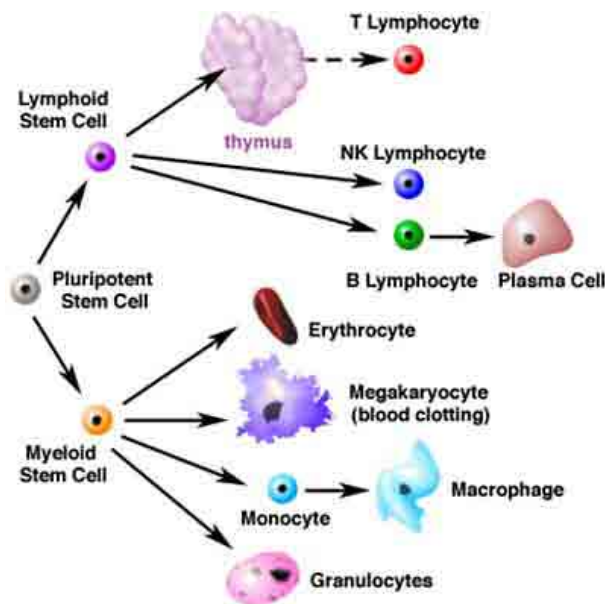


Target Discovery

Global profiling of kinases during hematopoietic differentiation

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

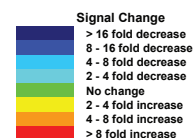
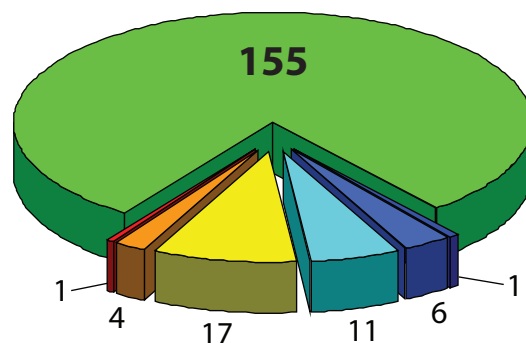
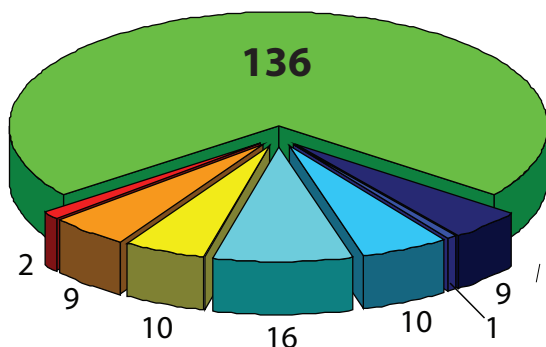
Profiling kinases in HL60 cells during PMA and RA differentiation

- 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



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

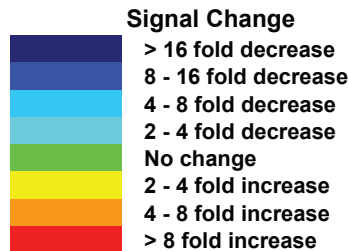
Kinase	Site	PMA	RA
AurA/B/C	ATP		
AurB	LYS1		
AurA	LYS2		
AurC	LYS2		
CHK1	LYS2		
CDK6	LYS2		
PLK1	LYS1		
PLK1	LYS2		
PLK1 like	LYS1		
Wee1	LYS2		
CDK2	LYS2		
CDK2	LYS1		
MYT1	LYS2		
CDC2	LYS2		

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		
NDR2	LYS1		
DYRK1B	LYS1		
IRAK3	LYS1		
MASTL	LYS1		
PKACa/g	LYS2		
CaMKK2	LYS1		
CaMKK1	LYS1		



Labeling site key
 LYS1 conserved lysine 1
 LYS2 conserved lysine 2
 ATP Loop ATP binding loop
 ACT activation loop

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)**

