Regulation of germ line stem cell homeostasis

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Abstract

Mammalian spermatogenesis is a complex process in which spermatogonial stem cells of the testis (SSCs) develop to ultimately form spermatozoa. In the seminiferous epithelium, SSCs self-renew to maintain the pool of stem cells throughout life, or they differentiate to generate a large number of germ cells. A balance between SSC self-renewal and differentiation is therefore essential to maintain normal spermatogenesis and fertility. Stem cell homeostasis is tightly regulated by signals from the surrounding microenvironment, or SSC niche. By physically supporting the SSCs and providing them with these extrinsic molecules, the Sertoli cell is the main component of the niche. Earlier studies have demonstrated that GDNF and CYP26B1, produced by Sertoli cells, are crucial for self-renewal of the SSC pool and maintenance of the undifferentiated state. Down-regulating the production of these molecules is therefore equally important to allow germ cell differentiation. We propose that NOTCH signaling in Sertoli cells is a crucial regulator of germ cell fate by counteracting these stimulatory factors to maintain stem cell homeostasis. Dysregulation of this essential niche component can lead by itself to sterility or facilitate testicular cancer development.

Keywords: germ cell homeostasis, NOTCH signaling, Sertoli cells, spermatogonial stem cell niche, spermatogonial stem cells.

Introduction

Stem cells are immature, undifferentiated cells that are able to self-renew to maintain their own pool, but also to differentiate into specialized cell types that will ultimately perform a biological function. In the embryo and fetus, stem cell activity is critical for organ development and growth. In adults, stem cells are essential for the maintenance, repair and regeneration of many tissues. In particular, a pool of actively self-renewing stem cells is necessary to sustain hematopoiesis in the bone marrow, to regenerate the intestinal epithelium and to maintain spermatogenesis in the seminiferous tubules, since in all these tissues differentiated cells are eliminated and must be replenished throughout life (Oatley and Brinster, 2008; Blanpain and Fuchs, 2009; Rossi et al., 2011; Arwert et al., 2012). Proper regulation of stem cell fate is therefore critical to maintain adequate cell numbers in health and diseases. Accumulating evidence suggests that stem cells behavior is regulated by both extracellular signals from their microenvironment, or niche, and intrinsic signals within the cells. Using diverse model organisms, much work has been done to understand how the niche controls stem cell self-renewal and differentiation and how in turn stem cells influence their environment. This mini-review focuses on recent findings pertaining to germ cell development and the relationship between spermatogonial stem cells of the testis and their niche.

Development of the male germ line

In the mouse, the germ line originates from a small population of primordial germ cells (PGCs), which arise from the pluripotent epiblast during gastrulation and are specified by BMP signaling at embryonic day 6.5 (E6.5; Ewen and Koopman, 2010). PGCs proliferate, migrate through the hindgut and colonize the genital ridges between E7.5 and E11. Once in the developing gonads, PGCs stop proliferating and initially develop according to cues based on the surrounding somatic cells. For example, studies have shown that irrespectively of their sex, germ cells will enter meiosis if they reside in a developing ovary, but not in a developing testis (Evans et al., 1977; Adams and McLaren, 2002), and that only subsequent gamete differentiation depends on the sex of the germ cells. In the developing ovary, between E12.5 and E14.5, meiosis entry is signaled by retinoic acid (RA), a powerful morphogen provided by the mesonephros (Rhinn and Dolle, 2012; Feng et al., 2014). In the developing testis, meiosis cannot proceed because of the activity of the enzyme CYP26B1, produced by Sertoli and interstitial cells, which catalyzes the oxidation of RA into inactive metabolites (White et al., 2000). Therefore once in the gonads, male PGCs, now called spermatogonia, or gonocytes, do not further differentiate due to depleted levels of RA and instead undergo mitotic arrest. They re-enter the cell cycle at postnatal day 3 (P3), then migrate to the basement membrane of the seminiferous epithelium to become spermatogonial stem cells (SSCs).
SSCs self-renew throughout life to maintain a pool of undifferentiated cells that are at the origin of spermatogenesis and ensure the permanent production of sperm (de Rooij and Griswold, 2012; Fig. 1). SSCs are single cells (A\textsubscript{single} spermatogonia, A\textsubscript{s}) that are not connected by intercellular bridges like the other more mature germ cells (Dym and Fawcett, 1971; Huckins, 1971; de Rooij and Russell, 2000). SSCs self-renew under the influence of glial cell line-derived neurotrophic factor (GDNF), which is produced by Sertoli cells and binds to the membrane receptor complex GFRA1/RET (Meng et al., 2000; Kubota et al., 2004; Hofmann et al., 2005; Naughton et al., 2006). When rodent SSCs differentiate, they divide into two daughter cells called A\textsubscript{paired} spermatogonia (A\textsubscript{pr}), which will in turn divide to form chains of 4, 8 or 16 A\textsubscript{aligned} spermatogonia (A\textsubscript{al}). This step is considered an amplification step that increases the number of progenitors, and A\textsubscript{single}, A\textsubscript{paired} and A\textsubscript{aligned} are often referred to as undifferentiated spermatogonia (de Rooij and Russell, 2000). A\textsubscript{aligned} cells will further differentiate into type A\textsubscript{1}-A\textsubscript{4} spermatogonia and B spermatogonia, which finally divide to form primary spermatocytes. Throughout this postnatal germ cell development period, the levels of CYP26B1 in Sertoli cells gradually decrease (Gaido and Lehmann, 2006), allowing the levels of retinoic acid to rise, which drives Stra8 expression in germ cells, meiosis entry and spermatocyte differentiation into haploid spermatids and spermatooza (Anderson et al., 2008). The molecular events underlying the regulation of CYP26B1 are not completely understood, nor are the cause and function of mitotic arrest in prospermatogonia.

**Figure 1.** Intrinsic markers essential for SSC self-renewal and differentiation. The transcription factor ID4 identifies a small population of A\textsubscript{single} spermatogonia that are believed to be the true stem cells (Chan et al., 2014). The membrane receptors GFRA1 and RET bind the ligand GDNF, which is crucial for stem cell self-renewal (Meng et al., 2000; Hofmann et al., 2005; Naughton et al., 2006). ZBTB16 and POU5F1 are transcription factors expressed by SSCs and undifferentiated spermatogonia (Ohbo et al., 2003; Buagas et al., 2004; Costoya et al., 2004; Dann et al., 2008). Upon downregulation of CYP26B1, increased retinoic acid levels will stimulate germ cell expression of STRA8, which will induce them to differentiate and enter meiosis (Anderson et al., 2008). SOHLH1/2 and NGN3 are expressed at the boundary between A\textsubscript{aligned} and A\textsubscript{1}-A\textsubscript{4} differentiating spermatogonia, while KIT is a known marker of differentiating spermatogonia (Schrans-Stassen et al., 1999; Yoshida et al., 2006; Suzuki et al., 2012). ZBTB16 is an inhibitor of KIT, which therefore contributes in maintaining the undifferentiated state (Filipponi et al., 2007). Molecules discussed more in depth in the present review are marked in blue.
The germline stem cell microenvironment

Stem cell behavior is regulated by specialized microenvironments, or niches, which maintain the size and homeostasis of the stem cell population. The role of the niche has been demonstrated in several tissues, in particular intestinal epithelium, bone marrow and epidermis (Singh, 2012). Many of the events determining germ cell development in the perinatal testis, including development of prospermatogonia into stem cells, and spermatogonial stem cell fate throughout life, are also orchestrated by their microenvironment, which is composed of several types of somatic cells (Fig. 2). Within the seminiferous epithelium, the Sertoli cell is an important cellular component of the testicular niche and is responsible for the production of several growth factors that influence the development of prospermatogonia and the fate of SSCs as described in the next section (Li et al., 1997; Meng et al., 2000; Miles et al., 2012; Garcia et al., 2013). Sertoli cells also contribute to the architecture of the seminiferous tubules before and after birth (van der Wee and Hofmann, 1999; Kim et al., 2007), provide structural support to the germ cells and ensure their proper differentiation along the basal-apical axis of the seminiferous epithelium (Su et al., 2013). Peritubular myoid cells, which surround the seminiferous tubules, also contribute to the niche by producing growth factors such as LIF (Piquet-Pellorce et al., 2000), and the CC-chemokine MCP-1 (Aubry et al., 2000). Together with Sertoli cells, peritubular myoid cells produce components of the basement membrane onto which they reside together with undifferentiated spermatogonia (Richardson et al., 1995; Siu and Cheng, 2008).

Other cellular components of the testicular niche are found in the interstitial space between the seminiferous tubules. Leydig cells, which are known to synthesize testosterone, also produce growth factors crucial for SSC self-renewal such as CSF-1 (Dirami et al., 1999; Kokkinaki et al., 2009; Oatley et al., 2009) and VEGF (Collin and Bergh, 1996; Ergun et al., 1997; Korpelainen et al., 1998; Anand et al., 2003; Caires et al., 2012; Lu et al., 2013). Lymph and blood vessels also occupy the interstitial space and must contribute to SSC maintenance and self-renewal since these cells are always located at the proximity of the testicular capillaries (Yoshida et al., 2007). Little is known about the factors that emanate from the vasculature, but it is thought that circulating hormones and VEGF may play an important role. All these components contribute together to create the SSC microenvironment, which regulates many aspects of stem cell function.

Figure 2. The spermatogonial stem cell niche. Depicted are the positive effects of FSH, FGF2 and other molecules on GDNF expression by Sertoli cells, which ensure SSC self-renewal (Tadokoro et al., 2002; Simon et al., 2007). Simultaneously, CYP26B1, by degrading retinoic acid, ensures maintenance of the undifferentiated state (Feng et al., 2014). Other cells belonging to the niche produce growth factors such as CSF-1, VEGF, LIF and MCP-1, which also contribute to positive induction of self-renewal (Aubry et al., 2000; Piquet-Pellorce et al., 2000; Oatley et al., 2009; Caires et al., 2012).
Niche factors produced by Sertoli cells

The Sertoli cell is arguably the most important component of the testis niche. In the past decade, a number of factors produced by Sertoli cells have been found that are crucial for gonocyte maintenance in the fetus and stem cell renewal after birth.

Low levels of the growth factor glial cell line-derived neurotrophic factor (GDNF) are detectable in the bipotential gonads as early as E11 (Beverdam and Koopman, 2006). Glial cell line-derived neurotrophic factor expression by Sertoli cells increases steadily during testis development and reaches a maximum at P3. Subsequently, GDNF expression decreases without completely disappearing since it is used by SSC to self-renew throughout life (Meng et al., 2000; Tadokoro et al., 2002; Shima et al., 2004; Miles et al., 2012). Therefore GDNF might be used by fetal prospermatogonia to ensure their survival (Miles et al., 2012; Garcia et al., 2013) in addition to be used by SSCs to maintain their pool after birth. In older animals, GDNF expression also varies across the stages of the seminiferous epithelium, and is highest at stages XIII–I, when undifferentiated spermatogonia proliferate (Johnston et al., 2011). It is lowest at stages VII and VIII, when most of the cells are quiescent and the majority of Aligned spermatogonia transition to the differentiating A1–A4 cells. These observations indicate that GDNF levels in the developing testis vary over time and are cyclic, and that its dosage is crucial for the regulation of perinatal germ cell fate and stage-specific proliferation of undifferentiated spermatogonia. However, the mechanisms responsible for this control are not understood.

When SSCs are established, GDNF binds to the RET/GFRA1 receptor complex to induce their self-renewal (Meng et al., 2000; Hofmann et al., 2005; Hofmann, 2008; Oatley and Brinster, 2008; Kanatsu-Shinohara and Shinohara, 2013). Absence of GDNF or its receptors RET and GFRA1 leads to a lack of SSCs (Naughton et al., 2006). Two signaling pathways triggered by GDNF binding to its receptors have been so far identified in SSCs (Fig. 3). In one scenario, RET phosphorylation induces SRC-family kinases (SFKs) and PI3K/AKT activation (Braydich-Stolle et al., 2007; Oatley et al., 2007) and ultimately the upregulation of Mycn (Braydich-Stolle et al., 2007; Lucas et al., 2012). Further analysis indicated that FYN kinase is probably the major SAK used by undifferentiated spermatogonia to proliferate (Braydich-Stolle et al., 2010). A recent study using spermatogonial transplantation confirmed that MYC family transcription factors are crucial for SSC self-renewal (Kanatsu-Shinohara et al., 2014). Another pathway activated by the binding of GDNF to the RET/GFRA1 complex is the canonical RAS pathway (He et al., 2008; Lee et al., 2009), which upregulates the expression of the transcription factor Fos (He et al., 2008). In turn, FOS increases the levels of the cyclin Ccna2, which is crucial for spermatogonial proliferation (Sunters et al., 2004; He et al., 2008; Wolgemuth et al., 2013). More recently the cyclin Ccne1 as a target of RAS signaling in germ cells has received some interest since its downregulation through siRNA inhibits SSC self-renewal (Kanatsu-Shinohara et al., 2014). However, it is not known whether both SRC and RAS, or only one of these pathways, specifically triggers self-renewal.

A molecule produced by fetal and neonatal Sertoli cells is the enzyme CYP26B1 as mentioned above. Levels of this enzyme are high enough in the male gonads before birth to degrade retinoic acid and prevent germ cell differentiation. Therefore, like GDNF, CYP26B1 contributes to the maintenance of the undifferentiated state of prospermatogonia in the perinatal testis. CYP26B1 expression starts decreasing around birth (Gaido and Lehmann, 2006), which allows retinoic acid production and activity. When retinoic acid reaches sufficient levels, it will induce the expression of the gene Stra8 (stimulated by retinoic acid 8), which increases in spermatogonia and eventually triggers meiosis in spermatocytes. Although the mode of action of Stra8 is still unknown, we know from knockout experiments that it is required for induction of the meiotic prophase (Anderson et al., 2008).

Control of germ cell development by Sertoli cells also requires the production of FGF2, which, together with GDNF, is crucial for the proliferation of prospermatogonia and SSC in cultures (Van Dessel-Emiliani et al., 1996; Kubota et al., 2004; Kubota and Brinster, 2008). PDGF, another factor produced by Sertoli cells, induces prospermatogonia proliferation after birth (Loveland et al., 1995; Li et al., 1997), probably in cooperation with estrogen (Thuillier et al., 2010). Disruption of cross-talks between PDGF and estrogen-triggered signaling pathways has been suggested to take place upon exposure to xenoestrogens in the environment, which could lead to alteration of spermatogonial behavior and preneoplastic states (Thuillier et al., 2003).

Control of growth factor production

Maintenance of spermatogenesis depends on SSCs and is sustained throughout life in a highly ordered manner. Despite the essential role of Sertoli cells within the niche, little is known about how the production of growth factors that influence the fate of SSCs is regulated. It is however evident that homeostasis within the stem cell compartment must be governed by positive and negative feedback regulation of these extrinsic signals.

E.1. Positive regulation

In 2002, the group of Nishimune demonstrated that follicle-stimulating hormone (FSH) was one of the major regulators of GDNF production by Sertoli cells and increased proliferation of undifferentiated
spermatogonia in vivo (Tadokoro et al., 2002). They treated steel/steel Dickie (Sl/Sld) mutant mice, which cannot produce KIT ligand (KITL) and lack A1-A4 differentiating spermatogonia, with a GnRH antagonist (Nal-Glu) for 5 days. This treatment significantly reduced GDNF expression and decreased the rate of proliferation of undifferentiated spermatogonia in the testes of these mice. Also, FSH stimulation induced GDNF expression in primary cultures of Sertoli cells. More recently, Simon and colleagues demonstrated that FGF2, TNFα and IL-1β also stimulated Gdnf expression in cultured Sertoli cells (Simon et al., 2007; Fig. 2).

Positive regulation of CYP26B1 in fetal Sertoli cells has been well documented. Transcription factor genes steroidogenic factor-1 (SF1) and Sry-related HMG box 9 (SOX9) are co-expressed with CYP26B1 in fetal Sertoli cells, and exogenous expression of SOX9 or SF1 increases Cyp26b1 mRNA levels (Kashimada et al., 2011). Conversely, in embryonic gonads deficient in Sox9 or Sf1, Cyp26b1 expression is decreased relative to wild-type controls (Kashimada et al., 2011). These results strongly suggest that SF1 and SOX9 are transcription factors that positively influence CYP26B1 production, ultimately downregulating retinoic acid under a critical level in the developing male gonad and blocking meiosis before birth.

**Figure 3.** Signaling pathways triggered by GDNF activation of the RET transmembrane receptor. GDNF promotes cell cycle progression via binding to RET and its co-receptor GFRA1. RET phosphorylation activates SRC kinase family members (SFKs), in particular FYN kinase (Braydich-Stolle et al., 2010). This in turn triggers the PI3K/AKT pathway to increase N-myc gene expression (Braydich-Stolle et al., 2007). Another pathway initiated by the interaction of GDNF with RET/GFRA1 is the RAS-dependent canonical signaling pathway. This pathway ultimately upregulates the transcription factors *Fos* and *ETV5*, which themselves induce the expression of cyclins and stem cell-specific genes (He et al., 2008; Wu et al., 2011).
E.2. Negative regulation

In order for the number of germ cells or stem cells to be properly regulated, negative regulators that antagonize growth factors and molecules essential for self-renewal and the undifferentiated state must exist. We recently demonstrated that NOTCH signaling within the SSC niche fulfills this function (Garcia and Hofmann, 2013; Garcia et al., 2013). NOTCH signaling is a highly conserved juxtacrine signaling pathway involved in a variety of cell fate decisions key to the development and maintenance of multiple organ and tissue systems (Bray and Bernard, 2010; Fig. 4). The NOTCH proteins (NOTCH1-4) are large cell-surface receptors that are activated by contact with membrane-bound ligands on neighboring cells, such as JAGGED (JAG1 and JAG2) and DELTA-like (DLL1, DLL3 and DLL4; Wang, 2011). Upon activation of the canonical pathway, the NOTCH intracellular domain (NICD) is cleaved and translocates to the nucleus where it associates with a DNA-binding protein called “recombining binding protein suppressor of hairless” (RBPJ), also called CBF1 (Tanigaki and Honjo, 2010). This displaces a repressor complex from the RBPJ protein and triggers the conversion of RBPJ from a transcriptional repressor to a transcriptional activator (Shao et al., 2012). In the canonical pathway, the Hes/Hey family of transcriptional repressors are targets of RBPJ (Iso et al., 2003; Bray and Bernard, 2010). HES/HEY proteins are bHLH transcription factors that inhibit the expression of other genes by forming complexes with corepressors (Iso et al., 2003; Fischer and Gessler, 2007). Mammalian HES/HEY proteins fulfill important roles during development and adulthood, but the result of their activation (proliferation, differentiation or lateral inhibition) depends on the cell type.

A number of NOTCH receptors, ligands and targets are expressed in the pre- and postnatal testis (Dirami et al., 2001; Hahn et al., 2009). Over the past two decades, constitutively active forms of the NOTCH pathway have been used to uncover its role in many model systems including Drosophila, C. elegans, and vertebrates (Coffman et al., 1993; Fortini et al., 1993; Rebay et al., 1993; Roehl and Kimble, 1993; Kopan et al., 1994). Indeed our gain-of-function mouse model that constitutively activates NOTCH1 signaling only in Sertoli cells led to a complete loss of germ cells around birth (Garcia et al., 2013). Interestingly, in this model E15.5 prospermatogonia, which are normally quiescent at this stage of development, re-entered the cell cycle and differentiated, as demonstrated by their de novo expression of the cyclins CCND1 and CCND3, and the markers STRA8 and cKIT (Garcia and Hofmann, 2013; Garcia et al., 2013). Further investigations demonstrated significant downregulation of Gdnf and Cyp26b1 in the mutant testes, indicating that germ cell differentiation occurred possibly because of a lack of niche molecules maintaining the undifferentiated state.

![Figure 4. Basic components of the NOTCH signaling pathway.](image-url)

Figure 4. Basic components of the NOTCH signaling pathway. Ligands of the JAGGED and DELTA-like families interact with NOTCH family receptors on an adjacent cell. Receptor-ligand interaction releases the NOTCH intracellular domain (NICD), which translocates to the nucleus, where it forms a complex with the RBPJ protein. This leads to the transcriptional activation of Notch target genes (Hey/Hes). Hey/Hes proteins are transcriptional repressors.
Functional studies of the NOTCH pathway in many tissues have been hampered by the fact that the NOTCH receptors are redundant. Therefore to ablate NOTCH signaling specifically in Sertoli cells, we disrupted the DNA-binding sequence of the RBPJ protein, which is at the intersection of the NOTCH1-4 pathways (Fig. 4). Loss-of-function of RBPJ in Sertoli cells led to an increase in Gdnf and Cyp26b1 expression resulting in a significant increase in SSCs and overall germ cell numbers (hyperplasia). In 20% of testes, meiotic arrest and bilateral testicular failure occurred, which we attribute to the inability of Sertoli cells to process all germ cells during spermiogenesis. This deficiency results in an increase in the number and size of residual bodies, which can be observed in unusual stages of the seminiferous epithelium, and epididymal occlusion. This phenotype of the NOTCH signaling knockout is in stark contrast and in many ways opposite - to the phenotype of the NOTCH signaling overactivation mutant.

Altogether, our results indicate that activation of NOTCH signaling in Sertoli cells upregulates the transcriptional repressors HES1 and HEY1, which inhibit GDNF and CYP26B1 production. This in turn could potentially be used as a mechanism to dampen germ cell proliferation when necessary and favor differentiation.

E2. Control of NOTCH signaling by germ cells

To identify which cells provide the ligand for NOTCH signaling in Sertoli cells, we specifically isolated pure populations of germ cells and Sertoli cells by FACS and demonstrated that the NOTCH ligand JAG1 was specifically expressed in undifferentiated spermatogonia. Further, only JAG1 induced an increase in Hes1, Hey1, and Hey1 expression by Sertoli cells in an in vitro assay, with concomitant decreased expression of Cyp26b1. However, Gdnf expression did not change in this context, indicating that the control of GDNF expression might be only indirectly dependent on the canonical NOTCH pathway. To demonstrate that germ cells are the signal-presenting cells, we isolated and cultured Sertoli cells from a Transgenic Notch Reporter GFP (TNR–GFP) mouse model (Duncan et al., 2005). In these mice, GFP expression depends on an active NOTCH signaling pathway, and in the testis GFP is exclusively expressed in Sertoli cells (Garcia et al., 2013). When Sertoli cells were isolated from TNR-GFP testes, their expression of GFP disappeared within 24 h. After 5 days of in vitro culture, we recombinated these Sertoli cells with germ cells isolated from CD1 mice that are not expressing GFP. Addition of germ cells induced GFP expression in Sertoli cells within 24 h, demonstrating that germ cells are the ligand-presenting cells activating NOTCH signaling in Sertoli cells. Altogether, these results indicate that germ cells might use JAG1 to activate the NOTCH pathway in Sertoli cells and control their own numbers, ensuring proper homeostasis and sperm output.

Conclusion

In the testis, as in other tissues belonging to multicellular organisms, stem cell homeostasis is governed by endocrine and paracrine communication via soluble factors including hormones, growth factors, and cytokines. Our laboratory is investigating spermatogonial stem cells homeostasis in the normal testis to gain a better understanding of deregulated growth patterns. Many characteristics of solid tumors, i.e., uncontrolled proliferation, deregulation of cellular and morphological differentiation, invasion, and colonization to distant organs, can be attributed, at least in part, to alterations in intercellular communication between neoplastic cells and normal cells in their microenvironment (Hanahan and Weinberg, 2000; Park et al., 2000). For example Sertoli cell-only syndrome and growth of testicular cancer cells following a genetic mutation might be viewed as a result of the disruption of homeostatic regulations, which determine whether spermatogonia and SSCs remain quiescent, proliferate, differentiate, or die. Interestingly, in two studies that examined only patients with germ cell maturation arrest, all were found to bear alterations in either the NOTCH1 receptor or its ligand JAG (Hayashi et al., 2001). In addition, alterations of NOTCH signaling have been observed in testicular tumors as well, and lack of NOTCH receptor expression seems associated to seminoma (Hayashi et al., 2004), a lesion developing from spermatogonia (Rajpert-De Meyts, 2006). Insights into the causes of germ cell maturation arrest and testicular cancer will only arise with a better understanding of the molecular mechanisms driving germ cell proliferation and differentiation in the perinatal testis. Indeed we do not yet fully understand what drives a spermatogonial into quiescence once it reaches the gonads, and what determines resumption of mitosis and differentiation after birth.

Our results indicate that Sertoli cells, the main component of the SSC niche, are able to regulate the levels of GDNF and CYP26B1 through FSH, FGF2 and NOTCH signaling. FSH and FGF2 upregulate GDNF production, while JAG1, a ligand for NOTCH, downregulates GDNF and CYP26B1 levels (Fig. 5). Interestingly, the signals activating the NOTCH pathway are probably produced by germ cells. An excess of spermatogonia/SSC will therefore increase NOTCH signaling in Sertoli cells, which will downregulate germ cell proliferation and promote differentiation through a negative feedback loop. Therefore, dysregulation of NOTCH signaling, by modifying components of the germ cell niche, will lead to maturation arrest and might possibly contribute to the growth of testicular cancer cells.
Figure 5. Integrated model showing how the effects of FSH/FGF2 are balanced by NOTCH activation and might regulate GDNF and CYP26B1 production in Sertoli cells to ensure stem cell homeostasis.

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