

**Name:** Surya Amarachintha,  
Dept of Biological Sciences,  
Bowling Green State University.

**Title:** Identification of PKC $\epsilon$  substrates in tumor cells by MALDI TOF-MS.

Protein Kinase C (PKC) is a family of rather similar isotypes. The first isotype discovered among the novel class of serine/threonine kinases is phorbol ester/diacylglycerol-sensitive and calcium-independent. It was called PKC $\epsilon$ <sup>1</sup>. Several physiological functions like activation of nervous, endocrine, exocrine, inflammatory, and immune systems are regulated by PKC $\epsilon$ . Depending on the type of activation PKC $\epsilon$  can play two different roles in disease processes, first, its controlled activation displays a protective role in postnatal myocardial development, and against ischemic damage and Alzheimer's disease. Secondly, its uncontrolled activation leads to malignant tumors and diabetes<sup>2</sup>. As PKC $\epsilon$  plays an important role in cancer progression, several inhibitors have been developed and tested in in vitro and in vivo cancer models trying to control the disease. Although PKC $\epsilon$  inhibitors down-regulate the activity of the enzyme, this down regulation could support the continuous low level of activity rather than making the enzyme inactive<sup>3</sup>.

Cells when exposed to phorbol 12-myristate 13-acetate (PMA) go through a series of morphological changes, which include stimulation of membrane ruffles, appearance of stress fibers, and remodeling of actin cytoskeleton that are also characteristic of oncogenic transformation<sup>4</sup>. Previous studies in Dr. Heckman's lab have demonstrated that PKC $\epsilon$  was the only isozyme to show decline in content correlated with increased stress fiber accumulation. This could take place either by directly turning over one of the stress fiber components or by regulating an upstream step affecting fiber organization<sup>5</sup>.

My study involves the consequences of PKC $\epsilon$  activation and identifying its substrates by gene knockout and protein degradation. I will be using both normal and cancer cell types of rat tracheal epithelial cells. It is known that PKC $\epsilon$  while degrading is still under activation and thus targets many substrates, which are yet to be determined. A matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF-MS) method is used in this study as it helps to identify the biomolecules with an accuracy of 0.01% of the total molecular mass of the sample. In conclusion, this study

will identify the substrates, which can ultimately aid in the evolution of potential targets for cancer therapy specifically against those which gain a phosphate moiety due to PKC $\epsilon$  activity.

#### References

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