Published in final edited form as:

Prostate. 2016 October ; 76(14): 1338–1341. doi:10.1002/pros.23220.

A genetic variation of *SOD2* does not determine duration of response to androgen deprivation therapy for prostate cancer

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Abstract

Background—Silencing *SOD2* expression up-regulates AR signaling and expression of *SOD2* is down-regulated in CRPC, compared with untreated tumors. The decreased *SOD2* activity could lead to AR gain-of-function and the development of castration-resistance.

Method—We genotyped *SOD2*-rs4880 in a cohort of 753 prostate cancer patients receiving androgen deprivation therapy (ADT) between 1996 and 2010. The rs4880 encodes Ala16Val in *SOD2* and the Val variant has been demonstrated to be functionally less efficient than the Ala variant. We assessed the impact of *SOD2*-rs4880 variants on the time to progression (TTP) and overall survival (OS) on ADT using multivariable Cox regression.

Results—432 out of 753 (57%) had metastases at the time of ADT initiation. Overall, median TTP on ADT was 18.4 (95% CI: 15.8, 20.9) months and median OS from ADT initiation was 6.3 (95% CI: 5.8, 6.8) years. In unadjusted and adjusted analyses, there was no association between *SOD2*-rs4880 and TTP or OS on ADT ($p > 0.05$). Results were similarly negative among patients with and without metastatic disease at ADT initiation.

Conclusions—Our result suggests that a functional genetic variant in *SOD2* does not determine the efficacy of ADT for prostate cancer. It is possible that the drastic down-regulation of *SOD2* in advanced prostate cancer cells may have overridden any influence of the genetic variation of *SOD2*. This study suggests the need for careful consideration about timing if the application of *SOD2* mimetics for prostate cancer therapy is considered.

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Conflicts of interest: The authors indicated no potential conflicts of interest.

Keywords

prostate cancer; MnSOD2

Introduction

Androgens and androgen receptor (AR) mediate prostate cancer (CaP) development and progression. Androgen deprivation therapy (ADT) has been the standard therapy for metastatic CaP patients. Unfortunately, CaP eventually becomes resistant to ADT and progresses to castration-resistant CaP (CRPC), leading to death. AR reactivation via a variety of mechanisms profoundly affects CRPC proliferation (1).

The mitochondrial antioxidant enzyme, superoxide dismutase 2 (*SOD2*), catalyzes the conversion of super-oxide to hydrogen peroxide (2), detoxifying the oxidative stress. The *SOD2* expression is commonly down regulated in cancer cells. Restoration of *SOD2* activity significantly inhibits tumor growth, including CaP. *SOD2* levels progressively decline during the transition from prostatic intraepithelial neoplasia to hormone sensitive CaP to CRPC. Strikingly, *SOD2* levels in one CRPC study are only 11% of that found in hormone sensitive CaP (2). Silencing *SOD2* expression in LNCaP induced AR binding to DNA and up-regulated AR-responsive genes. These data suggest that decreased *SOD2* activity may be mechanistically linked to AR gain-of-function and the castration-resistance development (3,4).

A single-nucleotide polymorphism (SNP) in exon-2 of *SOD2* (rs4880) causes Ala16Val substitution. The Val-variant causes partial arrest of the precursor protein within the inner mitochondrial membrane and decreases the active *SOD2*-tetramer formation. Thus, the Val-variant is functionally less active than the Ala-variant (5,6). Apparently, *SOD2*-rs4880 modifies the association between selenium level and risk of aggressive CaP at diagnosis in a hormone sensitive localized CaP cohort (7,8). Here, we investigated whether the *SOD2*-rs4880 is associated with overall survival (OS) and the response duration (TTP) to ADT in CaP.

Patients and Methods

The study cohort

Using our institutional clinical database, we identified 858 patients with biochemical recurrence (M0) or metastatic disease (M1) who received ADT from 1996 until 2010 for hormone sensitive CaP (9,10). We excluded patients who had insufficient follow-up data (n=86) or lacked genotyping data (n=19), leaving 753 patients for this analysis. Clinicodemographic data and dates of initiation and progression on ADT were reviewed. All patients were consented. Genomic DNA preparation and genotyping were as previously described (10).

Genotyping

Genomic DNA was prepared from blood using QIAamp DNA Blood mini kit (Qiagen Inc, Valencia, CA, USA). Genotyping was done using Sequenom iPLEX matrix-assisted laser desorption/ionization–time of flight mass spectrometry technology at the core facility of Boston Children Hospital. The *SOD2*-rs4880 genotype call rate was 98.8% and the genotype distribution followed Hardy Weinberg equilibrium (p -value= 0.78).

Statistical analysis

Progression was defined as a minimum of two rises in prostate-specific antigen (PSA), with the first rise date (nadir + >0.02 ng/ml) as the progression date. TTP during ADT was defined as the duration of time from ADT initiation to ADT progression or to initiation of a secondary therapy (for rising PSA before fulfillment of progression), or was censored at the last follow-up date or PSA value among patients who did not progress. OS was defined as the period from ADT initiation to patient death, or was censored on the last follow up day. The association of patient baseline characteristics with *SOD2*-rs4880 genotype was evaluated with chi-square test for categorical variables and Wilcoxon rank-sum test for continuous values. Distributions of TTP and OS were estimated using the Kaplan-Meier method; comparison between *SOD2*-rs4880 genotypes was conducted using the log-rank test or the Wald chi-square test by multivariable Cox regression model adjusted for prognostic factors including biopsy Gleason, primary therapy type, use of prior ADT with local therapy, metastatic status and PSA at ADT initiation. OS was similarly analyzed; age at ADT initiation was included as a covariate in multivariable models. All analyses were performed using SAS version-9.2 (SAS institute, Cary, NC) and $P < .05$ (two sided) was considered statistically significant.

Results and Discussion

The follow-up cutoff day was June 2012. Table 1 presents patient characteristics. Ninety-five percent of patients were Caucasian. Approximately 70% of patients received a local therapy (radical prostatectomy or radiation therapy); 57% had metastases at the time of ADT initiation. The Minor Allele Frequency (A) of rs4880 in our cohort is 0.4940, which is similar to those published data in the population with European Ancestry and in the 1000 genomes. The *SOD2*-rs4880 genotype did not correlate with patient baseline characteristics, including age at diagnosis, biopsy Gleason, type of primary therapy, PSA and metastasis status at ADT initiation (p values >0.05). Median follow-up time was 8.6 years (range 0.1–15.6 years). 592 patients experienced progression during ADT, with a median TTP on ADT of 18.4 (95%CI: 15.8, 20.9) months. 454 patients died and median OS from ADT initiation was 6.3 (95%CI: 5.8, 6.8) years.

In unadjusted and adjusted analyses, there was no association between *SOD-rs4880* and TTP on ADT ($p > 0.05$, Table 2). The adjusted hazard ratios were 0.91(95%CI: 0.75, 1.12) and 0.98(95%CI: 0.78, 1.24) for AG and GG carriers, respectively, when compared to AA carriers (adjusted $p = 0.595$). Additionally, *SOD*-rs4880 was not associated with OS after ADT initiation (adjusted HR: 1.03(95%CI: 0.81, 1.30) and 1.08(95%CI: 0.82, 1.41) for AG

and GG carriers, $p=0.855$). Results were similarly negative among patients with and without metastasis at ADT initiation.

Untreated localized CaP cells express a high level of *SOD2*, indicating its functional importance. Consistently, a prominent association of *SOD2* genetic variants between selenium level and CaP risk was observed in this population of CaP patients. The drastic down-regulation of *SOD2* in advanced CaPs may have overridden the influence of the *SOD2* genetic variation, explaining the absence of association of *SOD2*-rs4880 with OS and TTP on ADT.

Acknowledgments

The study was supported in part by funding from the Dana-Farber Prostate Cancer SPORE P50CA090381 (to P.W. Kantoff), a Prostate Cancer Foundation Challenge Award (to N. Sharifi) and grants from the National Cancer Institute (R01CA168899, R01CA172382, and R01CA190289; to N. Sharifi).

Abbreviations

ADT	androgen deprivation therapy
CaP	Prostate cancer
TTP	Time to progression
OS	Overall Survival
SOD2	MnSOD2

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Table 1

Patient and disease characteristics at diagnosis and at ADT initiation

	All	
	N	%
Clinical T stage at diagnosis	288	38.2
T1		
T2	184	24.4
T3–4	34	4.5
Tx/unknown	247	32.8
Clinical M stage at diagnosis	265	35.2
M0		
M1	121	16.1
Mx/unknown	367	48.7
Clinical N stage at Diagnosis	247	32.8
N0		
N1	56	7.4
Nx/unknown	450	59.8
Biopsy Gleason	115	15.3
6 or less		
7	238	31.6
8 or more	292	38.8
Unknown	108	14.3
Type of local therapy	292	38.8
RP +/-RT		
RT only/other	243	32.3
None	218	29.0
Use of ADT as part of local therapy	166	22.0
Age (years) at ADT initiation, median (IQR)	66	59–73
PSA (ng/mL) at ADT initiation, median (IQR)	13.6	5.0–55.1
Presence of metastases at ADT initiation	432	57.4
Received intermittent ADT	191	25.4
Received anti-androgen during ADT	520	69.1

Abbreviations: ADT, androgen deprivation therapy; RP, radical prostatectomy; RT, radiation therapy; M, metastatic disease

Table 2

Associations of *SOD2*-rs4880 genotypes with time to progression (TTP) and overall survival (OS) on androgen deprivation therapy

	N (%)	TTP		OS	
		Median (95% CI), Months	Adjusted Hazard Ratio (95% CI)*	Median (95% CI), Years	Adjusted Hazard Ratio (95% CI)*
All (N=753)					
AA	183(24.3)	18.1(14.5,23.6)	reference	6.4(5.1,7.2)	reference
AG	378(50.2)	18.5(15.4,22.5)	0.91(0.75,1.12)	6.1(5.3,7.0)	1.03(0.81,1.30)
GG	192(25.5)	19.4(12.8,24.0)	0.98(0.78,1.24)	6.4(5.2,7.5)	1.08(0.82,1.41)
<i>P</i> -value		0.739	0.595	0.667	0.855
By Metastatic Disease Status					
M0 (N=321)					
AA	77(24.0)	35.6(27.1,40.6)	reference	9.3(6.8,14.8)	reference
AG	167(52.0)	27.8(20.5,38.7)	1.08(0.78,1.49)	7.4(6.1,8.9)	1.49(0.98,2.26)
GG	77(24.0)	28.0(23.9,40.0)	1.22(0.84,1.77)	7.7(5.9,9.9)	1.34(0.84,2.14)
<i>P</i> -value		0.574	0.584	0.105	0.175
M1 (N=432)					
AA	106(24.5)	12.0(8.9,13.2)	reference	4.1(3.5,5.2)	reference
AG	211(48.8)	13.6(12.0,16.7)	0.83(0.64, 1.08)	5.0(4.2,6.1)	0.84(0.63,1.13)
GG	115(26.6)	12.6(11.0,16.8)	0.88(0.65, 1.18)	5.1(4.0,6.7)	0.94(0.67,1.33)
<i>P</i> -value		0.345	0.370	0.792	0.490

Abbreviations: TTP, time to progression; OS, overall survival; M, metastatic disease; CI, Confidence Interval

* adjusting for biopsy Gleason score, type of primary therapy, and use of prior ADT in conjunction with local therapy, metastatic status and PSA at initiation of ADT. Age at ADT initiation was also included as a covariate for OS analysis