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Original Contribution

Oxidative DNA damage in mouse sperm chromosomes: Size matters

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ABSTRACT

Normal embryo and foetal development as well as the health of the progeny are mostly dependent on gamete nuclear integrity. In the present study, in order to characterize more precisely oxidative DNA damage in mouse sperm we used two mouse models that display high levels of sperm oxidative DNA damage, a common alteration encountered both in *in vivo* and *in vitro* reproduction. Immunoprecipitation of oxidized sperm DNA coupled to deep sequencing showed that mouse chromosomes may be largely affected by oxidative alterations. We show that the vulnerability of chromosomes to oxidative attack inversely correlated with their size and was not linked to their GC richness. It was neither correlated with the chromosome content in persisting nucleosomes nor associated with methylated sequences. A strong correlation was found between oxidized sequences and sequences rich in short interspersed repeat elements (SINEs). Chromosome position in the sperm nucleus as revealed by fluorescent *in situ* hybridization appears to be a confounder. These data map for the first time fragile mouse sperm chromosomal regions when facing oxidative damage that may challenge the repair mechanisms of the oocyte post-fertilization.

1. Introduction

The burden of repairing sperm DNA damage, including the replacement of oxidized bases, falls onto the oocyte and the resulting embryo. If sperm DNA damage is not repaired, either because it is too extensive or because oocyte's repair capability is not sufficient (*e.g.*, advanced maternal age), the risk of producing *de novo* mutations in the offspring increases [1–9]. Of the characterized oxidative sperm DNA lesions, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is particularly at risk of promoting transversion mutations (G-C to T-A) if not properly repaired by the fertilized oocyte prior to the S phase of the first division [10]. Aggravating the situation, the zygote was shown to possess a limited capacity for 8-OHdG-repair which, consequently, may allow the transmission of unrepaired oxidative DNA damage to the embryo. Such unrepaired mutations will then be transferred to every cell of the fast developing embryo with the potential to alter the developmental programme itself as well as the life of the offspring [11–14]. To support these statements, studies recently suggested that sperm oxidative DNA damage directly affect zygotic development, embryo quality and may increase the susceptibility of the offspring to various pathologies such as lipid disorders, obesity, cancers and autism [11,15]. Exposure of spermatozoa to oxidation occurs under both physiological and non-physiological conditions. The pro-oxidative conditions of epididymal transit that participate in their structural maturation are required for spermatozoa to acquire their fertilizing capacity [16,17]. In particular, intra- and inter-molecular oxidative cross-linking of thiol-containing protamines occurs during epididymal transit to increase sperm nuclear compaction and ultimately prevent DNA damage [16,17]. This is a rather paradoxical situation given the high susceptibility of spermatozoa to oxidative attack. However, in order to ensure normal protamine cross-linking and prevent oxidative attack, the lumen and epithelium of the epididymis are equipped with a potent antioxidant system that exerts a tight control on these maturation-

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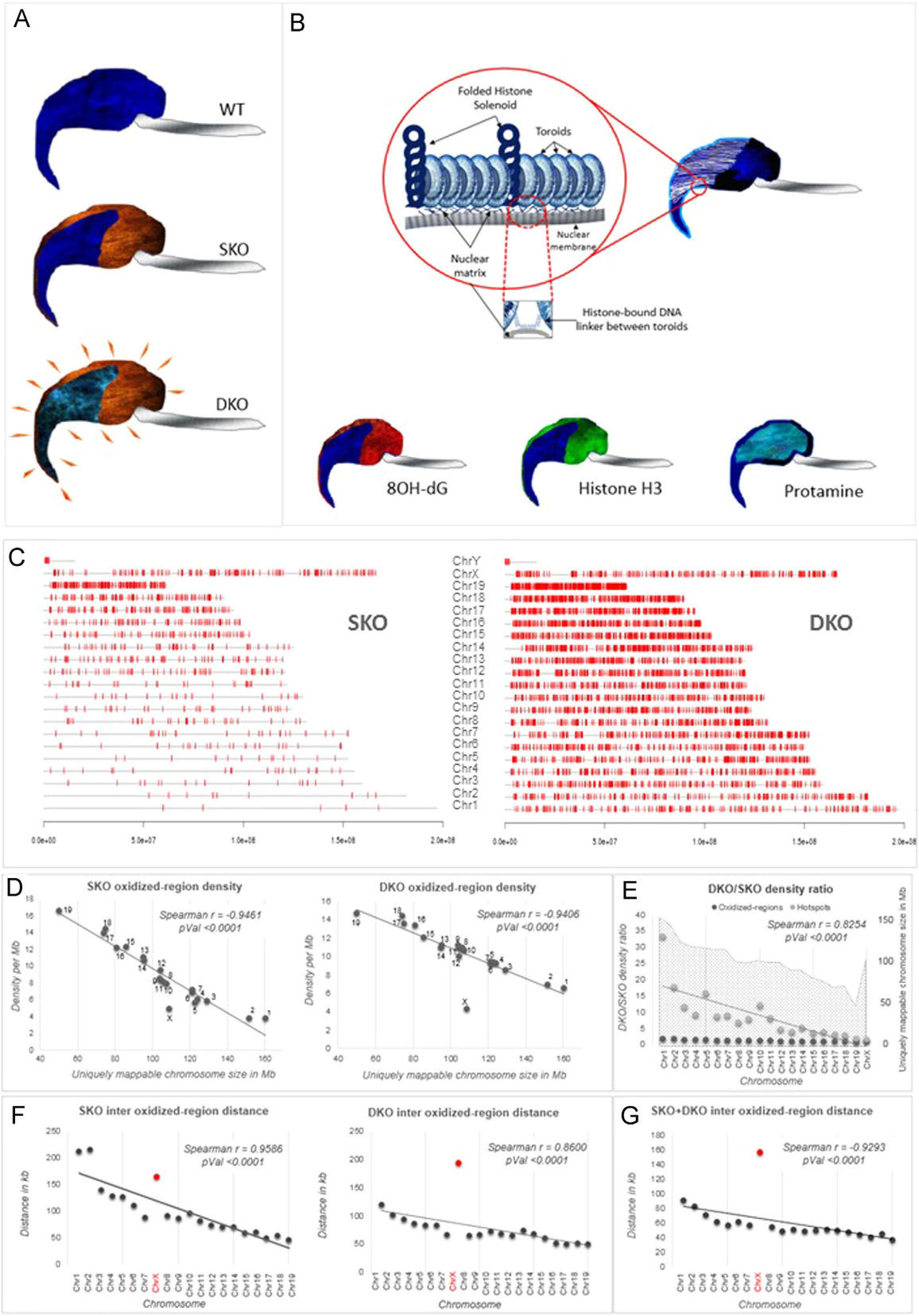
A
WT
SKO
DKO
B
Folded Histone Solenoid
Toroids
Nuclear membrane
Nuclear matrix
Histone-bound DNA linker between toroids
8OH-dG
Histone H3
Protamine
C
SKO
DKO
ChrY
ChrX
Chr19
Chr18
Chr17
Chr16
Chr15
Chr14
Chr13
Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
0.0e+00
5.0e+07
1.0e+08
1.5e+08
2.0e+08
D
SKO oxidized-region density
Spearman r = -0.9461
pVal <0.0001
Density per Mb
Uniquely mappable chromosome size in Mb
DKO oxidized-region density
Spearman r = -0.9406
pVal <0.0001
Density per Mb
Uniquely mappable chromosome size in Mb
E
DKO/SKO density ratio
Oxidized-regions
Hotspots
Spearman r = 0.8254
pVal <0.0001
DKO/SKO density ratio
Uniquely mappable chromosome size in Mb
Chromosome
F
SKO inter oxidized-region distance
Spearman r = 0.9586
pVal <0.0001
Distance in kb
Chromosome
DKO inter oxidized-region distance
Spearman r = 0.8600
pVal <0.0001
Distance in kb
Chromosome
G
SKO+DKO inter oxidized-region distance
Spearman r = -0.9293
pVal <0.0001
Distance in kb
Chromosome