High-Throughput LanthaScreen® Cellular Assays for Interrogating Post-Translational Modifications of p53 and Histone H3

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Overview

- Purpose: To advance drug discovery for epigenetic modifying enzymes by developing innovative high-throughput cellular screening tools
- Methods: BacMam-enabled LanthaScreen® cellular assays
- Results: A flexible and portable assay platform was developed for monitoring cell cycle-regulated Histone H3 Lys9 acetylation, Ser10 phosphorylation and Lys4 methylation, and DNA damage-induced p53 Lys382 acetylation and Ser15 phosphorylation

Introduction

Post-translational modifications such as phosphorylation, acetylation, methylation and ubiquitination play important roles in regulating the structure and functions of histones and transcription factors such as p53, which in turn regulate essential gene expression. Dysregulation of these post-translational modifications has been linked to cancer and metabolic diseases. High-throughput compatible cellular assays for monitoring epigenetic modifying enzymes are lacking. To enhance drug discovery research efforts for these modifying enzymes, we have developed a high-throughput TR-FRET-based LanthaScreen® technology to detect the resulting post-translational modifications of p53 and Histone H3 in various cell types.

Methods

- BacMam Particle Production: PCR-qualified bacmid DNA was transfected into SF9 insect cells and BacMam virus production was performed according to Bac-to-Bac® Baculovirus Expression Systems manual (Life Technologies).
- Cell Culture and BacMam Transduction: All cell culture media and supplements and primary human cells were from Life Technologies. U-2 OS, HCT116, HeLa and A549 cells were purchased from ATCC. For transduction of U-2 OS and human mammary epithelial cells, cells were mixed with BacMam virus at indicated concentrations (usually between 5 to 10% v/v) and plated onto a 384-well assay plate for 20 hours prior to the LanthaScreen assay. For BacMam-mediated expression of SIRT1, cells were transduced as described above but in the presence of serially-diluted BacMam SIRT1, with 20% (v/v) the highest concentration. For transduction of HeLa, A549 and HCT-116 cells, cells were mixed with BacMam virus at indicated concentrations (usually between 5 to 10% v/v) and plate onto a 6-well plate for 24 hours. Cells were harvested and plated onto a 384-well assay plate for 20 hrs prior to the LanthaScreen® assays.
- LanthaScreen® Cellular Assays: Cells on assay plate were lysed with lysis buffer containing Tb-anti-modification specific antibody and incubated at room temperature for 2 to 3 hrs prior to reading the plate on a plate reader (BMG Labtech PHERAstar) set to standard TR-FRET setting. Emission ratios were calculated using the time-gated fluorescence intensity at 520nm / 490nm (340nm excitation). The relative response ratios were calculated as the emission ratio values of each stimulated sample divided by the values of the unstimulated samples or samples following etoposide treatment.

Results

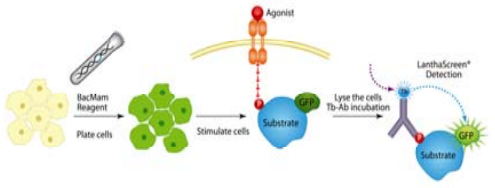

Figure 1. *Workflow of the BacMam-enabled cellular assay platform using LanthaScreen® TR-FRET technology.* For most cell types, cells are transduced with BacMam virus for expression of GFP-substrate fusions and incubated overnight for the LanthaScreen® cellular assay the following day. LanthaScreen® is a homogeneous immunoassay where GFP serves as the FRET acceptor for terbium (the FRET donor) on the modification-specific antibodies.

Measuring Histone H3 Phosphorylation (Ser10), Acetylation (Lys9) and Tri-methylation (Lys4) in U-2 OS Cells

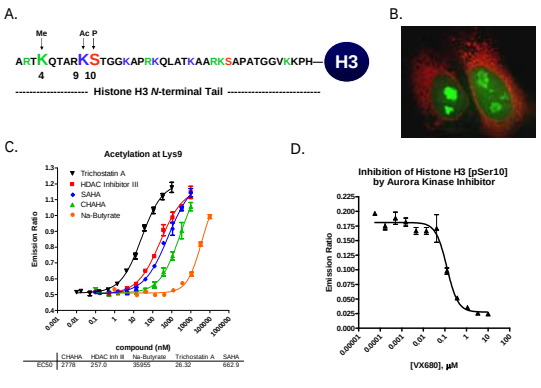

Figure 2. A: *Post-translational Modifications on Histone H3.* **B**: *GFP-Histone H3 localized in the nucleus.* U2OS cells were transduced with BacMam Histone H3 (GFP = green) overnight and stained with Mitotracker® red dye the following day. **C**: *Measurement of histone acetylation at Lys9* induced by multiple HDAC inhibitors. **D**: Detection of the inhibition of Histone H3 *phosphorylation at Ser10* by Aurora Kinase inhibitor VX680. **E**: Detection of *tri-methylation of Histone H3* at Lys4. DFO: Deferoxamine to induce hypoxic condition.

Measuring Histone H3 Acetylation at Lys9 induced by TSA in Various Cell Types

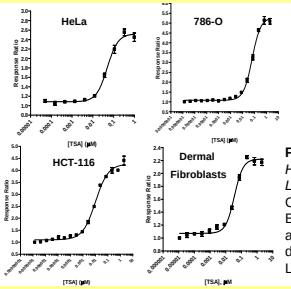

Figure 3. *Measuring Histone H3 Acetylation at Lys9 in various cell types.* Cells were transduced with BacMam GFP-Histone H3 and treated with indicated doses of TSA prior to the LanthaScreen® assay.

Measuring DNA Damage-Induced Phosphorylation (Ser15) and Acetylation (Lys382) of p53 in U-2 OS Cells

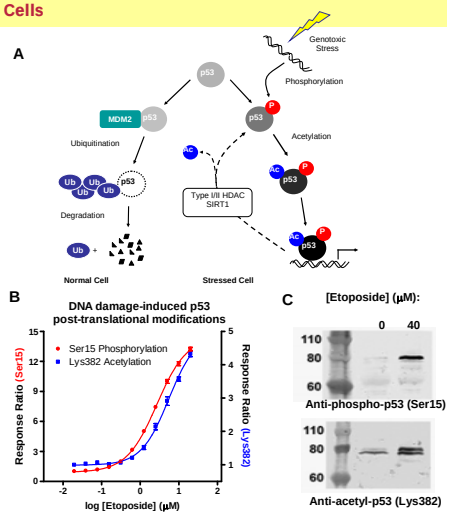

Figure 4. *Interrogation of DNA damage-induced post-translational modifications of p53.* **A:** Schematic of p53 signaling pathway. Under genotoxic stress, p53 becomes rapidly stabilized via phosphorylation at Ser15, followed by acetylation at Lys382. **B:** U-2 OS cells transduced with BacMam p53 were treated with a serial dilution of etoposide to induce phosphorylation and acetylation of p53. These modifications were detected via LanthaScreen® technology; **C:** Western blot validation of the LanthaScreen® TR-FRET results.

Involvement of SIRT1 in the Deacetylation of p53 Determined Using SIRT1 Specific RNAi, Small Molecule Inhibitor and Over-Expression of SIRT1 Protein

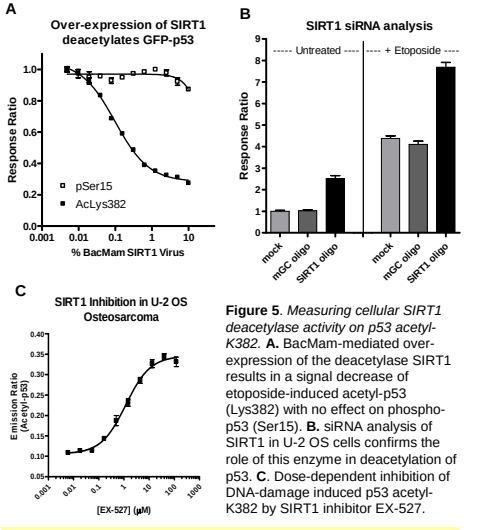

Figure 5. *Measuring cellular SIRT1 deacetylase activity on p53 acetyl-K382.* **A.** BacMam-mediated over-expression of the deacetylase SIRT1 results in a signal decrease of etoposide-induced acetyl-p53 (Lys382) with no effect on phospho-p53 (Ser15). **B.** siRNA analysis of SIRT1 in U-2 OS cells confirms the role of this enzyme in deacetylation of p53. **C**. Dose-dependent inhibition of DNA-damage induced p53 acetyl-K382 by SIRT1 inhibitor EX-527.

Conclusions

- BacMam and LanthaScreen® technologies together provide a powerful HTS-compatible assay platform for measuring post-translational modifications in a variety of cellular backgrounds.
- BacMam Histone H3 and BacMam p53 Cellular Assay Kits are broadly applicable in the field of epigenetic research to monitor the activity of kinases, acetyltransferases, HDACs, SIRTs, or histone methyltransferases.

Reference and Useful Weblinks

Robers, M.B.; Loh, C.; Carlson C.B.; Yang, H.; Frey, E.A.; Hermanson, S.B.; Bi, K. "Measurement of the cellular deacetylase activity of SIRT1 on p53 via LanthaScreen® technology" *Mol. Biosyst*., **2011**, *7, 59-66*

For more information on BacMam-enabled LanthaScreen® cellular assays, please visit: www.invitrogen.com/bacmamassay

To request information on custom assay development projects, please email discoveryservices@lifetech.com