Allegheny-Singer Research Institute

Annual Progress Report: 2007 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The Allegheny-Singer Research Institute received $237,838 in formula funds for the grant award period January 1, 2008 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Expressomic Approach to Enhanced Wound Healing - Cigarette smoking and tobacco use are known to significantly interfere with the body's ability to heal its wounds. In a wide variety of circumstances smokers are known to have significantly higher rates of complications following surgery than non-smokers, including after breast surgery, abdominal surgery, oral surgery etc., due to impaired wound healing. This poor wound healing results from a variety of causes which are still incompletely understood. In contrast, during developmental life all mammalian species (including humans) are able to heal their wounds flawlessly and without scar. It is likely that different genes are used by the body to heal itself during development than in adult life, that is, the developing organism uses a different "expressome" than an adult. This project seeks to better understand the genes in the "expressome" of developing mammals in the hopes that they may one day be used to improve wound healing in adult tissues.

Duration of Project

1/1/2008 - 12/31/2009

Project Overview

One of the most profound effects of tobacco use on human health is a marked impairment in the ability of tissues to heal their wounds properly. Multiple soft and bony tissue types demonstrate reduced wound healing with concomitant tobacco use. This may manifest both as delayed wound closure as well as increased scar deposition.

Whereas wound healing in adults is always accompanied by some scar formation, fetal mammals are able to heal wounds of their integument (and some other tissues) regeneratively, without scar. This property is intrinsic to fetal tissues, not simply the conferred benefit of the protected uterine environment. It persists until the middle of the third trimester, when a switch to the adult scirrhous phenotype occurs. The different pattern of wound healing in fetal tissues versus adults is likely the result of differential patterns of gene expression in these two tissue types.

We have established an animal model in which wound healing may be examined in both adult and fetal life. Tissues from fetal and adult wounds (as well as unwounded controls) have been examined for their expressomic content, that is, for the full range of the gene products expressed. Preliminary results indicate that fetal animals use a widely divergent gene set from adults to heal their wounds. A substantial number of the identified gene products are novel- either previously undescribed or known only as incomplete expressed sequence tags. This project aims to extend these preliminary analyses to more fully understand the differentially expressed gene products distinguishing scarless fetal wound healing from scar-forming adult wound healing.

Our specific aims in this regard are:

1) To complete expressomic analyses of fetal and adult wound and control (unwounded) tissues, including an examination of microRNAs.
2) To identify (through *in silico* and network analysis) candidate critical genes and pathways regulating scarless wound healing.
3) To verify observed differential gene expression by direct confirmatory assay (e.g., real-time RT-PCR); to determine the expressing cell types (e.g., fibroblasts, keratinocytes etc.) in the wound bed through *in situ* hybridization.
4) To characterize novel differentially regulated genes and microRNAs that may present attractive targets for molecular intervention to enhance wound healing and reduce scar.

Principal Investigator

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Other Participating Researchers

Phillip Gallo, Ph.D. – employed by Allegheny-Singer Research Institute

Expected Research Outcomes and Benefits

This research project undertakes basic molecular and cell biological investigations of a remarkable phenomenon, the scarless wound healing common to developing mammals, in the hopes that an understanding of this process can one day be used to enhance wound healing in adults.

This project will identify new genes that are preferentially used by developing organisms to control their regenerative healing. It will seek to gain a better understanding of their biological functions at the cellular and molecular level. It will set the stage for producing the reagents and materials that can be used for even further characterization of these genes, which may play important roles in other processes besides the wound healing being investigated here.

This project will for the first time seek to examine a new class of RNA called "microRNA" and its importance to wound healing. It will identify microRNAs used by developing animals to modulate their wound healing, allowing for their further study and experimental manipulation.

Our ultimate goal for this project is to identify the critical molecular pathways responsible for ideal wound healing as found in fetal organisms, in the hopes that controlling or targeting these pathways might allow us to intervene with molecular therapeutics to enhance the wound healing response in an adult organism.

The immediate benefits from this project will be a significant increase in our understanding of the way genes work to control wound healing. A downstream benefit will be the discovery of new molecular agents that may prove useful therapeutically to improve wound healing clinically. Improved wound healing can have a profound impact on health status in a huge array of patients, from trauma victims, to postsurgical patients, to those afflicted with chronic wounds from disease, etc.

Summary of Research Completed

Milestone: Complete expressomic comparison of microRNA complements between fetal and adult tissue types (either whole tissue or fibroblast cell).

The above passage represents what we hoped to accomplish in the past year of this award. However, these studies have proven to be the most technically problematic. We have determined that it will be impractical to conduct such an examination on whole tissue, due to the amount of starting nucleic acid material required. We are in the process of evaluating and refining protocols for miRNA isolation and characterization, but since we are working in rabbit, with vanishingly little information available regarding known miRNAs, this too is difficult.

Meanwhile, we have continued to make progress on other fronts. We have continued to characterize clone 11, which we now believe represents the rabbit homolog of Ero1-alpha, a protein involved in regulating the redox state and oxidative protein folding in the endoplasmic reticulum. We have now cloned the 5' UTR and 3' UTR of both spliced variants of rabbit Ero1L-alpha using RNA-ligase mediated rapid amplification of cloned ends (RACE) to yield full-length cDNAs from rabbit fetal tissue, and examination of multiple tissue Northern blots has revealed the putative presence of a third splice variant. These results for the first time implicate redox regulation of the endoplasmic reticulum compartment as an important contributing factor to the biology of wound repair; this work is currently being prepared for publication.

We have simultaneously been investigating a second previously uncharacterized candidate sequence. This sequence, previously noted as an expressed sequence tag without any attached function, has been cloned through the use of RNA-ligase mediated rapid amplification of cloned ends (RACE) to yield a full-length cDNA from rabbit and mouse. A polyclonal antibody detects native protein from various mammalian cell lysates on Western blots that we believe represent isoforms of the protein itself; this protein has a different molecular mass than our polyhistidine/V5 epitope tagged protein expressed in transient transfection in cell culture. Because the function of the protein is unknown, we sought to create a tagged version of the

protein with the tag located at the N-terminus (to ensure that the C-terminal tag is not interfering with the function of the protein.) Therefore, we have used site directed mutagenesis of this uncharacterized clone to introduce a FLAG tag into our mammalian expression construct; this construct protein should still be targeted to its native location when expressed transiently and stably in mammalian cell lines. We plan to compare the expression of these constructs before utilizing these in a two-hybrid system. Further experiments to evaluate the function/localization of this gene product are ongoing.

A third recovered and previously undescribed clone has been found to represent a family of at least four related sequences by Northern blot. We have begun using in situ hybridization with probes representing both orientations from our recovered clones to both confirm that this gene product is differentially regulated in healing wounds (versus control unwounded tissue) and more basically just to determine exactly which strand represents the predicted coding strand. We have developed several quantitative RT-PCR assays to assist in this process. We believe that one of family of sequences observed by Northern blot may represent an extended 3' UTR of a gene, similar to that observed with clone 11 / Ero1L-alpha described above, possibly pointing to a novel regulatory mechanism across gene families.

Research Project 2: Project Title and Purpose

Metabolic Perturbations of Adult Cardiac Progenitor Stem Cells - It is observed that there is a lack of significant improvement in the infarcted myocardium after stem cell grafting in humans. It is not clear why there is less improvement in humans while there is significant improvement in animal models. It is also known that patients that have ischemic heart disease either have diabetes as an underlying morbidity or develop insulin resistance after myocardial infarction. The purpose of this project is to study the effects of hyperglycemia in adult progenitor stem cells and apply new treatment modalities in-vitro to circumvent the detrimental effects of hyperglycemia, and to promote cell regeneration in-vivo and apply this to clinical medicine to prevent the development of diabetic and ischemic cardiomyopathy-related heart failure.

Duration of Project

1/1/2008 – 12/31/2009

Project Overview

Besides being the leading killer, coronary artery disease has a significant indirect impact on the United States economy. According to the American Heart Association's 2007 statistics, loss of productivity due to morbidity and mortality totaled $101.2 billion annually. Strategies targeting the replacement of hearts are limited, costly and associated with high mortality and morbidity. Therefore, treatment options focusing on the regeneration of injured myocardium appear to be the most logical approach.

The lack of significant improvement in the infarcted myocardium after stem cell grafting in humans is a major shortcoming. The exact cause is not clear when there are markedly better outcomes in animal models. It is also known that patients that have ischemic heart disease either

have diabetes as an underlying morbidity or develop insulin resistance after myocardial infarction. It has been observed by us and others that cytokine treatment of infarcted tissue corrects heart dysfunction and cytokine-conditioned media accelerates tissue growth. We have observed a paradoxical effect of high glucose on adult progenitor stem cell growth when incubated with fibroblasts only, suggesting a cytokine mediated paracrine effect. This finding may be used as a future treatment modality where infarcted hearts may be treated with cytokines and the stem cells that are obtained from patients can be grown in an accelerated fashion.

Our research will focus on treatment of hyperglycemic conditions to promote stem cell growth and alternative modalities to promote cell growth when there is no diabetes or hyperglycemia present.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Regenerative medicine has been a focus of cardiac research due to recent discoveries in the cardiac basic sciences. Previously, the heart was considered a terminal organ that did not have any regenerative capabilities. However, like any tissue in the body, the heart can renew itself under normal physiological conditions. Nonetheless, the aging body loses its regenerative capability; this impacts the elderly population where the majority of the morbidities arise. To date, the success of clinical trials that target regeneration of failing or infarcted myocardium has been limited; probably due to multifactorial causes. Pre-existing diabetes or insulin resistance in persons with failing hearts and after heart attacks has detrimental effects in the treatment and recovery of the effected myocardium. It is our hypothesis that hyperglycemia that occurs after myocardial infarction would be one of the major causes of limited success that is seen in clinical stem cell research, although this issue has not been entertained before. It is well known that stem cells in the diabetic heart behave like a senescent cell with limited regenerative capability. We have also shown clinical and basic research success in treating diseased hearts with GLP-1. We have asked the question whether the beneficial effect that we see is a combination of metabolic corrections in the effected cells and improvement in the regenerative capacity of the myocardium. GLP-1 has increased the regeneration capacity in one dose in the progenitor cells that we tested. We expect to see a similar response in the diabetic, myocardial infarction rat model after being treated with GLP-1. We are also expecting to see the beneficial effects that occur through the insulin cascade, specifically the Akt signaling cascade, and via p38 MAPK.

We are expecting to find that the increased number of progenitor cells in a high glucose group is a phenomenon of cytokines and related paracrine activity.

Summary of Research Completed

During the last six months of this project, there was no further laboratory work performed. Funds were spent on the project during the reporting period, but the principal investigator left the institution on December 31 2009 and would not provide a summary of progress for the period 7/1/09 – 12/31/09.