ANIMAL REPRODUCTION BIOTECHNOLOGY

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Annotation: Veterinary Reproductive Biotechnologies are used to obtain offspring from animals as an alternative to natural mating. The most common reproductive biotechnology in animals is artificial insemination (AI), in conjunction with sperm preservation/ cryopreservation

Keywords: testicular tissue, embedded compound, hematoxylin, eosin.

Histological analysis of testicular tissue was conducted using hematoxylin and eosin (H&E) staining, following the method outlined by Siregar. Testicular sections prepared from tissues fixed in 4% paraformaldehyde overnight at 4°C were sequentially submerged in increasing concentrations of sucrose (10% to 30%). The tissues were then embedded compound and sectioned at 10 µm thickness using a cryostat. Sections were air-dried on gelatin-coated slides and briefly rinsed with distilled water. The sections were stained with hematoxylin for one minute and then with eosin for five minutes. Subsequently, the sections underwent a graded series of ethanol dehydration (70% to 100% ethanol, three minutes each) and cleared in xylene. The slides were mounted using a permount mounting medium and examined under an Olympus microscope to capture images. Consistency was verified by analyzing five different sections from each sample. Our investigation into the expression of PDGFRα in testicular tissue of PDGFRαEGFP mice has provided detailed insights into its localization and potential functions. The maintenance of typical testicular morphology, as demonstrated by H&E staining, along with the presence of various cell types critical to spermatogenesis and hormonal regulation, sets the stage for a deeper understanding of PDGFRα's role in testicular function. The distinct visualization of PDGFRα+ cells in the interstitial spaces, specifically colocalizing PMC and Leydig cells, as highlighted by eGFP expression, points to PDGFRα's involvement in the testicular microenvironment. PDGFRα+ cells are engineered to express eGFP in the nucleus (Ha et al., 2017). Its co-localization with α-SMA further emphasizes its role in the structural and possibly functional aspects of myoid cells, which are integral to the

integrity and contractility of the seminiferous tubules .PDGFRα exhibits broad expression across mesenchymal tissues, including the testis, and serves distinct roles in various organs (Li et al., 2018). In the mouse testis, Sertoli, PMCs, and Leydig cells are the primary somatic types, distinguished by morphology and specific immunostaining markers. However, such markers are not exclusively specific, including 3βHSD for Leydig cells and α-SMA for PMCs . We opted for other Leydig cell markers, such as c-Kit and ANO-1, identified in previous studies.PDGFRα is a recognized marker for FLC , and our findings align with prior research indicating PDGFRα's presence in Leydig cells and PMCs, but its absence in germ cells, Sertoli cells, and vascular endothelial cells (Zhao et al., 2021). Stem Leydig cells differentiate into testosterone-producing Leydig cells under PDGFAA and luteinizing hormone and transform into PMCs in response to PDGFBB and TGFβ ,illustrating the effect of the microenvironment on cell differentiation. Given PDGFRα's activation by various PDGF ligands, it is inferred that the expression of PDGFRα in Leydig cells and PMCs is likely to play a role in response to PDGF signals . Our study highlights PDGFRα expression in 11-week-old mouse Leydig cells, which had previously been identified only in FLC, and prompts further investigation of its physiological functions.

In the gastrointestinal system, PDGFRα+ cells have distinct functions from c-Kit+ interstitial cells of Cajal (ICC), forming electrical synapses with ICCs and smooth muscle cells to regulate motility (Kurahashi et al., 2012). However, in the testis, the co-expression of both markers in a single cell may represent an integration of the functions of each protein. The observed c-Kit expression in PDGFRα+ testicular cells indicates that these cells, including Leydig cells and PMCs, might also participate in electroactivity. The co-localization of ANO-1 with PDGFRα+ cells, mirroring the c-Kit expression pattern, points to potential shared signaling and regulatory functions. Their co-localization suggests that the roles of PDGFRα+ cells in the testis could be more complex than in the gastrointestinal tract. These findings require further studies to understand the intricate networks governing testicular cell function and explore novel aspects of Leydig and PMC cell physiology.

The channel, a K+ channel member, is notably expressed in mouse PDGFRα+ cells .PDGFRα+ cells are known to express the small conductance Ca2+-activated K+ channels (SK3), which are implicated in the purinergic neuromodulation of colonic muscle .While this study did not directly investigate the functional expression of channels, it is plausible that PDGFRα+ cells across various tissues may express different types of channels. Analyzing the electrical properties of PDGFRα+ cells is, therefore, essential. The specific role channels in Leydig cells and PMCs remains unclear, but they are hypothesized to contribute to sperm motility and hormone secretion by facilitating electrical changes, potentially in conjunction with ANO-1.Further studies are needed to elucidate these roles. The co-expression of ANO-1 and TASK-1 with PDGFRα in Leydig cells and PMCs suggests a complex regulatory network influenced by these channels. These ion channels may play significant roles in cellular signaling and ionic homeostasis within the testis.

In conclusion, we demonstrate that PDGFRα is expressed in Leydig cells and PMCs within adult mouse testes. The observed expression pattern of PDGFRα offers valuable insights into its role in facilitating cell-cell interactions and signal transduction within the testicular environment. In particular, the co-localization of PDGFRα with c-Kit, ANO-1, and TASK-1 indicates that PDGFRα+ cells may be involved in regulating intracellular calcium signaling and ionic homeostasis.

Used literature:

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