ELSEVIER

Urologic Oncology: Seminars and Original Investigations ▮ (2017) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Original article

Synaptophysin expression on circulating tumor cells in patients with castration resistant prostate cancer undergoing treatment with abiraterone acetate or enzalutamide

Sumanta K. Pal, M.D.[a,1], Miaoling He, M.S.[a,1], Lin Chen, M.S.[b], Lixin Yang, M.S.[b], Raju Pillai, M.D.[b], Przemyslaw Twardowski, M.D.[a], JoAnn Hsu, M.S.[a], Marcin Kortylewski, Ph.D.[c], Jeremy O. Jones, Ph.D.[a,*]

[a] *Departement of Medical Oncology, City of Hope Comprehensive Cancer Center, Duarte, CA*
[b] *Department of Pathology, City of Hope Comprehensive Cancer Center, Duarte, CA*
[c] *Department of Cancer Immunotherapeutics & Tumor Immunology, City of Hope Comprehensive Cancer Center, Duarte, CA*

Received 3 August 2017; received in revised form 10 November 2017; accepted 11 December 2017

Abstract

Background: With the advent of secondary androgen receptor (AR)-targeted therapies in metastatic castration resistant prostate cancer (PC), nonadenocarcinoma PCs are becoming more prevalent. Many of these cancers express neuroendocrine markers, which may provide biomarkers for emergence of this disease state. We aimed to quantify the expression of synaptophysin (Syp) on circulating tumor cells (CTCs) from serial samples of patients being treated with abiraterone acetate or enzalutamide.

Methods: CTCs were isolated from 44 patients with castration resistant PC before starting abiraterone or enzalutamide, at 4, 8, and 12 weeks on therapy, and at progression. Patients were stratified into 3 groups: *de novo* resistance, short response, and long response. CTCs were enumerated on the CellSearch platform and Syp expression was quantified using the open fluorescent channel on the platform. Correlative analyses were performed.

Results: A baseline CTC count of 5 or greater was associated with a more rapid time to progression and increasing CTC counts correlated with emergence of drug resistance. Syp was readily detectable on the surface of CTCs, and baseline percentage CTC Syp expression was significantly associated with time to progression. Furthermore, in evaluable patients, percent CTC Syp expression increased with the emergence of drug resistance. We also found that prior exposure to AR-targeted therapies was inversely associated with progression free survival.

Conclusions: We have demonstrated that Syp can be quantified on CTCs and that Syp expression correlates with resistance to abiraterone and enzalutamide. Larger studies testing Syp as a biomarker of emergence of nonadenocarcinoma disease and as a marker of response to AR-targeted therapies are warranted.

Keywords: Prostate cancer; Circulating tumor cell; Androgen receptor; Synaptophysin; Abiraterone; Enzalutamide

1. Introduction

Two new androgen receptor (AR)-targeted therapies, enzalutamide and abiraterone acetate, have been approved for treatment of metastatic prostate cancer (PC) and more are in clinical trials [1]. Many patients respond to these agents, but both *de novo* and acquired resistance are common. Although resistance continues to be mediated by AR signaling in some patients, emerging evidence suggests that the strong AR inhibition mediated by these drugs is

Funding: Research reported in this publication included work performed in core facilities supported by the National Cancer Institute, United States of the National Institutes of Health, United States under award number P30CA033572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

[1] Both authors contributed equally to this work.

* Corresponding author. Tel.: +1-626-256-4673x80270; fax: +1-626-471-3902.

E-mail address: jjones@coh.org (J.O. Jones).

https://doi.org/10.1016/j.urolonc.2017.12.006
1078-1439/

giving rise to an increasing number of patients with truly AR-independent disease, often typified by a small cell appearance and expression of neuroendocrine (NE) markers [2,3]. Synaptophysin (Syp) is one of the most often used and pertinent markers of NEPC [3–5]. Although recent studies have suggested that there are multiple intermediate phenotypes of cells leading to NE disease, including variable AR expression, Syp expression is critical in defining the final transdifferentiated NE state [6,7]. These data suggest that expression of Syp on tumors is the ideal marker for the emergence of NE disease. Therefore, we have created an assay for identifying emergence of NE disease using Syp expression on circulating tumor cells (CTCs).

CTCs provide a powerful alternative to biopsies for determining the molecular characteristics of cancers that emerge following development of resistance. The presence of ≥5 CTCs measured with the CellSearch platform [8] is associated with poor survival in patients with mCRPC. Furthermore, increases in the number of CTCs following treatment with AR-targeted agents, abiraterone or enzalutamide, appears to be indicative of a lack of clinical response [9]. Such association with clinical outcome suggests that CTCs play an important role in metastatic process. In this study, we used the open channel on the CellSearch platform to quantify expression of Syp on CTCs from patients undergoing treatment with enzalutamide or abiraterone and found a correlation between increasing Syp-positive CTCs and the emergence of drug resistance.

2. Materials and methods

2.1. Patient selection

44 patients were enrolled between April 2014 and November 2016. To be eligible for this study, patients must have had a cytologically or pathologically verified diagnosis of PC, and radiographic evidence of metastatic disease. Patients must be defined by the clinician as having CRPC, typically constituting failure of combined androgen blockade with leuprolide and bicalutamide followed by antiandrogen withdrawal. With appropriate counseling regarding treatment options, patients must have opted to receive either abiraterone or enzalutamide with an anticipated monitoring plan that included serial collections of prostrate specific antigens (PSAs) simultaneous with collection of blood for correlative studies.

In line with Prostate Cancer Working Group 3 (PCWG3) guidelines [10], 3 cohorts of patients were identified: (1) patients who have primary or *de novo* resistance (Group A), patients who have a short response to treatment (Group B), and patients with an exceptional response (Group C). No firm criteria exist for delineating these groups; we therefore, propose that Groups A to C can be defined by the duration of abiraterone or enzalutamide being <3 months, 3 to 12 months, and >12 months, respectively. As emphasized in the guidelines, patients were maintained on abiraterone or enzalutamide until no longer clinically benefitting.

2.2. CTC enumeration and analysis of Syp expression

Blood from eligible patients with mCRPC were collected into CellSave tubes at baseline, at weeks 4, 8, and 12 of therapy, and at the time of progression. The standard CellSearch platform was used for enumeration by a trained operator. The CellSearch platform maintains an open channel for assessment of an additional marker on the CTCs, which was used to assess the expression of a FITC-conjugated Syp antibody (Biorbyt 16373). Staining and exposure times were optimized using HEK293 cells transfected with a Syp expression vector (Harvard repository clone ID# HsCD00338768) spiked into normal donor blood samples. The optimal exposure time was found to be 0.8 seconds and the optimal antibody dilution was 1:50. Positive events were determined by the trained CellSearch operator (L.C.). To test the accuracy of the assay, 150 untransfected and 150 Syp-transfected HEK293 cells were spiked into 7.5 ml of healthy donor blood in a CellSave tube. 264 CTCs were identified and 48.86% of them were determined to be SYP+, suggesting our optimized staining procedure was highly accurate (Fig. 1). Furthermore, a sampling of three patients demonstrated that no DAPI+/CD45+/CK− cells (leukocytes) were Syp positive, suggesting that background staining of contaminating white blood cells is absent.

2.3. Statistical analyses

A Kruskal-Wallis test was performed to test the association between baseline PSA or CTC count with group membership. A log-rank test was used to determine differences between CTC groups and time to progression (TTP) whereas regression analysis was used to determine an association between Syp-positivity and TTP; multivariate regression analysis was used to determine an association between Syp-positivity and TTP while controlling for age, baseline PSA, and baseline CTC count. A chi-squared test was used to determine differences between CTC Syp-positivity as a categorical variable and group membership.

3. Results

3.1. CTC numbers correlate with response to abiraterone and enzalutamide

The baseline characteristics of patients and treatments are shown in Table 1. Three general response patterns to abiraterone and enzalutamide have emerged in our practice: *de novo* resistance, typically with no decrease in PSA levels and progressive disease, short-term response, with only 1 or