REVIEW

Biological and clinical impact of imbalanced progesterone receptor isoform ratios in breast cancer

Caroline A Lamb[1], Victoria T Fabris[1], Britta M Jacobsen[2], Alfredo Molinolo[3] and Claudia Lanari[1]

[1]Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina
[2]University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
[3]Moore's Cancer Center, UCSD, La Jolla, California, USA

Correspondence should be addressed to C Lanari: lanari.claudia@gmail.com

Abstract

There is a consensus that progestins and thus their cognate receptor molecules, the progesterone receptors (PRs), are essential in the development of the adult mammary gland and regulators of proliferation and lactation. However, a role for natural progestins in breast carcinogenesis remains poorly understood. A hint to that possible role came from studies in which the synthetic progestin medroxyprogesterone acetate was associated with an increased breast cancer risk in women under hormone replacement therapy. However, progestins have also been used for breast cancer treatment and to inhibit the growth of several experimental breast cancer models. More recently, PRs have been shown to be regulators of estrogen receptor signaling. With all this information, the question is how can we target PR, and if so, which patients may benefit from such an approach? PRs are not single unique molecules. Two main PR isoforms have been characterized, PRA and PRB, which exert different functions and the relative abundance of one isoform with respect to the other determines the response of PR agonists and antagonists. Immunohistochemistry with standard antibodies against PR do not discriminate between isoforms. In this review, we summarize the current knowledge on the expression of both PR isoforms in mammary glands, in experimental models of breast cancer and in breast cancer patients, to better understand how the PRA/PRB ratio can be exploited therapeutically to design personalized therapeutic strategies.

Introduction

Nuclear estrogen receptors alpha (ERα) represent almost the first molecular targets used for breast cancer treatment, and two-thirds of these patients are treated with an endocrine therapy. Nuclear progesterone receptors (PRs) on the other hand, have been evaluated as surrogate markers of ERα integrity and functionality. There is nowadays emerging interest in understanding the interplay between the different members of the nuclear hormone receptor family and their role in tumor growth.

PRs are members of the nuclear steroid family of receptors, together with ER, androgen (AR), glucocorticoid (GR)/mineralocorticoid (MR) receptors and other less related molecules such as thyroid receptors, retinoic acid and orphan receptors (reviewed in Grimm *et al.* 2016).

They are ligand-activated transcription factors that can also act through ligand-independent mechanisms. Receptors other than the classical nuclear steroid receptor family are also involved in generating progesterone (P4)-mediated effects (reviewed in Mueck *et al.* 2014).

PR isoforms

The human PR gene consists of eight coding exons separated by seven non-coding introns. There are mainly two mRNA transcripts controlled by two different promoters, each one encoding a different protein. The mRNA regulated by the distal promoter encodes the full-length PR named PRB (116 kDa; 933 amino acids (aa)); the one regulated by the proximal promoter encodes the truncated version, named PRA (94 kDa), which lacks the first 164 aa starting its translation in the methionine 165 (reviewed in Abdel-Hafiz & Horwitz 2014). These two isoforms were originally described in the chick oviduct. Other mRNA PR variants with different deleted exons were detected in breast cancer tissues and cell lines (Cork *et al.* 2008). However, how these mRNAs influence PR signaling and if they are translated into functional proteins remains to be established. A third predicted truncated PR isoform, named PRC (60 kDa), results from an alternative translation site starting at methionine 595. This form should retain the ability to bind the ligand, but not DNA. It was first described in T47D cells (Wei *et al.* 1990), and it has been suggested that it may play a role in labor (Condon *et al.* 2006) where high PRC levels are associated with an increase in the PRA to PRB ratio (Renthal *et al.* 2015). A 78 kDa protein was also detected in breast cancer samples using PR antibodies (Graham *et al.* 1995). Since it is not reactive with an antibody that recognizes only PRB, it has been originally proposed as a PRA variant (Yeates *et al.* 1998).

In the next part of this review, we will focus on the two main PR isoforms, PRB and PRA, and the impact that the different PR isoform ratios might have on breast cancer progression.

PRA and PRB isoforms: localization, genomic and non-genomic effects

PRB isoforms have an amino terminal region named A/B, with a high degree of variability among the different nuclear receptors. Two transactivation domains: AF1 (aa 456–546) and AF3 (aa 1–164) located in this region participate in coactivator recruitment (Dobson *et al.* 1989). In addition, an inhibitory domain is involved in corepressor recruitment (Hovland *et al.* 1998). The DNA-binding domain consists of 78–80 aa and is composed of two type II zinc finger structures involved in the binding to specific cis-acting DNA sequences, the PR elements (PRE). The ligand-binding domain is located at the carboxy terminal end of the PR. It contains a third transactivation domain (AF-2) required for hormone-dependent coactivator recruitment, dimerization and interaction with chaperone proteins in the inactive state (reviewed in Hilton *et al.* 2015).

PRA differs from PRB in the lack of the AF3 transactivation site, located in the B-Upstream Segment (BUS) of PRB (Sartorius *et al.* 1994*b*). Similar affinities of PRA and PRB for the natural ligands and medroxyprogesterone acetate (MPA) have been reported (Ghatge *et al.* 2005); however, this is not valid for the progestins levonorgestrel or gestodene (Schoonen *et al.* 1998). After ligand activation, PR isoforms dimerize forming homo- or heterodimers. There is still no consensus on whether PR dimerization is necessary for binding to palindromic DNA sequences. A different model proposes that the activation of single monomers, which bind to PRE half sites (Jacobsen *et al.* 2009), might be thermodynamically favored (Heneghan *et al.* 2005). Many of the endogenous PR-binding sites detected by chromatin immunoprecipitation sequencing contain these PRE half sites (Ballare *et al.* 2013). More recently, it has been shown that in cells transfected with PRB, ligand activation promotes tetramerization of PR in a DNA-independent manner (Presman *et al.* 2016).

Another level of complexity is the fact that P4 may also elicit rapid non-genomic effects activating cytoplasmic signaling pathways (reviewed in Boonyaratanakornkit *et al.* 2018). These effects are mediated by PRB, as this isoform shuttles between nuclear and cytoplasmic compartments whereas, PRA is mainly localized in the nucleus (Guiochon-Mantel *et al.* 1994, Boonyaratanakornkit *et al.* 2008). In pathological contexts, the abnormal presence of cytosolic PRA may interfere with normal signaling.

PRA may also act as a ligand-dependent trans-dominant repressor of other steroid receptors including PRB, ERα, AR, MR and GR (reviewed in Chabbert-Buffet *et al.* 2005), but this effect may not be so relevant under physiological conditions (Scarpin *et al.* 2009). In most cases, PRB functions as an activator of progesterone-responsive genes in conditions in which PRAs are transcriptionally inactive (reviewed in Jacobsen & Horwitz 2012). Notwithstanding, genes regulated exclusively by PRA have been reported (Richer *et al.* 2002, Jacobsen *et al.* 2005). PRB competes with ERα for the same coactivators (McDonnell & Goldman 1994, Wen *et al.* 1994).

This coactivator squelching represents another mechanism through which progestins inhibit estrogen action. More recently, a rewiring of ERα and PR after progestin treatment was proposed to explain the inhibitory effects of progestins on breast cancer cell proliferation (Mohammed *et al.* 2015).

After ligand binding, PR aggregates in nuclear foci where the transcriptional activity takes place (Arnett-Mansfield *et al.* 2007). The activated PR exerts their genomic effects by binding either to PRE directly at the promoter of target genes or can be tethered to other transcription factors, thus regulating the expression of genes that lack PRE sites. PR isoform-mediated transcription has been reviewed in depth elsewhere (Jacobsen & Horwitz 2012). Post-translational modifications can modulate PR transcriptional activity. These include phosphorylations, acetylations, ubiquitinations, glycosylations and SUMOylations in serine and lysine residues located in the amino terminal end. The functional effects of these modifications are related to several factors such as modulation of the transcriptional activation or repression, DNA binding, cofactor recruitment, receptor turnover and changes in hormone responsiveness (reviewed in Qiu & Lange 2003, Abdel-Hafiz & Horwitz 2014, Diep *et al.* 2015). Several studies have suggested that, upon ligand binding, PRA is rapidly SUMOylated, while PRB becomes deSUMOylated. SUMOylation is a stabilizing modification and thus PRA results in a more stable isoform relative to PRB (Daniel *et al.* 2007). This biochemical difference in isoform SUMOylation, together with the increased turnover of PRB due to mitogen-activated protein kinase-dependent phosphorylation of serine 294 (Shen *et al.* 2001, Faivre & Lange 2007) may explain the PRA predominance observed in many human data sets reviewed herein.

Methods to determine PR isoform expression

PR isoform expression has been determined using PCR, western blots (WB) or immunohistochemistry (IHC)/immunofluorescence (IF) techniques. A critical issue in PR isoform mRNA detection is the correct primer design. Errors were detected in several studies by Aupperlee *et al.* (2005*a*). The levels of PRA are calculated by subtracting PRB from total PR adding a further degree of complexity.

Currently, WB is the most reliable method to evaluate PR isoforms since the ratio can be calculated in the same assay using one antibody. Notwithstanding, there are several limitations: (a) PRB splice variants with similar molecular weight (MW) to PRA might interfere with the

PRA band; (b) antibodies should have equal affinities for both denatured PR proteins and (c) WB is not in the routine practice in the clinic.

The detection of PR isoforms by IHC or IF is still controversial. A list of antibodies that did not recognize PRB by IHC in gelatin-embedded cells uniquely expressing PRB has been reported (Mote *et al.* 2001). A PRA-specific antibody is offered by Leica, a Novocastra antibody known as clone 16 or 312. The FDA approved this antibody to detect total PR in human tissue; however, the antibody is commercialized as a specific PRA antibody. Following these assumptions, many investigators have published results describing and quantifying PR isoform expression in different tissues using IHC (Kreizman-Shefer *et al.* 2014, Bartosch *et al.* 2015, Bonneterre *et al.* 2016). In xenografts, using cell lines modified to express either PRA or PRB, this antibody proved to be excellent to recognize both PR isoforms by IHC (Fabris *et al.* 2017).

PR isoforms in the mammary gland

PRA and PRB expression ratio may change in the different reproductive tissues. Even in the same organ, the PR ratio may differ according to the developmental stage or hormonal status (reviewed in Patel *et al.* 2015, Hilton *et al.* 2018).

Both PR isoforms are expressed in the mammary gland of most of the species studied. Equimolar ratios have been reported in human breast (Mote *et al.* 2002), higher levels of PRA than PRB (3:1) in mouse mammary glands (Schneider *et al.* 1991), and a single PRB isoform in the rabbit. Table 1 depicts in detail the PRA/PRB changes during the different stages of mammary gland development in different species.

PR isoforms in genetically modified mice

PRB is required for mammary morphogenesis during pregnancy (reviewed in Conneely *et al.* 2003). PR-knockout (KO) mice show decreased branching and lobuloalveolar differentiation. PRA-KO mice are infertile, but the mammary gland develops normally in response to P4, indicating that PRB is necessary and sufficient to induce mammary gland proliferation and differentiation during pregnancy. PRB-KO mice are fertile but the mammary gland is unable to differentiate (reviewed in Conneely *et al.* 2003). Conversely, transgenic mice overexpressing PRA show ductal hyperplasia (Shyamala *et al.* 2000) that was reversed by antiestrogen but not with antiprogestin treatment (Sampayo *et al.* 2013) and, mice overexpressing

Table 1 Expression of PR isoforms in different stages of development of normal mammary gland.

Species	Receptor	Puberty	Virgin adulthood	Pregnancy	Lactation	Antibody	Assay	References
Human	PR total	NAI	Luminal cells: 10–20% (constant throughout the menstrual cycle); PRA>PRB in BRCA1/2+	NAI	NAI	AB52 (from Dr K Horwitz) PR-86 hPRa3, NM*	IHC	Mote et al. (2002), Kariagina et al. (2008), Taylor et al. (2009)
	PRA	NAI	Luminal epithelial cells: 32.2%, co-expressed with PRB (1:1)	Luminal epithelial cells 6.2%; co-expressed with PRB	NAI	hPRa1, hPRa5 and hPRa7, NM Clone 16, cat NCL-PGR-312, NC* sc-538 (C-19) and sc-539 (C-20), SC*	IHC	
	PRB	NAI	Luminal epithelial cells/isolated myoepithelial cells: 23.5%. Co-expressed with PRA (1:1)	Epithelial cells 14.2% – also in isolated myoepithelial cells	NAI	hPRa6, NM NCL-PGR-B, NC	IHC	
Mouse	PR total PRA	3 weeks old: 55% epithelial cells 6 weeks old: 58% of epithelial cells internal layer of end buds and ducts	Ductal cells: 50% in 12 weeks old/28% in 17–20 weeks old (constant throughout the estrous cycle) PRA:PRB 3:1	11% ductal; 6% alveolar cells. BALB/c>C57BL/6	ND	hPRa7, NM C-19, SC C-20, SC	IHC, IF	Aupperlee et al. (2005b), Montero et al. (2007), Kariagina et al. (2008)
	PRB	ND	ND	Alveolar cells (mostly): 48%	ND	hPRa6, NM	IHC, IF, WB	
Rat	PR total PRA	30–40% epithelial cells in end buds, ducts and lobules (constant throughout the estrous cycle)	Ducts and lobules: 25% epithelial cells (constant throughout the estrous cycle). Co-expressed with PRB	Lobules: 5–10%; ducts: 20%	ND	A0098 (Dako) hPRa7 (NM)	IF	Kariagina et al. (2007)
	PRB	50–60% cells of cap cell layer of end buds, ductal and lobular cells. Expressed in epithelial (nuclei) and myoepithelial cells (nuclei and cytoplasm). Constant throughout the estrous cycle). Co-expressed with PRA (60%)	Epithelial and myoepithelial cells of ducts and lobules: 40–60%. Constant throughout the estrous cycle. Co-expressed with PRA (50%)	Ducts and lobules: 50–60%	Isolated myoepithelial cells (nuclear and cytoplasmic staining)	B15 raised against a synthetic Peptide N-t, AB*	IF	

Species	Isoform		Description		Antibody	Method	Reference
Monkey	PR total	NAI	Lobular cells: 4–17%; increases throughout the menstrual cycle; maximum in luteal phase. Ductal epithelial cells: 12–15%; constant throughout the menstrual cycle	ND	NCL-PGR-312, NC	IHC	Cheng *et al.* (2005)
	PRA	NAI	Ductal and lobular epithelial cells: 12–15%; constant throughout the menstrual cycle	ND	NCL-PGR-312, NC	IHC	
	PRB	NAI	Ductal epithelial cells: 12–15%; constant throughout the menstrual cycle; co-localization with proliferation marker (21% of cells)	ND	Clone SAN27, NC	IHC	
Dog	PR total	NAI	Intermediate and basal epithelial ductal cells. Co-expressed with proliferation markers	NAI	hPRa4, 5, 7, NM C-20, SC PR10A9, IM	IHC, WB	Lantinga-van Leeuwen *et al.* (2000), Gracanin *et al.* (2012)
	PRA	NAI	High expression in metaestrus, anoestrus, with progestin treatment	NAI		WB	
	PRB	NAI	Low expression; only in anoestrus; with progestin treatment	NAI		WB	

NAI, No Available Information; ND, not detectable; *AB, Affinity Bioreagents, Golden, CO; NC, Novocastra; IT, Immunotech; NM, Neomarkers; SC, Santa Cruz Biotech; PR, progesterone receptor; PRA, PR isoform A; PRB, PR isoform B; WB, Western blot; IHC, immunohistochemistry; IF, immunofluorescence.

PRB did not show ductal elongation and no ends buds were developed (Shyamala *et al.* 2000).

Transgenic *C/EBPB* mice showed an altered pattern of PR isoform expression (Grimm & Rosen 2003), suggesting that C/EBPB plays a role in the regulation of PR. PR, but not ERα, are also overexpressed in the mammary epithelial cells of *Brca1/p53*-deficient mice because of a defect in PR degradation (Poole *et al.* 2006), mirroring the data of human mammary glands from *BRCA+* patients showing increased levels of PRA/PRB (King *et al.* 2004). Furthermore, treatment of *Brca1/p53*-deficient mice with the PR antagonist, mifepristone (MFP; RU-486) prevented mammary tumorigenesis (Poole *et al.* 2006).

PR isoforms in human breast cancer cell lines

PR isoform expression and PR isoform ratio differentially affect breast cancer progression. Although controversial, *in vitro* (Lin *et al.* 1999, Khan *et al.* 2012, Wargon *et al.* 2015, Diep *et al.* 2016) and *in vivo* (Sartorius *et al.* 2003, Wargon *et al.* 2015) studies with breast cancer cell lines support this notion.

The *in vitro* evidence comes from studies using: (a) T47D cells with constitutive expression of both PR isoforms or modified to alter the PR isoform ratio (McGowan & Clarke 1999, Jacobsen *et al.* 2002, McFall *et al.* 2015, Wargon *et al.* 2015) or engineered to overexpress only PRA (T47D-YA) or PRB (T47D-YB) (Sartorius *et al.* 1994*a*); (b) MCF-7 cells in which endogenous PR is expressed after estradiol treatment or modified to express exogenous PR (Boonyaratanakornkit *et al.* 2001, McGowan *et al.* 2007); (c) ZR-75-1 cells in which PRB is the prevalent isoform (Diep *et al.* 2016); (d) IBH-6 cells that express low ERα and PR levels (Vazquez *et al.* 2004) and have been modified to overexpress PRA or PRB (Wargon *et al.* 2015); (e) MDA-MB-231, which are claudin low triple-negative cells, modified to express both PR isoforms or only PRB (Lin *et al.* 1999, 2000) or to conditionally express PRA, PRB or both isoforms (Khan *et al.* 2012, Bellance *et al.* 2013) and (f) BT-474 cells modified to express higher PRB levels as compared to the PRA isoform (Wu *et al.* 2004).

Experimental evidence points to PRB as the more proliferative isoform (Faivre *et al.* 2008) (reviewed in Lange *et al.* 2008). Progestins induce cell cycle progression in T47D-YB cells stably expressing PRB (Skildum *et al.* 2005). However, a biphasic effect of progestins has been reported in T47D cells showing a late inhibitory effect preceded by an increase in cell cycle progression (Musgrove *et al.* 1991). Conversely, in T47D cells with inducible PRA, there was no significant change in cell proliferation but

displayed reduced adherence to plastic, suggesting that increased PRA expression, may play a role in loss of adhesion observed in cancers (McGowan & Clarke 1999, Graham *et al.* 2005) and an increased ability to invade stromal tissue (McGowan *et al.* 2004).

The experimental data regarding the role of PR isoforms in tumor invasiveness and/or aggressiveness *in vitro* is still controversial. In some experimental models, PRA+ cells exhibit a more invasive and aggressive behavior (McGowan *et al.* 2003, Jacobsen *et al.* 2005), whereas in others, induction of PRB leads to an increase in cell migration (Ibrahim *et al.* 2008, Bellance *et al.* 2013).

The genomics of P4-induced effects on gene transcription mediated by PR isoforms has been studied in the T47D-YA/-YB model (Richer *et al.* 2002, Tung *et al.* 2006), in the MDA-MB-231 cells transfected with total PR or PRB (Leo & Lin 2008) or in the inducible systems (Graham *et al.* 2005, Jacobsen *et al.* 2005, Khan *et al.* 2012). In this review, we will focus on data obtained *in vivo*.

PR isoforms in experimental tumor growth

Mouse mammary carcinomas

There are few mouse mammary carcinoma models that express ERα and PR. MMTV-induced tumors were studied at a time that PR was detected by ligand-binding techniques and, to the best of our knowledge, no information has been reported regarding the prevailing PR isoforms in these models.

Our group developed MPA-induced mammary carcinomas, which expressed high levels of ERα and PR (reviewed in Lanari *et al.* 2009). With time, several different tumor variants arose which were classified according to their response to antiprogestin treatment. PR isoform expression revealed that responsive carcinomas had high PRA/PRB ratios while resistant carcinomas displayed the opposite ratio (Wargon *et al.* 2015). Two types of resistant variants were characterized: constitutive and acquired. The former carried a specific PRA promoter methylation and, upon demethylation, PRA expression and antiprogestin responsiveness were restored (Wargon *et al.* 2011). In acquired resistant variants, continuous antiprogestin treatment induced a decrease in PRA expression and estrogen or tamoxifen treatment restored antiprogestin responsiveness by increasing PRA expression, thus changing the PRA/PRB ratio (Wargon *et al.* 2009).

A large number of genetically engineered mice have also been developed in an attempt to model endocrine-responsive human breast cancer; however, few ER+

luminal mammary carcinomas have been generated (reviewed in Dabydeen & Furth 2014). PR isoforms were only briefly mentioned. In the *Wnt1* transgenic model, variable levels of PRB and PRA were observed among the different tumors (Zhang *et al.* 2005); whereas in the two cases shown of the *Stat1* KO model, high levels of both PRA and PRB were observed in WB, although they were not quantified (Chan *et al.* 2012).

Rat mammary carcinomas

To the best of our knowledge, there are no available data on PR isoform expression in rat mammary carcinomas.

Feline mammary carcinomas

The percentage of total PR+ cells increases in feline invasive mammary carcinomas as compared to normal mammary gland (67% vs 15%, respectively) (Millanta *et al.* 2005) and similar levels of PRA and PRB were observed by WB in mammary carcinomas (Gracanin *et al.* 2012).

Canine mammary carcinomas

In PR+ canine mammary carcinomas, higher levels of PRA than those of PRB were observed (Gracanin *et al.* 2012, Guil-Luna *et al.* 2014) and the antiprogestin aglepristone inhibited tumor growth (Guil-Luna *et al.* 2011). More recently, tumor samples excised before and after aglepristone treatment were analyzed for PR mRNA and Ki67 antigen labeling (Guil-Luna *et al.* 2017). In aglepristone-treated PRA+ tumors, both total PR and PRA mRNA expression levels decreased as well as the proliferation index, suggesting that PRA mediates the inhibitory effect.

Cell line-derived xenograft models

The PRA/PRB ratios in different breast cancer cell line-derived xenograft models are depicted in Table 2. T47D xenografts are strictly 17-β-estradiol (E2)-dependent for their *in vivo* growth. Bagatell *et al.* reported double bands for PRA and PRB using the PR MC243 antibody in WB (Bagatell *et al.* 2001), whereas Sartorius *et al.* showed increased PRB expression as compared with that of PRA in the T47D xenografts growing in nude female mice (Sartorius *et al.* 2003). We found similar levels of both PR isoforms, but occasionally there was an increase in PRB. T47D and T47D-YA tumors grow slower than the T47D-YB when inoculated into nude (Sartorius *et al.* 2003) or

NSG mice (Wargon *et al.* 2015). T47D-YA xenografts are tamoxifen and MFP sensitive while T47D-YB are resistant to both agents (Sartorius *et al.* 2003, Wargon *et al.* 2015). MPA induced only a slight increase in tumor growth of T47D xenografts; however, it did increase the expression of stem cell markers such as CK5 and BCL6 (Goodman *et al.* 2016). Others reported that P4 slightly decreased estrogen-induced tumor growth in MCF-7 and in T47D xenografts (el Etreby & Liang 1998, Mohammed *et al.* 2015) although in the latter, the decrease in growth rate was only significant when P4 was administered together with tamoxifen.

More recently, Singhal *et al.* reported that in T47D xenografts, the antiprogestin telapristone (TLP) induced transient inhibition of tumor growth, while the combined administration together with tamoxifen induced an almost complete tumor regression (Singhal *et al.* 2016). A similar effect was obtained with two other antiprogestins: CDB 4453 and EC313 (Singhal *et al.* 2018). These results are in line with the inhibitory effect previously observed in T47D xenografts treated with MFP (Wargon *et al.* 2015).

P4 (pellet; 25 mg) inhibited the growth of MDA-MB-231 cells transfected with PR (ABC28 clone) inoculated into ovariectomized NOD/SCID mice. Overall the tumors were too small, and no metastases were observed in any of the groups (Lin *et al.* 2001). Recently, we found that tumor growth and metastases were lower in MDA-MB-231 cells stably transfected with PRB inoculated into NSG mice as compared to the control counterparts. However, while MPA inhibited lung metastasis, MFP induced an increase in the number and size of the metastatic foci (Lanari *et al.* 2016).

IBH-4, IBH-6 and IBH-7 are ERα+ PR+ human breast cancer cell lines (Vazquez *et al.* 2004) that originate tumors when transplanted into nude mice (Bruzzone *et al.* 2009). IBH-4 and IBH-6 were able to grow without hormone supply and IBH-7 was strictly estrogen dependent (Bruzzone *et al.* 2009). Tumors express ERα and PR and although not quantified, higher levels of PRB than PRA were observed in WB (Bruzzone *et al.* 2009). MPA did not alter tumor growth; however, in the three cases the curves corresponding to MPA-treated mice showed a trend to decrease the growth rate as compared with their matched controls, either in the presence or absence of E2. Tamoxifen significantly inhibited the growth of IBH-4 and IBH-6 tumors.

IBH-6 tumors were not inhibited by antiprogestins such as MFP or TLP. However, these antiprogestins inhibited the growth of cells manipulated to overexpress PRA, whereas MFP stimulated those overexpressing PRB (Wargon *et al.* 2015).

Table 2 PR isoform imbalance in cell line xenograft breast cancer models.

Cell line/tumor	PRA/PRB ratio	Assay	Mice	Antibodies	Effect of hormones/ antagonists	References
T47D (ER+, PR+, AR+, GR+ (low), HER2−, P53 mutation)	PRA = PRB[1,2] PRB > PRA[3]	WB	NSG[1,6] SCID[2] *Nu/nu*[3,4,5,7]	MC243[#2]; AB-52* B-30*[3] H-190 (sc-7208, SC[€]) or Dako 1294[1]	MPA ↑ tumor growth[4] and stem cell markers[5] P4 ↓ slightly E2-induced tumor growth and improved TAM-induced inhibition[6] Antiprogestins (MFP[1], TLP[1,7]) ↓ tumor growth Antiprogestins (CDB4453 and EC313) ↓ tumor growth that is potentiated by TAM[8]	1: Wargon *et al.* (2015); 2: Bagatell *et al.* (2001); 3: Sartorius *et al.* (2003); 4: Liang *et al.* (2007); 5: Goodman *et al.* (2016); 6: Mohammed *et al.* (2015); 7: Singhal *et al.* (2016); 8: Singhal *et al.* (2018)
	T47D-YA Only PRA	WB	*nu/nu*[1] NSG[2]	AB-52; B-30[1]; H-190 (SC) or Dako 1294[2]	↓ growth rate as compared with -YB[1,2]; inhibited by TAM[1] and MFP[2]	1: Sartorius *et al.* (2003); 2: Wargon *et al.* (2015)
	T47D-YB only PRB	WB	*nu/nu*[1] NSG[2]	H-190 (SC) or Dako 1294	↑ growth rate as compared with wt and – YA[1,2]; TAM[1] and MFP[2] resistant	1: Sartorius *et al.* (2003); 2: Wargon *et al.* (2015)
MDA-MB-231 (ER−, PR−, AR−, HER2−, P53 mut)	Transfection of PRA/-B ABC28 clone	NAI	SCID	NAI	P4 ↓ tumor growth	Lin *et al.* (2001)
	PRB	WB	NSG	H-190 (SC)[1,2]; Clone 16 (NC[€])[2]; Clone 636 (NC)[2]	Fewer metastases than wt. MPA ↓ and MFP ↑ lung metastasis[1]	1: Lanari *et al.* (2016); 2: Fabris *et al.* (2017)
IBH-6 (ER+, PR+, HER2-)	PRB > PRA Wt	WB	*Nu/nu*[1,2]	C-19, sc-538 (SC) H-190 (SC)	Similar growth in E2- or MPA-treated or untreated mice. TAM ↓[1] and MFP ↑ tumor growth[2]	1: Bruzzone *et al.* (2009); 2: Wargon *et al.* (2015)
	PRA > PRB PRA transfection	WB	*Nu/nu*[1,2]	H-190 (SC)	MFP or TLP ↓ tumor growth Others not tested	Wargon *et al.* (2015)
	PRB > PRA PRB transfection	WB	*Nu/nu*[1,2]	H-190 (SC)	MFP ↑ tumor growth. TLP exerted no effect	Wargon *et al.* (2015)
IBH-4 (ER+, PR+, HER2-)	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	Similar growth in E2- or MPA-treated or untreated mice. TAM ↓ tumor growth. MPA ↓ E2-induced metastases	Bruzzone *et al.* (2009)
IBH-7 (ER+, PR+, HER2-)	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	E2-dependent. MPA ↓ E2-induced metastases	Bruzzone *et al.* (2009)
BT-474 (ER+, PR+, HER2+, p53 mut)	NAI				P4 ↑ tumor growth, MPA ↑ metastasis	Liang *et al.* (2010)
MCF-7 (ER+ PR inducible, RA+, GR+, HER2-P53 wt)	MCF-7 empty vector	WB (cell extracts[1])	NSG[1] *Nu/nu*[2]	1: NAI	P4 ↓ E2-induced tumor growth[1]. ONA, MFP or P4 ↓ as TAM tumor growth[2]	1: Mohammed *et al.* (2015); 2: el Etreby and Liang (1998)
	MCF-7 PRA	WB	NSG	NAI	Slower growth than wt	Mohammed *et al.* (2015)
	MCF-7PRB	WB	NSG	NAI	Slower growth than wt	Mohammed *et al.* (2015)

[#]Antibody provided by Dr D Toft, Mayo Clinic, Rochester, MN; *antibody developed by authors (Estes *et al.*, Biochemistry 26: 6250, 1987); [€]SC, Santa Cruz Biotech; NC, Novocastra; CS, Cell Signaling Technology; NAI, No Available Information; AR, androgen receptor; E2, 17-β-estradiol; ER, estrogen receptor; GR, glucocorticoid receptor; MFP, mifepristone; MPA, medroxyprogesterone acetate; ONA, onapristone; P4, progesterone; PR, progesterone receptor; PRA, PR isoform A; PRB, PR isoform B; TAM, tamoxifen; TLP, telapristone; WB, Western blot.

Patient-derived xenografts

Only in few xenograft models, PR isoforms were evaluated by WB. In three out of four tumors of the WHIM xenografts, the PRB band seems stronger than the PRA band (in the publication the bands are mislabeled; the upper band is PRB and the lower band is PRA). More recently, the growth of two ERα+, PR+ patient-derived xenografts (PDX), UCD4 and UCD65 was described (Finlay-Schultz *et al.* 2017). P4 and MPA similarly inhibited E2-induced tumor growth. In both cases, PRB expression was apparently higher than PRA by WB (Finlay-Schultz *et al.* 2017). Esber *et al.* (2016) showed WB data from the HBCx-19, -21, -22 and -34 PDX (Cottu *et al.* 2014); both PR isoforms were expressed with levels of PRB that seemed to be higher than those of PRA. The authors report a slight inhibition of tumor growth after treatment with ulipristal acetate, a selective PRs modulator, P4 or the novel antiprogestin APR19 using the HBCx-34 PDX (Table 3).

In summary, increased levels of PRB than PRA were seen in most models in which progestins showed inhibitory effects on tumor growth or metastasis. Taking into consideration that the ability to generate a PDX is by itself a bad prognostic factor (DeRose *et al.* 2011, Byrne *et al.* 2017, Shafaee & Ellis 2017), it can be speculated that higher levels of PRB than PRA are associated with worse prognosis.

PR isoforms in breast cancer cohorts

ERα, PR, HER2 and Ki67 are routinely evaluated as prognostic and predictive markers in breast cancer patients. According to the ER status, patients are categorized in ERα+ PR+; ERα+ PR−; ERα− PR+ or ERα− PR−. Whereas there is no doubt regarding the clinical benefit of measuring ERα, the benefit of measuring PR expression is more controversial (Olivotto *et al.* 2004). However, as pointed out by several studies and discussed by many authors, PR expression has been proposed as a surrogate marker for ERα integrity and endocrine response since high total PR levels correlate with an improved tamoxifen response, longer disease-free and overall survival (Fuqua *et al.* 2005, MacGrogan *et al.* 2005). Moreover, the ERα+PR− group has a worse prognosis, and this phenotype has been associated with impaired ERα function or aberrant growth factor signaling that could contribute to tamoxifen resistance (Arpino *et al.* 2005). On the other hand, the existence of the ERα−PR+ group of breast cancer patients remains controversial. It has been proposed that in selected samples, either the lack of ERα staining or the detection of false-positive PR are related to technical issues, since in many cases which had been revisited a change in diagnosis has been registered (reviewed in Kunc *et al.* 2018). However, others sustain that ERα−PR+ tumors represent a different subgroup with distinct molecular features and clinical course (Shen *et al.* 2015).

In our hands, from 352 samples with ERα scoring, 258 (82.10%) were ERα+ PR+, 59 (16.76%) were ERα− PR−; 31 (8.80%) were classified as ERα+ PR−, and only three samples were reported as ERα− PR+ (0.09%). In all three cases, these samples displayed a PR staining ≤40% and PR isoforms were not detected by WB (unpublished data).

Only few studies have addressed the expression of the different PR isoforms in breast cancer (Table 4). The first study evaluating PR isoforms in breast cancers was reported in 1995 (Graham *et al.* 1995). They used 202 cytosols from PR+ primary tumors and analyzed the pattern of PR bands in WB. Several bands were observed; one around 115/120 kDa equivalent to the PRB band of T47D cells used as controls, another ahead from the 81 kDa band observed in the controls considered as PRA and one of 78 kDa. Extra faint bands with lower MW were occasionally observed. The median PRA/B ratio observed was 1.26.

Bamberger *et al.* studied a total of 53 mammary carcinomas, 21 PR− and 32 PR+ (Bamberger *et al.* 2000) by WB. Band intensity was quantified as negative, weak, moderate, strong and very strong. In only two out of 32 cases, one was a recurrence, PRB showed to be increased, as compared with PRA. In this study, the authors conclude that PRB expression correlates with the absence of HER2. Overall, the authors conclude that higher PRB levels correlated with a more differentiated phenotype. The drawback is the small cohort and the fact that the authors included in the data analysis all PR+ and PR− patients.

Ariga *et al.* evaluated the expression of PR by IHC (Ariga *et al.* 2001). The samples included 47 cases of invasive ductal carcinoma, 40 ductal carcinomas *in situ* (DCIS), 27 atypical ductal hyperplasias and 27 cases of proliferative disease without atypia. The IHC scores for PRA in atypical ductal hyperplasias were higher than those in high histological grade DCIS or invasive carcinomas, whereas the scores for PRB were lower in invasive carcinomas with high histological grades, than in proliferative disease without atypia. Both scores correlated inversely with histological grade in invasive carcinoma and DCIS. A positive correlation between ERα, PRA and PRB was observed in invasive carcinomas. Again, as in the previous study, the analysis was performed on PR+ and PR− samples. From the 47 cases of invasive carcinomas, 16 were PR− and 31 PR+.

Table 3 PR isoform imbalance in breast cancer patient-derived xenograft models.

PDX	PRA/PRB ratio	Assay	Mice	Antibodies	Effect of hormones/antagonists	References
PDX WHIM 16 skin met	PRB > PRA	WB	NSG	#8757, CS[€]	E2 ↓ tumor growth	Li *et al.* (2013)
PDX WHIM 18 skin met	PRA > PRB	WB	NSG	#8757, CS	E2 has no effect on growth, FUL resistant	Li *et al.* (2013)
PDX WHIM 20 skin met	PRB > PRA	WB	NSG	#8757, CS	E2 has no effect on growth	Li *et al.* (2013)
PDX WHIM 24 skin met	PRB > PRA	WB	NSG	#8757, CS	E2 ↑ tumor growth	Li *et al.* (2013)
PDX UCD UCD4	PRB > PRA	WB	NSG	H-190 (SC); DAKO, 1294	E2 ↑ tumor growth; MPA or P4 ↓ E2-induced tumor growth	Finlay-Schultz *et al.* (2017)
PDX UCD UCD65	PRB > PRA	WB	NSG	H-190 (SC); DAKO, 1294	E2 dependent; MPA or P4 similarly ↓ E2-induced tumor growth	Finlay-Schultz *et al.* (2017)
HBCx 19	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	NAI	Esber *et al.* (2016)
HBCx 21	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	NAI	Esber *et al.* (2016)
HBCx 22	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	NAI	Esber *et al.* (2016)
HBCx 34	PRB > PRA	WB	*Nu/nu*	C-19 (SC)	E2 ↑ growth. P4, APR19, a novel antiprogestin and UPA ↓ tumor volume and/or tumor weight	Esber *et al.* (2016)

[€]SC, Santa Cruz Biotech; NC, Novocastra; CS, Cell Signaling Technology; NAI, No Available Information; E2, 17-β-estradiol; FUL, fulvestrant; MPA, medroxyprogesterone acetate; P4, progesterone; PR, progesterone receptor; PRA, PR isoform A; PRB, PR isoform B; UPA, ulipristal acetate; WB, Western blot.

The three previous studies did not analyze the role of PRA and PRB as predictive factors for endocrine therapies. However, in 2004, Hopp *et al.* presented data suggesting that high PRA levels identify a subgroup of women with a poorer response to tamoxifen (Hopp *et al.* 2004). They evaluated frozen primary breast tumor specimens from a cohort of 297 axillary lymph node-positive patients; 119 received no adjuvant therapy after surgery and 178 were treated only with tamoxifen. Eighty-nine per cent were ERα+ and 69% PR+ as determined by binding techniques. Total extracts were prepared for WB. PRA and PRB levels in tumors were normalized to PRB levels in the T47D-positive control lysate used in the same immunoblot. The primary outcome was disease-free survival. The authors reinforce the fact that it is not possible to distinguish between both isoforms by IHC and that stromal cells will not interfere with the balance between both of them. The median PRA/PRB ratio was 0.96. The PR isoforms showed a significant inverse correlation with tumor size, S phase and number of positive nodes. Only in the tamoxifen-treated group, PRA expression higher than PRB correlated with decreased disease-free survival, suggesting that the determination of PR isoforms could help to predict tamoxifen responsiveness.

Pathiraja *et al.* reported an association of *PRA* promoter methylation with worse outcome in ERα+ breast cancer patients (Pathiraja *et al.* 2011). They selected 500 patients who received tamoxifen treatment and 500 without endocrine treatment. Most of the tumors had a small size (48% < 2 cm) and 66% were node negative. Of the 227 ERα+, PR– tumors, *PRA* was methylated in 25.9% of the tumors and *PRB* in 28.5% suggesting loss of PRA or PRB was not a result of DNA methylation in regulatory regions of the PR genes. In the tamoxifen-treated group, overall survival was worse for patients with methylated *PRA* compared with non-methylated *PRA*. This finding conceptually contradicts the previous study (Hopp *et al.* 2004), since those patients with methylated *PRA* are supposed to be those with higher levels of PRB than PRA, and thus, better disease-free survival, although PR isoforms were not evaluated in this study.

Lindet *et al.* evaluated in 299 breast cancer samples the relation between mRNA expression of total (PRA+PRB) and PRB with different mRNAs of the HER/ERBB family and showed that they inversely correlated with aggressiveness (Lindet *et al.* 2012). Knutson *et al.* evaluated pSer294 expression in ten breast carcinomas. Phospho-Ser294 PRB was detected in five out of seven PR+ samples, suggesting that these tumors might be those with an active PR pathway (Knutson *et al.* 2012).

Mote *et al.* evaluated by IF the expression of both PR isoforms (Mote *et al.* 2015). PR expression was determined in tissue microarrays (TMAs) derived from the TransATAC cohort of patients (*n*=710) treated either with tamoxifen or with the aromatase inhibitor anastrozole, with a 10-year median follow-up. For cohort 1, sections of archival formalin-fixed and paraffin-embedded (FFPE) tissue were stained sequentially for PRB and then PRA, using a dual

Table 4 PR isoform ratio in breast cancer patients.

Study	Assay	Antibodies	Methods	Results	References
1	WB	Mix of hPRa7, hPRa0 (NM$^{€}$)	202 cytosols from PR+ primary tumors	PRA and PRB bands plus an extra one of 78 kDa. PRA/PRB ratio between 0 and 2 in 61.4%, and between 0 and 4, in 75.2% of tumors. PRA is the predominant isoform	Graham et al. (1996)
2	WB	NCL-PGR (NC$^{€}$)	53 mammary carcinomas (21 PR−; 32 PR+). Band intensity quantification: negative, weak, moderate, strong, and very strong	In 30/32 cases, PRA ≥ PRB. PRB correlated with better differentiation. The expression of both PR isoforms correlated with ERα expression	Bamberger et al. (2000)
3	IHC PCR (6 cases)	hPRa7 for PRA and hPRa2 for PRB (NM)	47 IDC* (31 PR+), 40 DCIS**, 27 ADH#, and 27 PDWA*** six cases were evaluated for (PRA + PRB) or PRB mRNA expression	IHC scores for PRB were lower in high-grade IDC than in the PDWA. Positive correlation between ERα, PRA and PRB in the IDC	Ariga et al. (2001)
4	Dual IF staining	hPRa6 for PRB and hPRa7 for PRA (own antibodies)	13 normal breast tissues (FFPE); archival FFPE blocks of 15 PDWA, 15 ADH; 15 DCIS, and 39 malignant carcinomas	IC: 51% equimolar, 10% PRB and 39% PRA predominance. Loss of control of relative PRA/PRB expression is an early event in breast cancer	Mote et al. (2002)
5	WB	#1294 (Dako)	297 frozen tumors from lymph node+ patients. 119: no adjuvant therapy; 178: TAM-treated. Isoforms normalized to PRB levels of the T47D control lysate. The primary outcome was disease-free survival	PRA/PRB ratio ranged 0–31 with 72% of the tumors showing a ratio between 0.5 and 2. The PR isoforms showed an inverse correlation with tumor size, S phase, and number of positive nodes. Only in the TAM-treated group PRA > PRB correlated with decreased free survival	Hopp et al. (2004)
6	PR methylation	NA	500 TAM-treated patients and 500 without treatment. All tumors were ERα+ and 77% PR+. Most of them had small sizes and low rates of metastasis	In 25.9% of PR− tumors, *PRA* was methylated. In the TAM-treated group, overall survival was worse for patients with methylated *PRA*. PRA is silenced in luminal breast cancer patients with worse prognosis	Pathiraja et al. (2011)
7	qPCR (PRA + PRB) and PRB	NA	299 samples. mRNA expression of total PR, PRB and mRNAs of the HER/ERBB family	PR isoforms inversely correlated with tumor aggressiveness. No differences were recorded between total PR or PRB isoform expression and HER/ERBB expression	Lindet et al. (2012)
8	Dual IF staining	PRB: hPRa6 (NM) or SAN27 (NC). PRA: hPRa7 (NM) and clone 16 (NC)	Tissue microarrays from the TransATAC cohort (n = 710) patients treated with TAM or anastrozole, (10-year median follow-up). Tumors were categorized into: PRA/PRB ratios between 0.8 and 1.2; lower than 0.8 and higher than 1.2	PRA = PRB (53%); PRA > PRB (29%); PRB > PRA (18%). A significant association between PRB predominance and previous or current use of MHT was observed. TAM-treated, but not anastrozole-treated patients had a shorter time to distant relapse if their tumors had high PRA/PRB ratios	Mote et al. (2015)
9	IHC	PRA: clone 16 (#312; NC). PRB: clone SAN27 H.1H (NC)	Archived samples from 789 women with IC were analyzed for PR isoforms. Tumors were considered as activated PR (APR)+ if they had an aggregated pattern of nuclear foci staining	From 79% of PR+ tumors, 25% were PRA+ and APR+, and 23% PRB+ and APR+. The APR+ score was associated with higher grade. The authors proposed this assay to select patients with aggregated PR expression as candidates for an antiprogestin treatment	Bonneterre et al. (2016)

(Continued)

Table 4 Continued.

Study	Assay	Antibodies	Methods	Results	References
10	WB	#1294 (Dako) and H-190 (SC$^\epsilon$)	Nuclear and cytosolic extracts from 222 frozen PR+ breast cancer samples were run in 8% gels for electrophoresis. Samples were categorized according to the PRA/PRB ratio Tumors were equimolar (ratio: 1.2–0.83), PRA high (PRA-H) if PRA/PRB ≥1.2 or PRB high (PRB-H) if PRA/PRB ≤0.83	PR+ tumors: 52.3% were PRA-H and 28.8% PRB-H. Molecular profiling and data mining associated PRA-H tumors with the luminal A and PRB-H with the luminal B tumors. PRB-H tumors were associated with lower total PR values, greater tumor size, higher histological grade, higher Ki-67 expression and HER-2 expression. 100% of PRA-H samples ($n=19$) were inhibited with 10 nM MFP in tissue cultures	Rojas *et al.* (2017)
11	WB, qPCR	8757 (Cell Signaling)	53 ER+/PR+ ductal carcinomas, from the Cooperative Human Tissue Network. T47D cells overexpressing PRA were used as controls	21 samples out of 53 showed PR isoform bands in WB. PRA is the prevailing isoform. A correlation between protein and mRNA is reported. PRA expression correlated negatively with miR92a3p and positively with miR-26b5p expression. A role for PRA inhibiting the inhibitory effect of ER on metastases is proposed	McFall *et al.* (2018)

#ADH, Atypical Ductal Hyperplasia; *IDC, Invasive Ductal Carcinomas; **DCIS, Ductal Carcinoma *In Situ*; ***PDWA, Proliferative Disease Without Atypia; $^\epsilon$NC, Novocastra; NM, Neomarkers; SC, Santa Cruz Biotech.
IC, Invasive Cancer; NA, NOT Applicable; MHT, Menopausal Hormone Treatment; ER, estrogen receptor; FFPE, formalin-fixed paraffin-embedded tissue; IHC, immunohistochemistry; MFP, mifepristone; PR, progesterone receptor; PRA, PR isoform A; PRB, PR isoform B; APR, activated PR; TAM, tamoxifen; WB, Western blot.

immunofluorescence technique, selective for PRA and PRB, that reflects relative levels of the two isoforms (Mote *et al.* 1999). In FFPE samples, the antibodies used for these studies had been previously recommended by this group to recognize only PRA (Mote *et al.* 2001) or PRB (Clarke *et al.* 1987). The mean PRA/PRB ratio was determined and 138 tumors were categorized into samples with PRA/PRB ratios between 0.8 and 1.2 (equimolar; $n=73$); PRA/PRB ratios lower than 0.8 (PRB predominance; $n=18$) and ratios higher than 1.2 (PRA predominance; $n=40$) in accordance with previous findings (Mote *et al.* 2002). A significant association between PRB predominance and previous or current use of menopausal hormone treatment was observed. Of the 16% (15/93) of patients who had never used menopausal treatment, almost half (7/15, 47%) had tumors expressing a high proportion of PRB. In addition, tamoxifen, but not anastrozole-treated patients had a significantly shorter time to distant relapse if their tumors had high mean PRA/PRB ratios, suggesting that the PRA/PRB ratio may be a discriminating factor in predicting response to these endocrine agents. The major drawback in this study is that the NCL-PGR-312 antibody used to detect PRA recognizes both PR isoforms in human xenografts (Fabris *et al.* 2017).

Bonnaterre and coworkers reported a novel immunohistochemical analysis to distinguish tumors in which PR is activated (Bonneterre *et al.* 2016). Based on previous studies (Mote *et al.* 2001), they proposed two subnuclear morphological PR distribution patterns indicative of transcriptional activation status: an aggregated pattern, formed by activated ligand-bound PR disposed in nuclear foci and a diffuse pattern. The authors propose that activated PR (APR) foci/aggregates in breast cancer cells can be used as a biomarker for antiprogestin responsiveness, namely onapristone (ONA). In this study, tumors are categorized according to their 'nuclear PR pattern' and correlate it with antiestrogen treatment outcomes. PR isoforms were detected by IHC in archived 801 specimens with previously determined ERα and PR status from women with early invasive breast cancer. APR– (APRneg) tumors are those that are either PR–, or, if they express PR, they have a diffuse staining. From 79% of PR+ tumors, 25% were PRA+ and APR+ and 23% were PRB+ and APR+. The APRA+ was associated with higher histological grade. The authors propose this assay to select patients with aggregated PR expression as candidates for an antiprogestin treatment. As mentioned before, the specificity of the PRA antibody has not been corroborated (Fabris *et al.* 2017).

Our laboratory has recently published results from a study involving 222 PR+ breast cancer samples (Rojas *et al.* 2017), which were categorized according to the PRA/PRB ratio, as measured by WB. Tumors were
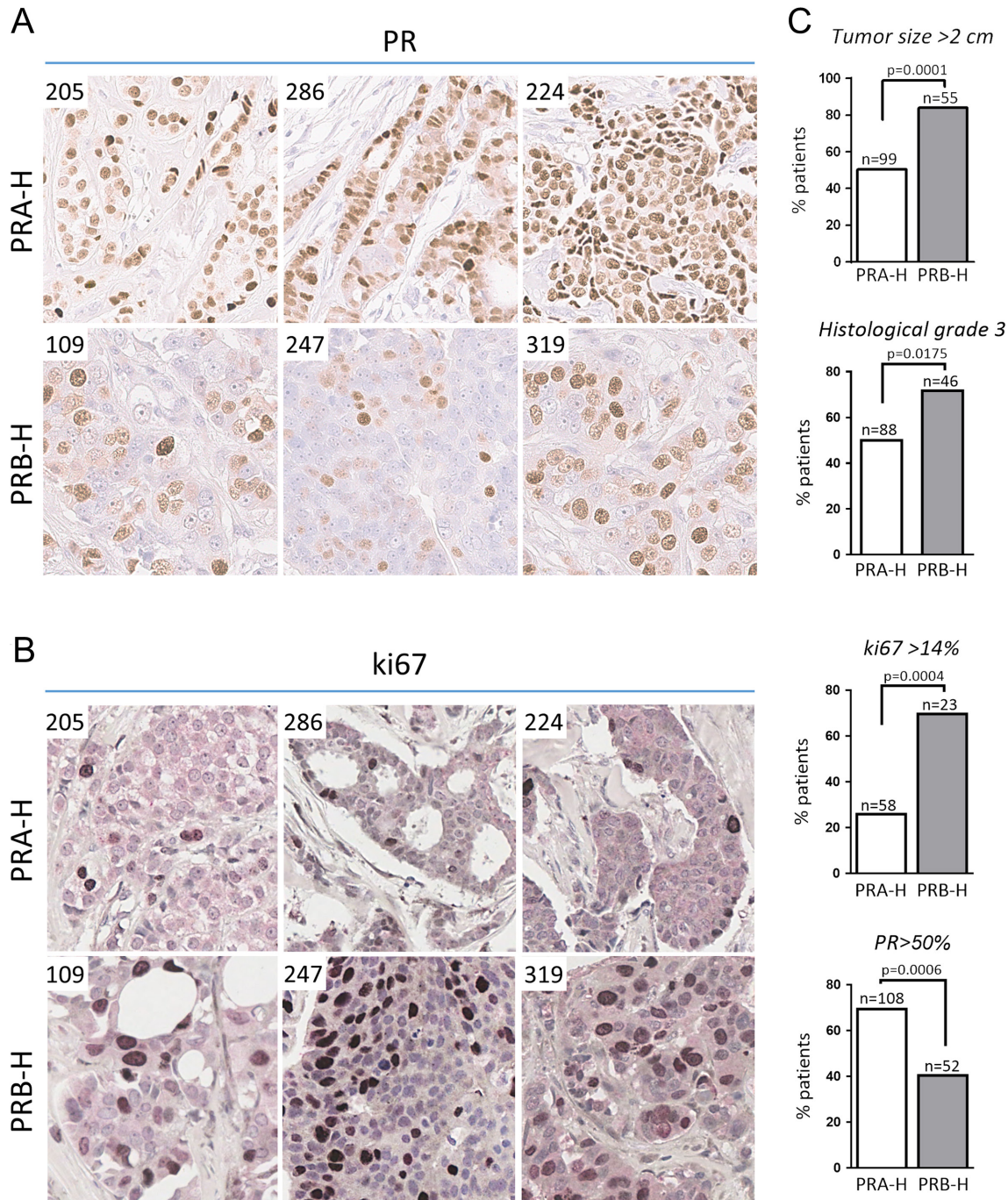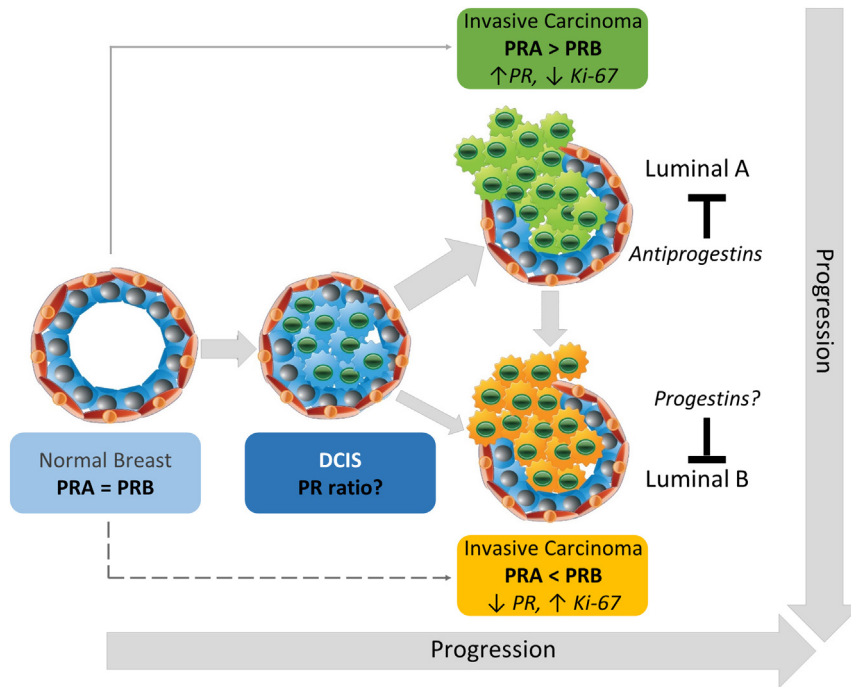
Figure 1

Quantification of clinicopathological parameters in PRA-H and PRB-H breast cancer samples. Immunohistochemical staining of PR (A) and ki67 (B) in three PRA-H and PRB-H breast cancer samples. (C) Quantification of the percentage of PRA-H or PRB-H patients with tumor sizes >2 cm, histological grade 3, Ki67 >14 or PR >50%. PR, progesterone receptor; PRA-H, breast cancer human sample with isoform A of PR/isoform B of PR ≥1.2; PRB-H, breast cancer human sample with isoform A of PR/isoform B of PR ≤0.83. The numbers on the corners in (A) and (B) correspond to the patient sample. Statistical analysis: Fisher exact test. Data obtained from Rojas et al. (2017). A full color version of this figure is available at https://doi.org/10.1530/ERC-18-0179.

Figure 2

Schematic model of PR expression in breast cancer tumor progression. Few mammary epithelial cells express PR (equimolar PRA and PRB levels in the normal breast). Most invasive carcinomas express higher levels of PRA than PRB. PRA-H tumors recapitulate luminal A molecular subtypes (high total PR levels, low ki67 levels and respond to antiprogestin therapy). We hypothesize that invasive PRB-H tumors arise mainly during tumor progression. PRB-H tumors recapitulate luminal B molecular subtypes (low total PR levels, high ki67 levels, are resistant to antiprogestin therapy and may respond to progestin therapy). Invasive carcinomas may arise from DCIS (Cowell *et al.* 2013) or directly from mammary stem cells (Wang *et al.* 2013). DCIS, ductal carcinoma *in situ*; PR, progesterone receptor; PRA-H, higher levels of PRA than PRB; PRB-H, higher levels of PRB than PRA. A full color version of this figure is available at https://doi.org/10.1530/ERC-18-0179.

considered equimolar if the ratio was within the range 1.2–0.83 or were classified as PRA high (PRA-H) if PRA/PRB ≥1.2 or PRB high (PRB-H) if PRB/PRA ≥1.2. In agreement with most of previous studies, PRA-H cases were predominant: 52.3% were PRA-H and 28.8% PRB-H, the rest being within the equimolar range. Molecular profiling and data mining of selected cases associated PRA-H tumors with the luminal A and PRB-H with the luminal B breast carcinomas. For this analysis, only ductal HER2− non-metastatic carcinomas were selected. This was corroborated by clinical parameters that associated the PRB-H with lower total PR values, greater tumor size, higher histological grade, Ki67 expression and HER-2 expression, supporting the data in molecular profiling (Fig. 1). Whereas in 100% of the PRA-H samples ($n=19$), a decrease in Ki67 staining was observed after incubation of tissue cultures with 10 nM MFP for 48 h, variable responses were observed in the 10 PRB-H, four equimolar or 3 PR− cases. This study is in line with that of Pathiraja *et al.* (2011), with observations of Knudson *et al.*, in modified T47D cells (Knutson *et al.* 2012) and with our preclinical studies suggesting that PRB-H tumors may be endocrine-resistant tumors (Lanari *et al.* 2012, Wargon *et al.* 2015).

Singhal *et al.* (2018) using the raw RNA-seq data from our study (Rojas *et al.* 2017) compared the list of differential genes expressed in T47D-YA and T47D-YB xenografts and those differentially regulated in PRA-H or PRB-H patients and concluded that a high PRA/PRB ratio might correlate with worse prognosis. Although the total number of

samples included in both studies coincides, for the PAM50 analysis, we excluded the HER2+ cases, a lobular tumor and a metastatic tumor from a premenopausal patient, to compare a homogeneous cohort of patients. In Singhal's study, two of the HER2+ patients and the lobular patient were included, and a luminal A case of the PRA-H group was excluded. A larger cohort will be necessary to confirm these findings.

In a recent study, a positive correlation between PRA and miR26b5p expression and a negative correlation with miR92a3p was observed in 21 PRA+ breast cancer samples, with these two miRNAs being involved in the ER–PR crosstalk and in the invasive and metastatic features of luminal breast cancer (McFall *et al.* 2018). MiR92a expression is inversely correlated to tumor grade, positive lymph node status and recurrence-free survival in breast cancer (Nilsson *et al.* 2012).

In all these studies, the consensus indicates that PRA is the prevailing isoform in breast cancers; however, the role of the PRA/PRB ratio as a prognostic marker needs to be further validated in larger cohorts of breast cancer patients.

Clinical trials

Progestins (reviewed in Carroll *et al.* 2017) and antiprogestins (reviewed in Klijn *et al.* 2000, Lanari *et al.* 2012) have been used in the past to treat breast cancers with isolated positive responses. Among current clinical trials aimed to test the effects of antiprogestins

or progestins in breast cancer treatment, only the MIPRA trial (NCT02651844) has included the evaluation of the PR isoform ratio as an inclusion criterion to select patients for MFP treatment during 14 days between biopsy and surgery. Only those with PRA/PRB >1.5 are eligible. One study uses MFP for prevention in patients carrying *BRCA1* or -2 mutations with a high risk/incidence of breast and ovarian cancer (NCT01898312). As mentioned previously, *BRCA* carriers usually have levels of PRA higher than those of PRB (Fuqua & Cui 2004, King *et al.* 2004).

Other antiprogestins, such as ONA or TLP, are currently being tested. Regarding ONA (NCT02052128), although the study was expected to be completed by April 2016, the website has not been modified since June 24, 2015. With respect to TLP, the drug is given orally once daily for 2–10 weeks between biopsy and surgical resection (NCT01800422); the status is *active, not recruiting*. The NCT02314156 trial will evaluate transdermal or oral TLP in *BRCA* carriers undergoing mastectomy. The status is *active, not recruiting*. The NCT02408770 trial is also aimed to test the effect of antiprogestins in prevention and compares the effect of ulipristal acetate in breast density using MRI and the status is *unknown*.

On the other hand, a study using progestins has recently started. The NCT03306472 clinical trial is designed to evaluate the effect of letrozole alone or combined with megestrol acetate before surgery. The status is *recruiting*. Alternatively, the NCT00123669 trial consists of a unique dose of hydroxyprogesterone prior to surgery and the rationale is to test the effect of P4 in operable breast cancer on overall and disease-free survival at 5 years. The status is *active, not recruiting*.

Concluding remarks

PR ligands may stimulate or inhibit breast cancer growth, and the challenge is to determine which patient will respond to either treatment. Breast cancers with PRA/PRB ratios may respond to antiprogestin treatments, as supported by preclinical assays (Wargon *et al.* 2009, 2015), clinical evidence in dogs (Guil-Luna *et al.* 2014) and *ex vivo* data in human breast cancer tissue cultures (Rojas *et al.* 2017).

On the other hand, progestins may be inhibitory in tumors in which PRB levels are higher than those of PRA, as seen in preclinical assays (Wargon *et al.* 2015), in xenografted human breast cancer cell lines, and in PDX studies (Mohammed *et al.* 2015, Finlay-Schultz *et al.* 2017).

It has been suggested that most of the models in which progestins stimulate mammary carcinoma growth are biased because of the lack of ERα expression, as it is

the case of the T47D-YA/YB models (Carroll *et al.* 2017) or because of the lack of estrogen supply. However, ERα levels in these models score higher than average ERα luminal breast carcinomas and experiments performed using syngeneic mice are performed under physiologic estrogen levels (Lanari *et al.* 2012).

Based on all the available information, malignant neoplastic transformation and the ability to invade neighboring tissues might be associated with an increased PRB activation resulting in PRB downregulation (Diep *et al.* 2015), inducing a switch from an equimolar stage to a preferential PRA-H stage in the human mammary gland. These tumors may be slow growing (low Ki67, luminal A) sensitive to ERα targeted therapy (and/or an antiprogestin treatment). As the tumor progresses and becomes insensitive to other treatments, there is an increase in cell proliferation, a decrease in total PR expression and mainly a decrease in PRA expression (which makes these tumors resistant to an antiprogestin therapy). We hypothesize that these high-grade tumors may now become sensitive to a progestin treatment (Fig. 2).

Future directions

More experimental work is necessary to better understand the role of PR isoforms in breast cancer growth and to exploit these receptors as therapeutic targets. Efforts should be geared to:

a. Design studies using homogeneous cohorts of patients to further define the PRA/PRB ratio as a prognostic factor.
b. Avoid the use of antibodies that supposedly recognize only PRA by IHC and develop specific antibodies to both PR isoforms.
c. Develop novel reliable methods to quantify the PR protein isoform ratio.
d. Determine whether there is a change in the PR isoform ratio between primary tumors and their metastatic spreads.
e. Determine whether PRA-H tumors will become PRB-H in recurrence.
f. Further explore mechanisms to re-express PRA in PRB-H tumors.
g. Design PRB selective inhibitors.
h. Dissect the role of GR and AR in the interplay with PR isoforms to design combined therapies.
i. Evaluate PR isoforms in breast cancer biopsies of patients undergoing clinical trials involving progestins/antiprogestins.

j. Better characterize metastatic tumor models to further evaluate the role of antiprogestins/progestins as therapeutic agents in tumor progression.

References

Abdel-Hafiz HA & Horwitz KB 2014 Post-translational modifications of the progesterone receptors. *Journal of Steroid Biochemistry and Molecular Biology* **140** 80–89. (https://doi.org/10.1016/j.jsbmb.2013.12.008)

Ariga N, Suzuki T, Moriya T, Kimura M, Inoue T, Ohuchi N & Sasano H 2001 Progesterone receptor A and B isoforms in the human breast and its disorders. *Japanese Journal of Cancer Research* **92** 302–308. (https://doi.org/10.1111/j.1349-7006.2001.tb01095.x)

Arnett-Mansfield RL, Graham JD, Hanson AR, Mote PA, Gompel A, Scurr LL, Gava N, de Fazio A & Clarke CL 2007 Focal subnuclear distribution of progesterone receptor is ligand dependent and associated with transcriptional activity. *Molecular Endocrinology* **21** 14–29. (https://doi.org/10.1210/me.2006-0041)

Arpino G, Weiss H, Lee AV, Schiff R, De Placido S, Osborne CK & Elledge RM 2005 Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *Journal of the National Cancer Institute* **97** 1254–1261. (https://doi.org/10.1093/jnci/dji249)

Aupperlee M, Kariagina A, Osuch J & Haslam SZ 2005*a* Progestins and breast cancer. *Breast Disease* **24** 37–57. (https://doi.org/10.3233/BD-2006-24104)

Aupperlee MD, Smith KT, Kariagina A & Haslam SZ 2005*b* Progesterone receptor isoforms A and B: temporal and spatial differences in expression during murine mammary gland development. *Endocrinology* **146** 3577–3588. (https://doi.org/10.1210/en.2005-0346)

Bagatell R, Khan O, Paine-Murrieta G, Taylor CW, Akinaga S & Whitesell L 2001 Destabilization of steroid receptors by heat shock protein 90-binding drugs: a ligand-independent approach to hormonal therapy of breast cancer. *Clinical Cancer Research* **7** 2076–2084.

Ballare C, Castellano G, Gaveglia L, Althammer S, Gonzalez-Vallinas J, Eyras E, Le Dily F, Zaurin R, Soronellas D, Vicent GP, *et al.* 2013 Nucleosome-driven transcription factor binding and gene regulation. *Molecular Cell* **49** 67–79. (https://doi.org/10.1016/j.molcel.2012.10.019)

Bamberger AM, Milde-Langosch K, Schulte HM & Loning T 2000 Progesterone receptor isoforms, PR-B and PR-A, in breast cancer: correlations with clinicopathologic tumor parameters and expression of AP-1 factors. *Hormone Research* **54** 32–37. (https://doi.org/10.1159/000063434)

Bartosch C, Monteiro-Reis S, Vieira R, Pereira A, Rodrigues M, Jeronimo C & Lopes JM 2015 Endometrial endometrioid carcinoma metastases show decreased ER-alpha and PR-A expression compared to matched primary tumors. *PLoS ONE* **10** e0134969. (https://doi.org/10.1371/journal.pone.0134969)

Bellance C, Khan JA, Meduri G, Guiochon-Mantel A, Lombes M & Loosfelt H 2013 Progesterone receptor isoforms PRA and PRB differentially contribute to breast cancer cell migration through interaction with focal adhesion kinase complexes. *Molecular Biology of the Cell* **24** 1363–1374. (https://doi.org/10.1091/mbc.e12-11-0807)

Bonneterre J, Bosq J, Jamme P, Valent A, Gilles EM, Zukiwski AA, Fuqua SA, Lange CA & O'Shaughnessy J 2016 Tumour and cellular distribution of activated forms of PR in breast cancers: a novel immunohistochemical analysis of a large clinical cohort. *ESMO Open* **1** e000072. (https://doi.org/10.1136/esmoopen-2016-000072)

Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT & Edwards DP 2001 Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. *Molecular Cell* **8** 269–280. (https://doi.org/10.1016/S1097-2765(01)00304-5)

Boonyaratanakornkit V, Bi Y, Rudd M & Edwards DP 2008 The role and mechanism of progesterone receptor activation of extra-nuclear signaling pathways in regulating gene transcription and cell cycle progression. *Steroids* **73** 922–928. (https://doi.org/10.1016/j.steroids.2008.01.010)

Boonyaratanakornkit V, Hamilton N, Marquez-Garban DC, Pateetin P, McGowan EM & Pietras RJ 2018 Extranuclear signaling by sex steroid receptors and clinical implications in breast cancer. *Molecular and Cellular Endocrinology* **466** 51–72. (https://doi.org/10.1016/j.mce.2017.11.010)

Bruzzone A, Vanzulli SI, Soldati R, Giulianelli S, Lanari C & Luthy IA 2009 Novel human breast cancer cell lines IBH-4, IBH-6, and IBH-7 growing in nude mice. *Journal of Cellular Physiology* **219** 477–484. (https://doi.org/10.1002/jcp.21694)

Byrne AT, Alferez DG, Amant F, Annibali D, Arribas J, Biankin AV, Bruna A, Budinska E, Caldas C, Chang DK, *et al.* 2017 Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nature Reviews Cancer* **17** 254–268. (https://doi.org/10.1038/nrc.2016.140)

Carroll JS, Hickey TE, Tarulli GA, Williams M & Tilley WD 2017 Deciphering the divergent roles of progestogens in breast cancer. *Nature Reviews Cancer* **17** 54–64. (https://doi.org/10.1038/nrc.2016.116)

Clarke CL, Zaino RJ, Feil PD, Miller JV, Steck ME, Ohlsson-Wilhelm BM & Satyaswaroop PG 1987 Monoclonal antibodies to human progesterone receptor: characterization by biochemical and immunohistochemical techniques. *Endocrinology* **121** 1123–1132. (https://doi.org/10.1210/endo-121-3-1123)

Condon JC, Hardy DB, Kovaric K & Mendelson CR 2006 Up-regulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor-kappaB may contribute to the onset of labor through inhibition of PR function. *Molecular Endocrinology* **20** 764–775. (https://doi.org/10.1210/me.2005-0242)

Conneely OM, Mulac-Jericevic B & Lydon JP 2003 Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. *Steroids* **68** 771–778. (https://doi.org/10.1016/S0039-128X(03)00126-0)

Cork DM, Lennard TW & Tyson-Capper AJ 2008 Alternative splicing and the progesterone receptor in breast cancer. *Breast Cancer Research* **10** 207. (https://doi.org/10.1186/bcr2097)

Cottu P, Bieche I, Assayag F, El Botty R, Chateau-Joubert S, Thuleau A, Bagarre T, Albaud B, Rapinat A, Gentien D, *et al.* 2014 Acquired resistance to endocrine treatments is associated with tumor-specific molecular changes in patient-derived luminal breast cancer

xenografts. *Clinical Cancer Research* **20** 4314–4325. (https://doi.org/10.1158/1078-0432.CCR-13-3230)

Cowell CF, Weigelt B, Sakr RA, Ng CK, Hicks J, King TA & Reis-Filho JS 2013 Progression from ductal carcinoma in situ to invasive breast cancer: revisited. *Molecular Oncology* **7** 859–869. (https://doi.org/10.1016/j.molonc.2013.07.005)

Chabbert-Buffet N, Meduri G, Bouchard P & Spitz IM 2005 Selective progesterone receptor modulators and progesterone antagonists: mechanisms of action and clinical applications. *Human Reproduction Update* **11** 293–307. (https://doi.org/10.1093/humupd/dmi002)

Chan SR, Vermi W, Luo J, Lucini L, Rickert C, Fowler AM, Lonardi S, Arthur C, Young LJ, Levy DE, *et al.* 2012 STAT1-deficient mice spontaneously develop estrogen receptor alpha-positive luminal mammary carcinomas. *Breast Cancer Research* **14** R16. (https://doi.org/10.1186/bcr3100)

Cheng G, Li Y, Omoto Y, Wang Y, Berg T, Nord M, Vihko P, Warner M, Piao YS & Gustafsson JA 2005 Differential regulation of estrogen receptor (ER)alpha and ERbeta in primate mammary gland. *Journal of Clinical Endocrinology and Metabolism* **90** 435–444. (https://doi.org/10.1210/jc.2004-0861)

Dabydeen SA & Furth PA 2014 Genetically engineered ERalpha-positive breast cancer mouse models. *Endocrine-Related Cancer* **21** R195–R208. (https://doi.org/10.1530/ERC-13-0512)

Daniel AR, Faivre EJ & Lange CA 2007 Phosphorylation-dependent antagonism of sumoylation derepresses progesterone receptor action in breast cancer cells. *Molecular Endocrinology* **21** 2890–2906. (https://doi.org/10.1210/me.2007-0248)

DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, Factor R, Matsen C, Milash BA, Nelson E, *et al.* 2011 Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nature Medicine* **17** 1514–1520. (https://doi.org/10.1038/nm.2454)

Diep CH, Daniel A, Mauro L, Knutson T & Lange C 2015 Progesterone action in breast, uterine, and ovarian cancers. *Journal of Molecular Endocrinology* **4** R31–R53. (https://doi.org/10.1530/JME-14-0252)

Diep CH, Ahrendt H & Lange CA 2016 Progesterone induces progesterone receptor gene (PGR) expression via rapid activation of protein kinase pathways required for cooperative estrogen receptor alpha (ER) and progesterone receptor (PR) genomic action at ER/PR target genes. *Steroids* **114** 48–58. (https://doi.org/10.1016/j.steroids.2016.09.004)

Dobson AD, Conneely OM, Beattie W, Maxwell BL, Mak P, Tsai MJ, Schrader WT & O'Malley BW 1989 Mutational analysis of the chicken progesterone receptor. *Journal of Biological Chemistry* **264** 4207–4211.

el Etreby MF & Liang Y 1998 Effect of antiprogestins and tamoxifen on growth inhibition of MCF-7 human breast cancer cells in nude mice. *Breast Cancer Research and Treatment* **49** 109–117. (https://doi.org/10.1023/A:1006098910000)

Esber N, Cherbonnier C, Resche-Rigon M, Hamze A, Alami M, Fagart J, Loosfelt H, Lombes M & Chabbert-Buffet N 2016 Anti-tumoral effects of anti-progestins in a patient-derived breast cancer xenograft model. *Hormones and Cancer* **7** 137–147. (https://doi.org/10.1007/s12672-016-0255-4)

Fabris V, Abascal MF, Giulianelli S, May M, Sequeira GR, Jacobsen B, Lombes M, Han J, Tran L, Molinolo A, *et al.* 2017 Isoform specificity of progesterone receptor antibodies. *Journal of Pathology: Clinical Research* **3** 227–233. (https://doi.org/10.1002/cjp2.83)

Faivre EJ & Lange CA 2007 Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. *Molecular and Cellular Biology* **27** 466–480. (https://doi.org/10.1128/MCB.01539-06)

Faivre EJ, Daniel AR, Hillard CJ & Lange CA 2008 Progesterone receptor rapid signaling mediates serine 345 phosphorylation and tethering

to specificity protein 1 transcription factors. *Molecular Endocrinology* **22** 823–837. (https://doi.org/10.1210/me.2007-0437)

Finlay-Schultz J, Gillen AE, Brechbuhl HM, Ivie JJ, Matthews SB, Jacobsen BM, Bentley DL, Kabos P & Sartorius CA 2017 Breast cancer suppression by progesterone receptors is mediated by their modulation of estrogen receptors and RNA polymerase III. *Cancer Research* **77** 4934–4946. (https://doi.org/10.1158/1538-7445.AM2017-4934)

Fuqua SA & Cui Y 2004 Estrogen and progesterone receptor isoforms: clinical significance in breast cancer. *Breast Cancer Research and Treatment* **87** (Supplement 1) S3–S10. (https://doi.org/10.1007/s10549-004-1577-4)

Fuqua SA, Cui Y, Lee AV, Osborne CK & Horwitz KB 2005 Insights into the role of progesterone receptors in breast cancer. *Journal of Clinical Oncology* **23** 931–932; author reply 932–933. (https://doi.org/10.1200/JCO.2005.05.152)

Ghatge RP, Jacobsen BM, Schittone SA & Horwitz KB 2005 The progestational and androgenic properties of medroxyprogesterone acetate: gene regulatory overlap with dihydrotestosterone in breast cancer cells. *Breast Cancer Research* **7** R1036–R1050. (https://doi.org/10.1186/bcr1340)

Goodman CR, Sato T, Peck AR, Girondo MA, Yang N, Liu C, Yanac AF, Kovatich AJ, Hooke JA, Shriver CD, *et al.* 2016 Steroid induction of therapy-resistant cytokeratin-5-positive cells in estrogen receptor-positive breast cancer through a BCL6-dependent mechanism. *Oncogene* **35** 1373–1385. (https://doi.org/10.1038/onc.2015.193)

Gracanin A, de Gier J, Zegers K, Bominaar M, Rutteman GR, Schaefers-Okkens AC, Kooistra HS & Mol JA 2012 Progesterone receptor isoforms in the mammary gland of cats and dogs. *Reproduction in Domestic Animals* **47** (Supplement 6) 313–317. (https://doi.org/10.1111/rda.12045)

Graham JD, Yeates C, Balleine RL, Harvey SS, Milliken JS, Bilous AM & Clarke CL 1995 Characterization of progesterone receptor A and B expression in human breast cancer. *Cancer Research* **55** 5063–5068.

Graham JD, Yeates C, Balleine RL, Harvey SS, Milliken JS, Bilous AM & Clarke CL 1996 Progesterone receptor A and B protein expression in human breast cancer. *Journal of Steroid Biochemistry and Molecular Biology* **56** 93–98. (https://doi.org/10.1016/0960-0760(95)00226-X)

Graham JD, Yager ML, Hill HD, Byth K, O'Neill GM & Clarke CL 2005 Altered progesterone receptor isoform expression remodels progestin responsiveness of breast cancer cells. *Molecular Endocrinology* **19** 2713–2735. (https://doi.org/10.1210/me.2005-0126)

Grimm SL & Rosen JM 2003 The role of C/EBPbeta in mammary gland development and breast cancer. *Journal of Mammary Gland Biology and Neoplasia* **8** 191–204. (https://doi.org/10.1023/A:1025900908026)

Grimm SL, Hartig SM & Edwards DP 2016 Progesterone receptor signaling mechanisms. *Journal of Molecular Biology* **428** 3831–3849. (https://doi.org/10.1016/j.jmb.2016.06.020)

Guil-Luna S, Sanchez-Cespedes R, Millan Y, De Andres FJ, Rollon E, Domingo V, Guscetti F & Martin De Las MJ 2011 Aglepristone decreases proliferation in progesterone receptor-positive canine mammary carcinomas. *Journal of Veterinary Internal Medicine* **25** 518–523. (https://doi.org/10.1111/j.1939-1676.2011.0723.x)

Guil-Luna S, Stenvang J, Brunner N, De Andres FJ, Rollon E, Domingo V, Sanchez-Cespedes R, Millan Y & Martin De Las MJ 2014 Progesterone receptor isoform A may regulate the effects of neoadjuvant aglepristone in canine mammary carcinoma. *BMC Veterinary Research* **10** 296. (https://doi.org/10.1186/s12917-014-0296-2)

Guil-Luna S, Millan Y, De Andres J, Rollon E, Domingo V, Garcia-Macias J, Sanchez-Cespedes R & Martin De Las MJ 2017 Prognostic impact of neoadjuvant aglepristone treatment in clinicopathological parameters of progesterone receptor-positive canine mammary carcinomas. *Veterinary and Comparative Oncology* **15** 391–399. (https://doi.org/10.1111/vco.12175)

Guiochon-Mantel A, Delabre K, Lescop P, Perrot-Applanat M & Milgrom E 1994 Cytoplasmic-nuclear trafficking of progesterone

receptor. In vivo study of the mechanism of action of antiprogestins. *Biochemical Pharmacology* **47** 21–24. (https://doi.org/10.1016/0006-2952(94)90433-2)

Heneghan AF, Berton N, Miura MT & Bain DL 2005 Self-association energetics of an intact, full-length nuclear receptor: the B-isoform of human progesterone receptor dimerizes in the micromolar range. *Biochemistry* **44** 9528–9537. (https://doi.org/10.1021/bi050609i)

Hilton HN, Graham JD & Clarke CL 2015 Minireview: progesterone regulation of proliferation in the normal human breast and in breast cancer: a tale of two scenarios? *Molecular Endocrinology* **29** 1230–1242. (https://doi.org/10.1210/me.2015-1152)

Hilton HN, Clarke CL & Graham JD 2018 Estrogen and progesterone signalling in the normal breast and its implications for cancer development. *Molecular and Cellular Endocrinology* **466** 2–14. (https://doi.org/10.1016/j.mce.2017.08.011)

Hopp TA, Weiss HL, Hilsenbeck SG, Cui Y, Allred DC, Horwitz KB & Fuqua SA 2004 Breast cancer patients with progesterone receptor PR-A-rich tumors have poorer disease-free survival rates. *Clinical Cancer Research* **10** 2751–2760. (https://doi.org/10.1158/1078-0432.CCR-03-0141)

Hovland AR, Powell RL, Takimoto GS, Tung L & Horwitz KB 1998 An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *Journal of Biological Chemistry* **273** 5455–5460. (https://doi.org/10.1074/jbc.273.10.5455)

Ibrahim YH, Byron SA, Cui X, Lee AV & Yee D 2008 Progesterone receptor-B regulation of insulin-like growth factor-stimulated cell migration in breast cancer cells via insulin receptor substrate-2. *Molecular Cancer Research* **6** 1491–1498. (https://doi.org/10.1158/1541-7786.MCR-07-2173)

Jacobsen BM & Horwitz KB 2012 Progesterone receptors, their isoforms and progesterone regulated transcription. *Molecular and Cellular Endocrinology* **357** 18–29. (https://doi.org/10.1016/j.mce.2011.09.016)

Jacobsen BM, Richer JK, Schittone SA & Horwitz KB 2002 New human breast cancer cells to study progesterone receptor isoform ratio effects and ligand-independent gene regulation. *Journal of Biological Chemistry* **277** 27793–27800. (https://doi.org/10.1074/jbc.M202584200)

Jacobsen BM, Schittone SA, Richer JK & Horwitz KB 2005 Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology. *Molecular Endocrinology* **19** 574–587. (https://doi.org/10.1210/me.2004-0287)

Jacobsen BM, Jambal P, Schittone SA & Horwitz KB 2009 ALU repeats in promoters are position-dependent co-response elements (coRE) that enhance or repress transcription by dimeric and monomeric progesterone receptors. *Molecular Endocrinology* **23** 989–1000. (https://doi.org/10.1210/me.2009-0048)

Kariagina A, Aupperlee MD & Haslam SZ 2007 Progesterone receptor isoforms and proliferation in the rat mammary gland during development. *Endocrinology* **148** 2723–2736. (https://doi.org/10.1210/en.2006-1493)

Kariagina A, Aupperlee MD & Haslam SZ 2008 Progesterone receptor isoform functions in normal breast development and breast cancer. *Critical Reviews in Eukaryotic Gene Expression* **18** 11–33. (https://doi.org/10.1615/CritRevEukarGeneExpr.v18.i1.20)

Khan JA, Bellance C, Guiochon-Mantel A, Lombes M & Loosfelt H 2012 Differential regulation of breast cancer-associated genes by progesterone receptor isoforms PRA and PRB in a new bi-inducible breast cancer cell line. *PLoS ONE* **7** e45993. (https://doi.org/10.1371/journal.pone.0045993)

King TA, Gemignani ML, Li W, Giri DD, Panageas KS, Bogomolniy F, Arroyo C, Olvera N, Robson ME, Offit K, *et al.* 2004 Increased progesterone receptor expression in benign epithelium of BRCA1-related breast cancers. *Cancer Research* **64** 5051–5053. (https://doi.org/10.1158/0008-5472.CAN-04-1283)

Klijn JG, Setyono-Han B & Foekens JA 2000 Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer. *Steroids* **65** 825–830. (https://doi.org/10.1016/S0039-128X(00)00195-1)

Knutson TP, Daniel AR, Fan D, Silverstein KA, Covington KR, Fuqua SA & Lange CA 2012 Phosphorylated and sumoylation-deficient progesterone receptors drive proliferative gene signatures during breast cancer progression. *Breast Cancer Research* **14** R95. (https://doi.org/10.1186/bcr3211)

Kreizman-Shefer H, Pricop J, Goldman S, Elmalah I & Shalev E 2014 Distribution of estrogen and progesterone receptors isoforms in endometrial cancer. *Diagnostic Pathology* **9** 77. (https://doi.org/10.1186/1746-1596-9-77)

Kunc M, Biernat W & Senkus-Konefka E 2018 Estrogen receptor-negative progesterone receptor-positive breast cancer – 'Nobody's land' or just an artifact? *Cancer Treatment Reviews* **67** 78–87. (https://doi.org/10.1016/j.ctrv.2018.05.005)

Lanari C, Lamb CA, Fabris VT, Helguero LA, Soldati R, Bottino MC, Giulianelli S, Cerliani JP, Wargon V & Molinolo A 2009 The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. *Endocrine-Related Cancer* **16** 333–350. (https://doi.org/10.1677/ERC-08-0244)

Lanari C, Wargon V, Rojas P & Molinolo AA 2012 Antiprogestins in breast cancer treatment: are we ready? *Endocrine-Related Cancer* **19** R35–R50. (https://doi.org/10.1530/ERC-11-0378)

Lanari C, Alvarez M, Giulianelli S, Riggio M, Sequeira G, May M, Novaro V, Sahores A & Lamb C 2016 The ratio of progesterone receptor isoform A to B determines the effect of antiprogestins on preclinical models of breast cancer metastasis. *Cancer Research* **76** P2-05-27. (https://doi.org/10.1158/1538-7445.SABCS15-P2-05-27)

Lange CA, Sartorius CA, Abdel-Hafiz H, Spillman MA, Horwitz KB & Jacobsen BM 2008 Progesterone receptor action: translating studies in breast cancer models to clinical insights. *Advances in Experimental Medicine and Biology* **630** 94–111.

Lantinga-van Leeuwen IS, van Garderen E, Rutteman GR & Mol JA 2000 Cloning and cellular localization of the canine progesterone receptor: co-localization with growth hormone in the mammary gland. *Journal of Steroid Biochemistry and Molecular Biology* **75** 219–228. (https://doi.org/10.1016/S0960-0760(00)00173-4)

Leo JC & Lin VC 2008 The activities of progesterone receptor isoform A and B are differentially modulated by their ligands in a gene-selective manner. *International Journal of Cancer* **122** 230–243. (https://doi.org/10.1002/ijc.23081)

Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, He X, Liu S, Hoog J, Lu C, *et al.* 2013 Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Reports* **4** 1116–1130. (https://doi.org/10.1016/j.celrep.2013.08.022)

Liang Y, Besch-Williford C, Brekken RA & Hyder SM 2007 Progestin-dependent progression of human breast tumor xenografts: a novel model for evaluating antitumor therapeutics. *Cancer Research* **67** 9929–9936. (https://doi.org/10.1158/0008-5472.CAN-07-1103)

Liang Y, Benakanakere I, Besch-Williford C, Hyder RS, Ellersieck MR & Hyder SM 2010 Synthetic progestins induce growth and metastasis of BT-474 human breast cancer xenografts in nude mice. *Menopause* **17** 1040–1047. (https://doi.org/10.1097/gme.0b013e3181d3dd0c)

Lin VC, Ng EH, Aw SE, Tan MG, Ng EH, Chan VS & Ho GH 1999 Progestins inhibit the growth of MDA-MB-231 cells transfected with progesterone receptor complementary DNA. *Clinical Cancer Research* **5** 395–403.

Lin VC, Ng EH, Aw SE, Tan MG & Bay BH 2000 Progesterone induces focal adhesion in breast cancer cells MDA-MB-231 transfected with progesterone receptor complementary DNA. *Molecular Endocrinology* **14** 348–358. (https://doi.org/10.1210/mend.14.3.0426)

Lin VC, Eng AS, Hen NE, Ng EH & Chowdhury SH 2001 Effect of progesterone on the invasive properties and tumor growth of

progesterone receptor-transfected breast cancer cells MDA-MB-231. *Clinical Cancer Research* **7** 2880–2886.

Lindet C, Revillion F, Lhotellier V, Hornez L, Peyrat JP & Bonneterre J 2012 Relationships between progesterone receptor isoforms and the HER/ErbB receptors and ligands network in 299 primary breast cancers. *International Journal of Biological Markers* **27** e111–e117. (https://doi.org/10.5301/JBM.2012.9198)

MacGrogan G, de Mascarel I, Sierankowski G, Mauriac L, Debled M, Durand M, De Lara CT, Avril A, Picot V & Mathoulin-Pelissier S 2005 Time for reappraisal of progesterone-receptor testing in breast cancer management. *Journal of Clinical Oncology* **23** 2870–2871; author reply 2871. (https://doi.org/10.1200/JCO.2005.05.241)

McDonnell DP & Goldman ME 1994 RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. *Journal of Biological Chemistry* **269** 11945–11949.

McFall T, Patki M, Rosati R & Ratnam M 2015 Role of the short isoform of the progesterone receptor in breast cancer cell invasiveness at estrogen and progesterone levels in the pre- and post-menopausal ranges. *Oncotarget* **6** 33146–33164. (https://doi.org/10.18632/oncotarget.5082)

McFall T, McKnight B, Rosati R, Kim S, Huang Y, Viola-Villegas N & Ratnam M 2018 Progesterone receptor A promotes invasiveness and metastasis of luminal breast cancer by suppressing regulation of critical microRNAs by estrogen. *Journal of Biological Chemistry* **293** 1163–1177. (https://doi.org/10.1074/jbc.M117.812438)

McGowan EM & Clarke CL 1999 Effect of overexpression of progesterone receptor A on endogenous progestin-sensitive endpoints in breast cancer cells. *Molecular Endocrinology* **13** 1657–1671. (https://doi.org/10.1210/mend.13.10.0356)

McGowan EM, Weinberger RP, Graham JD, Hill HD, Hughes JA, O'Neill GM & Clarke CL 2003 Cytoskeletal responsiveness to progestins is dependent on progesterone receptor A levels. *Journal of Molecular Endocrinology* **31** 241–253. (https://doi.org/10.1677/jme.0.0310241)

McGowan EM, Saad S, Bendall LJ, Bradstock KF & Clarke CL 2004 Effect of progesterone receptor a predominance on breast cancer cell migration into bone marrow fibroblasts. *Breast Cancer Research and Treatment* **83** 211–220. (https://doi.org/10.1023/B:BREA.0000014041.58977.80)

McGowan EM, Russell AJ, Boonyaratanakornkit V, Saunders DN, Lehrbach GM, Sergio CM, Musgrove EA, Edwards DP & Sutherland RL 2007 Progestins reinitiate cell cycle progression in antiestrogen-arrested breast cancer cells through the B-isoform of progesterone receptor. *Cancer Research* **67** 8942–8951. (https://doi.org/10.1158/0008-5472.CAN-07-1255)

Millanta F, Calandrella M, Bari G, Niccolini M, Vannozzi I & Poli A 2005 Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. *Research in Veterinary Science* **79** 225–232. (https://doi.org/10.1016/j.rvsc.2005.02.002)

Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Birrell SN, Bruna A, Saadi A, *et al.* 2015 Progesterone receptor modulates ERalpha action in breast cancer. *Nature* **523** 313–317. (https://doi.org/10.1038/nature14583)

Montero GG, Vanzulli SI, Cerliani JP, Bottino MC, Bolado J, Vela J, Becu-Villalobos D, Benavides F, Gutkind S, Patel V, *et al.* 2007 Association of estrogen receptor alpha and progesterone receptor A expression with hormonal mammary carcinogenesis: role of the host microenvironment. *Breast Cancer Research* **9** R22. (https://doi.org/10.1186/bcr1660)

Mote PA, Balleine RL, McGowan EM & Clarke CL 1999 Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **84** 2963–2971. (https://doi.org/10.1210/jcem.84.8.5928)

Mote PA, Johnston JF, Manninen T, Tuohimaa P & Clarke CL 2001 Detection of progesterone receptor forms A and B by immunohistochemical analysis. *Journal of Clinical Pathology* **54** 624–630. (https://doi.org/10.1136/jcp.54.8.624)

Mote PA, Bartow S, Tran N & Clarke CL 2002 Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. *Breast Cancer Research and Treatment* **72** 163–172. (https://doi.org/10.1023/A:1014820500738)

Mote PA, Gompel A, Howe C, Hilton HN, Sestak I, Cuzick J, Dowsett M, Hugol D, Forgez P, Byth K, *et al.* 2015 Progesterone receptor A predominance is a discriminator of benefit from endocrine therapy in the ATAC trial. *Breast Cancer Research and Treatment* **151** 309–318. (https://doi.org/10.1007/s10549-015-3397-0)

Mueck AO, Ruan X, Seeger H, Fehm T & Neubauer H 2014 Genomic and non-genomic actions of progestogens in the breast. *Journal of Steroid Biochemistry and Molecular Biology* **142** 62–67. (https://doi.org/10.1016/j.jsbmb.2013.08.011)

Musgrove EA, Lee CS & Sutherland RL 1991 Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-fos, and c-myc genes. *Molecular and Cellular Biology* **11** 5032–5043. (https://doi.org/10.1128/MCB.11.10.5032)

Nilsson S, Moller C, Jirstrom K, Lee A, Busch S, Lamb R & Landberg G 2012 Downregulation of miR-92a is associated with aggressive breast cancer features and increased tumour macrophage infiltration. *PLoS ONE* **7** e36051. (https://doi.org/10.1371/journal.pone.0036051)

Olivotto IA, Truong PT, Speers CH, Bernstein V, Allan SJ, Kelly SJ & Lesperance ML 2004 Time to stop progesterone receptor testing in breast cancer management. *Journal of Clinical Oncology* **22** 1769–1770. (https://doi.org/10.1200/JCO.2004.99.251)

Patel B, Elguero S, Thakore S, Dahoud W, Bedaiwy M & Mesiano S 2015 Role of nuclear progesterone receptor isoforms in uterine pathophysiology. *Human Reproduction Update* **21** 155–173. (https://doi.org/10.1093/humupd/dmu056)

Pathiraja TN, Shetty PB, Jelinek J, He R, Hartmaier R, Margossian AL, Hilsenbeck SG, Issa JP & Oesterreich S 2011 Progesterone receptor isoform-specific promoter methylation: association of PRA promoter methylation with worse outcome in breast cancer patients. *Clinical Cancer Research* **17** 4177–4186. (https://doi.org/10.1158/1078-0432.CCR-10-2950)

Poole AJ, Li Y, Kim Y, Lin SC, Lee WH & Lee EY 2006 Prevention of Brca1-mediated mammary tumorigenesis in mice by a progesterone antagonist. *Science* **314** 1467–1470. (https://doi.org/10.1126/science.1130471)

Presman DM, Ganguly S, Schiltz RL, Johnson TA, Karpova TS & Hager GL 2016 DNA binding triggers tetramerization of the glucocorticoid receptor in live cells. *PNAS* **113** 8236–8241. (https://doi.org/10.1073/pnas.1606774113)

Qiu M & Lange CA 2003 MAP kinases couple multiple functions of human progesterone receptors: degradation, transcriptional synergy, and nuclear association. *Journal of Steroid Biochemistry and Molecular Biology* **85** 147–157. (https://doi.org/10.1016/S0960-0760(03)00221-8)

Renthal NE, Williams KC, Montalbano AP, Chen CC, Gao L & Mendelson CR 2015 Molecular regulation of parturition: a myometrial perspective. *Cold Spring Harbor Perspectives in Medicine* **5** a023069. (https://doi.org/10.1101/cshperspect.a023069)

Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM & Horwitz KB 2002 Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *Journal of Biological Chemistry* **277** 5209–5218. (https://doi.org/10.1074/jbc.M110090200)

Rojas PA, May M, Sequeira GR, Elia A, Alvarez M, Martinez P, Gonzalez P, Hewitt S, He X, Perou CM, *et al.* 2017 Progesterone receptor isoform ratio: a breast cancer prognostic and predictive factor for antiprogestin responsiveness. *Journal of the National Cancer Institute* **109** djw317. (https://doi.org/10.1093/jnci/djw317)

Sampayo R, Recouvreux S & Simian M 2013 The hyperplastic phenotype in PR-A and PR-B transgenic mice: lessons on the role of estrogen and progesterone receptors in the mouse mammary gland and breast

cancer. *Vitamins and Hormones* **93** 185–201. (https://doi.org/10.1016/B978-0-12-416673-8.00012-5)

Sartorius CA, Groshong SD, Miller LA, Powell RL, Tung L, Takimoto GS & Horwitz KB 1994*a* New T47D breast cancer cell lines for the independent study of progesterone B- and A-receptors: only antiprogestin-occupied B-receptors are switched to transcriptional agonists by cAMP. *Cancer Research* **54** 3868–3877.

Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS & Horwitz KB 1994*b* A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. *Molecular Endocrinology* **8** 1347–1360. (https://doi.org/10.1210/mend.8.10.7854352)

Sartorius CA, Shen T & Horwitz KB 2003 Progesterone receptors A and B differentially affect the growth of estrogen-dependent human breast tumor xenografts. *Breast Cancer Research and Treatment* **79** 287–299. (https://doi.org/10.1023/A:1024031731269)

Scarpin KM, Graham JD, Mote PA & Clarke CL 2009 Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. *Nuclear Receptor Signaling* **7** e009. (https://doi.org/10.1621/nrs.07009)

Schneider W, Ramachandran C, Satyaswaroop PG & Shyamala G 1991 Murine progesterone receptor exists predominantly as the 83-kilodalton 'A' form. *Journal of Steroid Biochemistry and Molecular Biology* **38** 285–291. (https://doi.org/10.1016/0960-0760(91)90099-Q)

Schoonen WG, Dijkema R, de Ries RJ, Wagenaars JL, Joosten JW, de Gooyer ME, Deckers GH & Kloosterboer HJ 1998 Human progesterone receptor A and B isoforms in CHO cells. II. Comparison of binding, transactivation and ED50 values of several synthetic (anti)progestagens in vitro in CHO and MCF-7 cells and in vivo in rabbits and rats. *Journal of Steroid Biochemistry and Molecular Biology* **64** 157–170. (https://doi.org/10.1016/S0960-0760(97)00161-1)

Shafaee MN & Ellis MJ 2017 Breast cancer patient-derived xenografts: pros, cons, and next steps. *Journal of the National Cancer Institute* **109** djw307. (https://doi.org/10.1093/jnci/djw307)

Shen T, Horwitz KB & Lange CA 2001 Transcriptional hyperactivity of human progesterone receptors is coupled to their ligand-dependent down-regulation by mitogen-activated protein kinase-dependent phosphorylation of serine 294. *Molecular and Cellular Biology* **21** 6122–6131. (https://doi.org/10.1128/MCB.21.18.6122-6131.2001)

Shen T, Brandwein-Gensler M, Hameed O, Siegal GP & Wei S 2015 Characterization of estrogen receptor-negative/progesterone receptor-positive breast cancer. *Human Pathology* **46** 1776–1784. (https://doi.org/10.1016/j.humpath.2015.07.019)

Shyamala G, Yang X, Cardiff RD & Dale E 2000 Impact of progesterone receptor on cell-fate decisions during mammary gland development. *PNAS* **97** 3044–3049. (https://doi.org/10.1073/pnas.97.7.3044)

Singhal H, Greene ME, Tarulli G, Zarnke AL, Bourgo RJ, Laine M, Chang YF, Ma S, Dembo AG, Raj GV, *et al.* 2016 Genomic agonism and phenotypic antagonism between estrogen and progesterone receptors in breast cancer. *Science Advances* **2** e1501924. (https://doi.org/10.1126/sciadv.1501924)

Singhal H, Greene ME, Zarnke AL, Laine M, Al Abosy R, Chang YF, Dembo AG, Schoenfelt K, Vadhi R, Qiu X, *et al.* 2018 Progesterone receptor isoforms, agonists and antagonists differentially reprogram estrogen signaling. *Oncotarget* **9** 4282–4300. (https://doi.org/10.18632/oncotarget.21378)

Skildum A, Faivre E & Lange CA 2005 Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases. *Molecular Endocrinology* **19** 327–339. (https://doi.org/10.1210/me.2004-0306)

Taylor D, Pearce CL, Hovanessian-Larsen L, Downey S, Spicer DV, Bartow S, Pike MC, Wu AH & Hawes D 2009 Progesterone and estrogen receptors in pregnant and premenopausal non-pregnant normal human breast. *Breast Cancer Research and Treatment* **118** 161–168. (https://doi.org/10.1007/s10549-009-0322-4)

Tung L, Abdel-Hafiz H, Shen T, Harvell DM, Nitao LK, Richer JK, Sartorius CA, Takimoto GS & Horwitz KB 2006 Progesterone receptors (PR)-B and -A regulate transcription by different mechanisms: AF-3 exerts regulatory control over coactivator binding to PR-B. *Molecular Endocrinology* **20** 2656–2670. (https://doi.org/10.1210/me.2006-0105)

Vazquez SM, Mladovan A, Garbovesky C, Baldi A & Luthy IA 2004 Three novel hormone-responsive cell lines derived from primary human breast carcinomas: functional characterization. *Journal of Cellular Physiology* **199** 460–469. (https://doi.org/10.1002/jcp.10466)

Wang RA, Li ZS, Zhang HZ, Zheng PJ, Li QL, Shi JG, Yan QG, Ye J, Wang JB, Guo Y, *et al.* 2013 Invasive cancers are not necessarily from preformed in situ tumours – an alternative way of carcinogenesis from misplaced stem cells. *Journal of Cellular and Molecular Medicine* **17** 921–926. (https://doi.org/10.1111/jcmm.12078)

Wargon V, Helguero LA, Bolado J, Rojas P, Novaro V, Molinolo A & Lanari C 2009 Reversal of antiprogestin resistance and progesterone receptor isoform ratio in acquired resistant mammary carcinomas. *Breast Cancer Research and Treatment* **116** 449–460. (https://doi.org/10.1007/s10549-008-0150-y)

Wargon V, Fernandez SV, Goin M, Giulianelli S, Russo J & Lanari C 2011 Hypermethylation of the progesterone receptor A in constitutive antiprogestin-resistant mouse mammary carcinomas. *Breast Cancer Research and Treatment* **126** 319–332. (https://doi.org/10.1007/s10549-010-0908-x)

Wargon V, Riggio M, Giulianelli S, Sequeira GR, Rojas P, May M, Polo ML, Gorostiaga MA, Jacobsen B, Molinolo A, *et al.* 2015 Progestin and antiprogestin responsiveness in breast cancer is driven by the PRA/PRB ratio via AIB1 or SMRT recruitment to the CCND1 and MYC promoters. *International Journal of Cancer* **136** 2680–2692. (https://doi.org/10.1002/ijc.29304)

Wei LL, Gonzalez-Aller C, Wood WM, Miller LA & Horwitz KB 1990 5′-Heterogeneity in human progesterone receptor transcripts predicts a new amino-terminal truncated 'C'-receptor and unique A-receptor messages. *Molecular Endocrinology* **4** 1833–1840. (https://doi.org/10.1210/mend-4-12-1833)

Wen DX, Xu YF, Mais DE, Goldman ME & McDonnell DP 1994 The A and B isoforms of the human progesterone receptor operate through distinct signaling pathways within target cells. *Molecular and Cellular Biology* **14** 8356–8364. (https://doi.org/10.1128/MCB.14.12.8356)

Wu J, Richer J, Horwitz KB & Hyder SM 2004 Progestin-dependent induction of vascular endothelial growth factor in human breast cancer cells: preferential regulation by progesterone receptor B. *Cancer Research* **64** 2238–2244. (https://doi.org/10.1158/0008-5472.CAN-03-3044)

Yeates C, Hunt SM, Balleine RL & Clarke CL 1998 Characterization of a truncated progesterone receptor protein in breast tumors. *Journal of Clinical Endocrinology and Metabolism* **83** 4600–4467. (https://doi.org/10.1210/jcem.83.2.4531)

Zhang X, Podsypanina K, Huang S, Mohsin SK, Chamness GC, Hatsell S, Cowin P, Schiff R & Li Y 2005 Estrogen receptor positivity in mammary tumors of Wnt-1 transgenic mice is influenced by collaborating oncogenic mutations. *Oncogene* **24** 4220–4231. (https://doi.org/10.1038/sj.onc.1208597)