Mice Heterozygous for Germ-line Mutations in Methylthioadenosine Phosphorylase (MTAP) Die Prematurely of T-Cell Lymphoma

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Abstract

Large homozygous deletions of 9p21 that inactivate CDKN2A, ARF, and MTAP are common in a wide variety of human cancers. The role for CDKN2A and ARF in tumorigenesis is well established, but whether MTAP loss directly affects tumorigenesis is unclear. MTAP encodes the enzyme methylthioadenosine phosphorylase, a key enzyme in the methionine salvage pathway. To determine if loss of MTAP plays a functional role in tumorigenesis, we have created an MTAP-knockout mouse. Mice homozygous for a MTAP null allele (Mtapਬܠbeth) have an embryonic lethal phenotype dying around day 8 postconception. Mtap/Mtapβܠbeth heterozygotes are born at Mendelian frequencies and appear indistinguishable from wild-type mice during the first year of life, but they tend to die prematurely with a median survival of 585 days. Autopsies on these animals reveal that they have greatly enlarged spleens, altered thymic histology, and lymphoctic infiltration of their livers, consistent with lymphoma. Immunohistochemical staining and fluorescence-activated cell sorting analysis indicate that these lymphomas are primarily T-cell in origin. Lymphoma-infiltrated tissues tend to have reduced levels of Mtap mRNA and MTAP protein in addition to unaltered levels of methyldeoxycytidine. These studies show that Mtap is a tumor suppressor gene independent of CDKN2A and ARF. [Cancer Res 2009;69(14):5961–9]

Introduction

A quarter century ago, Toohey first recognized that certain murine malignant hematopoetic cell lines lacked methylthioadenosine phosphorylase (MTAP) activity (1). MTAP is a metabolic enzyme in the methionine salvage pathway that converts the polyamine byproduct 5'-dideoxy-5'-methylthioadenosine into adenine and methylthioribose-1-phosphate and is expressed in all tissues throughout the body (2, 3). Loss of MTAP has been shown to result in increased accumulation of 5'-dideoxy-5'-methylthioadenosine, a known inhibitor of $\delta$-adenosylmethionine-dependent methyltransferases (2, 4). In addition, loss of MTAP is known to affect the production of polyamines due to up-regulation of ornithine decarboxylase (5).

Today, we know that loss of MTAP is frequent in a large number of different human tumors including leukemias, lymphomas, mesothelioma, lung carcinoma, pancreatic carcinoma, squamous cell carcinoma, biliary tract cancer, glioblastoma, osteosarcoma, and neuroendocrine tumors (6–18). Loss rates range from 14% to 100% depending on the tumor type and the method used to assess MTAP loss.

MTAP is frequently inactivated in human tumors by large homozygous deletion of the 9p21 region where both MTAP and the CDKN2A/ARF tumor suppressor gene complex are located (19). Because these deletions generally inactivate CDKN2A/ARF as well as MTAP, it has been hypothesized that loss of MTAP in tumors was simply due to it being a coincident bystander. However, there is a growing body of data that suggests this may not be the case. Reexpression of MTAP in MTAP-deleted MCF-7 breast cells results in loss of anchorage-independent growth in vitro and loss of tumor formation in vivo when these cells were injected s.c. in severe combined immunodeficient mice (20). In addition, expression of MTAP in a MTAP-deleted melanoma cell line resulted in reduced invasion as measured in a Boyden chamber assay (21). Finally, in non–small-cell lung cancer and in astrocytomas, loss of MTAP has been observed in cells that retain CDKN2A/ARF (8, 9). Taken together, these observations suggest that MTAP may function as a tumor suppressor gene in its own right.

To test the idea that MTAP is a tumor suppressor gene, we have engineered a mouse with a germ-line mutation in the mouse MTAP gene (Mtap). We found that homozygosity for MTAP results in an early embryonic lethal phenotype, whereas heterozygosity often results in premature death by T-cell lymphoma. Our findings support the view that MTAP is a bona fide tumor suppressor gene.

Materials and Methods

Creation of MTAP-knockout mouse. RRK081 cells (SV129/Ola background) heterozygous for the Mtapβܠbeth allele were obtained from Bay Genomics6 and injected into C57BL/6 blastocysts by microinjection. Eighty 3.5-d-old blastocyte embryos were then implanted into pseudopregnant C57BL/6 mice. Four chimeric offspring were obtained, and two exhibited germ-line transmission. Heterozygous offspring from these mice were then used for intercrosses to generate homozygous offspring and were repeatedly backcrossed to C57BL/6. Mice were fed Harlan Tekland 2018SX diet ad libitum. For survival analysis, mice were monitored daily, and Kaplan-Meier analysis was done using GraphPad Prism 4.0. All studies were approved by the Fox Chase Cancer Center animal use committee.

Genotyping. Mtapβ埒beth was genotyped using two different methods. Initially, genotyping was done at the RNA level. RNA was extracted from tail snips using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Reverse transcription-PCR (RT-PCR) was done using the SuperScript One-step Reverse Transcription-PCR Kit (Invitrogen) and oligos

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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6 http://baygenomics.ucsf.edu
5'-CGGTGAAATGGAAATATGTTG-3' (sense), 5'-GAGGATCGGCTTCAAGGATCGT (antisense), and 5'-CTGTCAATGATGTTGGAATGAC- CATG (antisense) for the amplification of Mtap and MtaplacZ bands of 322 and 421 bp, respectively. After an initial reverse transcription phase at 52°C for 30 min, PCR amplification of CDNA was done with an initial denaturation step at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s with a final extension at 72°C for 5 min.

Later, a DNA-based assay was developed using primers 5'GACGACAAATTTGTTGGCT-TAAC-3' (forward), 5'-GCAAGAAGACGGCCAGAGACT- C (reverse 1), and 5'-ACTTGGACATCTTGTGGTCTTC-3' (reverse 2) to obtain either a Mtap band of 381 bp (with reverse 2) or a MtaplacZ band of 289 bp (with reverse 1). PCR reactions were carried out in a total volume of 25 μL reaction mixture containing 100 ng genomic DNA as a template, 1× PCR buffer [10 mmol/L Tris-HCl (pH 8.0), 50 mmol/L KCl, 1.5 mmol/L MgCl2], 250 μmol/L of each of four deoxynucleoside triphosphates, 10 ng each of sense and antisense primers, and 2.5 units of Taq DNA polymerase (Invitrogen). The thermal cycling conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 30 s.

**Timed matings.** Female and male animals were paired in the evening and checked early in the morning for the presence of a vaginal plug. If a plug was observed, animals would be sacrificed after 7.5, 8.5, 9.5, and 12.5 d.

**Immunohistochemistry.** Autopsied materials were fixed in buffered formalin and embedded in paraffin. The paraffin blocks were cut into 5-μm-thick sections that were placed on positively charged slides. The sections were dewaxed in xylene and hydrated through graded ethanol. Heat-induced antigen retrieval was then done in 10 mmol/L sodium citrate (pH 6.0) in a microwave for 10 min. The endogenous peroxidase activity was blocked by immersing the slides in 3% H2O2 in PBS for 30 min. After 30 min of incubation with goat blocking serum, slides were incubated with a 1:250 dilution of mouse monoclonal biotinylated antibodies (BD Biosciences) followed by streptavidin peroxidase for 30 min at room temperature. 3,3'-Diaminobenzidine (Sigma-Aldrich) substrate chromogen was applied for 4 min until the brown color developed. In the case of CD45, we used 1:200 dilution of mouse monoclonal biotinylated antibodies (BD Biosciences) and did not use secondary antibody. The slides were counterstained with hematoxylin and mounted with Permount (Fischer Scientific).

**Fluorescence-activated cell sorting analysis.** Fluorescence-activated cell sorting (FACS) analysis of spleen from euthanized animals was done as previously described (23). Analysis was done using a BD Biosciences LSR II/Diva flow cytometer, equipped with three laser excitations (405, 488, and 630 nm). Staining combinations were done on single-cell suspensions of spleen and included fluorescein anti-CD8 (53-6), phycoerythrin anti-CD3 (50A2-X2), allophycocyanin anti-CD4 (GK1.5), and Cy5-phyceroerythrin T-cell receptor β (TCRb; H57-597). All reagents were made in the laboratory of Richard R. Hardy, except for DX5 and TCRb, which were purchased from eBioscience.

**Southern blot analysis.** Genomic DNA was extracted using DNA isolation kit (Puregene) from spleens of diseased and normal animals. Approximately 20 μg of genomic DNA were digested using restriction enzyme Hin dIII (for T-cell lineage clonality assays of the TCRβ locus) and probed according to standard Southern blot protocol with TCRβ probe (5'Jβ2 probe for the Jβ2 cluster; ref. 24). Genomic DNA from tissue or cell lines with unarranged germ-line configuration of each antigen receptor locus was used as a negative control.

**Real-time PCR.** Mtap, p16, and β-actin were measured using TaqMan technology on an ABI Prism 7900 Sequence Detection System. Total RNA was extracted using the PicoPure RNA kit (Molecular Devices) according to the manufacturer's instructions. First-strand cDNA was generated from total RNA from each sample using the High Capacity cDNA kit (Applied Biosystems) according to the manufacturer's instructions. Reactions were prepared in triplicate for each gene using TaqMan Gene Expression Master Mix and the following TaqMan Gene Expression Assays (Applied Biosystems): Mtap (Mm01257901_m1), Cdkn2a/Arf (Mm00494491_m1), and β-actin (Mm00607939_s1). Plates were loaded and reactions were cycled using TaqMan universal cycling conditions according to the manufacturer's instructions. During thermal cycling, the threshold cycle (Ct) was determined for each sample by taking the average of the three replicates. The average Ct value for the control gene β-actin was subtracted from the average Ct value for each target gene (Mtap and cdkn2a/Arf) to normalize the amount of sample RNA added to the reaction. The comparative Ct method was used to determine the level of the target gene mRNA in heterozygous animals samples relative to the level found in the normal sample (Applied Biosystems User Bulletin #2, October 2001); relative quantification = 2^(-Ct), where C1 = average Ct (diseased) – average Ct (normal).

**MTAP enzyme assay.** MTAP enzyme activity in the liver extract was measured as described previously (20). A unit of 5'-deoxy-5'-methylthioadenosine phosphorylase activity is defined as the enzyme amount that catalyzes the formation of 1 μmol of adenosine per minute under the conditions of the assay described.

**Comparative genome hybridization analysis.** According to the manufacturer's protocol for Agilent Oligonucleotide array-based comparative genome hybridization (CGH) for Genomic DNA Analysis version 4.0 (Agilent Technologies), 3 μg of high quality genomic DNA were digested with restriction endonucleases Alu I and Bst X I, and digested genomic DNA was labeled using Agilent Genomic DNA Labeling Kit PLUS. Test and reference DNA samples were labeled with either Cy5-UTP or Cy3-UTP according to the manufacturer's protocol. Cy5- and Cy3-labeled DNA products were then purified using Microcon YM-30 filtration devices. The DNA yield and level of dye incorporation were measured using the ND-1000 Spectrophotometer. Appropriate Cy5- and Cy3-labeled DNA sample pairs were combined and then mixed with mouse Cot-1 DNA, Agilent 10X Blocking Agent, and Agilent 2X Hybridization Buffer. The labeled target solution was hybridized to Agilent 244K Mouse Genome CGH microarray (G4415A) using SureHyb chambers. After hybridization, the microarrays were washed and dried according to the procedures described in Agilent protocol. Microarray slides were scanned immediately using an Agilent microarray scanner. Data for individual features on the microarray were extracted from the scan image using Agilent Feature Extraction (FE) Software. Output files from FE were subsequently imported into Agilent CGH data analysis software, CGH Analytics for DNA copy number analysis.

**Methyldeoxycytidine quantitation.** Percent of methyldeoxycytidine present in genomic DNA was determined by liquid chromatography-tandem mass spectrometry as described (25).

**Results**

**Homozgyosity for MtaplacZ causes early embryonic lethality.** RRKO81 embryonic stem cells, containing a gene-trap vector inserted between exon 3 and exon 4 of the mouse Mtap locus (MtaplacZ; Supplementary Fig. S1), were injected into mouse blastocysts derived from C57BL/6 males and the embryos were implanted in pseudopregnant females. Four chimeric offspring were obtained and these animals were then bred to C57BL/6 animals to establish germ-line transmission. Two of the chimeras exhibited germ-line transmission.

Heterozygous MtaplacZ offspring were then intercrossed to determine the phenotype of a homozygous animal. We failed to obtain a single homozygous offspring of the 165 that were genotyped. Analysis of the heterozygous animals for MTAP enzyme activity in the liver showed that these animals had, on average, 31% activity compared with the wild-type animals (n = 6 per group; P < 0.0003), indicating that the insertion allele is a complete loss of function mutation. Taken together, these findings indicate that lack of MTAP activity results in an embryonic lethal phenotype.

We performed timed mating experiments to determine exactly when during development MtaplacZ homozygotes were being lost.
We failed to observe any homozygous embryos present in the uteri of postconception day 12.5 and day 9.5 mothers and also found a large number of empty uterine sacs. However, we were able to identify homozygous embryos present in the uteri of day 8.5 and day 7.5 animals. Homozygous embryos present at day 8.5 were much smaller and misformed compared with control heterozygous embryos (Fig. 1A). Both in size and appearance they seemed to resemble day 8 embryos (Theiler stage 12 as opposed to Theiler stage 13; ref. 26) and were probably in the process of reabsorption. Examination of day 7.5 embryos for MTAP expression by immunohistochemistry reveals that MTAP is expressed ubiquitously throughout the embryo at this time (Fig. 1B). These results indicate that MtaplacZ/MtaplacZ homozygotes are lost early in embryonic development, around postconception day 8.

MtaplacZ heterozygotes die prematurely of severe lymphoproliferative disease. MtaplacZ/+ heterozygotes are born at Mendelian frequencies and have no obvious abnormalities. We observed no difference in weight gain between heterozygotes and control animals during the first 6 months of life (Supplementary Fig. S2A). Because MTAP is involved in methionine and polyamine metabolism, we examined the serum amino acid profiles of overnight fasted MtaplacZ/+ and Mtap+/+ animals. We observed no significant differences in the 27 compounds that were measured (Supplementary Fig. S2B).

To determine if there might be any long-term detrimental effects of heterozygosity for MtaplacZ, we monitored a cohort of MtaplacZ/+ heterozygotes and sibling Mtap+/+ controls for up to 20 months and found that there was a significant difference in the survival of heterozygous animals compared with their wild-type siblings (Fig. 2A). Heterozygotes start dying as early as 200 days and have a median survival of 585 days. We performed necropsies on 21 of these animals and found that at least 60% of the animals showed marked splenomegaly and often enlargement of the liver and thymus as well (Fig. 2B). Examination of spleen, liver, and thymus sections by H&E staining indicated that the animals suffered from severe lymphoproliferative disease resembling lymphoma (Fig. 2C).

Characterization of lymphoma cells present in MtaplacZ heterozygotes. To more fully characterize the lymphoma present in MtaplacZ/+ animals, we performed immunohistochemical analysis of tissues from heterozygous and sibling wild-type animals. Spleen, thymus, and liver were stained with the T-cell marker CD3 or the B-cell marker CD45. In the spleens of all of the 14 heterozygous animals diagnosed with lymphoma at autopsy, we observed increased CD3+ cells compared with control spleen and reduced numbers of CD45+ cells (Fig. 3A). Infiltration of CD3+ cells was also observed in the livers of some of the heterozygous animals (Supplementary Fig. S3). In animals with enlarged thymuses, we observed an expansion of the CD3+ population and a decreased number in the livers of CD45+ cells (Fig. 3B). These results indicate that MtaplacZ/+ animals are succumbing to T-cell lymphoma.

To further characterize the T-cell expansion, we examined the T-cell population in the spleen of aged heterozygous and control animals for CD4 and CD8 content using FACS analysis. We sacrificed 11 MtaplacZ/+ heterozygotes (average age, 713 days) and six Mtap+/+ controls (average age, 684 days). In these sacrificed animals, we did not observe gross enlargement of

Figure 1. Mtap is essential during embryogenesis. A, photograph of sibling day 8.5 >MtaplacZ/+ and MtaplacZ/MtaplacZ embryos. Photographs taken at ×40 magnification. B, wild-type (WT) day 7.5 embryos sectioned and stained with H&E (left) or H&E staining of twice-embryos and thymus, taken at ×40 magnification.
the spleen as observed in the naturally deceased animals, and histopathologic examination of the spleen showed that most of these animals had much milder lymphoid hyperplasia. However, despite this fact, we found that 4 of the 11 heterozygous animals had CD4+ levels above the 95% confidence range determined by the mean of the control animals (Fig. 4A). We did not observe any animals with CD8 levels outside the control range. These results indicate that heterozygous animals are predisposed to develop expansions of a CD4+ T-cell population.

To determine if the T-cell expansions were monoclonal, we performed Southern blot analysis on spleen or thymus DNA isolated from 17 aged heterozygous and 4 sibling wild-type animals using a probe specific for the Db2-Jb2 cluster at the TCRβ locus (Fig. 4B). Five of the 17 heterozygous samples came from autopsy material derived from animals that had exhibited marked splenomegaly and had died of natural causes, whereas the remaining samples came from the healthy euthanized animals that had mild lymphoid hyperplasia used for the FACS analysis described above. In three of the five autopsy animals (60%), we found evidence of monoclonality, but we did not observe any evidence of monoclonality in the sacrificed animals. These findings show that the majority of heterozygous animals died of monoclonal T-cell lymphoma, but that the appearance of monoclonality occurs late in the disease process.

**Loss of MTAP expression in lymphoma cells.** We next examined if expression of the wild-type *Mtap* allele in *Mtap*^lacZ/+^ mice was reduced in tissues showing lymphoproliferative disease. First, we performed immunohistochemistry using an anti-MTAP polyclonal antibody on thymus, spleen, and liver from an animal with severe lymphoma and compared the staining to that of a wild-type healthy control animal (Fig. 5A). We observed significantly reduced MTAP staining in all three tissues compared with the control animal, showing that MTAP expression was reduced in all the lymphoid infiltrated tissue. In addition, we examined the spleens of nine other animals that died of lymphoma, and in all cases observed reduced MTAP staining compared with control animals (data not shown).

To determine if down-regulation of MTAP was caused by reduced *Mtap* mRNA, we quantitated *Mtap* RNA by quantitative RT-PCR. Initially, we examined MTAP expression in five 3-mo-old *Mtap*^lacZ/+^ and five *Mtap*^+/+^ control animals that showed no evidence of lymphoid hyperplasia in the spleen. As expected, we found that the heterozygous animals had significantly lower *Mtap* mRNA than did the *Mtap*^+/+^ controls (Fig. 6A). We then examined *Mtap* mRNA levels in the spleens of 13 older (sacrificed) *Mtap*^lacZ/+^ animals with...
lymphoid hyperplasia. In 12 of the 13 cases, we observed Mtap mRNA levels below the 95% confidence interval defined by the younger MtaplacZ/+ animals. To eliminate the possibility that Mtap mRNA levels decreased with age in healthy animals, we also measured Mtap mRNA in five older Mtap+/+ animals without evidence of lymphoid hyperplasia. We did not observe a difference in MTAP mRNA compared with the younger Mtap+/+ animals, indicating that MTAP expression is not lost simply due to aging.

Because chromosomal deletion is a major mechanism for inactivation of Mtap in human tumors, we performed CGH on DNA isolated from the spleen of three MtaplacZ/+ animals exhibiting severe lymphoid hyperplasia. In only one of the three samples (A7) did we see evidence for loss of the Mtap region of mouse chromosome 4 (Fig. 5B). This finding suggests that the reduced expression of Mtap mRNA observed above may be occurring mostly through epigenetic mechanisms.

**Loss of MTAP expression is independent from loss of CDKN2A expression.** Because MTAP and CDKN2A/ARF are often inactivated together in human tumors by large deletions, we also examined Cdkn2a/Arf mRNA in the spleens of animals showing lymphoid hyperplasia by quantitative RT-PCR. We found no difference in Cdkn2a/Arf mRNA levels in young sibling Mtap+/+ and MtaplacZ/+ animals, indicating that the MtaplacZ/+ insertion did not affect basal Cdkn2a/Arf expression. In older Mtap+/+ animals lacking hyperplasia, we observed a 20% decrease in Cdkn2a/Arf expression compared with the younger controls, suggesting some loss of Cdkn2a/Arf expression occurs as a result of aging. However, in the older MtaplacZ/+ animals, we found large variability in Cdkn2a/Arf expression. In seven animals, we observed significant elevations in Cdkn2a/Arf mRNA, whereas in seven others, we observed reduced expression. The lack of correlation between Cdkn2a/Arf and Mtap mRNA levels in these samples suggests that loss of Mtap expression is driving lymphoma formation independent of Cdkn2a/Arf in these mice.

**Loss of MTAP does not affect global deoxycytidine methylation.** One possible mechanism by which loss of MTAP might promote tumorigenesis is by affecting cellular methylation reactions. To determine if loss of MTAP affected cellular methylation, we measured the amount of methyldeoxycytidine present in the DNA isolated from the enlarged spleens of Mtap heterozygotes and

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**Figure 3.** Proliferation of CD3+ T cells in spleen and thymus. A, spleen of an aged control (sacrificed at 525 d) and MtaplacZ/+ animal (deceased at 260 d) stained with either antibody to CD3+ or CD45+ viewed at ×400 magnification. B, the thymus of the same two animals as above.
compared it to spleen DNA isolated from control animals. We observed a slight decrease in the percentage of DNA-incorporated methyldeoxycytidine in control versus Mtap heterozygous animals, but the difference was not statistically significant (n = 5; 4.3 ± 0.08 versus 4.04 ± 0.45; P = 0.33).

Discussion

In the experiments described above, we have characterized the phenotype of mice homozygous and heterozygous for a null allele of the Mtap locus. Aged mice heterozygous for Mtap\textsuperscript{lacZ} die prematurely with enlarged spleens, thymuses, and livers that have large numbers of CD3\textsuperscript{+} T cells invading the affected organs. These findings show that germ-line heterozygosity for Mtap\textsuperscript{lacZ} predisposes animals to develop T-cell lymphoma, and suggest that Mtap may be acting as a tumor suppressor gene. If Mtap were acting as a tumor suppressor gene, then according to Knudson's two-hit hypothesis (27), tumor cells should inactivate the remaining wild-type gene. Supporting this idea, we observed reduced expression of the wild-type CDKN2A/ARF mRNA by quantitative RT-PCR in the spleens of aged heterozygous animals. Examination of spleen DNA by CGH. These findings support the view that Mtap has tumor suppressor qualities independent of CDKN2A/ARF and that inactivation of the wild-type Mtap allele is occurring primarily by epigenetic mechanisms in these animals. Epigenetic silencing of Mtap via promoter hypermethylation has been previously shown to occur in human gastric and lung cancers (28, 29).

In humans, loss of MTAP has been observed in a wide variety of tumors, including both solid and hematologic malignancies. With the exception of a hepatocellular carcinoma observed in one animal, Mtap/Mtap\textsuperscript{lacZ} animals only developed T-cell lymphomas. In older heterozygous animals that have not yet died, we observed tumors, including lymphoma, lung adenocarcinoma, hepatocellular carcinoma, and sarcomas (30).

Despite the fact that human and mouse cells lacking MTAP seem to grow well in culture, mice homozygous for Mtap\textsuperscript{lacZ} are inviable and die early in embryogenesis, around postconception day 8. Just before this time (postconception day 7.5), Mtap seems to be expressed in all cells throughout the embryo. The day 8 homozygous embryos are severely deformed and much smaller than wild-type...
embryos, suggesting that loss of MTAP may be affecting processes involved in mouse organogenesis. Early embryonic lethality in mice homozygous for null mutations has been observed for several mouse tumor suppressor genes including Rb, Wt1, Apc, Nf2, and Brca1 (30). Our findings are also consistent with those of Williamson and colleagues (31), which also found that Mtap was an essential gene in mice.

A possible mechanism by which loss of MTAP promotes tumorigenesis may be related to its effect on altered methionine metabolism and methylation. Tumor cells seem to have disrupted methionine methylation as manifested by their inability to grow in medium in which methionine is replaced by its immediate precursor homocysteine (32). It has been hypothesized that the increased methionine requirements of tumor cells may be due to the high levels of transmethylation observed in tumor cells (33). Interestingly, drugs that inhibit DNA methylation reactions, such as ethionine or 5-azacytidine, are also protumorigenic. In theory, loss of MTAP could affect DNA methylation by two different mechanisms: either through intracellular accumulation of the methyltransferase inhibitor 5'-dideoxy-5'-methylthioadenosine or by reduction in the levels of S-adenosylmethionine due to the lack of a functional methionine salvage pathway. In the experiments

Figure 5. Evidence for loss of wild-type Mtap in lymphoma-infiltrated tissue. A, thymus, spleen, and liver of an aged control (sacrificed at 525 d) and Mtap<sup>lox<sup>Lox</sup></sup> animal (deceased at 260 d) stained with antibody to MTAP viewed at ×400 magnification. B, DNA from wild-type spleen and the enlarged spleen of a Mtap<sup>lox<sup>Lox</sup></sup> animal were hybridized using Agilent CGH chips and data were then analyzed using DNA analytics software. Regions of chromosome of copy number loss as determined by Z-score >3 are indicated by dark vertical bars on the left side. The location of the Mtap is shown by the arrow.
reported here, we did not find a significant difference in the methylation status of enlarged spleen DNA isolated from Mtap+/- heterozygotes and Mtap+/+ control animals. However, these studies are limited by the fact that DNA samples were isolated from total tissue and presumably contain a mixture of Mtap+/- stromal cells and Mtap-/+ tumor cells. Although not statistically significant, it is interesting to note that, on average, the DNA from Mtap+/- spleens had slightly reduced methylation and more variability than did DNA from the control animals.

In summary, our studies support the idea that Mtap, which encodes a basic metabolic enzyme, is a tumor suppressor gene that is essential for normal mouse development. The mice developed should be useful tools in elucidating the mechanism by which loss of Mtap contributes to tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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