Gynecologic Oncology 138 (2015) 663–667

Contents lists available at ScienceDirect

Gynecologic Oncology

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

Development of a technique to detect the activated form of the progesterone receptor and correlation with clinical and histopathological characteristics of endometrioid adenocarcinoma of the uterine corpus

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HIGHLIGHTS

- Hormonal therapy use for endometrial cancer treatment is rare.
- Current diagnostics cannot reliably identify potential responders to hormonal therapy.
- A modified diagnostic technique may be able to identify potential responders to anti-progestin therapy.

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 25 June 2015
Accepted 26 June 2015
Available online 2 July 2015

Keywords:
Endometrial cancer
Activated progesterone receptor
Onapristone
Progestin
Anti-progestin

ABSTRACT

Objective. Hormonal therapy is generally reserved for patients with endometrial cancers that fail cytotoxic chemotherapy, but there is a lack of sufficiently sensitive diagnostics to identify potential responders. We sought to develop a diagnostic technique to detect activated progesterone receptors (APR) in endometrial cancers using routine immunohistochemistry (IHC) and to correlate the presence of APR with other histopathological features and clinical disease stage.

Methods. Seventy-two tumor block specimens from patients with endometrial cancer were processed with conventional IHC methods for estrogen receptor-α (ERα), progesterone receptor (PR) and Ki67, a marker of proliferation. Tumor specimens were analyzed for the PR nuclear distribution patterns in individual tumor cells: APR positive (APR^{pos}) tumors were prospectively defined as any tumor with >5% countable malignant cells with an aggregated nuclear pattern. Tumor APR status was analyzed against other biomarkers including ERα expression, Ki67 and tumor grade.

Results. Fifty-six of 72 samples were endometrioid. Twenty-six of 49 PR-positive endometrioid tumors (53%; 95% CI 39–67%) were APR^{pos}. Percent of ER^{pos} cells correlated with % PR^{pos} malignant cells ($p = 0.001$, rho = 0.44). APR positivity did not correlate with % PR^{pos} cells in a given tumor, nor did it correlate with % Ki67 positivity; APR positivity was independent of disease stage and tumor grade (p = NS).

Conclusions. In this study, approximately half of endometrioid tumors were APR^{pos}. APR is independent of histopathological and other known risk factors. Refining conventional PR detection has the potential to prospectively identify patients with endometrial cancer who may benefit from anti-progestin therapy.

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http://dx.doi.org/10.1016/j.ygyno.2015.06.037
0090-8258/

1. Introduction

Endometrial carcinoma is the third most common cause of gynecologic cancer death, behind ovarian and cervical cancer, and accounted for 319,605 new cases and 76,155 deaths worldwide in 2012 [1]. In many societies, the incidence is increasing due to the growing prevalence of obesity and an aging population. The most common histology is endometrioid adenocarcinoma, with the majority being estrogen (ERα) and progesterone (PR) receptor positive [2]. Most cases of endometrioid cancers are cured at an early stage [3]. However, when diagnosed in more advanced stages, or if recurrent, endometrioid cancer is generally fatal: half of all patients with stage III disease, and 85% of those with stage IV disease, die within 5 years of diagnosis [3]. The standard treatment of metastatic disease is usually cytotoxic (carboplatin and paclitaxel) and the results of this are poor. Because adjuvant chemotherapy is used in high risk patients, hormonal therapy is generally reserved for later use. Importantly, hormonal therapy is better tolerated than chemotherapy and with the appropriate biomarker, might be used preferentially to chemotherapy. There are few curative options for patients with recurrent disease, highlighting a clear unmet medical need for novel therapeutics [4].

In endometrial cancer the agonistic action of progesterone exerts anti-tumor effects [5]. The actions of both progestins and antiprogestins are mediated in target tissues via the nuclear PR, which belongs to the steroid receptor family [6–9]. There are two PR isoforms: PRA and PRB, which originate from two separate promoter sites located on the single PR gene [10]. The PR is a ligand-activated transcription factor that plays a key role in hormonally-regulated tissues, such as breast and endometrium [8]. Upon progestin ligand binding, PR undergoes a sequence of conformational changes: it dimerizes and translocates to the nucleus, where it interacts with cofactors to form a complex (Fig. 1a) [11]. This complex becomes functional and binds to specific DNA promoter sequences of PR-dependent genes, termed Progesterone Response Elements (PREs) [12–14]. When not bound to a ligand, PR is distributed in a diffuse (D) pattern across the nucleus (Fig. 1bi) [15]. Using immunofluorescence, ligand-bound PR can be seen as a distinctive aggregated (A) pattern of discrete sub-nuclear foci in normal (Fig. 1bii) and malignant endometrial cells (Fig. 1biii and biv) [15]. PR foci tend to be larger in endometrial cancers, with a median length greater than 1.0 μm, whereas PR foci in normal tissue have a median length of 0.65 μm [11]. PR foci co-localize with nascent RNA transcripts and transcriptional inhibitors prevent their formation, showing that they are sites of active transcription involving PR (Fig. 1a) [11].

Progesterone is thought to oppose estrogen effects in the endometrium, and this is the basis of using synthetic progestins to treat endometrial cancer. This action is likely to be mediated by effects on stromal cells, as recombination studies have shown that stromal PR is required for progesterone to inhibit the proliferation of epithelial cells [16]. Response to progestins has been correlated with PR positivity, although not all PR-positive endometrial tumors respond to treatment with progestins [16]. Progestin agonists are more active in dysplasia or low-grade tumors and their activity declines in higher grade or later stage tumors [16]. Well-differentiated, advanced/recurrent disease, treated with progestins as second line therapy has exhibited response rates of 18–25% [17]. Despite their activity, progestin agonists are not commonly used in the treatment of endometrial cancer [5]. This may be in part due to the lack of a sufficiently sensitive diagnostic technique available to identify patients a priori that are likely to respond to hormone therapy [5]. This highlights a clear need to identify reliable biomarkers in patients with recurrent and advanced endometrial cancer in order to select the most appropriate treatment and improve outcomes [5]. Currently there is no diagnostic test to identify the activated form of the steroid nuclear receptors, and steroid therapies are prescribed based on the presence of ER or PR. Identification of APR^{pos} tumors would enable personalized anti-progestin therapy.

APR indicates that the PR present in cells is in a transcriptionally active state. Thus, APR detection on malignant cells is likely to suggest direct involvement of active PR in the biology of these cells. Previous studies have observed the presence of APR in a significant subpopulation of postmenopausal breast cancers, and in the epithelial cells of both normal and malignant endometrial tissues [11,15]. The presence of APR in endometrioid tumors would indicate that there is a strong rationale for investigating the potential clinical benefit of anti-progestins. The detection of APR could in the future be used as a biomarker to select patients more likely to respond to anti-progestin treatment. However, immunofluorescence methods of detecting APR are not amenable to

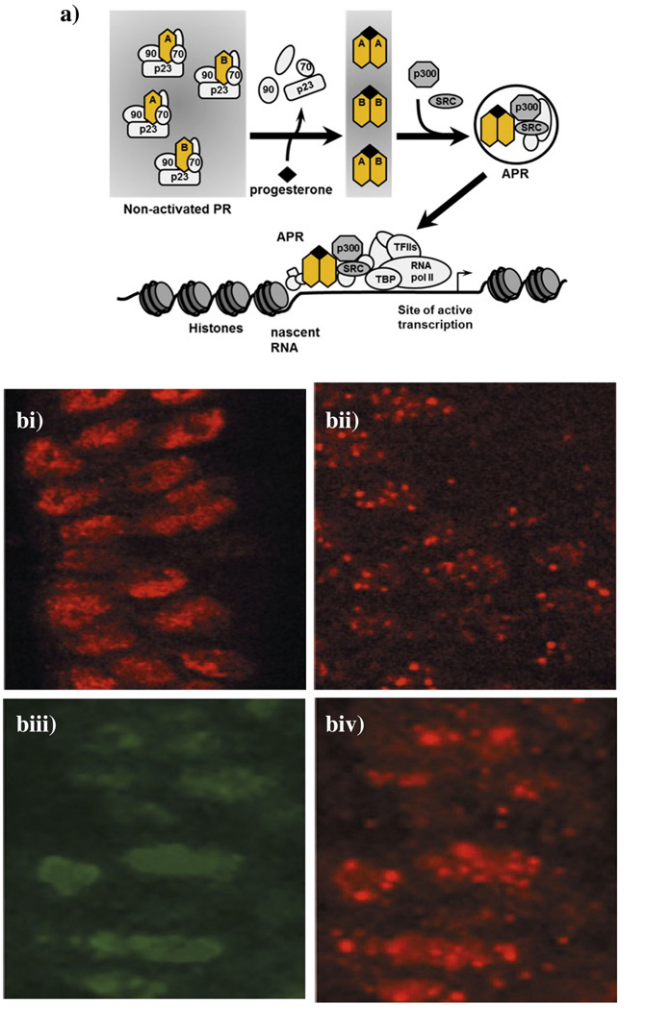

Fig. 1. a. Subnuclear distribution and activation of PR isoforms. Schematic representation of cell nucleus and PR nuclear distribution. A, PRA; B, PRB. Unliganded PR is evenly distributed in the nucleus and associates with heat shock proteins and other chaperones. Upon binding of ligand, chaperones dissociate, and PR forms dimers and is distributed into nuclear foci. The transcriptional factor p300 colocalizes with PR in nuclear foci, inhibition of SRC-1 recruitment abolishes foci, and foci associate with nascent RNA in the presence of ligand, indicating that subnuclear distribution of PR into foci is required for active transcription. Reproduced from Arnett-Mansfield 2007 [15]. b. PRA and PRB distribution in the normal endometrium during the menstrual cycle and endometrial cancer. i) PRB follicular, normal endometrium. ii) Luteal, normal endometrium. iii) PRA, endometrial cancer. iv) PRB endometrial cancer. PRA and PRB distribution in the normal endometrium during the menstrual cycle and endometrial cancer. PR isoform distribution was determined by dual immunofluorescence IHC and confocal microscopy in the glands of the normal endometrium. i, PR distribution in the proliferative phase of the menstrual cycle, endometrial gland with nuclei containing predominately even PRA and PRB distribution; Texas red (PRB). ii, PR distribution in the secretory phase of the menstrual cycle, endometrial gland with nuclei predominantly containing PR localized into discrete foci; Texas red (PRB). iii, Endometrial cancer expressing PRA and PRB; fluorescein isothiocyanate (PRA). iv, Endometrial cancer expressing PRA and PRB; Texas red (PRB). Reproduced from Arnett-Mansfield 2004 [11].