Oestrogen receptors and the male genital tract

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Oestrogens bind to oestrogen receptors (ERs) that act as ligand-activated transcription factors within cell nuclei. Two ERs, usually known as ERα and ERβ, have been cloned from mammals. Species-specific expression of splice variant isoforms of ERs has been reported. In the testis ERα is consistently detected in Leydig and peritubular cells in rodents but not in the human or primate. ERβ is expressed in both somatic and germ cells; expression of variant forms has been reported. Highest levels of expression of ERα are found in the epithelial cells lining the efferent ductules, these cells are also ERβ positive. Throughout the rest of the male reproductive tract most ERα positive cells are detected in the stromal compartment and in some basal epithelial cells. In contrast, ERβ is expressed by the majority of cells (both stromal and epithelial) in the epididymis, vas deferens, seminal vesicles and prostate. In conclusion, oestrogen receptors are widely expressed in the reproductive system of males and provide further evidence that locally produced oestrogens can act on multiple cell types. Further studies are required to identify the cell-specific alterations in signalling/gene expression that occur as a consequence of ER expression.

Key words: oestrogen receptor alpha, oestrogen receptor beta, splice variant, Sertoli, efferent ductile, spermatid

Studies over the last ten years have firmly established that oestrogens are locally produced within the male reproductive system (Carreau S., p. 99-105). Oestrogen action within the male reproductive system is mediated via receptors expressed in target cells. In the following sections the structure and function of the genomic oestrogen receptors is described and their pattern of expression in the male reproductive systems of rodents, primates and the human is reviewed.

Oestrogen receptors structure and function

The first ER cDNA was cloned and sequenced in 1986 [1]. Identification of this gene represented a major step forward in studies on the target sites of oestrogen action. However, reports that oestrogen binding could still be demonstrated in tissues that appeared to lack significant expression of ER suggested that alternative receptors existed and in 1996 this was confirmed by the cloning of a second receptor cDNA [2]. These two receptors, now usually known as ERα and ERβ, are the products of different autosomal genes (ESR1 and ESR2 respectively). Mice with targeted deletions in Esr1 (ERαKO/ERKO, [3, 4] and Esr2 (ERβKO/βERKO [4, 5]) have been generated and have also been cross-bred to prepare mice with disruptions in both genes (ERαβKO/DERKO, [4]). The phenotypic characteristics of the males are summarised in Table 1.

In common with other members of the steroid receptor superfamily of genes, ERα and ERβ proteins contain a number of key domains including a central DNA binding domain (with two zinc finger motifs), a domain towards the C-terminus which forms a ligand-binding pocket and two trans-
activation domains (AF-1 and AF-2) that contain target sites for phosphorylation and co-factor recruitment (Figure 1). Although ERα and ERβ exhibit significant sequence homology careful analysis of the structure of their ligand binding domains has resulted in the development of a number of ER subtype-selective ligands [6]. Signalling via oestrogen receptors can occur in a number of different ways (reviewed in [7]). Classically, following binding to an oestrogenic ligand both ERs undergo a conformational change, dimerise with a second ER, and then interact with regulatory regions (oestrogen response elements, EREs) on DNA causing up- or down-regulation in gene expression. ERs can also change patterns of gene expression without directly binding to DNA and a number of pathways have been identified [7]. In vitro studies have demonstrated that homodimers (ERα-ERα or ERβ-ERβ), or heterodimers (ERα-ERβ) can be formed and it has been suggested that one way in which ERβ may influence cell function is by blunting the activity of ligand-activated ERα [8]. Both ERs can be phosphorylated, either following steroid ligand activation, or in the absence of steroids via a number of different pathways including the MAPK phosphorylation cascade that is induced by peptide growth factors [7]. Rapid signalling via ERs associated with the plasma membrane, as well as a novel membrane associated oestrogen receptor, have been reported (Luconi M, p. 119-127).

A number of different ERα mRNA transcripts have been identified; several of these include untranslated exons that do not alter the final protein sequence. A novel ERα mRNA consisting of a previously unidentified 5′-sequence and the exons 4-8 of the ERα gene has been isolated from a human testicular cDNA library [9]. An ERα splice variant mRNA that is predicted to lack the ligand-binding domain, has been found in several mouse tissues including the testis [10]. In ERαKO mice residual oestrogen-binding activity has been detected and may in part be due to expression of a 61Kd ERα variant protein [11].

Messenger RNAs encoding splice variant isoforms of ERβ appear to be very widely expressed in man, primates and rodents [12-15]. Notably, species differences exist with the variants routinely detected in human and primate not being found in rodents and vice versa [15, 16]. One of these human ERβ variants known as ERβ2/CX, [12] (Figure 1) is identical to the wild type ERβ protein except at the C-terminus where the variant has a unique peptide encoded an alternatively spliced eighth exon. This protein lacks the AF-2 domain and does not have an intact ligand-binding domain however it is still able to form homo- or heterodimers with wild type ERβ [17].

### Table 1. Expression of oestrogen receptors in mice and the phenotypes reported for the mouse knockouts

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Testis</th>
<th>Efferent Ductules</th>
<th>Epididymis</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα protein</td>
<td>Leydig and peritubular myoid cells</td>
<td>Epithelium only</td>
<td>Normal**/rare epithelial cells</td>
<td>Basal cells, stroma</td>
</tr>
<tr>
<td>ERβ protein</td>
<td>Most somatic cells, some germ cells</td>
<td>Epithelial and stromal cells</td>
<td>Epithelial and stromal cells</td>
<td>Epithelial and stromal cells</td>
</tr>
<tr>
<td>ERαKO</td>
<td>Infertile, seminiferous tube dilation</td>
<td>Dilated, epithelium abnormal</td>
<td>Normal!</td>
<td>Normal!</td>
</tr>
<tr>
<td>ERβKO</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal!</td>
<td>Hyperplasia/normal*</td>
</tr>
<tr>
<td>ERαβKO</td>
<td>Infertile, seminiferous tube dilation</td>
<td>Abnormal!</td>
<td>Normal!</td>
<td>Hyperplasia/normal*</td>
</tr>
</tbody>
</table>

** Different results have been reported by different groups.
Data from: [4, 5, 20, 39, 52].

Figure 1. Diagramatic representation of the domain structure of human oestrogen receptors. Wild type, full-length ERα and ERβ are 66 and 59.2 Kd respectively, both contain a central DNA binding domain consisting of two zinc fingers, the ligand binding domain is towards the C-terminus. Phosphorylation sites and regions involved in co-factor recruitment are found in the AF-1 and AF-2 domains. The ERβ2/CX contains an alternative coding exon at the C-terminus, removal of the 5th exon by alternative splicing results in a truncated protein due to introduction of a premature stop codon.
heterodimers with full-length ERs [13]. Another variant lacks the 5th exon and as a result a truncated protein is formed (figure 1); it has been proposed that splice variants may act as dominant negative ‘receptors’ [17].

**Testicular expression of oestrogen receptors**

**Oestrogen receptor alpha**

ERα mRNA has been detected in extracts from rodent testes [18, 19]. In the testes of adult mice and rats ERα has been immunolocalised to the nuclei of Leydig and peritubular myoid cells but not to Sertoli or germ cells [20, 21]. In agreement with these findings ERα mRNA has not been detected in a murine Sertoli cell line [22] and germ cells transplanted from ERαKO males develop normally in recipients [23]. Male ERαKO mice become infertile as they age and the current thinking is that this is due to fluid accumulation resulting from deficiencies in the function of the efferent ductules rather than because of a primary deficit in testicular function (see below).

Studies using testicular tissues from human and primate have reported contradictory findings with regard to ERα expression. For example, an ERα variant containing an unidentified N terminus and exons 4 to 8 of ERα has been cloned from a Clontech human testis cDNA library [9]. However, using primers directed against exons 1 to 3, although an ERα-specific DNA has been amplified from commercial cDNA pools and cDNA libraries, it was not detected in those prepared from testicular biopsies taken so as to avoid contamination by efferent ductules or epididymis [24]. Using RTPCR, Carreau et al. identified wild type, exon 1 and exon 4 deleted forms, of ERα in immature germ cells purified from human ejaculates [25] however in mature spermatocytes isolated from the same samples only mRNA corresponding to the exon 1 deleted isoform was detected [25].

On Western bots, using a monoclonal antibody directed against the C-terminus of ERα Aquila et al. [26] detected a positive band corresponding to full length protein (66Kd) in human spermatozoa whereas Carreau et al. found protein with a molecular weight of 46Kd [25]. In contrast, no evidence for expression of ERα in testes from humans and primates was obtained using Western analysis or immunohistochemistry with two antibodies directed against the N terminal domain of wild type ERα [27, 28]. This is in agreement with studies on testes from rhesus and cynomolgous macaques where no specific immunostaining was visualised and no significant E2 binding was detected in a binding assay [29]. Differences in the results reported for human testes could be due to contamination of testicular cDNAs with efferent ductules, expression of truncated variants that are not recognised by some primers/antibodies, or genuine differences between primates and rodents.

**Oestrogen receptor beta**

In adult rats and mice ERβ mRNA has been detected in testicular extracts using RTPCR [18, 19]. In rats ERβ mRNA has been localised to pachytene spermatocytes (stages VII to XIV) and round spermatids (stages I-VIII) using in situ hybridisation with a non-radioactive probe [30]. Complementary cDNAs for full length ERβ, as well as several splice variants, have been amplified from human testis cDNA libraries and these have all been detected in extracts of human testes by RTPCR [24, 31]. Messenger RNAs corresponding to wild type ERβ and one of these variants (ERβ2/CX) have also been identified in primate testes [15]. Radioactive in situ hybridisation using human testes resulted in localisation of grains within the seminiferous epithelium with an intense signal in spermatids [32].

In rats and mice ERβ protein has been immunolocalised to nuclei in multiple cell types including Sertoli cells, Leydig cells and peritubular cells [20, 30, 33, 34]. The reported pattern of expression in different germ cells has varied between studies and may depend upon the method of fixation as well as antibody specificity. For example, in mice expression was not detected in spermatids in one study [20] whereas immunopositive staining can be seen in the image shown in figure 2 (see panel A); rat spermatids are reported to be immunopositive [30]. Using an antibody raised against a peptide located in the hinge domain of the protein (figure 1) positive immunostaining was detected multiple cell types, including Sertoli cells, Leydig cells and peritubular myoid cells in testes from adult humans, and primates (macaque and marmoset) [27]. In germ cells the intensity of immunostaining for ERβ was variable with intense staining of pachytene spermatocytes and round spermatids whereas staining in preleptotene, leptotene and zygote spermatocytes was low/absent and elongate spermatids were consistently immunonegative [27]. On Western blots more than one size of ERβ protein was detected in testicular extracts using this antibody that cannot discriminate between full length ERβ and several of the ERβ variants [27]. Subsequently, using isotype-specific monoclonal antibodies, the pattern of expression of ERβ1 (full length wild type protein) and the ERβ2/CX variant within the adult human testis was compared (figure 2C, D). Notably, although these proteins are both encoded by the same gene their patterns of expression are not identical with the most intense immunostaining of ERβ1 being detected in round spermatids whereas the ERβ2/CX variant appeared to be most abundant in Sertoli cells [24, 35]. The pattern of expression of full length ERβ1 protein in the testes of marmosets and macaques is similar to that in human testes with most intense staining in pachytene spermatocytes and round spermatids [15]. Pentikainen et al. [36] reported that incubation of isolated human seminiferous tubules with oestrogens reduces levels of germ cell apoptosis.
Treatment of *hpg* mice with oestrogens is reported to stimulate spermatogenesis [37] and in androgen binding protein overexpressing rats overexpression of ERβ was detected in degenerating pachytene spermatocytes [38]. These data suggest that a link may exist between the amount of ERβ expressed in germ cells and germ cell survival, furthermore in the human and primate the iso-type of ERβ in germ cells may also be important.

Figure 2. Immunolocalisation of oestrogen receptors in the male reproductive tract. Panel A) adult mouse testis, ERβ; B) macaque efferent ductules, ERα; C) human adult testis, ERβ1 (wild-type, stained using isotype-specific monoclonal), the most intense staining was detected in germ cells, Sertoli cells (arrows) were weakly immunopositive; D) human adult testis ERβ2/CX (truncated variant, stained using isotype-specific monoclonal), the most intense staining is seen in Sertoli cells (arrows); E) mouse epididymis, ERβ; F) mouse vas deferens, ERβ; G) adult human prostate, ERα, note that most immunopositive nuclei are in the stroma (S) although a few immunopositive basal cells can be detected in the epithelium lining the glands (G); H) human prostate ERβ, within the stroma (S) and glandular epithelium the majority of nuclei are immunopositive.
Expression of ERs in the efferent ductules

The efferent ductules (ED) are located immediately adjacent to the rete testis and the spermatzoa pass through them en route to the initial segment of the epididymis. In rats, Fisher et al. [21] have detected intense ERα positive immunopositive staining in the rete testis. The EDs are lined by columnar epithelial cells [39]. We, and others, have reported that nuclei within the cells of the epithelium of the ED in man, primates and rodents contain intense immunopositive staining for ERα [20, 27, 35, 40] (e.g. figure 2B). These epithelial cells are also immunopositive for ERβ suggesting that ERα-ERβ heterodimers could be formed [20, 35]. The majority of cell nuclei within the stromal compartment are reported to be ERα+/ERβ− [20, 27]. In a comprehensive series of studies Hess and colleagues have demonstrated that the ED are important site of fluid resorption and have shown that oestrogens are key regulators of ED epithelial cell function [39]. For example, alterations in ED cell function appear to be the key factor in the development of late onset infertility in ERαKO male mice (reviewed in [39]) table 1. Further evidence that oestrogens have a direct impact on the ED has been obtained by administration of anti-oestrogens to rats. This resulted in ED tubule dilation, an increase in testis weight and down-regulation in expression of ERα [41]. Expression of ERβ can be detected in the ED of ERαKO and appears unaffected in the ED of anti-oestrogen treated rats suggesting that expression of this receptor is not sufficient to maintain normal oestrogen-dependent function of EDs and that the impact of oestrogens is mediated via ERα in this part of the reproductive tract [19]. At present the functional significance of ERβ expression in the ED remains to be established.

Expression of ERs in the epididymis and vas deferens

ERα and ERβ mRNAs have been detected in extracts of epididymes from mice [19] and rats [18]. Expression of ERα is generally reported to be more abundant in stromal cells than in epithelial cells although this varies between species and upon the region of the epididymis being examined [20, 42]. ERα expression has been detected in epithelial cells within the initial segment in stump-tailed macaques and the marmoset monkey but in other parts of the epididymis immunopositive epithelial cells were rare [27, 35]. In the mouse immunopositive staining has been detected in most epithelial cells in the caput epididymis but only in rare clear cells within the epithelium in the corpus and caudal regions [20]. In contrast, the majority of the epithelial cells lining all regions of the epididymis and many of the stromal cells are immunopositive for ERβ [20, 27, 35] (figure 2E).

ERα and ERβ proteins have both been detected in extracts of human vas deferens using Western blotting; the amount of ERβ appeared higher than that of ERα [27]. ERβ has been immunolocalised to epithelial cells lining the vas deferens in the mouse [20] (figure 2F), rat [43], marmoset [27] and human [31]. Immunoreaction of two of the variant isoforms of hERβ (ERβ2 and ERβ4) has also been detected using isotype-specific antibodies [31]. In all species expression of ERα was limited to a few basal epithelial cells and to cells within the stroma [20, 27].

Expression of ERs in the seminal vesicles and prostate

ERα and ERβ mRNAs have both been detected in prostate tissues from multiple species [19]. It is notable that ERβ was first cloned from a rat prostate cDNA library [2] and that ERβ mRNA appears to be more abundant than ERα in this organ [18, 44]. ERα and ERβ were both detected in extracts from prostates and seminal vesicles of marmoset monkeys using Western blotting [27]. In the adult prostate expression of ERα protein appears to be largely confined to the stromal compartment [42, 45] although staining of occasional cells within the epithelium can be detected (figure 2G). In contrast ERβ has been immunolocalised to multiple cell types within the prostates of rats [42, 43], primates [35] and human [7, 45] (figure 2H) and is expressed in the majority of the cell nuclei within the epithelium. No significant change in the prostates of ERαKO mice has been reported [19] and the phenotype of the prostates in ERβKO/ERαβKO males is the subject of debate (table 1). Whereas Chambon and colleagues [4] did not find evidence of prostatic hyperplasia in single or compound ER knockouts, Gustafsson and colleagues have described foci of epithelial hyperplasia [5, 7]. Some studies on the prostates of ERβKO have reported that there is an increase in androgen receptor expression compared to normal controls and have concluded that ERβ plays a role in restraining epithelial growth [46]. In addition there is considerable interest in a potential role for ERβ in the development and/or progression of human prostate cancers and it has been suggested that the relative expression of wild-type and the ERβ2/CX splice variant might be prognostic predictors of survival [47].

There have been relatively few investigations on the expression of ERs within the seminal vesicles (SV). In agreement with studies on the prostate expression of ERα in primate SV appears largely confined to the stromal compartment whilst ERβ is expressed in multiple cell types [27, 35]; ERβ immunoreaction has also been detected in SV epithelium using human tissue (unpublished observations). In rats ERα-positive cells detected within the epithelium of the SV following oestrogen treatment have...
been characterised as basal cells [48]. Notably, treatment of hpg mice with oestradiol implants for 6 weeks resulted in multilayering of basal cells in the epithelia of both the prostate and seminal vesicles as well as changes in the stromal compartment consistent with α expression in these cells [49]. An age-dependent increase in the weight of the seminal vesicles in ERαKO mice has been reported but attributed to increased levels of androgen in these animals [19].

**Conclusion**

Oestrogen receptors are widely expressed within the male reproductive system. The pattern of expression of ERα and ERβ is not identical which is consistent with their mRNAs being encoded by different autosomal genes. In contrast to numerous papers describing the reproductive phenotypes of male mice with deletions in genes encoding ERα and/or ERβ [reviewed in (19, 39)] relatively little information exists on the reproductive phenotypes of patients with alterations in ERs. For example, to date no individuals with deletions in the ERβ gene have been identified although it has been reported that the frequency of the Rsal AG polymorphism in the ERβ gene was three times higher in infertile men compared with fertile controls [50]. This polymorphism does not change the protein sequence but may alter splice site selection [50] thereby influencing the proportions of ERβ wild type and ERβ variant proteins within the human testis [24, 31]. A single male patient with a mutation in his ERα gene resulting in a premature stop codon has been described [51]. This man had experienced puberty at the normal time; semen analysis revealed a normal sperm density but with decreased viability. The challenge for the future is the use of the information provided by studying patterns of expression of ERα and ERβ to advance our understanding of changes in cell function and gene expression which occur as a result of ER-mediated signalling events in the male reproductive system.

**References**


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