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Review

Novel regulators of spermatogenesis

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ABSTRACT

Spermatogenesis is a multistep process that supports the production of millions of sperm daily. Understanding of the molecular mechanisms that regulate spermatogenesis has been a major focus for decades. Yet, the regulators involved in different cellular processes of spermatogenesis remain largely unknown. Human diseases that result in defective spermatogenesis have provided hints on the molecular mechanisms regulating this process. In this review, we have summarized recent findings on the function and signaling mechanisms of several genes that are known to be associated with disease or pathological processes, including CFTR, CD147, YWK-II and CT genes, and discuss their potential roles in regulating different processes of spermatogenesis.

Contents

1. Introduction

Spermatogenesis is a unique process that supports the production of millions of sperm daily in the testis. It is a sophisticated multistep process involving three major phases, mitotic, meiotic and post-meiotic, as well as other cellular events such as cell migration, apoptosis and differentiation. These events are tightly regulated and the understanding of the molecular mechanisms that regulate the three phases of spermatogenesis has been a major focus for decades. Transcriptome analysis by microarray and next generation sequencing have revealed more than 10,000 genes differentially expressed during spermatogenesis [1,2]. However, the functions of these transcripts remain largely unknown. The major hurdle in deciphering the molecular events in spermatogenesis is the lack of *in vitro* model systems for highly specialized germ cells such as primary spermatocyte (tetraploid) and spermatid (haploid).

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Thus, most of the functional genomic studies in spermatogenesis mainly rely on the use of transgenic mice. Recently, significant progress has been made in studying the mitotic phase of spermatogenesis. The establishment of primary spermatogonial stem cell (SSC) cultures and spermatogonial stem cell line [3–5] as well as functional transplantation model [6] have become valuable tools for elucidating the mechanisms underlying the regulation of stem cell self-renewal and proliferation. Using these models, a number of cytokines and their corresponding signaling pathways including GDNF/PI3k-Akt, FGF2/MAP2K1, Nodal, Wnt5a, and CXCL12-CXCR4 have been demonstrated to regulate spermatogonial stem cell maintenance [7–11]. In addition, studies characterizing SSC niche are also emerging [12,13]. However, since it is not feasible to propagate tetraploid and haploid cells *in vitro*, studies in meiotic and post-meiotic phase are limited. Recent study by Sato et al. has reported an *ex vivo* organ culture method for production of functional sperm *in vitro* [14]. However, the efficiency for the haploid cells generation is low and gene manipulation in a specific cell type of the organ culture is technically difficult. Therefore, developing models of *in vitro* spermatogenesis for meiotic and post-meiotic phases remains a major challenge in the field.

Apart from the frequently used gain or loss-of-function approaches in model systems, human diseases that result in defective spermatogenesis have also provided hints on the molecular mechanisms regulating this process. Examples of these diseases included but not limited to non-obstructive azoospermia, oligozoospermia, cryptorchidism, cystic fibrosis and cancer. In non-obstructive azoospermia and oligozoospermia, the number of sperm produced is significantly lowered or even absent due to multi-faceted defects in spermatogenesis including developmental arrest and abnormal apoptosis of germ cells [15–17]. In cryptorchidism, the undescended testis is exposed to a higher temperature than that in the scrotum [18]. It is shown that hyperthermia reduces spermatogenesis and leads to germ cell apoptosis [19]. Cystic fibrosis is a common genetic disease that presents a multitude of clinical manifestations including male infertility and defective spermatogenesis [20–23]. Although cancer development is not directly related to spermatogenesis, both processes share common features such as unlimited proliferation/immortalization and cell migration/metastasis [24]. Moreover, there is a set of genes known as cancer/testis (CT) genes that are specifically expressed in the testis but not in normal tissues except during cancer development [25–27], suggesting common regulatory mechanisms shared between spermatogenesis and cancer development. This review summarizes the recent findings revealing unexpected roles of several disease-related genes in spermatogenesis and discusses their possible involvement in the regulation of spermatogenesis.

2. CFTR

Cystic fibrosis transmembrane conductance regulator (CFTR), is a cAMP activated Cl^- and HCO_3^- conducting channel, mutations of which result in cystic fibrosis (CF), a regressive human disease with multi-organ defects, including male infertility [21–23]. While over 97% CF males exhibit congenital bilateral absence of the vas deferens (CBAVD) [28–30], increased frequency of mild CFTR mutations has also been reported in patients with CBAVD as the only manifestation in absence of other typical CF symptoms [20,31–36]. Interestingly, the epididymal sperm taken from CBAVD patients with CFTR mutations showed reduced quality and lower IVF rates [37,38], suggesting that mutations of CFTR, in addition to causing CBAVD, might also affect sperm quality and/or quantity directly or indirectly. In fact, increased frequency of CFTR mutations or impaired CFTR expression in men with non-obstructive azoospermia or oligospermia as compared to the fertile men or a general population has been reported [33,39–45], although some studies claimed no significant difference [46–48]. Similarly, early studies using testicular histological analysis yielded controversial results showing either normal or impaired spermatogenesis in CF or CBAVD men [49–51]. It remained an open question whether CFTR plays a role in spermatogenesis until recently.

2.1. CFTR and FSH-signaling in Sertoli cells

Sertoli cells are the somatic cells in seminiferous tubules of the testis, which are essential to spermatogenesis by providing structural and nutritional support for the developing germ cells [52]. The function of Sertoli cells is tightly regulated by hormones, particularly FSH [53,54]. By activating the FSH receptor and subsequently the membrane-bound adenylyl cyclase (mAC) in Sertoli cell membranes, FSH triggers a cAMP/PKA dependent signal transduction and hence the expression of the downstream transcription factors, such as CREB, which drives genes expression and proteins synthesis necessary for spermatogenesis [53–55]. Although CFTR was first detected in the testis more than two decades ago [56], its exact role remained unclear. We recently demonstrated CFTR/HCO_3^--dependent activation of soluble adenylyl cyclase (sAC) and the cAMP/PKA/CREB pathway in cultured rat Sertoli cells [45]. Moreover, the FSH-induced cAMP production and CREB phosphorylation can be potentiated by the activation of CFTR and sAC [45], suggesting that CFTR may modulate the FSH signaling in Sertoli cells during spermatogenesis. Consistent with this notion, CFTR knockout mice show down-regulated CREB expression, smaller testis size and reduced sperm production, indicating impaired spermatogenesis [45]. Furthermore, we detected decreased expression levels of CFTR, phosphorylated CREB and total CREB in the Sertoli cells in testicular samples collected from non-obstructive azoospermia patients [45]. These results have suggested a role of CFTR in regulating the FSH/cAMP/CREB pathway during spermatogenesis (Fig. 1), which might provide an explanation to the observed association of CFTR mutations with non-obstructive azoospermia and oligospermia [33,39–45].

2.2. CFTR and cryptorchidism

Cryptorchidism, defined as failed descending of one or both testes from the abdomen to the scrotum, is found in 1% men and results in impaired spermatogenesis and reduced fertility [57]. The temperature in the abdomen is much higher in the scrotum, which is believed to be the major factor leading to defective spermatogenesis in cryptorchidism [58]. Interestingly, it has been well established that the post-translational processing of CFTR protein including its folding and membrane insertion favors a low temperature and a high temperature inhibits these steps for CFTR maturation leading to its degradation in cells [59–61]. Thus, under cryptorchidism, the level of CFTR in the undescended abdomen testis is expected to be decreased. Indeed, using hyperthermia and cryptorchidism mouse models, we observed that CFTR was down-regulated in 43 °C-heatshocked and in surgical-cryptorchid mouse testes [62]. Accompanied with the reduced CFTR level, the cryptorchid testes also showed over-activation of NF-kB, up-regulation of COX-2, down-regulation of tight junction proteins (ZO-1 and occludin) and vacuoles presence in Sertoli cells, with reduced number of matured germ cells [62], indicating defective spermatogenesis. Moreover, in the same study, elevating culture temperature resulted in down-regulation of CFTR, up-regulation of COX-2 in cultured rat Sertoli cells; inhibition or knockdown of CFTR in the Sertoli cell cultures caused increased production of PGE_2 and decreased expression of ZO1 and occludin proteins, which was reversed by NF-kB and COX-2 inhibition [62]. These results suggest that reduced CFTR in cryptorchidism may lead to

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