**CONCLUSION**

- The combination of iCell Hepatocytes with HTRF cell signaling assays enables convenient, robust and pertinent analysis of T2D-related pathways.
- As expected, insulin and H$_2$O$_2$ treatments induced a significant activation of AKT and AMPK/ACC signaling pathways respectively. Furthermore, cell exposure to pro-inflammatory cytokines triggered stress responses, with a marked effect of IL-1β compared to TNF-α.
- The next step would be to study the dysregulation of insulin signaling in diabetic conditions on diabetic patient-derived cells.

**ASSAY WORKFLOW**

- **iCell Hepatocytes**
- **HepG2**

**INSULIN/AKT SIGNALING**

- Phospho/mTOR (S2448)
- Phospho-GSK-3β (S9)
- Phospho-NF-κB (S536)
- Phospho-c-Jun (S63)
- Phospho-AMPK/ACC (S79, T172)
- Phospho-p38 MAPK (T180/Y182)
- Phospho-STAT3 (Y705)

**AMPK/ACC SIGNALING**

- Phospho-ACC (S79)
- Phospho-AKT (S473)
- Phospho-γ tubulin

**PRO-INFLAMMATORY CYTOKINE SIGNALING**

- Phospho-IL-1β (S12)
- Phospho-IL-6 (S63)
- Phospho-IFN-γ (Y705)

Graph legend: HTRF signal is calculated using the following formula: (665 nm signal/620 nm signal) x 10,000. Grey dotted lines represent the non-specific signal of the negative control lysis buffer with detection antibodies. Experiments were performed in triplicate. Error bars represent SEM.

**BACKGROUND**

Hepatic insulin resistance, which is defined as impaired action of insulin on the liver, plays a pivotal role in the development of type 2 diabetes (T2D).

Increased pro-inflammatory cytokine levels associated with obesity (e.g., TNF-α, IL-1β and IL-6) have been associated with this syndrome, but the exact molecular mechanisms leading to the disruption of the insulin-signaling cascade remain to be elucidated.

Deciphering the cell signaling pathways involved in glucose/lipid metabolism and its alteration is therefore of great interest for the development of more potent therapeutics.

**PURPOSE OF THE STUDY**

For many years, researchers have utilized primary rodent cells or immortalized cell lines as in vitro models. In order to move closer to the pathophysiology, Cellular Dynamics International (CDI) is developing innovative cellular models which are engineered from human induced pluripotent stem cells (iPSC) and can be derived into various terminally differentiated cell types, including iCell® Hepatocytes. The large scale production capability of these models represents a great advantage for long-term projects requiring a high batch-to-batch reproducibility.

Activation of intracellular signal-transduction proteins is usually analyzed by Western Blot, but this labor-intensive method often reveals a lack of accuracy and reproducibility. To overcome these limitations, Cisbio Bioassays is developing HTRF® (Homogeneous Time-Resolved Fluorescence) cell-based sandwich immunoassays to analyze protein phosphorylation status and expression level. These homogeneous TR-FRET assays are quantitative, sensitive and HTS-compatible.

Here, we demonstrate that iCell Hepatocytes in combination with HTRF phospho-total protein assays, represent a straightforward and physiologically-relevant solution for the investigation of insulin resistance in T2D. Using both sets of tools, we dissected the insulin/AKT and AMPK/ACC pathways that are involved in glucose/fatty acid metabolism. The numerous stress pathways activated by pro-inflammatory cytokines, such as JNK, NF-κB, p38 MAPK and STAT3, were also analyzed. Results obtained on iPSC-derived hepatocytes were compared with those obtained on the well-known immortalized HepG2 cell line.