RESEARCH EQUIPMENT RESERVE FUND (RERF) REPORT

1. Instrument: Nucleofector® II Transfection System

PI: Siva Kumar Kolluri, Department of Environmental and Molecular Toxicology, College of Agricultural Sciences.

Award Date: February 2011

Amount of award: $11,991

2. Final Budget statement: Entire award ($11,991) was used towards the purchase of Nucleofector Transfection System.

3. A brief summary of the scholarly work made possible as a result of the RERF funding.

Efficient gene expression or gene knockdown is an important technique in modern molecular biology research. It is critical to identify molecular pathways that are dysregulated in pathological conditions in order to target and rapidly develop new therapeutics. Once a candidate therapeutic has been identified, it is equally important to understand its mechanism of action by gene expression or gene knockdown methodologies. Nucleofector transfection system is a gene delivery method designed for primary cells and difficult-to-transfect cell lines. Certain cancer cells are very difficult to introduce genes of interest to test the introduced gene effects on their growth. Nucleofection device is enabling us to perform mechanistic studies in understanding the role of target proteins in carcinogenesis.

4. A brief summary of any additional scholarly activities the RERF funding made possible for the investigator(s).

The following experiment conducted in our laboratory demonstrates the efficiency of Nucleofector Transfection System in introducing a new gene (Green Fluorescent Protein (GFP)) into a tumor cell line. The tumor line used in this study normally resists taking up new genes by other experimental methods.
Figure 1: Expression of a new gene (Transfection) into a cancer cell line using Nucleofector Transfection System: A) Transfection condition codes employed for expression of GFP with the indicated buffer (SF). B) Transfection of GFP at different conditions indicated in A resulted in different degree of GFP positive (+) cells. Several conditions resulted in successful introduction of a GFP gene into the tumor cell line as indicated by the shift of blue histogram to right in panels 1-10 and 13-15. However, some experimental conditions didn’t allow efficient introduction of GFP into the tumor cell line as indicated by the blue histogram that remained on the left in panels 11, 12 and 16. C) Quantitative results indicating percentage of cells positive for GFP using three different buffers SF, SE, and SG with the conditions indicated in panel A.
5. Successful external funding proposals that have been submitted as a result of the RERF funding.

1. *Aryl Hydrocarbon Receptor Modulators for the Treatment of Hepatocellular Carcinoma.*
   
   RES019000A Kolluri (PI) 09/01/2011 – 08/31/2014 $402,000
   
   National Institutes of Health

2. *Small Molecules that Convert Bcl-2 from Protector to a Killer for Breast Cancer Treatment cancer cells.*

   Kolluri (PI) 09/01/2012 - 08/31/2014 $547,500
   
   Department of Defense/Breast Cancer Research Program

3. *AhR as a novel pathway for the induction of regulatory T cells*

   1 R01 ES016651-01A1 Kerkvliet (PI); Kolluri (co-I) $1,825,000
   
   06/01/2013 - 06/30/2018
   
   National Institutes of Health