Epigenetic toxicity measured by high-throughput mass-spectrometry based histone analysis

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PARTNERS AND COLLABORATIONS

Organisation

Name of the organisation Ghent University (UGent)

Department Pharmaceutics

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Environment, Human health
The Method is situated in	Basic Research, Regulatory use - Routine production
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Human
Type of cells/tissues/organs	All cell lines, depending on the tissue under study

DESCRIPTION

Method keywords

Histone-PTMs

Toxicoepigenetics
Pharmacoepigenetics
SWATH
Liquid chromatography
mass spectrometry

Scientific area keywords

Proteomics
LC-MS
cancer research
Toxicology
screening
Drug safety

Method description

Epigenetics have taken centre stage in the study of diseases such as cancer, diabetes, and neurodegeneration. A new field is emerging that targets epigenetic modifications for therapeutic intervention: pharmacoepigenetics. Particularly in oncology, inhibitors of epigenetic-modifying proteins, i.e. epidrugs, have been successfully used in treatment. For nearly thirty years, it has been known that cancer cells exhibit abnormal DNA methylation patterns. However, the large scale analysis of histone posttranslational modifications (hPTMs), has lagged behind because classically, histone modification analysis has relied on site specific antibody-based techniques. Mass spectrometry (MS) is a technique that holds the promise to picture the histone code comprehensively in a single experiment. We have recently adapted the data-independent acquisition (DIA) strategy, i.e. SWATH, for the specific and accurate study of the histone fingerprint (hSWATH). In 2020, we will make the transition from hSWATH as a fundamental research tool to a novel toxicoepigenetic assay. We are currently focusing on (i) increasing sample throughput during data acquisition and (ii) during data analysis, (iii) building a compound library on different cell types and (iv) adapting the assay to GLP. This novel toxicoepigenetic assay and the data it generates holds great potential for application in pharmaceutical industry, food science, clinical diagnostics,...

Lab equipment

High resolution LC-MS.

Method status

Still in development
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

High-throughput screening assay;
Personalized medicine;
Not limited to pharmaceuticals but also useful in the context of food safety and environmental toxicity;
Large scale hPTM screening is currently a void in drug development.

Challenges

Reducing the instrument-time needed per sample is the biggest challenge.

Modifications

Increasing sample throughput is an ongoing effort at the lab.

Future & Other applications

In a first stage, this method will be developed with a focus on toxicoepigenetics. For example, we will be able to detect (toxic) changes in hPTMs during drug development through this assay. But in a later stage, this assay will be further developed as a prognostic test (will a given treatment be effective for a particular patient?) and additionally for the discovery of new epidrugs. The potential of this assay extends beyond pharmaceutical industry or clinical diagnostics and also offers opportunities in the context of food safety and environmental toxicity screening.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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Untargeted histone profiling during naive conversion uncovers conserved modification markers between mouse and human. Laura De Clerck, Jasin Taelman, Mina Popovic, Sander Willems, Margot Van der Jeught, Björn Heindryckx, Petra De Sutter, Hendrik Marks, Dieter Deforce, Maarten Dhaenens. Scientific Reports, accepted

Associated documents

Links

ProGenTomics

Maarten Dhaenens Publications

Other remarks

Monitoring the DNA methylation status or sequencing miRNA are increasingly scalable to assay large sample batches for application as toxicoepigenetic assays. They yield rich datasets that allow broad biological interpretation. Histone modification analysis on the other hand, has classically relied on site specific antibody-based techniques such as western blotting, immunofluorescence and ChIP(seq). These approaches are confined by i) the limited number of targets that can be studied in a single experiment, ii) antibody cross-reactivity, iii) a lack of combinatorial information on hPTM co-occurrence and iv) epitope occlusion. In recent years, mass spectrometry (MS)-based approaches like multiple Reaction Monitoring (MRM) and Parallel Reaction Monitoring (PRM) have become viable

alternatives to these classic techniques for analysing hPTM. However, they still require the user to make a limited selection of specific targets of interest and do not allow for retrospective analysis of novel targets because only the initial targets are acquired. In fact, in fundamental research, data-dependent acquisition (DDA) is the MS technique of choice to study histones in an untargeted way, as it does not rely on prior hypotheses. It has become a promising complementary option allowing discovery-driven histone analysis.

This technique has already proven its merit in many areas, such as e.g. identification and quantification of differential proteins of whole cell lysates and monitoring of phosphorylations over time in phospoproteomics. However, histone extracts are rather unique when compared to traditional samples, as they generally contain just five small basic proteins (11 to 21 kDa) and a few co-extracted proteins and contaminants. However, more than twenty different types of hPTMs present on the histone protein backbone structure have already been described. Theoretically, this allows for these five proteins to have $7 \cdot 10^{17}$ different proteoforms with different hPTM combinations. Taken the high amount of lysines and arginines of histone peptides into account, this translates into $5 \cdot 10^{7}$ different histone peptidoforms with ArgC-like specificity. This vast amount of highly similar peptides leads to several unconventional situations, including more co-elution of isobaric peptidoforms during acquisition. Consequently, this results in a higher amount of chimeric MS/MS spectra and ambiguous spectrum annotations, leading to problematic identification. At the same time, the total histone protein abundance can be considered constant from a quantitative perspective, as it is proportional to the amount of DNA present in a cell. It is in fact the hPTMs which induce changes in peptide abundance, thereby creating an intrinsic peptide-centric setting for histone analysis. Correctly quantifying even the smallest of these differences is of crucial importance, as small changes at specific regions in the genome can have large implications in terms of gene (in)activation. Unfortunately, the quantification of histone peptidoforms in DDA has to be performed at MS1 level and is therefore very susceptible to interference of the many co-eluting isobaric peptides. Recently, sequential window acquisition of all theoretical fragment ion spectra (SWATH) emerged as an intermediate MS technique between targeted approaches such as MRM and PRM on the one hand and untargeted approaches such as DDA on the other. This technique has great potential to address many of the issues mentioned above. SWATH is a methodology that combines data-independent acquisition (DIA)

with peptide spectral library matching, originally developed for MRM-like, i.e. transition-centric, quantification of large sets of proteins across multiple samples. This implies that compared to DDA, it provides the indispensable specificity extracted from the ion traces to distinguish and thus quantify the many isobaric and (near-)coeluting histone peptides, while the acquisition itself remains untargeted, as opposed to MRM and PRM. Following 3 years of optimization, we have recently published a manuscript unlocking SWATH's full potential to get an untargeted perspective on histones (hSWATH).

In this study we show that hSWATH provides very accurate and untargeted measurement of the histone code. We illustrate the power of this approach on a benchmark experiment, wherein we deacetylated commercial histones in a time-lapse manner using HDAC1. Finally, we performed a proof-of-principle toxicoepigenetic screening of breast cancer cell lines which are either sensitive or resistant to Panobinostat, a pharmacoepigenetic epidrug. Because SWATH is a data-independent acquisition methodology, it comprises all formative peptide ions in a sample. This makes it (i) particularly fit for generating a (pharmacoepigenetic) compound library for toxicoepigenetic interpretation, while (ii) allowing retrospective targeting of already acquired data with novel targets. Taken together, these features make hSWATH the perfect stepping stone to develop a high throughput hPTM assay that can address the urgent need for a toxicoepigenetic assay that can move the field of pharmacoepigenetics forward.

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