The relevance of acetylated histone H4 at lysine 12 (H4K12ac) for RNA transcripts in human spermatozoa, mouse pronucleus formation and parthenogenetic activation of murine oocytes

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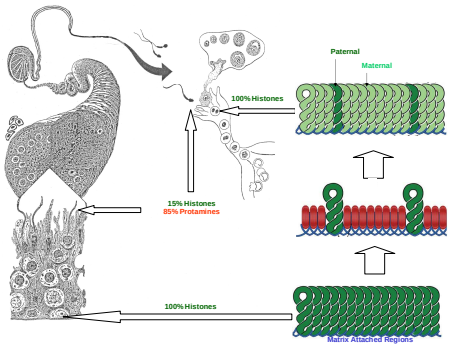
INTRODUCTION

- Sperm histones are highly acetylated normally a characteristic feature of transcriptional active cells.
- Spermatozoa are known to represent transcriptional inactive cells however mRNA transcripts stored in spermatozoa may be important for the embryo development

Hypothesis: Histone acetylation acts as an epigenetic mark that is transmitted from sperm to oocyte and involved in the regulation of transcriptional start and develpoment in the early embryo.



AIMS

- We aimed to investigate whether promoter association with H4K12ac in specific promoters is possibly correlated to mRNA transcripts stored in mature in spermatozoa of fertile and subfertile men.
- Furthermore, we studied the role of paternally derived H4K12ac for the embryo development in murine model.

MATERIAL UND METHODS

- Comparision of the data set of genes interacting H4K12ac in sperm of fertile and subfertile men (Chromatin Immunoprecipitation with H4K12ac in combination with promoter microarray HG18 NimbleGen) with genome wide expression profiling of mRNAs from human sperm of fertile and subfertle patients (CodeLink Human Whole Genome Bioarray, Applied Microarrays).
- The immunofluorscence with H4K12ac antibodies was carried out after in vitro fertilisation or parthenogenetic activation of oocytes.

RESULTS

We found 144 mRNA transcripts out of 525 genes promoters which have been identified as binding sites for H4K12ac. mRNA genome wide profiling provided expression levels of investigated genes between 7 and 16. H4K12ac interacting genes display high level of mRNA transcripts in spermatozoa (10-15). The highest expressed mRNAs are coding testis specific proteins such as PHF7 (PHD finger protein 7 (Testis development protein NYD-SP6) (mRNA level 15), EHD1 (EH domain-containing protein 1 (Testilin) (hPAST1) (mRNA level 14).

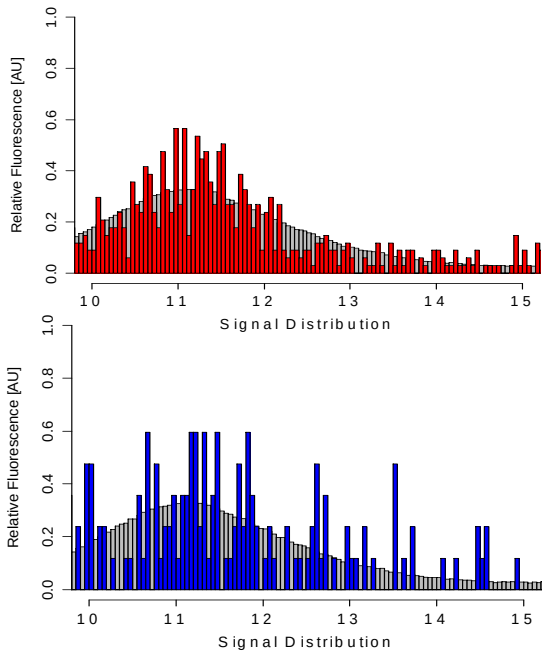


Figure 1: mRNA level of H4K12ac interacting genes in spermatozoa. Plot demonstrating the distribution of mRNA level in the group of H4K12ac bound genes (red bars) against the mRNA level in the group of all 20400 genes (grey bars) as mesured by transctptomic aproach from A ferlile donors and B. infertile patients

140 differentially expressed genes which promoters interacting with H4K12ac in sperm chromatin were subjected to gene ontology classification and clustering with the DAVID2010 database. Functional terms such as "cellular protein molecular process" (35 out of 140 P value=3.1e-4), "positive regulation of transcription, DNA-dependent" (11 out of 140; p value=5.4e-3), "embryonic development" (11 out of 140; p value 6.4e-2) are overrepresented in the group of genes expressed possibly through acetylation of H4K12 during spermatogenesis.

Immunofluorescence with H4K12ac antibodies showed a strong compact signal over the male pronuclus compared to a weaker one in female pronucleus. During pronuclei migration and at the point of pronuclei fusion, there was observed an increasing H4K12ac signal in female pronucleus as well, however, the male pronucleus remained strongly labelled After parthenogenetic oocyte stimulation, both established female pronuclei were positively labelled for H4K12ac.

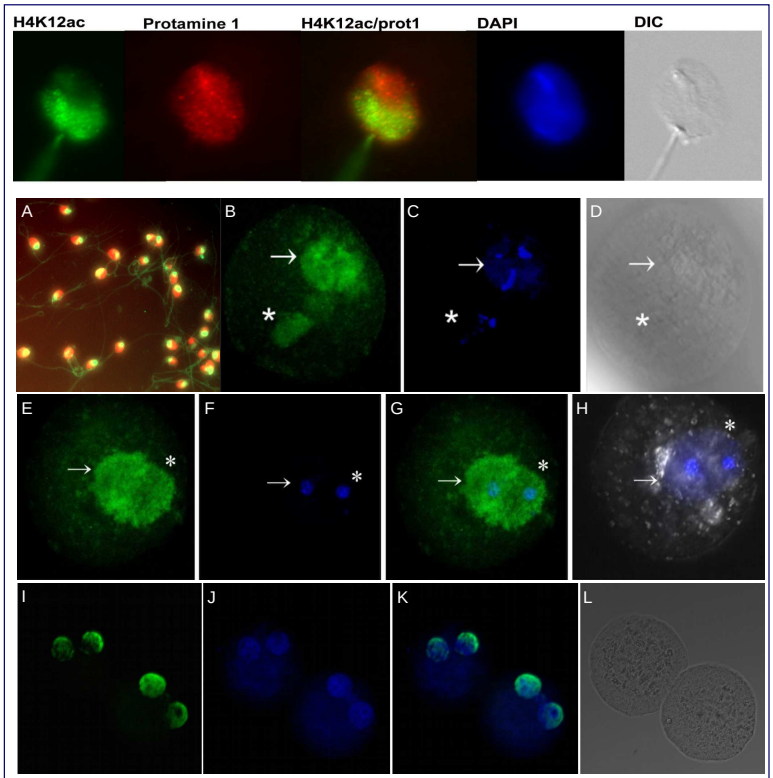


Figure 2: Immunofluorescent labelling of H4K12ac. (A) Mouse spermatozoa. (B-D) Early stages of pronuclei establishment; (E-H) Pronuclei fusion; (I-L) Partheno-geneticaly activated oocytes; A, D) H4K12ac staining (Green), Male pronucleus, shown by arrow, gives strong positive signal compares to a smaller female pro-nucleus shown by asterisk; I) female pronuclei after parthenogenetic activation display identical positive signal. C, F, H) DNA stained by DAPI (Blue), D, L) DIC. Photos are merged for each displayed colour labelling from several sequenced slices taken by a Leica confocal microscope.

CONCLUSIONS

H4K12ac, activating epigenetic mark, and mRNAs stored in mature spermatozoa may both represent the fingerprint for monitoring past events, especially the development profile of gene expression during spermatogenesis or spermiogenesis . This may also lead to inappropriate transfer of epigenetic information to the zygote. We demonstrated different binding pattern of H4K12ac sperm chromatin from fertile and subfertile patients. Proper occupancy of H4K12acac in developmentally important genes may serve potential predictors of sperm fertilising capacity and furthermore may help to predict embryo quality of ICSI outcomes.

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