CHARACTERIZATION OF SUMOYLATED PROTEINS IN HUMAN SPERM

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INTRODUCTION:

SUMOylation is a post-translational protein modification involved in the regulation of essential cell functions. Recently our group investigated the expression of SUMOylated proteins in human ejaculated spermatozoa (Marchiani et al, Int J Androl 2011). We found several SUMO1 and SUMO2/3ylated proteins in spermatozoa in a molecular weight range of 25-85 kDa. Moreover we showed that SUMO1 is mainly present in live spermatozoa and the percentage of SUMOylated spermatozoa was inversely correlated with total and progressive motility. Such correlations become stricter when only asthenospermic subjects were included in the analysis. By immunoconfocal fluorescence analysis and electron microscopy, we demonstrated that SUMOylated proteins are mainly located in the nucleus and in the midpiece.

AIM OF THE STUDY (1):

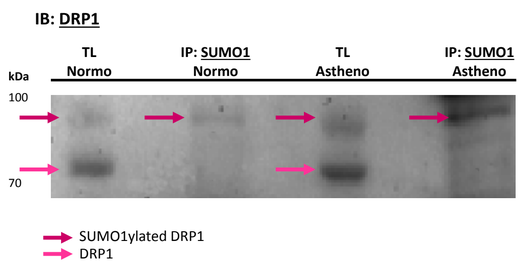
To characterize possible SUMO1 target proteins in human spermatozoa. In particular we evaluated whether SUMOylation occurs in the three proteins:

1. DRP1 (Dynamin-related protein 1): main substrate of SUMO in mitochondria of somatic cells; studies in somatic cells demonstrated that silencing of SENP5 (an enzyme which desumoylates DRP1) leads to stabilization of DRP1, to a fragmented mitochondrial morphology and to an increased production of ROS (Harder et al, Curr Biol. 2005; Zunino et al, J Cell Science. 2007)
2. RanGAP1 (Ran GTPase-activating protein 1): first documented substrate for SUMO in somatic cells; implicated in nuclear-cytoplasmic transport both in somatic cells and spermatidis (Kierszenbaum et al, Mol Reprod Dev. 2002)
3. Topoisomerase IIα: involved in introducing double strand DNA breaks in remodeling sperm chromatin and replacement of histones by protamines during spermiogenesis (Zhao et al, Genesis. 2004); oxidative and heat stress increased SUMO-Topoisomerase II levels in germ cells (Shrivastava et al, Reproduction. 2010)

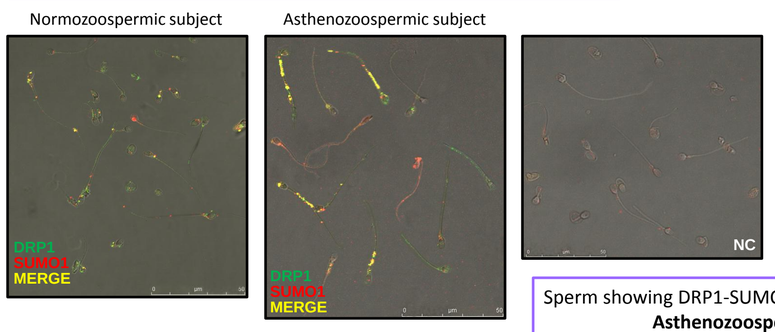
RESULTS & CONCLUSIONS (AIM 1):

➢ DRP1: Immunoprecipitation and western blot experiments

DRP1 in TL (total sperm lysate) and SUMO1 immunoprecipitated (IP) sperm proteins in normo- and astheno-spermic pools



➢ DRP1: Confocal microscopy experiments

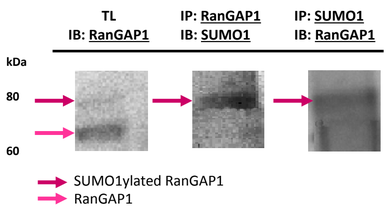


Sperm showing DRP1-SUMO1 midpiece colocalization /Total DRP1 positive sperm (%):
Asthenozoospermic subjects vs Normozoospermic subjects:
50,03±6,32 (n=5) vs 30,41±20,09 (n=5), p=0,07

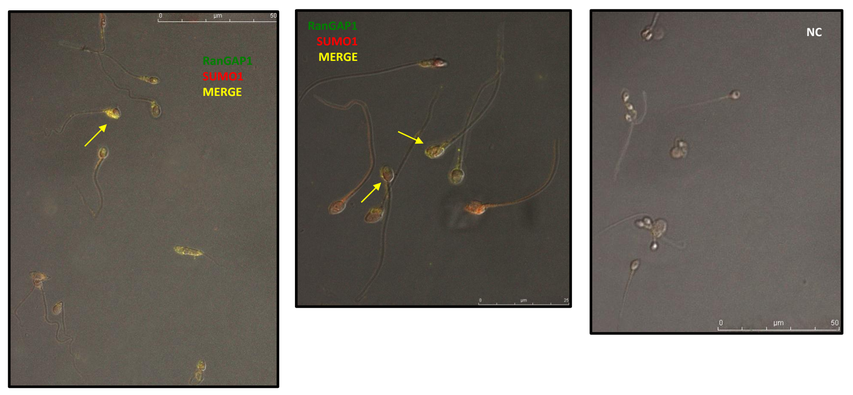
Sperm with **normal morphology**/Total SUMO1 positive sperm (%) vs
Sperm with **abnormal morphology**/Total SUMO1 positive sperm (%):
20,74±9,99 vs 80,07±9,6 (n=10), p<0,001

- DRP1 results to be sumoylated in human sperm
- The colocalization of DRP1 and SUMO1 at midpiece and tail principal piece level and the greater presence of such colocalization in asthenozoospermic subjects supports the hypothesis that SUMOylation of DRP1 could impair mitochondrial function and consequently decrease the sperm motility
- Sperm with morphological defects seem to express higher SUMO fluorescence in agreement with a previous study on human spermatozoa (Vigodner et al, Hum Reprod. 2012)

➢ RanGAP1: Immunoprecipitation and western blot experiments

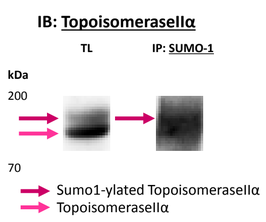


➢ RanGAP1: Confocal microscopy experiments

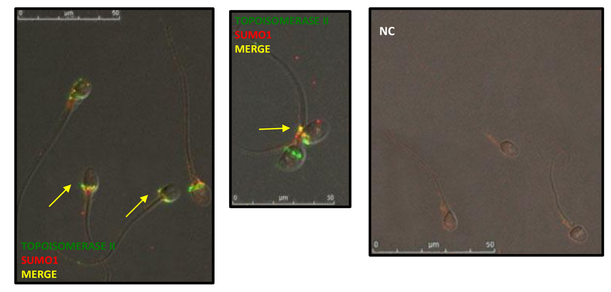


- RanGAP1 results to be sumoylated in human spermatozoa
- RanGAP1 and SUMO1 mainly localized at nucleus level
- SUMOylation of RanGAP1 could be implicated in nuclear-cytoplasmic transport, consistent with its role in somatic cells but it could also contribute to histone-protamine transition and transcription repression, as suggested by Vigodner group (Vigodner et al, Developmental Biology. 2005)

➢ TopoisomeraseIIα: Immunoprecipitation and western blot experiments



➢ TopoisomeraseIIα: Confocal microscopy experiments



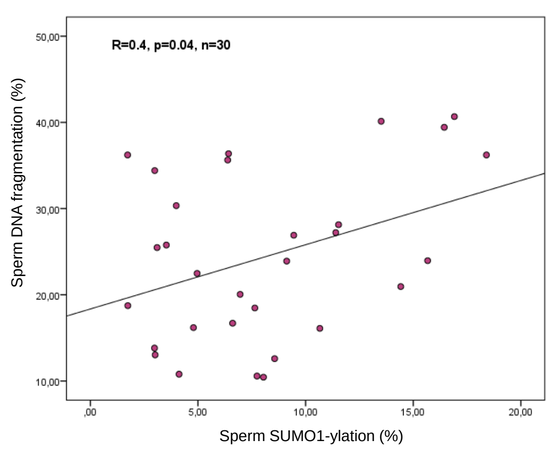
- TopoisomeraseIIα is a SUMO substrate in mature spermatozoa
- SUMOylation of TopoisomeraseIIα could be implicated in remodeling of sperm chromatin and in the replacement of histones by protamines during spermiogenesis (Laberge and Boissonneault, BOR, 2005)
- Moreover the modification of this nuclear protein could play a role in the re-organization of sperm chromatin after fertilization (Bizzaro et al, Zygote, 2000)

AIM OF THE STUDY (2):

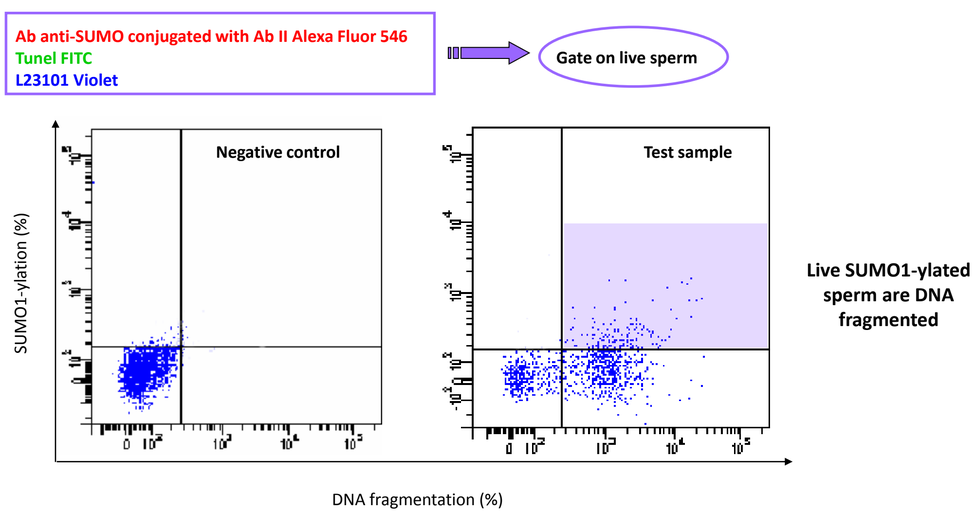
To investigate the relationship between SUMO1ylation and sperm DNA fragmentation, since SUMO-modified proteins have been detected close to DNA strand breaks in nuclei of somatic cells and mouse spermatocytes

RESULTS & CONCLUSIONS (AIM 2):

➢ Signifcant positive correlation between SUMO1 and DNA fragmentation



➢ SUMO1 and DNA fragmentation are simultaneously found in live sperm



- The positive relation found between SUMOylation and DNA fragmentation supports a possible role of SUMOylation in the process of DNA fragmentation. The concomitant occurrence of SUMOylation and DNA damage in live sperm appears to confirm the data on the localization of SUMO close to the sites of DNA breaks in mouse spermatocytes (Shrivastava et al, Reproduction. 2010)
- Overall these data suggest a possible involvement of SUMOylated proteins in formation or repair of DNA breaks, with a mechanisms to be clarified