

FOURTH WORKSHOP ON VALIDATION AND QUALIFICATION OF
NEW IN VITRO TOOLS AND MODELS FOR THE
PRE-CLINICAL DRUG DISCOVERY PROCESS

MARCH 6-7, 2014 | LISTER HILL AUDITORIUM, NIH CAMPUS | BETHESDA, MD



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Quantitative Profiling of Environmental Chemicals and Drugs for Farnesoid X Receptor Activity

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Farnesoid X receptor (FXR), a bile acid sensor, exerts protective function in numerous diseases including cholestasis, diabetes, liver regeneration, and cancer. Despite the extensive interests in FXR ligands in drug discovery, little is known regarding potential FXR-mediated toxicity effects from xenobiotic chemicals. Here we describe the profiling of approximately 10K environmental chemicals and drugs in modifying FXR signaling and associated cytotoxicity. The FXR beta-lactamase assay was used to screen the Tox21 10K compound library, containing environmental chemicals, clinically-approved drugs and known bioactive small molecules, in a 1536-well plate format at 15 concentrations in triplicate runs. 435 potent and reproducible hits were identified and grouped into several clusters based on their chemical structure similarity and known biological function. Many environmental chemicals including synthetic hormones, pesticides, and industrial chemicals showed FXR antagonist activity. Some drugs acted as agonists, antagonists, or partial agonists of FXR. Several clusters of compounds identified from the screening were also found to be active against other functionally related nuclear receptors. These results not only provide directions for prioritizing chemicals for further testing FXR-mediated toxicity but also suggest novel signaling pathways for future mechanistic studies.



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Engineered Cellular Models of Heart and Lung: A Novel Analysis of Electrical Recordings for Cardiotoxicity Studies and a Biomimetic Microfluidic Lung Model

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Improved in vitro cellular models which more closely emulate human physiological response are needed to accelerate the development of safe drugs. A multidisciplinary team led by RTI International is developing engineered cellular models for the heart, lung, and blood brain barrier. This presentation will focus on the heart and lung models.

Cardiotoxicity remains a leading cause of drug failure in clinical trials and withdrawal from the market. The recent availability of human induced pluripotent stem (iPS) cell derived cardiomyocytes with proven pharmacological relevance enables improved preclinical cardiotoxicity screening. We are leveraging the high content data available from non-invasive monitoring of spontaneously beating iPS cardiomyocytes using microelectrode arrays (MEAs). We have developed a novel approach for analysis of the rich data set produced by a commercial 48-well MEA recording instrument. While other measurements such as impedance detection and Ca-flux achieve higher throughput than 48 wells, MEAs provide multiple parameters of drug response related to signal morphology, timing relationships, and conduction properties. One of these parameters of particular importance is the action potential duration which is a marker of QT interval. We have detected changes in QT interval from compounds that have little effect on beat rate, and are therefore not detected by screening methods which rely on rate changes. Standard MEA software provides parameters as single averaged values, while our approach includes analysis of temporal and spatial variations with the purpose of determining the added predictive value of these features.

The lung is a primary site of exposure to pathogens and an attractive drug delivery route. We have developed a biomimetic multicellular lung model. For the lung tissue, primary cells are the closest in vitro representation of in vivo physiology however they are typically in monocultures. We have developed a co-culture model of normal bronchial epithelial cells cultured at an air-liquid interface, fibroblasts to mimic the lung interstitium and a polarized microvascular endothelial cell layer. The cells are cultured in a multi-compartment microfluidic device that emulates the tissue microarchitecture and enables cells interaction by paracrine signaling. This airways model provides a new tool for modeling diseases and investigating drug response.



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A Novel Single Cell Based DNA Damage/Repair Assay for In vitro Chemosensitivity Testing

Liyuan Ma

Introduction

Tumors are heterogeneous in their ability to repair DNA damage. Due to tumor heterogeneity at pathological, cellular and molecular levels, traditional chemotherapies do not have the same effectiveness for different patients. Selection of chemotherapies is crucial due to toxicity of drugs, which can cause side effects. However, evaluation of a population of tumor cells can only give a statistical average that can mask single cell behaviors due to tumor heterogeneity. There is a need to screen drugs reliably and rapidly for an individual patient. We have developed a single cell based HaloChip assay that can detect and quantify DNA damage and examine drug response without population interference, therefore predict therapeutic effect of chemotherapy.

Materials and Methods

Human cancer cell line (HeLa) obtained from the American Type Culture Collection is cultured in standard conditions (5% CO₂ in air at 37°C) in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin. Cells are trypsinized with 0.25% trypsin/0.53mM EDTA solution. Cell viability is determined by staining with Trypan Blue. Cell number is counted with a hemacytometer. Etoposide (VP-16) and irinotecan hydrochloride (CPT-11) are from Sigma-Aldrich. DNA damage/repair ability at single cell level is quantified by using a HaloChip method that involves micropatterned single cell array and fast alkaline halo assay.

Results and discussion

The HaloChip assay uniquely combines the state-of-the-art microfabrication technique and classical halo assay. Single cell array generated on silicon substrate is embedded in thin agarose gel film, and exposed to drug of certain concentration. Fluorescent image confirms boundary between nucleus and halo after staining DNA with ethidium bromide, where the symmetric shapes of halos and nuclei and non-overlapping cells/halos allow accurate and objective determination of size/dimension of halo. The two drugs used in this work are genotoxic, which can cause damage of cell DNA through either topo I (camptothecin) or topo II (VP16) mechanism. We have examined these drug actions with HaloChip Method by examining DNA damage/repair ability. Figure 1A shows drug titration data of HeLa cells. Both drugs can induce DNA damage at low concentration. The halos expand with increasing drug inputs, based on rNDF values (0 to 8.0). The rNDF values are highly sensitive at low drug inputs (<1 μ M). The DNA repair ability upon drug action with HaloChip has also been tested. Figure 1B shows the representative images of halos collected at different times after exposure to VP16, where the repair effect can be seen clearly.

Conclusions

The HaloChip method can be used to measure drug-induced DNA damage and will be a promising technique to stratify patients into sensitive and non-sensitive subgroups. The preliminary results have shown the feasibility of using HaloChip as a metric to quantify therapy induced DNA damage and repair in tumors. It is anticipated that this method will be useful in a broad range of clinical, epidemiological, and experimental settings.

Acknowledgements

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References

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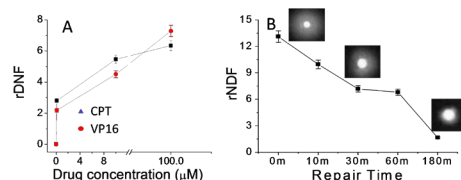


Figure 1. HaloChip assay with Topo drugs and DNA repair/recovery. **Panel A:** drug titrations with CPT or VP16 are shown. **Panel B:** cells were treated by VP16 for 90min and the drug was washed out and cells were allowed to recover prior to HaloChip analysis. Actual images are shown above selected data points.



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From Bench to FDA: What it takes to Bring Advances in the Science of Toxicology into Industry.

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Most experts agree that the current preclinical safety tests are not adequate to ensure safety of patients in clinical trials and in the market. With major advancement in the science of mechanistic toxicology, there has appeared a number of technologies used in the industry to different degrees to predict mechanisms of human toxicities. What is missing is a step that gets these technologies from discovery lead optimization stage to preclinical use. How, who, how and when does validation of these new technologies? Perhaps, there is an answer. We brought together academic groups, industry, regulatory and government organizations to develop a new validation path for in vitro human biology-based toxicity assays and to advance them into preclinical development. We are presenting the design of one such new validation approach. The approach is based on selection of pairs of marketed drugs with certain clinical toxicities, which are paired to structural analogs that did not cause the same adverse events. The compound pairs selected by Safer Medicines Trust with the advice of the scientific advisory panel, are now included in Phase 3 of ToxCast program and results will be analyzed by an independent organizations: FRAME (UK) and OpenTox (Switzerland). The results are expected to be released for analysis in Q2 2014 and results available in Q3. Simultaneously we are initiating the follow-up study, focused on DILI and done in collaboration with and with the advice from FDA Tox21 program and is using the same paired compound approach, applied to FDA's Liver Toxicity Knowledge base.



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Hierarchically-Organized Microvessels for Vascularized Tissue Models

Michael A. Daniele, Stella H. North, Kathryn Radom, Jawad Naciri, Frances S. Ligler and André A. Adams

Comprehensive models of bioagent infection and pathogenesis will rely upon engineered tissue constructs that mimic cardiopulmonary behavior. Presented here are results from recent efforts that demonstrate the design, synthesis and fabrication of bio-hybrid, free-standing microvessels that mimic microvasculature. The ability to generate microvessels from biocompatible polymer/biochemical hybrids provides a key resource for the improvement of in vitro tissue models as well as the understanding of cell-cell and cell-material interactions. To these ends, we synthesized and characterized a tissue scaffold that could be rapidly polymerized while harboring the desired cell type. The material's chemistry was tailored to approach properties of soft tissue, such as bioactivity, porosity, chemical functionality, and mechanical toughness. The resultant hydrogel was a bio/synthetic composite of gelatin methacrylamide and poly(ethylene glycol). By exploiting a cytocompatible click chemistry, gelatin with methacrylate groups and poly(ethylene glycol) with thiol or alkyne groups, were rapidly crosslinked by mild-UV exposure. The gelatin methacrylamide was selected for the presence of peptide binding sites to facilitate cellular ingrowth and native extracellular matrix formation; whereas, the poly(ethylene glycol) was chosen to provide mechanical support during formation and matrix remodeling. The composite promoted cell adherence and matrix remodeling. The flexible chemistry also allows for the future inclusion of instructive biological components.

During the course of exploring novel polymer chemistries for these bio/synthetic composites, we employed a microfluidic method for the production of microscale fibers and tubes from macromer solutions. By inducing spatial control of material and cellular composition by hydrodynamic focusing, the macromer precursor fluids can be arranged into complex patterns at the microscale. The method entails hydrodynamic shaping of multiple fluid flows to direct the assembly of multi-walled structures, solidified by in situ photopolymerization. By employing this shaping procedure, we generated microtubes made of synthetic scaffolding that support cellular proliferation. Microtubes have been fabricated on the scale of arterioles and venules; "capillary-sized" fibers can also be achieved with this method. Microfluidic production of individual blood vessels will provide the necessary level of architectural and cellular control at the microscale for building large scale 3D tissue models with complex vascular networks that recapitulate the given human physiology. This technology may afford new insights into studying the efficacy of potential medical counter measures (MCM) and may be used to identify biomarkers.



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Relevant Biology and Genetic Diversity in the Research Laboratory: Large Scale Generation and Use of Human Tissue Cells Derived from Induced Pluripotent Stem Cells

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Cellular Dynamics (CDI) was founded on the principle of leveraging recent advances in induced-pluripotent stem cell (iPSC) technology toward manufacturing human cell models that provide the necessary contextual relevance for successful drug development, basic biology research, and tissue engineering. To date CDI has produced multiple terminal and progenitor cell types from all three primary germ layers derived from an isogenic iPSC background. More importantly, all these human cell types have been made at industrial quantities ('scale up') under rigorous Quality Control regulations so as to enable robust and repeatable use across academic and industrial research programs. Concomitant with scale-up, the manufacturing process has been adapted for parallel reprogramming of multiple biological samples to iPSCs as well as parallel differentiation of multiple iPSC lines down different lineage pathways. Together these processes provide relevant human material across panels of individuals with varied genetic backgrounds for tissue engineers, drug discovery teams, and academic scientists. This presentation will highlight these cell types and their scale out; providing characterization data as to their in vivo-like functionality, demonstrating their utility across multiple research disciplines, and their establishing their readiness for validation as in vitro tools and their use in bioengineering efforts.



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High Throughput Arrhythmogenic Liability Assessment Utilizing Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

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Human induced pluripotent stem cell derived cardiomyocytes (HiPS-CMs) may be used as a powerful in vitro model for predictive cardiac safety assessment in the early drug discovery process. To enable HT arrhythmogenic testing in HiPS-CMs, we have developed the Kinetic Image Cytometer (KIC) for high throughput, automated cell-by-cell analysis of intracellular calcium transient dynamics allowing for an integrated assay to screen for compound effects across multiple ion channel types and is also capable of detecting multi-channel effects. Using a library of 50 compounds we demonstrate the ability of the assay to identify a wide range of proarrhythmia mechanisms.



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Examining the Predictive Capabilities of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells (HiPS-CMs) for Arrhythmogenic Liability Testing in a High Throughput Assay

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Human induced pluripotent stem cell derived cardiomyocytes (HiPS-CMs) may be used as a powerful in vitro model for predictive cardiac safety assessment and could allow for better identification of compounds with poor arrhythmogenic liability profiles in the early drug discovery process. To enable HT arrhythmogenic testing in HiPS-CMs, we have developed the Kinetic Image Cytometer (KIC) for high throughput, automated cell-by-cell analysis of intracellular calcium transient dynamics. Calcium transients integrate the electrochemical signals of the action potential with the molecular signaling pathways that regulate contraction. Drug induced alterations in the shape and duration of the cardiomyocyte action potential result in changes to the shape and duration of the intracellular calcium transient. Therefore, by examining calcium transient dynamics in hiPS-CMs a single assay can be used to screen for compound effects across multiple ion channel types and is also capable of detecting multi-channel effects. In the present study, we used KIC technology to assess the predictive values of hiPS-CMs using a library of 55 compounds known to alter the cardiac ion channels/receptors and action potentials (APs). Our data indicate that using hiPS-CMs, KIC is able to detect known drug-induced changes in Ca²⁺ transients and therefore, may potentially predict drug-induced arrhythmogenic liabilities in early drug discovery.



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A Pumpless 10-Organ System for Drug Discovery and Toxicological Evaluations.

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Our overall goal is to construct a low cost, easy to use platform to emulate human response to drug and chemicals with 10 organ systems. The platform will be “pumpless” and use a rocker platform with a valve and gravity for the fluid motion force. Since many units can be placed on a rocker platform and platforms can be stacked, the system can potentially be used for moderately high throughput studies. Initial studies have demonstrated that a platform can be constructed that is operational and can sustain viable cell cultures of HepG2/C3A cells in all compartments. We have developed tissues constructs for several organs that can be integrated with the 10-organ platform. For the GI tract system we have constructed silicon chip with an integrated membrane (from SU8) that contains both macrovilli and microvilli formed by Caco-2 cells. We are in the process of initiating studies using primary human intestinal gut cultures for the GI tract mimic. For the liver component we have used the 3D scaffold from Regenamed with human non-parenchymal cells and human hepatocytes to test our ability to sustain such a system for at least 21 days. For the circulatory system we have demonstrated our ability to make an artificial vessel network of branched vessels (up to 4 cm long vessels) that are fully lined with endothelial cells that form tight junctions and express appropriate marker proteins. For the nervous component, we have developed a blood brain barrier model. We have integrated electrodes to measure on-line the transendothelial resistance of brain endothelial cells growing in our platform. We have demonstrated the functionality of this model with mouse endothelial cells and are now in the process of transitioning to iPS cells. Successful culture of functional motoneurons and myotubes from human sources has also been confirmed using this system. We are currently working towards integrating these cell types with BioMEMS technologies to facilitate in situ measurements of functionality within our custom housings. We have also developed a cardiac module utilizing two sources of human stem cell derived cardiomyocytes in which we have measured conduction velocity, beat frequency, amplitude, QT interval and force in a serum-free defined system for 5 different drugs which have shown similar effects as that found in vivo.



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