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INVITED RESEARCH HIGHLIGHT

Male Health

Regulation of cell adhesion in the testis: a new role for p73

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The dramatic changes that male germ cells in the adult testis undergo in gene expression profile and morphology as they transition from spermatogonial stem cells through to mature spermatozoa is dependent upon their association with Sertoli cells. Sertoli cells are crucial for survival and maturation of male germ cells. Two recent papers, Holembowski *et al.*[1] and Inoue *et al.*[2] have described a surprising role for the p53 family member, p73, in regulation of germ cell-Sertoli cell adhesion.

Mitotic germ cells (spermatogonial stem cells and spermatogonia) are located on the basal side of the blood testis barrier (BTB), composed of basal tight junctions between Sertoli cells, whereas pachytene spermatocytes and postmeiotic spermatids are located on the adluminal side of the barrier. The BTB serves to ensure that only premeiotic germ cells are exposed to specific factors secreted from the niche required for stem cell maintenance and spermatogonial proliferation as well as to segregate meiotic and postmeiotic germ cells from immune surveillance. The tight junctions of the BTB must be dissolved and re-formed in order to allow passage of germ cells from the basal to adluminal compartments during germ cell maturation.[3]

In addition to the Sertoli-Sertoli cell junctions, the germ cells also maintain constant contacts with Sertoli cells. These take the form of desmosomal-like junctions that connect intermediate filament networks between germ cells and Sertoli cells up to the late spermatid stage. A specialized actin microfilament junction, ectoplasmic specializations (ES), form basal connections at the BTB, but are found as apical junctions in elongating and elongated spermatids.[3] p73 has now been identified as a critical transcriptional regulator of adhesion related molecules in germ cells.[1,2]

p73 and p63 are both paralogs of p53, well-known for its role in responding to cellular stresses by activating expression of genes that trigger cell cycle arrest or apoptosis and hence as a "genomic gatekeeper" that is frequently mutated during tumorigenesis.[4] p73 and p63 are the most ancient members of the family and the single family members present in *Cnidaria* and insects represents a p63/p73-like ancestral protein. Expression and functional studies in both organisms has suggested that the family evolved to protect against DNA damage in gametes by promoting elimination of germ cells with damaged genomes.[5–7]

Data from a variety of sources have indicated that vertebrate p53/p63/p73 proteins physically interact. Analysis of p63/p73 gene structure indicates that they share similar domains with p53: transactivation (TA), DNA-binding, and oligomerization domains. p63 and p73 encode multiple splice forms, some of which contain C-terminal sterile alpha motif and transcriptional inhibitory domains. Several N-terminally truncated isoforms (ΔN forms) exist that do not contain a TA domain.[4] Evidence that p63 is required for regenerative proliferation came with the observation that ΔNp63α is expressed almost exclusively in the basal cells of epithelia, where the stem cells reside. In fact, ΔNp63α has been shown to be expressed in epithelial stem cells with the highest proliferative capacity.[8] The p73 gene also has TA and ΔN forms that function in a similar manner to p63 but has many more unique splice forms (>28).[9] p53 family proteins are functionally active as tetramers. ΔNp63/ΔNp73 is endogenous dominant-negative proteins that oligomerize as part of the tetramer and disrupt p53 family activity. Although various members of the p53/p63/p73 family undoubtedly have unique functions they also act in an integrated manner so high levels of ΔNp73 not only impede the transcriptional activity of TAp73, but also of TAp63 and TAp53, thereby acting in the manner of a triple knockout. ΔNp73 is expressed from an alternative promoter (P2) to the TA isoform (P1), so is not generated by alternative splicing but via transcriptional control.[10,11]

Previous studies have indicated that TAp63 functions to eliminate damaged oocytes from the female germline and that TAp73 knockout mice exhibit infertility of both sexes. Poor oocyte quality, including multiple spindle abnormalities, was associated with the loss of TAp73[12] but the male infertility phenotype was not reported until studies of Inoue *et al.*[2] and Holembowski *et al.*[2] were published this year. The combined studies reported that p73 (global KO) and TAp73 (isoform-specific KO), but not Δp73 (isoform-specific KO) mice exhibited phenotypes in adult testes, indicating that only the TA isoform is required for spermatogenesis. It would be interesting to determine if overexpression of TAp73 has a testicular phenotype and if the ΔN isoforms of p63 may be modulating TAp73 activity as p63 has been reported to be expressed in murine male germ cells.[13] Histological examination of testes from TAp73 KO mice showed that many seminiferous tubules lacked developing germ cells although numbers of basal proliferating spermatogonia were normal. Inoue *et al.*[2] reported an increase in DNA damage and apoptosis of spermatogonia that curiously suggests that TAp73 protects spermatogonia from apoptosis. This is in marked contrast to the previously described pro-apoptotic

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functions of TAp73 in a variety of cellular contexts whereas the ΔNp73 isoforms have a protective role.[4] Loss of TAp73 causes germ cells to prematurely slough away from the seminferous epithelium and undergo apoptosis, primarily resulting in loss of round and elongated spermatids and spermatozoa from tubules. Both studies demonstrated that TAp73 KO testes contained degenerating Sertoli cells with disorganization of the ES layers that modulate attachment of germ cells.[1,2] Holembowski *et al*.[1] determined that this phenotype is a result of a cell autonomous defect in germ cell adhesion and that the defects observed in Sertoli cell morphology were secondary to loss of germ cell attachment. This conclusion was based on their observation that TAp73 expression was localized in germ cells, but not Sertoli cells (although Inoue *et al.*[2] show that TAp73 is also strongly expressed in Leydig cells). This was further supported by expression profiling of germ cells, which demonstrated upregulation of a number of genes associated with regulation of cell adhesion and migration in TAp73 KO cells. This included protease inhibitors, serine peptidase inhibitors, proteases, and integrins and ChIP showed these to be direct targets of TAp73. All of these genes were upregulated in the absence of TAp73, which is interesting as TAp73 is defined by the presence of a transcriptional activation domain. Holembowski *et al*.[1] performed a clever experiment to conclusively demonstrate the cell autonomous function of TAp73 in germ cells. They transduced rat germ cells with a mix of lentiviruses expressing five of the target genes (*Serpina3n*, *Timp1*, *Itga5*, *Serping1* and *Tnfrsf12a*). The transduced germ cells had much lower levels of adherence to Sertoli cells in co-cultures compared to wild-type germ cells. Injection of the viral mix into testes also produced a phenotype similar to TAp73 KO.[1]

These studies not only demonstrate the importance of germ cell-Sertoli cell adhesion for regulated spermatogenesis, but also add another function to the varied roles of the p53/p63/p73 family of transcriptional regulators. The balance of TA and ΔN isoforms in this family of proteins appears to be crucial for regulation of apoptosis and development of a number of tissues. Although, ΔNp73 does not appear to be required in male germ cells it will be interesting to observe if interactions with other family members contribute to regulation of spermatogenesis.

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