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Platelet-Synthesized Testosterone in Men with Prostate Cancer Induces Androgen Receptor Signaling[1]

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Abstract

Platelets have been long postulated to play a critical role in the pathogenesis of prostate cancer, although relatively little is known regarding the precise mechanisms involved. Androgen deprivation therapy (ADT) for prostate cancer eventually fails with relapse occurring in the form of castration-resistant prostate cancer (CRPC). CRPC tumors typically overexpress androgen receptor (AR), demonstrating continued dependence upon AR signaling. Platelets have been previously demonstrated to contain androgens, and we sought to explore the contribution of platelet-derived androgens in CRPC. In this study, we examined the role of platelet-derived androgens *in vitro* using platelets from men with CRPC, men with high-risk prostate cancer, and healthy male donors. A series of *in vitro* assays was performed to elucidate the impact of platelet-derived androgens on androgen-sensitive prostate tumor cells. By examining platelet-derived androgen effects on AR signaling in prostate tumor cells, we found that platelets, from men with CRPC and on ADT, strongly induce AR target genes and tumor cell proliferation. Moreover, we show a fully intact testosterone (T) biosynthetic pathway within platelets from its precursor cholesterol and demonstrate that platelets of CRPC patients with ADT resistance are able to generate T. Overall, our findings reveal an unknown capacity of platelets to synthesize T at functionally relevant levels in patients with lethal prostate cancer. Importantly, it suggests a novel paracrine mechanism of T production that may act to sustain CRPC state and potentiate therapeutic resistance.

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Abbreviations: ADT, androgen deprivation therapy; AR, androgen receptor; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone; HR, high-risk disease; MS, mass spectrometry; T, testosterone

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Introduction

The androgen receptor (AR)–mediated cellular program is a driving force in the process of prostate carcinogenesis [1]. For men with advanced disease, initial therapy consists of androgen-ablative strategies. Unfortunately, despite an initial response, most tumors relapse in the form of castration-resistant prostate cancer (CRPC), a lethal condition that accounts for approximately 30,000 deaths in the United States annually [2]. Notably, despite systemic androgen ablation, CRPC tumors exhibit continued cellular dependence on AR signaling. A variety of mechanisms have been demonstrated to support the castrate-resistant state including amplification and mutations of the AR gene, ligand-independent activation of AR, and dysregulation of AR gene coactivators and repressors, among others [3,4]. In addition, intratumoral prostate cancer cell production of androgens has also been shown to play an important role in the sustenance of CRPC tumors [5,6]. It is important to note that the current generation of androgen-ablative therapies (e.g., abiraterone) seeks to block this source of androgen synthesis [7].

Platelets have been postulated to perform a critical function in the pathogenesis of prostate cancer for decades [8,9], yet the precise nature of this interaction remains poorly understood. Proposed ways platelets may induce prostate cancer progression include the local delivery of growth factors and the protection of circulating tumor cells from immune surveillance [10–12]. Intriguingly, platelets have also been shown to produce the androgen dehydroepiandrosterone [13], raising the possibility of an additional mechanism through which platelets might support prostate cancer growth. This observation motivated us to assess intraplatelet testosterone (T) levels in men with advanced prostate cancer; interrogate the capacity of platelets to produce T from its precursor cholesterol, specifically in the castrate-resistant state; and test the ability of platelet-derived T to induce androgen signaling within prostate cancer cells. The findings presented in this work provide novel insight into a previously unknown role for platelets in the pathogenesis of prostate cancer and importantly identify platelets as both potentially new therapeutic targets as well as markers of extragonadal androgen biosynthesis.

Material and Methods

Reagents

All metabolites were from Sigma-Aldrich (St. Louis, MO): ^{13}C-T (cat no. 73610), ^{13}C-cholesterol (cat no. 749478), and ^{13}C-pregnenolone (cat no. 740985). Bicalutamide was a kind gift of Dr. Chinnaiyan (University of Michigan). Abiraterone acetate (cat no. SC-207240) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture

Human prostate cancer cell lines LNCaP and PC3 were obtained from the American Type Culture Collection (Manassas, VA), and LNCaP-AR cells were a kind gift from Dr. C. Sawyers (Memorial Sloan Kettering Cancer Center, New York, NY). All cells were authenticated by the University of Michigan DNA Sequencing Core using short tandem repeat DNA fingerprinting. Cells were maintained in the RPMI 1640 medium (Gibco; Life Technologies) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate.

Platelet Isolation

Written informed consent in accordance with the Declaration of Helsinki was received from all participants before inclusion in the study. All patient samples were obtained under an institutional review board–approved protocol at the University of Michigan following informed consent. Human platelets were isolated from venous blood from healthy subjects or prostate cancer patients not taking antiplatelet drugs (e.g., aspirin, long-term nonsteroidal anti-inflammatory drugs, clopidogrel). Separation of platelets from whole blood was achieved by centrifugation (1000 rpm for 20 minutes at room temperature). Platelet-rich plasma was then transferred into a separate tube, and prostaglandin-1 (500 ng/ml) was added to prevent platelet activation. Platelets were then precipitated by centrifugation at room temperature for 5 minutes at 2200 rpm and washed extensively in wash buffer (140 mM NaCl, 5 mM KCl, 12 mM trisodium citrate, 10 mM glucose, 12.5 mM sucrose, pH 6.0). Harvested platelets were then counted with a Coulter counter (Beckman Coulter, Fullerton, CA) and resuspended in platelet buffer (PB) (10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 140 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, 5 mM $NaHCO_3$, 10 mM glucose) before use.

Cell Proliferation

Cells were seeded into Poly-L-Lysine (Sigma-Aldrich)–coated 12-well plates at a density of 5×10^3 cells per well in RPMI 1640 containing 10% FBS. Medium was replaced 18 hours later with platelet buffer alone (control) or with human platelets (10×10^6/ml) resuspended in platelet buffer (treatment). Cell counts were done using a Coulter counter (Beckman Coulter) at indicated time points in triplicates. At least three independent experiments were performed.

Quatitative reverse transcriptase polymerase chain reaction

Cells were seeded into six-well plates (CellBIND) from Corning (Corning, NY) at a density of 5×10^5 cells per well in RPMI 1640 containing 10% FBS. Approximately 18 hours later, attached cells were androgen deprived for the next 48 hours by replacing the media with phenol red–free RPMI supplemented with 1% charcoal-stripped serum (CSS) from Life Technologies (Grand Island, NY). CSS containing media was replaced every 12 hours for the next 48 hours. For bicalutamide experiments, drug (10 μM) was added for 10 hours in 10% CSS phenol red–free RPMI and then added again in platelet buffer immediately before platelet addition. Either platelet buffer alone or platelets (150,000/μl) in 3 ml of platelet buffer were added to the cells.

After 24-hour incubation with platelets in a cell incubator, cells were washed 2× with phosphate-buffered saline (PBS) and lysed in Trizol (Life Technologies). RNA extraction from Trizol was performed according to the manufacturer's protocol; BioRad iScript cDNA synthesis kit was used for reverse transcriptase polymerase chain reaction (PCR) (BioRad, Hercules, CA). Quantitative PCR was performed using BioRAD SYBR Green Mastermix on an Applied Biosystems 7300 Real-Time PCR system. All reactions were performed in triplicates. Fold mRNA expression was calculated using $2^{-\Delta\Delta CT}$ method [14]. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Primers used were as follows:

PSA: (forward) 5'-TGACCAAGTTCATGCTGTGT-3' and (reverse) 5'-GTCATTTCCAAGGTTCCAAG-3'; TMPRSS2: (forward) 5'-CAGGAGTGTACGGGAATGTGATGGT-3' and (reverse) 5'-GATTAGCCGTCTGCCCTCATTTGT-3'; GAPDH: (forward) 5'-GCACCGTCAAGGCTGAGAAC-3' and (reverse) 5'-TGGTGAAGACGCCAGTGGA-3'.

Immunoblot

Platelet protein extracts were prepared using cell lysis buffer (Cell Signaling; cat no. 9803S) with protease inhibitors (Thermo Scientific; cat no. 78410). Protein concentrations were determined by