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Rhox in mammalian reproduction and development

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Homeobox genes play essential roles in embryonic development and reproduction. Recently, a large cluster of homeobox genes, reproductive homeobox genes on the X chromosome (*Rhox*) genes, was discovered as three gene clusters, α, β, and γ in mice. It was found that *Rhox* genes were selectively expressed in reproduction-associated tissues, such as those of the testes, epididymis, ovaries, and placenta. Hence, it was proposed that *Rhox* genes are important for regulating various reproductive features, especially gametogenesis in male as well as in female mammals. It was first determined that 12 *Rhox* genes are clustered into α (*Rhox1-4*), β (*Rhox5-9*), and γ (*Rhox10-12*) subclusters, and recently *Rhox13* has also been found. At present, 33 *Rhox* genes have been identified in the mouse genome, 11 in the rat, and three in the human. *Rhox* genes are also responsible for embryonic development, with considerable amounts of *Rhox* expression in trophoblasts, placenta tissue, embryonic stem cells, and primordial germ cells. In this article we summarized the current understanding of *Rhox* family genes involved in reproduction and embryonic development and elucidated a previously unreported cell-specific expression in ovarian cells.

Keywords: Embryonic development, Gametogenesis, Homeobox gene, Reproduction, Rhox, Stem cells

Introduction

Approximately 200 homeobox genes have been identified in rodents and one third of them are expressed in the gonads. The homeobox is a sequence that encodes transcription factors containing the DNA binding motif or "homeodomain" composed of 60 amino acids. Homeodomain-containing transcription factors regulate diverse developmental and physiological events.

The best-known homeobox gene family is the Hox family. The Hox gene cluster was first identified by Lewis [1] in *Drosophila melanogaster* approximately 30 years ago. It is a group of genes that control the body plan development of the embryo along the head-tail body axis. The main characteristics of the Hox family are that it encodes transcription factors containing the homeobox and more particularly, in mammals, it displays colinearity, in that the organization of Hox genes on the chromosome is the same order as their expression along the heal-tail body axis during embryonic development [2]. This temporal and spatial expression feature, that is, the colinearity of these genes, is related to the proper regulation of the development of their target tissues.

In 2005, approximately 20 years after the discovery of the homeobox, MacLean et al. [3] reported the discovery and characterization of new homeobox genes. These new homeobox genes, reproductive homeobox genes on the X chromosome (*Rhox*) are expressed selectively in male and female reproductive tissues in a cell type- and region-specific manner and play pivotal roles in embryonic development and adult reproduction [4,5]. In this review, we summarize the expression and roles of this new family of transcription factors *Rhox* in relation to reproduction and development.

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Rhox family genes

1. Genomic structure of *Rhox*

It was first determined that 12 *Rhox* genes are clustered into α

(*Rhox1-4*), β (*Rhox5-9*), and γ (*Rhox10-12*) subclusters, and more recently, *Rhox13* has been found. At present, 33 mouse genes have been found in this region and it comprises the largest homeobox gene cluster known in the mouse genome [5]; in the rat, 11 such genes have been identified, and three in the human. *Rhox* genes are extended to the large genomic region within an around 0.7 Mb segment in the A2 region of the mouse X chromosome [3]. The *Rhox* genes in the cluster have been classified by number according to their positional order on the X chromosome; the closest one to the centromere was named *Rhox1*, and the farthest, *Rhox12*.

The mouse *Rhox* gene family contains α, β, and γ subclusters [5]. The subcluster was originally defined as having four genes (*Rhox1-4*), but subsequent scrutiny of the X chromosome by genomic mapping and sequencing led researchers to understand that tandem replications of *Rhox2*, *Rhox3*, and *Rhox4* make a total of 23 genes in the 350 kb A2 genomic region (position 29780 K to 30100 K) between *Rhox1* and *Rhox5*. The replicated copies, namely paralogs, of each of these three genes are almost identical in sequence (more than 95% similarity). The α subcluster consists of eight paralogs of *Rhox2* and *Rhox3* and seven paralogs of *Rhox4*. At first, *Rhox4* was called *Ehox*, since it was initially identified in embryonic stem cells [6]. The β subcluster has five genes (*Rhox5-9*), and four of them were previously known by other names, specifically, *Pem* (*Rhox5*), *Psx1* (*Rhox6*), *Tox* (*Rhox8*), and *Psx2* or *Gpbox* (*Rhox9*). Finally, the γ subcluster is identified as four genes (*Rhox10-13*) [3,7,8], and *Rhox13* was discovered most recently [9].

The rat *Rhox* gene clusters are smaller than those in the mouse because it has only a single copy of α subcluster paralogs without *Rhox1*. Another difference from the mouse *Rhox* gene cluster is that the rat genome does not contain one of the β cluster genes, *Rhox6*, or *Rhox13* [10]. The dispositions of *Rhox* genes in the mouse and the rat are almost the same, suggesting the gene arrangements occurred by the mouse/rat split [5].

The human *RHOX* gene cluster is much smaller than those of rodents. Only two human *RHOX* orthologs have been detected on the region corresponding to the rodent X chromosome: *RHOXF1* (*PEPP1/OTEX*) and *RHOXF2* (*PEPP2*) [3]. Recently, Niu et al. [11] reported that: 1) 11 nonhuman primate species have one *RHOXF2* copy, 2) humans and 4 Old World monkey species have 2 copies, *RHOXF2* and *RHOXF2B*, and 3) chimpanzees have at least 6 copies of *RHOXF2*.

2. Expression of RHOX protein

The homeobox genes encode transcription factors containing a DNA-binding domain, namely the homeodomain, of 60 amino acids that has three α-helices. It is an important concept that homeodomain proteins bind not only to specific sequences on DNA, but also to specific proteins or RNAs [12,13]. This demonstrates the pivotal role of homeodomain transcription factors in various biological processes. Most mouse RHOX proteins have a very similar length and their homeodomains are located at the conserved position. Figure 1 depicts a schematic diagram of the RHOX protein products based on the data from the MacLean group [3] and Geyer and Eddy [9]. It shows that the RHOX proteins are similar in size and contain a single homeodomain near the C-terminus.

Homeobox gene product RHOX5 has been suggested to be a transcription factor that regulates a set of genes [3,4]. Prosaposin (PSAP), menin (MEN1), inhibitor of MyoD family (I-MFA), and cell division cycle 37 (CDC37) have been identified as interacting partners of RHOX5 [5]. The *Rhox8* gene encodes the protein with a length of 293 amino acids, and its homeodomain is close to the carboxy terminal region. Exceptionally, RHOX8 is highly glutamic acid rich and has two long glutamic acid repeats (25 and 26 amino acids, respectively). This makes RHOX8 a very acidic protein (pI = 3.75) due to 35% of the total protein being composed of glutamic acids [14]. The newly discovered gene, *Rhox13*, encodes a 232-amino acid protein. It was predicted to be a 25.3 kDa protein, but the rabbit antisera against the N-terminus of RHOX13 RHOX13 detected band at around 45 kDa from adult mouse testis homogenates using Western blot analysis [9]. To clarify the pos-

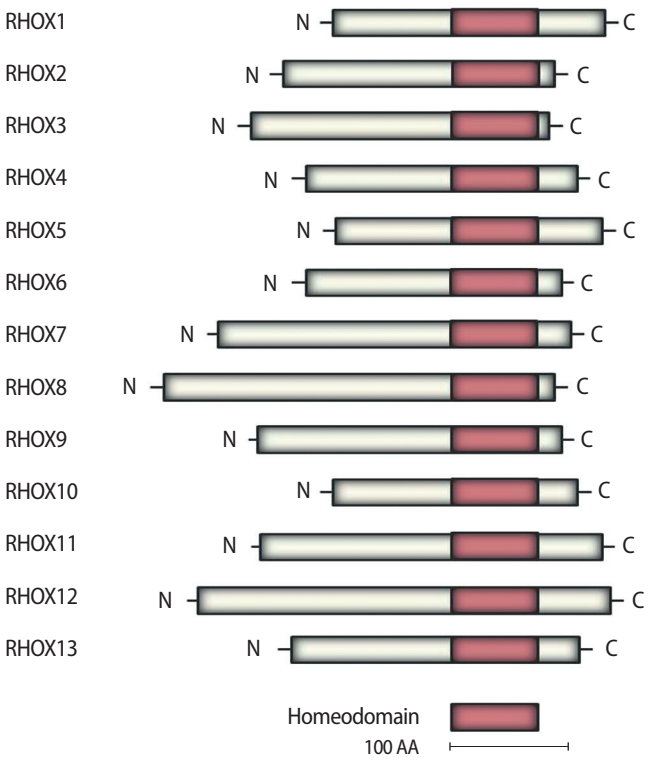

Figure 1. Schematic diagram of the reproductive homeobox genes on the X chromosome (*Rhox*) proteins. RHOX protein family members have a similar length, with a single homeodomain (pink box) near the C terminus. N, N-terminus; C, C-terminus.

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sible explanations for this size difference, Geyer and Eddy [9] generated a recombinant RHOX13 fusion protein with an N-terminal hexahistidine-maltose binding protein (His-MBP) tag, and found that the primary sequence of RHOX13 was responsible for its aberrant migration. The regulation of RHOX protein expression and its modification is not yet well understood. In addition, the function of RHOX as a transcription factor, and whether it acts as an activator or repressor, has not yet been determined.

Characteristics of *Rhox* gene expression

1. Cell type-specific expression of *Rhox*

Rhox genes are selectively expressed in male and female reproductive tissues: in the testes, epididymis, ovaries, and placenta. MacLean et al. [3,10] examined the pattern of *Rhox* gene expression in the mouse and rat in 2005 and 2012, respectively, by using real-time reverse transcription PCR (RT-PCR) analysis. Tissue-specific types of *Rhox* expression and their relative expression levels are summarized in Table 1. Most *Rhox* family members are expressed in reproductive-associated tissues. MacLean et al. [3] confirmed by Northern blot analysis the major expression of *Rhox*1 in the ovary and *Rhox3*, *8*, and *11* in the testis; while *Rhox2, 4, 5, 6, 9, 10*, and *12* were found in the placenta. Tissue expression of *Rhox13*, discovered by Geyer and Eddy [9] in 2008, could not be included in their expression studies due to lack of knowledge of this gene in the mouse in 2005 and no ortholog yet found in the rat.

Table 1. Tissue-specific expression pattern of *Rhox* genes

Species	Mouse	Rat
References	2005 [3]	2012 [10]
Method	Real-time RT-PCR	Real-time RT-PCR
Rhox1	**P O** T E	N/A
Rhox2	**P O T E**	**P** O T E
Rhox3	P **O T E**	**P** O T E
Rhox4	**P O T** E **Thy**	**P** O T **E**
Rhox5	**P O T E**	**P O T E**
Rhox6	**P O** T E	N/A
Rhox7	P **O T E St**	N O T E
Rhox8	P O **T E In**	P **O T E**
Rhox9	**P** O T **E**	**P** O T **E**
Rhox10	**P O** T E	P O T **E**
Rhox11	P O T **E**	**P** O T **E**
Rhox12	**P** O T E	**P O T E**

Underlined bold letters mean a value of more than 10 units and/or greater expression. Plain letters indicate a *Rhox* level of less than 10 units. Data are from Figure 3 of reference [3] and Figure 5 of reference [10].
Rhox, reproductive homeobox genes on the X chromosome; RT-PCR, real-time reverve transcription polymerase chain reaction; P, placenta; O, ovary; T, testis; E, epididymis; N/A, not available due to not finding the orthologs; Thy, thymus; St, stomach; In, intestine.

Additionally, mouse *Rhox4, 7*, and *8* are expressed in non-reproductive organs, such as the thymus, stomach, and intestine, respectively (Table 1). Daggag et al. [15] analyzed the expression of nine murine *Rhox* genes during mouse embryonic gonad development by means of real-time RT-PCR, and found the remarkable expression of *Rhox8* at the somatic counterpart of the embryonic gonad between E12.5 and E15.5.

Some of the *Rhox* genes are sexually biased; *Rhox6* and *Rhox9* are predominantly expressed in embryonic female germ cells, whereas *Rhox10* is only present in embryonic male germ cells. Transcripts of *Rhox3*, *Rhox8*, and *Rhox11* are found in the adult mouse testes, while *Rhox10* is detectable at the fetal testes. Expressions of *Rhox1*, *Rhox6*, and *Rhox7* mRNA are detected in the fetal ovaries, while *Rhox2a*, *Rhox4a*, *Rhox5*, and *Rhox9* are detectable in both the fetal ovaries and testes [15]. Song and colleagues analyzed human *RHOX* gene and protein expression patterns in 11 fetal and 8 adult tissues. They found that *RHOXF1* and *RHOXF2/B* mRNA are highly expressed in the human testes but marginally in the ovaries and non-reproductive tissues. In the testes, early stage germ cells (spermatogonia and early spermatocytes) express RHOXF2/2B, while later stage germ cells (pachytene spermatocytes and round spermatids) express RHOXF1. In the ovaries, RHOXF1 and RHOXF2/2B proteins are exclusively found in the oocytes rather than the whole ovary, where low levels of *RHOX* mRNA expressed [16].

2. Temporal and quantitative colinearity of *Rhox* expression

Expressions of *Rhox* genes show dynamic changes depending on developmental status. Expression of the α subcluster genes exhibit temporally and quantitatively unidirectional expression patterns, namely colinearity, corresponding to their position within the subcluster. For instance, *Rhox1*, the closest gene to the centromere of the α subcluster, is expressed first between postnatal days 7 and 12; after that, its expression was found to have disappear in the mouse testes. Around postnatal day 12, the expression of the next gene, *Rhox2*, is at its peak. The expression of *Rhox3* and *4* reach a peak between postnatal days 20 and 22. This phenomenon has been termed "colinearity" in temporal or quantitative expression [3].

In the case of β subcluster genes, colinearity in temporal expression is not observed, but *Rhox5*, *7*, and *8* do show a quantitative unidirectional expression. Interestingly, the expression of *Rhox6* or *Rhox9* is completely silent in the testis [3]. Similar to the α subcluster, γ subcluster genes show both temporal and quantitative colinearity. First, *Rhox10* exhibits its peak expression around postnatal day 12, and then *Rhox11* and *12* initiate their expression at the time that *Rhox10* expression begins to decrease around day 18. In summary, subclusters α and γ showed both temporal and quantitative colinearity, while subcluster β showed only quantitative colinearity [3].

Expression and function of the *Rhox* family

1. *Rhox* in the male reproductive system

The selective expression of the *Rhox* genes in reproductive-associated tissues suggests its important regulatory roles in reproductive features, such as in gametogenesis and fertility. Although little is known about which cells in adult reproductive organs express individual *Rhox* genes, relatively substantial data have been reported about *Rhox5*, and it is the only mammalian homeobox gene known to play a role in spermatogenesis [3,4,17]. However, conflicting results in male fertility have been reported. Male subfertility has been reported in *Rhox5*-null mice, characterized by increased germ cell apoptosis, reduced sperm number and sperm motility, and small litter size [4]. These findings conflictied with previously reported data that showed no obvious defects in *Pem* homeobox gene-deficient mice (*Pem* was the original name for *Rhox5*) [18].

Expression of *Rhox* genes in the Sertoli cells that nourish and regulate diverse characteristics of male germ cells, such as proliferation and differentiation, imply the sex hormone regulation of *Rhox* expression [19-21]. It has been reported that androgen induced *Rhox5* expression and that modulated the expression of androgen receptor (AR)-regulated genes such as phospholipid transfer protein (*Pltp*), transmembrane protein 47 (*Tmem47*), *Tmem176b*, ganglioside-induced differentiation-associated protein1 (*Gdap1*), and frizzled homolog 2 (*Fzd2*) in *Rhox5*-positive 15P-1 Sertoli cell clones [21]. In that study, they identified genes downstream of *Rhox5* in the testis, such as CD24a antigen (*Cd24a*), Kruppel-like factor 9 (*Klf9*), carboxypeptidase 1 (*Cpxm1*), *Tmem176a*, and *Tmem176b*.

By using a gain-of-function approach and microarray analysis in 15P-1 cells, Hu et al. [4,17] found the list of *Rhox5*-regulated genes. *Rhox5* negatively regulates the transcription of *Unc5c* proapoptotic receptor with tumor suppressor activity in Sertoli cells both *in vivo* and *in vitro*, and the *Unc5c* 5′-UTR has *Rhox5*-responsive *cis*-regulatory elements. Similar activity of *Rhox2* and *Rhox3* to *Rhox5* suggests the possible redundancy in Sertoli cells. This *Unc5c* repression by mouse *Rhox2* and *Rhox3* is elicited by human *RHOXF2*.

The epididymis is a highly segmented organ and is divided into three regions: the caput (proximal), corpus, and cauda (distal region). In general, mouse *Rhox* genes are abundant in the caput region and show gradient expression in the direction from the caput to the cauda [8]. *Rhox5*-null mice display an altered expression of the majority of the other *Rhox* genes, mostly lower in the caput, with only *Rhox6* higher in both the caput and cauda of the epididymis in *Rhox5*-null mice [8]. These results imply a compensation mechanism indicating that *Rhox* genes could reciprocally interact with each other and the nearest gene, *Rhox6*, could be an alternative in the absence of *Rhox5*. In addition, these results show that *Rhox5* is a master regulator of many other members of the *Rhox* family of genes in the murine epididymis.

Transcription of *Rhox5* mRNA is regulated by two promoters, a distal promoter (*Pd*) and a proximal promoter (*Pp*), which are each independently regulated [5,22]. While the *Pp* is restricted to somatic cells in the testes and epididymis, the *Pd* is expressed in the early embryo and somatic cells in adult female reproductive tissues, specifically the ovary and placenta, and is co-expressed within the *Pp* in the testes [23]. In addition, the *Pd* is widely found in many cells such as primary granulosa cells, mesenchymal stem cells, and tumor cells originateing from various cells or tissue lineages [5]. *Rhox5* transcription is regulated not only by hormonal regulation but also the site-specific methylation of cytosine and guanine in the *Pp* promoter. Androgen and AR act cooperatively on the AR-response element, and AR is recruited to the *Pp* in a region-specific and time-dependent manner. GATA-binding sites are also crucial for *Pp*-dependent *Rhox5* mRNA expression in the epididymis. These androgen and androgen receptor system, and GATA factors collaborate to regulate the expression of *Rhox5* mRNA in the epididymis [24].

Rhox10, a member of mouse γ subcluster *Rhox* genes, is abundantly expressed in immature male germ cells, such as fetal gonocytes, spermatogonial, and spermatocytes. Production of *Rhox10* mRNA of spermatogonia cells is dramatically induced by treatment with retinoic acid, wich provides an extrinsic cue to germ cells to enter meiosis [25]. When we measured mRNA expression in the testes and dissected cells from the female gonad, including oocytes, cumulus cells, and granulosa cells, we also found that *Rhox10* mRNA was the most abundantly expressed type of *Rhox* mRNA in the ovaries as well as the testes (Figure 2).

Rhox13 mRNA and protein expression have been detected in the ovaries from embryonic day 13.5 (E13.5), but RHOX13 protein expression is suppressed until postnatal day 3 in male mice [26]. Geyer et al. [26], who first found the *Rhox13* gene and its expression in germ cells in the fetal testis and ovary, reported that NANOS2 localized in P-bodies has the activity of deadenylase and degradation of specific mRNA. The RHOX13 protein was detectable in *NANOS2*-null mice at E15.5 but not in heterozygous mice. This suggests that the RHOX13 protein is regulated by an RNA-dependent mechanism.

RHOXF1 and *RHOXF2/2B* are the most abundant in the human testis. Early-stage germ cells (spermatogonia and early spermatocytes) express *RHOXF2/2B*, while later-stage germ cells (pachytene spermatocytes and round spermatids) express *RHOXF1*. *RHOXF1* and *RHOXF2/2B* mRNA expression increases gradually with the gestational days of the fetal testes [16]. In summary, the *Rhox* genes of the male reproductive system are tightly regulated in a temporal, spatial, and hormone-dependent manner.

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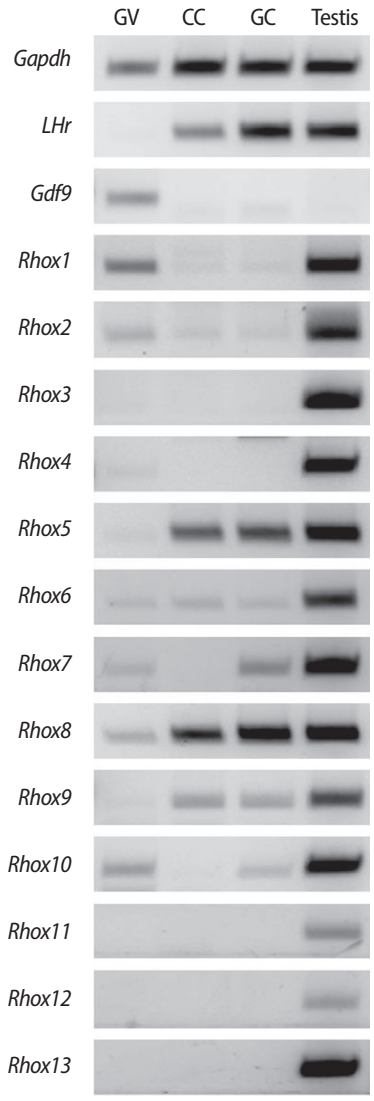

Figure 2. Expression of reproductive homeobox genes on the X chromosome (*Rhox*) family members in dissected mouse ovarian tissues, specifically oocytes, cumulus cells, granulosa cells, and testes. *Gdf9* and *LHr* were used as markers for oocytes and granulosa cells, respectively. *Gapdh* was used as an internal control. In the case of the oocytes, PCR was carried out by using cDNAs equivalent to a single-oocyte. These pictures are representative of the experiments, which were repeated five times. GV, denuded germinal vesicle oocytes; CC, cumulus cells; GC, granulosa cells.

2. *Rhox* in the female reproductive system

Rhox genes also play key roles in the female reproduction system. MacLean et al. [3] previously showed that adult mouse ovaries express significant amounts of *Rhox1, Rhox2, Rhox5*, and *Rhox7* by real-time RT-PCR and *Rhox1* using northern blot analysis. The same authors found a slightly different expression pattern in the rat ovaries, in which *Rhox* expression was relatively lower [10]. Transcripts of *Rhox1, 2a, 4a, 5, 6, 7,* and *Rhox9* are detectable in female germ cells between E12.5 and E15.5 [15].

We measured the expression of all 13 members of *Rhox* family genes by RT-PCR in germinal vesicle (GV) oocytes, cumulus cells, and granulosa cells dissected out from 21-day-old mice 48 hours after equine chorionic gonadotropin (eCG) stimulation (Figure 2). All 13 members were expressed in the testes, whereas, interestingly, only some of them were expressed in the oocytes and follicular cells. Expression of *Rhox3, 4, 11, 12,* and *13* was not detectable in the ovarian cells. Only expression of *Rhox1* and *2* was detectable in the adult germ cells, but not in the follicular cells, whereas *Rhox5* and *9* were detectable in the follicular cells but were only very slightly expressed in the oocytes. Facinatingly, *Rhox7* and *10* were detectable in the oocytes and granulosa cells but not in the cumulus cells, since the cumulus cells are a cluster of granulosa cells closely surrounging the oocyte. It strongly suggests that the reciprocal interactions and powerful effects between oocyte and cumulus cells on their each others' gene expression. Finally, only *Rhox5* and *9* were constitutively expressed in ovarian cells. As mentioned earlier, the *Rhox* promoter, especially the distal promoter *Pd* was substantially transcribed in female reproductive tissues in the ovary and placenta; therefore, based on our results in Figure 2, it would be a good model for studying the tissue- and stage-specific and aberrant regulation of Rhox gene expression.

Promoter *Pd* for *Pem* (*Rhox5*) is transcriptionally regulated by the Ets transcription factors, Gabp and Elf1, and the ubiquitous zinc finger transcription factors, Sp1 and Sp3, in normal granulosa cells [27]. The Ets family transcription factors are closely related to diverse tumor cell types for tumor cell growth, invasion, and metastasis, and to the proper control of proliferation and differentiation of normal cells. Adult human ovaries express a significant amount of RHOXF1 and RHOXF2/2B proteins primarily in oocytes, despite low levels of *RHOX* mRNA in the whole ovary [16]. However, little is known about their function in the human reproductive organs.

The gene expression of *Rhox2, 5, 6,* and *8* is affected by the ovulatory cycle, but their downstream genes are unknown in the female reproductive tissues [28]. Researchers have observed that *Rhox8* displayed a unique expression pattern with the progesterone response element within the *Rhox8* promoter in granulosa cells. In addition, they found that *Rhox8* mRNA expression in *Rhox5*-null mice was reduced after hCG administration but recovered with follicular development. *Rhox8* exhibited normal stimulation by eCG but failed to reach its peak mRNA level at 8 hours post-hCG, as found in wild-type ovaries, in progesterone receptor knockout (PRKO) mice. Based on these results, they conclude that *Rhox8* transcription is dependent on *Rhox5* during early folliculogenesis and on progesterone during the periovulatory window [28]. The same researchers also found that the mouse *Rhox* α and β subcluster genes are induced by FSH and LH, whereas *Rhox7* and the γ subcluster genes remained silent in superovulation.

3. *Rhox* in stem cells and embryonic development

In addition to the roles of *Rhox* genes in reproductive biology, *Rhox* genes are also involved in the differentiation and/or regulation of several types of stem cells, including embryonic stem cells, primordial germ cells, and trophoblast stem cells. *Rhox4* (*Ehox*) expression is found in embryonic stem cells and *in vitro* differentiation of embryonic stem cells with transient knocked down of *Rhox4* without leukemia inhibitory factor impairs hematopoietic, endothelial, and cardiac differentiation. Thus *Rhox4* is considered essential for the earliest stages of murine embryonic stem cell differentiation [6]. As mentioned earlier, at present, 33 *Rhox* genes have been found in mice, 11 in rats, and three in humans. Jackson et al. [7] postulated that this unique clustering of *Rhox* genes among mice, rats, and humans may explain species differences in embryonic stem cell derivation and maintenance. They found important comparable functions of two *Rhox* genes within the duplicated region of the cluster of *Rhox2* to *Rhox4*, in regulating the initial stage of embryonic stem cell differentiation and maintenance. It has been reported that *Rhox4* is also expressed in trophoblast stem cells, supporting a role for *Rhox4* in the placental stem cells in the developing placenta [3,29].

It has been suggested that *Rhox5* plays a vital role in the differentiation of embryonic stem cells. *Rhox* genes are abundant in E9.5 trophoblasts and *Rhox5*, which is localized in the X chromosome, is expressed predominantly in female mouse blastocysts rather than male blastocysts [30]. Embryonic stem cells with constitutively expressing *Rhox5* (*Pem*) are not differentiated into the primitive endoderm nor embryonic ectoderm, but embryonic stem cells with constitutively expressing *Rhox5* (*Pem*) developed into the teratoma containing only undifferentiated embryonic carcinoma-like cells. This differentiation inhibition of embryonic stem cells with forced expression of *Rhox5 in vitro* and *in vivo* suggests a role for *Rhox5* in the transition between undifferentiated and differentiated cells in the early mouse embryo [31].

All *Rhox* genes, except *Rhox8*, showed germ cell-specific expression in the embryonic testes and ovaries [15]. *Rhox8* was found to be expressed within the somatic compartments. In addition, *Rhox* expression in embryonic gonads showed dynamic and sexually dimorphic patterns with a tendency toward higher expression in female germ cells, with the single exception of *Rhox10*, which showed exclusive expression in male embryonic germ cells. These results imply an important developmental role for *Rhox* genes in embryonic gonads [15].

Meanwhile, *Rhox6* is abundantly expressed in the placenta and the post-migratory primordial germ cells. Continuous knock-down of *Rhox6* disturbed the primordial germ cell differentiation process, implying that *Rhox6* is necessary in the regulation of germ line differentiation [32]. *Rhox9* is 91% identical to *Rhox6* in the protein-coding nucleotide sequence and has been identified in the primordial germ cells, placenta, female embryonic stem cells, and in the ovary [33]. The histone demethylase Lysine (K)-specific demethylase 6A (*KDM6A*, also known as *UTX*; ubiquitously transcribed tetratricopeptide repeat, X chromosome), that removes H3K27me3 from *HOX* genes to restore their activity, is encoded by an X-linked gene that escapes X inactivation in somatic tissues of the mouse and human [34]. This suggests that KDM6A may be important for female-specific junctions [35]. Berletch et al. [33] found that *Rhox6, Rhox9*, and *Kdm6a* are expressed in a sexually dimorphic manner. Expression of *Rhox6* and *Rhox9* are significantly higher in undifferentiated female embryonic stem cells, and this female bias was due to KDM6A, which occupied the 5′ end of *Rhox6* and *Rhox9* to regulate their expression. It has been found that *Rhox6, Rhox9,* and *Kdm6a* are highly expressed in the ovary, showing the consistent paternal imprinting of these genes previously reported in the placenta and embryonic stem cells [36].

Future directions

The *Rhox* family of genes are recently identified homeobox genes, and similar to other homeobox genes, these genes are selectively expressed in embryonic and adult reproductive tissues and stem cells. In light of their spatially and temporally limited expression pattern, their functions in reproduction and development have been proposed and confirmed one by one.

The *Rhox* genes have been studied for the last two decades; however, much remains to be clarified regarding the biological significance and function of individual paralogs, as well as their regulation. It is evident so far that *Rhox* expression and regulation are important during embryogenesis and gametogenesis as well as in the reproductive physiology of adults. The regulatory mechanism of *Rhox* genes and their roles in reproduction and stem cell biology should be scrutinized in the future.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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