Annals of Diagnostic Pathology 35 (2018) 21–26

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Analytical and clinical performance of progesterone receptor antibodies in breast cancer

Benjamin C. Calhoun[a,*], Brian Mosteller[b], Daniel Warren[b], Margie Smith[b], J. Jordi Rowe[a], Christopher P. Lanigan[a], Karen C. Mrazeck[a], Espen Walker[b], Amy Hanlon Newell[b], Raymond Jones[b,**]

[a] Department of Pathology, Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH, USA
[b] Ventana Medical Systems, Inc., Tucson, AZ, USA

ARTICLE INFO

Keywords:
Progesterone receptor
Hormone receptor
Breast cancer
Diagnosis
Pathology
Monoclonal antibody
Clone 1E2
Clone 636
Clone 1A6
Clone 16

ABSTRACT

Objective: Comparison of analytical and immunohistochemical performance of progesterone receptor (PR) antibodies with correlation to recurrence of invasive breast cancer treated with endocrine therapy.
Methods: The binding-affinity kinetics of PR clones 1E2, 1A6, 16 and 636 were compared using synthetic peptides derived from identified epitopes on a Biacore T200. A cohort of 351 cases (Hormone Receptor (HR) +/HER2−) were stained for PR expression with immunohistochemistry (IHC) and scored according to ASCO/CAP criteria.
Results: The stability of the antigen/antibody complex was greater for the 1E2 clone compared to 1A6, 16 and 636 clones. PR IHC on archival tissue resulted in 94.3% (299/317) concordance with clones.
Conclusion: Clones evaluated in this study had a high level of concordance with IHC despite PR (1E2) demonstrating higher analytical binding properties than other clones. In a minority of cases (1.3% for 1E2 and 2.5% for 636) IHC results could convert estrogen receptor (ER)−/PR− to ER−/PR+ tumors, making these patients potentially eligible for endocrine therapy.

1. Introduction

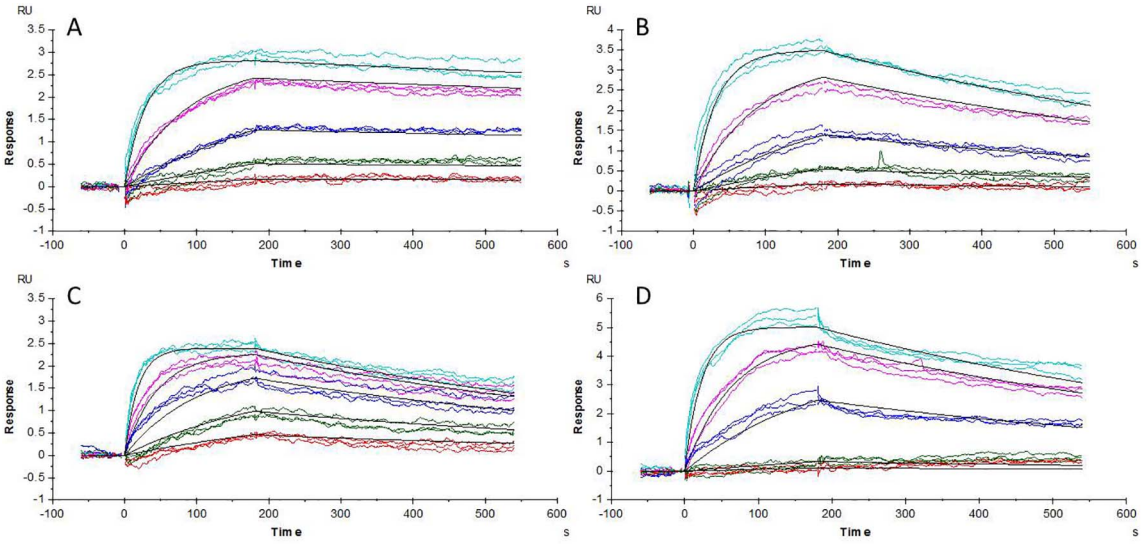
Approximately 75% of breast cancers are ER-positive and 65% are PR-positive [1]. HR IHC is part of the routine evaluation of newly diagnosed invasive-breast carcinoma [2]. Several studies support the importance of PR as a prognostic marker in breast cancer in general [3-7] and specifically, in ER-positive breast cancer [3,8-13]. IHC for ER and PR is also predictive of benefit from endocrine therapy with selective estrogen receptor modulators (SERMs), such as Tamoxifen (TAM), or aromatase inhibitors (AI) in patients with HR-positive tumors [14,15]. The main determinant of eligibility for a SERM or AI is typically ER expression. However, some studies suggest that PR is an independent predictor of outcome in patients treated with endocrine therapy [3,10,16,17]. Conversely, in a large study of AI versus TAM in ER+ breast cancer, PR expression was associated with prognosis, but not with benefit from treatment with an AI [18]. PR expression is often inversely correlated with HER2 expression [19,20] and the absence of PR in ER-positive tumors may be an indicator of aberrant growth factor signaling that may be related to resistance to endocrine therapy [21,22]. However, a large meta-analysis of 20 clinical trials including over 20,000 patients showed that PR was not a useful predictor of response to TAM in ER-positive breast cancer [14].

The current recommendation in the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) guidelines are that all newly diagnosed invasive breast cancers should be tested for both hormone receptors with a cutoff of 1% for positive results for either ER or PR [2]. Prior to the publication of the guidelines in 2010, there were several reports of false negative and false positive IHC results for hormone receptors [23,24].

The majority of PR antibody clones reportedly react with the A and B isoforms of the receptor and routine immunohistochemical studies provide an assessment of total PR protein [25]. The ratio of PRA to PRB (PRA:PRB ratio) is known to be higher in tumor with a worse prognosis [26]. A recent retrospective immunofluorescence IHC study of specimens from the TransATAC trial [27] found, that a high PRA:PRB ratio predicted earlier relapse in women treated with a SERM but not with an AI [28]. PR expression may be an indicator of intact ER-signaling pathways and the absence of PR may be related to aberrant growth

* Department of Pathology and Laboratory Medicine, University of North Carolina - Chapel Hill, 160 North Medical Drive, Campus Box #7525, Chapel Hill, NC 27599-7525, USA.
** Correspondence to: R. Jones, Systems Integration, Ventana Medical Systems, Inc., 1910 E. Innovation Park Dr., Tucson, AZ 85755, USA.
E-mail addresses: ben.calhoun@unchealth.unc.edu (B.C. Calhoun), raymond.jones.rj2@roche.com (R. Jones).

https://doi.org/10.1016/j.anndiagpath.2018.02.007



Antibody	Kon (1/Ms)	SE (ka)	Koff (1/s)	SE (kd)	Rmax (RU)	SE (Rmax)	KD (nM)	Half life
Ventana 1E2	2.25E+05	2.10E+02	2.67E-04	1.80E-06	2.8	0.0013	1.2	43
Dako 636	2.05E+05	2.20E+02	1.34E-03	2.30E-06	3.6	0.0018	6.5	9
Leica 1A6	1.40E+05	1.90E+02	1.35E-03	2.70E-06	5.2	0.0029	9.7	9
Leica 16	5.75E+05	8.50E+02	1.51E-03	3.10E-06	2.5	0.0016	2.6	8

Fig. 1. Specific binding curves for the PR antibodies and their associated peptides. The upward slope (K_{on}) indicates the rate at which the antibody binds the peptide. Top of the curve indicates equilibrium. Downward slope is an indicator of dissociation (K_{off}). Each color is a different concentration of peptide. (A) clone 1E2 (B) clone 636 (C) clone 16 (D) clone 1A6. Kinetic coefficients, binding quantities, and standard errors for all PR clones shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

factor signaling or promoter methylation [21,22]. Methylation of the PRA promoter has been associated with worse outcomes in patients treated with a SERM. In current practice, laboratories are not called upon to provide isoform-specific data for ER or PR and the role of hormone receptor isoforms in breast cancer remains an area of active investigation. While none of the studied antibodies discern isoform A from B, this suggests the importance of evaluation of PR expression in breast cancers.

Several PR antibody clone comparison studies have shown variability in the performance of commercially available antibody clones [29–31], with recent studies showing concordance between the PR clones despite tumor heterogeneity of PR expression [32,33]. Multiple isoforms of PR have been identified and most commercially available antibody clones recognize PRA and PRB [34] (Supplemental Table 1). The potential for false-negative results in patients who may otherwise benefit from endocrine therapy highlights the need for robust quality assurance schemes to assure inter-laboratory reproducibility for HR IHC testing [35,36].

Since ER/PR status determines the treatment regimen for new breast cancer cases, performance of both antibodies are valuable. Post translational modifications to PR can lead to variation in antibody binding and protein-protein interactions [37]. Binding studies of estrogen have elucidated the mechanisms for receptor formation [38] and alterations due to the presence of ER agonists/antagonists [39,40]. These findings support the need to assess the analytical performance of antibodies intended for clinical use. Analytical characterization techniques, including the measurement of binding kinetics via surface plasmon resonance are well documented in the literature [41–43]. The goal of this study was to compare IHC results for commercially available PR clones and to explore potential differences in sensitivity in a well-characterized cohort of breast cancer cases with outcome data to determine whether clone selection influences PR IHC testing results.

2. Materials and methods

2.1. Epitope mapping of PR clones

Epitope sequences for clones 16, 1E2, 636 and 1A6 were determined through peptide sequence analysis performed by JPT and NimbleGen. In brief, 13–16 amino acid peptide sequences comprising PR isoform B, in single amino acid shifts were queried using the four different clones. Antibodies bound to the peptide array were tagged with species specific, fluorophore conjugated secondary antibodies. In turn these were detected via fluorescent signal. Alignment of the bound peptides was used to determine the overlapping region of bound peptide sequence. The overlapping sequence is representative of the epitope in the peptide.

2.2. Affinity assay

Kinetic analysis was performed on a Biacore T200 using Series S CM5 sensor chips (GE Healthcare, BR100530) with the Amine Coupling kit (GE Healthcare, BR100050). The rabbit antibody capture surface was prepared to a target immobilization level of 15,000 RU with 50 ng/mL goat anti-rabbit IgG Fc (Jackson Labs, 111-005-046) in 10 mM Acetate pH 5. Conditioning was performed with 50 nM whole molecule rabbit IgG (Jackson Labs, 011-000-003) for 120 s at 75 μL/min for five cycles. Surface regeneration was accomplished using a four step process: 15 s of HBSN (10 mM HEPES and 150 mM NaCl) + 0.5% Tween (Sigma, P9416) at 20 μL/min, 20 s of Glycine pH 1.5 (GE Healthcare,