged according to the American Joint Committee on Cancer staging system for cutaneous melanoma (33, 34, 39). All tumors were reviewed by light microscopic examination of the original hematoxylin and eosin-stained section for accuracy of the original diagnosis and pathological stage (SY and JBB). The most representative tumor area was carefully selected. For cases with small tumor size, a single core was taken for examination. For 63% of the specimens, we included two or three cores representative of the tumor, depending on the size of the lesion. A total of 361 tissue cores (0.6 mm in diameter), representing 110 primary melanomas, 19 melanoma metastases and 59 benign nevi, were examined. Primary melanomas were from initial biopsies of patient with primary melanoma. Melanoma metastases were from metastatic lesions derived from different sites: lymph node, cutaneous, or internal organ. The 110 primary melanomas included 35 cases which subsequently developed metastatic disease, 55 cases that had not metastasized at the time of follow-up, and 20 cases for which there was no follow-up information. The dates of the initial melanoma diagnosis ranged from 1994 to 2008. The mean follow-up of this cohort was 9.8 years, with a median follow-up of 10 years. The histological and prognostic factors analyzed were established from sections of the initial tumor biopsy or excisional specimen. Information regarding sentinel lymph node status, as well as local or distant metastatic status, was available for 90 out of the 110 melanomas. This research was approved by the Institutional Review Board of Human Research at Dartmouth College (CPHS#21764).

Immunohistochemistry. Immunohistochemical studies were performed on 4-μm TMA sections using an automatic immunostainer (Biogenex I-6000; Biogenex Laboratories, Inc., Fremont, CA, USA) according to the manufacturer’s instruction. The sections were incubated for 30 min at 37°C with primary antibodies of ProEx C antibody cocktail (prediluted; TriPath Imaging Inc, Burlington, NC, USA) and GLUT1 (dilution 1:200; ABCAM, Boston, MA, USA). The immunostaining by ProEx C was visualized using Super Sensitive Link/label Immunohistochemical Detection system with 3,3’-diaminobenzidine chromogen (Biogenex Laboratories, Inc.). Antibody binding to GLUT1 was visualized using Leica Bond Polymer Refine Red Detection kit with Fast Red chromogen (Leica Biosystems Inc., Buffalo Grove, IL, USA).

Evaluation of immunohistochemical staining. Each tissue core was analyzed at 400× magnification under light microscopy. For ProEx C, positive nuclear staining within dermal melanocytes was counted as a positive result (Figure 1) and the index (percentage of positively stained cells) was calculated. Functional melanocytes and lymphocytes, as could be identified morphologically, were excluded from analysis. No fewer than 100 and up to 500 dermal melanocytes were counted for each tissue core. For cores in which more than 500 dermal melanocytes were present for evaluation, areas with the highest percentage of positively stained cells were counted. Tissue cores without sufficient tissue were excluded from analysis. For cases in which multiple cores were available, an average staining index of all cores was taken. For GLUT1, membranous staining of dermal melanocytes was measured by semiquantitative assessment of positively stained tumor cells as described previously (35). Scoring of GLUT1 included both the percentage of positively stained dermal melanocytes (<1%, 0 point; 1-10%, 1 point; 11-50%, 2
points; 51-80%, 3 points; and ≥80%, 4 points) as well as the intensity of staining (weak, 1 point; moderate, 2 points; strong, 3 points). Fewer than 1% positively stained cells was considered negative regardless of staining intensity. The points for both were totaled and specimens were assigned to four groups according to their overall score (total of 0-1 points, negative, overall score=0; total of 2-3 points, weak, overall score=1; total of 4-5 points, moderate, overall score=2; total of 6-7 points, strong, overall score=3). Infrequently, multiple cores from the same cases received different scores and an average score was used for further analysis. Scoring was performed in a double-blind manner by two investigators (SY, BNC).

Statistical analysis. Statistical Analysis System (SAS) 9.4 (SAS Institute Inc., Cary, NC, USA) software was used for data analyses. Comparison of ProEx C index in melanoma and nevi was analyzed using t-test. A proportional odds model was used to study the expression of GLUT1, an ordinal variable, in different groups of melanocytic lesions. Cumulative odds ratio for stronger GLUT1 expression and its 95% confidence interval were calculated. p-Values came from Wald Chi-square test in the proportional odds model. The association between ProEx C index and GLUT1 expression in melanocytic lesions was analyzed using a proportional odds model. The association between these markers and the clinicopathological parameters of primary melanoma cases was assessed using regression for continuous variables and logistic regression for dichotomous variables separately. Disease-specific survival data were analyzed using the Kaplan–Meier method, and the Mantel–Cox log rank score was used to assess statistical significance. For survival analysis, the original predictors were recorded based on the following cutoff points: ProEx C staining index <40% and ≥40% (average ProEx C index in melanomas was close to 40%), GLUT1 with positive expression (overall score of 1-3) versus negative expression (score of 0). Deaths not caused by melanoma were treated as censored data. Univariate and multivariate survival analyses using Cox regression models were conducted to assess the effect of prognostic factors on survival. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ProEx C and GLUT1 for detecting melanoma were calculated using different thresholds according to traditional methods (36).

Results

Immunohistochemical staining of TMAs. In order to assess staining by ProEx C and for GLUT1, we used immunohistochemical assays of a TMA consisting of melanoma and benign nevus specimens from patients seen at the Dartmouth-Hitchcock Medical Center. Clinical and histological features of the patients and their tumors are described in Table I. Of the 59 benign nevi, 56 (94.9%) out of the ProEx C- and 55 (93.2%) out of the GLUT1-stained sections were sufficient for analysis. Of the 129 malignant melanomas included in this evaluation, 110 (85.3%) out of the ProEx C-stained sections and 117 (90.7%) out of the GLUT1-stained sections were adequate for analysis.

Evaluation of the immunohistochemical staining revealed variable nuclear staining by ProEx C and membranous staining of GLUT1 in benign and malignant melanocytic lesions. Figure 1 shows examples of immunohistochemical staining in cases of malignant melanoma and benign nevus. Strong nuclear ProEx C staining (Figure 1A) and strong membranous GLUT1 expression (Figure 1B) was observed in a malignant melanoma, while staining using these antibodies was essentially negative in a benign nevus (Figure 1C and D).
The ProEx C index and GLUT1 immunostaining data for each group of lesions are summarized in Table II and III. ProEx C index and GLUT1 positivity were both statistically significantly higher in melanoma than in benign nevi \((p<0.0001)\). Neither marker significantly differed between primary and metastatic melanoma. The ProEx C index was significantly higher in primary melanomas developing subsequent metastases in follow-up than that in those that did not \((p=0.048)\). Similarly, higher GLUT1 expression was more frequently observed in primary melanomas developing subsequent metastases in follow-up \((p=0.021)\).

**Clinicopathological correlation (Tables IV and V).** Dermal mitosis was found to correlate positively with both ProEx C and GLUT1 \((p=0.003\) and \(p<0.001\) respectively). Ulceration and tumor thickness positively correlated with GLUT1 expression \((p=0.013\) and \(p=0.033\), respectively), but not with ProEx C index. Neither of these biomarkers was associated with factors such as tumor-infiltrating lymphocytes, regression, tumor pigmentation, and tumor-associated nevus (all \(p>0.05\)). Overall ProEx C index was significantly positively associated with stronger GLUT1 expression \((p<0.001)\).

**Survival analysis.** In order to further evaluate the impact of these two biomarkers on survival, Kaplan–Meier disease-specific survival curves were prepared to examine the effect of dermal mitosis, ProEx C index and membranous GLUT1

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**Figure 1.** Examples of ProEx C and glucose transporter isoform 1 (GLUT1) immunohistochemical staining in melanoma and benign nevus. A: Strong nuclear staining with ProEx C in a melanoma case (index 38.5%). B: Strong membranous expression of GLUT1 in a melanoma case (with intensity score of 3 and percentage score of 4). C: Negative staining with ProEx C in a benign nevus (index 0%). D: Negative expression of GLUT1 in a benign nevus (with intensity score of 0 and percentage of 0%).
expression in patients with primary melanoma. Since the mean ProEx C index was near 40%, 40% was chosen as a cut-off for ProEx C in survival analysis. Our evaluation indicated that patients with dermal mitoses (≥1) in primary melanomas had a significantly lower rate of disease-specific survival compared to patients without (p=0.004) (Figure 2A). A high ProEx C index (≥40%) in primary melanomas did not have a significant impact on melanoma-specific survival compared to a low ProEx C index (<40%) (p=0.83) (Figure 2B). When 30% was used as cut-off for ProEx C index, there was also no difference in disease-specific survival. However, patients with negative GLUT1 expression (score 0) in their primary melanomas appeared to have prolonged disease-specific survival compared to those with positive GLUT1 expression (score >0) (p=0.039) (Figure 2C). These data indicate that dermal mitosis and GLUT1

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Table III. Glucose transporter-1 (GLUT1) immunostaining in melanocytic lesions.

<table>
<thead>
<tr>
<th>Overall score for GLUT1 expression</th>
<th>0 (Negative)</th>
<th>1 (Weak)</th>
<th>2 ( Moderate)</th>
<th>3 (Strong)</th>
<th>Cumulative OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant melanoma</td>
<td>53/117 (45.3%)</td>
<td>27/117 (23.1%)</td>
<td>29/117 (24.8%)</td>
<td>8/117 (6.8%)</td>
<td>4.75 (2.34-9.65)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Benign nevi</td>
<td>42/55 (76.4%)</td>
<td>13/55 (23.6%)</td>
<td>4/55 (7.3%)</td>
<td>0/55 (0%)</td>
<td>0.5 (0.19-1.33)</td>
<td>0.165</td>
</tr>
<tr>
<td>Melanoma metastasis</td>
<td>7/18 (38.9%)</td>
<td>4/18 (22.2%)</td>
<td>4/18 (22.2%)</td>
<td>0/18 (0%)</td>
<td>2.66 (1.16-6.12)</td>
<td>0.021</td>
</tr>
<tr>
<td>Primary melanoma</td>
<td>46/99 (46.5%)</td>
<td>24/99 (24.2%)</td>
<td>25/99 (25.3%)</td>
<td>4/99 (4.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma with metastasis</td>
<td>9/32 (28.1%)</td>
<td>12/32 (37.5%)</td>
<td>8/32 (25.0%)</td>
<td>3/32 (9.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma without metastasis</td>
<td>28/50 (56.0%)</td>
<td>11/50 (22.0%)</td>
<td>10/50 (20.0%)</td>
<td>1/50 (2.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: Odds ratio; CI: confidence interval.

Table IV. Association of ProEx C with histopathological variables.

<table>
<thead>
<tr>
<th>ProEx C</th>
<th>LSM (%)</th>
<th>(95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathological variable</td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td>40.7 (32.8-48.6)</td>
<td>34.6 (28.7-40.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>35.9 (28.1-43.6)</td>
<td>37.4 (31.3-43.4)</td>
<td>0.76</td>
</tr>
<tr>
<td>Regression</td>
<td>40.8 (30.0-51.5)</td>
<td>35.84 (30.5-43.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>Tumor-infiltrating lymphocytes</td>
<td>37.4 (31.3-43.6)</td>
<td>35.89 (28.4-43.4)</td>
<td>0.75</td>
</tr>
<tr>
<td>Associated nevus</td>
<td>32.2 (21.8-42.7)</td>
<td>38 (32.7-43.3)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

LSM: Least square means; CI: confidence interval.

Table V. Association of ProEx C and glucose transporter-1 (GLUT1) with histopathological variables.

<table>
<thead>
<tr>
<th>Histopathological variable</th>
<th>ProEx C</th>
<th>GLUT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Increase (95% CI)</td>
<td>p-Value</td>
<td>Cumulative OR (95% CI)</td>
</tr>
<tr>
<td>Tumor thickness (1 mm increase)</td>
<td>0.08 (–1.35-1.51)</td>
<td>0.91</td>
</tr>
<tr>
<td>Mitotic rate (increase 1/mm²)</td>
<td>1.02 (0.34-1.69)</td>
<td>0.003</td>
</tr>
<tr>
<td>Ulceration (present vs. absent)</td>
<td>2.7 (1.2-6.0)</td>
<td>0.013</td>
</tr>
<tr>
<td>Pigmentation (present vs. absent)</td>
<td>1.2 (0.5-3.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Regression (present vs. absent)</td>
<td>0.7 (0.3-1.7)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

OR: Odds ratio; CI: confidence interval.
expression are significant prognostic factors for survival, but ProEx C index is not.

The effect of these two biomarkers on survival probability of patients with primary melanoma was further evaluated by multivariate analysis using a Cox regression model (Table VI). Shorter survival was significantly associated with thicker tumor, high tumor mitotic count and the presence of ulcer \( (p=0.005, p=0.004 \text{ and } p=0.002, \text{ respectively}, \) indicating these factors as independent predictors of survival. ProEx C index and GLUT1 expression were not predictive of survival \( (p=0.47 \text{ and } p=0.96, \text{ respectively}) \), therefore these two biomarkers are not independent prognostic markers.

**Diagnostic value of ProEx C and GLUT1 (Table VII).** We also evaluated the diagnostic value of these two biomarkers in distinguishing malignant melanoma from benign nevi. With a discriminatory threshold of \( \geq10\% \), ProEx C index correctly categorized 80 out of 92 primary melanomas, with a sensitivity of 87.0% and specificity of 98.2%. With an arbitrary decrease or increase of the threshold, the sensitivity and specificity changed accordingly. Moderate to high GLUT1 expression \( (\text{score}=2 \text{ or } 3) \) was only observed in melanoma, not in benign nevi. With a discriminatory score of \( \geq2 \), GLUT1 categorized only 29 out of 99 primary melanomas, with a sensitivity of 29.3% but with specificity and PPV both of 100%. Using expression score \( \geq1 \) as the cut-off, the sensitivity increased, but the specificity and PPV decreased. Overall, moderate or strong membranous GLUT1 staining supports a malignant diagnosis, although a negative or weak staining pattern does not exclude melanoma. In summary, ProEx C is sensitive and specific, while high GLUT1 expression is a highly specific, but not sensitive marker for distinguishing malignant melanoma from benign nevi.

**Discussion**

Histological differentiation of malignant melanoma from benign melanocytic lesion can be challenging. As an immunohistochemical marker of cellular proliferation, Ki67 has been used in conjunction with other histological and clinicopathological features to help differentiate between malignant melanoma and benign nevus (37, 38). Although newer to the scene of investigative pathology, the ProEx C cocktail of antibodies directed against MCM2 and TOP2A proteins has also garnered recognition for its usefulness in highlighting cellular proliferation in malignant or pre-malignant transformation of cells, primarily in the uterine cervix, but also in other tissues (15, 18).

In our study, ProEx C demonstrated significantly different proliferative rates in benign versus malignant melanocytic lesions. Few studies evaluating ProEx C staining in melanocytic lesions are available for comparison with our study. Boyd et al. reported using antibodies direct against MCM2, one of the targets of ProEx C, in the evaluation of a variety of melanocytic lesions, including benign nevi (39). Their results showed that the percentage of positively staining nuclei in benign nevi \( (1.2\%) \), dysplastic nevi \( (6.1\%) \), primary melanomas \( (49.1\%) \), and melanoma metastases \( (40.9\%) \) differed significantly \( (p<0.0001) \) among the four groups, similar to the ProEx C indices seen in our study \( (1.9\% \text{ in benign nevi vs. } 37.5\% \text{ in primary melanoma vs. } 40.9\% \text{ in metastatic melanoma}) \) although no significant difference among the latter two groups was observed in our study. Walts et al. demonstrated variable ProEx C staining in malignant melanoma of the perineum, with over 50% tumor cells showing positive staining in 43% of perineal melanoma cases (19). In other studies, ProEx C appears to be more sensitive for highlighting proliferating cells than Ki67 (40, 41). MCM2 protein is highly expressed in actively proliferating cells and cells with the potential to replicate. It is expressed as cells enter the G1 phase from G0 phase even before they engage in DNA synthesis, therefore ProEx C can detect pre-malignant cells, and is more sensitive than Ki67 according to literature (15, 42, 43).

Although the ProEx C index was significantly higher in primary melanomas developing subsequent metastases than in those without metastases in the follow-up, ProEx C index had no prognostic significance in disease-specific survival by
Figure 2. Kaplan–Meier analyses of disease-specific survival according to mitotic frequency (<1 vs. ≥1) (A), ProEx C index (<40% vs. ≥40%) (B) and glucose transporter isoform 1 (GLUT1) expression (overall score of 0 vs. 1, 2 or 3) (C) in patients with primary melanoma.
Kaplan–Meier analysis. The prognostic significance of the Ki67 proliferative index in malignant melanoma is controversial in literature. The use of Ki67 as a significant indicator of survival in patients with melanoma has been demonstrated in multiple studies (11, 13), although other studies showed no significant association between high Ki67 index and survival (12, 44). In our study, ProEx C did not have prognostic significance in disease-specific survival in patients with primary melanoma and was not an independent prognostic factor in multivariate study.

There was a high ProEx C index for primary and metastatic melanomas, but staining was rarely detected in benign nevi. These data are in accordance with literature. Low or undetectable expressions of proliferative markers in benign nevi confirmed their biological behavior. These results indicate that ProEx C potentially provides an additional tool for distinguishing melanoma from its malignant counterpart. Some melanomas occasionally have low proliferative activity, and some nevi, especially Spitz nevi, have been shown to have an increased Ki67 index, as high as 7-12% (7, 45).

Therefore, the proliferative index must be used as an adjunct to histopathology to assist diagnosis. Overall as a diagnostic tool, ProEx C seems to be useful in distinguishing melanoma from benign with high sensitivity and specificity, although its expression in atypical nevi needs to be further evaluated.

Malignant neoplasms have enhanced glycolytic metabolism (20). In our study, moderate to high GLUT1 expression was only observed in melanomas, and not in benign nevi (specificity of 100%), indicating the usefulness of this marker in assisting diagnosis. GLUT1 expression was negative in 45.3% of melanomas. These tumor cells may express other subtypes of glucose transporters instead of GLUT1. A prior study has shown variable expression of GLUT1 in different melanoma samples, with a 22-fold variation in the level of GLUT1 detected by immunoblotting, quantified by using enhanced chemiluminescence (30). Additional studies to evaluate other glucose transporter family members will be helpful.

Patients with positive expression of GLUT1 in their primary melanomas appear to have poorer prognosis compared to those with negative expression of GLUT1 (p=0.039), indicating the prognostic value of this biomarker. Although GLUT1 was not an independent prognostic indicator in multivariate analysis, this may be accounted for by the very close association between GLUT1 and the other significant risk factors such as tumor thickness, ulceration, and mitotic count.

Tissue microarrays have been widely accepted as an efficient method of examining high-throughput tumor cases on a single glass slide (46, 47, 48, 49). One study shows that the probability of one, two and three cores correctly representing the expression of three markers in the whole-slide sections of ovarian carcinoma was 91%, 96% and 98%, respectively (48). Multiple validation studies have also shown that TMA with three cores per case is sufficient for immunohistochemical profiling compared to whole-slide sections (49). Nevertheless, further validation study of these two biomarkers in whole tissue sections of melanocytic lesions is needed.

In summary, ProEx C and GLUT1 are potentially useful immunohistochemical markers in the evaluation of melanocytic lesions of the skin. Additional studies to include atypical nevi and other nevus variants, evaluation of the expression of other glucose transporter subtypes, and evaluation of these markers on whole sections of tumors are needed to further refine the potential role for these biomarkers in the diagnosis and prognostication of patients with melanocytic lesions. Nowadays, whole slide imaging is becoming standard and image processing software is more sophisticated in digital pathology. This may help establish more feasible quantitative analysis of biomarkers with high reproducibility and reliability.

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