Neutrophil Elastase in the capacity of the “H2A-specific protease”

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Introduction

The amino-terminal tail of histone proteins and the carboxy-tail of histone H2A protrude from the nucleosome and can be modified by many different posttranslational modifications, thereby mediating chromatin dynamics. Fundamental changes in the epigenetic status of histones from hematopoietic stem cells might be one of the driving forces behind many malignant transformations and subsequent leukemia development 1.

A specific kind of histone modification that has received only little attention in epigenetics until now is histone clipping. The specific clipping product of the C-tail of histone H2A at V114 (ch2A) has been already described in the context of leukemia in the late 70’s and is still being referenced today 2. The responsible enzyme was annotated as the “H2A specific protease” (H2Asp), but it was never sequenced nor identified 3.

Results

Histone H2A is clipped by neutrophil elastase at V114

In Vitro:

- In a histone extract with H2Asp activity only one identified protease is able to cleave histone H2A at V114.
- NE cleaves all histones in a dose dependent manner.

In Vivo:

- Isolated leukocytes from WT and NE Null mice were compared using AQUA-peptides.
- No H2A clipping could be detected in NE Null mice.

A high throughput AQUA approach for absolute detection of specific histone H2A clipping

- Based on two isotopically labeled synthetic peptides an approach was optimized to specifically quantify H2A V114 clipping in one MS run.

- AQUA1: Tryptic H2A N-terminal peptide (m/z 475.7) used to quantify the total amount of histone H2A present in the sample and to compensate for protein composition of the histone extract.

- AQUA2: Semi-tryptic peptide (m/z 743.4) to quantify H2A clipping.

Conclusion

While the epigenetic potential of histone clipping is gradually gaining interest we have identified Neutrophil Elastase as the H2A specific protease that has been uncharacterized for over 35 years. Despite several reports on histone clipping in leukemia samples, we refute its use as a prognostic marker in CLL.

The clipping of histone H2A C-tail shows a remarkable parallel to the recently described clipping of the H3 N-tail 4. Retinoic acid-induced differentiation of THP-1 promonocytes into macrophages is briefly accompanied by ch2A V114 formation, just as H3 clipping is induced by differentiating embryonic stem cells 5. Thus, we emphasize the potential role of H2A clipping in hematopoietic differentiation.