Case Report

Transcription factor 4 expression in circulating tumor cells from castration-resistant prostate cancer

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Abbreviations & Acronyms
ABI = abiraterone
ADT = androgen deprivation therapy
AR = androgen receptor
AR-V7 = AR splice variant 7
BCL = bicalutamide
CBZ = cabazitaxel
CRPC = castration-resistant prostate cancer
CTC = circulating tumor cell
DOC = docetaxel
ENZ = enzalutamide
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HER2 = human epidermal growth factor receptor 2
mRNA = messenger ribonucleic acid
NED = neuroendocrine differentiation
PC = prostate cancer
PSA = prostate-specific antigen
qPCR = quantitative polymerase chain reaction
TCF4 = transcription factor 4

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Introduction: Neuroendocrine differentiation is partly caused by antiandrogen therapy and exhibits an androgen receptor-independent growth mechanism. We hypothesized that the expression of transcription factor 4, an inducer of neuroendocrine differentiation, in circulating tumor cells is related to drug resistance in castration-resistant prostate cancer.

Case presentation: We evaluate the messenger ribonucleic acid expression of transcription factor 4 in circulating tumor cells from 17 patients with castration-resistant prostate cancer and compared these levels between patients receiving antiandrogen therapies and those who were resistant to antiandrogen therapies and receiving chemotherapies. The expression of transcription factor 4 in circulating tumor cells was significantly higher among patients receiving chemotherapies.

Conclusion: This study shows that transcription factor 4 is higher in the group of patients who were judged by their physicians to need chemotherapy treatment.

Key words: androgen receptor, castration-resistant prostate cancer, circulating tumor cells, neuroendocrine differentiation, transcription factor 4.

Keynote message

Our result suggests TCF4, an inducer of NED, expression in CTCs is associated with the resistance to antiandrogen treatment in CRPC.

Introduction

Most patients with advanced PC respond initially to ADT, but majority eventually develop fatal CRPC. The main mechanisms underlying antiandrogen resistance include splice variants and point mutations of the AR as well as NED.[1,2]

NED, an AR-independent growth mechanism, is commonly observed in advanced PC and is correlated with a poor prognosis.[2,3] A study conducted by Lipianskaya *et al*. suggested NED development during ADT and/or treatment with inhibitors that target the AR signaling pathway.[4] It is important to detect PC including neuroendocrine cancer cells during treatment, enabling an earlier transition from a less effective treatment modality. However, PC is often a heterogeneous mixture of neuroendocrine and adenocarcinoma tumor components, increasing the difficulty of an early diagnosis. Although serum neuroendocrine markers, such as chromogranin A and neuron-specific enolase, have a high sensitivity and negative predictive value for the detection of neuroendocrine tumors, they lack specificity.[5]

Previously, we revealed that TCF4 mediates ENZ resistance in PC cells by inducing NED through a canonical Wnt-independent mechanism.[6] Here we analyzed the correlation between TCF4 expression in CTCs and clinical features of PC patients.

Case presentation

Patient selection

Between March 2019 and August 2019, the CTCs of 17 patients with histologically proven PC and a diagnosis of CRPC based on the European Association of Urology guidelines were

analyzed at Juntendo University Hospital.[7] This study was approved by the institutional review board at Juntendo University (admission number: 14-052). All patients provided written informed consent before participation, and all experiments were carried out in accordance with the Declaration of Helsinki.

CTC analysis

AdnaTest Prostate Cancer (QIAGEN, Hilden, Germany) was used to isolate and enrich tumor cells from blood samples collected from patients with CRPC according to the following procedures. Epithelial cells were separated from blood samples (7.0 mL) using antibody-labeled binding beads specific for the epithelial cell adhesion molecule and HER2. The captured cells were lysed and treated with Dynal Oligo-dT beads to isolate mRNA. Subsequently, 20 μL cDNA was generated from the mRNA using the Sensiscript Reverse Transcriptase Kit (QIAGEN).

Real-time PCR

One microliter of cDNA was subjected to qPCR using a StepOnePlus™ thermocycler (Applied Biosystems, Foster City, CA, USA) and SYBR Green ROX QPCR Mastermix (QIAGEN, Valencia, CA, USA). The following PCR primers for specific targets were used: human TCF4 (forward: CCTGGCTATGCAGGAATGTT, reverse: CAGGAGGCGTACAGGAAGAG); human AR-FL (forward: AGGTGGAAGATTCAGCCAAG, reverse: TTCTGGAAAGCTCCTCGGTA); human AR-V7 (forward: AACAGAAGTACCTGTGCGCC, reverse: TCAGGGTCTGGTCATTTTGA); human PSA (forward: GATGACTCCAGCCACGACCT, reverse: CACAGACACCCCATCCTATC); and human GAPDH (forward: CTCCACCTCCTGCACCTAAG, reverse: CTGGGTGGCAGTGTAGGAAT). The presence of CTCs was determined by the positive expression of PSA. GAPDH was used as a normalizer, and the $2^{-\Delta CT}$ (ΔCT = CT value of GAPDH – CT value of AR, AR-V7 or TCF4) method was used to compare gene expression levels.

Statistical analysis

Continuous variables were compared using the Mann–Whitney *U* test. All statistical analyses were performed using R Statistical Software (version 3.5.2).[8] All statistical tests were two-sided; *P*-values < 0.05 were considered statistically significant.

Result

Patient characteristics

The blood samples of 17 CRPC patients contained detectable CTCs with positive mRNA PSA expression. Figure 1 presents the patient clinical characteristics, including age, serum PSA concentration, Gleason sum score (sum of the two most prevalent Gleason grades), metastases sites, time since PC diagnosis, treatment administered for advanced PC, and currently administered treatment type. Patients were chemically castrated during analysis and had received BCL as a first-line therapy.

Patient No.	Age (yrs)	PSA (ng/dL) at CTC analysis	Gleason Sum Score	Metastasis at CTC analysis	Time from diagnosis (m)	Prior Treatment: BCL	ENZ	ABI	DOC	CBZ	Treatment Line
1	59	0.9	8	Bone	42						1
2	78	6.2	N/A	Bone	41						1
3	78	40	8	Bone	9						2
4	72	11.8	8	Bone	11						2
5	81	66	9	LN · Bone	29						2
6	72	36.2	8	None	14						2
7	75	200	7	LN · Bone	90						2
8	71	270	8	Bone	56						3
9	68	0.1	7	LN · Bone	16						2
10	54	5.1	9	Bone	21						2
11	67	1.551	10	Bone	16						2
12	70	126.9	9	None	12						2
13	75	25	8	LN · Lung	20						3
14	70	5.1	7	Bone	204						3
15	72	885	8	Bone	20						3
16	71	87	7	LN · Lung · Bone	30						4
17	74	10	8	None	136						4

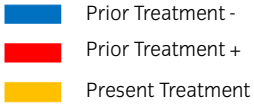

Fig. 1 Patient clinical characteristics. The clinical background information of the 17 patients, including age, serum PSA concentration, Gleason sum score, sites of metastases, time since the diagnosis of PC, type of treatment administered for advanced PC, and type of treatment currently administered. Treatments that were and were not previously administered are indicated in red and blue, respectively. The treatment administered at the time of the CTC analysis is indicated in yellow.

Nine patients received chemotherapies after exhibiting resistance to antiandrogen therapies; of these, seven received DOC and two received CBZ. The remaining eight patients received a first-line, second-line, or third-line antiandrogen therapy during CTC analysis (BCL, ENZ, or ABI). No guideline has clearly indicated the appropriate timing of chemotherapy for CRPC; in our institution, chemotherapy was administrated when no antiandrogen therapy response was expected. The time-to-CRPC (duration of response to initial ADT), high Gleason score (9 or 10), visceral metastasis at diagnosis, pain due to bone metastasis, and high lactate dehydrogenase and alkaline phosphatase levels during ADT were used to determine when the attending physician starts chemotherapy. We developed two groups: patients receiving antiandrogen therapies (n = 8) and those who developed resistance to antiandrogen therapies and were receiving chemotherapies (n = 9). There were no significant differences between the two groups in age, Gleason sum score, serum PSA, or the time since the PC diagnosis (Table 1).

mRNA expression in CTCs

In the qPCR analysis, no significant differences were observed in the expression levels of the genes encoding AR-V7 (Table 2). However, the TCF4 expression was significantly higher in CTCs of patients receiving chemotherapies than those receiving antiandrogen therapies. However, the AR expression was significantly lower in CTCs of patients receiving chemotherapies than those receiving antiandrogen therapies (Fig. 2; Table 2).

Table 1 Patients clinical background according to treatment line

	Receiving antiandrogen therapy (n = 8)			Receiving chemotherapy (n = 9)			
	Median	Min	Max	Median	Min	Max	P
Age (years)	73.5	59.0	81.0	70.0	54.0	75.0	0.066
Gleason sum score	8	7	9	8	7	10	0.954
PSA (ng/dL) at CTCs analysis	38.1	0.90	270	10.0	0.10	885	0.441
Time from diagnosis (min)	35.0	9.00	90.0	20.0	12.0	204	0.923

Table 2 Expression of AR, AR-V7, and TCF4 in CTCs according to treatment line

	Receiving antiandrogen therapy (n = 8)			Receiving chemotherapy (n = 9)			
	Median	Min	Max	Median	Min	Max	P
AR (unit)	7.68	0.02	21.1	0.23	0.01	2.05	0.012
AR-V7 (unit)	1.83	0.10	27.1	0.20	0.06	3.15	0.102
TCF4 (unit)	0.03	0	1.76	2.02	0	47.9	0.015

Unit = $2^{-\Delta CT}$ (ΔCT = CT value of GAPDH − CT value of AR, AR-V7 or TCF4).

Discussion

In this study, we found a significant increase in TCF4 mRNA expression and significant decrease in AR mRNA expression in CTCs from patients with CRPC who had developed resistance to antiandrogen therapies and received chemotherapies.

NED has been implicated as a mechanism of antiandrogen resistance in PC. Aggarwal *et al*. reported treatment-emergent small-cell prostate carcinoma (t-SCNC) in 17% of advanced metastatic CRPC cases.[5] Studies have reported associations of ENZ therapy with the transcriptional dysregulation and genetic abnormalities causing NED.[9–11] We also previously revealed that TCF4 induces NED during resistance acquisition to ENZ therapy.[6] Therefore, the expression of TCF4 in PC cells could monitor antiandrogen-mediated neuroendocrine modulation, contributing to treatment resistance. The analysis of TCF4 expression in CTCs may indicate NED and provide new insights to support clinical decision-making processes. This study's results did not show a significant difference in the expression level of AR-V7 between the two groups. However, previous prospective study has shown a relationship between AR-V7 and resistance to novel antiandrogens.[12] No conclusion could be drawn regarding the relationship between AR-V7 and resistance to antiandrogen therapy from this study.

This study had several limitations. This study shows that TCF4 is higher in the group of patients who were judged by their physicians to need chemotherapy treatment. However, the reasons for the physician's determination of the need for chemotherapy based on the clinical course may vary. Also, the interaction between TCF4 expression and chemotherapy was not determined. Furthermore, patient 8 is ENZ resistant,

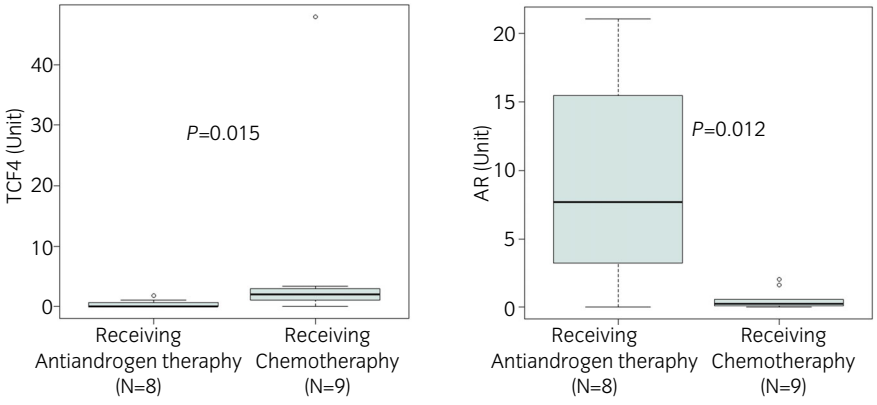

Fig. 2 Expression of the gene encoding TCF4 and AR. Significantly higher expression of TCF4 was detected in the CTCs of patients receiving chemotherapies, compared to those receiving antiandrogen therapies ($P < 0.05$). Significantly lower AR expression was detected in the CTCs of patients receiving chemotherapies than those receiving antiandrogen therapies ($P < 0.05$).

but the expression of TCF4 was not high. The mechanism of ENZ resistance in this patient does not appear to be due to TCF4.

In our opinion, there is a need to analyze the relationship between TCF4 expression and progression-free survival in patients treated with novel antiandrogens in future studies in order to demonstrate the relationship between the effect of novel antiandrogens and TCF4 expression. Furthermore, we must investigate the NED in tumors during CTC sampling, such as protein, RNA, and methylation, to clarify how AR and TCF4 expression in CTCs is involved in the NED in CRPC.

Conclusions

This study shows that TCF4 is higher in the group of patients who were judged by their physicians to need chemotherapy treatment.

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Conflict of interest

The authors declare no conflict of interest.

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