

Philadelphia College of Osteopathic Medicine

Annual Progress Report: 2006 Formula Grant

Reporting Period

July 1, 2010 – December 31, 2010

Formula Grant Overview

The Philadelphia College of Osteopathic Medicine received \$15,859 in formula funds for the grant award period January 1, 2007 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Nutritional Factors in Prostate Cancer - The purpose of this research project is to use the effects of resveratrol (RSV, a molecule particularly abundant in grapes and red wine) on prostate cancer as a model system to decipher the chemo-preventive mechanisms of nutritional factors in prostate cancer development (initiation, progression, and metastasis). Results from this research will be helpful in determining whether RSV should be clinically tested as a dietary supplement. In addition, the knowledge obtained from this research could also be applied in the exploration of newer and more efficient nutritional factors against prostate as well as other cancers.

Duration of the Project

1/1/2007 – 12/31/2010

Project Overview

The long term goal of the PI's research is to elucidate the underlying molecular mechanisms of the chemopreventive effects of dietary and nutritional factors, and to apply this knowledge in the identification of newer, more effective means of cancer prevention. The specific focus of this study will be resveratrol (RSV), a substance found primarily in the skins of red grapes, which is a notable component in red wine. Due to its oral bioavailability, low toxicity, and especially its cancer-preventing and longevity-enhancing properties, RSV has been identified as one of the most promising preventive agents against various cancers. However, our knowledge about the molecular mechanisms behind RSV's effects remains vague. Prostate cancer has been used as a model system in studying RSV's chemopreventive effects. Androgen receptor (AR) is a transcriptional factor involved in transcriptional regulation of a multitude of genes, and it also plays an essential role in prostate cancer tumorigenesis.

The immediate goal of this research project is to decipher the molecular mechanisms of RSV's chemopreventive effects through alteration of AR transcriptional activity. Specific Aim 1: To demonstrate that RSV exerts its effects on AR transcriptional activity: (i) Demonstrate that RSV

antagonizes androgen and affects AR target gene expression by RT-PCR or real-time PCR. (ii) Use ARE-driven reporter gene assays to demonstrate RSV effects on AR activity. (iii) Use the siRNA technique to generate AR(-) LNCaP cells to demonstrate the essentiality of AR in regulation of AR target gene expression. Specific Aim 2: To demonstrate that RSV affects AR activity by affecting recruitment of co-regulators in AR target gene promoter/enhancer regions. Chromatin immunoprecipitation (ChIP) assays will be used as the main methodology to determine the RSV effects on AR-mediated recruitment of co-regulators. Results from the research project will enable us to elucidate the AR-mediated RSV effects in prostate cancer prevention. In addition to providing information on the potential usefulness of RSV as a specific dietary supplement for prostate cancer prevention, this work will also provide valuable insights into the design of new strategies for directly combating the essential co-regulators in prostate cancer.

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Other Participating Researchers and Employers

Ellen Cho, Joseph Farrell - employed by Philadelphia College of Osteopathic Medicine

Expected Research Outcomes and Benefits

Prostate cancer is the most prevalent form of cancer among American men. About 234,460 new cases of prostate cancer will occur in the United States in 2006. In Pennsylvania, about 13,290 men will develop prostate cancer in 2006. This is the fourth highest number of new cases in the 50 states. Prostate cancer is a leading cause of male cancer deaths. The high occurrence rate of prostate cancer and the relatively slow progression to significant disease make it an ideal target for chemoprevention. Chemoprevention is the use of dietary changes or supplements to prevent the development of or slow the progression of cancer. Previous research has found dietary factors linked to a possible increase or decrease in prostate cancer risk. Some studies have specifically identified food components that may be useful in prostate cancer chemoprevention. Identifying nutritional factors to apply in chemoprevention may therefore help to improve health status by preventing prostate cancer or helping men who develop this disease to avoid surgery. In addition, a chemoprevention approach to prostate cancer could greatly improve health status by serving as a model for other types of cancer. Access will be improved by providing information for physicians and other health professionals to use when counseling their patients about diet and nutrition. The U.S. Preventive Services Task Force recommends that counseling “be tailored to the individual risk factors, needs, preferences, and abilities of each patient.” Information on dietary factors that could help patients to lower their prostate cancer risk or slow disease progression would be an important component of access to such individualized counseling.

Summary of Research Completed

Mechanisms of RSV repressive effects

Previously, we have demonstrated that resveratrol (RSV) represses androgen receptor (AR) transcriptional activity by inhibiting AR target gene PSA expression and reporter luciferase activity. In order to understand the mechanisms of the RSV effects on AR transcriptional activity, we first examined the hormone-stimulated nuclear translocation of AR with and without RSV treatment for two hours. We found that without R1881 stimulation, most AR protein resides in the cytoplasm; after two hours treatment with R1881 most of AR protein was found in the nucleus. Therefore, RSV treatment itself did not affect AR subcellular location. Further tests showed that treatment of cells with a combination of R1881 and RSV did not affect hormone-induced AR nuclear translocation. In addition, treatment of the AR(+) cells with R1881 and RSV did not affect AR protein levels. This further demonstrated that in the AR(+) cells, AR is expressed constitutively, and the effects of RSV on AR target gene expression are the reflection of AR transcriptional activity.

We then focused effort on the effect of RSV on the AR and ARE interaction. The results from ChIP assay (Figure 1) show that the artificially expressed AR interaction with ARE is, as expected, induced by the synthesized androgen R1881. The DNA bands in the inputs and in the precipitation of IgG indicate that the ChIP assay was successful. But the RSV has no effect on androgen induced AR/ARE interaction. This excluded the possibility that RSV represses AR activity by affecting AR/ARE interaction, and indicated that the effects are post AR/ARE interaction.

RSV inhibits AR activity and prostate cancer cell growth

We have successfully shown that RSV affects AR function by down-regulating AR transcriptional activity. One of the ultimate goals of our research is to demonstrate that RSV can inhibit AR activity and repress prostate cancer development. Next, we conducted an experiment to show that RSV not only inhibited AR activity but also cell growth in AR-dependent prostate cancer cells (LNCaP). The cells were treated overnight with or without RSV, and mRNA was purified for RT-PCR. First, we found that RSV inhibited prostate cancer cell growth in a dose-dependent manner (Figure 2A). Second, since PSA is the best biomarker and has been used as the gold standard of prostate cancer screening, we showed in Figure 2B that RSV inhibited the mRNA levels of both AR and the AR-target gene, PSA. Therefore, our research results not only confirmed the effects of RSV on prostate cancer growth but also dissected the underlying molecular mechanism.

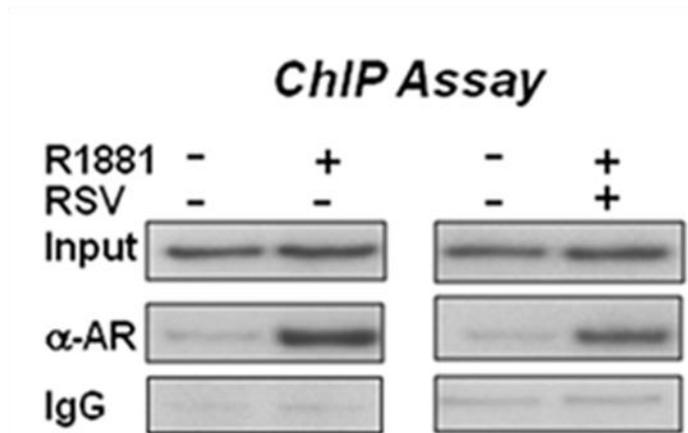


Figure 1, Effect of RSV on AR/ARE interaction. Cells were treated with either R1881 or RSV alone, or in combination of both overnight as indicated. ChIP assays were conducted and PCR was done with specific primers for PSA promoter.

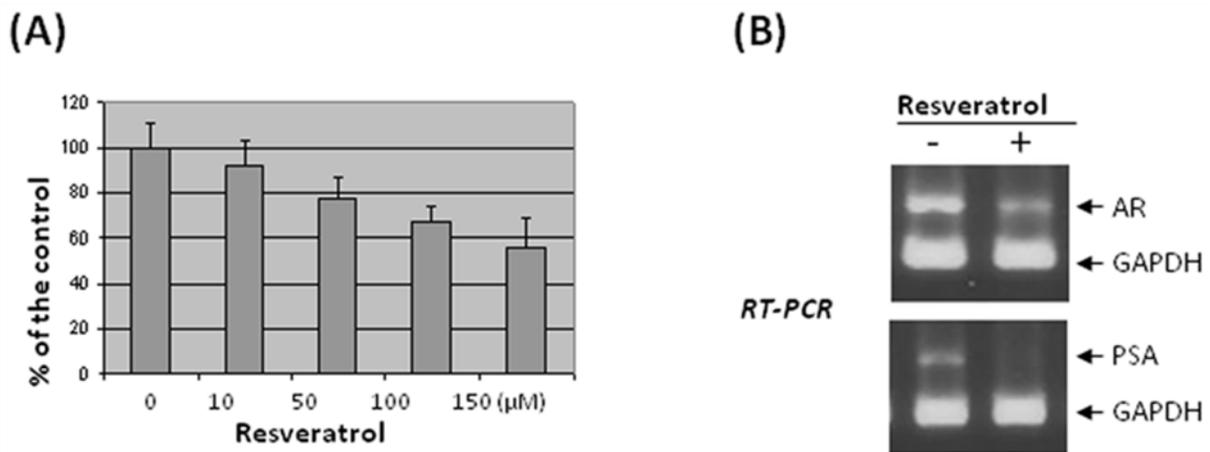


Figure 2. RSV Effects on LNCaP cells. (A) RSV inhibits LNCaP cell growth in a dose-dependent manner. Equal numbers of cells were seeded in growth medium containing 10 nM R1881, and different concentrations of RSV as indicated. After 3 days of treatment, cells were collected and cell numbers were determined. The numbers of cells in different RSV treatments were expressed as percentage of the control (0 uM RSV). The experiment was conducted in triplicate and the averages were plotted and shown in (A). (B) RSV down-regulates the mRNA levels of AR and the AR target gene PSA. Total RNA was isolated from cells treated with 50 μM of RSV for 3 days. AR and PSA mRNA levels were estimated by RT-PCR. In order to obtain more accurate measurements, the internal control GAPDH was amplified together with genes of interest in the same PCR reaction.