**Imaging, Diagnosis, Prognosis**

**Homogeny Deletion of MTAP Gene as a Poor Prognosticator in Gastrointestinal Stromal Tumors**

Hsuan-Ying Huang,1 Shau-Hsuan Li,4 Shih-Chen Yu,1 Fong-Fu Chou,2 Ching-Cherng Tzeng,6 Tsung-Hui Hu,5 Yih-Huei Uen,7 Yu-Fang Tian,7 Yu-Hui Wang,6 Fu-Min Fang,3 Wen-Wei Huang,8 Yu-Ching Wei,1 Jing-Mei Wu,1 and Chien-Feng Li6,9

**Abstract**

**Purpose:** Chromosome 9 is frequently deleted in high-risk gastrointestinal stromal tumors (GISTs), whereas its specific tumor suppressor genes (TSGs) are less understood. We did an integrative study of MTAP gene at 9p21 to analyze its implication in GISTs.

**Experimental Design:** To search TSGs on chromosome 9, we used ultrahigh-resolution array comparative genomic hybridization to profile DNA copy number alterations of 22 GISTs, with special attention to MTAP gene. MTAP immunohistochemistry was assessable for 306 independent GISTs on tissue microarrays, with 146 cases analyzed for MTAP homozygous deletion, 181 for mutations of KIT and PDGFR receptor tyrosine kinase genes, and 7 for MTAP hypermethylation.

**Results:** Array comparative genomic hybridization identified 11 candidate TSGs on 9p and six on 9q. MTAP and/or CDKN2A/CDKN2B at 9p21.3 were deleted in one intermediate-risk (11%) and seven high-risk (70%) GISTs with two cases homozygously codeleted at both loci. MTAP homozygous deletion, present in 25 of 146 cases, was highly associated with larger size and higher mitotic rate, Ki-67 index, and risk level (all $P < 0.01$) but not with receptor tyrosine kinase genotypes. Whereas MTAP homozygous deletion correlated with MTAP protein loss ($P < 0.001$), 7 of 30 GISTs without MTAP expression did not show homozygous deletion, including three MTAP-hypermethylated cases. MTAP homozygous deletion was univariately predictive of decreased disease-free survival ($P < 0.0001$) and remained multivariately independent ($P = 0.0369$, hazard ratio = 2.166), together with high-risk category ($P < 0.0001$), Ki-67 index >5% ($P = 0.0106$), and nongastric location ($P = 0.0416$).

**Conclusions:** MTAP homozygous deletion, the predominant mechanism to deplete protein expression, is present in 17% of GISTs. It correlates with important prognosticators and independently predicts worse outcomes, highlighting the role in disease progression. (Clin Cancer Res 2009;15(22):6963–72)

Gastrointestinal stromal tumors (GISTs) are characterized by constitutive activation of receptor tyrosine kinase (RTK) resulting from gain-of-function mutations of KIT and PDGFR receptor tyrosine kinase (1). Although NIH consensus scheme proved prognostically useful (2–5), the associations of tumor location and RTK genotypes with outcomes underscore the need to assess new adjuncts to better risk-stratify GISTs (4, 6–13). Furthermore, identification of novel genes altered in tumor cells is critical for better understanding tumorigenesis, developing diagnostic tests, and designing effective therapies.

The prospect of genome-wide approaches is now encouraging efforts to characterize cancer genomes. Decreased gene dosage by hemizygous and/or homozygous deletion can lead to inactivation of tumor suppressor genes (TSGs), which may open a new avenue for derived targeted therapies (14). Nonrandom losses of chromosome 9p and/or 9q have been recurrently

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Identification of cancer-associated genes by genomic profiling enables the elucidation of tumor development and progression. KIT or PDGFRA receptor tyrosine kinase gene mutations are primary events in the tumorigenesis of gastrointestinal stromal tumors (GISTs). However, chromosomal imbalances may play pivotal roles in promoting clinical aggressiveness. Nonrandom losses of chromosome 9p and/or 9q have been observed in advanced GISTs. Except for p16INK4A, little is known about other candidate tumor suppressor genes in chromosome 9.

Using ultrahigh-resolution array comparative genomic hybridization, the authors profiled DNA copy number alteration in chromosome 9 for 22 GISTs. Several candidate tumor suppressor genes, preferentially deleted in high-risk cases, were delineated, including MTAP at 9p21.3 telomeric to p16INK4A. Detected in 17% of cases from independent cohorts by quantitative PCR, MTAP homozygous deletion was highly associated with adverse prognosticators and worse disease-free survival but not with receptor tyrosine kinase genotypes. Immunohistochemistry substantiated MTAP homozygous deletion as the predominant mechanism of protein depletion, whereas 23% of MTAP protein–deficient GISTs were not homozygously deleted. These cases, in part attributable to promoter hypermethylation, behaved favorably like MTAP-expressing counterparts. Given MTAP-depleted cells becoming wholly dependent on de novo AMP synthesis, MTAP-directed agents may be a therapeutic alternative for high-risk, imatinib-resistant GISTs devoid of MTAP expression.

As a major mechanism to deplete protein expression, MTAP homozygous deletion was associated with important prognosticators and independently predictive of worse outcomes.

**Translational Relevance**

Identification of cancer-associated genes by genomic profiling enables the elucidation of tumor development and progression. KIT or PDGFRA receptor tyrosine kinase gene mutations are primary events in the tumorigenesis of gastrointestinal stromal tumors (GISTs). However, chromosomal imbalances may play pivotal roles in promoting clinical aggressiveness. Nonrandom losses of chromosome 9p and/or 9q have been observed in advanced GISTs. Except for p16INK4A, little is known about other candidate tumor suppressor genes in chromosome 9. Using ultrahigh-resolution array comparative genomic hybridization, the authors profiled DNA copy number alteration in chromosome 9 for 22 GISTs. Several candidate tumor suppressor genes, preferentially deleted in high-risk cases, were delineated, including MTAP at 9p21.3 telomeric to p16INK4A. Detected in 17% of cases from independent cohorts by quantitative PCR, MTAP homozygous deletion was highly associated with adverse prognosticators and worse disease-free survival but not with receptor tyrosine kinase genotypes. Immunohistochemistry substantiated MTAP homozygous deletion as the predominant mechanism of protein depletion, whereas 23% of MTAP protein–deficient GISTs were not homozygously deleted. These cases, in part attributable to promoter hypermethylation, behaved favorably like MTAP-expressing counterparts. Given MTAP-depleted cells becoming wholly dependent on de novo AMP synthesis, MTAP-directed agents may be a therapeutic alternative for high-risk, imatinib-resistant GISTs devoid of MTAP expression.

observed in advanced GISTs (15–20). To pinpoint potential TSGs associated with tumor progression in chromosome 9, we used oligonucleotide-based array comparative genomic hybridization (aCGH) with ultrahigh-resolution for profiling copy number alterations in 22 GISTs. There is mounting evidence that DNA losses of chromosome 9 in cancers, either spanning the entire chromosome or restricted areas of p arm, oftentimes encompass p9p21 that harbors several candidate or established TSGs, such as CDKN2A (also known as p16INK4A/p14ARF), CDKN2B (also known as p15INK4B), and MTAP, etc. (20–26).

MTAP (methylthioadenosine phosphorylase) encodes a key enzyme in the catabolism of methylthioadenosine, which is a by-product of polyamine biosynthesis in the methionine salvage pathway (22, 23, 25, 27, 28). Recently, MTAP has been proposed as a functional TSG, prompting us to select this gene for further validation of aCGH findings (26–29). Using large independent cohorts of GISTs, we assessed MTAP protein expression by tissue microarray (TMA)–based immunohistochemistry and precisely quantified MTAP gene dosage by coupling laser capture microdissection (LCM) with real-time quantitative PCR. The MTAP gene status, dichotomized as homozygously deleted or not, was successfully determined in 146 primary localized GISTs. In addition, promoter hypermethylation of MTAP gene was identified in three of seven cases deficient in protein expression but devoid of homozygous deletion. As a major mechanism to deplete protein expression, MTAP homozygous deletion was associated with important prognosticators and independently predictive of worse outcomes.

**Materials and Methods**

**Patients and tumor materials.** The institutional review board had approved this study (97-2211A3). We first used aCGH to profile somatic copy number alterations of 22 fresh GIST specimens (screening set), including 3 low-risk, 9 intermediate-risk, and 10 high-risk tumors. To independently validate aCGH results in formalin-fixed tissues, MTAP gene dosage of LCM-isolated tumor cells and MTAP immunoexpression were successfully determined for 146 and 306 primary GISTs by using real-time quantitative PCR and TMA technologies, respectively. The mutation variants of RTK genes were successfully genotyped for 181 cases. All patients enrolled for quantitative PCR assay, immunostain, and RTK genotyping were independent of the screening set and did not receive imatinib treatment before disease relapses. Details on clinicopathologic characteristics of cohorts for aCGH analysis and for validation of MTAP gene status and immunoexpression were summarized in Supplementary Tables S1 and S2, respectively.

**DNA preparation, hybridization, and data analysis of aCGH.** For each fresh sample, 1 μg of genomic DNA was extracted for hybridization against oligonucleotide microarrays. Each chip has 385K probes with a median spacing of 6 kb and variable lengths to achieve a melting temperature of 76°C (NimbleGen Systems). The reference DNAs were obtained from normal lymphocytes of gender-matched donors. The procedures of DNA labeling, hybridization, normalization of oligonucleotide arrays, window averaging of contained probes, and data acquisition were done by the facility of manufacture, as reported (30). The Cyt3 and Cyt5 signal intensities were normalized to one another using Qspline normalization. The circular binary segmentation algorithm proposed by Olshen et al. was used for segmentation of the averaged log2 ratio data (31). Each segment was then assigned a log2 ratio that was the median of the contained probes, and the data were centered by the tallest mode in the distribution of the segmented values.

To finely delineate the breakpoints of whole array probes, we defined gains and losses as log2 ratios of ≥0.20 or ≤−0.20, respectively. To unravel candidate TSGs showing copy number alteration–driven deregulation associated with disease progression, we searched for hemizygous (log2 ratio between −0.20 and −0.50) and/or homozygous (log2 ratio<−0.50) deletions at identical DNA segments among cases, which were recurrently present in at least 40% of high-risk samples but not in >25% of non-high-risk samples. Java TreeView software10 was used to generate a colorimetric graph for genomic profiling of chromosome 9 (Fig. 1A).

**Immunohistochemistry.** The TMA sections were prepared as previously described for antigen retrieval (3), followed by overnight incubation with the primary antibody of MTAP (MGC:31876, 1:100, Proteintech) and detection with ChemMate EnVision kit (K5001, DAKO). The percentage of tumor cells with cytoplasmic immunoreactivity was scored for multiple cores from the same patient and averaged to obtain a mean labeling index (LI). MTAP LI of <10% was used to define aberrant loss of protein expression in keeping with various cutoff values adopted previously (23, 27, 32). Most TMA blocks had also been previously used for Ki-67 staining in our earlier report (3), wherein we used a mean LI of >5% to define Ki-67 overexpression.

**Determination of MTAP gene dosage.** Approximately 3,000 tumor cells in each sample were microdissected by Veritas LCM machine (Arcturus Engineering) to extract genomic DNAs as previously described (33). Given that aCGH showed very few copy number alterations spanning PFKLI gene at 21q22.3, this gene was chosen as the reference to measure MTAP gene dosage. Because the comparative computed

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10 http://jtreeview.sourceforge.net is the website address of Java TreeView software.
Fig. 1. aCGH analysis of 22 GISTs. A, profiling of DNA copy number alterations in chromosome 9 for GISTs of various NIH risk categories. Intensities of red and green coloration generated by Java TreeView indicate an increased or decreased signal ratio for each averaged window of 10 probes, respectively. Each row corresponds to an individual average window, and each column represents the array CGH profile of chromosome 9 in a tumor sample. Left, cytoband pattern of chromosome 9; middle, recurrent regions of alterations (vertical black lines) with deletions of candidate TSGs (in brackets); right, close-up views of loci harboring genes of most interest. B, representative horizontal karyograms of chromosomal region 9p21.3. GISTs with DNA deletions (left) are compared with those without DNA alterations (right). Cases displaying homozygous deletions at loci harboring MTAP and CDKN2A/CDKN2B are encircled by red and green ellipses, respectively. H, high risk; I, intermediate risk; L, low risk.
tomography method may result in skewed values when analyzing low-copy templates (e.g., ICM-isolated cells from paraffin-embedded tissue; refs. 34, 35), we first constructed standard curves for the reference PFKL and target MTAP genes for real-time PCR assays as detailed in Supplementary Method S1 and Supplementary Fig. S1. The sequences of primer pairs and probes targeting PFKL and exon 8 of MTAP gene were described in Supplementary Method S1. We adopted a cutoff of average MTAP/ PFKL ratio at <0.2 to define homozygous deletion in two independent assays with duplicate samples for each case, based upon the assumption that the normal tissue contamination and intratumoral heterogeneity should collectively account for ≤20% of experimental deviation.

Mutation analysis of the RTK genes. We had previously described the methods of DNA extraction, PCR amplification, direct sequencing of KIT exon 11, and screening by denatured high-performance liquid chromatography for exons 9, 13, and 17 of KIT gene and exons 12 and 18 of PDGFRα gene with confirmatory sequencing (11).

Methylation-specific PCR to detect MTAP promoter methylation. Following manufacturer’s instructions, we used Puregene kit (Genta Systems) to extract genomic DNA from formalin-fixed tissue, 1 µg of which was then modified with sodium bisulfite for each sample using Her- 
mam’s method (36). To maximize success in the amplification of bisul- 
fous parameters were evaluated by 

\( \chi^2 \) Test. Wilcoxon rank sum, and 

Student’s t tests as appropriate for MTAP gene status. Follow-up data were available in 306 and 146 cases with informative MTAP immuno- 

histochemical scores (median, 49.9 mo) and MTAP gene dosage ratio (median, 56.3 mo), respectively. The end point was disease-free survival 

(al), which would not be confounded by imatinib therapy for patients with disseminated disease. RTK genotypes were dichotomized into two prognostically different groups, as detailed in our previous report (11). Kaplan-Meier curves were plotted to compare prognostic differences by log-rank tests. The 146 cases with informative MTAP gene status formed the basis for multivariate survival analysis. Significant 

prognosticators at univariate level were analyzed by Cox regression model except for the strong dependent covariate of MTAP gene status (i.e., MTAP immunoeexpression) and component factors of NIH risk scheme (i.e., tumor size, mitotic activity; ref. 2). For all analyses, two-sided tests of significance were used with \( P < 0.05 \) considered significant.

Results

Genomic profiling of chromosome 9 by ultrahigh-resolution aCGH. As shown in Fig. 1A, complete and/or partial loss in both 9p and 9q was recurrently detected in 7 of 10 high-risk GISTs, including two cases showing a number of interstitial de- 

lentions. However, another high-risk case revealed complete loss of 9p alone. Conversely, among 12 non–high-risk GISTs tested, there was only one intermediate-risk case showing almost complete loss of chromosome 9, whereas no large-scale alterations were detected in the remaining cases.

The recurrent regions of DNA losses on 9p were mapped to five major deletion cores and collectively spanned 11 hemizy- 
gously and/or homozygously deleted candidate TSGs (Fig. 1A). These included DOCK8, ANKRDI5 (also known as KANK1), and SMARCA2 at 9p24.3; PTPRD at 9p24.1; SH3GL2 at 9p22.2; MTAP, CDKN2A, CDKN2B, TUSCL, TOPORS at 9p21; and RECK at 9p13.3. As for the nonrandom losses on 9q, there were six potential TSGs with hemizygous and/or ho- 
mozygous deletions within three major deletion cores where TLE1 (9q21.32) and DAPK1 (9q21.33) at 9q21.3; DEC1 (9q33.1), DBC1 (9q33.1), and DAR2IP (9q33.2) at 9q33; and MRPLA1 at 9q34.3 were identified. Noticeably, one intermediate-risk (11%) and seven high-risk (70%) GISTs clearly showed deletions at the chromosome band 9p21.3. The latter encompassed the most frequent loci of homozygous losses (Fig. 1B), affecting MTAP in three cases (two high-risk cases, one intermediate-risk case) and CDKN2A/CDKN2B in four (three high-risk cases, one intermediate-risk case). Moreover, two cases were homozygously codelated at both loci.

MTAP immunoeexpression and its gene status. The cohort for MATP immunohistochemical analysis (Fig. 2; Supplementary Table S2) consisted of 306 GISTs from 153 males and 153 females, including 115, 96, and 95 cases classified as very low or low risk (Fig. 2A), intermediate risk (Fig. 2B), and high risk (Fig. 2C) based on the NIH consensus scheme, respective- 

ly. MTAP immunoeexpression displayed a wide variation in LI from 0 to 100% and was aberrantly lost (LI < 10%) in 58 cases (19%; Fig. 2D-F). Among cases with immunohistochem- 

ical results, normalized MTAP gene dosage was successfully determined in 146 GISTs, consisting of 44 very low-risk or low-risk, 49 intermediate-risk, and 53 high-risk cases. Twenty- 

five GISTs (17%) showed homozygous deletion with MTAP/ 

PFKL ratio of <0.2 (Table 1). No essential difference in the clin- 

icopathologic characteristics and RTK genotypes was found be- 

tween the cohorts examined for immunoeexpression and gene status (Supplementary Table S2).

Genotyping of RTK genes. In this study, analysis of RTK genes was successfully done in 181 GISTs with mutations detected in 

158 cases (87%). The trends of survival curves for individual RTK genotypes were generally in keeping with our, as well as others’, earlier reports (4, 6, 8, 9, 11). According to our previous grouping rationale (11), these 181 cases were further dichotomized as fa- 

vorable (n = 88) versus unfavorable (n = 93) genotypes as de- 

tailed in Supplementary Fig. S2. The prognostically favorable genotypes comprised (a) PDGFRα mutation involving exon 12 or 18 in nine cases, (b) 3′ tandem insertion of KIT exon 11 with or without point mutation in 11 cases, and (c) single-point mutation of KIT exon 11 in 68 cases. The group of unfavorable genotypes included (a) Ala502-Tyr503 insertion of KIT exon 9 in three cases, (b) wild type for both KIT and PDGFRα genes in 23 cases, and (c) 5′ deletion of KIT exon 11 with or without point mutation in 67 cases. Of the KIT exon 11–deleted subgroup, there were 42 and 26 cases with deletions involving codons 557 to 558 and other codons, respectively. No mutation of KIT exon 13 or 17 was detected in this series.

Correlations of MTAP homozygous deletion with immunoeexpression and other clinicopathologic and molecular parameters. As shown in Table 1, a strong correlation was subst- 

sianted between MTAP homozygous deletion and loss of pro- 

tein expression (\( P < 0.001 \)) in GISTs. In cases showing homozygous deletion, the vast majority (92%, 23 of 25) showed MTAP protein deficiency, whereas this aberrant loss was only observed in 6% (7 of 121) of cases without homozygous deletion. MTAP homozygous deletion, more frequently involving GISTs that occurred in the nongastric location (\( P = 0.041 \)), showed presence of epithelioid histology (\( P = 0.003 \)) and displayed higher mitotic rate (\( P = 0.008 \)). More interestingly, it was also highly related to larger tumor size (\( P < 0.001 \)), increasing NIH risk levels.
The findings of strong associations between homozygous deletion of the MTAP gene and several adverse clinicopathologic prognosticators suggested its crucial role in tumor progression of GISTs. However, the correlation of this genetic aberration with RTK genotypes could not be confirmed.

**Survival analyses.** Correlations of clinical outcomes with various clinicopathologic, immunohistochemical, and molecular
Gusously deleted in genomic DNA. We found that promoter hypermethylation as an alternative mechanism to homozygous deletion also identified in three non-high-risk (two low-risk case, one intermediate-risk case) cases but undetected in another four high-risk counterparts (Fig. 4). More intriguingly, GISTs with homozygously deleted MTAP behaved more aggressively with significantly shorter DFS than those MTAP protein–deficient cases without homozygous deletion (P = 0.0493; Fig. 3D). The latter was actually not different from MTAP-expressing GISTs in the DFS rate (P = 0.9695).

**Discussion**

Whereas oncogenic mutations of RTK genes are primary events in GIST tumorigenesis, chromosomal imbalances are considered to play pivotal roles in promoting clinical aggressiveness (18, 37). Previous studies using conventional CGH and/or array-based genomic profiling indicated that loss of chromosome 9, especially 9p, is a specific indicator of unfavorable outcomes in GISTs (16–19). More intriguingly, GISTs with homozygously deleted MTAP behaved more aggressively with significantly shorter DFS than those MTAP protein–deficient cases without homozygous deletion (P = 0.0493; Fig. 3D). The latter was actually not different from MTAP-expressing GISTs in the DFS rate (P = 0.9695).

**Table 1. Correlations of MTAP homozygous deletion with immunoexpression and other clinicopathologic and molecular parameters**

<table>
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<th>Parameter</th>
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<td>Epithelioid and mixed</td>
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<td>24</td>
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<tr>
<td>Tumor Size (cm)</td>
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<td>5.972 ± 3.5988*</td>
<td>11.128 ± 6.1347*</td>
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<td>Mitotic count (50 HPFs)</td>
<td>146</td>
<td>6.89 ± 15.255*</td>
<td>25.28 ± 31.172*</td>
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<tr>
<td>Ki-67 LI†</td>
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<tr>
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Abbreviation: Homo. del., homozygous deletion.

*Expressed as mean ± SD.
†Statistically significant.
‡Analyzed by Wilcoxon rank sum test.
TSGs. Many of these were recently reported to implicate other cancers, such as PTPRD located at 9p24.1 in neuroblastomas and cutaneous squamous cell carcinomas (30, 39); MTAP, TUSC1, and TOPORS at 9p21 in lung or colorectal cancers (26, 40, 41); DAPK1 at 9q21.3 in renal cell carcinomas (42); and D1C1, DBC1, and DAB2IP at 9q33 in esophageal (43), lung (44), and breast carcinomas (45), respectively.

Prior studies on tumor specimens and/or cell models have shown that MTAP depletion, rendering cells dependent on de novo synthesis of purine derivatives, is not uncommon in human malignancies (21, 22, 25–29), such as melanoma (27, 29), non–small cell lung cancers (26), and T-cell acute lymphoblastic leukemia (21). Nevertheless, there exist conflicting thoughts on whether loss of MTAP activity is indeed pathogenically relevant, given frequent codeletion of MTAP with proximate CDKN2A and/or CDKN2B. However, evidence has emerged that MTAP may represent as a genuine TSG with unique tumor-suppressive effects and functional basis. First, MTAP can be lost independently of CDKN2A in non–small cell lung cancers, with an even higher rate of homozygous deletion in the former (38% versus 18%; ref. 26). Second, reexpression of MTAP in MTAP-deleted breast cancer cells (MCF-7) enables dramatic inhibition of anchorage-independent growth in vitro and tumorigenicity in vivo because of decreased ornithine decarboxylase and polyamines, such as putrescine (28). Third, through accumulation of 5′-deoxy-5′-(methylthio)adenosine (the MTAP substrate), MTAP depletion may enhance the invasive and vasculogenic capability of melanoma cells by inducing expression of matrix metalloproteinases and angiogenic growth factors, respectively (29).

Integrating various methodologies, we have first unraveled the gene status and protein expression of MTAP at 9p21.3 in GISTs and provided compelling evidence of MTAP homozygous deletion as a critical event in disease progression. Unfavorable RTK genotypes, especially KIT exon 11 deletions, were recently found to predict aggressiveness of GISTs (4, 6, 8, 9, 11). Nevertheless, these genotypic variations did not correlate with MTAP homozygous deletion, further supporting that the

<table>
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<td>39</td>
<td>16</td>
</tr>
<tr>
<td>&gt;10</td>
<td>44</td>
<td>33</td>
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<tr>
<td>NIH consensus</td>
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<tr>
<td>Very low/low</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Mutation type</td>
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<tr>
<td>Favorable type</td>
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<td>17</td>
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<tr>
<td>Unfavorable type</td>
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<td>48</td>
</tr>
<tr>
<td>Ki-67 LI</td>
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<tr>
<td>≤5</td>
<td>205</td>
<td>50</td>
</tr>
<tr>
<td>&gt;5</td>
<td>80</td>
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<td>MTAP gene</td>
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<tr>
<td>No homozygous deletion</td>
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<td>33</td>
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<tr>
<td>Homozygous deletion</td>
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<td>17</td>
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<td>MTAP expression</td>
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<td>Expressed</td>
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<td>67</td>
</tr>
<tr>
<td>Deficient</td>
<td>58</td>
<td>28</td>
</tr>
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</table>

Abbreviation: 95% CI, 95% confidence interval.
*Statistically significant. HR, hazard ratio.
†Tumor size, mitotic activity, and MTAP immunoexpression were not introduced in multivariate analysis, because the former two were component factors of NIH risk scheme and MTAP immunoexpression was a strong dependent covariate of MTAP gene status.
latter was secondarily acquired during cytogenetic evolution rather than being driven by activated RTKs.

In GISTs, MTAP homozygous deletion showed strong concordance with loss of immunoexpression, confirming this aberration (77%) as the main inactivating mechanism leading to MTAP deficiency. As a candidate TSG, MTAP was epigenetically silenced via promoter hypermethylation in three cases, whereas the underlying cause of MTAP deficiency was not identified in

![Graph A](image1.png)

**Fig. 3.** Kaplan-Meier plots to predict DFS according to (A) genotypes of *KIT* and *PDGFRA* genes, (B) MTAP protein immunoexpression, and (C) MTAP gene dosage. **D**, in subgroup analyses, GISTs with homozygously deleted MTAP gene have a significantly shorter DFS than those MTAP protein–deficient cases without homozygous deletion. The latter shows an essentially similar DFS rate compared with MTAP-expressing GISTs.

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

![Methylation-specific PCR](image5.png)

**Fig. 4.** Methylation-specific PCR for analysis of MTAP promoter methylation in MTAP protein–deficient GISTs without homozygous gene deletion. PCR products amplified from MTAP promoter with methylation-specific primers can be distinctly visualized in three GISTs, G92 (low risk), G176 (low risk), and G80 (intermediate risk), whereas the MTAP gene promoter of a representative high-risk GIST, G22, is unmethylated. TE, TE buffer alone; M.SssI, a healthy donor's DNA treated with M.SssI methyltransferase; HD-1, a healthy donor's DNA without treatment of M.SssI methyltransferase; M, methylation-specific primers; U, unmethylation-specific primers.
another four MTAP-immunonegative GISTs. However, alternative genetic defects contributing to MTAP downregulation could not be completely excluded, such as point mutations or small deletions involving more 5’ exons and/or promoter of MTAP. Recently, promoter hypermethylation was found preponderant in MTAP-deficient hepatocarcinomas, implying histotype-dependent selectivity in its inactivating mechanisms (46, 47).

MTAP homozygous deletion in GISTs not only correlated with larger size and higher mitotic rate, Ki-67 index, and NIH risk level but also independently predicted worse DFS with 2-fold increased risk. These findings corresponded with the similar prognostic effect observed in mantle cell lymphomas (48), indicating that this genomic loss may provide a survival or proliferation advantage. Although only a small proportion (17%) of cases were homozygously deleted for MTAP, approximately a third of high-risk GISTS carried this aberration and would be eligible for alternative MTAP-directed therapy (e.g., 1-alanosine) to completely switch off AMP supply to tumor cells (21, 25, 48, 49) once resistance to imatinib occurs. However, in vitro studies could not validate the proliferation-promoting effect of MTAP deficiency in melanoma- and hepatoma-derived cell lines with promoter hypermethylation (27, 47). More intriguingly, MTAP promoter hypermethylation, albeit a small proportion, was all seen in non-high-risk cases, consistent with occurrence of this epigenetic alteration even as early as in precancerous liver cirrhotic tissues (46). Furthermore, non-homozygously deleted, MTAP-deficient GISTs behaved as indolently as MTAP-expressing GISTs, unlike those aggressive, homozygously deleted tumors. Accordingly, it is possible that the timing of MTAP gene abrogation may be different among various inactivating mechanisms in the evolution of GISTs, thereby culminating in different prognostic effect.

In summary, MTAP homozygous deletion, present in 17% of GISTs, represents the predominant mechanism to deplete protein expression. Notably, it not only correlates with important adverse parameters but also provides independent prognostic effect, further highlighting its role in disease progression. Our findings may warrant prospective validation in future studies to justify the potentiality of MTAP-directed agents as an alternative therapy in high-risk, imatinib-resistant GISTs devoid of MTAP expression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Hsuan-Ying Huang, Shau-Hsuan Li, Shih-Chen Yu, et al.


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