9th International Symposium on
Focal Therapy and Imaging in prostate & kidney cancer
June 23-25, 2016
Washington Duke Inn Hotel, Durham NC, USA
Presented by: Duke University School of Medicine
In collaboration with:

PP47

ROLE OF ENDOCYTIC TRAFFICKING IN LOCAL GENE DELIVERY VIA ELECTROTRANSFECTION

L. D. Cervia, F. Yuan

Duke University, Durham, North Carolina, USA

Introduction: Gene therapy has the potential to improve local control of prostate cancer. It can be more effective when it is combined with other treatment modalities. However, the main limitation of all gene therapies is the ability to introduce therapeutic genes into the cell nucleus safely and efficiently. A non-viral method for gene delivery is called electrotransfection, which involves the application of a pulsed electric field to introduce naked plasmid DNA (pDNA) into cells. Although the method has great potential in clinical applications, mechanisms by which the electric field delivers pDNA through the plasma membrane, cytosol, and nuclear membrane remain unknown. A widely-held theory was that the electric field permeabilizes the plasma membrane by forming pores allowing pDNA to enter the cell, referred to as electroporation. However, several key experiments illustrated the timeframe of pDNA uptake exceeds the lifetime of transient pores. We hypothesized that endocytosis was responsible for the uptake and guidance of pDNA to the nucleus. To test the hypothesis, we studied the role of endosomal escape of pDNA in electrotransfection as well as effects of inhibiting specific endocytic transitions to identify the optimal point of endosomal release of pDNA.

Methods: Endosomal release was investigated by means of photochemical internalization (PCI), which involved embedding photosensitizer in endosomal membranes such that upon light treatment, the membrane was ruptured. To study the essential point of release, chemical treatments (Bafilomycin A1, chloroquine, and ammonium chloride) were utilized to inhibit progression of pDNA at different stages of endosomal trafficking.

Results: Early endosomal accumulation with Bafilomycin A1 resulted in a decrease in electrotransfection efficiency (eTE), demonstrating that progression in endocytic trafficking was essential for delivery. Surprisingly, all photochemically- and chemically- induced endosomal escape resulted in decreased eTE.

Conclusion: The negative impact of endosomal accumulation and endosomal escape upon eTE provided evidence that endosomes were crucial for transport and protection of naked pDNA. The effect of endosomal release on electrotransfection provided insight into the delivery mechanism and suggested that pDNA should be protected to improve gene delivery efficiency for clinical use.

Sources of Funding: This research was supported by generous funding from National Institutes of Health (GM098520) and National Science Foundation (BES-0828630), and the Duke University Pharmacological Sciences Training Program (PSTP) (T32 GM 007105).