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: Lehigh University**

2. **Reporting Period (start and end date of grant award period): 1/1/2009 – 6/30/2011**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Nicole M. Corali, BS, BA**

4. **Grant Contact Person's Telephone Number: 610-758-4585**

5. **Grant SAP Number: SAP #4100047638**

6. **Project Number and Title of Research Project: 1 – Targeted Killing of Cancer Cells**

7. **Start and End Date of Research Project: 1/1/2009 – 6/30/2011**

8. **Name of Principal Investigator for the Research Project: Dr. Lynne Cassimeris**

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:

   $ 109,602.94

   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

Carney	Graduate Assistant	25% Yr 1-2, 0% Yr 3	$12,359.46
Dupuis	Graduate Assistant	25% Yr 1, 0% Yr 2-3	$6,089.64
Maradeo	Graduate Assistant	25% Yr 1, 0% Yr 2-3	$6,089.64
Ringhoff	Graduate Assistant	25% Yr 1, 0% Yr 2-3	$6,089.64
Mushock	Technician	<1% Yr 2	$82.72

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
Cassimeris	P.I.	25% Yrs 1 -3
Caruso	Graduate Assistant	25% Yrs 2 - 3
Carney	Graduate Assistant	25% Yr. 3

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
None		

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____X_____ No_____

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:
Microtubule Stability and Cell Proliferation	XNIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	July, 2010	$1,599,413 total costs	Not Funded
Signal Transduction by the Microtubule Destabilizer, Stathmin/Oncoprotein 18	XNIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	Feb, 2011	$480,716 total costs	Pending
Microtubule Control of Metabolism in Prostate Cancer	☐NIH x Other federal (specify:_CDMRP _____ _____)	May, 2011	$122,834 total costs	Pending

	☐ 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: If necessary, will resubmit NIH proposal.

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

Why is stathmin (a protein) only required in cancer cells and not normal cells? We will continue research to identify the mechanisms underlying stathmin's requirement for cancer cell survival.

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			2	
Female			3	
Unknown				
Total			**5**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			5	
Unknown				
Total			**5**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			5	
Black				
Asian				
Other				
Unknown			5	

Total				

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_____X____ No_____

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

Having an active lab group enhances the research environment within the department of Biological Sciences and within the University by contributing to the intellectual environment locally.

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 for the entire grant award period. 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.

Introduction: The ultimate goal of cancer therapy is to induce cancer cells to undergo apoptosis, while minimizing death of the normal cell population. The difficulties in reaching this goal are twofold: specificity of the treatment to destroy only cancer cells and heightened resistance of most cancer cells to agents inducing apoptosis. We are studying a pathway which shows promise in specifically inducing apoptosis in those cancer cells lacking p53, a critical tumor suppressor protein, by reducing the level of stathmin, a microtubule regulatory protein (reviewed by Murphy and Cassimeris, 2006).

Stathmin, also known as oncoprotein 18, is a soluble, 18 kD protein first identified by its phosphorylation in response to a number of extracellular signals and its high expression in leukemias. Stathmin is highly expressed in a number of cancers and in many of these cancers, higher stathmin levels are correlated with cancer stage progression and reduced patient survival. In addition to its potential use as a cancer biomarker, stathmin represents a novel therapeutic target. Recent experiments have demonstrated that stathmin depletion is sufficient to activate apoptosis in several cancer cell lines. In breast cancer cell lines, those lines requiring stathmin for survival are correlated with mutations in p53. In contrast to these cancer cells, stathmin knockout mice are viable, suggesting that potential therapies based on stathmin depletion will not be deleterious to non-cancerous cells. These studies have raised interest in stathmin as a therapeutic target, but to take advantage of stathmin-dependent cell survival in cancer, we need to understand the context(s) under which it is required for cell survival and the pathway(s) where stathmin acts to promote survival or block apoptosis.

Drugs that stabilize microtubules (e.g. taxol) or destabilize microtubules (e.g. vinblastine) have been used successfully to treat many cancers, but their toxicity to non-cancer cells limits their usefulness and treatment course. Finding alternative targets that show specificity for cancer cells could be used in combination with microtubule-targeted drugs to increase the therapeutic potential of drugs such as taxol and vinblastine.

Our overall goal is to find the pathway activated by stathmin depletion that leads cancer cells to delay in the cell cycle and to activate apoptosis causing cell death. Our first objective was to rigorously test the hypothesis that stathmin depletion synergizes with loss of p53 and induces cell death. Results from these experiments were published in 2010 (Carney and Cassimeris). The next goals of this grant were to find the pathway activated by stathmin depletion in cancer cells. During the course of the grant we deviated from the original aims as we focused on areas that evolved during the course of the research, but were not known to us when the proposal was written. Here I first describe the original aims and then describe progress beyond those original goals.

ORIGINAL SPECIFIC AIMS

Aim 1: Develop a cancer cell line having stable expression of GFP-tagged cytochrome C. The release of cytochrome C from mitochondria serves as a marker for the net activities of pro- and anti-apoptotic proteins regulating outer mitochondrial membrane integrity.

Aim 2: Examine whether stathmin depletion leads to cytochrome C release from mitochondria, as predicted for loss of anti-apoptotic Bcl2 proteins. Stathmin will be depleted by siRNA-based methods, cytochrome C release will be followed by fluorescence microscopy.

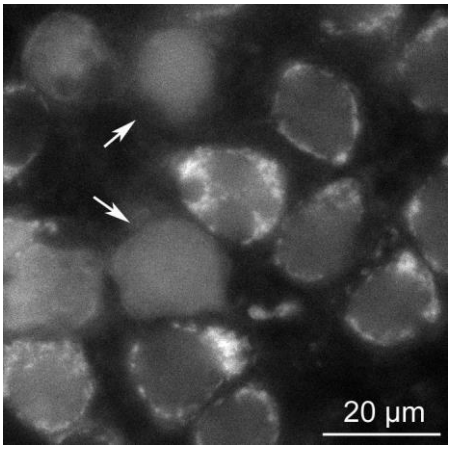
Aim 3: Determine whether stathmin over-expression protects cells from the drug ABT-737, a drug that blocks anti-apoptotic (Bcl-2 family) proteins. We will also address whether stathmin depletion sensitizes cells to ABT-737.

PROGRESS ON ORIGINAL AIMS

Aim 1. Develop a cancer cell line having stable expression of GFP-tagged cytochrome C.

We were able to obtain a cell line from Douglas Green's lab at St. Jude Children's Research Hospital in Memphis Tennessee, making it unnecessary to generate our own cell line. We confirmed that cytochrome C is released into the cytoplasm in response to cytoplasmic stress.

Figure 1 shows a field of cells after treatment with staurosporine, an inducer of apoptosis. Several cells have released cytochrome C from mitochondra (marked by arrows). Other cells show mitochondrial localization of cytochrome C (punctate signal in surrounding cells).



Aim 2. Examine whether stathmin depletion leads to cytochrome C release from mitochondria.

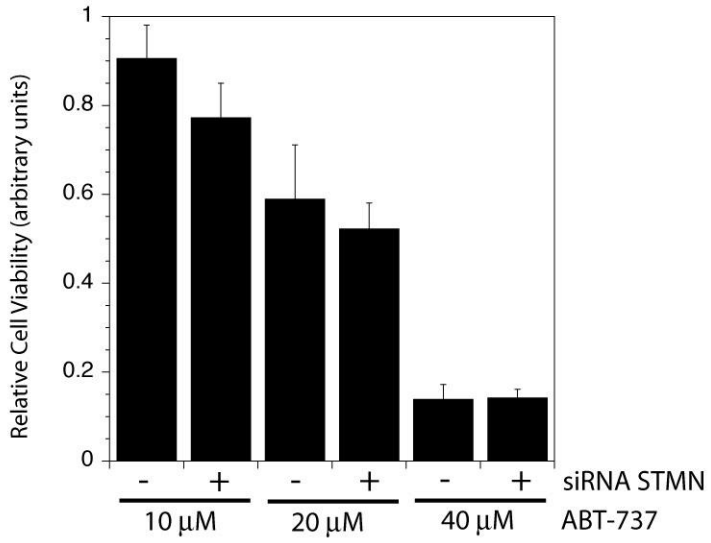
Based on results from Aim 3, and new results described below, we did not pursue this aim because we did not expect the results to be informative, at least over the course of the current grant. Instead, we focused attention on more definitive experiments described under Progress Beyond Original Aims.

Aim 3. Determine whether stathmin over-expression protects cells from the drug ABT-737, a drug that blocks anti-apoptotic (Bcl-2 family) proteins. We will also address whether stathmin depletion sensitizes cells to ABT-737.

We did not find evidence for reproducible rescue or synergy between stathmin level and ABT-737 in regulating cell survival. Specifically, we did not find significant synergy between stathmin depletion and ABT-737, a drug that blocks the function of anti-apoptotic proteins at mitochondria. By blocking anti-apoptotic proteins, ABT-737 favors apoptosis by shifting the balance toward pro-apoptotic proteins, resulting in cytochrome C release from mitochondria.

A representative experiment is shown below in Figure 2. At each ABT-737 concentration, depletion of stathmin did not significantly decrease cell viability over that observed with ABT-737 alone. In several additional experiments we also failed to detect significant synergy between stathmin depletion and ABT-737. Cell viability measurements were made based on a commericial kit that detects ATP levels by enzymatic generation of a colored reaction product.

Figure 2.



PROGRESS BEYOND ORIGINAL AIMS

Overview (taken from the abstract of Carney and Cassimeris, 2010):
Stathmin, a microtubule regulatory protein, is over-expressed in many cancers and required for survival of several cancer lines. In a study of breast cancer cell lines, Alli et al. (Oncogene. 26:1003-12) proposed that stathmin is required for survival of cells lacking p53, but this hypothesis was not tested directly. Here we tested their hypothesis by examining cell survival in cells depleted of stathmin, p53 or both proteins. Comparing HCT116 colon cancer cell lines differing in TP53 genotype, stathmin depletion resulted in significant death only in cells lacking p53. As a second experimental system, we compared the effects of stathmin depletion from HeLa cells, which normally lack detectable levels of p53 due to expression of the HPV E6 protein. Stathmin depletion caused a large percentage of HeLa cells to die. Restoring p53, by depletion of HPV E6, rescued HeLa cells from stathmin-depletion induced death. Cleaved PARP was detected in HCT116$^{p53-/-}$ cells depleted of stathmin and cell death in stathmin-depleted HeLa cells was blocked by the caspase inhibitor Z-VAD-FMK, consistent with apoptotic death. The stathmin-dependent survival of cells lacking p53 was not confined to cancerous cells because both proteins were required for survival of normal human fibroblasts. In HCT116 and HeLa cells, depletion of both stathmin and p53 leads to a cell cycle delay through G2. Our results demonstrate that stathmin is required for cell survival in cells lacking p53, suggesting that stathmin depletion could be used therapeutically to induce apoptosis in tumors without functional p53. These results were published (Carney and Cassimeris, 2010) and are summarized below under **Finding 1**.

We are now examining the pathway activated by stathmin depletion. Stathmin's only demonstrated function is to regulate the MT cytoskeleton, acting as a MT destabilizing protein. Depletion of stathmin leads to more stable microtubules. Whether stable microtubules activate a cell cycle checkpoint in G2, rather than M phase, has been controversial in the literature and we have devoted considerable effort to determine whether stathmin-depletion induced microtubule

stability is responsible for the observed cell cycle delay. These experiments, testing whether microtubule stability is the signal downstream of stathmin depletion that delays cells during G2 of the cell cycle, are nearing completion, are described below and should be submitted for publication within the next several months. Results from these experiments also eliminate two other stathmin binding partners, STAT3 (a signal intermediate and transcription factor) and p27Kip1 (a cyclin-dependent kinase inhibitor that, when active, can block cells in G1 or G2) as the potential signal relay downstream of stathmin depletion. These results are summarized below under **Finding 2**.

Finding 1. Stathmin depletion is "synthetically lethal" with loss of p53, since it is only when both proteins are reduced or eliminated that cells undergo a delay in G2, slowing cell proliferation, and an increase in apoptotic cell death.

Figure 3 (below) shows an example of our results from Hela cells. Panel A shows the increased cell death for cells lacking stathmin and p53. Depletion of the HPV protein, E6, restores p53 in this cell type and rescues cells from stathmin-depletion induced apoptosis. Panel B demonstrates that an shRNA, targeting a separate region in stathmin mRNA, also increases cell death. Panel C documents depletion of stathmin protein level by either si- or shRNA. Data from Carney and Cassimeris, 2010.

Figure 3. Trypan blue uptake is an indicator of cell death.

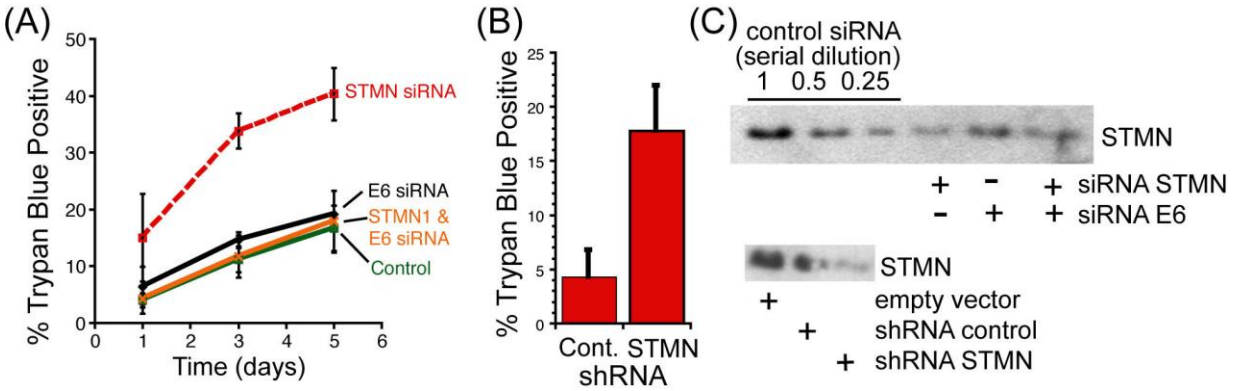


Figure 4 below demonstrates that stathmin depleted cells die via apoptosis. These cells show an increase in the percentage of cells having cleaved PARP (a target of active caspases). Cell death is greatly reduced by addition of Z-VAD-FMK, a caspase inhibitor.

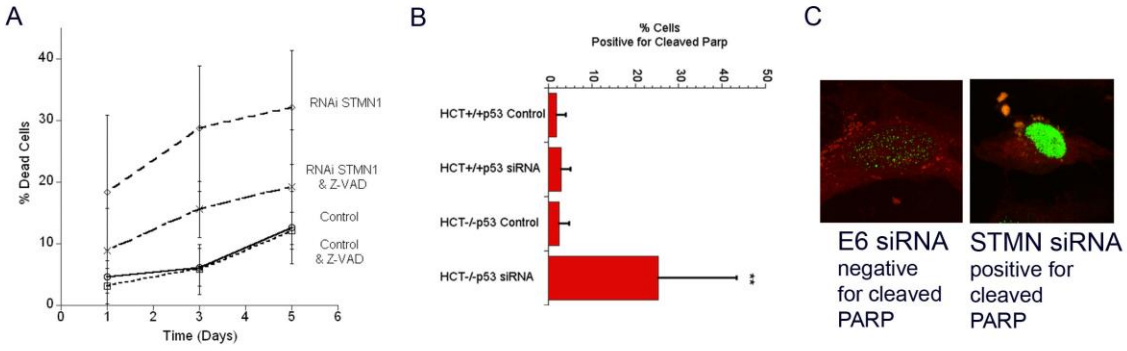
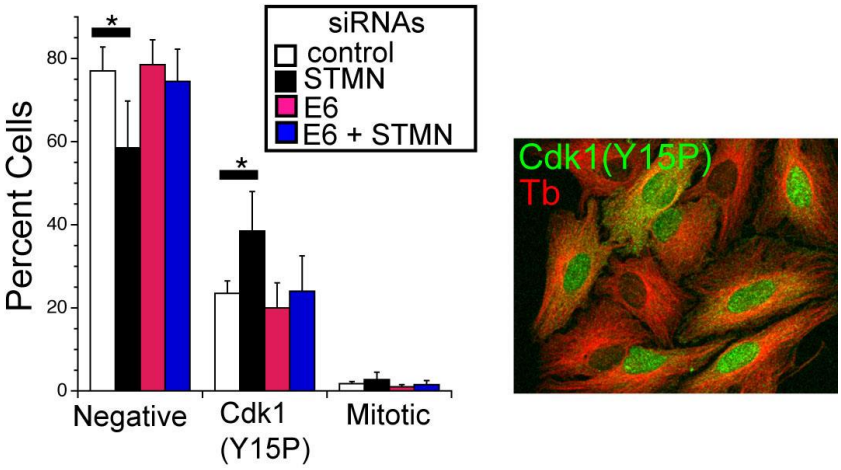


Figure 5 (below) shows an example of the cell cycle delay observed in cells lacking both stathmin and p53. Stathmin depletion increases the population of cells in G2. In this example from Hela cells, stathmin depletion increases the percent interphase cells positive for Cdk1 Y15P, an inhibitory phosphorylation. Restoring p53 reverses the G2 delay. Data from Carney and Cassimeris, 2010.

Figure 5. One example of G2 cell cycle delay in stathmin depleted cells. The percent of cells staining positive for CDK1(Y15P) are shown. The Y15 phosphorylation inhibits CDK1 activation and prevents mitotic entry. Microtubule staining was used to differentiate cells in G2 from those in mitosis. Two other markers were used to confirm the results shown below.

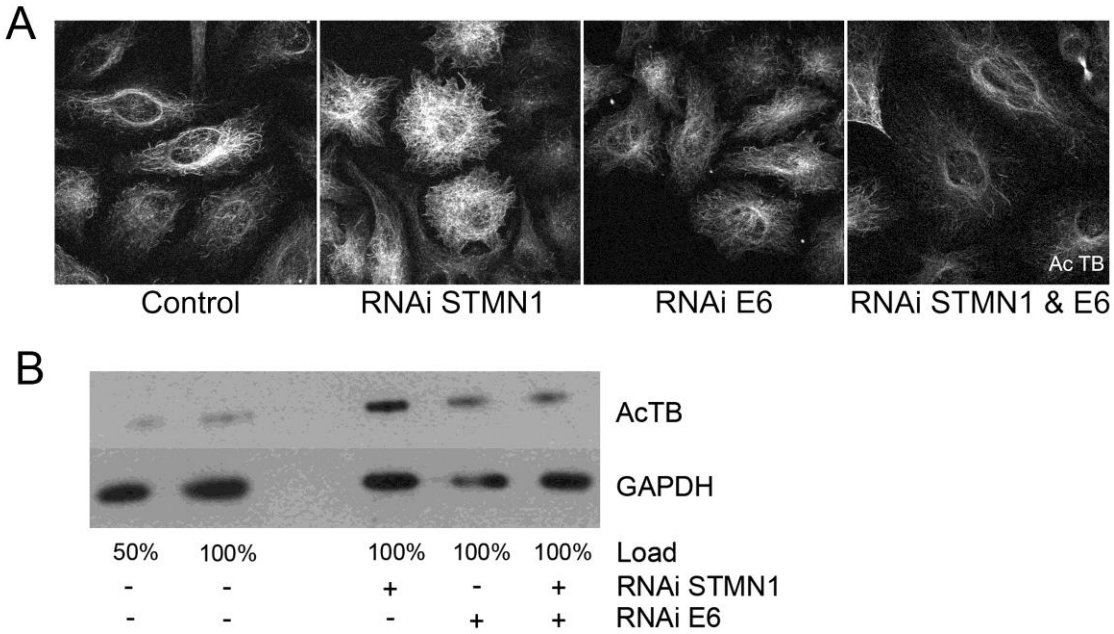


Finding 2. Here we probed the signal intermediate downstream of stathmin depletion, focusing on the cell cycle delay because this delay occurs before apoptosis. These experiments demonstrate that stathmin depletion relays a signal via increased microtubule stability.

Figure 6 (below) demonstrates that microtubules are stabilized to the greatest extent in cells lacking both stathmin and p53. Restoring p53 in Hela cells, by depletion of HPV E6, restores microtubules to their normal stability level. Here stability is represented by the amount of a post-translational modification, acetylated alpha tubulin (AcTb), associated with microtubule stability. We found the same results in a second set of cell lines, matched HCT116 colon cancer cell lines

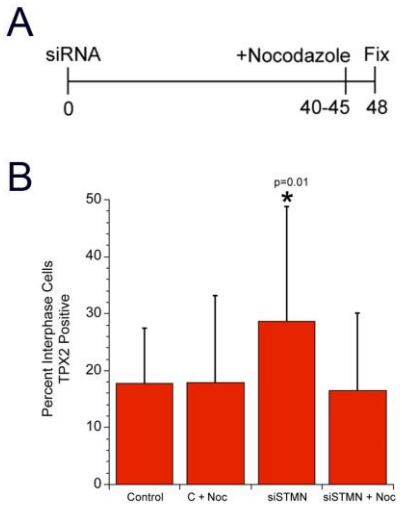
+/+ or -/- for the p53 gene. Additional results (not shown) demonstrate that cells lacking both p53 and stathmin have a greater rate of microtubule nucleation from the centrosome (microtubules are "born" at a greater rate).

Figure 6. Panel A shows representative images of cells stained with an antibody recognizing acetylated tubulin. Panel B shows a western blot confirming that the amount of acetylated tubulin is increased significantly after stathmin depletion.



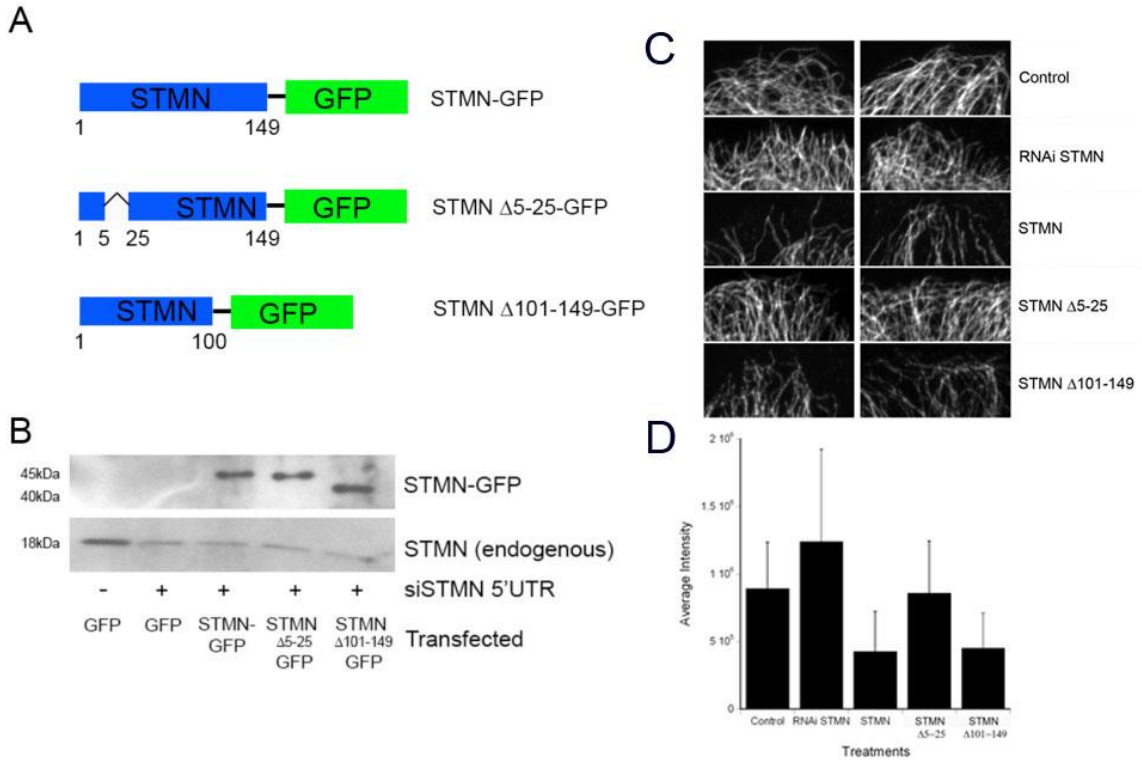
To test more directly whether microtubule stability is causing a G2 cell cycle delay, we asked whether microtubule depolymerization would rescue cells from this delay. Here we used nocodazole incubation for 3 - 5 hours prior to cell fixation. Nocodazole depolymerizes microtubules and blocks cells in mitosis. If stable microtubules are the cause of the G2 cell cycle delay, we expected nocodazole to abrogate the delay and then lead to a mitotic block. Figure 7 (below) shows that this expected result is exactly what we observed.

Figure 7. Panel A outlines the experimental protocol. During the first 40 - 45 hours after transfection of stathmin siRNA the population of cells in G2 will double. Nocodazole is then added to depolymerize microtubules and cells fixed 3- 8 hours later. One example is shown for cell populations stained with TPX2, a protein present in the nucleus from late S phase through mitosis. Microtubule staining allowed easy differentiation between G2 and mitotic cells.



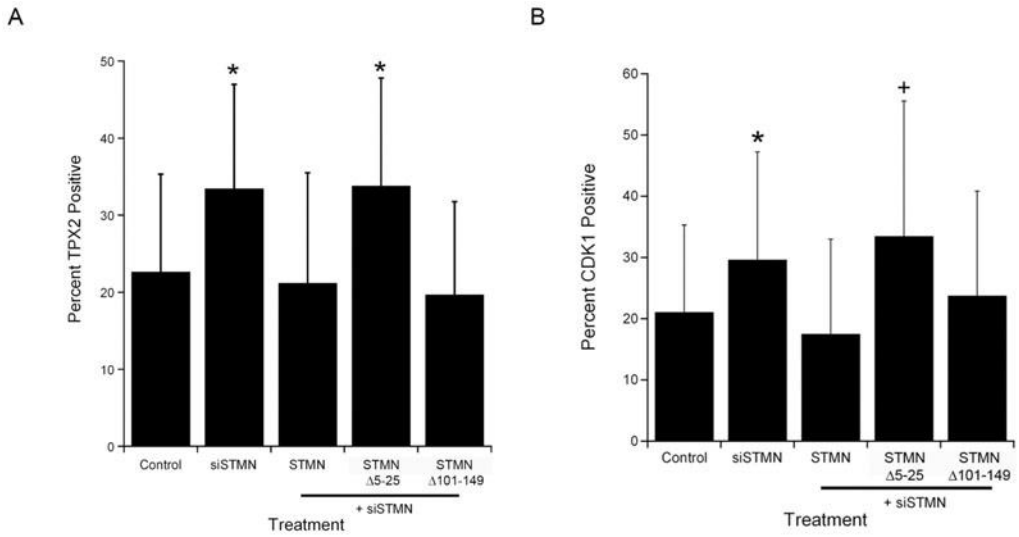
We also performed rescue experiments to express GFP-tagged stathmin or stathmin truncations while simultaneously depleting cells of the endogenous stathmin protein. We developed and optimized an siRNA targeting the 5'UTR of the endogenous mRNA and found that both protein depletion and cell cycle delay in G2 were as efficient as that observed using siRNA's targeting the coding sequence. Control experiments also demonstrated that the GFP-tagged stathmin and truncations behaved as expected based on published results. Both the full length stathmin and the N-terminal half of the protein were able to destabilize microtubules when expressed in cell. A construct lacking amino acids 5 - 25 was unable to destabilize microtubules.

Figure 8 (shown on the next page). Expression of stathmin-GFP or truncated stathmins and microtubule density (a measure of microtubule stability). Panel A shows a representation of the three fusion proteins generated. Panel B shows western blots demonstrating that (a) the siRNA targeting the 5'UTR of stathmin mRNA depletes the endogenous protein level and (b) that the fusion proteins are expressed at the correct molecular weight. Panel C shows that siRNA depletion increases microtubule density, while the full length and N-terminal half of stathmin are sufficient to depolymerize many microtubules. The truncation missing amino acids 5 - 25 does not change microtubule density. Panel D shows image intensity measurements of microtubule density at the cell periphery, quantifying images shown in C.



Using these tagged stathmin proteins, we found that expression of either the full length protein or the N-terminal half was sufficient to rescue cells from a cell cycle delay (Figure 9 below). In contrast, the construct lacking amino acids 5 - 25 did not rescue the delay.

Figure 9. Graphs of the percentage of interphase cells staining positive for TPX2 (Panel A) or CDK1(Y15P). Each protein serves as a marker of cells in G2 of the cell cycle.

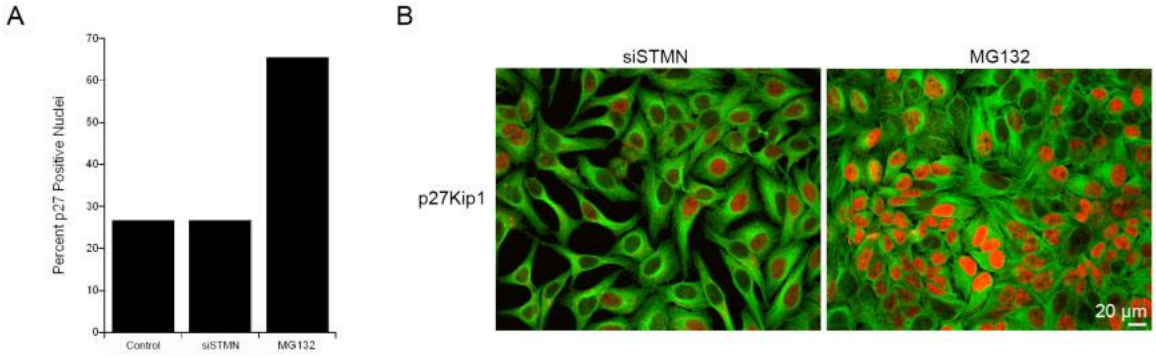


Given that only the truncation able to depolymerize microtubules was also able to rescue the G2 delay provides strong support for microtubule stability serving as a signal intermediate. STAT3

and p27Kip1, stathmin's other known binding partners, bind to the C terminal half of stathmin. Note that this region is not required for rescue, providing evidence that neither of these proteins is the critical signal relay downstream of stathmin depletion.

We confirmed that p27Kip1 was not likely serving as a signal intermediate by examining whether stathmin depletion was sufficient to activate p27Kip1. Activation of p27Kip1 results in the protein's accumulation and transport to the nucleus. As a positive control, we treated cells with MG132, a proteosome inhibitor. Cells treated with MG132 for 3 - 5 hours showed a large increase in cells staining positive for p27Kip1 in the nucleus. In contrast, stathmin depleted and control transfected cells showed indistinguishable staining for p27Kip1 (Figure 10).

FIGURE 10. Stathmin depletion does not activate p27Kip1. A. Percentage of cells staining positive for p27Kip1 in the nucleus. MG132 treatment serves as a positive control. Data shown is from a representative experiment. An additional experiment also failed to show an increase in p27Kip1 positive cells after stathmin depletion. B. Examples of cells stained for tubulin/microtubules (green) and p27Kip1 (red).



SUMMARY

During the funding period we established that several cancer cell lines delay during G2 of the cell cycle and die by apoptosis only when lacking the tumor suppressor p53 and depleted of the microtubule regulatory protein stathmin. The cell cycle delay is mediated by microtubule stability and not by activation of STAT3 or p27Kip1. Future experiments will examine how microtubule stability is able to relay a signal. Possibilities include delayed mitotic entry because the microtubule cytoskeleton cannot be reorganized quickly to form the spindle, or that excess microtubules bind, sequester and inhibit signaling molecules. Support for the latter model comes from numerous studies showing that a large number of signaling intermediates are bound to microtubules and that microtubule binding can inhibit or localize their activity. We are also exploring the mechanism responsible for delaying mitotic entry in cells with stabilized microtubules to understand which enzymes controlling mitotic entry are sensitive to microtubule stability.

Published Abstracts and Presentations:

Carney, B. and L. Cassimeris. 2010. Stathmin/Oncoprotein 18, a microtubule regulatory protein, is required for survival of both normal and cancer cell lines lacking the tumor suppressor, p53.

Presented by B.C. at the annual meeting of the Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA

Carney, B. and L. Cassimeris. 2010. Increasing stable microtubules leads to apoptosis in human cell lines lacking p53. Mol. Biol. Cell. 21: 4299 (abstracts). Presented by BC at the annual meeting of the ASCB, December, Philadelphia, PA

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
_____ 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, listed in the table, in a PDF version 5.0.5 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. Stathmin/oncoprotein 18, a microtubule regulatory protein, is required for survival of both normal and cancer cell lines lacking the tumor suppressor, p53	Bruce Carney and Lynne Cassimeris	Cancer Biology and Therapy	January, 2010	☐Submitted ☐Accepted xPublished
2.				☐Submitted ☐Accepted ☐Published
				☐Submitted

3.				☐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:
A second manuscript is in preparation and should be submitted by September 2011

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.

None

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.*

NIH Biosketch on next page

NAME	POSITION TITLE
Lynne Cassimeris	Professor of Biological Sciences
eRA COMMONS USER NAME (credential, e.g., agency login)	
CASSIMERIS	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Springfield College, Springfield, MA	B.S.	1980	Biology
Univ. North Carolina, Chapel Hill, NC	Ph.D.	1988	Cell Biology
Univ. Pennsylvania, Philadelphia, PA	Post-doc	1988-1992	Cell Biology

B. Positions and Honors

Positions and Employment

1992 – 1996 Assistant Professor, Molecular Biology, Biological Sciences, Lehigh University, Bethlehem, PA
1996 – 2004 Associate Professor, Biological Sciences, Lehigh University
2000 Visiting Associate Professor, Dept. Cell Biology, Duke University, Durham, NC
2004 - Professor, Biological Sciences, Lehigh University

Other Experience and Professional Activities

Editorial Boards:
Cytoskeleton
Cellular and Molecular Bioengineering

Study Sections:

Dept. of Defense. Breast Cancer Research Program.	Member (1998)
American Cancer Society. Cell Cycle and Growth Control	Member (2004-2008)
NIH Biology 2	Member (1999)
NIH CDF4	Member (2000, 2002)
NICH Cell dev. and function (NRSA)	Member (2005; 2007)
NIH NICHD R03	Member (2005 -06)
NIH ZRG-1-CB-N02	Member (2008)
NSF. Cell Biology.	Panel Member (2000, 2009)

Honors (1992 - present)

1992 – 1996 Dana Foundation Faculty Fellow
1999 – 2001 Distinguished Associate Professor, Endowed by Lehigh Univ. Class of '61
2002 Distinguished Graduate Alumnus, University of North Carolina, Chapel Hill
2004 – 2007 Keith R. Porter Fellow
2006 Eleanor and Joseph F. Libsch Research Award, Lehigh University

C. Selected peer reviewed publications (of 72 total)

Most relevant to the current application. Members of my group are underlined.

1. B.K. Carney and L. Cassimeris. 2010. Stathmin/oncoprotein 18, a microtubule regulatory protein, is required for survival of both normal and cancer cell lines lacking the tumor suppressor, p53. Cancer Biol. & Therapy. 9: 699 - 709.

2. Ringhoff, D.N. and L. Cassimeris. 2009. Stathmin regulates centrosomal nucleation of microtubules and tubulin dimer/polymer partitioning. Mol. Biol. Cell. 20: 3451-3458.

3. Segerman, B., P. Holmfeldt, J. Morabito, L. Cassimeris and M. Gullberg. 2003. Autonomous and phosphorylation-responsive microtubule-regulating activities of the N-terminus of Op18/stathmin. J. Cell Sci. 116: 197-205.

4. Howell, B.J., H. Deacon and L. Cassimeris. 1999. Decreasing oncoprotein 18 levels reduces microtubule catastrophes and increases microtubule polymer in vivo. J. Cell Sci. 112: 3713-3722.

5. Howell, B., N. Larsson, M. Gullberg and L. Cassimeris. 1999. Dissociation of the Tubulin-Sequestering and Microtubule Catastrophe-Promoting Activities of Oncoprotein 18/Stathmin. M. Biol. Cell. 10: 105 - 118.

Additional recent publications of importance to the application

1. D.N. Ringhoff and L. Cassimeris. 2009. Gene expression profiles in mouse embryo fibroblasts lacking stathmin, a microtubule regulatory protein, reveal changes in the expression of genes contributing to cell motility. BMC Genomics. 10:343.

2. Cassimeris, L., B. Becker and B. Carney. 2009. TOGp Regulates Microtubule Assembly and Density during Mitosis and Contributes to Chromosome Directional Instability. Cell Motil. Cytoskel. 66: 535-545.

3. M. Murphy and L. Cassimeris. 2006. A novel cancer therapy approach targeting microtubule function. Cancer Biol. Ther. 5: 1721 – 1723.

4. J. Warren, A. Rutkowski and L. Cassimeris. 2006. Infection with Replication-Deficient Adenovirus Induces Changes in the Dynamic Instability of Host Cell Microtubules. Mol. Biol. Cell. 17: 3557 – 3568.

5. Piehl, M. U.S. Tulu, P. Wadsworth and L. Cassimeris. 2004. Centrosome maturation: Measurement of microtubule nucleation throughout the cell cycle using GFP tagged EB1. Proc. Natl. Acad. Sci. 101: 1584-1588.

6. L. Cassimeris and J. Morabito. 2004. TOGp, the human homolog of XMAP215/Dis1, is required for centrosome integrity, spindle pole organization and bipolar spindle assembly. Mol. Biol. Cell. 15: 1580-1590.

7. M. Piehl and Cassimeris, L. 2003. Organization and dynamics of growing microtubule plus ends during early mitosis. Mol. Biol. Cell. 14: 916 - 925.

8. L. Cassimeris. 2002. The oncoprotein 18/stathmin family of microtubule destabilizers. Curr. Opin. Cell Biology. 14: 18 - 24.

9. Holmfeldt, P., N. Larsson, B. Sergerman, B. Howell, J. Morabito, L.Cassimeris and M. Gullberg. 2001. The catastrophe-promoting activity of ectopic oncoprotein18/stathmin is required for disruption of the mitotic spindle. M.Biol. Cell. 12: 73 – 83.