Advances in Experimental Medicine and Biology 1166

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Genetic Damage in Human Spermatozoa

Second Edition

Springer

Advances in Experimental Medicine and Biology

Volume 1166

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-21663-4 ISBN 978-3-030-21664-1 (eBook)
https://doi.org/10.1007/978-3-030-21664-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

This book is dedicated to Prof. Gianni Forti, who guided our activities with great sapience.

Preface

The goal of the male gamete is to deliver a fully intact and functioning paternal genome to the oocyte. To fulfill this aim, the process of chromatin maturation during spermiogenesis must be correctly completed to guarantee DNA protection during the long journey to reach the oocyte and to properly decondense and form the male pronucleus after fertilization. Genetic abnormalities in spermatozoa can be generated in any phase of the sperm production and life and may be due to endogenous and exogenous conditions, the latter including in vitro manipulation for assisted reproduction and gonadotoxic therapies. In addition, emerging studies point out the importance of the damage to the sperm epigenome and address the mechanisms involved in generating it. All these abnormalities may have profound consequences for male fertility status and even for the health of the progeny. This book presents an updated overview of the various types of damage that may affect sperm chromatin. Besides the main mechanisms involved in the generation of de novo mutations and DNA strand breaks and oxidation, two chapters of the book are dedicated to sperm epigenome and epigenetic damage and their consequences for the progeny. In addition, as one of the most important issues regards the possible medical interventions to reduce or prevent sperm DNA fragmentation, one chapter faces the important aspect of pharmacological and surgical treatments, lifestyle modifications, and prevention against exposure to environmental and occupational toxicants.

We wish to thank all the authors for their invaluable contributions to the book. They are all expert scientists in the field, and we appreciate their willingness to offer their knowledge in this important branch of reproductive medicine. We hope that this book will help the researchers in the topics of reproduction and serve as a reference for medical and technical staff working in assisted reproduction laboratories.

Florence, Italy

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Genetic Factors Affecting Sperm Chromatin Structure

1

Mélina Blanco and Julie Cocquet

Abstract

Spermatozoa genome has unique features that make it a fascinating field of investigation: first, because, with oocyte genome, it can be transmitted generation after generation; second, because of genetic shuffling during meiosis, each spermatozoon is virtually unique in terms of genetic content, with consequences for species evolution; and finally, because its chromatin organization is very different from that of somatic cells or oocytes, as it is not based on nucleosomes but on nucleoprotamines which confer a higher order of packaging. Histone-to-protamine transition involves many actors, such as regulators of spermatid gene expression, components of the nuclear envelop, histone-modifying enzymes and readers, chaperones, histone variants, transition proteins, protamines, and certainly many more to be discovered.

In this book chapter, we will present what is currently known about sperm chromatin structure and how it is established during spermiogenesis, with the aim to list the genetic factors that regulate its organization.

Keywords

Spermatozoa · Chromatin · Protamine · Nucleosome · Histone · Gene expression · Nucleus · Spermatids · Spermiogenesis

Introduction

Spermatozoa are produced through a multi-step process called spermatogenesis, during which spermatogonial stem cells at the base of the seminiferous tubules enter the differentiation pathway to ultimately give rise to spermatozoa, released in the lumen of the testicular seminiferous tubules. Spermatogenesis can be divided into three phases: mitotic phase, meiosis, and post-meiotic phase or spermiogenesis. During mitotic phase, spermatogonial stem cells undergo mitotic divisions to maintain the spermatogonial stem cell pool; some of them differentiate into primary spermatocytes. Each primary spermatocyte undergoes DNA replication and meiotic division to produce four haploid round spermatids. Round spermatids then differentiate into elongated spermatids in a process that involves dramatic morphological changes including cytoplasm removal, acrosome biogenesis, development of flagellum for motility, accumulation of mitochondria in the midpiece, and extensive chromatin remodeling that results in nuclear condensation and transcriptional silencing (Russell et al. 1990). The

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E. Baldi, M. Muratori (eds.), *Genetic Damage in Human Spermatozoa*, Advances in Experimental Medicine and Biology 1166, https://doi.org/10.1007/978-3-030-21664-1_1

post-meiotic differentiation of round spermatids into spermatozoa is called spermiogenesis. During this step, spermatid chromatin is extensively modified and remodeled to give rise to a chromatin organization only found in spermatozoa. Indeed, in all other cells (somatic cells, female germ cells, and male germ cells until spermatid stage), the nucleosome is the core particle of chromatin structure (Luger et al. 1997). Histone proteins H2A, H2B, H3, and H4 assemble into an octamer around which 146 base pairs of DNA are wrapped, and this nucleosome structure occurs every 200 base pairs in the eukaryotic genome (Mcghee and Felsenfeld 1980; Luger et al. 1997). In sperm chromatin, the basal unit is not the nucleosome but the nucleoprotamine, formed of smaller, more basic proteins (richer in arginine) than histones: the protamines. Sperm chromatin is organized as toroids containing ~50–100kb of DNA, leading to a chromatin structure 5–10 times more condensed than nucleosome-based chromatin (Ward and Coffey 1991; Balhorn 2007). This tight compaction is essential to allow DNA to fit into a nucleus that is seven times smaller than an interphasic somatic cell nucleus (Ward and Coffey 1991) and to protect the paternal genome from physical and chemical damages. It is also possible that a small nucleus is a hydrodynamic advantage that confers a higher speed to spermatozoa during their transit (Braun 2001).

Briefly, the process of replacement of histones by protamines requires (i) opening of the histone-based chromatin structure facilitated by histone posttranslational modifications (PTM) – in particular histone hyperacetylation – and incorporation of histone variants, (ii) binding of bromodomain proteins to acetyl residues and recruitment of chromatin-remodeling proteins and of transition proteins, (iii) formation and repair of DNA breaks, and (iv) incorporation of protamines leading to a protamine-based compact chromatin structure. At the end of this process, most histones have been replaced by protamines. A small portion of histones (~1% in mice, ~10% in humans) is retained in the spermatozoa genome and contributes to the epigenetic program of the embryo (Balhorn et al. 1977; Gatewood et al. 1990; Hammoud et al. 2009; Brykczynska et al. 2010; Erkek et al. 2013; Ihara et al. 2014; Carone et al. 2014; Samans et al. 2014; Royo et al. 2016; Yoshida et al. 2018; Yamaguchi et al. 2018). [For review, see Champroux et al. (2018).]

Studying animal models (mostly knockout mice) and patient cases, researchers and clinicians have found many genes involved in histone-to-protamine transition, and many more will certainly be discovered. Each of them is a genetic factor which could alter chromatin structure when mutated. In this review, we will present their known or predicted roles while describing the key steps leading to the transition from a histone-based chromatin to protamine-based chromatin (see also Table 1.1).

Regulation of Spermatid Gene Expression

The differentiation of round spermatids into spermatozoa involves profound morphological and functional changes and requires a very specific genetic program with thousands of genes only expressed at that time and regulated at the transcriptional and post-transcriptional levels (Steger 1999; White-Cooper and Davidson 2011; Kleene 2013). Studies of gene expression dynamic throughout spermatogenesis have shown that this program starts as early as the pachytene phase of meiosis [see, for instance, da Cruz et al. (2016) and Chen et al. (2018)].

Among the genes of which expression is activated/upregulated during spermiogenesis are those required for histone-to-protamine transition such as histone variants, chaperones, histone-modifying enzymes, transition proteins, and, of course, protamines themselves. Hence, transcription regulators which control the spermatid gene expression program can indirectly impact on sperm chromatin structure via deregulating key genes of this process.

This is particularly true for regulators of *Protamine 1* (*Prm1*) and *Protamine 2* (*Prm2*) gene expression: in the mouse, *Prm1* and *Prm2* are transcribed into mRNAs that can be detected

Table 1.1 List of genes of which mutations have been shown to result in abnormal sperm chromatin structure

Gene name	Protein	Molecular role in spermiogenesis	Phenotype when mutated	Evidence of role in sperm chromatin structure	References
Genes encoding chromatin proteins					
Prm1/2	*Protamine 1/ 2*	DNA compaction in male germ cells	*Prm1*$^{+/-}$ and *Prm2*$^{+/-}$ chimeric male mice are infertile with abnormal chromatin compaction and sperm DNA damage Another study found that *Prm2*$^{+/-}$ males are fertile and that *Prm2*$^{-/-}$ males are infertile with chromatin compaction defect	Acridin orange assay on *Prm1*$^{+/-}$ and *Prm2*$^{+/-}$ chimeric mice and Comet assay on *Prm2*$^{+/-}$ chimeric mice sperm Electron microscopy on sperm from *Prm2*$^{+/-}$ chimeric mice and *Prm2*$^{-/-}$ mice	Cho et al. (2001, 2003); Schneider et al. (2016)
Tnp1/2	*Transition protein 1/2*	Intermediates in histone-to-protamine transition	*Tnp1*$^{-/-}$ and *Tnp2*$^{-/-}$ *mice* are hypofertile but present chromatin compaction defect and high level of unprocessed PRM2-precursor in sperm *Tnp1*$^{-/-}$ *Tnp2*$^{-/-}$ double knockout mice are infertile with chromatin compaction defect and unprocessed PRM2 precursor protein	Electron microscopy and western blot on spermatids at different stages	Yu et al. (2000); Zhao et al. (2001); Zhao et al. (2004)
H1fnt (H1t2)	*Testis-specific histone H1*	Testis specific Histone H1	Knockout male mice are infertile with sperm chromatin compaction defect, nuclear abnormalities in spermatids, and low protamine level in sperm	Quantification of propidium iodide in sperm DNA by FACS, western blot on sperm and electron microscopy on elongated spermatids	Tanaka et al. (2005); Martianov et al. (2005)
Th2a (Hist1h2aa) and Th2b (Hist1h2ba)	*TH2A (histone cluster 1 H2A family member a, Hist1h2aa) and TH2B (histone H2B type 1-A, Hist1h2ba)*	Testis specific Histone 2 variants	In *Th2b*$^{-/-}$ mouse, fertility is not altered. The absence of TH2B is compensated by the overexpression of H2B in testes However, transgenic mice, in which TH2B is fused to a C-terminal tag, are infertile, and elongating spermatids fail to differentiate and to compact their chromatin. TH2B is incorporated into chromatin but is not replaced by transition proteins or protamines in elongating spermatids In *Th2a*$^{-/-}$ *Th2b*$^{-/-}$ double mutant mice, TNPs and PRMs also fail to incorporate into chromatin, and H2B is overexpressed	Electron microscopy on sperm, MNase digestion in condensed spermatids, and immunostaining of spermatids at different stages Histone liquid chromatography and mass spectrometry	Montellier et al. (2013); Shinagawa et al. (2015)

(continued)