Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Fox Chase Cancer Center

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2011

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Minko Gill

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100047634

6. **Project Number and Title of Research Project:** 2 – Characterization of the Role of MTAP Gene in Tumorgenesis

7. **Start and End Date of Research Project:** 1/1/2009 – 12/31/2010

8. **Name of Principal Investigator for the Research Project:** Warren D. Kruger, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $528,943

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost

Kruger	PI	20% YR01-02	$76,210.68
Tang	Research Associate	55% YR01-02	$87,163.68
Kadariya	Research Associate	25% YR01-02	$57,104.92

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___ _____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

I will use the data generated by this funding as preliminary data for a new R01 application

12. Future of Research Project. What are the future plans for this research project?

Our future research will focus on understanding the mechanism by which MTAP expression alters specific downstream gene expression.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				1
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				2
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				2
Other				
Unknown				
Total				**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No___X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u>

Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The original aims of this project were modified six months after the project began to avoid overlap with an R01-funded NIH application entitled "The role of Methylthioadenosine Phosphorylase Loss in Tumorigenesis" that was awarded in May of 2009. These modified aims and the progress made toward completion of each of them are listed below.

Aim 1) Characterization of MTAP-deficient tumor cells.

In our preliminary data we showed that mice heterozygous for MTAP-deficiency die prematurely of lymphoma. In this aim we will characterize the lymphoma cells present in these animals. In addition, we will characterize isogenic MTAP+ and MTAP- HT1080 fibrosarcoma cells for tumorigenicity in SCID mice and will examine the levels of the MTAP-substrate methylthioadenosine.

Progress:

Characterization of lymphoma cells present in *Mtap*^{lacZ} heterozygotes. To more fully characterize the lymphoma present in *Mtap*^{lacZ}/+ 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 to control spleen and reduced numbers of CD45+ cells (Fig. 1a). Infiltration of CD3+ cells was also observed in the livers of some of the heterozygous animals (data not shown). In animals with enlarged thymuses, we observed an expansion of the CD3+ population and a large decrease in the number of CD45+ staining cells (Fig. 1b). The increase in CD3+ cells and the decrease in CD45+ cells indicates that *Mtap*^{lacZ}/+ animals are succumbing to T-cell lymphoma.

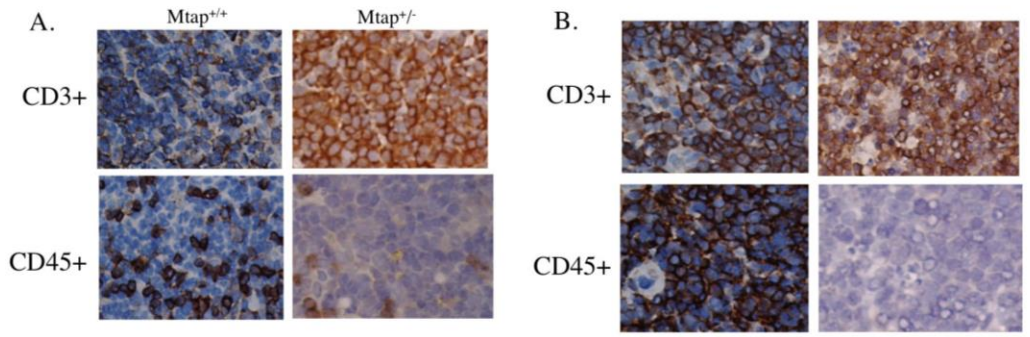

Figure 1. Proliferation of CD3+ T-cells in spleen and thymus. *A*, Spleen of an aged control (sacrificed at 525 days) and *Mtap*^{lacZ}/+ animal (deceased at 260 days) stained with either antibody to CD3+ or CD45+ viewed at 400x magnification. *B*, The thymus of the same two animals as above.

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. For these experiments we sacrificed 11 *Mtap*^{lacZ}/+ heterozygotes (average age 713 days) and six +/+ controls (average age 684 days). In these sacrificed animals, we did not observe gross enlargement of the spleen as observed in the naturally deceased animals, and histopathological 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. 2a). 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 TCR-β locus (Fig. 2b). Five of the

17 heterozygous samples came from autopsy material derived from animals that had exhibited marked splenomegaly and had died of natural causes, while 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.

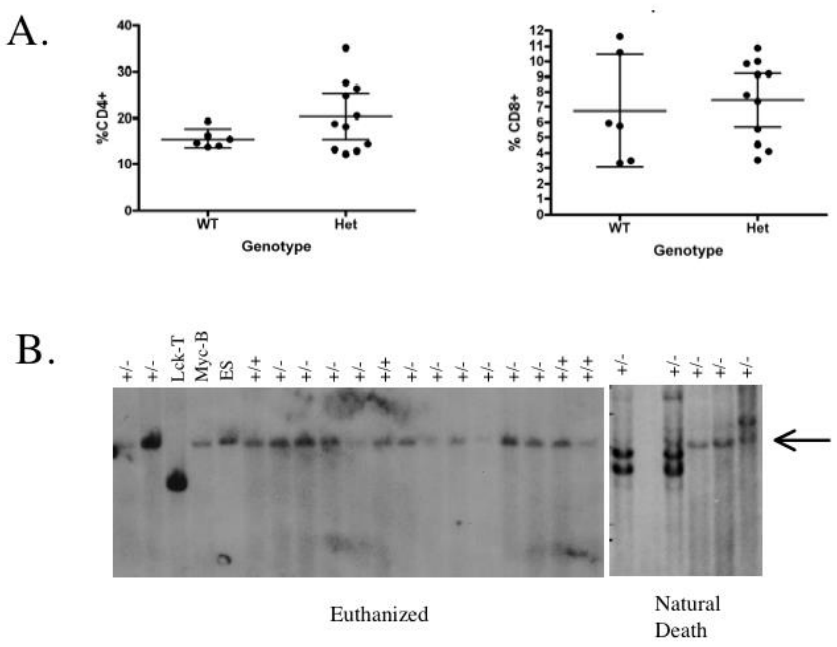

Figure 2. Evidence for T-cell clonality. *A*, FACS analysis of CD4+ and CD8+ present in the spleen of aged wild type and heterozygous animals. Horizontal bar shows mean percentage and error bars show 95% confidence intervals. *B*, Southern blot analysis of spleen DNA of representative heterozygous and wild type animals. The blot is probed with Jβ2 to detect TCRβ rearrangement. Arrow at right shows size of germline (unrearranged band). Controls include DNA from RRK081 embryonic stem cells (ES), DNA from a T-cell lymphoma from a Lck-T mouse, and DNA from a B-cell lymphoma from a Eμ-myc transgenic mouse.

Comparison of growth kinetics of MTAP+ and MTAP- HT1080 subcutaneous tumors using biofluorescent imaging.

Previously our lab created GFP labled versions of our isogenic MTAP+ and MTAP-HT1080 cells. A total of 10 mice were injected subcutaneously with either $2x10^6$ HT1080:MTAP+:GFP or HT1080MTAP-:GFP and tumors were monitored weekly using an IVIS Spectrum Bioluminescent/fluorescent imager. We found that after 28 days three of the five animals injected with HT1080MTAP-:GFP cells had detectable tumors, while none of the five animals injected with HT1080MTAP+:GFP had tumors (Figure 3). These data support the idea the MTAP behaves as a tumor suppressor.

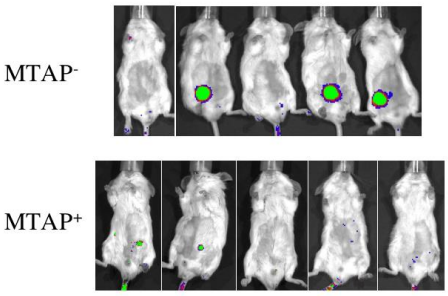

Figure 3. Tumor formation *in vivo* by MTAP+ and MTAP-HT1080 cells.

Measurement of MTA levels in HT180 cells lacking MTAP.

In collaboration with Dr. Yibai Chen at FCCC, we have developed a LC-MS/MS assay to measure intracellular and extracellular MTA. Cells and media from a 10 cm dish containing confluent HT1080:MTAP+ or HT1080:MTAP- cells were collected, washed with phosphate buffered saline, and lysed and extracted with 0.4 M perchloric acid. The PCA-insoluble material was removed by centrifugation and the supernatant was neutralized to pH 7 with 5 M KOH. The supernatant was then be applied to a Agilent 1100 HPLC system with an Xterra MS C18 column and the MTA was purified by elution in isocratic mode with 25% MeOH and 75% H_2O. The collected fraction containing MTA was subsequently collected, dried down in a Speedvac and resuspended in 10 μl of 30% MeOH, 75% H_2O and 0.1% formic acid.

For LC-MS/SM analysis a Waters 2690 Alliance HPLC system and an LCQ Classic ion trap mass spectrometer was used. The purified sample (10 μl) was separated on a C18 column in isocratic mode with 30% ethanol, 75% H_2O and 0.1% formic acid with a flow rate of 50 μl/min. The LCQ ion trap mass spectrometer is triggered by the HPLC and operated using Xcalibur software.

To quantitate MTA in cell lysates we will use selected reaction monitoring mode. This scan type monitors for both the parent ion (298 m/z) and the product ions (136.1 and 162.8). For Quantitation we used a [5′-$^2H_3$] MTA internal standard to the samples. We found that loss of MTAP resulted in a 60-fold increase in MTA in the media, but only a 3-fold increase in MTA

inside the cell (Figure 4). This finding suggests that the cell can efficiently excrete MTA such that it does not build up inside the cell to concentrations necessary to inhibit methyltransferase function.

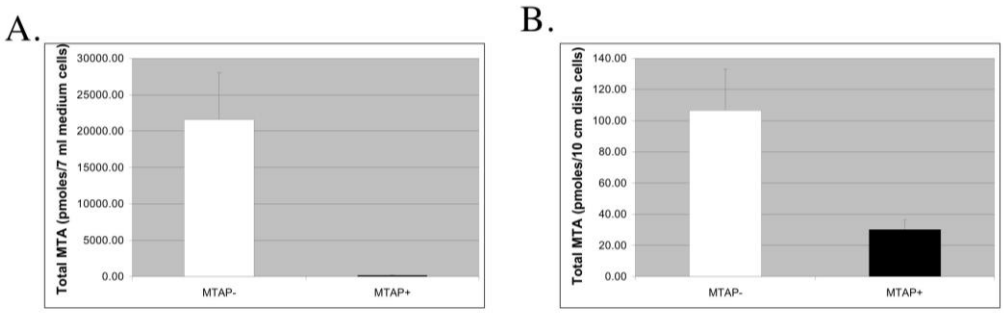

Figure 4. MTA levels in medium and pellets. A. Total MTA in media. B. Total MTA in cell pellet.

Aim 2) Create a Tet-inducible MTAP expressing cell line.

Our preliminary data utilizing isogenic HT1080 cell lines indicate that constitutive MTAP-expression affects the steady-state mRNA levels of many hundreds of genes involved in a variety of cancer related processes. However, it is unclear which of these changes represents direct MTAP-mediated effects and which are secondary effects. In order to address these questions, we will make an MCF-7 cell line with a Tet-On::MTAP fusion that only expresses MTAP upon exposure to Dox. The creation of this cell line will allow us to perform the experiments described in Aim 3.

Progress: The pTRE-Tight:MTAP plasmid was created by inserting a DNA fragment corresponding to the coding region of human MTAP from pCR:sMTAP into pTRE-Tight plasmid (Clontech, Palo Alto, CA), between BamHI and EcoRV sites, under the control of tetracycline regulated-promoter (TRP). The vector was then linearized and combined with a linear puromycin selection maker (clontech) at 20 to 1 ratio and transfected into MCF-7 Tet-on Advanced Cells (Clontech) by using Lipofectamine Transfection agent (Invitrogen). The MCF-7 Tet-ON advanced cells were then cultured in DMEM medium supplemented with 2 mM glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum, 250 μg/ml G418, and puromycin (1 μg/ml). After 3 weeks, puromycin resistance clones were selected and cultured in the absence and presence 1 μg/ml of Dox. The stable expression of human MTAP gene was confirmed by western blot analysis and MTAP enzyme activity assay. The 7 clones with the highest MTAP expression were combined and the MTAP expressing cell line is named MCF-7-TT/M. Similarly, pTRE-Tight plasmid was used as vector control and transfected into MCF-7 Tet-On Advanced Cells. The 7 puromycin resistance clones were combined to form the vector control cell line, named MCF-7-TT/C. These pools were then used to examine the kinetics and strength of MTAP induction. We found that in the absence of Dox, MTAP protein

was undetectable by western blot analysis (Figure 5). Upon exposure to1μg/ml Dox we found that MTAP levels were initially detectable at eight hours, and continued to rise through 40 hours (Figure 5a). We also performed a dose response experiment by exposing cells to doses of Dox between 0.008 and 5 μg/ml for 40 hours. We found induction with as little as 0.2 mg/ml Dox

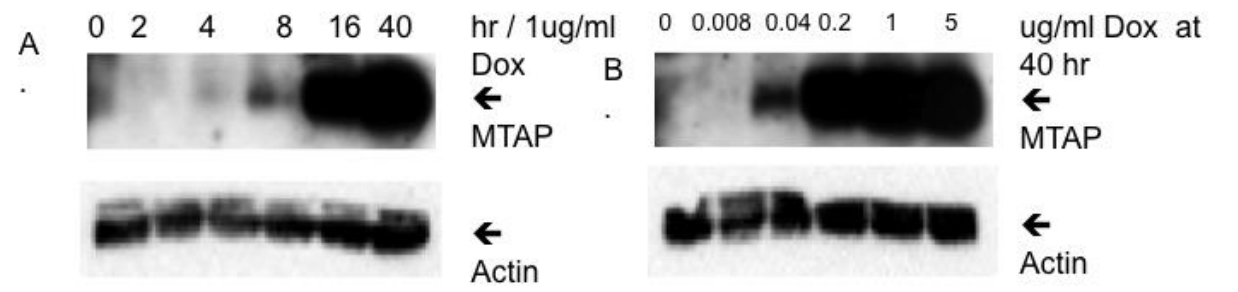

Figure 5. Inducible MTAP expression in MCF-7 cells. *A*, MCF-7-TT/M cells were exposed to Dox for the indicated period of time and MTAP expression was monitored by immunoblot. *B*, Same as A except dose was modified as opposed to time.

and maximum induction with 1 mg/ml (Figure 5b).

Aim 3) Kinetic analysis of MTAP regulated transcripts in response to MTAP induction.

Using the cell line created in Aim 2, we will perform a time course experiment in which cells are harvested at various time points after induction and gene expression are examined by microarray. These studies will determine which genetic pathways are directly affected by MTAP expression in a breast cancer cell line.

Progress:

Microarray studies of a Tet-inducible MTAP expressing cell line.

We performed global mRNA expression studies on the Tet-inducible MTAP expressing MCF-7 cell line which we described above. A total of four plates were grown, two with Dox and two without. Dox treatment was at a dose of 1 μg/ml for 48 hours. RNA was then isolated, labeled and hybridized to Agilent G4112A chips according to manufacturers instructions. Data for each array was captured using an Agilent G2565 scanner. Data for each array was normalized using Agilent Feature Extraction software.

We identified over 283 genes whose expression levels changed at least 50% with a p-value <0.05. We

Gene	Array M+/M-	qPCR M+/M-
AGPAT4	6.21	13.84
COL8A1	5.10	nd
FGF18	0.51	0.46
GALNTL1	2.61	3.26
IGFBP5	0.45	0.38
MGP	0.55	0.61
MUC20	1.55	1.69
SEC16B	61.17	nd
TIMP3	0.49	0.92
TFF1	0.64	0.43

Figure 6. MTAP regulated Genes. Number indicates fold-increase in mRNA in doxycycline treated (M+) versus non-treated (M-) cells. Column labeled Array is data form microarray while column labeled qPCR was assessed using Taqman RT-PCR.

then performed pathway analysis on these changed genes to identify pathways that were enriched in this subset of genes using the Gene Set Analysis Toolkit V 2.0. We observed significant enrichments for genes with functions in the extracellular matrix, genes involved in bone development, and genes encoding for oxidoreductases.

We next confirmed the microarray analysis on a series of regulated genes using quantitative reverse-transcription PCR (qRT-PCR). A total of ten genes were examined; five that were up-regulated by MTAP expression and five that were down regulated (Figure 1). For each of these genes we obtained probes from Applied Biosystems and measured mRNA levels by Taqman assay. Using this approach, we were able to confirm differential regulation in eight of the ten genes assessed.

In the microarray analysis we observed that the gene with the greatest level of induction was SEC16B, with a 61-fold induction after a 48 hour exposure to doxycycline. However, we were unable to confirm this induction by qPCR. To understand these findings, we carefully examined the structure of the SEC16B gene and found that the Taqman assay used for RT-qPCR was directed at sequences in exon boundary 12 and 13, while the probe used to detect SEC16B on the microarray was directed at the 3'untranslated exon (exon 26). Examination of the UCSC genome database indicated that there was an alternative transcript which initiated downstream of exon 12 and thus lacked the Taqman probe region. To determine if this transcript was responsible for the differential regulation we obtained a new Taqman probe designed to detect the exon 25 and 26 boundary region. Using this probe, we confirmed that SEC16B was upregulated at least 50-fold within four hours of MTAP induction by doxycycline (Figure 2). These findings suggest that MTAP expression results in the production of an alternate form of the SEC16B message.

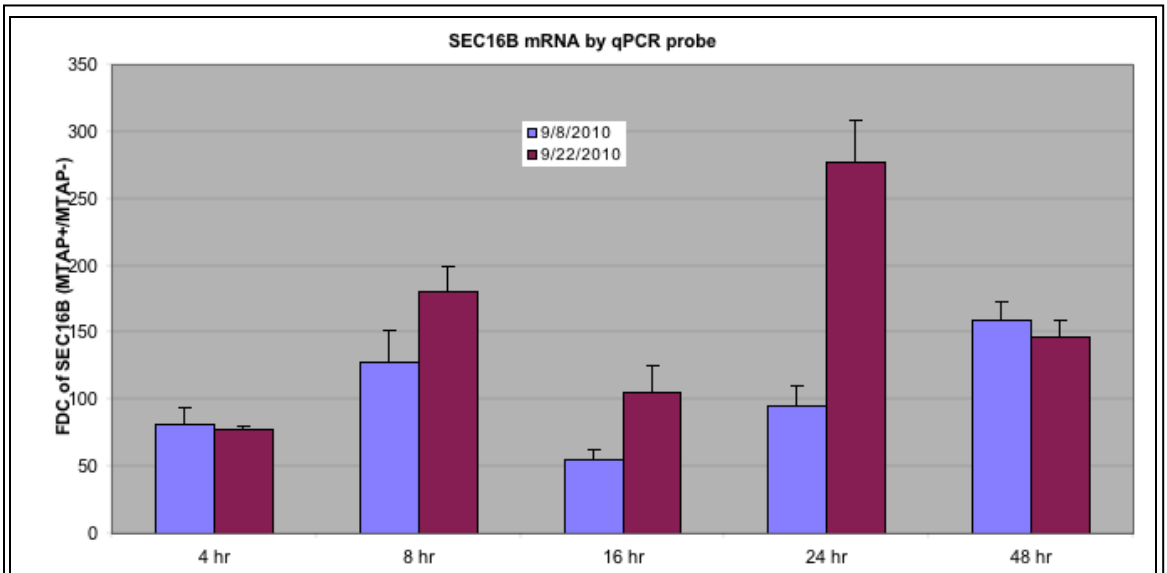

Figure 7. Induction of SEC16B mRNA assessed by Taqman. MCF-7$^{MTAP:Teton}$ cells were treated with doxycycline for the specified period of time and assessed for SEC16B message using a probe specific to the exon 25-26 junction. The experiment was performed on biological replicates grown on two separate occasions. Fold-increase was determined by comparing to mRNA levels present in non-doxycycline treated cells.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
___X__ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Mice Heterozygous for Germ-line Mutations in Methylthioadenosine Phosphorylase (MTAP) Die Prematurely of T-cell Lymphoma	Yuwaraj Kadariya, Bu Yin, Baiqing Tang, Susan A. Shinton, Eoin P. Quinlivan, Xiang Hua. Andres Klein-Szanto, Tahseen I. Al-Saleem, Craig H. Bassing, Richard R. Hardy, and Warren D. Kruger	Cancer Research	March 2009	☐Submitted ☐Accepted ☑Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans: I plan to submit a paper describing the gene profile changes induced by MTAP expression in the engineered MCF-7 cells described in this report.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The major discovery described here is that our data show that MTAP is a novel tumor suppressor gene and that its loss in cancer cells is likely to be due to its having a direct role in tumor formation. The discovery that induction of MTAP causes the alteration of the gene expression profile in as little as 48 hours indicates that these effects are likely to be direct as opposed to indirect. In addition, the finding that germ-line mutations in MTAP cause T-cell lymphoma is also novel. Taken together our findings suggest that MTAP may play a key role in tumorigenesis.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No __X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Biosketches are included for Drs. Kruger, Kadariya, and Tang.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kruger, Warren D., Ph.D.	Professor
eRA COMMONS USER NAME wdkruger	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.A.	06/85	Biology
University of California, San Francisco, CA	Ph.D.	07/91	Biochemistry
University of California, San Francisco, CA	Fellowship	1991-95	Genetics

A. PERSONAL STATEMENT

I have been studying the genetics of hyperhomocysteinemia and sulfur metabolism since establishing my lab in 1995. I have published over 60 papers specifically on sulfur amino acid metabolism and homocysteine-related diseases. My lab has made several key advances in the field including the cloning of the remethylation enzyme methionine synthase, identification of the regulatory domain on the CBS enzyme, the discovery that it is possible to restore function to mutant CBS proteins by manipulating the cellular chaperone environment, and the development of yeast and mouse models to study the effects of homocystinuria. I am a recognized leader in the 1-carbon metabolism field, having been an invited speaker and session chair several times at both the International Homocysteine Meeting and the FASEB summer conference on Folate, Methionine, and B_{12} Metabolism. I am also well versed in the area of chromatin modification and transcriptional regulation as a consequence of my doctoral work in the lab of Dr. Ira Herskowitz. During this time I made the key discovery that missense mutations in histone and histone-related HMG proteins could alleviate the need of the SWI/SNF complex for transcription of the HO endonuclease. This work set the stage for later work showing that chemical modification of histones could affect transcription.

B. POSITIONS

Positions and Employment

1984-1985	Undergraduate research, Laboratory of Dr. Mariana Wolfner, Cornell University
1986-1987	Graduate student, Laboratory of Dr. Rudolph Grosschedl, University of California at San Francisco
1987-1991	Graduate student, Laboratory of Dr. Ira Herskowitz, University of California at San Francisco
1991-1995	Postdoctoral Fellow, Laboratory of Dr. David R. Cox, Stanford University
1995-2001	Associate Member, Fox Chase Cancer Center, Philadelphia
2001-2008	Member with Tenure, Fox Chase Cancer Center
2008-date	Professor (adjunct), Department of Pharmacology, Temple University, Philadelphia
2008-date	Professor, Fox Chase Cancer Center
2009-date	Professor (adjunct), Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia

C. SELECTED PEER-REVIEWED PUBLICATIONS:

Shan, X.. and **Kruger, W.D.** Correction of disease-causing *CBS* mutations in yeast. *Nature Genet*. **19**:91-94, 1998.

Tang, B., Li, Y.N., **Kruger, W.D.** Defects in methylthioadenosine phosphorylase are associated with but not responsible for methionine-dependent tumor cell growth. *Cancer Res*. **60**:5545-5547, 2000.

Subhi, A.L., Diegelman, P., Porter, C.W., Tang, B., Lu, Z.J., Markham, G.D., **Kruger, W.D.** Methylthioadenosine phosphorylase regulates ornithine decarboxylase by production of downstream metabolites. *J. Biol. Chem*. **278**:49868-49873, 2003.

Tang, B., **Kruger, W.D.**, Chen, G., Shen, F., Lin, W.Y., Mboup, S., London, W.T., Evans, A.A. Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers. *J. Med. Virol.* **72**:35-40, 2004.

Subhi, A.L., Tang, B., Balsara, B.R., Altomare, D.A., Testa, J.R., Cooper, H.S., Hoffman, J.P., Meropol, N.J., **Kruger, W.D.** Loss of methylthioadenosine phosphorylase and elevated ornithine decarboxylase is common in pancreatic cancer. *Clin. Cancer Res*. **10**:7290-7296, 2004.

Wang, L., Chen, X., Tang, B., Hua, X., Klein-Szanto, A., **Kruger, W.D.** Expression of mutant human cystathionine beta-synthase rescues neonatal lethality but not homocystinuria in a mouse model. *Hum. Mol. Genet*. **14**:2201-2208, 2005.

Chen, X., Wang, L., Fazlieva, R., **Kruger, W.D.** Contrasting behaviors of mutant cystathionine beta-synthase enzymes associated with pyridoxine response. *Hum. Mutat*. **27**:474-482, 2006.

Tang, B., Kadariya, Y., Murphy, M.E., **Kruger, W.D.** The methionine salvage pathway compound 4-methylthio-2-oxobutanate causes apoptosis independent of down-regulation of ornithine decarboxylase. *Biochem. Pharmacol.* **72**:806-815, 2006.

Gupta, S., Wang, L., Hua, X., Krijt, J., Kozich, V., **Kruger, W.D.** Cystathionine β-synthase p.S466L mutation causes hyperhomocysteinemia in mice. *Hum. Mutat*. **29**:1048-1054, 2008.

Gupta, S., Kühnisch, J., Mustafa, A., Lhotak, S., Schlacterman, A., Slifker, M.J., Klein-Szanto, A.,. High, K.A., Austin, R.C., **Kruger, W.D.** Mouse models of cystathionine β-synthase deficiency reveal significant threshold effects of hyperhomocysteinemia. *FASEB J.* **23**:883-893, 2009.

Kadariya, Y., Yin, B., Tang, B., Shinton, S.A., Quinlivan, E.P., Hua, X., Klein-Szanto, A., Al-Saleem, T.I., Bassing, C.H., Hardy, R.R., **Kruger, W.D.** Mice heterozygous for germline mutations in methylthioadenosine phosphorylase (MTAP) die prematurely of T-cell lymphoma. *Cancer Res*. **69**:5961-5969, 2009.

Tang, B., Mustafa, A., Gupta, S., Melnyk, S., James, S.J., **Kruger, W.D.** Methionine-deficient diet induces post-transcriptional downregulation of cystathionine β-synthase. *Nutrition* **26**:1170-1175, 2009.

Kadariya, Y., Tang, B., Myers, C.B., Fukui, J., Peterson, J.R., **Kruger, W.D.** Chemical genetic screening for compounds that preferentially inhibit growth of methylthioadenosine phosphorylase (MTAP) deficient Saccharomyces cerevisiae. *J. Biomol.* **16**:44-52,2011.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kadariya, Yuwaraj M.D., Ph.D.	Research Associate
eRA COMMONS USER NAME ykadariya	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Tribhuvan University Institute of Medicine, Nepal	M.D.	11/95	Medical Science
Mie University, Japan	Ph.D.	04/04	Laboratory Molecular Medicine

A. PERSONAL STATEMENT

I studied about a gene "Methythioadenosine Phosphorylase (MTAP)" that is frequently deleted or inactivated in various human cancers. I created MTAP knock-out mice in Kruger lab and demonstrated that MTAP heterozygosity can predispose animals to T-cell lymphoma, thereby showing MTAP is important tumor suppressor gene. I demonstrated that MTAP heterozygosity can also enhance tumor formation in other mouse model like *c-myc* and .*Pten.* Recently, I have been studying about the development, pathogenicity and mechanism of Malignant Mesothelioma in mouse model upon asbestos exposure. I am trying to understand how inflammasome can enhance tumor formation in mice with asbestos exposure. Also I am trying to understand the effect of cytokines produced by inflammasome in Asbestos induced tumor formation.

B. POSITIONS AND HONORS

Medical laboratory technician Kanti Children's' Hospital Kathmandu, Nepal	1986 – 1993
Medical internship training Tribhuvan University Teaching Hospital Kathmandu, Nepal	1994 – 1995
House Officer (Medical Officer) Tribhuvan University Teaching Hospital Kathmandu, Nepal	1995 – 1996
Medical Officer, worked as a primary health care provider Nepal Government Ministry of Health, Nepal	1996 – 1999
Medical Assistant and research Assistant Mie University School of Medicine	2001 – 2004
Postdoctoral Associate at Fox Chase Cancer Center	2004 - 2010
Research Associate at Fox Chase Cancer Center	2010- present

HONORS

Monbusho Fellowship from JAICA, JAPAN 1999 - 2004

C. SELECTED PEER-REVIEWED PUBLICATIONS

Kadariya, Y., Nishioka, J., Nakatani, K., Nakashima, K.. Nobori. T. Deletion of Dinucleotide Repeat (D14 Allele) in the Methylthioadenosine Phosphorylase (MTAP) Promoter and Allelotype of MTAP Promoter in the Japanese Population. *Jpn. J. Cancer Res.* **93**, 369-373, 2002.

Kadariya, Y., Nishioka, K., Nakamura, A., Kato-Nakagawa, K., Nobori, T. Molecular Characterization of 5'-deoxy-5'Methylthioadenisine Phosphorylase-deficient Mutant Clones of Murine Lymphoma Cell Line R1.1. *Cancer Science*, **94**, 519–522, 2003.

Nishioka, J., **Kadariya, Y.**, Nobori. T. Southern blotting and Northern blotting Method. *Gene Analysis* **128**, 149–162, 2004.

Katsura, N.. Nakatani, K., Yoshikazu, T., Yoshitaka, M., **Kadariya**, **Y.**, Nobori. T., Analysis of Proinsulin, *Analysis and Methodology*, **32**, 293–296, 2004.

Kadariya Y., Nakatani K., Nishioka J., Fujikawa T., Kruger W.D**.**, Nobori T. Regulation of human methylthioadenosine phosphorylase gene by the CCAAT binding factor CBF/NF-Y. *Biochem. J.* **387**:175-183, 2005.

Tang B., **Kadariya Y.**, Murphy M.E., **Kruger W.D.** The methionine salvage pathway compound 4-methylthio-2-oxobutanate causes apoptosis independent of down-regulation of ornithine decarboxylase. *Biochem. Pharmacol.* **72**:806-815, 2006.

Kadariya, Y., Yin, B., Tang, B., Shinton, S.A., Quinlivan, E.P., Hua, X., Klein-Szanto, A., Al-Saleem, T.I., Bassing, C.H., Hardy, R.R., **Kruger, W.D**. Mice Heterozygous for germline mutations in methylthioadenosine phosphorylase (MTAP) die prematurely of t-cell lymphoma. *Cancer Res*. **69**:5961-5969, 2009.

Kadariya, Y., Tang, B., Myers, C.B., Fukui, J., Peterson, J.R., **Kruger, W.D**. Chemical genetic screening for compounds that preferentially inhibit growth of methylthioadenosine phosphorylase (MTAP) deficient Saccharomyces cerevisiae. *J. Biomol.* **16**:44-52, 2011.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Tang, Baiquing, Ph.D.	Research Associate
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Sichuan Medical College, China	B.M.	1982	Medicine
University of Pharmaceutical Science of China	M.S.	1986	Medicine
Mount Sinai School of Medicine, New York, NY	Ph.D.	1993	Biomedical Science

A. PERSONAL STATEMENT

I have been a biomedical researcher since I graduated with a PhD degree in Biochemistry from Mount Sinai Medical School in New York. I have extensive research experience in a wide variety of fields including signal transduction, cell proliferation, differentiation, apoptosis, and cancer biology. Over the last five years, my primary interest has been understanding the role of the methylthioadenosine phosphorylase (MTAP) gene in tumorigenesis. My research has demonstrated that MTAP is a tumor suppressor gene and that loss of MTAP affects a variety of processes involved in tumor formation. My current interest is in understanding the precise molecular mechanism by which MTAP inhibits tumor formation, and using loss of MTAP as a means to develop a therapy that can selectively target tumor cells.

B. POSITIONS AND HONORS

Postdoctoral Fellow, Yunnan Institute of Pharmaceutical Sciences, China	1982-83
Senior Scientist, National Inst. for the Control of Pharmaceutical and Biological Products, China	1986-87
Research Fellow, Mount Sinai Medical Center, New York	1987-88
Postdoctoral Fellow, Division of Endocrinology, Memorial Sloan-Kettering Cancer Center	1993-96
Postdoctoral Fellow, Division of Hematology, Memorial Sloan-Kettering Cancer Center	1996-98
Postdoctoral Associate, Fox Chase Cancer Center, Philadelphia	1998-2001
Research Associate, Fox Chase Cancer Center, Philadelphia	2001-date

HONORS

The Outstanding Young Scientist Award of the Pharmaceutical Society of China	1986
The Charles H. Revson Foundation Fellowship	1993
PHS Fellowship	1994-1996

C. PUBLICATIONS

Tang, B., Guller, S., and Gurpide, E. (1993). Mechanisms involved in the decidualization of human endometrial stromal cells. *Acta Eur Fertil*. 2**4**:221-223, 1993.

Tang, B. and Gurpide, E. Direct effect of gonadotropins on decidualization of human endometrial stromal cells. *J Steroid Biochem Molec Biol*. **47**:115-121, 1993.

Tang, B., Guller, S. and Gurpid,e E. Mechanism of human endometrial stromal cells decidualization. *Ann NY Acad Sci*. **734**:19-25, 1994.

Tang, B., Jeoung, D. and Sonenberg, M. Effect of human growth hormone and insulin on [3M-thymidine incorporation cell cycle progression, and cyclin D expression in 3T3-F442A preadipose cells. *Endocrinology* **136**:3062-3069, 1995.

Jeoung, D., **Tang, B.** and Sonenberg, M. Effects of tumor necrosis factor α on antimitogenicity and cell cycle-related proteins in MCF-7 cells. *J Biol Chem*. **270**:18367-18373, 1995.

Jeoung, D., **Tang B.** and Sonenberg, M. Induction of tumor suppressor P21 protein by kinase inhibitors. *Biochem Biophys Res Com*. **214**:361-366, 1995.

Jeoung D, **Tang B** and Sonenberg M. Mitogenic response to TGF-β in 3T3-F442A cells. *Biochem Biophys Res Com*. **216**:964-969, 1995.

Lingueros, M., Jeoung, D., **Tang, B.**, Reidenberg, M.M. and Sonenberg, M. (Gossypol inhibition of mitosis, cyclin DI and Rb protein in human mammary cancer cells and cyclin DI transfected human fibrosarcoma cells. *British J Cancer*. **76**:21-28, 1997.

Tang, B., Li, Y.N., Kruger, W.D. Defects in methylthioadenosine phosphorylase are associated with but not responsible for methionine-dependent tumor cell growth. *Cancer Res*. **60**:5545-5547, 2000.

Subhi, A.L., Diegelman, P., Porter, C.W., **Tang, B**., Lu, Z.J., Markham, G.D., Kruger, W.D. Methylthioadenosine phosphorylase regulates ornithine decarboxylase by production of downstream metabolites. *J. Biol. Chem*. **278**:49868-49873, 2003.

Tang, B., Kruger, W.D., Chen, G., Shen, F., Lin, W.Y., Mboup, S., London, W.T., Evans, A.A. Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers. *J. Med. Virol*. **72**:35-40, 2004.

Subhi, A.L., **Tang, B**., Balsara, B.R., Altomare, D.A., Testa, J.R., Cooper, H.S., Hoffman, J.P., Meropol, N.J., Kruger, W.D. Loss of methylthioadenosine phosphorylase and elevated ornithine decarboxylase is common in pancreatic cancer. *Clin. Cancer Res*. **10**:7290-7296, 2004.

Wang, L., Chen, X., **Tang, B**., Hua, X., Klein-Szanto, A., Kruger, W.D. Expression of mutant human cystathionine beta-synthase rescues neonatal lethality but not homocystinuria in a mouse model. *Hum. Mol. Genet*. **14**:2201-8, 2005.

Tang, B., Kadariya, Y., Murphy, M.E., Kruger, W.D. The methionine salvage pathway compound 4-methylthio-2-oxobutanate causes apoptosis independent of down-regulation of ornithine decarboxylase. *Biochem. Pharmacol*. **72**:806-815, 2006.

Kadariya, Y., Yin, B., **Tang, B**., Shinton, S.A., Quinlivan, E.P., Hua, X., Klein-Szanto, A., Al-Saleem, T.I., Bassing, C.H., Hardy, R.R., Kruger, W.D. Mice heterozygous for germline mutations in methylthioadenosine phosphorylase (MTAP) die prematurely of T-cell lymphoma. *Cancer Res*. **69**:5961-5969, 2009.

Tang, B., Mustafa, A., Gupta, S., Melnyk, S., James, S.J., Kruger, W.D. Methionine-deficient diet induces post-transcriptional downregulation of cystathionine β-synthase. *Nutrition* **26**:1170-1175, 2009.

Kadariya, Y., **Tang, B.,** Myers, C.B., Fukui, J., Peterson, J.R., Kruger, W.D. Chemical genetic screening for compounds that preferentially inhibit growth of methylthioadenosine phosphorylase (MTAP) deficient Saccharomyces cerevisiae. J. Biomol. **16**:44-52, 2011.