Tissue Microarray Analysis of Methylthioadenosine
Phosphorylase Protein Expression
in Melanocytic Skin Tumors

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Background: Using tissue microarrays, we investigated whether methylthioadenosine phosphorylase (MTAP) protein expression is associated with clinicopathologic variables in benign and malignant melanocytic skin tumors.

Observations: Cytoplasmic MTAP expression was detected in 227 (72.1%) of 315 informative cases. Expression was significantly reduced in primary malignant melanomas and in melanoma metastases compared with benign nevi (P<.001 for both). No difference was noted in MTAP expression between primary malignant melanomas and melanoma metastases. In primary malignant melanomas, a Ki67-labeling index less than 5% was associated with MTAP expression (P=.04), suggesting that loss of MTAP expression is associated with proliferation. No other variables had significant associations with MTAP expression. Lymph node metastases demonstrated significantly higher MTAP expression compared with skin metastases (P=.01). In the overall cohort, MTAP expression was not associated with prognosis. Among 26 patients with MTAP-positive melanomas and tumor recurrence, 18 patients who received interferon therapy had a significant benefit compared with 8 patients who did not receive interferon therapy (P=.009). This was not seen in the patients with MTAP-negative tumors.

Conclusion: Methylthioadenosine phosphorylase protein expression may be a predictive marker of interferon therapy resistance in patients with melanoma and disease progression.

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METHYLTHIOADENOSINE phosphorylase (MTAP) catalyzes the phosphorylation of methylthioadenosine, a byproduct of the synthesis of polyamines that acts as a potent inhibitor of polyamine aminopropyltransferase and methyltransferase. Methylthioadenosine phosphorylase is abundantly expressed in normal cells and tissues. Many malignant cells lack MTAP activity because of chromosomal loss or epigenetic regulation. The reason for the frequent loss of MTAP activity is the chromosomal location of MTAP. Starting from the centromeric end, the gene order on human chromosome 9p21 is p15, p16, MTAP, interferon α, and interferon β. Tumors, especially malignant melanomas, have selective deletions in this region. MTAP gene deletions have been identified in endometrial cancer, osteosarcoma, and hematologic neoplasias. In malignant melanomas, loss of MTAP expression is also caused by promoter hypermethylation. Using immunohistochemistry (IHC), a significant inverse association between MTAP protein expression and progression of melanocytic tumors was recently demonstrated, with the amount of MTAP staining decreasing from benign nevi to melanoma metastases. In contrast, MTAP was not expressed in normal human colon epithelium but was strongly up-regulated in colon carcinomas. In addition, MTAP expression has been shown to have a significant effect on STAT1 (signal transducer and activator of transcription 1) activity. STAT1 is essential for activation of interferon γ signaling pathways. Mowen et al have suggested an association between MTAP activity and interferon sensitivity via STAT1. Results of in vitro experiments have recently shown that reexpression of MTAP in melanoma cell lines leads to responsiveness to interferon.

Tissue microarrays (TMAs) are highly efficient tools for the investigation of large series of tumor cases with defined clinical characteristics, including disease outcome. The objective of the present study was to investigate if MTAP expression is associated with clinicopathologic variables in benign and malignant melanocytic skin tumors.

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METHODS

TISSUE MICROARRAYS

Tissue microarrays were constructed as described previously and contained a total of 350 formalin-fixed, paraffin-embedded human tissues (88 primary malignant melanomas [25.1%], 101 metastases [28.9%], and 161 benign nevi [46.0%]). In patients with multiple subsequent neoplasms, only initial and unifocal malignant melanomas were included. Hematoxylin-eosin-stained slides of all tumors were evaluated by 2 of us (T.V. and M.L.). Clinical follow-up data from the Central Tumor Registry were available for all patients with primary malignant melanomas. The median follow-up for all patients was 54 months (range, 0-135 months). The University of Regensburg institutional review board granted approval for the project.

To prevent structural imbalance between patients with recurrence and patients without recurrence over time, a staggered matching algorithm based on tumor thickness was used. Initial matching criteria were 1:2 match for tumor thickness.

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Table 1. Methylthioadenosine Phosphorylase (MTAP) Expression Among 350 Tissue Microarrays

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Analyzable (n = 312)</th>
<th>MTAP Immunoreactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (n = 85)</td>
<td>1 (n = 149)</td>
</tr>
<tr>
<td>Primary malignant melanomas†</td>
<td>80</td>
<td>31</td>
</tr>
<tr>
<td>Melanoma metastases</td>
<td>95</td>
<td>38</td>
</tr>
<tr>
<td>Benign nevi</td>
<td>137</td>
<td>16</td>
</tr>
</tbody>
</table>

*Immunoreactivity of 0 indicates negative; 1, weak positive; and 2, strong positive.
†Only initial and unifocal malignant melanomas were included. P < .001 (2-sided Fisher exact test) for primary malignant melanomas only; others were nonsignificant.

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Table 2. Clinicopathologic Variables and Methylthioadenosine Phosphorylase (MTAP) Expression Among 312 Analyzable Tissue Microarrays

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Analyzable</th>
<th>MTAP Immunoreactivity*</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Primary Malignant Melanomas (n = 80)‡</td>
<td>45</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>35</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>46</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Sex</td>
<td>34</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Clark level§</td>
<td>51</td>
<td>18</td>
<td>20</td>
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<tr>
<td>II</td>
<td>12</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>3</td>
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</tr>
<tr>
<td>IV</td>
<td>37</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Tumor thickness, mm</td>
<td>29</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>&lt; 2.0</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Growth pattern</td>
<td>36</td>
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<td>11</td>
</tr>
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<td>33</td>
<td>14</td>
<td>11</td>
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<tr>
<td>SSM</td>
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</tr>
<tr>
<td>NMM</td>
<td>37</td>
<td>15</td>
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</tr>
<tr>
<td>LMM</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>K 67-labeling index</td>
<td>29</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>&lt; 5%</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>≥ 5%</td>
<td>36</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Melanoma Metastases (n = 95)</td>
<td>41</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Lymph node</td>
<td>54</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Skin</td>
<td>12</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Compound and junctional</td>
<td>52</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>Dermal</td>
<td>35</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Congenital</td>
<td>50</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>

Abbreviations: ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; NMM, nodular malignant melanoma; NOS, not otherwise specified; SSM, superficial spreading melanoma.

*Immunoreactivity of 0 indicates negative; 1, weak positive; and 2, strong positive.
†Fisher exact test (2-sided); boldface indicates statistical significance.
‡Only initial and unifocal malignant melanomas were included.
||Data are missing.
less than 2.00 mm (ie, 1 patient with recurrence was matched to 2 patients without recurrence) and 1:1 match for tumor thickness between 2.00 and 4.20 mm. Among patients with tumors exceeding 4.20 mm in thickness, 12 patients with tumor recurrence were compared with 9 patients without recurrence. For the 9 patients without recurrence, no exact match was possible because patients with advanced tumor stages almost always had a history of melanoma recurrence. Based on follow-up data through March 1, 2005, there was no significant difference in melanoma thickness between patients with recurrence and patients without recurrence using the Mann-Whitney test. Characteristics of the TMAs are summarized in Table 1 and in Table 2.

IMMUNOHISTOCHEMICAL ANALYSIS

Paraffin-embedded preparations of tissues from patients with primary malignant melanomas, melanoma metastases, and benign nevi were screened for MTAP protein expression using IHC as described previously. In brief, tissues were deparaffinized, rehydrated, and incubated with primary polyclonal chicken anti-MTAP antibody (1:1 500) overnight at 4°C. The secondary antibody (biotin-labeled antichicken, 1:1 000; Jackson ImmunoResearch Laboratories, Ltd, West Grove, Pa) was incubated for 30 minutes at room temperature, followed by incubation with streptavidin-peroxidase (Dako Cytomation GmbH, Hamburg, Germany) for 30 minutes. The primary antibodies used were anti-MTAP (provided by David Carson, MD) and anti-Ki67 (rabbit monoclonal clone MIB1, 1:10; final concentration, 5 µg/mL; Dako Cytomation GmbH). As an internal positive control for MTAP IHC, normal squamous epithelium of the epidermis (asterisk in A) serves as an internal positive control. A and B, Negative immunoreactivity (score, 0). C and D, Weak immunoreactivity (score, 1). Asterisk in C indicates epithelium. E and F, Strong immunoreactivity (score, 2) (original magnification ×100 [A, C, and E] and ×400 [B, D, and F]).

STATISTICAL ANALYSIS

All specimens on the TMAs were considered independently. Contingency table analysis and 2-sided Fisher exact tests were used to study the statistical association between clinicopathologic and immunohistochemical variables. Retrospective overall survival (OS) and recurrence-free survival (RFS) curves comparing patients with and those without any of the variables were calculated using the Kaplan-Meier method, with significance
evaluated using 2-sided log-rank statistics. For the RFS analysis, patients were censored at the time of their last tumor-free clinical follow-up appointment. For the OS analysis, patients were censored at the time of their last clinical follow-up appointment or at their date of death unrelated to the tumor. \( P < .05 \) was considered significant. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, Ill). For multiple testing, the closed-test principle was used.

### RESULTS

#### IMMUNOHISTOCHEMICAL ANALYSIS

Investigation of MTAP protein expression in a large series of skin tumors using TMA technology was informative in 312 (89.1%) of 350 cases (data are missing for 18 cases). Expression of any intensity was detected in 227 (72.8%) of 312 informative cases. Representative MTAP immunostaining patterns are shown in Figure 1. Table 1 summarizes the results of IHC for each tumor entity on the TMAs. Expression of MTAP was significantly reduced in primary malignant melanomas and in melanoma metastases compared with benign nevi \( (P < .001 \) for both).

Among the TMAs, clinicopathologic variables were compared relative to MTAP expression on IHC (Table 2). In primary malignant melanomas, a high Ki67-labeling index was associated with loss of MTAP expression \( (P = .04) \). No other variables were significantly associated with MTAP expression. Skin metastases demonstrated significantly weaker MTAP expression compared with lymph node metastases \( (P = .01) \). No differences in MTAP expression were observed among the various types of benign nevi on the TMAs.

### PROGNOSTIC RELEVANCE

Overall survival and RFS were compared between MTAP-positive and MTAP-negative cases using univariate log-rank statistics. Expression of MTAP was not associated with OS \( (P = .8) \) or RFS \( (P = .4) \) in patients with primary malignant melanomas (Table 3). Considering the work of Mowen et al and Behrmann et al, a subgroup analysis was performed among 26 patients with MTAP-positive melanomas and tumor recurrence. In this subgroup, 18 patients who received interferon therapy had a significant benefit compared with 8 patients who did not receive interferon therapy \( (P = .009) \) (Figure 2A). Among the 18 patients, 8 received interferon therapy as a primary adjuvant regimen, whereas the other 10 received interferon therapy after tumor recurrence. In a subgroup of 13 patients with MTAP-negative melanomas and tumor recurrence, no such survival benefit associated with interferon therapy was found \( (P = .8) \) (Figure 2B).

#### COMMENT

To our knowledge, this is the first study demonstrating that MTAP protein expression of primary malignant melanomas is predictive of interferon therapy response in patients with disease progression. A significant inverse association between MTAP protein expression and progression of melanocytic tumors was recently demonstrated by immunohistochemical staining of 38 tissue samples of malignant melanomas, melanoma metastases, and benign nevi that revealed decreasing amounts of MTAP protein staining in the progression from benign nevi to metastatic melanomas. It was postulated that

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**Table 3. Univariate Analysis of Clinicopathologic Variables Relative to Recurrence-Free Survival (RFS) and Overall Survival (OS) Among Tissue Microarrays From Patients With Primary Malignant Melanomas**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RFS Total</th>
<th>RFS Events</th>
<th>P Value†</th>
<th>OS Total</th>
<th>OS Events</th>
<th>P Value†</th>
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</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis, y</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤60</td>
<td>48</td>
<td>25</td>
<td>.7</td>
<td>48</td>
<td>7</td>
<td>.6</td>
</tr>
<tr>
<td>&gt;60</td>
<td>40</td>
<td>18</td>
<td></td>
<td>40</td>
<td>7</td>
<td></td>
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<tr>
<td><strong>Sex</strong></td>
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<tr>
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<td>15</td>
<td>.06</td>
<td>39</td>
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<td>.4</td>
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<td></td>
<td>49</td>
<td>9</td>
<td></td>
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<td></td>
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<tr>
<td>II</td>
<td>5</td>
<td>0</td>
<td>.4</td>
<td>5</td>
<td>0</td>
<td>.3</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>8</td>
<td></td>
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<td>2</td>
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<tr>
<td>IV</td>
<td>54</td>
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<tr>
<td>V</td>
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<td>7</td>
<td></td>
<td>13</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor thickness, mm</strong></td>
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<tr>
<td>&lt;2.00</td>
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<td>14</td>
<td>.03</td>
<td>38</td>
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<td>.2</td>
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<tr>
<td>≥2.00</td>
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<td>29</td>
<td></td>
<td>50</td>
<td>10</td>
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<tr>
<td>&lt;5%</td>
<td>33</td>
<td>17</td>
<td>.7</td>
<td>33</td>
<td>7</td>
<td>.9</td>
</tr>
<tr>
<td>≥5%</td>
<td>36</td>
<td>16</td>
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<td>36</td>
<td>7</td>
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<tr>
<td><strong>MTAP immunoreactivity‡</strong></td>
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<tr>
<td>0</td>
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<td>13</td>
<td>.4</td>
<td>31</td>
<td>5</td>
<td>.8</td>
</tr>
<tr>
<td>1-2</td>
<td>49</td>
<td>26</td>
<td></td>
<td>49</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviation: MTAP, methylthioadenosine phosphorylase.

*Only initial and unifocal malignant melanomas were included.

†Log-rank test (2-sided); boldface indicates statistical significance.

‡Immunoreactivity of 0 indicates negative; 1, weak positive; and 2, strong positive.
inactivation of MTAP in melanoma progression might affect tumor response to interferon treatment. Mowen et al\(^1\) suggested an association between MTAP activity and interferon sensitivity via the transcription factor STAT1 after demonstrating that MTAP expression modified STAT1 activity. Reduced activity of MTAP in cells leads to accumulation of methylthioadenosine, which acts as an inhibitor of methyltransferases. Mowen et al identified the methylation of arginine 31 in STAT1 by the protein arginine N-methyltransferase 2 as an important modification, with this modification leading to enhanced binding of protein inhibitors of activated STAT to STAT1 and to inhibition of STAT1 DNA-binding activity. Because STAT1 is essential for activation of interferon \(\gamma\) signaling pathways, loss of MTAP expression was expected to reduce the response of the cells to interferon treatment. Some investigators recently questioned this model.\(^1\) However, other research in colon carcinoma supports a correlation between MTAP expression and responsiveness to interferon.\(^7\)

Based on the hypothesis by Mowen et al,\(^8\) we analyzed MTAP expression as a predictive marker, as opposed to its prognostic relevance. Patients with melanoma recurrence were evaluated because these patients require further treatment (eg, with interferon). Consistent with the findings of Mowen et al, patients with MTAP-negative melanomas with tumor recurrence and interferon treatment showed a significantly reduced OS compared with patients with MTAP-positive melanomas with tumor recurrence and interferon treatment \((P = .009)\). In contrast, MTAP immunoreactivity was not associated with prognosis (ie, OS and RFS) in patients with primary malignant melanomas.

These data supporting the previously suggested association between MTAP activity and interferon sensitivity may be of great clinical significance because biological markers predictive of tumor response to interferon therapy have not yet been defined.\(^9\) In adjuvant therapy for malignant melanoma, interferon alfa is to date the only agent demonstrating a significant (metastasis-free) survival benefit in prospective randomized clinical trials.\(^13\) Therefore, according to the current guidelines of the Dermatological Cooperative Oncology Group (http://www .ado-homepage.de/EV_Home/Guidelines/guidelines .html), all patients with melanoma and high risk of recurrence (tumor thickness \(>1.5\) mm or lymph node metastasis) should be offered adjuvant therapy with interferon alfa if there are no contraindications. Because any adjuvant treatment with interferon may have serious adverse effects and reduce the quality of life, the indications must be carefully assessed. Hence, immunohistochemical profiling of primary malignant melanomas responding to interferon treatment would be of significant clinical interest for selection of high-risk patients who might benefit from interferon therapy.

Our findings demonstrating an association between MTAP expression in primary malignant melanomas and response to interferon therapy needs to be validated in prospective clinical trials. Based on data presented herein, MTAP may represent a potential immunohistochemical marker to predict a patient’s response to interferon therapy.

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Wild, Hofstaedter, and Bosserhoff. Statistical analysis: Wild and Pauer. Obtained funding: Hofstaedter and Bosserhoff. Administrative, technical, and material support: Bataille, Ameres, Klinkhammer-Schalke, Hofstaedter, and Bosserhoff. Study supervision: Wild, Hofstaedter, and Bosserhoff. Drs Wild and Meyer contributed equally to this work. Financial Disclosure: None. Funding/Support: This study was supported by a grant from the Harry J. Lloyd Charitable Trust, Kansas City, Kan (Dr Bosserhoff). Acknowledgment: We thank Susanne Wallner, Frank van Rey, Lydia Kuenzel, and Rudolf Jung for excellent technical assistance; and David Carson, MD, for providing the primary anti-MTAP antibody.

REFERENCES


