Expression of Activated Extracellular Signal-Regulated Kinases 1/2 in Malignant Melanomas: Relationship with Clinical Outcome

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ABSTRACT

Purpose: The purpose of the present work was to analyze the protein expression of activated extracellular signal-regulated kinases 1 and 2 (ERK1/2) in a panel of superficial spreading melanomas (SSM) and nodular metastatic melanomas, and to correlate the expression level with clinicopathological parameters.

Experimental Design: Expression of activated ERK1/2 was examined by immunohistochemistry in 172 primary melanomas (108 SSM and 64 NMs), 67 metastatic lesions, and in 41 benign nevi.

Results: Fifty four percent of primary and 33% of metastatic melanomas expressed variable levels of activated ERK1/2. No immunoreactivity was detected in benign nevi. In 21% of the primaries only cytoplasmic expression was detected, whereas 3% and 30% showed positive immunoreactivity in either nucleus or cytoplasm and nucleus, respectively. Activated ERK1/2 expression varied significantly with the thickness of superficial spreading melanomas, with lower expression in thinner lesions (P = 0.016). A significant correlation between activated ERK1/2 and cyclin D1 (P = 0.031) in nodular, as well as between activated ERK1/2 and cyclin D3 (P = 0.030) in SSMs were observed. The protein level of p27Kip1 correlated with activated ERK1/2 (P = 0.048) in the nucleus. Furthermore, a strong inverse correlation between activated ERK1/2 and membrane-bound β-catenin (P = 0.004) in nodular melanomas was revealed. Activation of ERK1/2 did not have any impact on relapse-free or overall survival.

Conclusion: Our results suggest that activation of ERK1/2 may be involved in cell cycle regulation in SSMs. Moreover, the inverse association between membrane-bound β-catenin and ERK1/2 in NMs suggest that ERK1/2 activation may play a role in decreasing homotypic interactions through destabilization of β-catenin.

INTRODUCTION

MAPKs represent a family of serine-threonine kinases activated by a number of external stimuli. Depending on the cellular context, activation of MAPKs have been correlated with different responses, including proliferation, differentiation, and cell survival (1). The ERKs, ERK1 (p44) and ERK2 (p42), are the most thoroughly studied MAPKs in a group also including p38 MAPK, c-Jun NH2-terminal kinase/stress-activated protein kinase MAPK, and Big MAPK (2). Activation of Ras at the plasma membrane initiates a sequential activation of a series of protein kinases such as Raf-1, which, in turn, phosphorylates and activates MEK1/2. ERK1/2 is activated subsequently by dual phosphorylation of MEK1/2 (3). Activated ERK1/2 phosphorylates cytoplasmic proteins like pp90rsk (4), epidermal growth factor receptor (5), Raf-1 (6), and MAPK kinase (7), and on translocation to the nucleus, phosphorylates and activates transcription factors such as c-jun, c-myc (8), elk-1/p62TCF (9), and RNA polymerase II (10).

Many studies have revealed that aberrant regulation of the Ras-Raf-MEK-ERK cascade is involved in malignant progression (11, 12). Constitutively active MEK1 has been associated with malignant transformation in the human A375 malignant melanoma cell line (13), and activated Ras mutations have been observed in ~20% of primary malignant melanomas, and at even higher rates in metastatic and recurrent lesions (14, 15). Furthermore, B-Raf mutations have been reported recently in 66% of malignant melanomas as well as in a high percentage of benign nevi (16, 17).

It is well established that the MAPK signaling pathway is a key regulator of cell proliferation (18). Expression of cyclin D1 in early G1 phase has in several studies been demonstrated to depend on sustained ERK1/2 activation (19), whereas induction of the cdk-inhibitor p21WAF1/CIP1 requires a strong ERK signal (18). Furthermore, constitutive ERK1/2 activation leads to deregulation of the cdk-inhibitor p27Kip1 either by promoting its degradation or by sequestering p27Kip1 into cyclin D1/cdk4 complexes (20). Finally, regulation of the p53 tumor suppressor protein has in some systems been shown to be under control of the Ras-Raf-MEK-MAPK pathway (21).

Elevated levels of activated ERK1/2 have been detected in carcinomas of the kidney (22), liver (23), prostate (24), and breast (25, 26). In this study, we have investigated the expres-

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2 The abbreviations used are: MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, mitogen activated protein kinase kinase/extracellular signal-regulated kinase kinase; cdk, cyclin-dependent kinase; SSM, superficial spreading melanoma; NM, nodular melanoma.
sion of activated ERK1/2 in human melanoma biopsies using immunohistochemistry, and assessed the relationship between activated ERK1/2 and known prognostic variables, cell cycle factors, and progression in human malignant melanomas.

MATERIALS AND METHODS

Specimens. Formalin-fixed, paraffin-embedded tissue sections were obtained from 172 primary melanomas, 67 metastases, and 41 benign nevi. Of the primaries, 108 were classified as SSM and 64 as NM. Clinical follow-up was obtained for all of the patients.

Immunohistochemical Analysis. Sections of formalin-fixed, paraffin-embedded tissue were immunostained using the biotin-streptavidin-peroxidase method (Supersensitive Immunodetection System, LP000-UL; Biogenex, San Ramon, CA) and the optiMax Plus Automated Cell Staining System (Biogenex). Deparaffinized sections were microwaved in 10 mM citrate buffer (pH 6.0) to unmask the epitopes. After treatment with 1% hydrogen peroxidase for 10 min to block endogenous peroxidase, the sections were subsequently incubated with monoclonal phospho-p44/42 MAPK (Thr202/Tyr204) E10 antibody, dilution 1:300 (Cell Signaling Technology Inc., Beverly, MA), for 30 min at room temperature. The sections were then incubated with biotin-labeled secondary antibody (1:30) and streptavidin peroxidase (1:30) for 20 min each. Tissue was stained for 5 min with 0.05% 3,3’- diaminobenzidine tetrahydrochloride freshly prepared in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.024% hydrogen peroxidase, and then counterstained with hematoxylin, dehydrated, and mounted in Diatex. All of the dilutions of antibody, biotin-labeled secondary antibody and streptavidin-peroxidase were made with PBS (pH 7.4) containing 1% BSA. To ensure that negative immunostaining was because of lack of phosphorylated ERK1/2, 20 negative cases were stained with a monoclonal antibody targeting both phosphorylated and unphosphorylated ERK2, dilution 1:75, (Santa Cruz Biotechnology Inc., Santa Cruz, CA). All of the series included positive controls. Negative controls included substitution of the primary antibody with mouse myeloma protein of the same subclass and concentration as the monoclonal antibody. All of the controls gave satisfactory results. Four semiquantitative classes were used to describe the number of stained cells: (−) none; (+) <5% of the cells, (++) 5–50% of the cells, and (+++) >50% of the cells. Both nuclear and cytoplasmic staining were examined. Staining for cyclin D1, D3, A, p21\(^{WAF1/CIP1}\), p27\(^{KIP1}\), and β-catenin was performed as described previously (27–31).

Statistical Analysis. The relationship between activated ERK1/2 expression and mean tumor thickness was evaluated nonparametrically using the Mann-Whitney two sample test. A comparison between the expression of activated ERK1/2 and other markers of cell cycle progression was performed using the \(\chi^2\) test. Kaplan-Meier estimates and the log-rank test were used to evaluate the survival data statistically. \(P < 0.05\) was considered statistically significant.

RESULTS

Expression of ERK1/2 in Primary and Metastatic Melanoma Lesions. Formalin-fixed, paraffin-embedded tissue from 172 primary (108 SSM and 64 NM) and 67 metastatic melanomas, as well as 41 benign nevi were analyzed by immunohistochemistry for protein expression of activated ERK1/2. The results are summarized in Table 1. A heterogeneous cytoplasmic and/or nuclear staining pattern was observed (Fig. 1). Of 172 primary tumors, 93 (54%) expressed detectable levels of activated ERK1/2 in either cytoplasm, nucleus, or both. In 37 (21%) of the primaries activated ERK1/2 was observed only in the cytoplasm, whereas 5 (3%) and 51 (30%) showed positive immunoreactivity in either nucleus or cytoplasm and nucleus, respectively. There was a clear decline in the number of tumors expressing activated ERK1/2 in the metastases as compared with the primaries, because only 22 of 67 metastases (33%) showed positive immunostaining in either nucleus or cytoplasm and nucleus, respectively. The same tendency was observed also when primary and metastatic tumors from the same patient were examined. Whereas 12 of 33 (36%) primaries lacked ERK1/2 expression, 21 of 33 (64%) of the metastases showed no ERK1/2 immunoreactivity. Only 4 metastases expressed more ERK1/2 than the corresponding primary melanoma.

No ERK1/2 immunoreactivity was observed in 41 benign nevi or in normal skin adjacent to tumor. To ensure that negative immunostaining was due to lack of phosphorylated ERK1/2, 20 negative cases were stained with an antibody targeting both phosphorylated and unphosphorylated ERK2. All of the cases
showed positive staining in both cytoplasm and nucleus, supporting the idea that the ERK1/2 antibody is detecting the activated form of the protein.

**Expression of ERK1/2 in Relation to Clinical Parameters.** When examining the number of SSMs and NMs expressing activated ERK1/2 in the cytoplasm and/or nucleus, no difference could be observed; 58 of 108 (54%) of the SSMs were positive for ERK1/2, whereas the corresponding numbers for NMs were 35 of 64 (55%).

Activated ERK1/2 expression varied significantly with the thickness of SSM, with lower expression in thinner lesions ($P = 0.016$; Table 2). Although not significant, in NMs, lack of ERK1/2 activation was associated with thicker tumors ($P = 0.112$). Activation of ERK1/2 did not have any impact on relapse-free or overall survival, neither for SSMs ($P = 0.09$; $P = 0.50$) nor for NMs ($P = 0.31$; $P = 0.14$). Evaluating cytoplasmic and nuclear staining individually did not change the statistical outcome (data not shown).

**Relationship between ERK1/2 Expression and Cell Cycle Markers.** The panel of malignant melanomas has been examined previously for expression of cell cycle proteins (27–30). Because activation of ERK1/2 has been shown to affect cell proliferation (18) it was of interest, therefore, to study the association between ERK1/2 activation and expression of proteins involved in cell cycle regulation (Table 3). A significant correlation between activated ERK1/2 levels in the cytoplasm and/or nucleus and cyclin D1 ($P = 0.031$) in NMs as well as between activated ERK1/2 and cyclin D3 ($P = 0.030$) in SSMs was observed. Moreover, a close to significant association between activated ERK1/2 and cyclin A ($P = 0.064$), and between activated ERK1/2 and Ki-67 ($P = 0.16$) was found in SSMs. The protein level of p27Kip1 in SSMs correlated with activated ERK1/2 ($P = 0.048$) in the nucleus (data not shown), but not when analyzing the expression of activated ERK1/2 in the cytoplasm and/or nucleus. No association between

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**Table 2** Relationship between activated ERK1/2 and depth of tumor growth of primary melanomas

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Marker</th>
<th>Expression type</th>
<th>No. of patients</th>
<th>Depth of growth (mm)</th>
<th>$P$</th>
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<td>1.39</td>
<td>0.016</td>
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<td></td>
<td>Positive</td>
<td>57</td>
<td>2.08</td>
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<tr>
<td>Nodular</td>
<td>ERK1/2</td>
<td>Negative</td>
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<td>5.50</td>
<td>0.112</td>
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<td></td>
<td></td>
<td>Positive</td>
<td>35</td>
<td>3.81</td>
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</table>

$^{a}$ Expression level was scored as described in "Materials and Methods": $-$, negative, $+/-/+;+++;++$, positive.

$^{b}$ Tumor thickness was available for a total of 168 patients (105 superficial and 63 nodular melanomas).

$^{c}$ Measured as the mean thickness in each group.
Activated ERK1/2 was revealed for any of the subgroups.

Activated ERK1/2 Is Associated with Low Levels of Membrane-Bound β-Catenin. It has been reported that activation of ERK1/2 plays a role in disassembling of adherens junctions, which includes decreased expression of cell adhesion proteins. In this regard, stabilization of β-catenin protein following ERK1/2 inactivation has been demonstrated (32–34). Because our panel of melanoma specimens has been examined recently for protein expression of β-catenin (31), it was of interest to evaluate the panel for an association between activated ERK1/2 and β-catenin (Table 3). Interestingly, we found a significant inverse correlation between activated ERK1/2 in cytoplasm and/or nucleus and membrane-bound β-catenin (P = 0.004) in NMs. No association was revealed for the superficial spreading subtype.

DISCUSSION

In the present study we used immunohistochemistry to examine the level of activated ERK1/2 in a panel of primary and metastatic human malignant melanomas, and to evaluate to what extent activation had an impact on clinical outcome. Because our panel of melanoma specimens has been analyzed previously for expression of the cell cycle markers p21<sub>WAF1/CIP1</sub>, p27<sub>Kip1</sub>, cyclin A, D1, D3, and Ki-67, as well as the adhesion molecule β-catenin (27–31) we examined whether activation of ERK1/2 was associated with expression of these proteins.

In agreement with studies on other tumor forms (22–26), a heterogeneous cytoplasmic and/or nuclear staining pattern was found in a substantial percentage of primary and metastatic melanocytic lesions, but in no benign nevi. Of particular interest is our finding demonstrating a significant decline in ERK1/2 activation in metastases as compared with activation in primary melanomas. Our results are supported by studies showing an inverse correlation between ERK1/2 activation and progression of prostate cancer (35, 36). Although we have not examined the melanomas for Akt phosphorylation, it is of interest to note that in both of the above articles, disease progression was accompanied by inactivation of ERK1/2 by the phosphatidylinositol 3'-kinase pathway. Moreover, it has been shown that phospho-Akt is able to inactivate Raf and thereby the Raf-MEK-MAPK signaling pathway (37).

### Table 3: Relationship between expression of activated ERK1/2 and cell cycle/adhesion factors in cytoplasm, nucleus, or both

<table>
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<tr>
<th>Type of lesion</th>
<th>Number of tumors examined</th>
<th>Immunohistochemistry&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Expression of ERK1/2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Expression level</th>
<th>Negative</th>
<th>Positive</th>
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<sup>a</sup> Expression level was scored as described in “Materials and Methods”: −/+, low expression, +/+++/+++; high expression, except for p27<sub>Kip1</sub> and β-catenin: −/++, low expression, +++, high expression.

<sup>b</sup> Expression level was scored as described in “Materials and Methods”: −, negative, +/+++/++++, positive.

<sup>c</sup> β-cat-cyt, cytoplasmic β-catenin; β-cat-mem, membrane-bound β-catenin.
No correlation between ERK1/2 activation and relapse-free or overall survival was observed for this cohort of patients. Our results are in contrast with studies on prostate, kidney, liver, and breast cancer demonstrating an association between activation of ERK1/2 and tumor progression (22–25), but agrees with the studies of Pawelz et al. (35) and Malik et al. (36) on prostate cancer. Furthermore, in vitro studies suggest that sustained activation of ERK1/2 may lead to transformation in some but not all of the cell types (38). Together, these results suggest that the importance of ERK1/2 activation in tumor progression may be tumor specific.

Ras, Raf-1, and B-Raf mutations have been shown to result in activation of the MAPK (ERK1/2) signaling cascade (3, 16, 39, 40). Ras mutations are observed in ~20% of all of the melanomas, and recently Davies et al. (16) found point mutations in the B-Raf gene in 66% of the examined cases, suggesting ERK1/2 activation in nearly all of the melanomas. This is in contrast to the present study demonstrating ERK1/2 activation in only 54% and 33% of primary and metastatic lesions, respectively. Furthermore, during the course of this study, Pollock et al. (17) revealed that B-raf mutations are very frequent also in benign nevi. In agreement with the study of Satyamoorthy et al. (41) we did not detect ERK1/2 activation in the benign nevi, strongly suggesting that B-raf mutations alone are not sufficient to activate the ERK1/2, at least in melanomas in vivo. Our findings can be explained by the fact that not all of the Ras and Raf mutations may give rise to ERK1/2 activation (11, 37, 42). Furthermore, it is well accepted that the MAPK signaling pathway is activated in response to growth factors (43). Thus, it may be speculated that the heterogeneous activation of ERK1/2 in melanoma tissue is because of paracrine/autocrine growth factor stimulation rather than Ras or Raf mutations, a hypothesis also put forward by others (41, 44). Although our panel of melanomas has not been examined for overexpression or mutations in the Ras genes, it is of interest to note that a mutation in codon 18 of exon 1 in the N-Ras gene was shown recently to be associated with better clinical outcome for this group of patients (45). This and the fact that overexpression of the Ras oncogene has been found to induce apoptosis in tissues and immortalized cell lines (46, 47) may help to explain the observed decline in ERK1/2 activation in the metastases.

Several studies have shown that the MAPK (ERK1/2) signaling pathway is of major importance in regulating cyclin D1 expression (18, 19). Whereas most studies have focused on the regulation of cyclin D1, little is, however, known about the interplay between cyclin D3 and signaling in human cancer. We have reported recently an association between the protein level of cyclin D3, cell proliferation, and disease progression in SSMs (28). Here we show that ERK1/2 activation is also significantly associated with cyclin D3 protein levels and, furthermore, with the thickness of the primary tumors, suggesting that in SSMs, ERK1/2 activation may induce cyclin D3 expression, thereby leading to increased cell proliferation. As an additional support of ERK1/2 activation being involved in regulation of cell proliferation, we observed a tendency of increased expression of cyclin A and Ki-67 in tumors with activated ERK1/2. Interestingly, however, no association between cyclin D1 and ERK1/2 activation was observed for SSMs, a finding in agreement with Davies et al. (16) showing that in a melanoma cell line incubated with a MAPK inhibitor, expression of cyclin D1 remained unchanged. It has been demonstrated that induction of cyclin D1 requires only moderate ERK activity, but the activity has to be sustained for several hours (19). Therefore, it may be speculated that the duration of MAPK activation is not long enough to induce cyclin D1. Taken together, our results support observations suggesting that other pathways, like the phosphatidylinositol 3'-kinase pathway, may contribute in regulation of translation and stability of cyclin D1 (39).

In NMs, activated ERK1/2 was found to be associated with cyclin D1 expression. In agreement with our previous study demonstrating an inverse correlation between cyclin D1 levels and tumor thickness (28) we observed, although not significant, a trend toward activated ERK1/2 in thinner primary lesions. No association between ERK1/2 activation and other markers of cell cycle progression (Ki-67, cyclin A, p21WAF1/CIP1, and p27Kip1) was observed, suggesting that ERK1/2-induced expression of cyclin D1 in NMs is not associated with increased cell cycle progression.

Evidence has been provided indicating the involvement of ERK1/2 in regulating p21WAF1/CIP1 (18). In our study, no such association was observed in biopsies from SSMs nor NMs. It has been suggested that induction of p21WAF1/CIP1 requires a strong ERK1/2 signal (18), and it may be speculated, therefore, that the degree of ERK1/2 activation in the melanoma biopsies is not strong enough to induce p21WAF1/CIP1. Furthermore, activation of other signaling pathways like the c-Jun NH2-terminal kinase/stress-activated protein kinase have been shown to induce elevated p21WAF1/CIP1 protein levels (48). The p27Kip1 protein level was found to be associated with ERK1/2 activation in nucleus and only in SSMs. In contrast to studies showing increased p27Kip1 degradation following ERK1/2 activation (49) we observed that lack of ERK1/2 was associated with low levels of nuclear p27Kip1. Our result also differ from the study by Kortylewski et al. (13) showing accumulation of p27Kip1 after inhibition of ERK1/2. On the other hand, Cheng et al. (20) found that ERK1/2 activation reduced the ability of p27Kip1 to inhibit cdk2/cyclin E/A complexes through sequestration of p27Kip1 into cdk4/cyclin D complexes, rather than by initiating protein degradation.

We demonstrated recently a correlation between cytoplasmic β-catenin and cyclin D1/D3 in this panel of melanoma specimens (31). This spurred us to examine the melanoma panel for an association also between β-catenin protein levels and ERK1/2 activation. Interestingly, we observed a strong significant inverse association between ERK1/2 and membrane-bound β-catenin in NMs, suggesting that activated ERK1/2 either directly or indirectly may lead to phosphorylation of β-catenin subsequent to its degradation. In agreement with this, inhibition of MEK1 has been shown to increase the protein level of β-catenin (32–34). On the other hand, our results do not support results demonstrating phosphorylation of cytoplasmic p90Rsk by activated ERK1/2, leading to suppression of glycogen synthase kinase 3β and stabilization of β-catenin (50, 51). The β-catenin protein contains multiple potential ERK phosphorylation sites (31), and a direct regulation of β-catenin levels by ERK1/2 can, therefore, not be excluded.

In summary, we have shown that ERK1/2 is activated in a substantial percentage of primary melanomas, whereas the de-
gree of activation declines in metastatic lesions. No ERK1/2 activation was detected in benign nevi, a finding indicating that the high percentage of B-Raf mutations observed in malignant melanomas and nevi (16, 17) is not sufficient to activate ERK1/2. Furthermore, our results suggest that activation of ERK1/2 may be involved in cell cycle regulation in SSMs, whereas in the nodular subtype, ERK1/2 activation may lead to decreased homotypic interactions through destabilization of β-catenin. ERK1/2 activation did not have an impact on the clinical outcome for this cohort of patients, suggesting that in vivo more than one signaling pathway plays a role in the development and progression of a malignant phenotype.

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REFERENCES


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