Lehigh University

Annual Progress Report: 2008 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

Lehigh University received $108,901 in formula funds for the grant award period January 1, 2009 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Targeted Killing of Cancer Cells - Cancers harboring mutations in the tumor suppressor protein p53 are typically among the most difficult to treat and make up at least 50% of all human cancers. Tumor cells lacking a functional p53 are resistant to common chemotherapies, which induce a cell death process called apoptosis. These cancer cells require a protein called stathmin for their survival, while normal cells live happily without stathmin. Future development of drugs to mimic stathmin depletion holds the promise to kill cancer cells without producing side effects in normal tissues. To take full advantage of stathmin as a drug target, we need to understand where and how it functions to promote cancer cell survival. This project examines how stathmin blocks apoptosis in cells lacking p53.

Anticipated Duration of Project

1/1/2009 - 12/31/2011

Project Overview

Many cancer cells lacking p53 require stathmin, a microtubule cytoskeleton regulatory protein, for survival. When stathmin is depleted from these cells, they activate apoptosis and die. The p53-independent pathway responsible for apoptosis is not known, nor is it known why stathmin depletion is sufficient to trigger apoptosis in these cells. Our broad research objective is to determine where and how stathmin functions to promote cancer cell survival, or why its depletion induces apoptosis in some cancers. Understanding how stathmin functions will be critical to development of novel chemotherapies based on stathmin function. We cannot simply target stathmin directly because it is a poor target for development of a small molecule inhibitor. In addition, the general use of siRNA-based therapies to deplete specific proteins also remains an elusive challenge at the organism level.

Hypothesis: Our working model is that cells lacking p53 are primed for death, but normally resist death signals because they over-express anti-apoptotic proteins of the Bcl-2 family. Stathmin is

required to maintain these anti-apoptotic signals and its depletion results in loss of anti-apoptotic proteins, tipping the balance toward cell death.

We will test our hypothesis by completion of the following aims: (1) Develop a cancer cell line having stable expression of GFP-tagged cytochrome C allowing us to follow mitochondrial integrity in response to possible apoptotic signals; (2) Examine whether stathmin depletion leads to cytochrome C release from mitochondria, as predicted for loss of anti-apoptotic Bcl-2 proteins; and (3) Determine whether stathmin over-expression protects cells from the drug ABT-737, a drug that blocks anti-apoptotic (Bcl-2 family) proteins.

Principal Investigator

Lynne Cassimeris, PhD
Lehigh University
Department of Biological Sciences
111 Research Dr., B-217
Bethlehem, PA 18015

Other Participating Researchers

Robert Skibbens, PhD – employed by Lehigh University

Expected Research Outcomes and Benefits

Completion of the project will allow us to define a potentially novel pathway to induce death in cancer cells. We predict that stathmin functions similar to anti-apoptotic proteins and protects cells from a normal death pathway that starts by disruption of the cell's mitochondrial membrane. The results of this project could lead to new targets for chemotherapy development for the treatment of cancer. Stathmin protein regulates the microtubule cytoskeleton, a system that is also the target of several successful chemotherapies. Therefore, the results of the project may also suggest novel combinations of chemotherapeutics.

Summary of Research Completed

The control of cell proliferation and survival are critical factors in maintaining healthy cell populations and eliminating those cells defective in proper cell cycle control. A key protein regulating the cell cycle and apoptosis is p53, a protein mutated in >50% of human cancers. We recently found that the combined loss of both p53 and stathmin, a microtubule destabilizing protein, results in cell death by apoptosis (Carney and Cassimeris, 2010). These initial results indicate that stathmin, or the pathway where it functions, could be a target for specific killing of those cancer cells harboring mutations in p53. We are investigating the pathway responsible for apoptosis for cells lacking both stathmin and p53 to understand why stathmin is required for their survival.

Research completed during reporting period:
Depletion of both stathmin and p53 leads to a cell cycle delay during G2. This delay is observed

prior to significant cell death in both Hela (cervical cancer) and HCT116 (p53-/- line; colon cancer). The G2 delay was detected by the presence of several markers: TPX2, a protein synthesized beginning in S phase and degraded during mitosis; cyclin B, a cofactor necessary for mitotic entry and synthesized to high levels during G2; and phosphorylated CDK1 (Y15), a kinase necessary for mitotic entry, held in inactive form by phosphorylation on tyrosine 15. An example from Hela cells is shown below. In these cells we manipulate stathmin level through siRNA-mediated depletion. Hela cells normally lack detectable p53 because they express the HPV viral protein E6. We restore p53 in these cells by depletion of viral E6. As shown below, a G2 delay is observed only in cells lacking both stathmin and p53.

The cell cycle delay was confirmed by following individual cells lacking both stathmin and p53. Cells were followed by phase contrast microscopy and images collected every 5 minutes for 65 hours. The duration of interphase for cells lacking stathmin and p53 is increased significantly over that in control cells, while the mitotic duration is unchanged. Assuming the 8 hour interphase delay in stathmin depleted cells is due to a longer G2, our data from living cells predicts an increase in the percent cells in G2 matching our measurements from fixed cell populations. Together these data point to mitotic entry as the likely step delayed by stathmin depletion.

We also examined whether stathmin functions at mitochondria to regulate apoptosis. Here we looked for a possible synergy between stathmin depletion and addition of the drug ABT-737. This drug blocks the function of anti-apoptotic proteins such as Bcl2, tipping the balance toward cell death. We hypothesized that stathmin could normally function as an anti-apoptotic protein and if so, its depletion would synergize with ABT-737 addition. We did not find support for this model. We find that cell survival is unchanged between cells depleted of stathmin and those both depleted of stathmin and treated with ABT-737.

Research during this funding period was included in the publication:
Carney, B.K. 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. Ther. 9: 699-709.

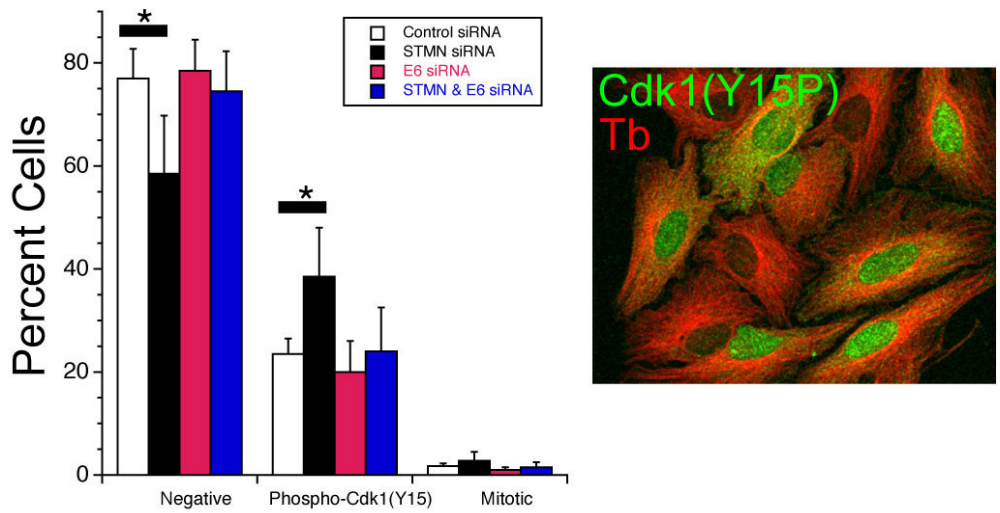

Figure 1: Stathmin depletion increases the population of cells in G2. In this example from Hela cells, stathmin depletion increases the percent cells positive for Cdk1 phosphorylated on tyrosine 15, an inhibitory phosphorylation. Restoring p53 (E6 depletion) reverses this delay.

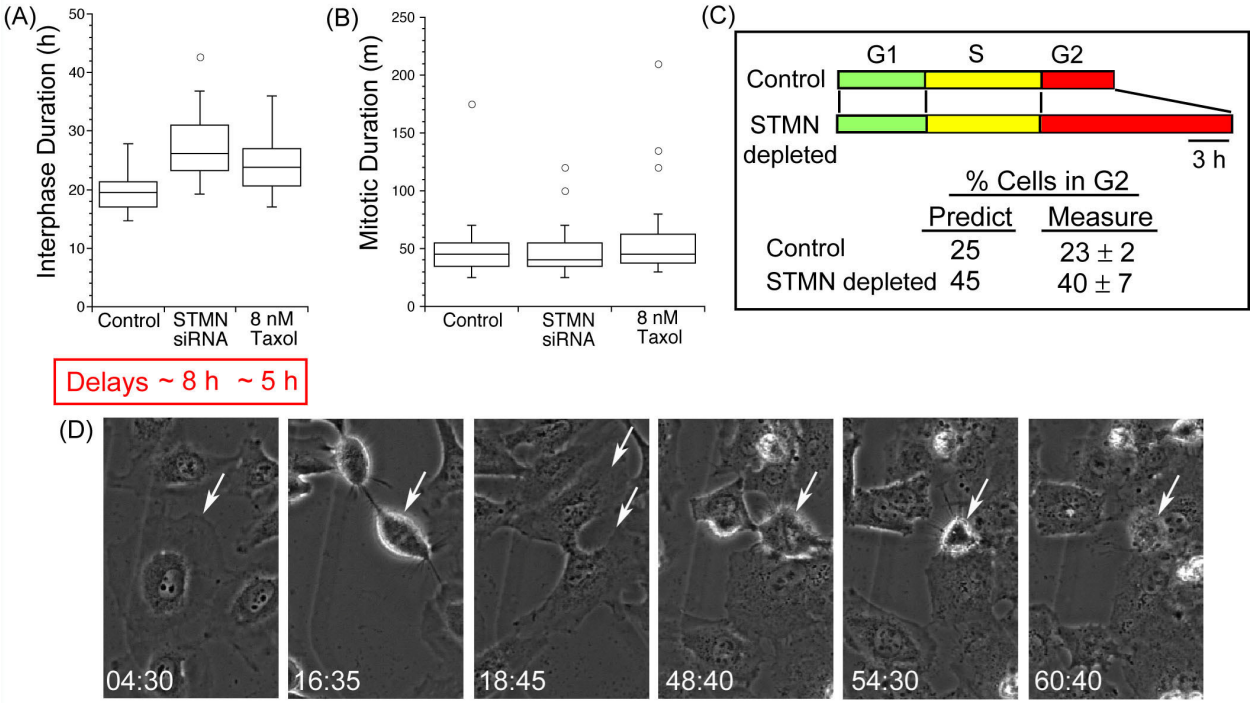

Figure 2: (A,B) Interphase duration is lengthened in stathmin-depleted or 8 nM taxol-treated Hela cells. Cells were imaged at 5 min intervals for 65 hours. Durations of interphase and mitosis are shown in box plots for 30 – 50 cell cycles per condition. (C) The approximate times for interphase stages in control or stathmin-depleted Hela cells, assuming the 8 hour delay in stathmin depleted cells results from a longer G2. The estimates from live cell imaging match those from population studies summaried in Figure 1. (D) Phase contrast image series of a stathmin-depleted Hela cell and its descendants (arrows). After a first division, one daughter entered mitosis after 32 hours, then died another 8 hours later. Time in hrs:min.