Nu.Q® Immunoassay enables fast and reproducible monitoring of EZH2 inhibitor performance.

Introduction

EZH2 (Enhancer of Zeste Homolog 2) is the key enzymatic subunit of the Polycomb Repressive Complex 2 (PRC2), a highly conserved histone methyltransferase that catalyzes the trimethylation of lysine 27 on histone H3 (H3K27Me3). H3K27Me3 is a well-established epigenetic mark associated with gene silencing and transcriptional regulation. This epigenetic mark plays a crucial role in tumorigenesis, cell cycle progression, and dysregulated proliferation.[1]

Hyperactivation of EZH2, either through overexpression or mutations, is frequently observed in various cancers, including prostate, breast, uterine, renal, and gastric cancers, as well as melanoma where it often correlates with advanced tumor stages and poor prognosis.[2–4] Given its critical role in cancer progression, targeting EZH2 and modulating H3K27Me3 levels is a promising therapeutic approach. With the increasing importance of biomarkers in drug discovery and development, tracking H3K27Me3 mark as a biomarker could play a crucial role in advancing the development of drugs targeting the PRC2 complex from drug screening and dose optimization to monitoring treatment efficacy, thus streamlining the process from preclinical to clinical trials.

To address this need, we developed and analytically validated an automated chemiluminescent immunoassay (ChLIA), Nu.Q®H3K27Me3, for the robust quantitative measurement of circulating H3K27Me3-nucleosomes in K2EDTA plasma samples. This fully automated sandwich assay utilizes an anti-histone H3K27Me3 antibody in capture on the solid phase, consisting of magnetic particles, combined with a conformational anti-nucleosome antibody coupled with acridinium ester molecules in detection (Figure 1).[5] The analytical validation of this Nu.Q®H3K27Me3 confirms that this cutting-edge immunoassay enables highly specific and precise quantification of H3K27Me3-nucleosome in human K2EDTA plasma samples. Moreover, we have demonstrated their remarkable potential across various sample types, including mouse blood, as well as tissue and cell extracts, opening exciting new possibilities for broader applications.

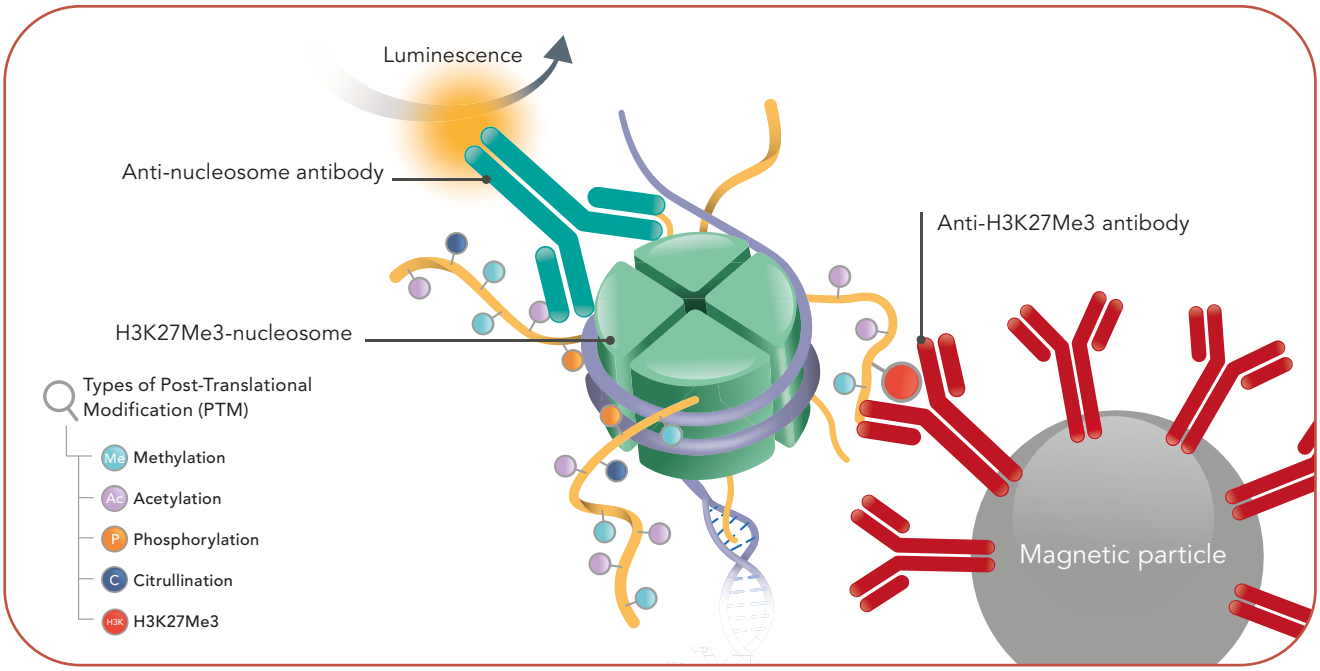

Figure 1: Visual Representation of Nu.Q®H3K27Me3 mechanism. (antibody reference: NIAID Visual & Medical Arts. (07/10/2024). Antibody. NIAID NIH BIOART Source. bioart.niaid.nih.gov/bioart/17)

Case studies

a) Leveraging H3K27Me3 Biomarkers to Assess EZH2 Inhibitor Drug Efficacy: A Case Study in Cellular Model

In vitro experiments were conducted to evaluate the effectiveness of the EZH2 inhibitor (EZH2i) specifically examining its effect on H3K27Me3 levels.[6] Following cells exposure to the EZH2i or its vehicle (DMSO), chromatin was extracted at different time points (4h, 24h, or 48h), and H3K27Me3 levels were determined by Nu.Q®H3K27Me3 levels normalized to the global level of nucleosome thanks to the Nu.Q®H3.1 immunoassay (Figure 2A) and by Western blot (Figure 2B). Results demonstrated that the EZH2i drug induced a time-dependent reduction in H3K27Me3 levels. Furthermore, these results highlighted the potential usefulness of Nu.Q® Immunoassay to monitor epigenetic drug efficiency in cellular models.

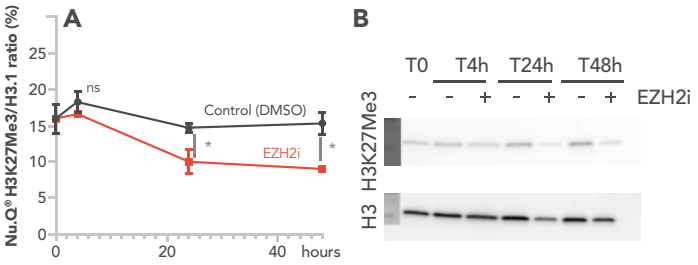

Figure 2: Longitudinal assessment of H3K27Me3- nucleosome level in cells lines throughout the EZH2 inhibitor (EZH2i) treatment by Nu.Q® immunoassay (Nu.Q®H3K27Me3 / Nu.Q®H3.1 ratio) (A) or anti-H3K27Me3 and anti-H3 Western blot (B) after EZH2i exposition (red) or DMSO used as Vehicle (black).

b) Nu.Q®H3K27Me3-Guided Evaluation of PRC2 inhibitor Drug Efficacy: A Case Study in a Murine Model

During the preclinical stage of drug development, 22Rv1-tumor-bearing mice were treated with a potent and selective allosteric inhibitor of the PRC2 (PRC2i) and K2EDTA plasma samples were analyzed with Nu.Q® assays.[7] Nu.Q®H3K27Me3 normalized to Nu.Q®H3.1 was then employed to track and measure the effectiveness of the treatment. First, a titration of the PRC2i was performed to determine the appropriate drug dosage by comparing the vehicle administration with 10–30 or 100 mg/kg of PRC2i. The Nu.Q®H3K27Me3/Nu.Q®H3.1 ratios were then evaluated in plasma on Day 7 (Dx7) post-administration. In this way, the drug developer demonstrated that the PRC2i tested decreased the H3K27Me3 level in a dose-dependent manner (Figure 3A). The highest dose (100 mg/mL) was then applied, with Nu.Q®H3K27Me3/Nu.Q®H3.1 ratios being monitored at multiple time points (Dx3, Dx5, and Dx7) following administration (Figure 3B). This demonstrated that the PRC2i drug induced a time-dependent reduction of circulating H3K27Me3-nucleosome levels. Interestingly, the reduction in H3K27Me3-nucleosomes levels was selectively observed in the tumor-bearing mice treated with PRC2i, with no such effect in the control group (non-tumor-bearing) (Figure 3C).

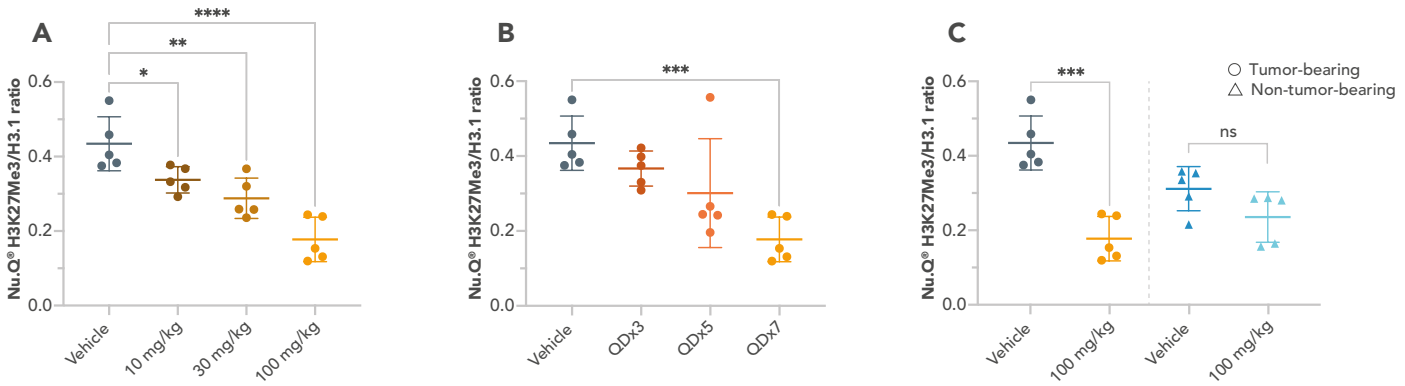

*Figure 3: Nu.Q®H3K27Me3-Guided Evaluation of PRC2 inhibitor (PRC2i) drug efficacy in preclinical mouse model. (A) 22Rv1-tumor-bearing mice were treated with Vehicle or an increasing dose of PRC2i (10–30 or 100 mg/kg) and the level of Nu.Q®H3K27Me3/Nu.Q®H3.1 ratios were then evaluated in plasma on Day 7 (Dx7) post-administration. (B) Vehicle or 100 mg/mL of PRC2i was applied, with Nu.Q®H3K27Me3/Nu.Q®H3.1 ratios being monitored at multiple time points (Dx3 , Dx5 and Dx7) following administration. (C) Nu.Q®H3K27Me3/Nu.Q®H3.1 ratios was measured after vehicle or 100mg/kg of PRC2i administration to non-tumor-bearing mice (circles) or tumor-bearing mice (triangles). One-way ANOVA, followed by Dunnett's multiple comparison test, was used to compare PRC2i groups to Vehicle. *, <0.05 ; ** ,p<0.01 ; ***, p<0.001 ; ****, p<0.0001.*

Conclusion

The analytical validation of this Nu.Q®H3K27Me3 assays confirms its reliability as a cutting-edge immunoassay for precise quantification of H3K27Me3-nucleosome in various biological samples. Its effectiveness has been confirmed in human plasma K2EDTA samples, murine samples, chromatin extracts from cells or tissues, and other biological sources. With the inclusion of the Nu.Q®H3K27Me3 immunoassay into the Nu.Q® Discover platform, we are able to provide an advanced service for the robust analysis of H3K27Me3 mark as well as other epigenetic modifications. By normalizing the Nu.Q®H3K27Me3 level using the Nu.Q®H3.1 assay, which measures the global level of nucleosomes, this approach enables a more accurate assessment of epigenetic changes. This makes it an invaluable tool for monitoring the efficacy of epigenetic drug treatments such as EZH2i or PRC2i in preclinical models. Furthermore, the incorporation of the Nu.Q® immunoassay into the Nu.Q® Discover platform offers significant advantages in clinical phases, particularly when transitioning from preclinical to clinical development. By using a blood-based test (liquid biopsy), it allows for continuous, non-invasive monitoring of patients during clinical trials, enabling real-time assessment of treatment efficacy. This approach not only provides more frequent and less invasive sampling compared to tissue biopsies, but it also allows for the tracking of systemic epigenetic changes across various tissues and organs, providing a more comprehensive understanding of the therapy's impact.

In conclusion, the potential of using H3K27Me3 as a biomarker also positions Nu.Q®H3K27Me3 technology as a key asset for clinical trials, offering critical insights for drug development approval and therapy monitoring. Altogether, the Nu.Q® Discover program enables drug developers and scientists to access a range of state-of-the-art, market ready assays.

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