Lincoln University

Annual Progress Report: 2009 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2010

Formula Grant Overview

Lincoln University received $23,171 in formula funds for the grant award period January 1, 2010 through June 30, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Polymorphisms in the 1,25D$_3$-MARRS Receptor: Characterization and Association with Serum Vitamin D Levels - The purpose of this project is to expand the scope of current research activities to include investigations on existing tissue and blood samples to determine the prevalence of polymorphisms in the 1, 25D$_3$-MARRS receptor, which is a membrane receptor for vitamin D. Lower incidences of cancer have been correlated with increasing levels of vitamin D, but the mechanism of action is not well understood. It is possible that different forms of both vitamin D receptors cause vitamin D to have different effects. Therefore, we will examine the occurrence of polymorphisms (different forms) in three different genes associated with activity of 1α, 25 (OH)$_2$-vitamin D.

Anticipated Duration of Project

1/1/2010 - 6/30/2012

Project Overview

Human studies of dietary vitamin D intake and serum vitamin D levels have not reported consistent associations with cancer risk. Several recent meta-analyses have reported that genetic polymorphisms in the nuclear vitamin D receptor (VDR) are associated with either increased or decreased risk of certain cancers. It is possible that polymorphisms in the membrane-binding protein, 1,25D$_3$-MARRS, may also influence 1,25D$_3$ effects on cancer. Thus, this project will determine the frequency of specific 1,25D$_3$-MARRS polymorphisms, in a healthy population, and molecularly characterize the interactions between genetic variants of VDR and 1,25D$_3$-MARRS in a lung carcinoma cell line (H1299) as they influence metabolism of vitamin D. We will also study the effects of interactions between VDR and 1,25D$_3$-MARRS on specific gene promoters that may provide necessary information to better design treatments for specific cancers.

The Specific Aims are to:
1. Identify existing and novel polymorphisms and estimate their prevalence in a healthy population through screening a sub-sample (N=25) and genotyping a larger sample (N=100), and
2. Determine whether there are differences in transactivation of vitamin D-responsive genes related to polymorphisms in the $1,25D_3$-MARRS receptor.

Aliquots of genomic DNA that was previously isolated from dried blood spot cards from 25 randomly selected African American participants will be sequenced within a 400 bp region encompassing exons I and VI of $1,25D_3$-MARRS. Polymorphisms will be identified by visual inspection of the chromatogram and BLAST alignment with the wild-type sequence. Genotypes of 100 participants will be measured on genomic DNA isolated from frozen lymphocytes of all 100 participants, using a SNP genotyping assay designed for identified polymorphisms. The H1299 cells will be transfected with combinations of the two VDR polymorphic variants, wild-type 1,25D3-MARRS, and siRNA plasmids for each of these constructs. The measured endpoint will be activation of 24-hydroxylase as measured by a luciferase reporter construct.

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Other Participating Researchers

Robin T. Wilson, PhD – employed by Penn State College of Medicine

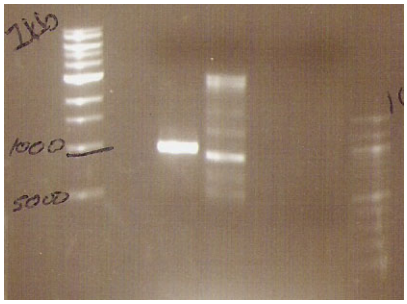
Expected Research Outcomes and Benefits

This research is expected to show the effects of specific, known polymorphisms on efficiency of $1,25D_3$ binding and stimulation of biological activity. $1,25(OH)_2$-D_3 regulates both membrane and nuclear events. Polymorphisms found in both the nuclear receptor (VDR) and membrane receptor ($1,25D_3$ – MARRS) for 1, $25(OH)_2$-D_3 will be investigated. There are two polymorphisms in VDR which result in different lengths of the vitamin D receptor (VDR-short and VDR-long). Because of this change in the protein that has demonstrated significant differences in gene transactivation, and because meta-analyses of several studies have identified an increased association with cancer among those individuals homozygous for the VDR-long form, it is expected that the two forms of VDR will show significant differences in efficacy of stimulating the promoter for 1, 25 hydroxylase. Polymorphisms expressed in $1,25D_3$ – MARRS are expected to affect binding, as the largest number of identified polymorphisms is found in the proposed binding region for 1,25 D_3. Individuals expressing polymorphic $1,25D_3$ – MARRS would be expected to bind $1,25D_3$ less efficiently, and therefore $1,25D_3$ - stimulated membrane activities would either be altered, or not occur at all. The results of this study will improve our understanding of the effect of vitamin D status on various health and disease outcomes.

Summary of Research Completed

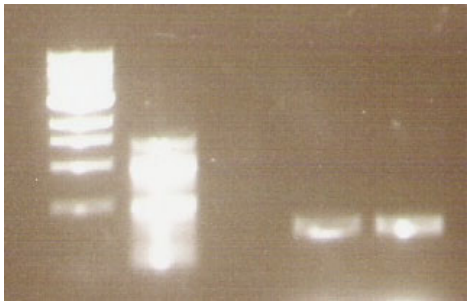
Specific Aim 1

Primers were designed to amplify Exons I and VI of 1,25D_3-MARRS (also known as PDAI3, Erp57, GRP58) using the Primer 3 tool available from the NCBI website. The primers were checked for potential problems such as presence of hairpins, possibility of annealing with random sequences, and homo- and heterodimerization using the oligoanalyzer tool available on IDT (Integrated DNA Technologies) website. Resulting primers were tested in a polymerase chain reaction (PCR) using a human DNA test sample. The PCR was done with and without DMSO as a protectant against self-folding, using Phusion technology (New England Biolabs). The PCR reactions resulted in a single band of the expected size from each primer. Exon I, which has the larger PCR product (Figure 1), requires DMSO as PCR without DMSO resulted in multiple bands. The PCR results for Exon VI were identical with and without DMSO (Figure 2).The PCR products were gel purified, dried down, and sent with the primers to Penn State University's core sequencing lab to test the efficacy of the primers in providing the appropriate DNA sequence. We are waiting on those results before proceeding.



1 2 3 4

Figure 1. Lanes 1 and 4 show molecular weight markers, 1000 bp and 100 bp, respectively. Lane 2 shows PCR product of Exon I with DMSO and Lane 3 shows PCR product of Exon I without DMSO.



1 2 3 4

Figure 2. Lanes 1 and 2 show molecular weight markers, 1000 bp and 100 bp, respectively. Lane 3 shows PCR product of Exon VI with DMSO and Lane 4 shows PCR product of Exon VI without DMSO.