



## 2009 Research Grant Program Winning Abstract

### A Novel High-Content Assay for Renal Cell Biology

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Diabetic nephropathy (DN) is the most common cause of chronic kidney failure and accounts for 40% of new cases of end-stage kidney disease (ESRD) in the United States. DN is the primary cause of morbidity and mortality in type 1 diabetes mellitus (T1DM) patients, and affects approximately 30% of these patients. Chronic elevated glucose levels associated with diabetes adversely affects kidney function and leads to dysfunction in several types of renal cells. To elucidate the mechanisms contributing to hyperglycemia-induced cell damage, a variety of renal cell culture systems have been utilized. Primarily as a consequence of availability, most existing studies have focused on a small subset of renal cell types, whereas the kidney contains more than 26 types of cells, including multiple types of tubular epithelial cells, glomerular cells, interstitial cells, and those of the vasculature. Our approach utilizes a high-content imaging strategy that has been applied to highly heterogeneous cell populations. Such a methodology has applications to high-throughput cell-based screening and for biomarker analysis of biopsy material.

This proposal will optimize cell-based assays on a commercial source of primary renal cells prepared from human donor kidneys. The advantages include the cells have not been transformed as in immortalized cell lines, and thus a more faithful system for studying processes such as replication, survival, and differentiation. Also, optimization of the assays with human renal cell culture will benefit future studies where biopsy material may be available.

A variety of renal cell sources are available, including differentiated stem cells, immortalized cell lines, and primary cultures from nephrectomized kidney tissue. This proposal will optimize cell-based assays on a commercial source of primary renal cells prepared from human donor kidneys. The advantages include the cells have not been transformed as in immortalized cell lines, and thus a more faithful system for studying processes such as replication, survival, and differentiation. Typically, a heterogeneous population of cells would be considered a practical disadvantage when assessing effects of treatments. However, the ability to measure effects on a cell-by-cell basis alleviates this disadvantage. Automated cell imaging methods facilitate a rapid and unbiased acquisition from a rather large number of cells and can measure multiple parameters from single cells. Simultaneously assessing a diversity of cell types adds a layer of multiplexing, where the most susceptible cell types to 'diabetic' culture conditions can be identified from the mixed cell population. Utilizing a 7x9 montage with a 20X High NA objective, an entire 3x3mm 384 well can be acquired with 4 multiplexed readouts using the BD Pathway 855 Bioimager. Individual cells are assessed for presence or absence of differentially expressed proteins which are used to discriminate cell types. Cells are next challenged with hyperglycemic conditions to mimic the diabetic phenotype, and the susceptible subpopulations are identified.



Thus from a heterogeneous cell population, hyperglycemia-sensitive cells are identified and pursued with additional functional assays of proliferation (Ki-67), NFκB activation (luciferase assays; TNF IHC) and measurement of reactive oxygen species.

Assays will be amenable for evaluating pharmacologic compound, RNAi, and overexpression screens in the context of hyperglycemia effects. Biomarkers observed to associate with hyperglycemia effects in this study have the potential for development into diagnostic tools in biopsy tissue. Although the focus of this proposal is to develop high-content assays for evaluating the mechanisms of diabetic nephropathy, these assays can readily be adapted for other chronic kidney diseases and the study of renal cell carcinomas. In addition, the cell identification algorithms and functional assays developed here will have diverse applications in other examples of heterogeneous cell preparations.

BD Biosciences instrumentation and reagents will play vital roles in this project. The BD Pathway high-content bioimager has proven a formidable tool for acquiring the mixed cell images using the 384 well plate format. BD Biosciences antibodies (ie, TNF, Ki-67), Argutus test kits, as well as 384 well microplates will be key components to this proposal.

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