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Consequences of irradiation on adult spermatogenesis: Between infertility and hereditary risk

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ABSTRACT

DNA damage response in adult spermatogenic cells should limit the propagation of mutations to the offspring, without being detrimental to fertility. In differentiating spermatogenic cells, the genomic instability is limited in time, whereas in spermatogonial stem cells it can be maintained all along life. Spermatogonial stem cells are long-lived cells that support normal germ cell differentiation and must be preserved throughout life. However after irradiation spermatogenesis recovery can be impaired as a consequence of the radiation-induced decline in spermatogonial stem cell. In this review, we summarize the differential sensitivities to DNA damage of spermatogenic cell populations, and the DNA repair mechanisms activated in these cells that paradoxically might favour the maintenance of cells with impaired genomic integrity. We describe how the testis tissue collapses in response to irradiation and we discuss the molecular pathways involved in the control of DNA damage response and homeostasis in spermatogonial stem cells.

1. Introduction

Impaired DNA integrity in spermatogenic cells and reduced fertility are important consequences of radiotherapy in patients with cancer [1–3]. In adult spermatogenic cells, ionizing radiation (IR) produces DNA lesions leading to gene mutations and chromosomal damage that are very harmful for fecundity and/or the progeny. Moreover the use of haploid cells with genomic instability in assisted *in vitro* fertilization strategies could amplify the hereditary risk. DNA damage response (DDR) pathways and the DNA repair machinery are necessary to preserve the genetic integrity of spermatogenic cells and limit the hereditary effects of IR exposure. Nevertheless the toxicity of misrepaired DNA damages is limited by cell death that results in sub-fertility or infertility. The molecular pathways that regulate the balance between apoptotic death and cell survival, especially in spermatogonial stem cells (SSCs), are not fully understood. The improvement of SSC and spermatogonia characterization in mice and of genetically engineered murine models begins to enlighten DDR pathways in spermatogenic cells.

2. Adult spermatogenesis

After the first postnatal cycle, adult spermatogenesis is a continuous and cyclic process, in which clones (syncytia) of spermatogenic cells differentiate in a wave-like manner along the testis seminiferous tubules [4,5]. This process initiates from SSCs with the expansion of spermatogonia, followed by meiosis and

Abbreviations: ABCG2, ATP binding cassette subfamily G member 2; alt-EJ, alternative end joining; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; A_{undiff}, undifferentiated spermatogonia; DDR, DNA damage response; BAX, BCL2-associated X protein; DNA-PK, protein kinase DNA activated catalytic polypeptide; DSBs, DNA double-strand breaks; IR, ionizing radiation; GSCs, germinal stem cells; HSCs, hematopoietic stem cells; NHEJ, non-homologous end joining; MDC1, mediator of DNA damage 1; MRE11, MRE11A homolog A double strand break repair nuclease; PARP1, poly (ADP-ribose) polymerase family member 1; PUMA/BBC3, BCL2 binding component 3; RAD51, RAD51 recombinase; SP, side population; SPO11, SPO11 meiotic protein covalently bound to DSB; SSCs, spermatogonial stem cells; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1; TNFRSF10/TRAIL, tumor necrosis factor (ligand) superfamily member 10; TNFRSF10B/DR5, tumor necrosis factor receptor superfamily member 10b; BMI1 Bmi1, polycomb ring finger oncogene; CDKN1a, cyclin-dependent kinase inhibitor 1A (P21); $p19^{Arf}$, $p16^{Ink4a}$ cyclin-dependent kinase inhibitor 2A; MDM2, transformed mouse 3T3 cell double minute 2; CDK4, cyclin dependent kinase 4; CXCL12, chemokine (C-X-Cmotif) ligand 12; CSF1, colony stimulating factor 1; c-KIT, CD117 receptor tyrosine kinase; KITL, KIT ligand; PI3K-AKT, phosphatidylinositol 3-kinase-thymoma viral proto-oncogene 1; mTOR, mammalian target of rapamycin; ROS, reactive oxygen species; Ddit4/Redd1, DNA-damage-inducible transcript 4.

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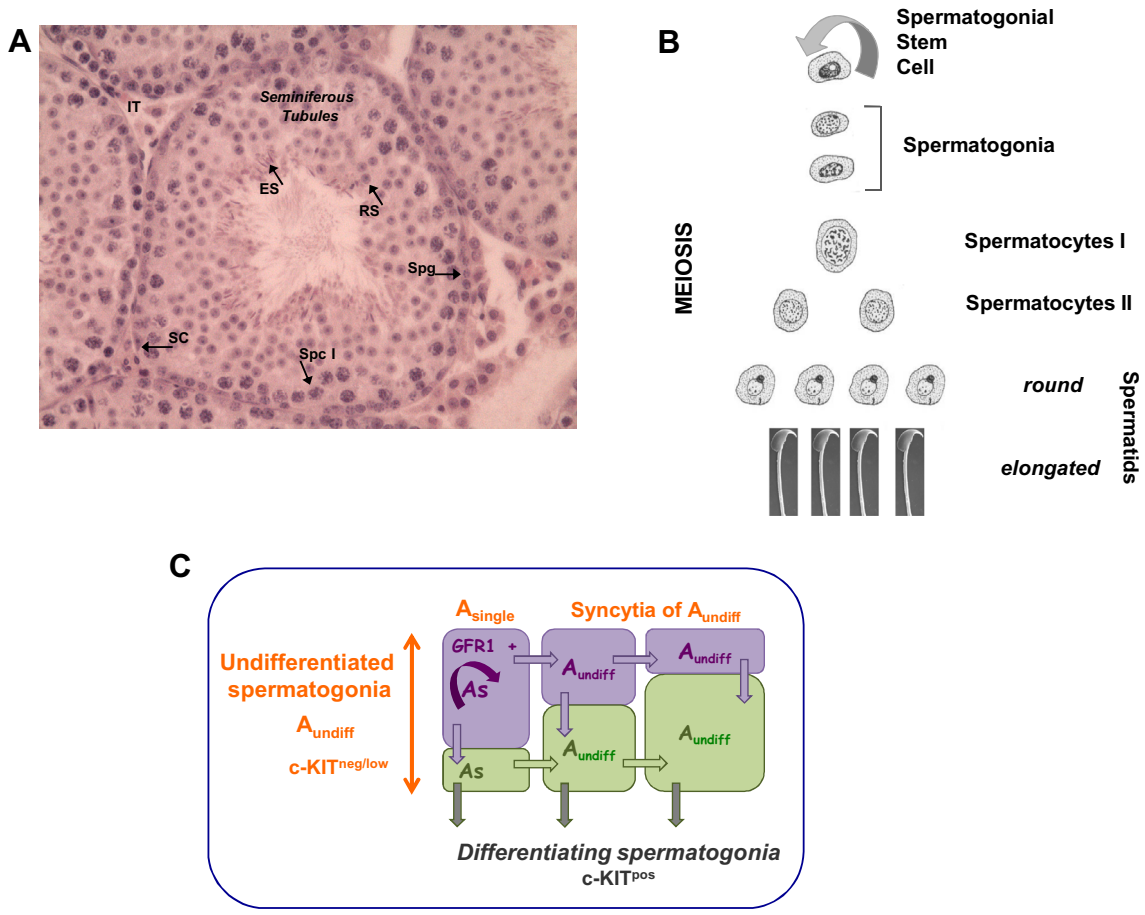

Fig. 1. Spermatogenesis in adult mice.
A. Testicular cross-section stained with hematoxylin-eosin: Seminiferous tubules are separated by interstitial tissue (IT), formed by Leydig cells and vasculature. Sertoli cells (SC) are somatic cells located inside the tubules and support spermatogenic cell differentiation. Spermatogonia (Spg) are the premeiotic cells in contact with the basal membrane and Sertoli cells. In the adluminal compartment progress meiosis with formation of Spermatocytes I (SpcI) and then Spermatocytes II. Haploid cells mature from round (RS) to elongated (ES) spermatids that are released in the lumen.
B. Schematic of spermatogenic cell differentiation, from the self-renewing SSC pool to production of spermatozoa. After a last replication, spermatogonia progress to spermatocytes I. These meiotic cells pass through Leptotene, Zygotene, Pachytene and Diplotene stages before the MI division; this progression with the long pachytene duration, allows distinguishing between, early and late spermatocytes I.
C. Undifferentiated spermatogonia in adult mice
Spermatogonia are divided into undifferentiated (A_{undiff}), and differentiating that expresses the c-KIT receptor. The undifferentiated population is composed of single spermatogonia, and their cell progeny is connected by intercellular bridges (syncytia). A_{undiff} is a heterogenous population that express various molecular markers, such as the receptors for GDNF, c-RET and GFR1α. The stem-cell potential is considered to be limited to the GFR1α positive-cells (in purple, mainly single A_{undiff}), and it progressively decreases with the elongation of syncytia. By contrast, most of the GFR1α negative-A_{undiff} (in green) directly progress to differentiating spermatogonia. Some A_{undiff} might maintain the ability to detach from syncytia and revert to the stem-cell state. Syncytia fragmentation is observed during steady-state spermatogenesis and occurs frequently during regeneration following tissue injury.

ends with the production of spermatozoa (Fig. 1A and B). In adult mice, premeiotic diploid cells are divided in undifferentiated and differentiating spermatogonia. Undifferentiated spermatogonia (A_{undiff}) include isolated single cells that divide with incomplete cytokinesis, giving rise to syncytia of A_{undiff} cells. Within the A_{undiff} population, SSCs undergo self-renewal to maintain the SSC pool or differentiate to produce transit-amplifying (T/A) progenitors [4,5]. Based on the expression of specific markers, A_{undiff} constitute a heterogeneous population and the stem-cell functionality is mostly concentrated in single spermatogonia. However it is also observed in some (T/A) progenitors within syncytia, that maintain the potential to revert to a self-renewing state (Fig. 1C). The SSC pool is considered to be in a proliferative state [6,7]. The more committed A_{undiff} cells further progress to differentiating spermatogonia that enter meiosis after several rounds of synchronous division. After DNA replication that produces tetraploid primary spermatocytes, meiosis proceeds by two successive divisions. During the first, reductional meiosis (MI), the two copies of individual chromosomes are segregated in spermatocytes II. In the second division (MII) sister chromatid pairs are separated between haploid spermatids. This marks the entry into spermiogenesis, during which round spermatids mature into elongated spermatids that will then produce spermatozoa.

The differentiation of spermatogenic cells is strictly dependent on the somatic environment [8,9]. Specifically, Leydig cells, which are located in the spaces between seminiferous tubules, trigger steroidogenesis. Inside tubules, spermatogenic cells are associated with Sertoli cells that provide nutrients, growth factors and cytokines necessary for their differentiation.

3. Testis collapse and recovery after high dose irradiation

Like other tissues characterized by high cell turnover, adult testis is very sensitive to radiation, as shown by the data collected using rodent models. This is correlated with the high sensitivity of differentiating spermatogonia (mean lethal dose D_0, 0.5 Gy) established by quantification of repopulated seminiferous tubules post-irradiation [10]. The consequences of testis irradiation are