

## Background

Despite their success in biological studies, 2D cell cultures fail to accurately reproduce the tumor microenvironment. On the other hand, 3-dimensional (3D) cell cultures more accurately mimic the tumor microenvironment and serve as effective *in vitro* models of the avascular stage of tumor growth to study novel hypoxic markers, targeted therapy and drug resistance. We have developed a multi-cellular tumor spheroid model which is of intermediate complexity between that of the tumor micro-environment and monolayer cultures. A high throughput Kinome RNAi screening was performed utilizing our 3D spheroidal model.

## Objectives

The objective of this study was to compare the profile of kinases regulating the activity of the hypoxia inducible factor 1 alpha (HIF-1 $\alpha$ ) between 2D and 3D cell cultures.

## Methods

The pancreatic cancer cell line Panc-1 stably expressing the HRE-Luc construct (Panc-1 HRE) was used in this study. Prior to performing the screen we identified the optimum conditions for both spheroid formation and siRNA transfection. A high throughput RNAi screen was performed on the Panc-1-HRE cells using a pooled human kinome RNAi library (4 siRNA targeting each gene) purchased from Dharmacon. Growth of the spheroids for 6 days following transfection with siRNA resulted in the development of a hypoxic inner core identified by an luciferase increase in rase expression, an indication of HIF-1 $\alpha$  activation. Luciferase expression was determined in the spheroids by adding luciferin into the media, incubating the plates at 37°C for 5 minutes and imaging the plates in a xenogen imager. On the other hand induction of HIF-1 $\alpha$  in the 2D cell culture was achieved by culturing the cells in normoxia for 48 h following transfection followed by growth in 1% oxygen for 18 – 20 h. HIF-1 $\alpha$  activity was correlated to the extent of luciferase expression in our luciferase reporter assay. The relative luciferase units were determined by the ratio of average luciferase expression per well/ average spheroid volume per well in the 3D system and the ratio of luciferase expression/cell viability in the 2D system.

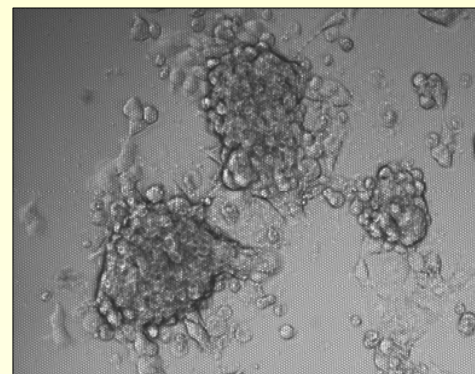
## Results

### Nano Culture Plate for 3D Spheroid Cultures



**Figure 1.** The nano material based scaffold is layed out in a honeycomb pattern and square pattern (SCIVAX Inc.)

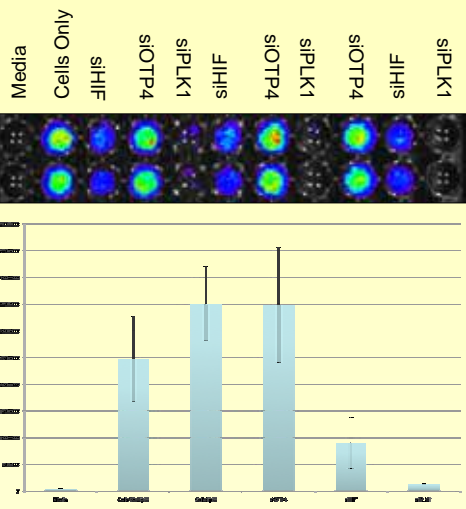
### 3D Spheroids of Panc-1 HRE cells



**Figure 2 .** Panc-1HRE cells were plated at a cell density of 20,000 cell per well of the 96 well plate. Well defined 2spheroids were formed by 6 days of growth

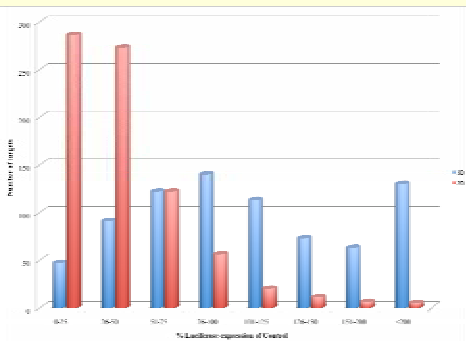
## Results

### Optimization of Transfection efficiency



**Figure 3.** Panc-1 HRE cells were transfected with siRNA targeting HIF-1 $\alpha$  (assay specific control, siPLK1 (+ve control) and siOTP4 (-ve control). The z-factor was 0.76.

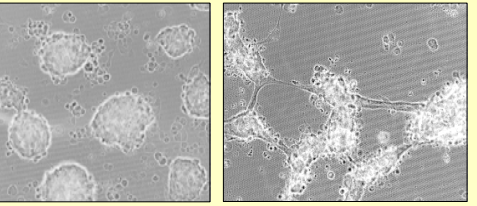
### Comparison between the 3D and 2D systems in the regulation of HIF-1 $\alpha$ activity



**Figure 4.** Comparative data of HIF-1 $\alpha$  regulation in both a 2D and 3D cell culture system. A high throughput Kinome RNAi screen was carried in both systems. Relative luciferase units were determined in both systems and presented as a percentage of control.

## Results

### Silencing of a group of kinases leads to a spreading morphology



**Figure 5.** Morphological changes observed in the 3D cell culture system. The silencing of a panel of kinases resulted in a significant change in morphology. In most of these cases this alteration in morphology was associated with a significant increase in HIF-1 $\alpha$  activity.

## Conclusion

We observed a significant difference in the ability of kinases to regulate HIF-1 $\alpha$  activity between the 2D and 3D cell cultures systems. HIF-1 $\alpha$  activity was inhibited by greater than 70% following the silencing of over 50% of the targeted kinases in 2D cell culture. On the other hand, the inhibition of HIF-1 $\alpha$  activity in the 3D cell spheroidal system demonstrated a more wider distribution. We did however note that there were some kinases that regulated the activation of HIF-1 $\alpha$  to a similar degree in both the 2D and 3D cell cultures. In addition, the silencing of a panel of about 200 kinase resulted in a significant increase in HIF-1 $\alpha$  activity in the 3D cell culture resulting in the formation of a spreading morphology indicative of invasion.

## Future Directions

- Normalize the data utilizing statistical, bioinformatic and image analysis.
- Validate selected targets
- Determine the therapeutic significance of silencing the selected targets in an animal model