Lehigh University

Annual Progress Report: 2008 Formula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

Lehigh University received $108,901 in formula funds for the grant award period January 1, 2009 through June 30, 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.

Duration of Project

1/1/2009 - 6/30/2011

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

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Other Participating Researchers

Robert Skibbens, PhD – employed by Lehigh University

Expected Research Outcomes and Benefits

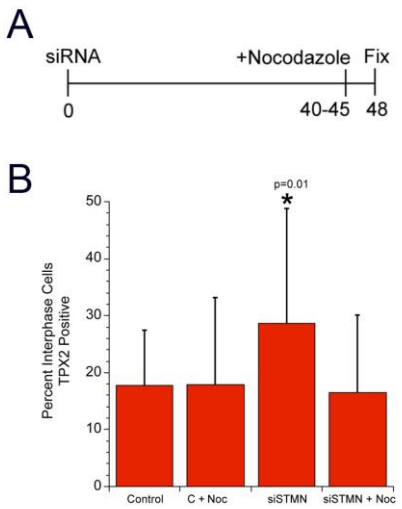
Completion of the project will allow us to define a potentially novel pathway to induce death in cancer cells. We predict that stathmin functions similar to anti-apoptotic proteins and protects cells from a normal death pathway that starts by disruption of the cell's mitochondrial membrane. The results 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

During the final year of the grant we conducted experiments to test whether stathmin depletion causes a cell cycle delay by one of the three possible mechanisms: 1. increasing microtubule stability within cancer cells, 2. activation of the transcription factor STAT3 or 3. activation of the cyclin-dependent kinase inhibitor, p27Kip1. We used drug treatment to depolymerize microtubules and expression of stathmin truncations to differentiate among these models. The data demonstrate that stathmin depletion delays cell cycle progression because the microtubules are hyperstabilized. We are currently writing a manuscript describing these findings. The results will be the first to show a link between stathmin depletion and a cell cycle delay. While we and others have previously shown that stathmin is required for cancer cell survival and cell cycle progression, we are now the first to make inroads in to the mechanism of action.

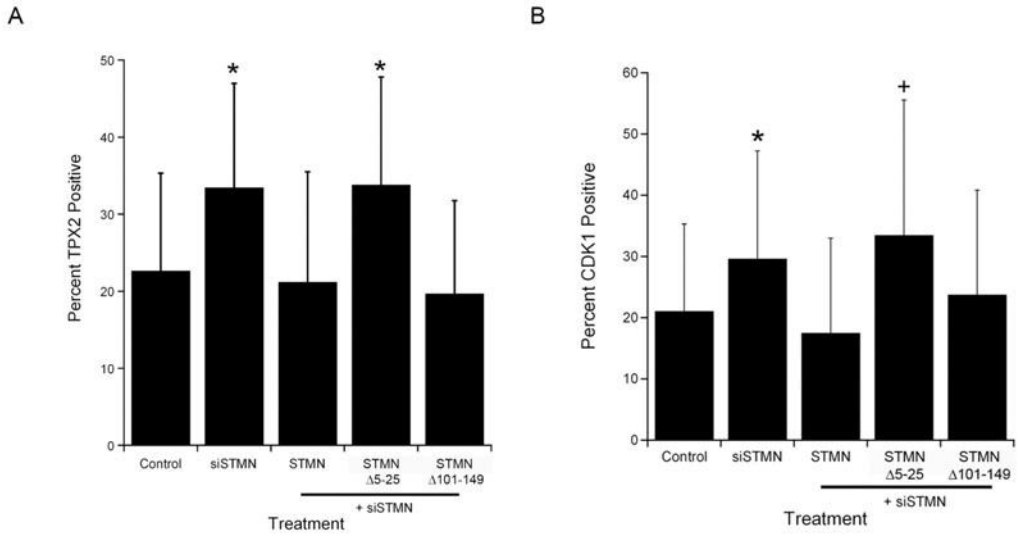
The major results are summarized below.

Figure 1 (below) demonstrates that microtubule depolymerization in a 3 - 5 window is sufficient to abrogate the G2 cell cycle delay observed in stathmin depleted cells. 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 (as shown in the third bar in the graph shown in B). Nocodazole is then added to depolymerize microtubules and cells fixed 3- 5 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 cells. A construct lacking amino acids 5 - 25 was unable to destabilize microtubules.

Figure 2 (below) shows that expression of full length GFP-tagged stathmin or the N terminal half of stathmin are sufficient to rescue cells from a cell cycle delay. Deletion of a small N terminal region (amino acids 5 - 25) results in a mutant protein that cannot depolymerize microtubules (not shown here) and that cannot rescue the cell cycle delay.



The results are shown for two markers used to identify cells in G2. Both STAT3 and p27Kip1 bind to stathmn's C terminus. The ability of the N terminus to rescue cells from the delay also eliminates a role for either of these proteins.

Future experiments, funded through a separate mechanism, will explore the signal transduction pathway activated by microtubule stability.