MAPKAP Kinase 2 Overexpression Influences Prognosis in Gastrointestinal Stromal Tumors and Associates with Copy Number Variations on Chromosome 1 and Expression of p38 MAP Kinase and ETV1

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Abstract

Purpose: ETV1 has been proposed to be activated by KIT mutations in gastrointestinal stromal tumors (GIST). The aim of the study was to evaluate the clinical role of ETV1 and associated proteins in GIST.

Experimental Design: Expressions of ETV1, MAPKAP kinase 2 (MAPKAPK2), phosphorylated p38 MAP kinase (pp38), phosphorylated MSK1 (pMSK1), phosphorylated RSK1, COP1, and KIT protein were determined immunohistochemically in 139 GISTs. Sequence analysis of KIT, PDGFRA, and MAPKAPK2 and FISHs of ETV1 as well as chromosomes 1 and 7 were done.

Results: Prominent ETV1 expression was seen in 50% of GISTs, but no correlation with clinical outcome was found. Correlation of ETV1 expression and KIT mutation was seen in 60% of cases. MAPKAPK2 overexpression (n = 62/44.6%) correlated with pp38 expression (P = 0.021, χ² test) and alterations of chromosome 1 (n = 17, P = 0.024, χ² test). In one of 20 sequenced cases with high MAKAPK2 expression, an putative damaging MAPKAPK2 gene mutation was found. All relapsing GISTs with very low/low risk according to Fletcher showed high MAPKAPK2 and KIT expression. MAPKAPK2 overexpression was an independent prognostic factor for disease-free survival (P = 0.006, Cox regression).

Conclusion: ETV1 is not universally overexpressed in GIST and seems to also be induced by pathways other than KIT mutation. Nevertheless, its clinical relevance is low. Overexpression of ETV1 inhibitor MAPKAPK2 is associated with shorter survival in GIST, indicating a clinically relevant role of this gene not reported previously. Patients with low-risk GISTs showing MAPKAPK2 overexpression might profit from early adjuvant tyrosine kinase inhibitor therapy. Clin Cancer Res; 18(7): 1–9. ©2012 AACR.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract (1). Radical surgery seems to be the best treatment option for localized GIST tumors (2). The recurrence rate after radical surgery seems to depend mainly on tumor localization, size, and mitotic activity and ranges between 5% and 75%, with a poor clinical outcome in relapsed patients (3, 4). Current classifications take into account tumor size, mitotic rate, and tumor location, but they do not include mutational data nor protein expressions of tumor cells (5–7). Due to the wide range of behavior, it is important to identify further factors providing a prognostic value in predicting the risk of relapse of fully resected tumors.

The finding of KIT and PDGFRA activating mutations in the majority of GIST represents a significant progress in understanding the biologic behavior with significant influence on their treatment (8). However, the relevance of the mutational status of KIT and PDGFRA as a prognostic factor remains controversial (9, 10), although its predictive value on tyrosine kinase inhibitors response is now well established (11, 12). It has recently been suggested that mutated and activated KIT enhances ETV1 stability and together promote tumorigenesis (13). ETV1 belongs to the well-known ETS family transcription factors and oncogenes and cooperates with other pathways, as the p38 and MSK1 (14, 15). RSK1 (also known as 90-kDa ribosomal S6 kinase 1) has been shown to phosphorylate ETV1 and to enhance ETV1-dependent transcription (16). Recently reported negative regulators of ETV1 included COP1 and MAPKAPK2 that degrades ETS transcription factors or phosphorylates...
ETV1 within their central inhibitory domain, respectively (14, 17). However, these up- and downregulators of ETV1 were evaluated in vitro and would need confirmation by in vivo approach.

In this work, we investigated protein expression of ETV1 and ETV1-interacting genes MAPKAPK2, p38, COP1, RSK1, and MSK1 in a large single-center cohort of GISTs and tried to correlate these data with genetic findings and clinical outcome. Our study shows that MAPKAPK2 is a valuable predictive marker for relapse, is superior to other well-established classifications, and may thus have the potential of a new therapeutic target.

Materials and Methods

Cases

We studied 139 consecutive cases of GIST treated at the Medical University of Vienna between 08/1992 and 02/2011. All cases were restaged according to UICC tumor–node–metastasis (TNM) classification of malignant tumors seventh edition and risk evaluation according to Fletcher and Miettinen (5–7). Institutional review board approval was obtained.

Immunohistochemical analysis

In all 139 cases, protein expression of KIT, ETV1, MAPKAPK2, MAPKAPK2 phosphorylated at Thr334 (n = 62), p38 (phosphorylated at Thr180 and Tyr182) (pp38), MSK1 phosphorylated at Serine 376 (pMSK1), RSK1 phosphorylated at pThr 359 (pRSK1), and COP1 (n = 136) were investigated in formalin-fixed paraffin-embedded (FFPE) tumor tissue. Detailed information about all applied antibodies is provided in Supplementary Table S1. Expressions of ETV1, pp38, pMSK1, and MAPKAPK2 were evaluated by light microscopy on full slides and scored as described previously (18, 19): an immunostaining score [immunohistochemistry (IHC) score: 0–300] was calculated as the product of the staining intensity [1 (weak), 2 (moderate), or 3 (strong) expression] and the staining rate (percentage of positive tumor cells, 0%–100%). Tumors with scores exceeding the median were considered showing high expression, tumors equal or below the median as showing low expression. In cases of pp38, a specimen was considered as positive when 1% or more of tumor nuclei showed distinct expression. A specimen was considered as positive for KIT, pRSK1, or COP1 expression, when 90 or more of cells showed distinct cytoplasmic or nuclear staining (Fig. 1J and K).

Apart from the 139 GIST tumors, the expression of MAPKAPK2 and ETV1 was evaluated in the interstitial cells of Cajal—the presumed cell of origin of GIST—of 2 normal gastric tissues. Cajal cells were identified by KIT and Nestin. Confocal laser scanning microscopy was done on a Zeiss LSM 510.

Sequence analysis

DNA was isolated from archival FFPE GIST tissues. A total of 138 cases were tested successfully for mutations of KIT (exons 9, 11, 13, and 17) and PDGFRA (exons 12 and 18). Twenty cases with high MAPKAPK2 protein expression were sequenced for mutations of MAPKAPK2 (exons 2–10). Primer sequences are provided in Supplementary Table S2. Sequencing analysis was done as described previously (20, 21).

FISH analysis

Tissue microarrays from all 139 cases were constructed for FISH. ETV1 status was investigated with a dual-color, break-apart rearrangement probe to screen for translocations and copy number variations (CeGaT). ETV1 was considered as amplified if the ratio ETV1/centromere 7 signals was more than 2. Furthermore, aneuploidies of centromere 7 and chromosomal bands 1p36 (TP73), and 1q25 (ABL2) (Abbott) were analyzed using standard protocols.

Statistical analysis

Mann–Whitney, Fisher, and $\chi^2$ tests were used as appropriate.

Disease-free survival (DFS) was defined from the day of surgery until first evidence of progression of disease if complete surgical resection was possible. Patients showing advanced disease at the time of initial diagnosis were excluded from analysis of DFS. Univariate analysis of survival was done as outlined by Kaplan and Meier or by univariate Cox regression in case of patients’ age.

Simple Cox regression was used for multivariate analysis of survival. A 2-tailed P value of 0.05 or less was considered as significant.

Results

Clinical data in correlation with known risk factors

Clinical characteristics of patients (n = 139) are summarized in Table 1. Mean follow-up time was 51 ± 4 (SE) months. Twenty-two patients showed recurrent disease and 12 died from their GIST. Tyrosine kinase inhibitors were administered in 27 patients, in 7 of them palliatively. Risk factors according to Fletcher and Miettinen were good predictors of recurrence (Table 3), but nevertheless, 4 of
67 (6%) GISTs classified as very low or low risk according to Fletcher and 3 of 55 (5.5%) GISTs scored as none or very low risk according to Miettinen developed recurrent disease. The vast majority of cases showed expression of KIT protein (n = 121; 87.1%), whereas KIT mutations were found in 85 cases (61.6%) and mutations of PDGFRA in 20 cases (14.5%); 2 cases showed a combined mutation of KIT and PDGFRA, and 35 cases (25.4%) showed no mutations. Although in several collectives of GIST higher rates of KIT mutations have been reported (8, 11, 22), our gene mutation spectrum is in concordance with reports from other groups (10, 23–28). Despite the high number of patients in our study, the rate of recurrent disease and death of disease was relatively low. This observation might be explained by the fact that only surgically resected cases were included, which were in about 50% of cases small, mostly accidentally diagnosed tumors.

**ETV1 is not generally upregulated in GIST**

In a first step, we evaluated the total number of ETV1-positive cases in our cohort. Seventy cases (50.4%) were considered as positive for ETV1 expression (Table 1, Fig. 1F). High ETV1 expression was more common in cases with KIT overexpression compared with those without (53.7% vs. 27.8%, P = 0.046, exact chi-squared test, Table 2). Next we correlated ETV1 expression with KIT mutation status. In the 85 cases with KIT mutation, high ETV1 expression was significantly more common (n = 50/58.8%) compared with cases without KIT mutations (n = 20/37.7%; P = 0.016, chi-squared test, Table 2).
Despite the significant correlation between ETV1 expression and KIT mutation, we observed a lack of KIT mutations in 20 of 70 cases (28.6%) with high ETV1 expression. To investigate whether a possible ETV1 translocation or amplification analogic to prostate cancer (29) and melanoma (30) might attribute to ETV1 protein overexpression...
independently of KIT mutation status, FISH was done and successful in 116 cases. We observed ETV1 translocations in none of the cases and low-copy amplification (6 signals for ETV1 and 2 signals for centromere 7) in only one case without KIT mutation but high ETV1 expression (Fig. 1H). We concluded that ETV1 rearrangements do not significantly contribute to ETV1 overexpression in our series of patients.

ETV1 correlates with pMSK1 but not with pp38, pRSK1, and COP1

Due to the fact that a considerable number of ETV1-positive cases were negative for KIT mutations, we correlated the expression of ETV1 with its activators pp38 and pMSK1 in all cases. Both antigens showed a nuclear staining pattern in IHC. Thirty-eight cases (27.3%) were considered as positive for pp38 expression (Fig. 1O), and 69 cases (49.6%) showed high pMSK1 expression (Fig. 1R and S, Table 1). No association of ETV1 with pp38 expression was observed ($P > 0.05$, $\chi^2$ test). When investigating all cases, ETV1 correlated with pMSK1 expression ($P = 0.001$, $\chi^2$ test), but subgroup analysis for KIT mutation status revealed that this was only evident in the group with KIT mutations ($P < 0.001$, $\chi^2$ test).

Distinct nuclear staining for ETV1 activator pRSK1 was found in only one GIST, which was also positive for ETV1.

Table 2. Protein expression and gene status

<table>
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<tr>
<th>Protein expression</th>
<th>MAPKAPK2+</th>
<th>ETV1+</th>
<th>pMSK1+</th>
<th>pp38+</th>
<th>KIT+</th>
<th>COP1 (n = 136)+</th>
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<tr>
<td>MAPKAPK 2</td>
<td>Low (n = 77)</td>
<td>—</td>
<td>31 (40.3)a</td>
<td>34 (44.2)</td>
<td>15 (19.5)a</td>
<td>65 (84.4)</td>
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<td>High (n = 62)</td>
<td>—</td>
<td>39 (62.9)a</td>
<td>35 (56.5)</td>
<td>23 (37.1)a</td>
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<td>23 (33.3)a</td>
<td>24 (34.8)a</td>
<td>16 (23.2)</td>
<td>56 (81.2)a</td>
<td>61 (88.4)</td>
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<td></td>
<td>High (n = 70)</td>
<td>39 (55.7)a</td>
<td>45 (64.3)a</td>
<td>22 (31.4)</td>
<td>65 (92.9)a</td>
<td>64 (95.5)</td>
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<td>pMSK1</td>
<td>Low (n = 70)</td>
<td>27 (38.6)</td>
<td>25 (35.7)a</td>
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<td>13 (18.6)a</td>
<td>64 (91.4)</td>
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<tr>
<td></td>
<td>High (n = 69)</td>
<td>35 (50.7)</td>
<td>45 (65.2)a</td>
<td>—</td>
<td>25 (36.2)a</td>
<td>57 (82.6)</td>
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<td>Negative (n = 101)</td>
<td>39 (38.6)a</td>
<td>48 (47.5)</td>
<td>44 (43.6)a</td>
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<td>Positive (n = 38)</td>
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<td>Positive (n = 121)</td>
<td>56 (46.3)</td>
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<td>COP1 protein</td>
<td>Negative (n = 24)</td>
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<td>KIT gene (n = 138)</td>
<td>No mutation (n = 53/38.4%)</td>
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<td>20 (37.7)a</td>
<td>31 (58.5)</td>
<td>16 (30.2)</td>
<td>39 (73.6)a</td>
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<td>Mutation (n = 85/61.6%)</td>
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<td>1 (100)</td>
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<td>1 (100)</td>
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<td>PDFRA gene (n = 138)</td>
<td>No mutation (n = 118/85.5%)</td>
<td>53 (44.9)</td>
<td>60 (50.8)</td>
<td>55 (46.6)</td>
<td>29 (24.6)</td>
<td>106(89.8)a</td>
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<td>Mutation (n = 20/14.5%)</td>
<td>9 (45)</td>
<td>10 (50)</td>
<td>14 (70)</td>
<td>9 (45)</td>
<td>14 (70)a</td>
</tr>
<tr>
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<td>3 (75)</td>
<td>2 (50)</td>
<td>3 (75)</td>
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<tr>
<td></td>
<td></td>
<td>6 (37.5)</td>
<td>8 (50)</td>
<td>11 (68.8)</td>
<td>6 (37.5)</td>
<td>11 (68.8)</td>
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<td>MAPKAPK2 gene (n = 20)</td>
<td>No damaging mutation (n = 19/95%)</td>
<td>19 (100)</td>
<td>11 (57.9)</td>
<td>6 (31.6)</td>
<td>0 (0)</td>
<td>18 (94.7)</td>
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<td>Possibly damaging mutation (n = 1/5%)</td>
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<td>1 (100)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

NOTE: Values in parenthesis are given in percentage.
aSignificant difference
expression and KIT wild type. Furthermore, we tested COP1, a negative regulator of ETV1 expression. Staining was possible in 136 cases, of which 125 (91.9%) showed strong expression (Table 1, Fig. 1K and L). Downregulated COP1 was not associated with ETV1 overexpression (P > 0.05, χ² test) or deletion of chromosomal band 1q25 located next to COP1 (data not shown) in all cases as well as in subgroups with or without KIT mutations (data not shown; Fig. 1M).

Expressions of pMSK1, ppp38, and COP1 in the cases with overexpression of ETV1 and negativity for KIT mutations are summarized in Supplementary Table S3. Taken our data together, we found no prevailing influence of p38, pRSK1, and COP1 on ETV1 expression in GIST, whereas pMSK1 seems to influence ETV1 expression at least in KIT mutated GISTs.

**MAPKAPK2 is frequently overexpressed in GIST**

MAPKAPK2 was reported to be activated directly by p38 and counteracts the p38-dependent ETV1 activation pathway (14, 31, 32). Therefore, MAPKAPK2 was regarded as an interesting candidate and tested in our series. First, we investigated MAPKAP2 and ETV1 expression in Cajal cells, the presumed cell of origin of GIST, in 2 normal gastric tissues. Both ETV1 (Fig. 1A–1E) and MAPKAP2 were weakly expressed in Cajal cells compared with tumors. Next, we screened our 139 GIST cases. Sixty-two GISTs (44.6%) showed a high MAPKAP2 expression evident by distinct cytoplasmic staining pattern (Table 1, Fig. 1N and P). At immunostaining for MAPKAP2 phosphorylated at Thr334, in no case cytoplasmic staining was seen, and moderate or strong nuclear staining in only 5 cases.

High MAPKAP2 expression was associated with pp38 (P = 0.021, χ² test) and ETV1 expression (P = 0.008, χ² test). The association between MAPKAP2 and ETV1 was observed only in cases without KIT mutations (P = 0.007, χ² test).

Because 39 cases with high expression of MAPKAP2 (62.9%) showed no expression of pp38, we tried to find alternative explanations for MAPKAP2 activation in these patients. MAPKAP2 is located on chromosome 1 (chromosomal band 1q32). Chromosome 1 is reported to be frequently altered in GIST using comparative genomic hybridization and chromosomal banding (33–35). We correlated our FISH data of chromosome 1 with MAPKAP2 expression in 110 cases. Aberrations of chromosome 1 were found in 17 cases [1p− (n = 10), 1q+ (n = 2), 1q−+ (n = 1), 1q− (n = 1), 1p++ 1q+ (n = 1)] and associated with high MAPKAP2 expression (P = 0.024, χ² test). The correlation was even stronger in cases without pp38 expression (n = 79; P = 0.002, χ² test, 26.5% vs. 72.7%). With regard to cases with high MAPKAP2 protein expression and no expression of pp38, we carried out sequence analysis of MAPKAP2 in all 17 cases with inconspicuous FISH findings for chromosome 1. Three further cases with high MAPKAP2 expression were also tested. A possibly damaging mutation was found [c.782C > T heterozygous (p.P261L)] in one patient, and a benign mutation in another case [c.1081G > T heterozygous (p.A361S); Supplementary Table S4]. We concluded that MAPKAP2 mutations are unlikely a common cause for MAPKAP2 overexpression.

**MAPKAPK2 is a strong prognostic factor in GIST**

After the evaluation of protein expressions and gene status, we looked for a clinical significance of the investigated proteins in relation to established risk classifications (Table 1). High ETV1 expression was associated with lower risk factor according to Fletcher and lower pT stage (P = 0.037 and P = 0.04, respectively, Mann–Whitney test). Also a significant association of high ETV1 expression with low mitotic rate according to UICC was seen (P = 0.003, χ² test). No association of COP1 or pp38 expression with clinical risk factors was found, but high pMSK1 expression was associated with low mitotic rate according to UICC (P = 0.013, χ² test). No significant influence of ETV1, pp38, COP1, and pMSK1 expression on DFS was seen (P > 0.05, log-rank test and Cox regression).

High MAPKAP2 expression was associated with metastases already at time of diagnosis (P = 0.034, Fisher exact sign test). The association was even stronger in cases without KIT expression (P = 0.009, Fisher exact sign test).

### Table 3. Survival analysis of 133 patients with resectable GIST

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value univariate</th>
<th>P value multivariate</th>
<th>Relative risk (95% CI)</th>
</tr>
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<tr>
<td>Patients’ age</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>—</td>
</tr>
<tr>
<td>pT Stage (UICC)</td>
<td>0.04</td>
<td>0.039</td>
<td>1.752 (1.03–2.979)</td>
</tr>
<tr>
<td>Grading UICC</td>
<td>0.001</td>
<td>&gt;0.05</td>
<td>—</td>
</tr>
<tr>
<td>MAPKAPK2</td>
<td>0.003</td>
<td>0.006</td>
<td>3.872 (1.488–10.073)</td>
</tr>
<tr>
<td>Patients’ age</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>—</td>
</tr>
<tr>
<td>Risk Fletcher</td>
<td>0.005</td>
<td>0.002</td>
<td>2.086 (1.302–3.34)</td>
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<tr>
<td>MAPKAPK2</td>
<td>0.003</td>
<td>0.007</td>
<td>3.739 (1.443–9.691)</td>
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<tr>
<td>Patients’ age</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>—</td>
</tr>
<tr>
<td>Risk Miettinen</td>
<td>0.036</td>
<td>0.021</td>
<td>1.501 (1.063–2.120)</td>
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<td>MAPKAPK2</td>
<td>0.003</td>
<td>0.007</td>
<td>5.744 (1.629–20.251)</td>
</tr>
</tbody>
</table>

Disease-free survival (UICC)

Disease-free survival (Fletcher)

Disease-free survival (Miettinen)

**Factor**

- **Patients’ age**
- **pT Stage (UICC)**
- **Grading UICC**
- **MAPKAPK2**
- **Patients’ age**
- **Risk Fletcher**
- **MAPKAPK2**
- **Patients’ age**
- **Risk Miettinen**
- **MAPKAPK2**

**P** values

- Univariate: >0.05
- Multivariate: >0.05

**Relative risk (95% CI)**

- Disease-free survival (UICC): 1.752 (1.03–2.979)
- Disease-free survival (Fletcher): 2.086 (1.302–3.34)
- Disease-free survival (Miettinen): 3.739 (1.443–9.691)
expression (B).

-associated with signiﬁcant shorter DFS compared with those with low expression (B).

test) and development of recurrent disease (P = 0.004, Fisher exact test), but not with risk according to Fletcher and Miettinen or UICC staging and grading (Table 2, P > 0.05). High MAPKAPK2 expression was associated with shorter DFS (P = 0.003, log-rank test, Fig. 2B) at investigation of all patients with totally resectable tumor (including also cases with totally resectable metastases): 5 years DFS rate was 94% in patients with low MAPKAPK2 expression, whereas it was 67% in patients with high expression.

When investigating DFS only in cases with resectable GISTs and without any metastases at time of diagnosis (n = 126, 17 recurrences), MAPKAPK2 expression was a strong prognostic factor in univariate (P = 0.007, log-rank test) and multivariate analysis (P = 0.013, relative risk = 3.963, Cox regression). MAPKAPK2 expression remained also an independent prognostic factor for DFS (P = 0.023, Cox regression), when the use of tyrosine kinase inhibitors (yes vs. no) was introduced into the model (P = 0.024, Cox regression). Interestingly, all 4 very low/low-risk GISTs according to Fletcher and all 3 none/very low-risk GISTs according to Miettinen that developed recurrent disease showed high MAPKAPK2 expression. This association of high MAPKAPK2 expression with recurrent disease reached signiﬁcance in the group of very low/low-risk tumors according to Fletcher, even despite of the low number of cases (P = 0.041, Fisher exact test). All those MAPKAPK2-positive relapsing cases were also positive for KIT expression. In multivariate analysis, MAPKAPK2 expression remained an independent prognostic factor for DFS (P = 0.006, Cox regression; Table 3). When conducting survival analysis using risk scores according to Fletcher or Miettinen, high MAPKAPK2 expression was a stronger independent prognostic factor for DFS than these risk scores (Table 3).

Discussion

In this study, we investigated the clinical relevance of ETV1 and associated proteins in a large collective of GISTs. Our results show that ETV1 is not activated exclusively by KIT mutations in GIST. ETV1 has been proposed as diagnostic marker in this tumor entity (13), but it is not superior to KIT for this purpose. In addition, ETV1 overexpression seems of only low clinical relevance in GIST.

In contrast to ETV1, MAPKAPK2 seems to play a key role in progression of GISTs. Although in our cohort multivariate survival analysis must be interpreted with caution because of the relatively low number of recurrences, it indicates that the influence of MAPKAPK2 is independent from established prognostic factors.

Interestingly, we observed a positive correlation between ETV1 and MAPKAPK2 GISTs in the absence of KIT mutations. MAPKAPK2 has been described as an inhibitor of ETV1 in a cell type–speciﬁc manner and seems to have no effect on ETV1 when it is activated through the extracellular signal–regulated kinase–mitogen-activated protein kinase (ERK–MAPK) pathway (14). In KIT mutated GISTs MAPK–ERK signaling is activated in a KIT-dependent manner (36). Consequently, MAPKAPK-2 might not interact with ETV1 in an inhibitory manner in GISTs with KIT mutation. In cases without KIT mutations, MAPKAPK2 overexpression in ETV1-positive cases might also be driven by a—currently unknown—compensative mechanism deregulating ETV1 expression. To investigate this subject in detail, additional functional studies would be needed.

Multiple residues of MAPKAPK2 are generally phosphorylated in vivo in response to stress, but only 4 residues (Thr25, Thr222, Ser272, and Thr334) are phosphorylated by p38 MAPK in vitro (37). Using a phosphorylation-speciﬁc antibody, we were able to show that in GIST, MAPKAPK2 activation by phosphorylation at Thr334 has no relevance.

Only few in vitro data and no clinical data for that matter have been reported so far about the relevance of MAPKAPK2 in human malignant disease. It has been shown recently that MAPKAPK2 increased cell invasion in human prostate cancer cells via modulation of matrix metalloproteinases (MMP) 2 and 9 activity (38). Only few data exist on MMPs in GIST (39, 40). Nevertheless, MAPKAPK2-driven MMPs activation, followed by increased invasiveness, might be a possible mechanism also with regard to GISTS. In addition, it has also been shown recently that MAPKAPK2 plays a critical role in cancer cells in the posttranscriptional regulation of gene expression in response to DNA damage (41). A possible correlation between MAPKAK2 overexpression and impaired cellular response of GIST cells to DNA damage should be investigated in further functional studies.

MAPKAPK2 overexpression seems to be induced by several mechanisms: activation by p38 has been reported in the literature (14, 31, 32). Few data are available on the inﬂuence of p38 on expression levels of MAPKAP. So it has been shown that MAPKAPK2 expression is lost in p38 knockout mice, and that p38 modulates MAPKAPK2 expression both...
transcriptionally and posttranscriptionally in murine cell lines and embryos (42).

In our study, a strong correlation between these 2 factors was only seen in about 40% of cases. Although we did not investigate the MAPKAPK2 gene location on chromosome 1q32 directly using FISH, high MAPKAPK2 expression seemed to be associated with copy number variations on chromosome 1. These data indicate that MAPKAPK2 expression might be dysregulated by chromosome 1 alterations that are frequently observed in GISTs. In some other cases, MAPKAPK2 overexpression might be induced by MAPKAPK2 gene mutations. In conclusion of above findings, we did not find a uniform pathway for MAPKAPK2 overexpression. Despite the fact that the oncogenic effects of MAPKAPK2 are only partly understood activation mechanisms, high MAPKAPK2 expression is of substantial clinical relevance in GIST, as it was strongly associated with recurrence of disease in our study. All GIST cases that were considered to have low or even none risk of recurrence, but nevertheless relapsed, showed high MAPKAPK2 expression.

The identification of MAPKAPK2 as a putative early predictor of relapse is of particular interest in GIST. About 50% of GISTs are small, asymptomatic tumors mostly detected accidentally, and they are considered at low risk for relapse. Affected patients do not receive adjuvant tyrosine kinase inhibitors, but about 5% of them develop metastases, which are associated with dismal prognosis. MAPKAPK2 might serve as a factor for risk adjustment especially in small GISTs, as when only considering MAPKAPK2-positive tumors with very low/low (Fletcher) or none/very low risk (Miettinen), about 12% relapse. This is a recurrence rate comparable with early-stage gastric (43) or breast cancer (44), indicating that MAPKAPK2-positive small GIST patients might be currently undertreated with surgery alone. It might be speculated that adjuvant tyrosine kinase inhibitor therapy in patients with small, but MAPKAPK2 overexpressing, GISTs reduces the rate of recurrences.

Because MAPKAPK2 plays an important role in inflammatory diseases like rheumatoid arthritis (45), selective inhibitors have been developed (46, 47). In addition to tyrosine kinase inhibitors in KIT-positive GISTs, such MAPKAPK2 inhibitors in tumors overexpressing this protein might be of benefit for patients. The inclusion of MAPKAPK2 in future clinical studies may help to improve therapy schemes and justify early potential therapeutic intervention in a clinically seemingly low-risk group.

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S.F. Schoppmann received an unrestricted research grant from Pfizer. The other authors disclosed no potential conflicts of interest.

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Analysis and interpretation of data (e.g., statistical analysis, biostatis-
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References


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MAPKAP Kinase 2 Overexpression Influences Prognosis in Gastrointestinal Stromal Tumors and Associates with Copy Number Variations on Chromosome 1 and Expression of p38 MAP Kinase and ETV1

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