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Gonocyte transformation in a congenitally cryptorchid rat is normal and may be similar to the situation reported in human acquired cryptorchidism

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ABSTRACT

Background: In congenital undescended testis (UDT) in humans, thermal insult damages early germ cell development during mini-puberty (3–6 months) causing increased risk of both cancer and infertility. In rodents however, UDT causes infertility but not cancer. In the TS rat with congenital UDT we hypothesized that early germ cell development would be normal as UDT only becomes manifest at 3–4 weeks (and the germ cells only become sensitive to thermal injury) after minipuberty is complete at 1 week.
Methods: Normal testis and potential UDT from unilateral cryptorchid TS rats were collected at week 1 and 4 and processed into paraffin sections labeled for Sertoli cells (AMH), early germ cells (MVH) and spermatogonial stem cells (PLZF). Confocal microscopic images and Fiji Image J were used to count cells in testicular tubules with paired T-test statistical analysis.
Results: Total germ cells/tubule, basement membrane-bound germ cells/tubule, and Sertoli cells/tubule were unchanged between normally descending and future UDT at 1–4 weeks old ($P > 0.05$) Total germ cells/tubule and spermatogonial stem cells/tubule increased dramatically between weeks 1 and 4.
Conclusion: Rat gonocyte transformation is normal in both normally descending and future UDT. This suggests that congenitally cryptorchid rats may not develop testicular cancer because gonocytes (the putative origin of malignant degeneration) normally transform into spermatogonial stem cells before UDT occurs and the risk of thermal injury develops. This suggests the TS rat may be a good model for acquired UDT in human where the abnormal testicular position develops after gonocyte transformation is completed in the first year.

Cryptorchidism, or undescended testes (UDT), occurs in 2%–4% of all newborn males and in up to a third of premature boys [1,2]. The resultant dual pathologies of germ cell development, malignancy and infertility, have been well described, although optimum management strategies are still debated [3]. The pathophysiology of both these disorders has been hypothesized to be thermal injury to the UDT at 35–37 °C compared with the scrotum (33 °C) [4], or a primary dysfunction of the hypothalamic–pituitary–gonadal axis [5,6]. Regardless of etiology, cryptorchidism causes gross and microscopic changes to the testis and impacts its histological, physiological, biochemical and functional properties.

In humans about half of the neonatal germ cells, called gonocytes, transform postnatally into spermatogonial stem cells (SSCs). Those that fail to transform undergo apoptosis, or programmed cell death, leaving no gonocytes left in the testis. Proper postnatal maturation of germ cells into SSC requires the exquisitely maintained microenvironment of the testis [7,8], including its lower-than-body temperature. Inadequate numbers of gonocytes undergoing transformation cause insufficient SSC, and lead to infertility, while persisting gonocytes may undergo malignant transformation after puberty. Gonocytes mature during mini-puberty, which is a transient surge of gonadotrophins and androgens at 3–6 months in human [9] or in the first week of life in rodents [10]. Many studies have linked abnormalities of mini-puberty to gonocyte transformation into SSC [6,11].

Unlike the human, testicular descent in rodents is not complete until after mini-puberty (2–3 weeks in mouse and 3–4 weeks in rats) [12], which is at the beginning of puberty, so gonocytes should transform or undergo apoptosis and disappear normally despite the higher temperature in the descending testis before it reaches the scrotum. This suggests that the reprogramming of the testicular physiology to be optimal at the scrotal temperature of 33 °C is likely to occur at birth in children,

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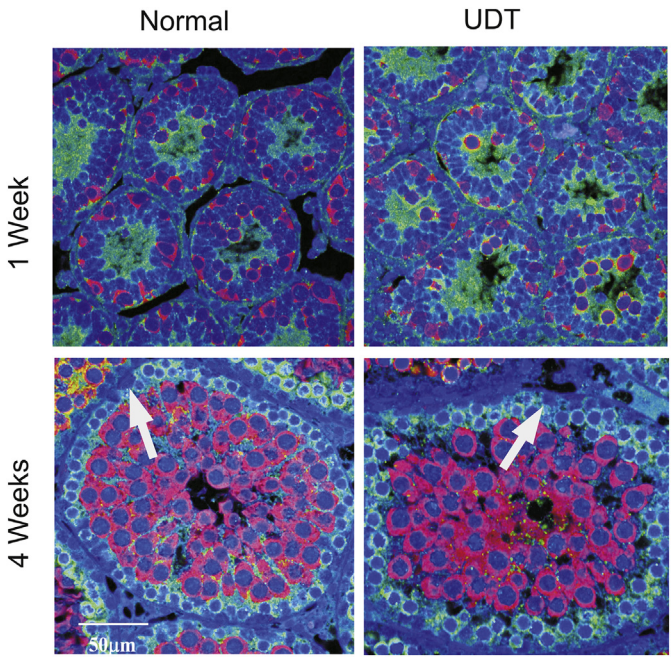

Fig. 1. Cross-sections of testicular tubules labeled with AMH (green), MVH (red) and DAPI (blue). At one week almost all MVH+ germ cells are on the basement membrane in both normal and undescended testes. At four weeks of age MVH+ germ cells fill the lumen and AMH+ Sertoli cells line the basement membrane; AMH−/MVH− cells are located at basement membrane (arrow). Bar = 50 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

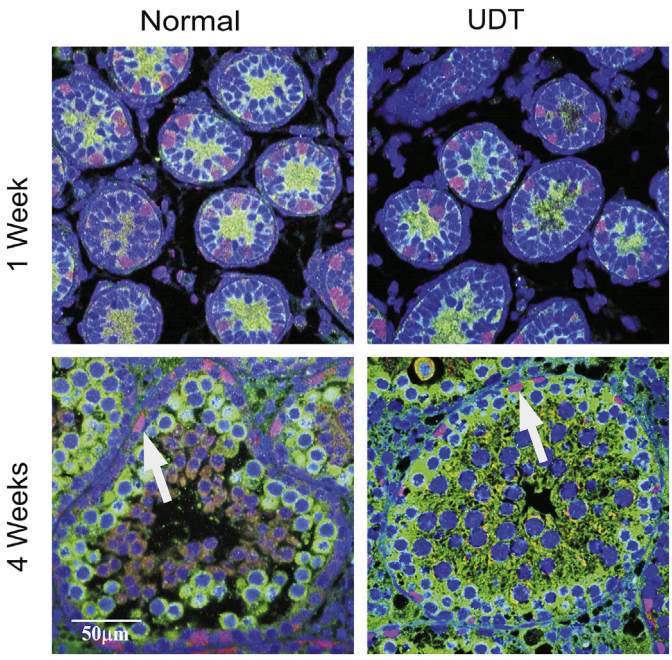

Fig. 2. Cross-sections of testicular tubules labeled with AMH (green), PLZF (red) and DAPI (blue). PLZF+ germ cells are on the basement membrane at one week and remain there at four weeks (arrow), albeit in a less dense distribution. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

but at 3–4 weeks of age in a rat. This means that onset of a thermal injury should begin at puberty, rather than at birth, in the rodent with UDT after normal gonocyte transformation or apoptosis, leading to well-documented infertility in adult rats with UDT, but no reports of malignancy [13].

To determine the effects of congenital UDT on germ cell maturation and the cellular makeup of the rodent testis, we used the congenital UDT rat strain 'Trans-scrotal' (TS) to compare the number and localization of cell types in future UDT to those in the normal testis just after mini-puberty (1 week) and during the peripubertal period (4 weeks).

1. Methods

1.1. Animals and tissue collection

Trans-scrotal (TS) rats were bred from founder animals and maintained in the animal research laboratory at our Research Institute. TS rats develop congenital unilateral or bilateral UDT in approx. 80% of males [14]. The testosterone levels are normal in TS rats, and the cause of UDT in this animal is an abnormal genitofemoral nerve (GFN) [15–19]. Rats were housed in Techniplast individually ventilated cages (Westchester, Pennsylvania) with irradiated FibreCycle bedding and environmental enrichment with 12 h of light and darkness at 21 °C. Rats were fed a diet of irradiated rat and mouse breeder cubes (Barastoc, Ridley Corp.). All animal procedures were performed with the approval of Institutional animal ethics committee (A734).

Male rats with one undescending and one normally descending testis were culled at one week (n = 6) and four weeks (n = 4) old. Animals were anesthetized with Isofluorane (Abbvie, Melbourne, Australia) and sacrificed using 0.5 ml pentobarbital sodium intraperitoneal injection (Lethabarb, Virbac, Milperra, Australia), followed by division of their diaphragm. Testes were collected and fixed in 4% paraformaldehyde at 4 °C overnight before being processed in graded alcohol and embedded in paraffin. The normally descending testis was distinguished from the future UDT by the direction of its migrating gubernaculum, as described previously [13], as the normally descending gubernaculum points towards the scrotum, while the future undescended testis has a gubernaculum pointing laterally towards the femoral region. The normally descending testis of the TS rat with unilateral congenital UDT was considered 'normal' control, as germ cell development is identical to that seen in normal rats [13].

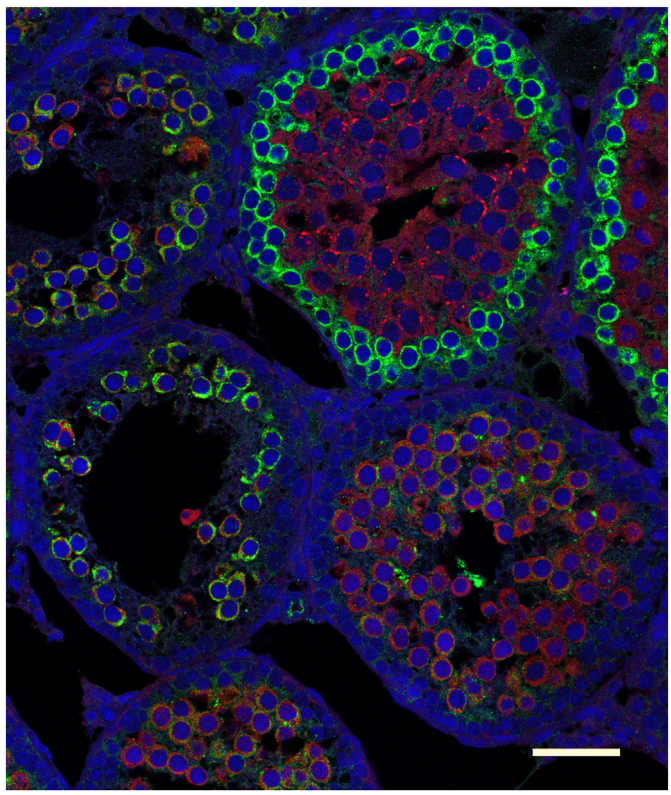

Fig. 3. Cross-section of tubules of four-week-old testes showing varied expression of AMH (green) and empty tubule. Red = AMH, Bar = 50 µm.

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