Cremaster Muscle Myogenesis in the Tip of the Rat Gubernaculum Supports Active Gubernacular Elongation During Inguinoscrotal Testicular Descent

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Abbreviations and Acronyms

E = embryonic day

GFN = genitofemoral nerve

P = postnatal day

PBS = phosphate buffered saline

Study received ethical approval.

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Purpose: Cryptorchidism is a common abnormality and normal testicular descent is controlled by the gubernaculum. The cremaster may originate from abdominal muscles during gubernacular eversion or alternatively it may develop inside the gubernaculum. We studied cremaster myogenesis to determine how it develops.

Materials and Methods: Coronal sections of the pelvis were prepared from male Sprague-Dawley® rats and from males treated prenatally with the antiandrogen flutamide at embryonic day 19, and postnatal days 10, 19 and 35 after receiving ethical approval. Immunohistochemical stains were prepared for Ki67, Pax-7, myogenin, myosin heavy chain 7, Myh1, Myh2, Myh4, embryonic myosin, and slow and cardiac troponin T. Cell counts of the 1) gubernacular tip, 2) proximal gubernaculum/cremaster muscle and 3) adjacent abdominal wall are shown as a percent of positive fibers or positive cells per area.

Results: Throughout embryonic day 19, and postnatal days 10 and 19 proliferation (Ki67) was maximal at the gubernacular tip ($p < 0.001$), as were muscle stem cells markers (Pax-7 $p < 0.05$), early myogenesis (myogenin $p < 0.001$) and immature muscle (Myh7, and slow and cardiac troponin T $p < 0.0001$). In contrast, secondary (fast twitch, Myh1, 2 and 4) fibers were more common in abdominal muscles ($p < 0.0001$). Differences in muscle maturity and composition decreased with time. Flutamide treated rats showed more cellular proliferation than controls postnatally on postnatal day 10 ($p < 0.001$) as well as persistent immature embryonic myosin at the tip from postnatal day 19 ($p < 0.05$).

Conclusions: Results show that the rat cremaster muscle is more immature at the gubernacular tip, consistent with myogenesis occurring in the gubernaculum during migration to the scrotum, as proposed in humans.

Key Words: testis; cryptorchidism; muscle development; fetal development; rats, Sprague-Dawley

Undescended testis, or cryptorchidism, is a common congenital defect in boys. Its etiology is not wholly understood since the mechanism of normal testicular descent remains incompletely defined. During embryonic development the testis must find its way from its initial intra-abdominal location to its final position in the scrotum. A biphasic model of descent is commonly accepted, comprising 2 distinct phases, including the transabdominal and the inguinoscrotal phase.[1]

Since the gubernaculum, or genitoinguinal ligament, ends in the inguinal abdominal wall at the start of the

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inguinoscrotal phase,[2] it is thought to actively migrate into the scrotum, pulling the testis behind it.[3,4] There is controversy over how inguinoscrotal descent is achieved. Some groups suggest that intra-abdominal pressure pushes the gubernaculum to the scrotum, creating a pulsion diverticulum of peritoneum, that is the processus vaginalis.[5–7] In this hypothesis androgens alter the viscoelastic properties of the gubernaculum, allowing it to be passively pushed through the masculinized inguinal canal by abdominal pressure.[6,7] Others propose that the gubernaculum grows actively toward the scrotum, directed via release of a neurotransmitter (calcitonin gene-related peptide) from the GFN and aided by cremaster muscle contraction.[1,8,9] In this theory the gubernaculum elongates to the scrotum like an embryonic limb bud.[3]

The cremaster muscle in the adult human and the rat contains skeletal muscle fasciculi surrounded by loose connective tissue in the cremasteric fascia. Its primary origin is the inferomedial border of the internal oblique muscle, with which its fibers appear continuous, suggesting that it may have formed as a stretching out of the internal oblique during testicular descent.[10] However, the cremaster muscle has a separate nerve supply from the anterior abdominal wall muscles (GFN) as well as differences in firing frequency, composition and voluntary control, and it has unique functions quite different from those of the abdominal wall.[10,11]

Muscle fibers develop initially from primitive mesenchyma that originated in the somites. Myogenesis is regulated by a family of factors that act in a coordinated, sequential manner.[12] Early myogenic stem cells express Pax-7 and become more committed to muscle development with the expression of Myf-5, MyoD and then myogenin, by which time Pax-7 is no longer expressed in the cells, which are now wholly committed myoblasts.[13] Primary muscle fibers then form, marked by embryonic myosin and slow myosin expression. With time embryonic myosin decreases, and myosins 1, 2 and 4 increase. Secondary fibers then begin to form around the primary fiber with progressive loss of embryonic myosin (Myh3) and neonatal myosin (Myh8), and an increase in fast myosins, that is the myosin heavy chains 1, 2 and/or 4, which are labeled by My-32 antibody. The cremaster muscle has predominately slow twitch striated muscle with some smooth muscle[11] and mostly type 1 (slow twitch) fibers[14] but in adult rats and humans there are some type IIB (very fast twitch) fibers.[15]

To address questions about the origin of the cremaster muscle we investigated myogenesis in the cremaster and the expression of different muscle proteins in it, which are now well known.

MATERIALS AND METHODS

After receiving ethical approval Sprague-Dawley rats were maintained at the Animal Research Laboratory, Murdoch Children's Research Institute. They were housed in standard rat shoebox cages with free access to commercial rat chow and water with a 12-hour light-dark cycle and a temperature controlled environment. Some time mated dams were treated with the antiandrogen flutamide (catalogue F9397, Sigma-Aldrich®) at a dose of 75 mg/kg in sunflower oil/ethanol (80%/20% volume per volume) subcutaneously on E16 to E19 with the vaginal plug day defined as E0. This is the critical time window of androgenic effects on inguinoscrotal descent.[16]

Male offspring were sacrificed using standard operating procedures at E19, and postnatal days P10, P19 and

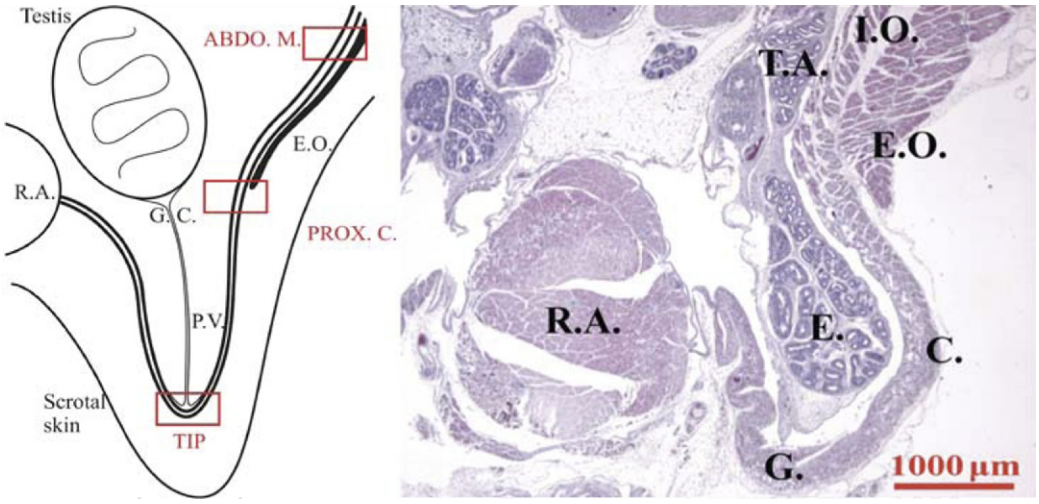

Figure 1. Investigated sites. Photomicrograph was taken at P10. *ABDO. M.*, abdominal muscles. *E.O.*, external oblique muscle. *R.A.*, rectus abdominis muscle. *G.C.*, gubernacular cord. *PROX. C.*, proximal cremaster. *P.V.*, processus vaginalis. *I.O.*, internal oblique muscle. *T.A.*, transverse abdominal muscle. *E.*, external oblique muscle. *C.*, cremaster muscle. *G.*, gubernacular tip. H & E.