Minireview

The novel estrogen receptor-β subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens

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Abstract The recent discovery that an additional estrogen receptor (ER) subtype is present in various rat, mouse and human tissues has advanced our understanding of the mechanisms underlying estrogen signalling. The discovery of a second ER subtype (ERβ) suggests the existence of two previously unrecognised pathways of estrogen signalling: via the ERβ subtype in tissues exclusively expressing this subtype and via the formation of heterodimers in tissues expressing both ER subtypes. Various models have been suggested as explanations for the striking cell- and promoter-specific effects of estrogens and anti-estrogens, all on the basis of the assumption that only a single *ER* gene exists. This minireview describes several of these models and focuses on the potential role which the novel ERβ subtype might have in this regard.

Key words: Estrogen; Estrogen receptor; Nuclear receptor; Anti-estrogen; Breast cancer; Tamoxifen

1. Estrogens: mechanism of action

The steroid hormone estrogen influences the growth, differentiation and functioning of many target tissues. These include tissues of the male and female reproductive systems, such as mammary gland, uterus, vagina, ovary, testis, epididymis and prostate [1]. Estrogens also play an important role in bone maintenance, and in the cardiovascular system where estrogens have certain cardioprotective effects [2,3]. Estrogens are mainly produced in the ovaries and testis. They diffuse in and out of cells, but are retained with high affinity and specificity in target cells by an intranuclear binding protein, termed the estrogen receptor (ER). Once bound by estrogens, the ER undergoes a conformational change allowing the receptor to interact with high affinity with chromatin and to modulate transcription of target genes [4–6]. The ER is a member of the nuclear receptor superfamily of genes which consists of a surprisingly large number of genes [6]. It includes receptors for the steroids estrogen, progesterone, cortisol, aldosterone and testosterone. In addition it also includes receptors for thyroid hormone, vitamin D, retinoic acids and ecdysone. Cloning by various means has identified a large number of previously unknown genes having extensive sequence homology to the steroid/thyroid/retinoic acid receptor family [7]. For several of these so-called 'orphan receptors', which are putative receptors interacting with unknown compounds, ligands or activators have recently been identified [8,9]. Biochemical and mutational analysis of estrogen receptors and other nuclear receptors indicated that they can be subdivided into several functional domains (Fig. 1) ([1,6] and references therein). The N-terminal A/B domain is highly variable in sequence and length, and usually contains a transactivation function, which activates target genes by interacting with components of the core transcriptional machinery ([10] and references therein). The C domain contains two type II zinc fingers, which are involved in specific DNA-binding and receptor dimerization. The ligand-binding domain is relatively large and is functionally complex. It does not only harbour regions important for ligand binding but also regions involved in receptor dimerization, nuclear localization and interactions with transcriptional co-activators and co-repressors [6,10].

The ER-encoding cDNAs were cloned during 1986 from several species [11–15]. Since that time there has been the general acceptance that only one ER gene existed, as is also accepted for the other steroid hormone receptors. This contrasts with other members of the nuclear receptor superfamily where multiple receptor subtypes have been identified, for instance for thyroid hormones and retinoic acids [6]. The proposed existence of only a single ER gene was also surprising in view of the striking differences in biocharacter that some synthetic estrogens, anti-estrogens and their analogs show in terms of certain responses in different target cells and tissues [16].

2. Cloning and characterisation of ERβ

At the end of 1995 a novel estrogen receptor (ERβ) was cloned from a rat prostate cDNA library [17]. The ERβ subtype protein is highly homologous to the sofar known estrogen receptor protein (consequently ERα), particularly in the DNA-binding domain and in the ligand-binding domain (Fig. 1). Saturation ligand-binding experiments reveal high affinity and specific binding of estradiol and the ERβ protein is able to stimulate the transcription of an estrogen receptor target gene in an estradiol-dependent manner [17,18]. Some synthetic or naturally occuring ligands have different relative affinities for ERα vs ERβ, although many ligands (including various anti-estrogens) bind with very similar affinities to both ER subtypes [18]. The rat tissue distribution and/or the relative level of ERα and ERβ-mRNA expression is quite different; that is moderate to high expression in uterus, testis, pituitary, ovary, kidney, epididymis, adrenal for ERα and prostate, ovary, lung, bladder, brain, testis for ERβ [18]. Examination of ERβ mRNA expression at the cellular level, by in situ hybridisation, shows that in the rat prostate ERβ is highly expressed in the epithelial cells of the secretory alveoli, where-

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as in the ovary the granulosa cells of primary, secondary and preovulatory follicles show expression of ERβ [17,19]. In contrast, examination of ERα expression in the ovary reveals expression at a low level throughout the ovary with no particular cellular localization [19]. Additional experiments with immature rats undergoing exogenous hormonal challenges demonstrate that the preovulatory luteinizing hormone surge down-regulates ERβ-mRNA in the ovary [19]. These results clearly implicate the physiological importance of ERβ in female reproductive functions. In rat brain ERβ appears to represent a significant fraction of ER RNA [18], and examination of ERβ mRNA expression by in situ hybridisation shows expression within various regions of the hypothalamus [20]. The mouse [21] and human [22] homologs of rat ERβ have been cloned in the meantime, and high expression in ovary and testis among other tissues was reported.

None of the reports published sofar have compared ERα and ERβ expression in different tissues at the protein level. The development of specific ERβ antibodies and specific ERα antibodies is of great importance for the analysis of the protein expression pattern, needed also to confirm that the RNA levels of both subtypes parallel their protein expression level.

3. ERβ in the ERα knock-out mouse

Knock-out mice in which the ERβ gene has been disruptedwould be very informative in elucidating the unique endocrine and physiological functions of ERβ. These mice are not yet available, but in the meantime a lot can be learned from the ERα knock-out mice. Homozygous mutant mice with the ERα gene disrupted [23] were made at the time when it was thought that only a single ER gene exists. These mice appear healthy, and with the exception of fertility problems in male as well as female animals, there are no obvious problems in pre-natal sexual development [23,24]. This was quite surprising because of the known importance of estrogens in breast and uterine development and in preventing bone loss after menopause and after ovariectomy in mice [1,3,5]. Furthermore, the presence of ER in pre-implantation mouse embryos, and the absence of reported human ER mutations have been interpreted as indications for an essential role of estrogens during embryonic development [25]. This view was challenged not only by the survival of the ERα knock-out mice but also by the rather recent discovery of a male with (partial) estrogen

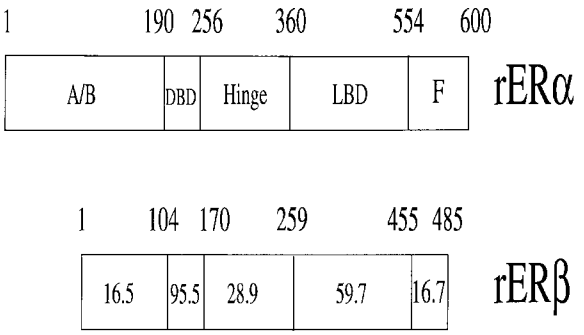

Fig. 1. Comparison between rat ERα and ERβ protein. Percentage amino-acid identity in the domains A/B (N-terminus), DBD/C (DNA-binding), hinge and LBD/F (ligand binding, dimerization and ligand-dependent transactivation) are depicted. Similar homologies are present in the respective domains of the human and mouse ER subtypes.

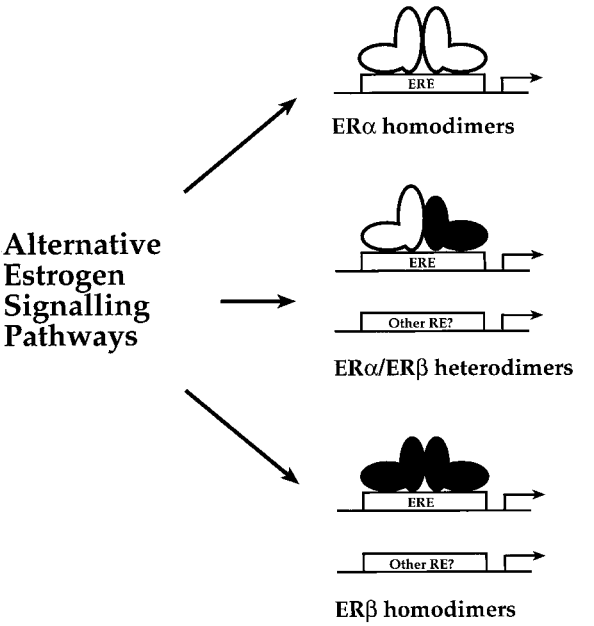

Fig. 2. Alternative estrogen signalling pathways. The existence of two ER subtypes and their ability to form DNA-binding heterodimers, suggests the existence of three potential pathways of estrogen signalling. In cells expressing only the ERα or ERβ subtype, homodimers of either subtype can interact with response elements in target genes promoters and influence transcription levels. In cells expressing both subtypes, heterodimers can be formed depending on the ratio of the subtypes. It cannot be excluded at this stage that unique response elements exist within the context of target gene promoters which interact preferentially with ERβ homodimers or ERα/ERβ heterodimers. An open question in this regard is whether estrogen target genes exist which are exclusively regulated by either of the homodimers or the heterodimer.

resistance caused by a mutation in the ERα gene [26]. With the discovery of the ERβ subtype, the unexpected viability of the ERα knock-out mouse and human with non-functional ERα protein, could also be explained by complementation through ERβ protein during embryonal development. On the other hand the existence of a karyotypically female patient with pseudohermaphroditism caused by a null mutation in the aromatase cytochrome P-450 gene [27], the enzyme which is essential for estradiol biosynthesis, raises further questions about the exact role of estrogens and ER proteins during embryonal development.

To what extent the lack of a phenotype in the breast, bone and cardiovascular tissue of the ERα knock-out mouse is due to up-regulation and compensation by ERβ protein, or is a reflection of the normal physiological role of ERβ is unknown at the moment. Although no data on the expression level of ERβ-mRNA and protein in the ERα knock-out mouse has been published, it is clear that the presence of ERβ protein should be kept in mind when interpreting experiments using the ERα knock-out mice.

4. Tissue-specific effects of ER agonists and antagonists and potential role of ERβ

It has been known for long that human breast cancers are hormone-dependent (i.e. estrogen- and progesterone-dependent) and that they undergo regression when deprived of these supporting hormones by ovariectomy or hypophysectomy [5].

With the development of anti-estrogens as tamoxifen, and derivatives thereof, an alternative to endocrine ablation became available for the palliative treatment of hormone-dependent breast cancer ([5] and references therein). Anti-estrogens bind with high affinity to the ER, but thereafter their effects differ from those of the physiological ligand estrogen. Most anti-estrogens exhibit curious pharmacological behaviour; depending on the species, the tissue and the dose administered they can act as either agonists and antagonists [5,16]. For example, tamoxifen therapy in post-menopausal women with breast cancer has shown estrogen-like actions on bone mineral density and lipoprotein levels as well as estrogen-like effects in the uterus, while in contrast tamoxifen inhibits the development and recurrence of breast tumors ([16] and references therein). Various explanations, until recently based on the assumption that only a single ER gene exists, have been suggested for this puzzling pharmacological behaviour of certain anti-estrogens:

4.1. Co-activators and co-repressors

Transfection of estrogen-responsive promoter-reporter constructs into different cells enables one to study the regulation of specific genes in different cellular backgrounds. Studies using different cell types in such assays have provided clear evidence that, as expected from the clinical data, cell background influences the ERα-mediated transcriptional response to different estrogens and anti-estrogens [28]. In addition to contacting the basal transcriptional machinery directly, steroid receptors inhibit or enhance transcription by recruiting an array of co-activator and co-repressor proteins to the transcription initiation complex. These co-regulatory proteins, which are present in limiting amounts in the cell, are believed to be interposed between the steroid receptor and the basal transcriptional machinery. In the last two years not less than 10 different co-activators and co-repressors that can interact with the ER have been described ([10] and references therein). Although, no study has yet addressed the exact physiological relevance of each individual interacting protein, it is possible that they constitute a factor in the cell type- and promoter-specific action of ER agonists and antagonists. The ratio and concentration of co-activators and co-repressors could be different in various cell types. Depending on the ligand (agonist or antagonist) bound to the ER and the structure of the target gene promoter different sets of co-activators and co-repressors could be associated with the ER [10]. The observation that tamoxifen acts as an antagonist in the breast and as an agonist in the uterus could be explained by assuming that the balance of ER bound interacting proteins shifts from excess co-repressor to excess co-activator in the different cells.

4.2. Antagonist-specific binding sites

An extra layer of complexity is added by the observation that in addition to competing for the estrogen-binding site, the anti-estrogen 4-hydroxytamoxifen reacts with a second binding site in the ER, which is not recognised by estrogen [5,29]. Differences in the relative affinities for the two putative binding sites have been suggested to provide a clue as to how 4-hydroxy-tamoxifen can function as an agonist or antagonist in a concentration-dependent manner in breast cancer cells [30].

4.3. ER activation of target gene expression via AP-1 sites

In the 'classical' estrogen response ER activates transcription after binding to estrogen response elements in the promoter region of estrogen-responsive genes. An alternative pathway has been reported in which the ER appears to be able to stimulate transcription from promoters that contain an AP-1 site, the cognate binding site for the transcription factors Jun and Fos, rather than an estrogen response element [31]. The detailed mechanism by which the ER stimulates transcription in this AP-1 site-dependent pathway is unknown. It is believed to involve protein-protein rather than protein-DNA interactions, since it is partly independent of the ER DNA-binding domain [32]. Interestingly, it was found that the anti-estrogen tamoxifen is a potent activator of ER-mediated induction of transiently transfected promoter reporter constructs regulated by AP-1 sites in cell lines of uterine origin, but not in cell lines of breast origin [32]. It thus parallels tamoxifen agonism in vivo. However, to what extent the stimulation of uterine endometrial growth by tamoxifen is mediated by AP-1 site-containing ER target gene promoters remains to be investigated.

4.4. Multiple ER subtypes

The recent discovery that an additional estrogen receptor subtype (ERβ) is present in various rat [18] mouse[21] and human [22] tissues has advanced significantly our understanding of the mechanisms underlying estrogen signalling. It suggests the existence of two previously unrecognised pathways of estrogen signalling; via ERβ in cells exclusively expressing this subtype and via the formation of heterodimers in cells expressing both ER subtypes (Fig. 2). The ERβ protein interacts in vitro as a homodimer with similar estrogen response element (ERE) oligonucleotides as the ERα protein, and in subsequent experiments it was shown that ERα and ERβ proteins form heterodimeric complexes with ERE oligonucleotides (Pettersson et al., submitted). At the moment the possibility cannot be excluded that ERβ homodimers interact with novel response elements, apart from the known EREs. The existence of two ER subtypes greatly expands the physiological regulatory potential of estrogenic hormones. Different target cells may respond differently to the same hormonal stimulus due to alternative composition of receptors. Varying ratios of ERα and ERβ proteins in different cells, resulting in different populations of homo- and heterodimers could constitute a hitherto unrecognised mechanism involved in the tissue- and cell type-specific actions of not only estrogens but also of anti-estrogens. The anti-estrogen 4-hydroxytamoxifen inhibits estradiol-stimulated transcriptional activity of ERα as well as of ERβ. However, while 4-hydroxytamoxifen alone displays partial agonistic activity with mouse ERα on a basal promoter linked to ERE-elements in COS-1 cells, this effect is not observed with mouse ERβ [21], showing that indeed the molecular mechanisms regulating the transcriptional activity of ERα and ERβ can differ under appropiate conditions. No data has yet been presented on the actions of estrogens and anti-estrogens in cells expressing both ER subtypes.

5. Concluding remarks

The described mechanisms, proposed to explain the tissue- and cell-specific actions of estrogens and anti-estrogens, are by no means mutually excluding. The challenge for the future will be to unravel for each particular situation the relative importance of these mechanisms and to exploit this knowl-

edge for the development of ER antagonists with improved therapeutic profiles.

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