

Neutrophil Elastase in the capacity of the "H2A-specific protease"

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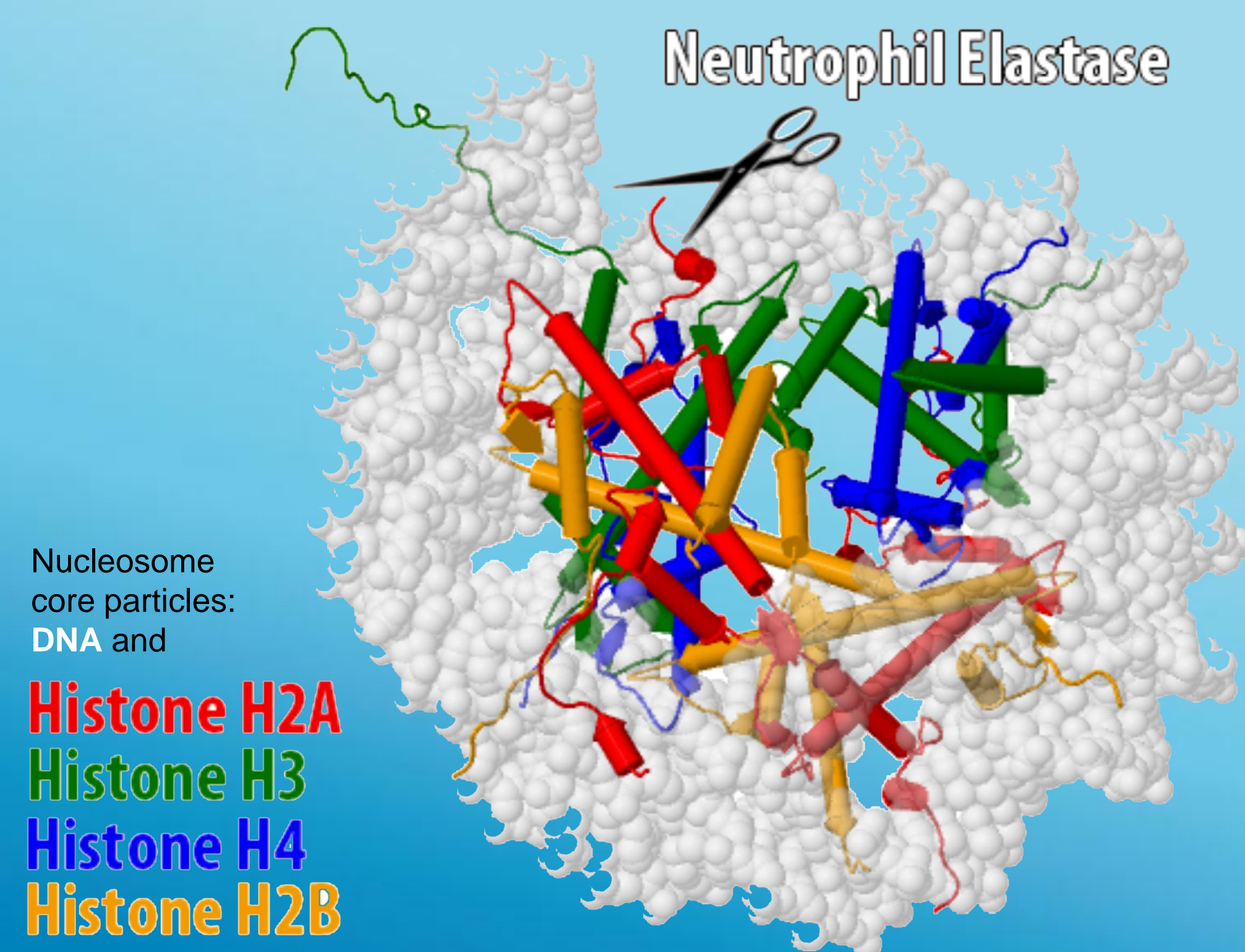
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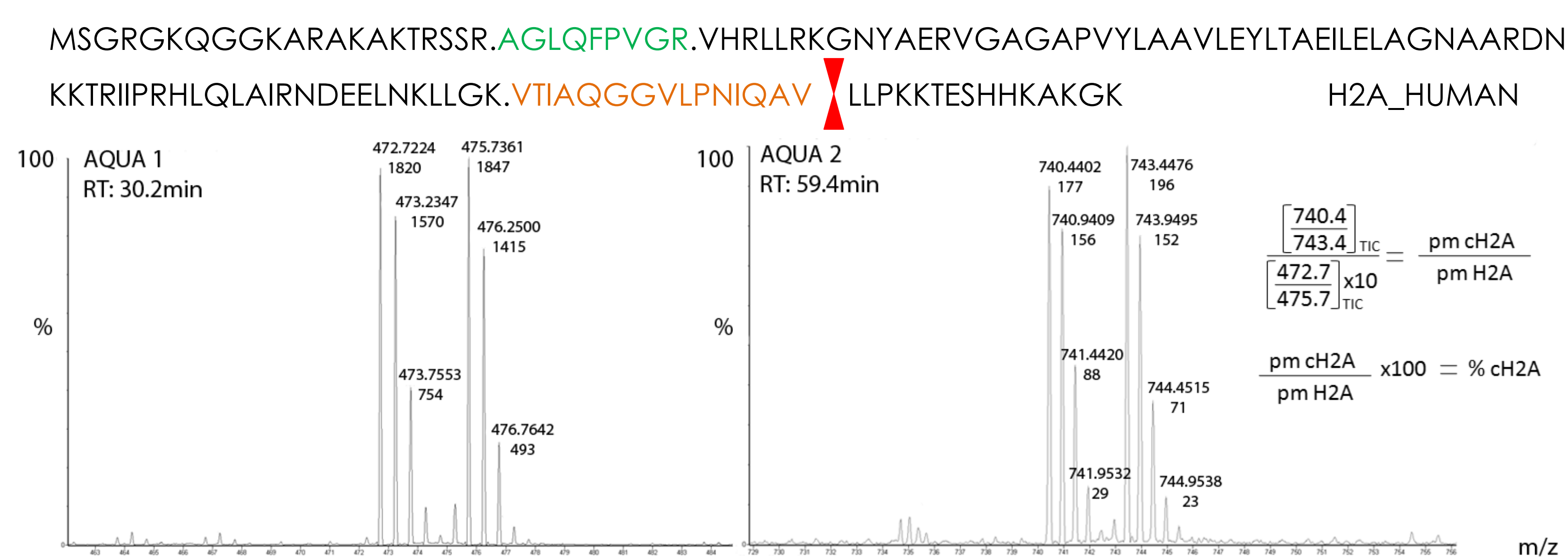
Introduction

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 development¹.

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 today². The responsible enzyme was annotated as the 'H2A specific protease' (H2Asp), but it was never sequenced nor identified^{3,4}.

Results

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



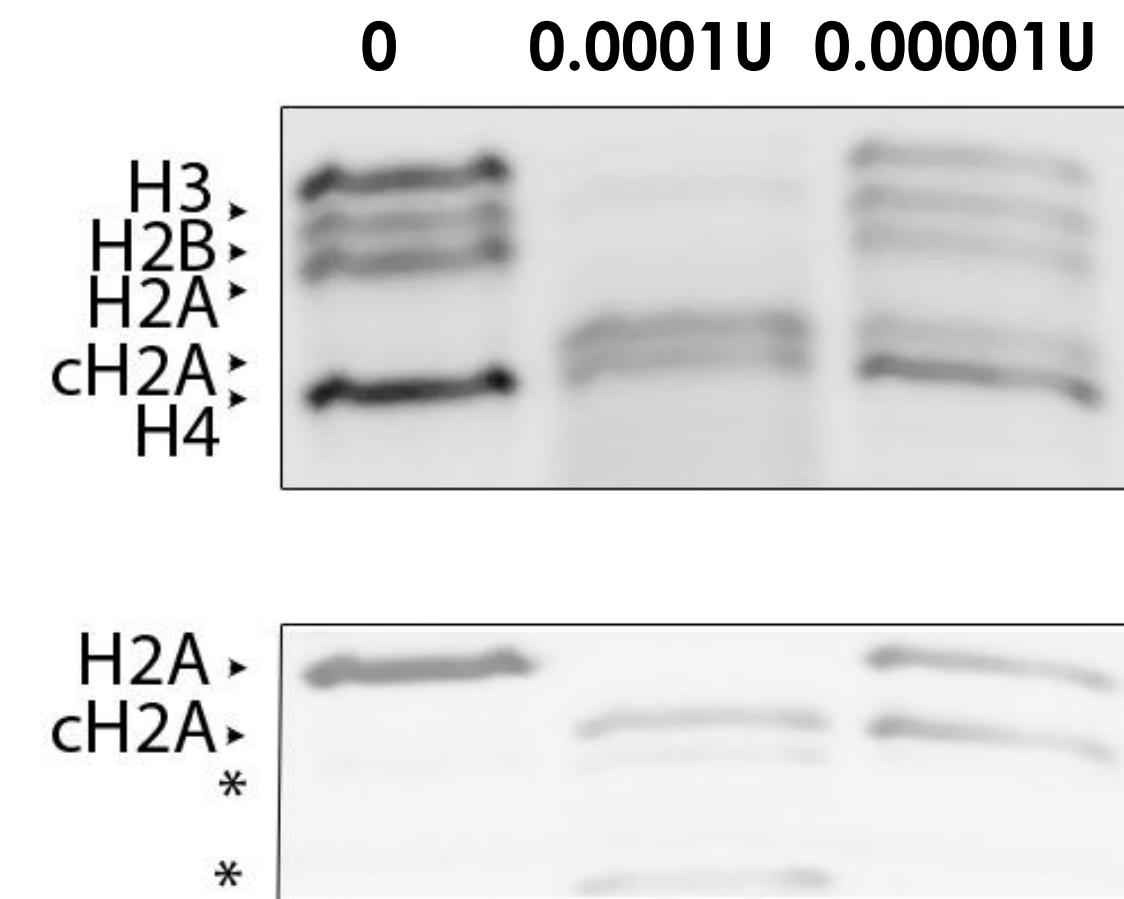
- Based on **two isotopically labeled synthetic peptides** an approach was optimized to specifically quantify H2A V114 clipping in one MS run.

- **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.

Histone H2A is clipped by neutrophil elastase at V114

In Vitro:



- In a histone extract with H2Asp activity only one identified protein is a serine protease with known specificity for valine: **Neutrophil Elastase (NE)**.

- NE cleaves all histones in a dose dependent manner.

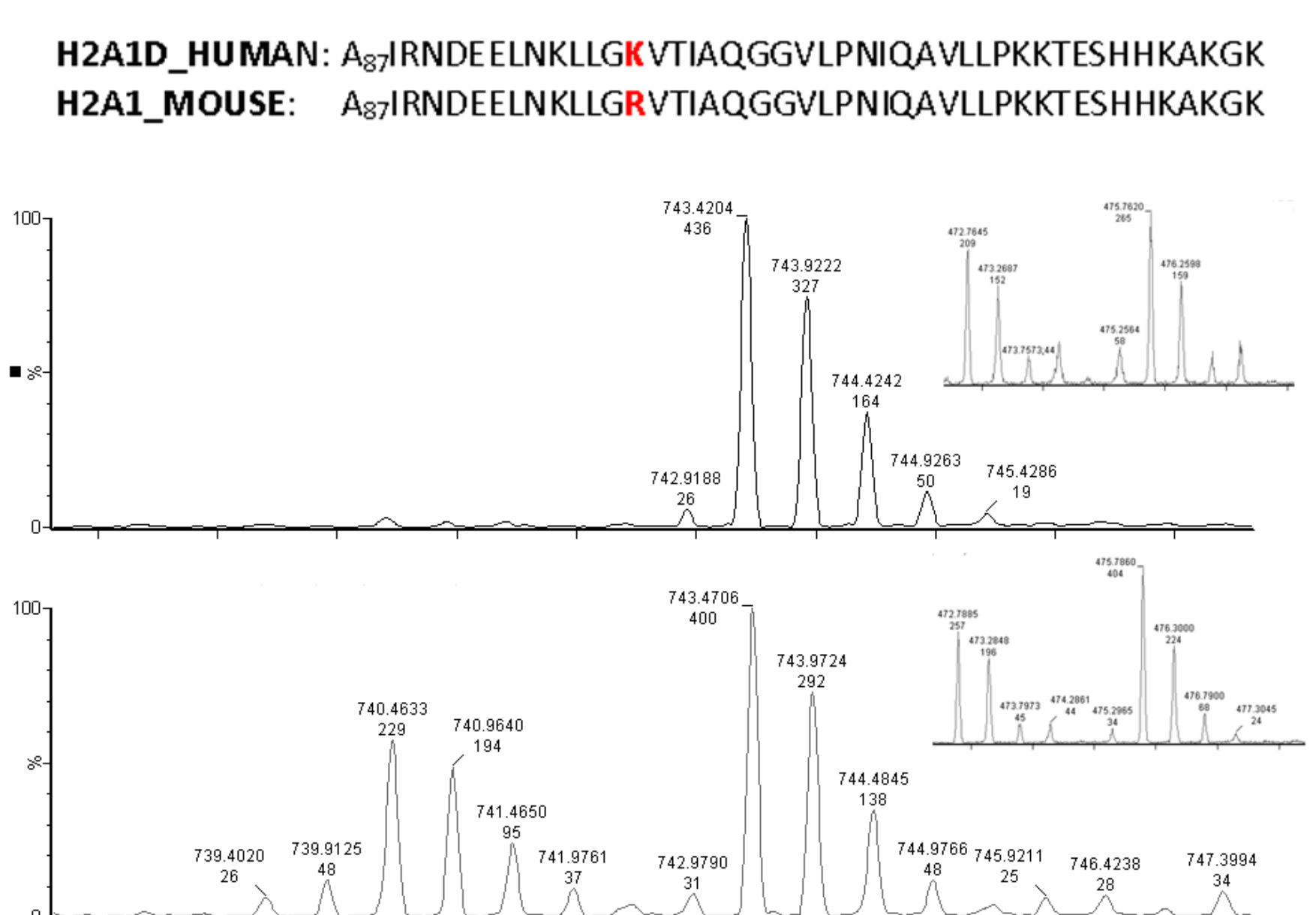
Upper panel: Sypro gel. Commercially purified NE clips all bovine histones when incubated at only 0.0001U. *Lower panel*: H2A blot. Histone H2A V114 is the only substrate at 0.00001U. Asterisks: additional degradation fragments.

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.

Upper panel: Strong sequence homology between mouse and human histone H2A allows for the use of AQUA detection of cH2A in mice. *Lower panel*: No clipping could be detected in NE null mice as confirmed with AQUA-peptides.

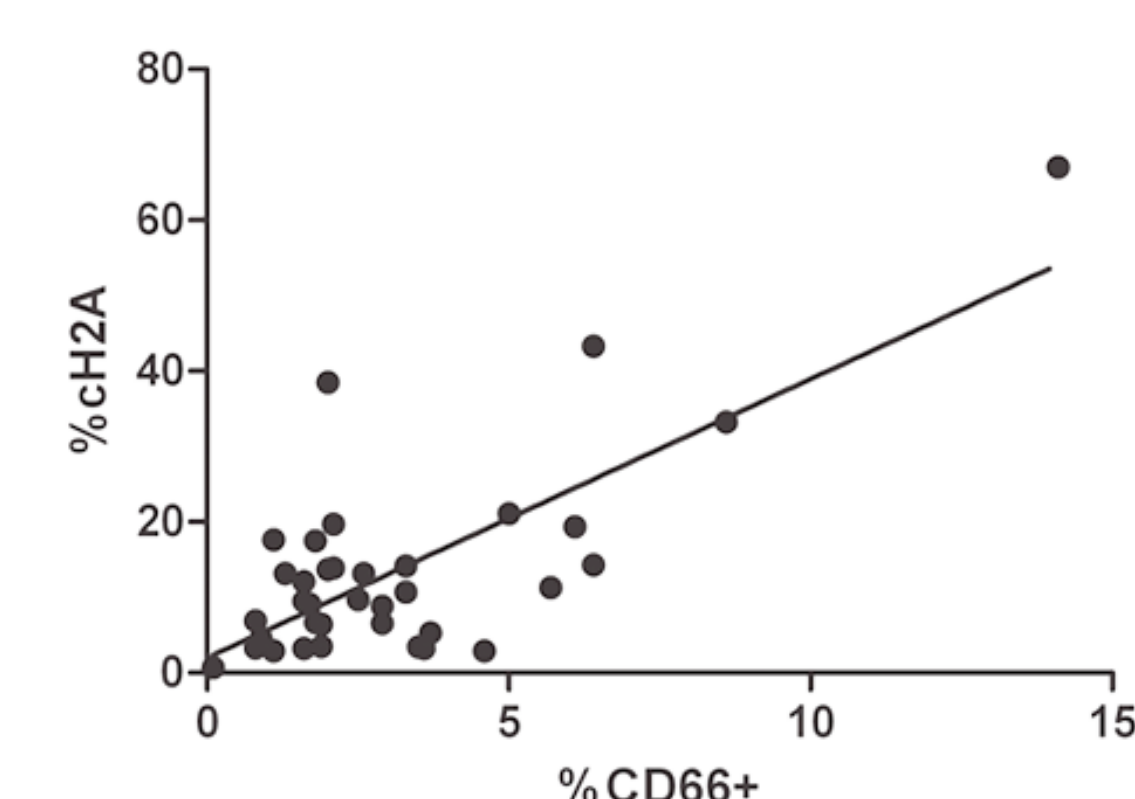


Has no prognostic value in chronic lymphatic leukemia (CLL)

- Screening of **36 differently diagnosed CLL patients**.

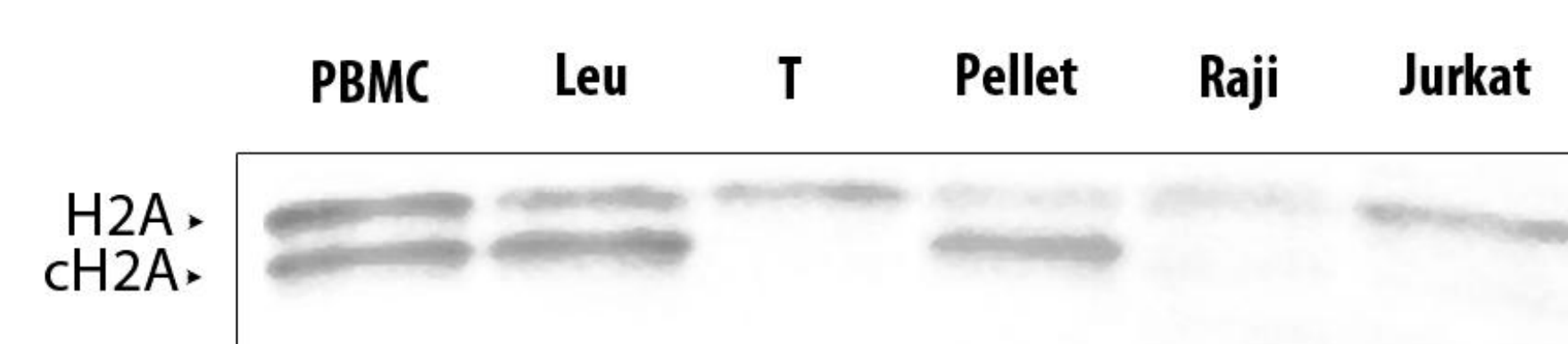
- No correlation between %cH2A and established CLL prognostic markers.

- Only significant correlation: %CD66+. Flowcytometry results reveal that cH2A prevalence is more a prominent **myeloid characteristic**.



Clipping of Histone H2A :

Occurs in the myeloid lineage and in healthy donors

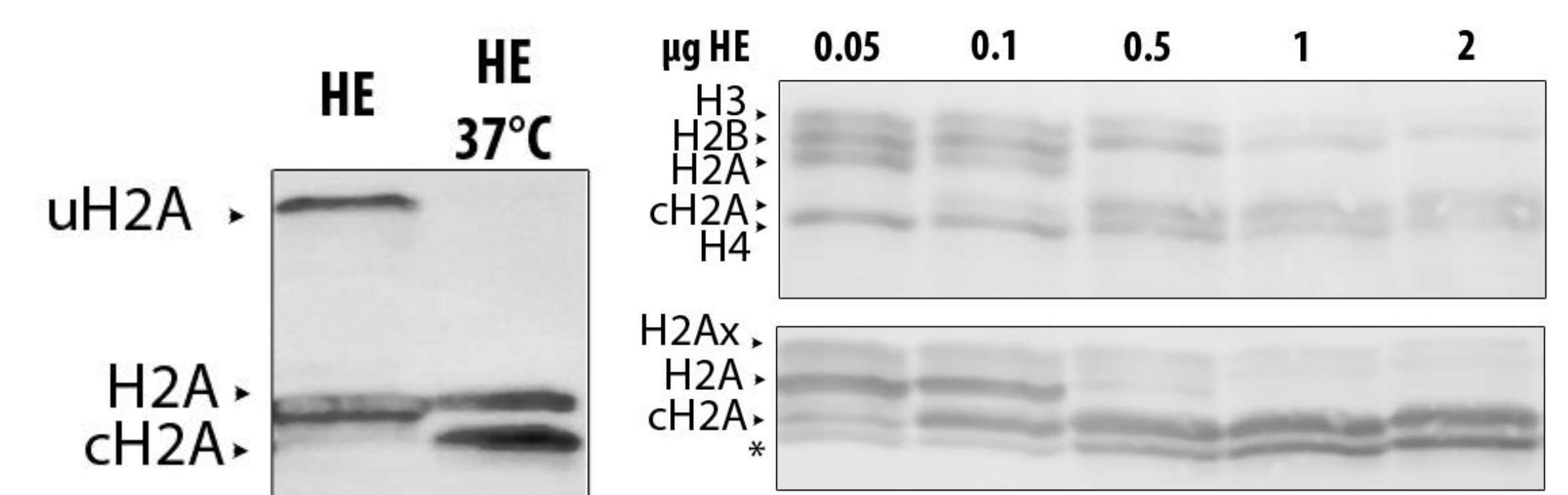


Samples from healthy donors. Western blot with H2A antibody revealed that cH2A is present in the total leukocytes (Leu) obtained by red blood cell lysis. Both in the PBMCs (the buffy coat) and the pellet from a ficoll paque separated cell population the cH2A was detected. Purified T-cells do not show any detectable H2A clipping, nor do Raji or Jurkat cell lines of B- and T-cell origins. V114 clipping was confirmed by AQUA in all samples.

H2Asp is co-extracted during an acid histone extract

- **Ubiquitinated H2A** is a substrate of the H2Asp.

- H2A V114 is the **most sensitive** H2A-specific protease clipping site.



Left panel: As shown by Western blot, salt activation of extracted histones can clip H2A². *Right panel*: 0.05 to 2µg of leukocyte histone extract incubated with 10µg of buffered bovine histones for 2h at 37°C. Sypro stain (*upper part*) and H2A western blot (*lower part*) illustrate that the H2Asp indeed is active and co-extracted during histone extraction. Results confirmed with AQUA-peptides LCMS.

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**⁵. 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 cells⁶. Thus, we emphasize the potential role of H2A clipping in hematopoietic differentiation.