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Letter to the Editor

E2F1 deficiency impairs murine spermatogenesis and augments testicular degeneration in SCP3-nullizygous mice

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Dear Editor,

E2F1 is a strong activator of transcription and DNA synthesis and can promote S phase entry and induce apoptosis in a p53-dependent or -independent manner.[1] Both depletion and overexpression of E2F1 in mice result in testicular atrophy at 36 or 6 weeks of age, respectively.[2,3] Whereas testicular atrophy in mice overexpressing E2F1 results from p53-independent apoptosis,[3] the cause of testicular atrophy in mice deficient in E2F1 remains unclear. We have previously reported that absence of the meiosis-specific synaptonemal complex protein 3 (SCP3) also gives rise to testicular atrophy in male mice. SCP3-null germ cells display extensive chromosomal pairing failures during early meiotic prophase I, triggering a p53-independent chromosomal pairing checkpoint that renders males infertile via spermatocyte apoptosis.[4,5] We have now investigated further the involvement E2F1 and SCP3 during spermatogenesis and male meiosis.

We show here that testicular atrophy in *E2f1*-deficient mice occurs as early as 12 weeks of age (Figures 1a and b), and we propose that this is due to spermatogonial cell loss (Figures 1e–g), rather than germ cell apoptosis (Figure 1c). Loss of a spermatogonial cell will result in the subsequent loss of spermatocytes as well as haploid cells (i.e. cells are lost in a clonal fashion). As expected, the lower numbers of spermatogonia in turn lead to an observed proportional reduction in the number of differentiated spermatocytes (Figures 1e–g), with a 67% lower epididymal sperm count compared with wild type (data not shown). The overall number of Sertoli cells is essentially unaltered (Figures 1d and gII).

The cell loss seen in $E2f1^{-/-}$ testes becomes more pronounced in $E2f1^{-/-}Scp3^{-/-}$ testes (Figures 2a–c), which exhibit an increase in the number of seminiferous tubules devoid of germ cells, so-called Sertoli-cell-only tubules (Figures 2b and c). To monitor whether lack of E2F1 affects meiotic progress, we investigated the expression of the synaptonemal complex protein 1, SCP1, a marker for chromosomal pairing that first appears in zygotene spermatocytes, and the centromeric marker CREST, in spermatocytes with the following genotypes: wild-type; $E2f1^{-/-}$; $Scp3^{-/-}$ and $Scp3^{-/-}E2f1^{-/-}$. We find that the SCP1-labelling pattern in *E2F1*-deficient zygotene spermatocytes does not differ from that of their wild-type counterparts, while spermatocytes null for both SCP3 and E2F1 are indistinguishable from $Scp3^{-/-}$ cells, in that both contain fragmented fibrillar structures (Figures 2d). E2F1 deficiency does not, therefore, rescue the prophase I defects seen in $Scp3^{-/-}$ testes.

In summary, we find that loss of E2F1 reduces the number of mitotic spermatogonia, i.e. the progenitors of spermatocytes, and aggravates degeneration of the seminiferous tubules when combined with SCP3 deficiency. We propose that the reduction in spermatogonial cell numbers in $E2f1^{-/-}$ testes is due to a lower proliferation rate of spermatogonial type A stem cells. Moreover, we report that the checkpoint monitoring the apoptotic mechanism responding to SCP3-null chromosome pairing is thus p53- and E2F1 independent, which provides as yet undocumented examples in regard to the role of the E2F family during meiosis.

Our proposals for reduced mitotic proliferation in spermatogonia are supported by two other studies on $E2f1^{-/-}$ mice. Cooper-Kuhn *et al.*[6] have previously shown that *E2F1*-deficient mice have significantly fewer neural stem cells and less progenitor division in the proliferative zones of the lateral ventricle wall and the hippocampus. D'Souza *et al.*[7] found that epidermal keratinocytes isolated from $E2f1^{-/-}$ mice exhibit altered patterns of proliferation, including significant delays in transit through both G1 and S phases of the cell cycle. Taken together, these findings are consistent with recent genome-wide analyses of cell-cycle-regulated genes, where a large fraction of E2F-regulated gene targets are found to encode proteins known to be involved in the progression of cells into G2 and through mitosis.[8]

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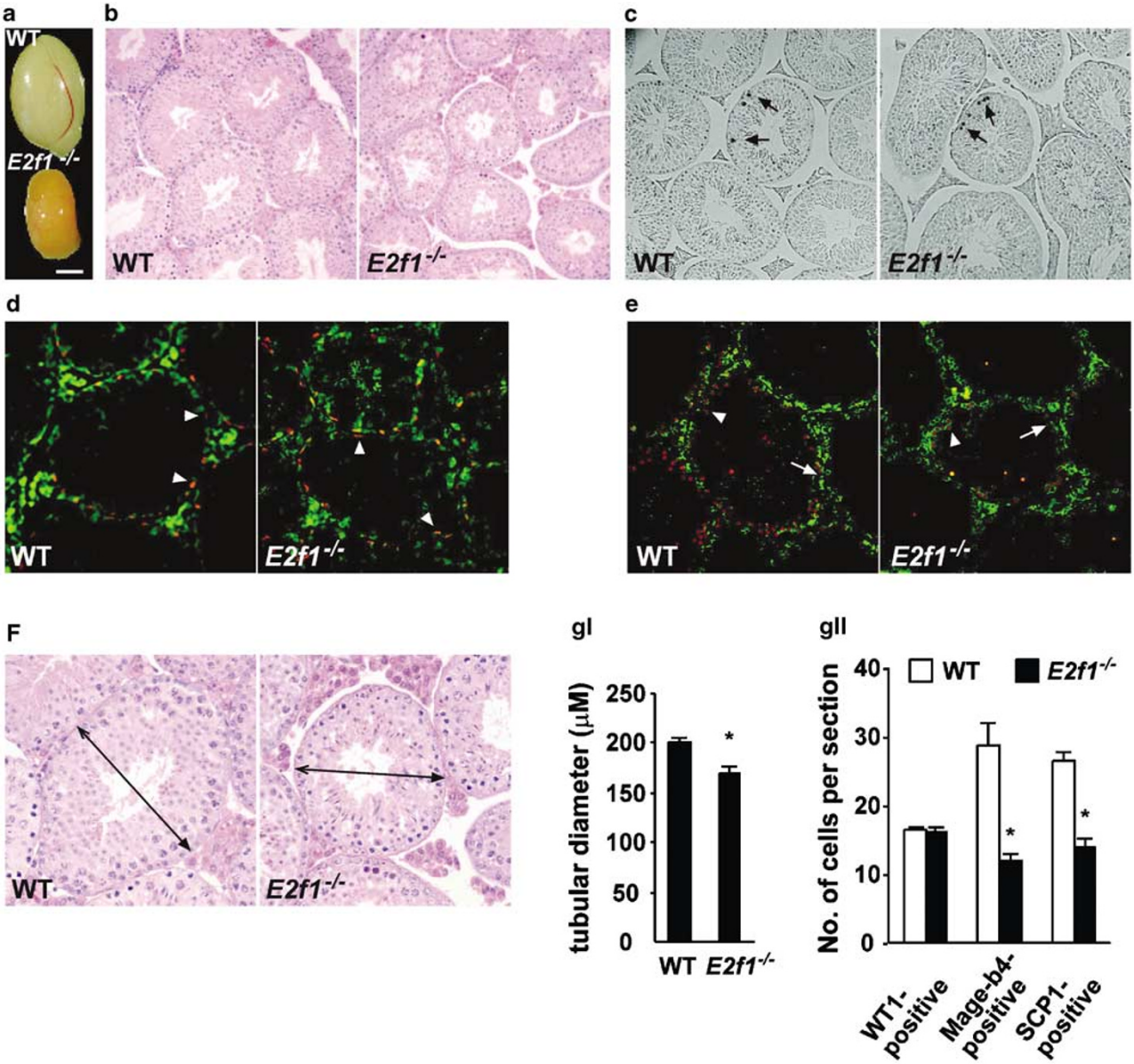

Figure 1 Testes from wild-type (WT) and *E2f1*$^{-/-}$ mice at 12 weeks of age. **(a)** Comparison of testis size in WT and *E2f1*$^{-/-}$ mice. Bar, 2 mm. **(b)** Sections of seminiferous tubules of WT and *E2f1*$^{-/-}$ littermates stained with hematoxylin–eosin. Magnification, $\times$ 200. Note the decrease in tubular size in the testis from *E2f1*$^{-/-}$ mouse compared to wild type. **(c)** Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay detects few apoptotic cells (arrows) in the seminiferous tubules of both WT and *E2f1*$^{-/-}$ testes (200 seminiferous tubules from four mice in each genotype are recorded). Magnification, $\times$ 200. **(d, e)** Immunohistochemical staining for Wilms' tumor gene (WT1), SCP1 and Mage-b4 in WT and *E2f1*$^{-/-}$ testes. WT1 and SCP1 are the markers for Sertoli cells and the chromosomal synapsis at pachynema, respectively, while Mage-b4 is highly expressed in the cytoplasm of spermatogonia and pre-pachytene cells in adult testis.[9] Magnification, $\times$ 400. (**d**) Merged image of WT1 (red) and Mage-b4 (green). The overall number of WT1-positive nuclei (arrowheads) in *E2f1*$^{-/-}$ testis is unaltered as compared to that in wild type. (**e**) Merged image of SCP1 (red) and Mage-b4 (green). The number of spermatogonia (green; arrows) as well as spermatocytes (red; arrowheads) is reduced in *E2f1*$^{-/-}$ testis compared to wild type. **(f)** Hematoxylin–eosin-stained sections of the seminiferous tubules from the same mice shown in (**b**). Magnification, $\times$ 400; note reduced diameter of the seminiferous tubules (double-headed arrows) in *E2f1*$^{-/-}$ testis due to reduced germ cell numbers. **(g)** Quantitative comparison between WT and *E2f1*$^{-/-}$ testes. (I) The tubular diameter (μm) of the cross-sections of seminiferous tubules shown in (**f**) (100 seminiferous tubules from six mice in four litters in each genotype are measured). Data represent mean $\pm$ standard error of the mean (S.E.M.). The tubular diameter of the seminiferous tubules of *E2f1*$^{-/-}$ males is significantly reduced compared to that in wild-type mice (*$P < 0.01$, Student's *t*-test). (II) The numbers of WT1-positive (Sertoli cells), Mage-b4-positive (spermatogonia) and SCP1-positive (spermatocytes) per cross-section of an average of 25 seminiferous tubules from two mice in two different litters in each genotype with immunohistochemical staining shown in (**d** and **e**). The reduction in the numbers of spermatogonia and spermatocytes in *E2f1*$^{-/-}$ mice is significant (*$P < 0.01$, Student's *t*-test), where the overall number of Sertoli cells in *E2f1*$^{-/-}$ testes is unaltered compared to that in wild type

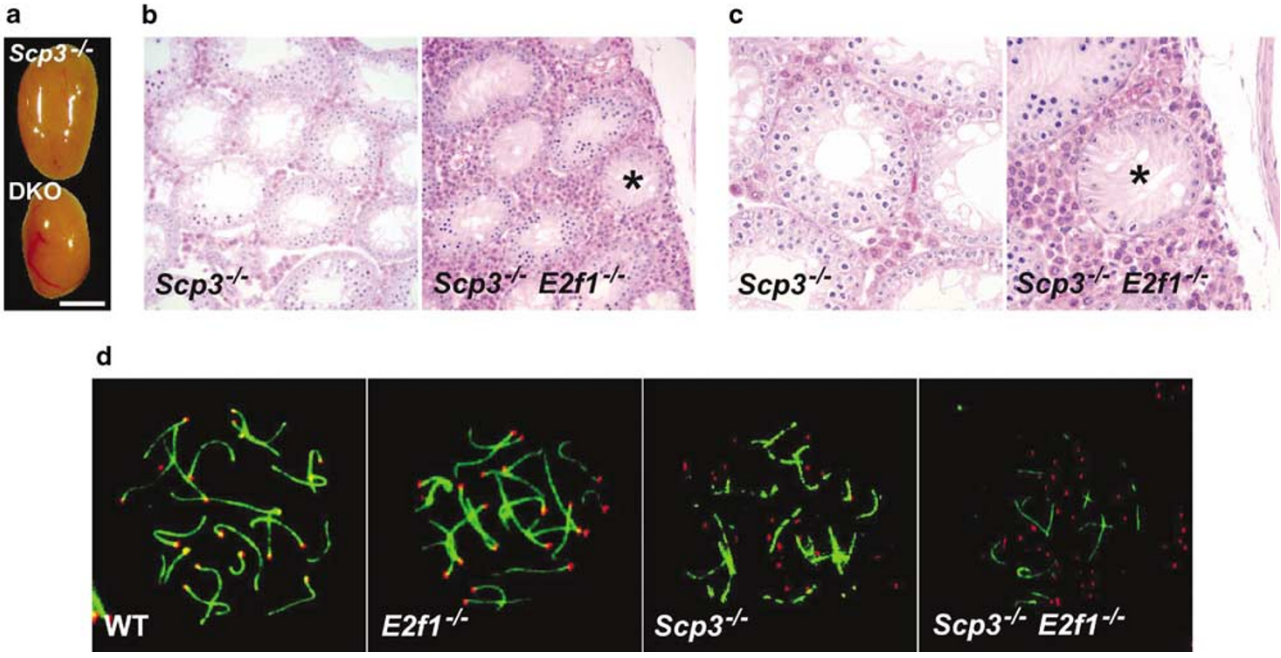

Figure 2 E2F1 ddeficiency aguments testicular degeneration in SCP3 nullizygous mice. **(a)** Comparison of testis size in *Scp3*$^{-/-}$ and *Scp3*$^{-/-}$*E2f1*$^{-/-}$ (DKO, double knockout) mice at 12 weeks of age. Bar, 2 mm. **(b)** Sections of seminiferous tubules of 12-week-old *Scp3*$^{-/-}$ and *Scp3*$^{-/-}$*E2f1*$^{-/-}$ littermates stained with hematoxylin–eosin (100 seminiferous tubules from six mice in each genotype are recorded). Magnification, $\times$ 200. **(c)** Hematoxylin–eosin-stained sections of the seminiferous tubules from the same mice shown in (**b**). Magnification, $\times$ 400. *Scp3*$^{-/-}$*E2f1*$^{-/-}$ testes are more severely impaired compared to their *Scp3*$^{-/-}$ counterparts. In many atrophic tubules, only Sertoli cells but no spermatogonia are seen (so-called Sertoli-cell-only tubules; star). **(d)** Fluorescent immunostaining of spermatocyte spreads from mice ($n = 3$ per genotype) with four different genotypes, i.e. WT; *E2f1*$^{-/-}$; *Scp3*$^{-/-}$ and *Scp3*$^{-/-}$*E2f1*$^{-/-}$. The cells are fixed and labelled using SCP1 (green fibrillar structures) and CREST (a centromere marker) antisera

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