Amplification of Multiple Receptor Tyrosine Kinase Pathways in a Patient with Metastatic Castration-Resistant Prostate Cancer with Disseminated Intravascular Coagulation (DIC)

Tingrui Wang[1], Zonghui Ding[2], Buqing Ni[3], Jeffrey Wang[3], Jue Wang[4*]

[1] *Department of Internal Medicine, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA*

[2] *Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ, USA*

[3] *Baylor University, Waco, TX, USA*

[4] *University of Arizona Cancer Center at Dignity Health St. Joseph's, Phoenix, AZ, USA*

*Corresponding Author: *Jue Wang, MD, FACP, University of Arizona Cancer Center at Dignity Health St. Joseph's, Phoenix, AZ, USA,* **E-mail:** *jue.wang@dignityhealth.org*

Abstract

Background: *Disseminated intravascular coagulation (DIC) has long been associated with prostate cancer development and prognosis is very poor. Understanding the molecular mechanisms of castration resistant prostate cancer (CRPC) and associated DIC may improve response to therapy and clinical trial designs.*

Methods: *A 72-year-old man with past medical history of metastatic castration resistant prostate cancer developed progressive disease with DIC. Patient was treated with mitoxantrone, flutamide and ketoconazole. The next day, PSA and D-dimer was reduced from 4697 ng/ml to 1842 ng/ml and 27, 645 to 7871 ng/ml, respectively. Guardant 360 biopsy-free^{TM} tumor sequencing showed AR mutation, AR, MET, EGFR, FGFR1 amplification mutations. Two months later, patient presented in emergency department with altered mental - status. His head CT scan showed large mixture of intraparenchymal and subarachnoid hemorrhage.*

Results: *We report a prostate cancer patient with DIC and additionally reviewed the reported clinical features of 81 more patients through a systematic literature search. We analyzed the characteristics and management of prostate cancer and DIC.*

Conclusions: *The molecular profiling of DIC with prostate cancer has not been reported to date. An in-depth analysis of tumor molecular profile will facilitate new drug development and provide new insights into CRPC and its related DIC.*

Keywords: *disseminated intravascular coagulation; molecular profiling; castration resistant prostate cancer; next generation sequencing; Circulating tumor DNA; gene amplification; androgen receptor; c-MET; receptor tyrosine kinase; Enzalutamide (Xtandi)*

Abbreviations: *Disseminated intravascular coagulation (DIC); castration resistant prostate cancer (CRPC); androgen receptor (AR); Androgen deprivation therapy (ADT); Next generation sequencing (NGS); circulating tumor DNA (ctDNA); Prothrombin time (PT); activated partial thromboplastin time (aPTT)*

1. INTRODUCTION

Disseminated intravascular coagulation (DIC) is characterized by activation of coagulation which results in thrombosis in vessels, organ failure and severe bleeding. DIC may be caused by infection, sepsis, solid cancers, leukemia, and obstetric diseases. The incidence rate of DIC is 13–30% in metastatic prostate cancer [1, 2]. Patients with prostate cancer has been observed to develop a life threatening DIC and may

develop systemic and intracranial bleeding due to excessive fibrinolysis. Current understanding on mechanisms of DIC in prostate cancer is limited and the knowledge linking prostate cancer progression and DIC is lacking.

Prostate cancer is the most commonly diagnosed male cancer and is the third leading cause of cancer-related deaths in men. Approximately 161,360 American men will be diagnosed with prostate cancer this year, and more than 26,000 can expect to die from their disease[3]. Androgen deprivation therapy (ADT) is considered first-line treatment, which provide initial benefits, however, progression to androgen independence. Castration-resistant prostate cancer (CRPC) is a progression of disease despite medical or surgical castration. The androgen axis plays an important role in the growth of CRPC. Understanding mechanism of resistance is essential to develop future treatment.

According to the molecular genetic evidence, CRPC is characterized by alterations in the androgen receptor hypersensitive pathway and growth factor receptors such as EGFR control survival pathways[4]. The molecular profiling of DIC with prostate cancer has not been reported to date, and this investigation provides insights into the expression of gene that may have fundamental importance to the development of DIC and progression of CRPC.

In this study, we described a prostate cancer patient with DIC presenting a quick progression and a poor prognosis. A genomic profile (Guardant 360 biopsy-free^{TM} tumor sequencing) was requested, with the goal of finding potential actionable molecular alterations. Our molecular findings of amplification both AR and multiple oncogenic receptor tyrosine kinases including c-Met and EGFR may shed some light on the association of prostate cancer and DIC. We additionally retrospectively analyzed the clinical presentation and management by searching the database for previous published journals of prostate cancer patients with DIC.

2. RESULTS

2.1. Case Report

A 72-year-old man had a known medical history of metastatic castration resistant prostate cancer with progressive disease after multiple lines of therapies including biclutamide, lupron, Provenge immunotherapy, enzalutamide, abiraterone (zytiga), docetaxel. In September 2016, patient presented with fatigue and generalized weakness. Patient clinically had no active bleeding. Physical exam was unremarkable for any significant abnormalities. Laboratory results showed hemoglobin (Hb) 6.8 gm/dl, hematocrit 20.1% (normal range: 40-52%) platelets 39 x10^9/L. Prothrombin time (PT) was 17.5 s (normal range: 10–12.3), activated partial thromboplastin time (aPTT) 36.3s (normal range: 25.5–36), fibrinogen 325 mg/dl, and D-dimer 27,645 ng/ml (normal range: 150-243). PSA was 4697 ng/ml (normal range: 0.05-4).

He developed laboratory evidence of disseminated intravascular coagulation (DIC). CT head showed no acute intracranial hemorrhage and no other acute intracranial abnormality. Patient was treated with blood transfusion, mitoxantrone 12 mg/m^2 intravenously monthly and flutamide 250 po mg q8hr and ketoconazole po 200mg q8hr. The next day, PSA and D-dimer was reduced from 4697 ng/ml to 1842 ng/ml and 27,645 to 7871 ng/ml respectively. Guardant 360 biopsy-free^{TM} tumor sequencing showed multiple genomic alterations including androgen receptor (AR) mutation, AR amplification, EGFR, FGFR1 and MET amplification. The findings from ctDNA suggest an extensive cross-talk between activation of inflammation and coagulation exists. Inflammatory mediators (such as cytokines) not only activate the coagulation system, but vice versa activate coagulation proteases and protease inhibitors which may modulate inflammation

through specific cell receptors. However, based on ctDNA result, none of the drugs are FDA approved for DIC associated with mCRPC. Two months later, patient was admitted to hospital for altered mental status. On assessment in the emergency department, his head CT scan showed large mixture of intraparenchymal and subarachnoid hemorrhage involving large portion of the left cerebral hemisphere. Associated severe mass effect upon the left lateral ventricle and left temporal lobe with prominent approximate 1.5 cm midline shift to the right (Figure 1). Laboratory results showed hemoglobin (Hb) 6.8 gm/dl, hematocrit 20.3% (40-52%), platelets 18 $x10^9$/L. PT was 22.2 s (10–12.3), activated PTT 31.9 s (25.5–36), fibrinogen 235 mg/dl, D-dimer 7463 ng/ml (150-243). The patient was intubated, admitted to the ICU with unresponsiveness and with multiple family members arriving with terminal extubation and comfort measures as per family members.
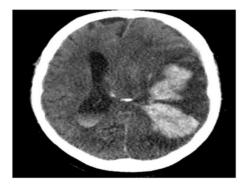

Figure1. *Head CT scan showed large mixture of intraparenchymal and subarachnoid hemorrhage involving large portion of the left cerebral hemisphere. Associated severe mass effect upon the left lateral ventricle and left temporal lobe with prominent approximate 1.5 cm midline shift to the right.*

2.2. Literature Review

Table1. *Baseline characteristics of the study cohort*

Parameters	Median	Range
Age (years)	68	43-92
PSA (µg/L)	614	0.8-8138
Median Survival (months)	4.5	0-45

n=81

Through the literature review by searching the database for previous published journals, we were able to additionally reviewed the reported clinical features of 81 prostate cancer patients with DIC (age range: 43-92 years, median age: 68 years, Table 1). 79 out of 81 patients were known to have stage IV prostate cancer. Ninety-one percent of the patients (74/81) had metastases when they developed DIC. The level of PSA at the diagnosis of DIC was at average of 614 µg/L. The median value of platelet counts was 69 $x10^3$/ µL. The median survival time of these patients was 4.5 months. Patients had elevated D-dimers, PT, aPTT and decreased fibrinogen and platelet (Table 2).

Table2. *Laboratory parameters of prostate cancer patients with DIC*

Parameters	Median	Range
PSA (µg/L)	614	0.8-8138
Platelets ($X10^9$/L)	69	3-205
PT (sec)	16	0.2-2415
aPTT (sec)	47.5	22-15032.6
Fibrinogen (mg/dl)	72	9-478
D-dimer (mg/l)	7.909	1-664.1

Bleeding sites induced by DIC included subcutaneous bleeding in 52 patients (64%), hematuria in 22 (27%), epistaxisin 21 (26%), invasive procedure (incision, biopsy, trauma, operation) bleeding in 20 (25%), gastrointestinal bleeding in 18 (22%), oral in 13 (16%), and cerebral bleeding in 6 (7%) (Table3)

Table3. *Sites of bleeding induced by DIC*

Sites	Patients (n=81)	
	n	%
Subcutaneous hemorrhage	52	64
Hematuria	22	27
Gastrointestinal	18	22
Cerebral	6	7
Epistaxis	21	26
Oral	13	16
Invasive procedures	20	25
Other	6	7

Management of DIC varies widely within this study. 69% (56/81) patients received anti androgen hormonal therapy, 64% (52/81) supportive care, 32.1% (26/81) fresh frozen plasma, 27.1% (22/81) platelet, 14.8% (12/81)

cryoprecipitate transfusion, 12.3% (10/81) vitamin K, 31% (25/81) heparin use, 26% chemotherapy(21/81), 11% radiation therapy (9/81), 4% immunotherapy (3/81), and 2.4% (2/81) fibrinolytic inhibitor (aminocaproic acid). (Table 4)

Table4. *Types of treatments for prostate cancer related DIC*

Treatments	Patients (n=81)	
	n	**%**
Supportive	52	64
Hormonal	56	69
Chemotherapy	21	26
Radiation	9	11
Immunotherapy	3	4
Fresh frozen plasma	26	32
Platelet	22	27
Cryoprecipitate transfusion	12	14.8
Vitamine K	10	12.3
Heparin	25	31
Fibrinolytic inhibitor	2	2.4

3. DISCUSSION

Prostate cancer is the most commonly diagnosed cancer in men. Androgen deprivation therapy is the standard treatment for locally advanced or metastatic prostatic cancer which provides initial benefits. Majority of patients will progress to castration resistant prostate cancer within three years. Disseminated intravascular coagulation has long been associated with prostate cancer development and prognosis is very poor. A retrospective analysis of prostate cancer revealed that DIC was mostly seen in patients with high-grade prostate cancer and 93% patients were castration resistant [5].

CRPC tumors release DNA into circulation and all mutations in tumor biopsies are accurately represented in cell free circulating tumor DNA (ctDNA). Genomic profiling is a useful tool for understanding the molecular mechanism and etiology of CRPC and cancer related DIC. The study has the potential to identify the novel molecular mechanisms of resistance to antitumor therapies.

Androgen axis plays an important role in the CRPC development. Five theories proposed on the causes of development of aggressive castration resistant cancer: hypersensitive, outlaw, promiscuous, coactivators and corepressors, bypass pathway[4]. Androgen receptor(AR) through development of amplifications may become sensitive to very small amounts of androgens (hypersensitive pathway) [4]. The amplification of AR promotes CRPC progression. Thirty to eighty percent of CRPC cell lines show amplification of the AR and twenty percent of CRPC metastases have evidence of AR amplification [6, 7]. Amplific - ation of AR may contribute to development of AR Splice variants of AR (AR-Vs), which lacks the ligand binding domain (LBD) and is constitutively active [8-11]. AR mutation lead to activation by molecules other than Androgens. Various AR point mutations have been identified that lead to increased AR activity in the presence of low androgen level which has been associated with more aggressive disease [12, 13]. AR recruits co-activators or co-repressors to enhance or repress transcriptional activity. Co-regulators identified as molecular chaperones modulate the protein through phosphorylation, acetylation, methylation. Growth factor IGF and KGF activate AR. Growth factor receptors such as EGFR control survival pathways, enhance AR activity and promote androgen independence [4].

ctDNA analysis provides a way to identify mutational mechanisms of drug resistance in CRPC. In our case, patient ctDNA demonstrates AR mutation, AR amplification, EGFR, FGFR1 amplification which explains his resistance to therapy. These newly identified mutations might offer novel target treatment that can overcome resistance.

DIC is a devastating complication of CRPC leading to excessive fibrinolysis and bleeding. It is characterized by hypofibrinogenemia with decreased platelet, increased D-dimer and prolonged PT/aPTT. If untreated, the hemorrhage risk is high and survival time is

very short. The most important principal to manage prostate cancer with DIC is to treat underlying disease. Improvement in DIC has been shown in androgen suppressive therapies. Ketoconazole can reduce testosterone level and not cause androgen surge. Several case reports have demonstrated the improvement of DIC with the use of ketoconazole [14, 15]. Combining ketoconazole with antiandrogen flutamide has been shown increase in efficacy in treatment of severe DIC[16]. Chemotherapy mitoxantrone has been reported for the reversal of DIC with greater than 50% decrease in PSA[17]. We combined three drugs together to put maximum therapeutic effect which resulted in both level of PSA and D-dimer tremendous reduction. Estrogen may show some benefit, however, it can also cause hypercoagulation which may worsen DIC [18-20]. GnRH agonist has been avoided in DIC because it causes androgen surge[21]. Supportive blood products aimed at reversing hemostatic derangements and reducing bleeding. In general, platelet transfusion is administered in DIC patients with active bleeding and a platelet count less than 50 x10^9/L. Fresh frozen plasma and cryoprecipitate replete clotting factor and fibrinogen respectively. Fibrinogen deficiency (less than 1g/dl) can be corrected with the administration of purified fibrinogen concentrates or cryoprecipitate. Fibrinolytic inhibitor aminocaproic acid is effective in treating bleeding. However, cases complicated with severe thrombosis have been documented [22]. Patients with DIC are at high risk of venous thromboembolism (VTE). The administration of heparin is beneficial in non-symptomatic DIC to prevent deep vein thrombosis (DVT), however should be avoided in active bleeding[23].

The mechanisms underlying the association between CRPC and DIC are multifactorial and under investigation. Expression of procoagulant proteins or cancer procoagulant may provoke thrombin generation which results in fibrinolysis and anti-fibrinolytic response. Tissue factor (TF) level is an important marker to predict the development of DIC in patients with cancer. A significant elevation of TF level was observed in DIC associated cancer, however no significant elevation in leukemia patients[24]. TF exposed to circulation is the culprit to initiate of ongoing DIC. The continuous exposure to TF exhausts the tissue factor pathway inhibitor (TFPI) which cause thrombin generation, and activation of factor XI and fibrin generation[25]. In glioblastoma multiforme (GBM), cancer cells overexpress tissue factor and interact with coagulation system which is driven by genetic transformation through expression of epidermal growth factor receptor (EGFR)[26]. *Rak J. et al.* demonstrated that upregulation of tissue factor is caused by oncogenic events including activation of K-ras and epidermal growth factor receptor (EGFR) [27]. The changes on tissue factor expression may cause a systemic hypercoagulable state and lead to tumor progression [28-31].

MET oncogene encodes a tyrosine kinase receptor for growth factor. MET and its ligand hepatocyte growth factor (HGF) play an important role in the development and progression of CRPC. Activation of the HGF/c-Met axis is a late event in prostate cancer progression. Prominent expression of c-Met was observed in advanced prostate cancer with bone metastases[32].

Recent investigation suggests genetic pathways, such as activation of oncogenes RAS or MET might induce the expression of genes controlling hemostasis and cause thrombotic event. Fibrin formation results in an advantage for prostate cancer cells by providing scaffold to promote tumor invasion and growth [33]. *Boccaccio et al.* study shows MET oncogene was thrombogenic. All mice injected with MET oncogene developed thrombi and later developed DIC [31]. MET oncogene activation and amplification results in up regulation of cyclooxygenase(COX-2) and plasminogen activator inhibitor type 1(PAI-1). The elevation

level of COX-2 and PAI-1 induced fibrinolysis and activation of platelets which results in DIC. Administration of inhibitors for PAI-1 and COX-2 caused decreased level of D-dimer[31]. Therefore, c-met may be target for prostate cancer with DIC. c-Met / HGF signaling pathway inhibition is a promising therapeutic target for the treatment of metastatic prostate cancer. Cabozantinib (XL184) is a predominantly MET tyrosine kinase inhibitor which demonstrates potent antitumor effects in CRPC. A variety of xenograft models demonstrated that cabozantinib inhibits MET signaling and induces apoptosis of cancer cells [34]. The study of phase II trial of cabozantinib showed resolution of bone metastasis in CRPC within six weeks[32]. MET amplification mutations may serve as biomarkers in prostate cancer with DIC which may help to guide patient selection and apply novel targeted therapy. By identifying patient's molecular profile, targeted therapy for CRPC and its related DIC would result in significant clinical improvement and further improve patient's survival and quality of life.

4. CONCLUSION

DIC is a devastating complication of prostate cancer and prognosis is extremely poor. The fundamental management for this condition is to control underlying prostate cancer and hemorrhagic support. This work indicates that investigation of DIC based on gene expression can identify genes that may help provide potential individualized therapeutic manage-ment. Our molecular findings of amplification both AR and multiple oncogenic receptor tyrosine kinases including c-Met and EGFR may indicate the association of prostate cancer and DIC. Such impressive findings have never been reported with any conventional or novel targeted treatment. New treatment strategies for disseminated intravascular coagulation may potentially emerge based on understanding of the pathophysiology.

REFERENCES

[1] Deme, D., et al., Metastatic prostate cancer complicated with chronic disseminated intravascular coagulopathy causing acute renal failure, mimicking thrombotic throm bocytopenic purpura and hemolytic uremic syndrome: pathomechanism, differential diagnosis and therapy related to a case. Magy Onkol, 2010. 54(4): p. 351-7.

[2] Smith, J.A., Jr., M.S. Soloway, and M.J. Young, Complications of advanced prostate cancer. Urology, 1999. 54(6A Suppl): p. 8-14.

[3] Siegel, R.L., K.D. Miller, and A. Jemal, Cancer Statistics, 2017. CA Cancer J Clin, 2017. 67(1): p. 7-30.

[4] Chandrasekar, T., et al., Mechanisms of resistance in castration-resistant prostate cancer (CRPC). Transl Androl Urol, 2015. 4(3): p. 365-80.

[5] Hyman, D.M., G.A. Soff, and L.J. Kampel, Disseminated intravascular coagulation with excessive fibrinolysis in prostate cancer: a case series and review of the literature. Oncology, 2011. 81(2): p. 119-25.

[6] Liu, W., et al., Homozygous deletions and recurrent amplifications implicate new genes involved in prostate cancer. Neoplasia, 2008. 10(8): p. 897-907.

[7] Taylor, B.S., et al., Integrative genomic profiling of human prostate cancer. Cancer Cell, 2010. 18(1): p. 11-22.

[8] Dehm, S.M. and D.J. Tindall, Alternatively spliced androgen receptor variants. Endocr Relat Cancer, 2011. 18(5): p. R183-96.

[9] Guo, Z., et al., A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res, 2009. 69(6): p. 2305-13.

[10] Hu, R., et al., Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res, 2009. 69(1): p. 16-22.

[11] Sun, S., et al., Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest, 2010. 120(8): p. 2715-30.

[12] Makridakis, N.M., E. di Salle, and J.K. Reichardt, Biochemical and pharmacogenetic dissection of human steroid 5 alpha-reductase type II. Pharmacogenetics, 2000. 10(5): p. 407-13.

[13] Scariano, J.K., et al., The SRD5A2 V89L polymorphism is associated with severity of

disease in men with early onset prostate cancer. Prostate, 2008. 68(16): p. 1798-805.

[14] Litt, M.R., W.R. Bell, and H.A. Lepor, Disseminated intravascular coagulation in prostatic carcinoma reversed by ketoconazole. JAMA, 1987. 258(10): p. 1361-2.

[15] Lowe, F.C. and W.J. Somers, The use of ketoconazole in the emergency management of disseminated intravascular coagulation due to metastatic prostatic cancer. J Urol, 1987. 137(5): p. 1000-2.

[16] Chakrapee-Sirisuk, S., et al., Ketoconazole and Flutamide in the treatment of disseminated intravascular clotting from prostate cancer: a case report and review. J Med Assoc Thai, 2001. 84(10): p. 1495-501.

[17] Smith, M.R., Successful treatment with mitoxantrone chemotherapy of acute disseminated intravascular coagulation due to metastatic androgen independent prostate cancer. J Urol, 2000. 163(1): p. 248.

[18] Doll, D.C. and J.W. Yarbro, Vascular toxicity associated with chemotherapy and hormonotherapy. Curr Opin Oncol, 1994. 6(4): p. 345-50.

[19] Al-Mondhiry, H., et al., Hemostatic effects of hormonal stimulation in patients with metastatic prostate cancer. Am J Hematol, 1988. 28(3): p. 141-5.

[20] Cornfield, D.B. and R.E. Rossman, Diethylstilbestrol-diphosphate-induced disseminated intravascular coagulation in prostatic carcinoma. South Med J, 1982. 75(2): p. 248-9.

[21] Bern, M.M., Coagulopathy, following medical therapy, for carcinoma of the prostate. Hematology, 2005. 10(1): p. 65-8.

[22] Brown, J.E., et al., All-trans retinoic acid (ATRA) and tranexamic acid: a potentially fatal combination in acute promyelocytic leukaemia. Br J Haematol, 2000. 110(4): p. 1010-2.

[23] Wada, H., T. Matsumoto, and Y. Yamashita, Diagnosis and treatment of disseminated intravascular coagulation (DIC) according to four DIC guidelines. J Intensive Care, 2014. 2(1): p. 15.

[24] Asakura, H., et al., Role of tissue factor in disseminated intravascular coagulation. Thromb Res, 1995. 80(3): p. 217-24.

[25] Osterud, B. and E. Bjorklid, The tissue factor pathway in disseminated intravascular coagulation. Semin Thromb Hemost, 2001. 27(6): p. 605-17.

[26] Magnus, N., D. Garnier, and J. Rak, Oncogenic epidermal growth factor receptor up-regulates multiple elements of the tissue factor signaling pathway in human glioma cells. Blood, 2010. 116(5): p. 815-8.

[27] Rak, J., P. Klement, and J. Yu, Genetic determinants of cancer coagulopathy, angiogenesis and disease progression. Vnitr Lek, 2006. 52 Suppl 1: p. 135-8.

[28] Yu, J.L., et al., Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood, 2005. 105(4): p. 1734-41.

[29] Rong, Y., et al., PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. Cancer Res, 2005. 65(4): p. 1406-13.

[30] Tallman, M.S., et al., Effects of all-trans retinoic acid or chemotherapy on the molecular regulation of systemic blood coagulation and fibrinolysis in patients with acute promyelocytic leukemia. J Thromb Haemost, 2004. 2(8): p. 1341-50.

[31] Boccaccio, C., et al., The MET oncogene drives a genetic programme linking cancer to haemostasis. Nature, 2005. 434(7031): p. 396-400.

[32] Varkaris, A., et al., The role of HGF/c-Met signaling in prostate cancer progression and c-Met inhibitors in clinical trials. Expert Opin Investig Drugs, 2011. 20(12): p. 1677-84.

[33] Boccaccio, C. and P.M. Comoglio, Genetic link between cancer and thrombosis. J Clin Oncol, 2009. 27(29): p. 4827-33.

[34] Yakes, F.M., et al., Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther, 2011. 10(12): p. 2298-308.