Steroids 103 (2015) 105–114

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Expression of steroidogenesis-related genes in murine male germ cells

Martine Culty [a,b,c,*], Ying Liu [d], Gurpreet Manku [a,b,c], Wai-Yee Chan [e], Vassilios Papadopoulos [a,b,c]

[a] The Research Institute of the McGill University Health Centre, McGill University, Montreal, Quebec, Canada
[b] Department of Medicine, McGill University, Montreal, Quebec, Canada
[c] Department of Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada
[d] Section of Experimental Atherosclerosis, Center of Molecular Medicine, NHLBI, NIH, Bethesda, MD, USA
[e] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 14 July 2015
Accepted 17 August 2015
Available online 21 August 2015

Keywords:
Testis
Germ cells
Steroidogenesis
Spermatogenesis

ABSTRACT

For decades, only few tissues and cell types were defined as steroidogenic, capable of de novo steroid synthesis from cholesterol. However, with the refinement of detection methods, several tissues have now been added to the list of steroidogenic tissues. Besides their critical role as long-range acting hormones, steroids are also playing more discreet roles as local mediators and signaling molecules within the tissues they are produced. In testis, steroidogenesis is carried out by the Leydig cells through a broad network of proteins, mediating cholesterol delivery to CYP11A1, the first cytochrome of the steroidogenic cascade, and the sequential action of enzymes insuring the production of active steroids, the main one being testosterone. The knowledge that male germ cells can be directly regulated by steroids and that they express several steroidogenesis-related proteins led us to hypothesize that germ cells could produce steroids, acting as autocrine, intracrine and juxtacrine modulators, as a way to insure synchronized progression within spermatogenic cycles, and preventing inappropriate cell behaviors between neighboring cells. Gene expression and protein analyses of mouse and rat germ cells from neonatal gonocytes to spermatozoa showed that most steroidogenesis-associated genes are expressed in germ cells, showing cell type-, spermatogenic cycle-, and age-specific expression profiles. Highly expressed genes included genes involved in steroidogenesis and other cell functions, such as Acbd1 and 3, Tspo and Vdac1-3, and genes involved in fatty acids metabolism or synthesis, including Hsb17b4 *10* and *12*, implying broader roles than steroid synthesis in germ cells. These results support the possibility of an additional level of regulation of spermatogenesis exerted between adjacent germ cells.

1. Introduction

For several decades, the expression of genes related to de novo steroid synthesis from cholesterol was believed to be limited to specialized cells in steroidogenic tissues, including the adrenal cortex and the gonads under the regulation of the hypothalamo-pituitary axis, and the placenta and glial cells for which the regulation is unknown [1]. Recently, several cell types have emerged as being able to produce steroids from cholesterol in tissues not traditionally considered to be steroidogenic, such as the adipose tissue [2,3], prostate [4,5] and thymus [6]. In earlier studies, we identified P450 17a-hydroxylase/17, 20-lyase (CYP17) in mouse spermatozoa [7–9], where it was found to act as a squalene monooxygenase (epoxidase), an activity different from its conventional role in the formation of dehydroepiandrosterone and androstenedione in steroidogenic cells. More recently, we reported the expression of translocator protein 18 kDa (TSPO) in rat male germ cells, where it localized mainly in nuclei, in contrast with its mitochondrial localization in steroidogenic cells [10]. TSPO is a high affinity cholesterol-binding protein found in a complex comprising both mitochondrial membrane proteins such as VDAC1, and cytosolic proteins, such as the steroidogenic acute regulatory protein (STAR), also called STAR-related lipid transfer domain-containing 1 (STARTD1) [11]. This complex regulates the transfer of cholesterol from the outer to the inner mitochondrial membranes, permitting its conversion to pregnenolone by cytochrome CYP11A1 in steroidogenic cells [11]. Besides its role in steroid formation, TSPO has been shown to participate in a variety of cellular functions in multiple tissues/cell types, from being a molecular sensor to modulating cell proliferation or apoptosis [12]. Another steroidogenesis-related gene expressed in rodent and human germ cells at different stages of the spermatogenic cycle and in spermatozoa is

* Corresponding author at: The Research Institute of the McGill University Health Center, 1001 Decarie Blvd, Bloc E, Room EM1-3218, Montreal, QC, H4A 3J1, Canada.
E-mail address: martine.culty@mcgill.ca (M. Culty).

http://dx.doi.org/10.1016/j.steroids.2015.08.011
0039-128X/

CYP19A1, an enzyme catalyzing the aromatization of testosterone to estradiol [13,14]. The estrogens produced by germ cells may act as autocrine/paracrine factors since the estrogen receptors ESR1 and ESR2 are expressed in different types of germ and Sertoli cells throughout the spermatogenic cycle [15]. Indeed, estrogens have been shown to play critical roles in early phases of germ cell development [16,17], various steps of spermatogenesis [18], spermiogenesis [19], and germ cell survival [20]. Progesterone was recently proposed to have a direct inhibitory role on spermatogenesis, besides its known suppressive effects on gonadotropin secretion [21]. Taken together, these findings alluded to the possibility that germ cells might have the ability to produce steroids with autocrine and/or paracrine regulatory functions. In the present study, we examined this hypothesis by analyzing the expression of genes associated with the steroidogenic machinery in rodent testicular germ cells at different ages.

2. Materials and methods

2.1. Animals

Male postnatal day (PND) 6 and PND60 mouse C57BL/6 strain, and PND3, 8 and 60 Sprague Dawley rats from Charles Rivers Laboratories (Senneville QC; Wilmington, MA) were euthanized according to institutional regulations at the indicated ages. Testes were collected and either fixed in formalin or frozen.

2.2. Germ cell isolation

PND3 gonocytes and PND6/8 spermatogonia were isolated as previously described by sequential enzymatic and mechanical tissue dispersion, overnight differential adhesion and separation on STA-PUT BSA gradient [22]. The cells were further used for protein and mRNA analyses. Fractions of PND60 enriched rat germ cells were also prepared by collecting seminiferous tubules after collagenase–hyaluronidase treatment of decapsulated testes, in order to remove interstitial cells released in the supernatant. Tubule preparations contained a majority of germ cells at all adult stages of development, and a small number of Sertoli and myoid peritubular cells.

2.3. Serial analysis of gene expression (SAGE)

Purified total RNA isolated from type A spermatogonia of PND6 mice and pachytene spermatocytes and round spermatid of PND60 mice were used to generate SAGE libraries using the I-SAGE kit (Invitrogen Life Technologies, Carlsbad, CA). Ten microgram of RNA was used to generate a SAGE library according to the manufacturer's instruction with minor modifications [23]. The anchoring enzyme is *Nla* III and the tagging enzyme is the type IIS restriction enzyme, *Bsm*F I. Transforming clones were sequenced with the help of an ABI PRIZM 377 DNA sequencer; the sequences were further analyzed using the SAGE2000 software. Each library was sequenced to equal depth with ~111,000 tags. The SAGE analysis software used automatically eliminated the duplicate ditags when the number of sequenced tags was compiled. Tags derived from linkers were also eliminated. The identity of the genes represented by the SAGE tags was determined using the SAGEmap (www.ncbi.nlm.nih.gov/SAGE) and UniGene (www.ncbi.nlm.nih.gov/UniGene) [23].

2.4. Laser capture microdissection

Frozen sections (6–7 μm) of adult testis from mouse C57BL/6 strain were prepared, fixed and stained. Laser capture microdissection (LCM) was performed as described [8,10,24]. For each sample, one hundred Leydig cells, spermatogonia/spermatocytes, pachytene spermatocytes and round spermatids were captured and pooled and total RNA was extracted.

2.5. Quantitative real-time RT-PCR (qPCR)

Total RNA from laser captured cells or from isolated cell preparations was extracted with PicoPure RNA isolation kit (Arcturus, Mountain View, CA, USA) and treated with DNase I (Qiagen, Valencia, CA, USA). Samples were then used for reverse transcription and qPCR analysis (TaqMan reagent, Applied Biosystems, Grand Island, NY, USA). DNA Engine Opticon 2 (MJ Research Inc/Bio-Rad, Hercules, California) was used with the default thermal cycling program (95 °C for 10 min followed by 40 cycles of 95 °C, 15 s, 60 °C, 1 min). 18S rRNA was used as an endogenous reference. The primers of steroidogenesis-related genes were designed by Primer Express (Applied Biosystems) (Table 1).

2.6. Gene expression arrays

Gene expression in rat PND3 gonocytes, PND8 spermatogonia and PND60 mixed germ cell populations was determined using the RatRef-12 Expression BeadChip microarrays for genome-wide expression analysis (Illumina, San Diego, CA, USA) by the McGill University's Genome Quebec facility, as previously described [22]. Three independent RNA preparations were analyzed for rat gonocytes (each sample generated from 60 to 90 pups) and spermatogonia (each sample from 10 rats). For PND60 enriched rat germ cells, a single preparation from the combined testes from 6 rats was used, to compare with neonatal (PND3) and infantile (PND6/8) germ cells. For PND3 and 8, gene expression analysis in the corresponding somatic cell fractions was also performed and confirmed the high purity of the germ cell fractions ([22]; data not shown). Genes with a relative signal intensity of $\geqslant$50 represent 35% of the genes in the arrays, whereas a signal intensity of $\geqslant$2000 was found in 1% of genes. For PND60 adult cells, germ cell enrichment was shown by the presence of minimal levels of genes specific/highly expressed in Sertoli cells, such as *Fshr* presenting a

Table 1
Mouse primer sets for quantitative real-time RT-PCR.

Gene name	Nucleotide ID	Forward primer	Reverse primer	Product length
Cyp17a1	NM_007809	AAGGCCAGGACCCAAGTGT	CCACCGTGACAAGGATATGCT	50
Tspo	NM_009775	CCCGCTTGCTGTACCCTTAC	AGTTGAGCACGGTGGCAAA	54
Cyp11a1	NM_019779	GGAGACATGGCCAAGATGGTA	TGATGCTGGCTTTGAGGAGTG	52
Hsd3b1	NM_008293	CCAGAACCTATTGGAGGCCCTG	AGAAGATGAAGGCTGGCACAC	53
hsd17b2	NM_008290	GGCCGTGGTTAACAATGCC	TGAGTTCCCCGTCGATAGGTA	53
Cyp19a1	D00659	CCCTGGTCTTGTTCGAATGGT	AATGCTGCTTGATGGACTCCA	53
Stard6	BC061022	CGGACACACTCATATGCCACA	AATTGAGCCCATGGCAAAAC	53
Scp2	M62361	CCTCAGTCGGCCTTCTTTCA	CCAGTCCCATGTTACCAGCAA	55
Acbd1	NM_007830	CTTGATTGCTGCTGCTTCTGA	GGTGATACGGATGCCGAAAG	54