Hematopoietic differentiation

- HL60s are promyeloblastic cells that can be terminally differentiated into either macrophages or granulocytes with phorbol myristate acetate (PMA) and retinoic acid (RA) respectively
- This differentiation process is an important model for myelocytic leukemia

Goals
The KiNativ™ platform was used to profile changes in activity/abundance of all protein kinases during this differentiation process:

- To better understand the molecular mechanism of differentiation
- To identify novel targets for the treatment of myelocytic leukemia

Profiling kinases in HL60 cells during PMA and RA differentiation

- The protein and lipid kinases in resting, PMA, and RA differentiated HL60 cells were analyzed by targeted mass spectrometry.
- A total of 194 kinase peptides comprising 175 unique protein and lipid kinases were accurately quantified

Distribution of probe labeling changes (number of kinases)

- PMA treatment
- Retinoic acid treatment

For more information about KiNativ™ call 858-526-2515 or visit www.kinativ.com
KiNativ™ Application Note
Target Discovery
Global profiling of kinases during hematopoietic differentiation

Changes in probe labeling for selected functional classes of kinases

**Decreased probe labeling for kinases involved in cell cycle progression, consistent with terminal differentiation induced by PMA and RA**

**Kinase Site** | **PMA** | **RA**
--- | --- | ---
AurA/B/C ATP |  |  
AurB LYS2 |  |  
AurA LYS2 |  |  
AurC LYS2 |  |  
CHK1 LYS2 |  |  
CDK6 LYS2 |  |  
PLK1 LYS1 |  |  
PLK1 LYS2 |  |  
P.LK1 like LYS1 |  |  
Wee1 LYS2 |  |  
CDK2 LYS2 |  |  
CDK2 LYS1 |  |  
MYT1 LYS2 |  |  
CDC2 LYS2 |  |  

**Changes in Src-like kinases suggest potential roles for these kinases in mature macrophages and granulocytes**

**Kinase Site** | **PMA** | **RA**
--- | --- | ---
FES LYS1 |  |  
FYN LYS1 |  |  
FGR ACT |  |  
HCK LYS1 |  |  
LYN LYS1 |  |  
PYK2 ACT |  |  
SYK LYS1 |  |  

**Altered probe labeling for kinases whose specific roles in differentiation are unknown — possible novel functions for these kinases**

**Kinase Site** | **PMA** | **RA**
--- | --- | ---
CaMK2b LYS1 |  |  
CaMK1a LYS1 |  |  
ND2 LYS1 |  |  
Dyrk1B LYS1 |  |  
IraK3 LYS1 |  |  
MASTL LYS1 |  |  
PKAca/g LYS2 |  |  
CaMKK2 LYS1 |  |  
CaMKK1 LYS1 |  |  

**Signal Change**
- > 16 fold decrease
- 8 - 16 fold decrease
- 4 - 8 fold decrease
- 2 - 4 fold decrease
- No change
- 2 - 4 fold increase
- 4 - 8 fold increase
- > 8 fold increase

**Changes in activity versus abundance**

To determine if the changes in probe labeling were due to changes in activity or abundance, the abundance of selected kinases was directly determined by Western blot. Changes in PKAc, IKKb, and CDK6 probe labeling did not correlate with changes in abundance.

The mechanism for reduced probe labeling of CDK6 during differentiation was determined by monitoring the abundance of CDK6 regulators.
- CDK6 probe labeling decreased 50- and 10-fold during PMA and RA differentiation, respectively
- By Western blot, CDK6 only decreased 2-fold during PMA differentiation, and was unchanged during RA differentiation
- The strong decrease in probe labeling of CDK6 correlated with increases in the known CDK inhibitors p27 (PMA and RA) and p21 (PMA)

**Distribution of probe labeling changes (number of kinases)**

**PMA** | **RA** | **Control** | **Diff.**
--- | --- | --- | ---
CDK6 |  |  |  
p27 (kip1) |  |  |  
p21 (WAF) |  |  |  

For more information about KiNativ™ call 858-526-2515 or visit www.kinativ.com