Review

Progesterone receptors – animal models and cell signaling in breast cancer

Expression and transcriptional activity of progesterone receptor A and progesterone receptor B in mammalian cells

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Abstract

Progesterone is an essential regulator of normal female reproductive function. Its effects are mediated by two nuclear progesterone receptor (PR) proteins, PRA and PRB, which are identical except for an additional 164 amino acids at the N-terminal end of PRB. Transcriptional analyses of the two receptor forms have assigned strikingly distinct functional signatures to the two PRs, despite their apparent physical similarity. The basis of these differences is yet to be fully understood. Furthermore, these differences are strongly influenced by the cell type and the promoter used. We review the mammalian transcriptional studies of PRA and PRB, and compare them with what is known about their expression and function in target tissues.

Keywords: progesterone receptor, progestin signaling, transcriptional activation

Introduction

Progesterone plays a pivotal role in normal female reproduction, in the uterus, the ovary, the mammary gland and the brain [1]. The number of cellular pathways regulated by progesterone reflects the complexity of its physiological role. In normal breast development, progesterone directs the formation of lobular-alveolar structures and also affects differentiation in the breast by modulation of milk protein synthesis [1]. In the human endometrium, progesterone directs glandular differentiation and glycogenesis, as well as stromal proliferation and development of predecidual cells [1]. These effects are mediated through the nuclear progesterone receptor (PR), expressed as two protein forms (PRA and PRB). There is increasing evidence to date that PRA and PRB are functionally different, and that it is the balance between these two forms that may make it possible for progesterone to affect such diverse physiological targets. Much of this evidence is conflicting and model specific, however, and the true differences between the receptor forms in normal tissues are yet to be fully understood.

The purpose of the present article is to review the current state of knowledge about the transcriptional differences between PRA and PRB from mammalian *in vitro* models, and to correlate this with the effects of altered PRA and PRB levels on target tissues.

Transcriptional regulation by the PR

The PR is a member of a large family of ligand-activated nuclear transcription regulators, which are characterised by organisation into specific functional domains and are conserved, to differing degrees, between species and family members. The PR is made up of a central DNA binding domain and a carboxyl-terminal ligand-binding domain. In addition, the receptor contains multiple activation function (AF) and inhibitory function elements, which enhance and

AF = activation function; PR = progesterone receptor; PRE = progestin response element.

repress transcriptional activation of the PR by association of these regions with transcriptional coregulators [2].

In most species examined, the PR exists as two distinct forms (PRA and PRB). The expression of human PR is controlled by two promoters, which direct the synthesis of two distinct subgroups of mRNA transcripts [3] encoding the two receptor proteins. The two PR forms are identical except that PRA lacks 164 amino acids contained at the N-terminal end of PRB. The region of the protein that is unique to PRB contains a transcription activation function, AF3 [4], in addition to AF1 and AF2, which are common to PRA.

Newly transcribed cytoplasmic PR is assembled in an inactive multiprotein chaperone complex that dissociates on ligand binding and receptor activation. Progestin binding to the PR causes a conformational change and dimerisation, resulting in association of the progestin-complexed PR dimer with specific coactivators and general transcription factors. The activated complex binds to progestin response elements (PREs) in the promoters of target genes, resulting in modulation of transcription of those genes (reviewed in [2,5]).

There is now considerable evidence for differences in the transcriptional activities of PRA and PRB from transient cotransfection into a variety of cell lines of PRA and/or PRB and reporter constructs containing progestin-responsive sequences. These constructs range from the simple PRE-tk-CAT (containing one copy of a palindromic PRE) to more complex constructs such as those incorporating the mouse mammary tumor virus long terminal repeat, which contains multiple hormone-responsive elements.

PRB exhibits hormone-dependent transactivation in all cell types examined irrespective of the complexity of the response elements, whereas the transcriptional activity of PRA is cell specific and reporter specific. With reporter constructs containing a single palindromic PRE, PRA displays similar transactivation activity to PRB [6]. However, this activity is reduced or inactive when more complex response elements such as the mouse mammary tumor virus long terminal repeat and PRE_2TATA_{tk} constructs are used [4,6,7]. Interestingly, PRA acts as a transdominant inhibitor of PRB in situations where PRA has little or no transactivational activity [6,7]. Moreover, PRA can regulate the transcriptional activity of other nuclear receptors such as glucocorticoid, mineralocorticoid, androgen and estrogen [6–9], suggesting that PRA may play a central role in regulation of activity of a number of nuclear receptors in addition to PRB. The ability of PRA to act as a transdominant repressor is highly model specific, however, and there is considerable variability between reports.

McDonnell and Goldman [9] reported that PRA but not PRB, in the presence of either progesterone or antiprogestins, lessened the ability of estrogen to induce an estrogen-responsive reporter when the two constructs were transfected into CV-1 or HS578T cells, but not into HepG2 cells. PRA had similar anti-estrogenic effects on endogenous estrogen receptor activation of a minimal estrogen-responsive reporter in MCF-7 breast cancer cells in the presence of RU 38486 [8]. When the estrogen-responsive region of the pS2 gene was used as a reporter in MCF-7 cells, however, PRB and not PRA repressed activation of the reporter by estrogen [10].

The mechanisms by which PRA and PRB exert such apparently different transcriptional activities in various cell and promoter systems remain largely unknown, although a number of possible scenarios have been proposed. The physical differences at the N-terminal end of the two receptors are clearly responsible for some transcriptional differences. In addition to the fact that AF3 is unique to PRB, the PRB-specific region has a distinct conformation in solution [11] and is likely to mask an inhibitory domain that is active in the N-terminus of the PRA protein [12]. This could act to enhance the transcriptional activity of PRB, as well as preventing it from acting as an inhibitor of other receptors.

The unique AF in PRB may confer a difference in affinities of the two PRs for coregulators. When compared in a phage display assay, the two PR forms bind to distinct subgroups of peptides. This suggests that coactivators may bind differently to the two PRs or that the two receptors bind to different subgroups of coactivators [13]. Motifs contained in AF3, with the same sequence as the NR boxes of coactivators, have been shown to be necessary for the transcriptional activity of the PRB-unique AF [14] and may form contacts between the receptor and a unique set of cofactors, or within the PR dimer itself. Given that the PR acts in combination with multiple other transcription factors to affect transcription, it is possible that variability of the tissue-specific expression of the components of this multiprotein complex may result in different PRA and PRB activities in the same cell. Furthermore, differential cofactor requirements between gene promoters may lead to differences in the transcriptional efficacy of the two PRs on the same promoter [2]. Further evidence is required to support these assertions since, although most cofactors are expressed at limiting levels, they have been demonstrated to be widely expressed throughout cell types. Furthermore, a degree of functional redundancy in PR coactivators seems to exist, with gene knockout studies of SRC-1 delivering only a mild phenotype [15] and the demonstration that TIF2 and SRC-1 are able to activate the PR to a similar extent in transfections [16].

The PR regulates the expression of a diverse population of transcriptional targets [1], and it would be expected that changes in the relative amounts of PRA and PRB would result in altered target gene expression patterns if the two

isoforms are transcriptionally distinct. When patterns of gene regulation were examined in T47D breast cancer cells expressing exclusively PRA or PRB, a remarkably small overlap was seen between the sets of genes regulated by the two receptors, with the subset of genes regulated by PRB far exceeding in number those regulated by PRA [17]. When the relative expression of PRA and PRB was varied in wild-type T47D cells that already express both isoforms, however, the impact on transcription was not dramatic unless PRA was in vast excess over PRB [18]. Furthermore, no evidence was seen of dominant transcriptional inhibition by PRA. These data suggest that coexpression of both isoforms at similar levels, which is common in normal progesterone target cells (see later), is associated with appropriate transcriptional response to progestins and that changes in relative PRA and PRB levels must be quite dramatic before physiological changes in progestin signaling are observed.

Expression and function of PRA and PRB in normal and malignant physiology

In general, PRA and PRB are coexpressed in the same target cells in the human [19], and their relative expression, where it has been examined, is generally close to unity [19,20]. In some normal physiological circumstances, and in some cell types, there is a predominance of one isoform. PRA is always the predominant isoform in the uterine stroma, and PRB is predominantly expressed in the epithelial glands in the mid-secretory phase of the menstrual cycle [19]. PRA is the predominant isoform in the rodent [1] and is widely expressed in the macaque reproductive system.

Predominance of one isoform also occurs in cancers. In breast cancers, the equivalent expression of PRA and PRB seen in normal cells is disrupted early in carcinogenesis, and predominance of one isoform is common [21,22]. Most endometrial cancers express only one PR isoform, and isoform predominance is associated with higher histological grade [23]. The association of PR isoform predominance with cancer is supported by the demonstration that transgenic mice overexpressing PRA exhibited features in their mammary glands that were abnormal and commonly associated with neoplasia [24]. The expression of PRA and PRB *in vivo* supports a role for both isoforms in normal physiology, particularly in the human breast. The fact that the equivalent levels of the two proteins seen in the normal breast become disrupted early in breast carcinogenesis, and that predominance of one PR isoform, usually PRA, is seen in cancers, suggests that disrupted progesterone signaling may play a role in development or progression of breast cancer.

Other mechanisms of PRA and PRB action

In addition to the ligand-activated transcriptional effects already discussed, which reflect the nuclear activity of this receptor, the PR also regulates transcription via alternative pathways. Ligand-independent activation of the PR can occur and provides evidence for regulation of the PR via membrane-generated signals [25]. The PR also interacts with cytoplasmic signaling pathways to activate c-Src family members [26], and PR interactions with the mitogen-activated protein kinase pathway are also described. Migliaccio *et al*. found that PR activation of signaling pathways was independent of the transcriptional activity of the receptor and was indirectly mediated by PRB, but not PRA, through interaction with the estrogen receptor [26]. Other studies have examined the ability of the PR to transiently activate mitogen-activated protein kinase pathways but have generally focused on PRB, which was more transcriptionally active than PRA in most settings [27] (CA Lange, personal communication).

Conclusion

The *in vitro* data on the relative activities of PRA and PRB tend to support the view that PRB is the active PR, whereas PRA is either inactive or acts as an inhibitor of PRB activity. However, this perspective is at odds with the demonstrated coexpression of both isoforms in normal physiology [1,19,20] and with the distinct roles ascribed to each protein from knockout studies discussed in the other sections of this review series. An explanation for the discrepancy between the *in vivo* and the *in vitro* data resides in the experimental protocols used to examine the question of relative PRA and PRB transcriptional activity. Most of the information has been obtained from transient transfection studies largely in cell lines that are not progestin targets, using exogenously transfected reporter sequences and each isoform in isolation. The relative levels of isoform protein coexpressed under these conditions cannot be known, and is probably highly relevant to interpretation of the data. There is little evidence *in vivo* that PRA is a dominant inhibitor of PRB. Moreover, when the data in human tissues and null animals are taken together, they suggest that the two isoforms either work cooperatively to mediate progesterone action or suggest that each isoform has distinct physiological roles that are probably cell specific and promoter specific. A combination of cooperative action and distinct activity is probably the best explanation for the complex and divergent pathways of progesterone action in normal and malignant physiology.

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