Failing to mature: somatic cell dysfunction in the spermatogenic niche among patients with Sertoli cell–only and Klinefelter syndromes

In this article, Saen et al. follow up on one of their previous findings that degeneration and fibrosis of the seminiferous tubules in patients with Klinefelter syndrome (KS) is absent during development and initiated in the peripubertal period (1, 2). The current study aims to add further granularity to these observations by describing alterations in the expression pattern of multiple extracellular matrix (ECM) proteins at different developmental time points in control and KS seminiferous tubules. Given that both Sertoli cells and peritubular myoid cells (PTMCs) express ECM proteins and contribute to the seminiferous tubule basement membrane, they explore how alterations in Sertoli cell and PTMC maturation may contribute to this profibrotic phenotype. Finally, these findings are placed in the context of not only control seminiferous tubules but also idiopathic forms of Sertoli cell–only (SCO) syndrome. This is more broadly significant because it furthers our understanding of the seminiferous tubule as a complex signaling hub in which both germ cells and somatic cells play an important role in spermatogenesis. Whereas germ cell dysfunction arising from aneuploidy may be an important driver of azoospermia in the KS population, dysfunction of the supporting somatic cells, such as Sertoli cells, Leydig cells, and PTMCs, is increasingly implicated.

The histologic hallmarks of KS are classically described as progressive hyalinization, fibrosis, germ cell depletion, and ultimately an SCO phenotype. These events appear to be initiated in the peripubertal period and are complete by adulthood. Although the Sertoli cells persist despite this altered architecture, it is clear that they are markedly different in both phenotype and function (3) from their control counterparts. This raises the question of whether Sertoli cell dysfunction precedes or follows the fibrotic events that lead to tubular degeneration. To better define the chronology of Sertoli cell dysfunction, Saen et al. examine antimüllerian hormone (AMH) and androgen receptor (AR) expression in Sertoli cells at multiple developmental time points. AMH expression is a well established marker of Sertoli cell development because it is expressed in prepubertal Sertoli cells but not in adult Sertoli cells. Mechanistically, this is thought to be a result of increased testosterone signaling from rising AR expression in Sertoli cells during puberty, combined with action from intratesticular androgens. In addition, to this juxtacrine effect, meiotic entry of germ cells appears to act independently from androgen activity to posttranscriptionally regulate AMH at the tubular level and decrease serum AMH levels. Consistent with previous findings, this paper describes decreasing AMH and increased AR immunostaining in control tubules with increasing age. Fetal and prepubertal KS and idiopathic SCO samples displayed staining patterns similar to those of control samples. Among peripubertal KS patients, there was statistically significant delay in loss of AMH expression and a trend toward diminished AR expression, albeit a nonstatistically significant one, compared with control subjects. A similar staining pattern is reflected in the idiopathic SCO samples. The authors then contrast these findings to those in patients with SCO due to deletion of the azoospermia factor gene (*AZF*) in which cytokeratin-18 (KRT18), a marker of Sertoli cell immaturity, regresses by adulthood. They therefore suggest that KS and other forms of SCO may exhibit failure of spermatogenesis due to somatic cell dysfunction as opposed to intrinsic germ cell dysfunction. Although these findings do indeed add to the growing body of descriptive evidence to this effect, they need to be put in the context of other cell types in the seminiferous tubules. Indeed, given that AMH is regulated by both input from Leydig cells through the testosterone-AR axis and germ cells through posttranscriptional means, it is unclear whether the failure of AMH down-regulation is a herald of Sertoli cell–driven dysfunction in the spermatogenic niche, or a result of this dysfunction.

Alpha-smooth muscle actin (ACTA) has been previously described as a marker of PTMC differentiation in a Rhesus monkey model, and its expression is driven by testosterone and FSH. Similarly, the current study was able to show that ACTA2 expression was absent in both control and KS PTMCs before puberty. The authors go on to describe that with the progression of puberty, ACTA2 expression rises and it forms an interrupted pattern surrounding the seminiferous tubules which then goes on to completely surround the tubule by adulthood in control patients. Although KS samples did not show a statistically significant difference in a semiquantitative analysis of staining patterns during puberty, there was a trend toward increased interrupted pattern by late puberty and a statistically significant difference in adults. Interestingly, this interrupted pattern was not seen in their adult samples with idiopathic SCO syndrome. These findings are of interest for two reasons. First, they provide ongoing support for the hypothesis that somatic cell dysfunction is central to the failure of the spermatogenic niche in various forms of nonobstructive azoospermia (NOA). Moreover, it points to the fact that different forms of NOA exhibit different types of somatic cell dysfunction. Second, it begs the question as to what role the undifferentiated PTMCs play in the progression of azoospermia. The authors speculate that PTMCs with low ACTA2 expression may exhibit this diminished expression due to a cell-fate switch toward a myofibroblast-type phenotype and thereby contribute to fibrosis. This being said, in addition to ECM components, PTMCs also produce key factors such as P-Mod-S, leukemia inhibitory factor, interleukins, glial-derived neurotrophic factor, and colony-stimulating factor 1 which interact with all the different cellular components of the spermatogenic niche (4). It is therefore plausible that the PTMCs that fail to mature do not express these necessary factors. Regardless, further work is needed to better understand the implication of low

ACTA2 expression in KS on the PTMC phenotype and how this contributes to disease progression.

ECM proteins play an important signaling role in the blood-testis barrier and are primarily expressed by PTMCs and Sertoli cells. The basement membrane lies in apposition to the spermatogonial stem cells and Sertoli cells with a thick layer of type I collagen fibrils underlying it. Although the basement membrane has many components, this study specifically examines fibronectin, laminin, and type IV collagen expression patterns and attempts to classify their expression levels and patterns into distinct subtypes to characterize how they change in KS. By systematically examining staining patterns, they were able to make a number of general observations. First, adult KS and SCO samples have thickened tubular walls containing aberrant ECM protein expression and concentric layers of PTMCs compared with control samples. Second, in both forms of NOA increased intensity and thickness of fibronectin staining is observed, and this is mirrored by a loss of laminin expression. Third, expression patterns of collagen I and IV were significantly different between KS and SCO patients. KS patients tended to have decreased expression of both types of collagen, with a tramtrack appearance. Whether these staining patterns actually correlate with the severity of NOA phenotype, and whether they have a material influence on signaling within the spermatogenic niche would be an interesting area for future study.

When viewed in aggregate, this study has attempted to profile three elements of the spermatogenic niche that are tightly interrelated. The basement membrane is largely composed of ECM and PTMCs and is thought to be integral to Sertoli cell and early spermatogenic cell regulation before germ cell migration from the basal compartment. The ECM is also critical for cell signaling, epithelial integrity, and scaffolding for cell organization. Thus, the potential impact of ECM dysregulation in KS and NOA testes may assert its impact through various potential mechanisms. Abnormal ECM is likely the reason for abnormal PTMCs, progenitors, or Sertoli cells and likely contributes to further somatic niche dysfunction; however, dismantling the observations into mechanistic insight will require additional research.

Although this study by Saen et al. is a largely descriptive study hampered by inconsistency of time points between control, SCO, and KS samples owing to limitations in acquiring human specimens, it provides further sequential evidence contributing to the overall narrative that in KS the somatic supporting cells are not functioning properly and thus may be contributing to the infertile phenotype.

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