MLL translocation in two castration-resistant prostate cancer patients

Rajasree Pia Chowdry, MD,[1] Elisa Ledet, PhD,[2] Lahiru Ranasinghe,[3] Alton Oliver Sartor, MD[2]

[1]Section of Hematology/Medical Oncology, Tulane University Hematology/Oncology. New Orleans, Louisiana, USA
[2]Department of Urology, Tulane Cancer Center, New Orleans, Louisiana, USA
[3]Tulane University Medical School, New Orleans, Louisiana, USA

CHOWDRY RP, LEDET E, RANASINGHE L, SARTOR AO. MLL translocation in two castration-resistant prostate cancer patients. *Can J Urol* 2016;23(5): 8483-8486.

The mixed-lineage leukemia (MLL) protein acts as a histone methyltransferase regulating multiple genetic elements. Rearrangements of the MLL gene result in expression of MLL-fusion proteins that occur in some acute leukemias and are associated with poor prognosis. The MLL protein complex has been shown to interact with the androgen receptor via the MLL-menin subunit, thus promoting gene activation. The presence of MLL translocation has not been previously reported in patients with castrate resistant prostate cancer (CRPC). We describe two cases of metastatic CRPC with a translocation in the MLL gene detected by a specific fluorescent in situ hybridization (FISH) assay. Both patients had an aggressive course and succumbed to the illness.

Key Words: prostate cancer, castration-resistant, MLL, bone marrow

Introduction

The mixed-lineage leukemia (MLL) protein acts as a histone methyltransferase that regulates multiple genetic elements. Chromosomal rearrangements of the MLL gene result in expression of MLL-fusion proteins that occur in a subset of acute leukemias and are associated with poor prognosis. The MLL protein complex has been shown to interact with the androgen receptor (AR) via the MLL-menin subunit.[1] The AR-MLL interaction promotes gene activation. In model systems, MLL-menin inhibition blocks castrate resistant prostate cancer (CRPC) growth.[1] To date there have been no documented cases in the literature of MLL translocation occurring in patients with CRPC. We describe two cases of metastatic CRPC with a translocation in the MLL gene.

Accepted for publication August 2016

Address correspondence to Dr. Rajasree Pia Chowdry, Section of Hematology/Medical Oncology, Tulane University School of Medicine, 1430 Tulane Avenue, SL-78, New Orleans, LA 70112-2699 USA

Case report

Case 1

A 45-year-old Caucasian male with no significant past medical history presented with bladder outlet obstructive symptoms. Prostate-specific antigen (PSA) at the time was 1.1 ng/mL. His symptoms did not improve with the use of alpha blockers or 5-alpha reductase inhibitors and eventually worsened leading to acute urinary retention. Computed tomography (CT) scan done at the time showed bilateral hydronephrosis and thickening of the trigone of the bladder. Cystoscopy was done and suggestive of direct prostatic cancer invasion into the bladder. He underwent transurethral prostatectomy (TURP) and pathology revealed poorly differentiated high grade prostatic adenocarcinoma, Gleason 3+5=8 with bilateral seminal vesicle extension and invasion of the base of the bladder. PSA was 0.36 after having been on a 5-alpha reductase inhibitor. Staging scans including CT scans and nuclear medicine (NM) bone scan did not reveal any distant or osseous metastases, however magnetic resonance imaging (MRI) pelvis did show direct invasion into the bladder. His clinical stage using the American Joint Committee on Cancer (AJCC) criteria was a T4N0M0, stage IV.

He was started on treatment with combined anti-androgen and androgen deprivation therapy (ADT) with bicalutamide and Lupron Depot. Additionally he received pelvic external beam radiation therapy (EBRT) and received a total of 7560 centigray (cGy) to the prostate and 4500 cGy to the pelvic lymph nodes. At the completion of radiation therapy, PSA was 0.07 and this had later dropped to 0. He tolerated radiation well with minimal side effects. He remained on complete androgen deprivation for 3 years.

At approximately 3.5 years after his initial diagnosis, he presented with hip pain. NM bone scan revealed osseous lesions in bilateral ribs, thoracic spine, lumbar spine, sacrum, and right femur. PSA was 0.4 and testosterone had recovered. CT abdomen and pelvis confirmed the bony metastases but did not reveal any visceral metastases. He was restarted on bicalutamide and Lupron and underwent palliative radiation to his spine and right hip. PSA rose to 1.76 over 3 months, however was still low indicating that his tumor was not well-differentiated.

The patient was started on chemotherapy due to concern that he had a poorly-differentiated tumor with rapid radiographic progression without considerable rise in PSA. He was started on carboplatin area under the curve (AUC) 4.5 and docetaxel 50 mg/m^2 every 3 weeks with growth factor support with pegfilgrastim. He completed six cycles of chemotherapy. PSA prior to starting was 1.76 and upon completion of chemo had dropped to 0.06. He was maintained on hormonal therapy concurrently with chemo. Restaging scans showed unchanged bony metastases. Approximately 3 months after completion of chemo he developed worsening back pain and was found to have a PSA of 2.42. Additionally, MRI revealed worsening osseous metastases in his lumbar spine. He underwent palliative radiation again. He was started on abiraterone and dexamethasone and remained on this regimen for about 6 weeks, however had biochemical progression with PSA rise to 51.1, therefore the abiraterone was stopped. Due to a positive family history of prostate cancer and his young age, an analysis of 28 genes associated with hereditary cancer was done and he was found to be negative.

The patient was restarted on chemotherapy due to rapidly rising PSA and progression. He had previously responded to carboplatin and docetaxel therefore this regimen was re-introduced. He received two cycles of carboplatin and docetaxel, however unfortunately he had a PSA rise to 97.8. Furthermore, his lactate dehydrogenase (LDH) had risen to 2564 units/L. Additionally, his platelet count had dropped to 46,000 whereas 2 months prior it was normal. While it was possible this could be chemotherapy related, he did have nucleated red blood cells on peripheral smear suggestive of a myelophthisic process, therefore prostate cancer with bone marrow involvement was on the differential. He was started on treatment with diethylstilbestrol (DES) 2 mg daily as well as oral cyclophosphamide 50 mg twice daily. He was unable to tolerate the cyclophosphamide and therefore stopped taking it 3 days later. He was next started on infusional 5-Fluorouracil (5FU) 200 mg/m^2. DES was continued. PSA at the start of treatment was 117. He did experience a clinical response after starting 5FU with a reduction in his pain. Additionally his LDH began to decline.

A bone marrow biopsy was done to work up his thrombocytopenia. Results revealed a hypocellular marrow with metastatic prostatic adenocarcinoma occupying less than 5% of the total marrow space. There was no evidence of myelodysplasia or blasts. Fluorescence in situ hybridization (FISH) of the bone marrow specimen revealed rearrangement of the MLL locus using a commercially available (Cytocell, Ltd.) MLL Breakapart probe, thus indicating an MLL translocation. This probe is routinely used in our institution in evaluation of marrow malignancies given the previous annotated importance of MLL translocations in leukemias.

The patient experienced biochemical response after infusional 5FU in that his PSA declined to 46.6 and LDH declined to 427. He did have considerable side effects including fatigue, diarrhea, and nausea therefore the 5FU was held. DES was stopped as well. Additional treatments following this included mitoxantrone and paclitaxel, however he developed worsening thrombocytopenia. This was followed by additional 5FU and then cabazitaxel, however the patient developed worsening pancytopenia and was ultimately referred to hospice. He expired shortly thereafter.

Case 2

A 72-year-old male with localized prostate cancer diagnosed 18 years earlier and initially treated with radical prostatectomy but given the initial medical records were not available. Six years after initial diagnosis, he had a PSA rise to 5.2 and underwent EBRT. Five years after completion of radiation he was noted to have a PSA rise and was placed on ADT with a LHRH agonist. Two years after this he was found to have a PSA rise to 34 and a lesion on his thoracic spine. Biopsy of this lesion showed metastatic prostate cancer to the bone. At this time he underwent an orchiectomy and was placed on bicalutamide. After 4 years of bicalutamide therapy he was noted to have a slight PSA rise from 0.44 to 1.3 over 4 months, therefore he was switched to nilutamide. Due to a reaction from the nilutamide, he was switched to flutamide. He remained on flutamide for approximately 4 months then experienced a rise in PSA, however without any radiographic progression. Flutamide was discontinued. He was then started on abiraterone and prednisone and remained on it for approximately 2 years, at which point he developed both biochemical and radiographic progression in the form of bone metastases. He was next switched to abiraterone and dexamethasone, but soon experienced a PSA rise therefore treatment was stopped. His subsequent treatment included enzalutamide plus radium-223. After a few months on this regimen he experienced a PSA rise and was placed back on abiraterone in combination with enzalutamide and radium-223. He completed six doses of radium-223. Unfortunately he experienced further rise in PSA and was eventually started on chemotherapy with docetaxel and completed five cycles. Following this, he had a PSA rise and carboplatin was added to his regimen. After three cycles of carboplatin in combination with docetaxel, he experienced further PSA rise and was switched to mitoxantrone. He could not tolerate mitoxantrone due to side effects and had a small PSA rise therefore it was discontinued. At this time due to his prior response to abiraterone, it was decided to place him back on abiraterone and dexamethasone.

He continued to have biochemical progression and was noted to have anemia requiring packed red blood cell transfusions as well as thrombocytopenia with a platelet count as low as 15,000. He was also noted to have immature white blood cells on his complete blood count differential. Additionally he had nucleated red blood cells on his peripheral smear indicating a myelophthisic picture. PSA continued to slowly rise. DES was added to his regimen of abiraterone and dexamethasone. A bone marrow biopsy was done due to his cytopenias and revealed a packed marrow with 100% cellularity, virtually replaced with metastatic prostate cancer. There was minimal evidence of residual hematopoietic activity. No blasts or dysplastic cells were identified. Furthermore, FISH studies revealed the presence of the MLL gene translocation in 32 out of 200 cells using a commercially available assay. There was no evidence of leukemia or myelodysplasia.

PSA continued to rise while on DES and abiraterone and imaging showed progression in bony metastases. The patient became symptomatic with worsening back pain and was sent for palliative radiation. He was next started on palliative chemotherapy with infusional 5FU, however this was stopped after two cycles due to a joint infection and concern for sepsis. Additionally he developed worsening pancytopenia. PSA at this point had risen quite dramatically to above 2000. His therapy was switched to enzalutamide at this juncture in an effort to provide treatment per his wishes, however he quickly deteriorated and succumbed to his disease.

Discussion

Prostate cancer is the second most common cancer diagnosed in men and the second leading cause of cancer death in men. Screening methods have led to a decline in the incidence of metastatic prostate cancer and most present with localized disease.[2] While treatment options have expanded with the approval of abiraterone and enzalutamide, as well as upfront docetaxel with ADT, a proportion of men will still succumb to the disease. Here we present two cases with particularly aggressive disease with bone marrow involvement. While the extent of involvement in the bone marrow varied between both men, they each had a translocation of the MLL gene as detected by a commercially available specific MLL translocation FISH assay (Cytocell Ltd, Cambridge, UK) performed as part of our routine malignant bone marrow evaluation. Thus, the findings were serendipitous. Primary tumor tissue was not available for study but would not change the conclusions noted herein. It is not clear that MLL translocations would

have been detected by less specific testing such as exome sequencing given that the breakpoint here is not known. Translocation of the MLL gene is well documented in leukemia but has not been previously described in CRPC. The clinical implications of this translocation in prostate cancer are not known, however, from our observation, both of these patients had an aggressive course of their disease.

Increased androgen receptor (AR) activity is one of the major drivers of CRPC. It has been shown that the MLL subunit interacts directly with the AR in human prostate cancer cell lines[1].1 This interaction occurs via the MLL subunit menin[1].1 Menin has an oncogenic role in MLL-mutated leukemias.[3] An oncogenic role of menin has been suggested in solid tumors as well, such as breast and hepatocellular carcinoma and high menin expression has been associated with poorer outcomes.[4-6] Grasso et al identified both mutations and copy number alterations in MLL3, as well as other members of the MLL complex in approximately 9% of metastatic CRPC patients. These previously described alterations do not include translocations. The MLL complex is involved in the epigenetic transcriptional activation in AR signaling. Specifically, the interaction between MLL and the AR, which may also harbor aberrations, helps promote gene activation.[7] Thus, in the presence of mutations and/or copy number alterations, AR signaling remains active even in the presence of anti-androgen therapy ultimately leading to CRPC.

The AR-MLL interaction serves as a potential therapeutic target. Small molecule inhibitors targeting the MLL-menin interaction have been studied in leukemia.[8,9] Recently Malik, Chinnaiyan, et al described a small molecule inhibitor of menin in CRPC xenograft cell lines.[1] The inhibitor MI-136 given at 40 mg/kg per day led to a significant decrease in growth of CRPC cell lines in mouse models.[1] Further studies need to be done in order to determine the prevalence of MLL-translocated CRPC and potential therapeutic benefits of small molecule inhibitors. Both of our patients had a particularly aggressive course and both had been treated with multiple lines of chemotherapy. It is not known what the relationship is between multiple lines of chemotherapy use and development of aggressive mutations in CRPC. Both of our patients had received the topoisomerase II inhibitor mitoxantrone. Topoisomerase II inhibitors have been associated with the development of therapy-related acute leukemia with MLL translocation.[10] It is not clear as to whether they have any effect on the development of MLL translocations in CRPC. Additional studies are warranted regarding the frequency of MLL translocation in CRPC and importance of this potential therapeutic target. □

References

1. Malik R, Khan AP, Asangani IA et al. Targeting the MLL complex in castration-resistant prostate cancer. *Nat Med* 2015;21(4):344-352.
2. Welch HG, Gorski DH, Albertsen PC. Trends in metastatic breast and prostate cancer--lessons in cancer dynamics. *N Engl J Med* 2015; 373(18):1685-1687.
3. Yokoyama A, Somervaille TC, Smith KS et al. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* 2005;123(2):207-218.
4. Dreijerink KM, Mulder KW, Winkler GS et al. Menin links estrogen receptor activation to histone H3K4 trimethylation. *Cancer Res* 2006;66(9):4929-4935.
5. Imachi H, Murao K, Dobashi H et al. Menin, a product of the MENI gene, binds to estrogen receptor to enhance its activity in breast cancer cells: possibility of a novel predictive factor for tamoxifen resistance. *Breast Cancer Res Treat* 2010;122(2):395-407.
6. Xu B, Li SH, Zheng R et al. Menin promotes hepatocellular carcinogenesis and epigenetically up-regulates Yap1 transcription. *Proc Natl Acad Sci U S A* 2013;110(43):17480-17485.
7. Grasso CS, Wu YM, Robinson DR et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 2012;487(7406):239-243.
8. Grembecka J, He S, Shi A et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. *Nat Chem Biol* 2012;8(3):277-284.
9. He S, Malik B, Borkin D et al. Menin-MLL inhibitors block oncogenic transformation by MLL-fusion proteins in a fusion partner-independent manner. *Leukemia* 2016;30(2):508-513.
10. Ohshima A, Miura I, Chubachi A et al. 11q23 aberration is an additional chromosomal change in de novo acute leukemia after treatment with etoposide and mitoxantrone. *Am J Hematol* 1996;53(4):264-266.