Experimental Hematology

Experimental Hematology 2017;49:34–38

Androgen receptor expression in mantle cell lymphoma: Potential novel therapeutic implications

Elahe A. Mostaghel[a,b], Paul S. Martin[a,b], Stephen Mongovin[a], Shani Frayo[a], Ailin Zhang[a], Kerstin L. Edlefsen[c], Oliver W. Press[a,b], and Ajay K. Gopal[a,b]

[a]*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA;* [b]*Division of Medical Oncology, Department of Medicine, University of Washington, Seattle, WA, USA;* [c]*Department of Laboratory Medicine, University of Washington, Seattle, WA, USA*

(Received 14 October 2016; revised 9 January 2017; accepted 11 January 2017)

Mantle cell lymphoma (MCL) affects approximately 4500 patients/year in the US and demonstrates a male to female ratio of approximately 4:1. While the pathobiology underlying this ratio is unknown, the hematopoietic system is characterized by sex-related differences in androgen receptor (AR) expression, leading us to hypothesize that the male-biased incidence of MCL may reflect sex-related differences in AR signaling during MCL lymphomagenesis. To explore the AR axis in MCL, we evaluated AR expression in MCL cell lines and human tumors, and tested the impact of androgen pathway inhibition on MCL proliferation. AR transcript levels ranged up to ~2^6 fold higher in MCL lines vs non-MCL NHL lines ($p = 0.006$) and were correlated with expression of the canonical AR-regulated gene, prostate-specific antigen (PSA; $r = 0.715$, $p = 0.001$), consistent with functional AR activity. Patient-derived MCL samples demonstrated a range of AR expression. Treatment of four different MCL lines with the potent AR antagonist enzalutamide demonstrated suppression of proliferation across both male and female-derived cell lines. These data suggest androgen-axis blockade may represent a novel therapeutic modality in MCL. This novel treatment approach is currently under investigation in a phase II clinical trial of AR inhibition in patients with relapsed/refractory MCL.

Although mantle cell lymphoma (MCL) is characterized by a nearly 80% male predominance, the biological underpinnings of this observation are unknown [1]. Sex-steroid receptors are widely expressed in the hematopoietic system and influence hematopoietic differentiation and activity directly: androgen exposure downregulates natural killer cells/macrophages in vitro, whereas estrogen exposure enhances differentiation of antigen presenting cells and expansion of regulatory T cells [2–5]. Sex-related differences in receptor expression include higher androgen receptor (AR) expression in leukocytes and macrophages from male donors [6–8]. Notably, disease-specific differences include hypermethylation of AR in follicular and some diffuse large B-cell lymphomas (DLBCL), whereas in MCL, AR is unmethylated, allowing gene transcription [9–13].

We hypothesized that the male-biased incidence of MCL may reflect sex-related differences in AR signaling during MCL lymphomagenesis and that AR may represent a unique therapeutic target. We evaluated AR expression in MCL cell lines and human specimens and investigated whether the potent AR antagonist enzalutamide inhibited MCL proliferation.

Methods

Cell lines and human MCL specimens

All studies were approved by the institutional review board of the Hutchinson Center. Prostate cancer (PCa) and MCL cell lines were obtained from American Type Culture Collection (ATCC, Rockville, Maryland, USA). Buffy-coat RNA was isolated from three MCL patients with circulating tumor cells for analysis of AR transcript expression. Formalin-fixed samples from 12 archival MCL tumor specimens were obtained for AR immunohistochemistry (IHC).

Offprint requests to: Dr. Ajay K. Gopal, MD, Seattle Cancer Care Alliance, G3-200, 825 Eastlake Ave. East, Seattle, WA 98109; E-mail: agopal@u.washington.edu

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.exphem.2017.01.001.

0301-472X/Copyright © 2017 ISEH - International Society for Experimental Hematology. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.exphem.2017.01.001

RNA isolation, quantitative RT-PCR, and IHC

RNA isolation, quantitative real-time PCR (qRT-PCR), and IHC staining for AR (clone C-19, Santa Cruz Biotechnology, Dallas, TX) were carried out as described previously [14,15]. Fold changes were determined by the $2^{-\Delta\Delta CT}$ method [16].

Proliferation assays

Cells were brought up in serum-free medium (DMEM/F12 with 5% charcoal-stripped FBS) and incubated under standard culture conditions (37°C with 5% CO_2) for 24 hours before being plated in triplicate in serum-free medium at 0.25×10^6 cells/mL with the AR antagonist enzalutamide (10 μmol/L), the synthetic AR agonist R1881 (1 nmol/L), or the combination for 96 hours. Proliferation was quantified using the CyQUANT Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Confirmatory experiments were repeated over a dose range of enzalutamide and the influence of 10 μmol/L enzalutamide was evaluated over a dose range of R1881 using the CellTiter 96 Aqueous Cell Proliferation Assay Kit (Promega, Madison, WI, USA).

Statistical analysis

Unpaired *t* tests were used to compare AR expression in MCL versus non-MCL or PCa cell lines and to compare mean levels of proliferation in cell lines treated with enzalutamide or R1881. Correlation of AR and prostate-specific antigen (PSA) expression was assessed by Spearman rank correlation. $p < 0.05$ was considered significant.

Results and discussion

AR expression in MCL cell lines and human tumors

We quantified AR expression in seven MCL and three non-MCL B-cell non-Hodgkin's lymphoma (B-NHL) lines. The median difference in cycle threshold (dCT) for AR (normalized to the housekeeping gene RPL13A) was −12 in MCL lines versus −18 in non-MCL lines ($p = 0.006$; Fig. 1A and B), representing a 64-fold higher level. AR in MCL lines was considerably lower than in AR-positive PCa lines (LNCaP and VCaP, $p < 0.0001$), yet clearly higher than AR-negative PCa cells (PC3 and DU145, median dCT = −17, $p = 0.0048$).

Expression of the canonical AR-regulated target gene PSA (Fig. 1A) and the tight correlation of PSA with AR ($r = 0.715$, $p = 0.001$; Fig. 1C) suggest that AR expression within these MCL lines is capable of driving transactivation of target genes.

We next studied a limited number of frozen, patient-derived MCL tumor cells. Two of three patients had AR and PSA levels similar to the MCL lines, whereas one was more similar to AR-negative PCa and non-MCL NHL lines (Fig. 1D). As before, PSA and AR levels tracked together. A range of AR expression was also observed in biopsies from 12 patients (clinical characteristics are summarized in Supplementary Table E1, online only, available from www.exphem.org), ranging from no staining (patients 1–3), to predominantly cytoplasmic AR staining of uncertain significance (patients 4–7), to varying degrees of scattered nuclear AR staining (patients 8–12) (Fig. 1E). There was no consistent association between AR expression and patient sex.

Impact of androgen-axis blockade on MCL proliferation

Treatment of four AR-positive MCL lines with the potent anti-androgen enzalutamide (10 μmol/L, similar to serum levels achieved with therapeutic dosing) showed statistically significant suppression in three lines (Grants, Jeko-1, and Maver-1) with a trend toward significance in the fourth (Rec-1; $p = 0.16$), whereas AR-negative Ramos cells showed no change ($p = 0.92$) (Fig. 2A) [17]. The AR agonist R1881 did not stimulate MCL growth (in contrast to stimulation of LNCaP growth), suggesting a possible ligand-independent function of AR in these cell lines, as has been reported in PCa cells [18].

Notably, the concurrent presence of R1881 did not dampen the suppressive effect of enzalutamide on MCL proliferation (Fig. 2B), suggesting that circulating androgen levels in male patients (12–25 nmol/L for testosterone) would not abrogate activity of enzalutamide in vivo. Importantly, most MCL cells lines (but not Ramos) showed a dose-dependent response to AR inhibition (Fig. 2C) that was maintained over a wide range of androgen concentrations (0.1–100 μmol/L; Fig. 2D). Of the four MCL lines evaluated, Jeko-1 is derived from a female donor and demonstrated a robust response to AR inhibition, suggesting possible antitumor efficacy in both male and female patients.

This first report of AR activity in MCL is hypothesis generating. Many questions remain regarding the potential role of AR in MCL pathogenesis and AR-associated inhibition of MCL proliferation. The ectopic expression of cyclin D1 in MCL is unlikely to be involved in driving AR activity because the primary cyclin D isoform in MCL tumors is D1a, which negatively regulates AR activity [19].

The behavior of established MCL cell lines may not accurately reflect developmental pathobiology. Therefore, whereas proliferation of MCL lines in vitro was not stimulated by ligand-mediated AR activation, this does not preclude an influence of circulating androgens on tumor pathogenesis in male patients. Conversely, whereas gender-related differences favoring AR activity may underlie the male predominance of this disease, this does not preclude the possibility that AR activity plays a role in female MCL patients, as suggested by sensitivity of the female-derived Jeko-1 cells to enzalutamide.

Castration-resistant prostate tumors express AR splice variants (ARVs) that lack the ligand-binding domain (LBD), rendering them resistant to enzalutamide, which binds AR in the LBD [20,21]. We did not detect transcripts