

Possible functions of P21 CDKN1A within the germ cells of the mouse seminiferous epithelium

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Abstract

The cyclin dependent kinase inhibitor P21 CDKN1A has been shown to be upregulated during differentiation and after DNA damage in somatic cells. We examined the expression of P21 CDKN1A and of γ -H2AX during the differentiation of germ cells in normal mouse testis. P21 CDKN1A was normally expressed in a variable mode in different cell types (spermatogonia, spermatocytes, spermatids, Sertoli cell) located since the basal till the lumen of the seminiferous tubules. Moreover, in agreement with γ -H2AX expression, P21 maybe involved in different cell cycle checkpoints in spermatocytes such as a pre-replication, XY body inactivation, a mid-pachytene and a metaphase I checkpoints. Finally, in comparison to control, the p21 deficient mouse testis show elevated numbers of apoptosis of elongated spermatids within stages VIII-IX but any difference in the number of spermatogonia mitosis. These results suggest that P21 also may take part in the regulation of the differentiation of the male germ cells and may have a role in spermatogonia not related with a mitotic checkpoint.

Keywords

P21 CDKN1A, Spermatogenesis

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The cyclin dependent kinase inhibitor P21 CDKN1A has been shown to be upregulated during differentiation and after DNA damage in somatic cells (1). Also, it was found that P21 is a factor which maybe important during the meiotic prophase in spermatocytes and repair mechanisms in these cells, but not in the spermatogonial cell cycle (2). Here we further study the role of P21 in the mouse seminiferous epithelium by analysing its expression in mouse testis and by performing apoptosis and proliferation studies in the mouse deficient for p21.

We started by analysing the expression of P21 CDKN1A in mouse testes paraffin cuts. P21 is expressed in a variable mode in different cell types (spermatogonia, spermatocytes, spermatids, Sertoli cell) located since the basal till the lumen of the seminiferous tubules (fig.1).

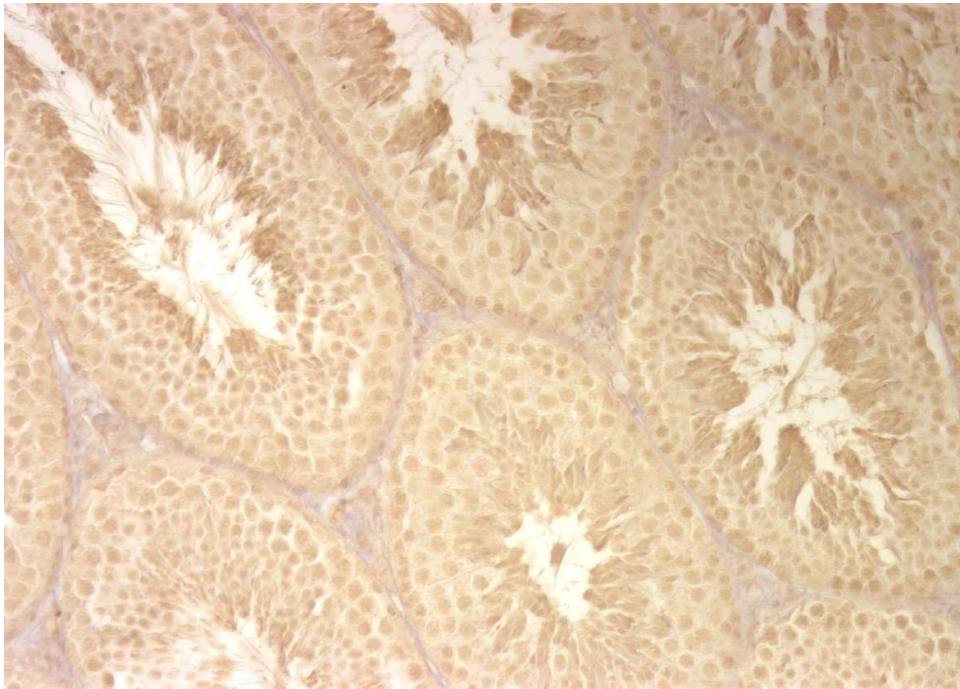


Figure 1: Expression of P21 CDKN1A in the mouse testes (POD, x40)

Further, we counted the number of spermatogonia mitotic figures in control and in p21 deficient testis and found there is no significant difference between the number of spermatogonia mitotic figures in control and in *p21* deficient testis (9) (not shown).

Next, we analysed the relationships of the expression of P21 CDKN1A with those of γ -H2AX in the mouse meiotic germ cells (fig.2). During preleptotene, when pre-meiotic replication takes place expression of P21 and γ -H2AX are similar, meaning P21 maybe halting cell cycle progression while γ -H2AX identifies sites of stalled replication forks (3). During leptotene, when meiotic double-strand breaks (DSBs) occur, γ -H2AX expression is maximal (4) while P21 is much lower, thus allowing cell cycle progression while meiosis is initiated. During zygotene and the zygotene-pachytene transition (Z-P), γ -H2AX starts decreasing while P21 raises to a peak of expression at Z-P, when γ -H2AX only appears covering the forming X-Y body (5), suggesting that P21 plays a role in XY body inactivation. Moreover, during pachytene as γ -H2AX continues to fade, P21 shows another peak of expression where it is supposed it works a mid-pachytene checkpoint monitoring those pachytene spermatocytes with meiotic recombination defects (6,7). γ -H2AX totally disappears during diplotene while P21 peaks again at metaphase I (MI), suggesting a role for P21 during a MI checkpoint (8).

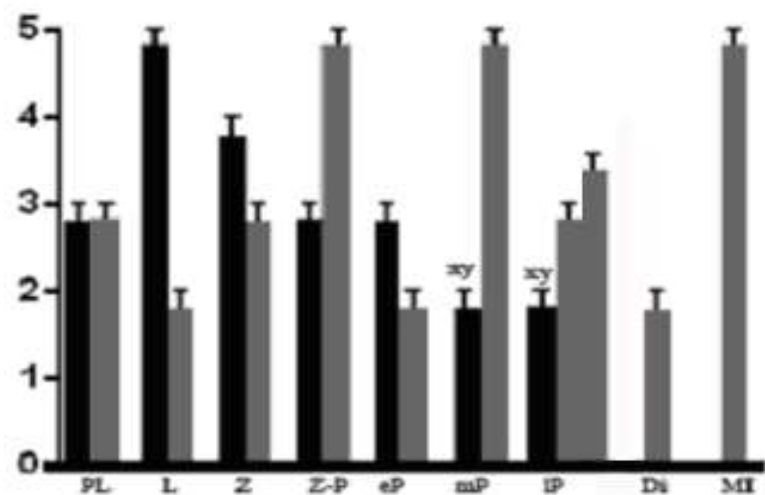


Figure 2: Variation of the expression of P21 (arbitrary units from 0 to 5) in relation with γ -H2AX in different cell types of mouse testes. Black bars- γ -H2AX, grey bars – P21. PL – preleptotenes, L –leptotenes, Z – zygotenes, Z-P – transition zygotene-pachytene, eP – early pachytene, mP – mid pachytene, IP – late pachytene, Di – diplotene, MI – metaphase I. xy- γ -H2AX is only present covering the XY body.

Finally, we quantified apoptosis in the mouse seminiferous epithelium (fig.3). We got to the conclusion that in the p21 deficient mouse there are more apoptosis of elongated spermatids at stages VIII and IX.

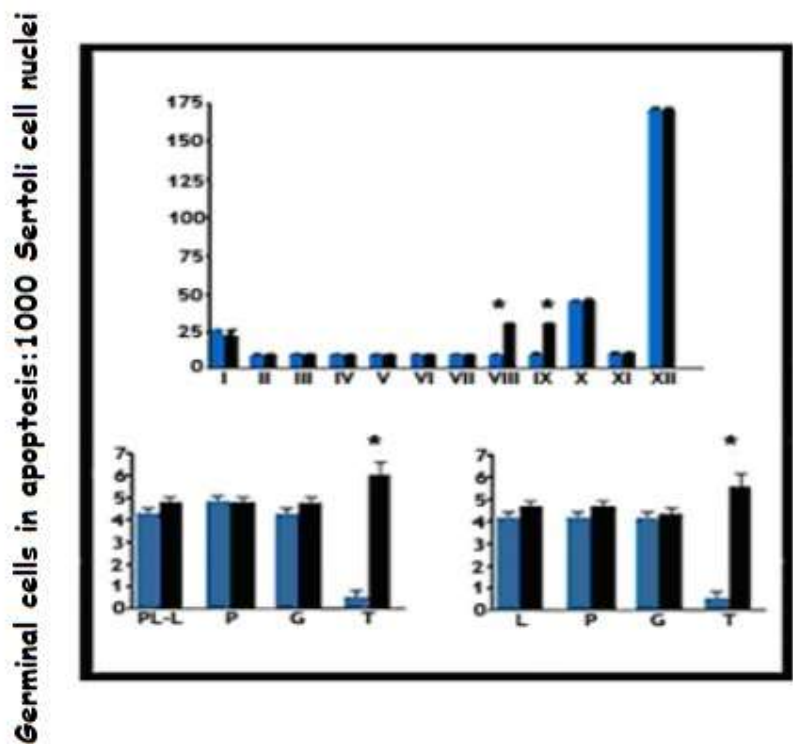


Figure 3: Apoptosis within germ cells of control (blue) and p21 deficient mice (black) per stage and cell types (germ cells in apoptosis per 1000 Sertoli cell nuclei). *, means significant statistical difference, $p < 0.05$. The lower diagrams represent apoptosis at stages VIII (PL-L -preleptotene, P- pachytenes, G, spermatogonias, T, elongated spermatids) and IX (L -leptotene, P- pachytenes, G, spermatogonias, T, elongated spermatids)

Conclusions

These results suggest that P21 may take part in the regulation of the differentiation of the male germ cells and may have a role in spermatogonia not related with a mitotic checkpoint. Also, P21 maybe involved in different cell cycle checkpoints in spermatocytes such as a pre-replication, XY body inactivation, a mid-pachytene and a metaphase I checkpoints.

Materials and methods

This study was performed within the scope of Ana Pedro's PhD Thesis approved on the 07/07/2009 by the Dean of University of Valladolid, Spain.

Mice

We used control and p21 deficient (9) mice with the same background (129sv/C57BL6; 50:50) kindly donated by Manuel Serrano, CNIO, Madrid. The mice testes were desiccated and included in paraffin blocks.

P21 CDKN1A and γ -H2AX staining

We used for brightfield P21 study the following antibodies: P21 CDKN1A Neomarkers, ab-9, #RB-032-P0, 1:150), Santa Cruz Biotechnology, Inc; Rabbit ABC Staining System; #sc-2018. For double immunofluorescence we used mouse anti- γ -H2AX, 1:2000 (Upstate, #05-636), P21 CDKN1A Neomarkers, ab-9, #RB-032-P0, 1:150), Santa Cruz Biotechnology, Inc., anti-rabbit Alexa Fluor ®488 , 1:500 (Molecular Probes, # A-11034), anti-mouse Alexa Fluor ® 488, 1:1500 (Molecular Probes, # A-21202), anti-rabbit Alexa Fluor ® 594, 1:1000 (Molecular Probes # A-21207), anti-mouse Alexa Fluor ® 594 (Molecular Probes, # A-21203)

Apoptosis labelling

For apoptosis labelling, we used the Roche, In situ Cell Death Detection Kit, POD, # 1 684 817).

Statistics

We used Prism program. For apoptosis quantification we analysed 10 paraffin cuts from p21 deficient mouse and 10 paraffin cuts from control mouse, counter-stained with PAS and count the number of apoptosis in 30 tubules of each stage in agreement with Oakberg (1956). For mitosis, we counted the number of mitotic figures of spermatogonia in 5 non-consecutive tubules of the stages *I, IV, VI, IX, X y XII both in control and p21 deficient mouse. We calculated the percentage of mitosis in relation to the average number of Sertoli cell in control and in p21 deficient mouse.*

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