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Review Article

A brief history of intracrine androgen metabolism by castration-recurrent prostate cancer

James L Mohler[1,2]

[1]Department of Urology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA; [2]University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA

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Abstract: This mini-review describes the evolution of the concept of intracrine androgen metabolism by prostate cancer during androgen deprivation therapy. Persistence of androgen receptor protein in the face of castrate circulating levels of testosterone could not be explained fully by hypersensitization or mutation of the androgen receptor. The hypothesis that castration-recurrent prostate cancer produced its own testosterone was proven using radioimmunoassay and mass spectrometry methods adopted for use in prostate tissue. Intracrine synthesis of testicular androgens led to FDA approval of abiraterone, an inhibitor of androgen metabolism. Further understanding of intracrine androgen metabolism may allow the development of more targeted agents that perform better and do not require co-administration of prednisone that may extend survival and diminish side effects from treatment of advanced prostate cancer.

Keywords: Prostate cancer, androgen deprivation therapy, intracrine androgen metabolism, Donald S. Coffey

Introduction-Tribute: Hail to The Chief

This mini-review is dedicated to the memory of Donald S. Coffey, PhD, hereafter The Chief. Recognition of the possibility that castration-recurrent prostate cancer could produce its own testicular androgens required out-of-the box thinking that was the cornerstone of The Chief's mentorship. Collaboration with experts in radioimmunoassay and mass spectrometry to provide irrefutable data supporting the concept was emblematic of The Chief's commitment to team science. So now that The Chief is gone, what should investigators in our field remember? The things that I have taken away and I will continue to think about often are to 1) question everything; 2) believe the person you talked to last; 3) follow your gut; and, my favorite, 4) if you can't dazzle them with data, baffle them with bulls__t. I miss you Chief, but all of us are extremely grateful that you shared yourself with us. You (and Eula) should be glad to know that you live on in all of us!

Cell motility, metastasis and mentorship

Alan Partin, MD, PhD has been an MSTP student, urology resident, faculty and Urology Chair at Johns Hopkins University School of Medicine. Dr. Partin and I intersected for 2 years while he was in the PhD portion of his MSTP training and I served a research fellowship under the tutelage of Donald S. Coffey, hereafter The Chief. Dr. Partin and I explored the hypothesis that the aggressiveness of prostate cancer was not apparent to pathologists because they examined dead, formalin-fixed, paraffin-embedded tissues, instead of examining living cancer cells. Dr. Partin and I filmed cancer cells from fresh operative specimens using time-lapsed video microscopy for approximately 20 of every 24 hours for nearly 2 years. We developed a visual scoring system that assessed membrane ruffling, pseudopodal extension, and translocation [1, 2]. His thesis progressed to a Fourier analysis-based system to more objectively determine whether the motility of cancer cells could predict the capacity for tissue invasion and metastases [3].

I was devastated when my first R01 on cancer cell motility, submitted from the University of North Carolina at Chapel Hill (UNC) where I was their first urologic oncologist, was not funded. The Chief told me he would be "right there" and

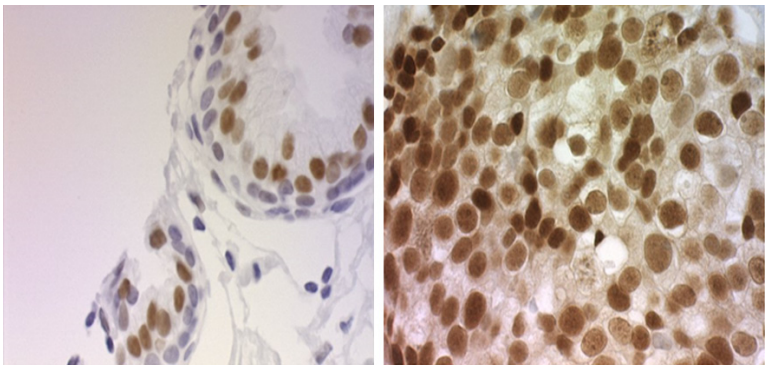

Figure 1. Androgen receptor expression in androgen stimulated benign prostate (left) and castration-recurrent prostate cancer (right).

I was to spread my grant proposal and its review on my dining room table. We would go over them page-by-page to see how this heinous crime could be addressed most effectively. When he arrived, he asked if we knew of this little barbeque place that was on the edge of Chapel Hill. I, my wife and our 3 year old spent about 2 hours at Allen and Sons and then returned to my house to review the grant. The Chief indicated that he'd left Eula at the Carolina Inn and they were on their way to Asheville for a weekend. He told me, which I'll never forget, "Jim you know more about what you're doing than I do, and I know you're going to be successful." He never looked at a single page of that grant. The Chief assured me that I'd learned how to think at Johns Hopkins and all I had to do was immerse myself in what was available at the "great University of North Carolina" and I would be fine.

The androgen receptor and "androgen-independent" prostate cancer

Elizabeth Wilson, PhD and Frank French, MD had cloned the androgen receptor at UNC near simultaneously with Shutsung Liao, PhD and Chawnshang Chang, PhD at University of Chicago. Their papers appeared back-to-back in Science. I'd already had some preliminary conversations with Drs. Wilson and French, who were in the Department of Pediatrics and interested primarily in androgen sensitivity syndrome. I could not pass up the opportunity to help them extend their studies of the androgen receptor to prostate cancer. They had produced a polyclonal antibody, AR-52, that worked only in frozen tissue. We worked to optimize the antibody for immunostaining of prostate tissues. Simultaneously, I banked prostate tissue from radical prostatectomy specimens and trans-urethral resections of patients who presented in urinary retention from local regrowth of prostate cancer during androgen deprivation therapy. The Pathology Department at UNC had reservations about my taking research samples from prostate specimens and so I purchased India ink from an art store and began inking the specimens myself under the observation of Susan Maygarden, MD. She soon determined that I wasn't compromising the Pathology Department's ability to examine the margins and so began my tissue bank of frozen samples of androgen-stimulated benign prostate, androgen-stimulated malignant prostate and castration-recurrent prostate cancer. Immunostaining of the androgen-stimulated benign prostate and prostate cancer samples with AR-52 produced the expected findings that the androgen receptor was expressed in the nucleus, nuclear androgen receptor expression was fairly homogeneous, and the strong, homogeneous expression was confined to the epithelial secretory cells of the prostate and was less apparent in stromal tissue and basal cells (**Figure 1**) [4]. Castration-recurrent prostate cancer exhibited immunostaining that was of similar mean intensity but was more variable and occurred in spite of the patient having undergone surgical castration. We were so surprised by this finding that we reevaluated AR-52, extensively controlled all aspects of the immunostaining, and developed additional polyclonal antibodies to verify these findings.

The working hypothesis in our group at UNC was that the androgen receptor had changed molecularly or biochemically to become hypersensitive to low levels of ligands, a concept that we'd been working on during the time we spent validating the immunohistochemical findings. Our group demonstrated that the androgen receptor was 10,000 times more sensitive in androgen-independent than androgen-sensitive prostate cancer cell lines [5], that AR coactivators in the AR transcription complex changed from SRC1 to TIF2 in cell lines, xenografts and clinical specimens [6] and that AR was phosphorolated by Ack1 tyrosine kinase [7].

Table 1. Tissue leves of testicular androgens measured using mass spectrometry and radioimmunoassay

	Benign prostate (n=18)		Castration-Recurrent CaP (n=18)		
	T (nM)	DHT (nM)	ADT T (nM)	DHT (nM)	
	3.4	23.6	LHRH+flu	1.6	0.0
	0	14.5	Orch	3.7	0.0
	1.2	16.8	Orch+flu	13.6	4.9
	1.8	11.3	LHRH	1.2	4.6
	2.5	12	LHRH+flu	1.7	0.0
	2.9	20.5	Orch	3.8	7.8
	13.0	17.1	LHRH	5.4	3.9
	1.2	13.2	Orch	8.6	6.7
	2.9	9.8	1°hypogonadism	9.8	2.8
	1.4	14.3	Flu	11.4	1.2
	1.6	11.2	Orch	1.1	0.0←
	2.0	6.5	Orch	2.5	0.4
	2.7	10.7	LHRH→DEs	7.2	1.3
	2.8	13.7	LHRH	0.0	0.0
	2.8	13.7	Orch	1.6	0.7
	3.2	20.3	Orch	6.7	5.2
	3.3	38.3	DEs→orch	9.1	1.5
	3.9	12.4	flu→DEs	1.1	0.0
Mass spec	2.8	13.7		3.8	1.3
RIA	3.2	8.1		2.8	1.5

ADT-androgen deprivation therapy; CaP-prostate cancer; DES-diethylstilbestrol; DHT-dihydrotestosterone; flu-flutamide; LHRH-luteinizing hormone-releasing hormone; mass spec-mass spectrometry; orch-orchiectomy; RIA-radioimmunoassay; T-testosterone.

Microenvironmental versus systemic response to androgen deprivation therapy

I questioned the assumption that the tissue contained androgen levels that were the same as the castrate levels in the serum. The Chief questioned whether I had "gone off the deep end in North Carolina". My UNC colleagues also thought this was a crazy idea but worth pursuing. Two important collaborations allowed testing of the hypothesis that tissue levels of androgens were different than serum levels of circulating androgens. Peter Petrusz, MD, PhD, an expert in measurement of estrogens and androgens in saliva, agreed to see whether his radioimmunoassays could be adapted for use in prostate tissue, which he doubted "because everyone knows that the prostate is a factory of proteases and whatever steroid hormones are present will probably be destroyed". We spent approximately 4 years optimizing the methodology that allowed for accurate measurement of tissue levels of testosterone (T), dihydrotestosterone (DHT), androstenedione (ASD), dihydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-SO_4) and sex hormone binding globulin (SHBG).

The findings remain startling to this day. The levels of T were approximately 3 nM (pMoles/g tissue) in both androgen-stimulated benign prostate and castration-recurrent prostate cancer [4]. T was 5 α-reduced to DHT in the androgen-stimulated benign prostate but something was amiss because T was not metabolized to DHT as well in castration-recurrent prostate cancer. The levels of adrenal androgens in the tissue were somewhat lower, but not statistically so, in castration-recurrent prostate cancer compared to androgen-stimulated benign prostate. Hence, it appeared that castration-recurrent prostate cancer was capable of producing T by intracrine metabolism. Even though 5 α-reduction was impaired, the levels of DHT were approximately 1.5 nM, which is sufficient to transactivate even a molecularly and biochemically normal androgen receptor, let alone an androgen receptor that is hypersensitive.

I shared these findings with The Chief prior to submitting for publication and he counseled me that "whatever the last person tells me, I believe until I don't believe it". He was quite skeptical of these results and cautioned me that most people will not believe them unless these surprising findings are confirmed using a second methodology. Little did The Chief know that I was working simultaneously with Kenneth Tomer, PhD, Laboratory of Structural Biology, National Institute of Environmental Health Sciences. Dr. Tomer was the world's expert in measuring levels of estrogens in water for studies of why male fish were becoming female that was causing depopulation of our estuaries. Like Dr. Petrusz, Dr. Tomer was skeptical that his mass spectrometry techniques that worked so well in fluids could be applied to tissue.

Table 2. Testicular androgen levels in prostate tissue

Mass spectrometry			Radioimmunoassay		
Titus 2005 [9]			Molher 2004 [4]		
	T	DHT		T	DHT
AS BP (n=18)	2.75	13.7	AS BP (n=30)	3.26	8.13
CR CaP (n=18)	3.75	1.25	CR CaP (n=15)	2.78	1.45
Montgomery 2008 [13]			Geller 1979 [11]		
	T	DHT		T	DHT
AS BP (n=6)	0.04	1.92	AS BP (n=17)	-	17.6
CR CaP (n=4)	0.23	2.75	CaP orch±DES (n=9)	-	4.47
CR Met CaP (N=8)	0.74	0.25	CaP DES 1 mg (n=6)	-	12.4
			Labrie 1989 [12]		
				T	DHT
			Human CAP (n=NR)	-	18.6
			Orch (n=5, 2-12 m)	-	9.29
			Orch+fl (n=4, 2 m)	-	ND

AS-androgen-stimulated; BP-benign prostate; CR-CAP-castration-recurrent prostate cancer; DES-diethylstilbestrol; DHT-dihydrotesterone; fl-flutamide; Met-metastasis; NR-not reported; orch-orchiectomy; T-testosterone.

However, the results obtained using mass spectrometry [8] were almost identical to those obtained using radioimmunoassay (**Table 1**; data extracted from **Table 1**, in reference 9) [9]. Mass spectrometry has been used by us to measure T and DHT levels in 47 cases of castration-recurrent prostate cancer. Only a single patient has not had detectable levels of either testicular androgen in this experience (arrow, **Table 1**). The quality of that tissue was problematic and it is more likely that the tissue was degraded than this is the only patient of the 47 that had "androgen-independent" prostate cancer.

The Chief indicated that he now "probably believed" that the castration-recurrent prostate cancer was making its own testicular androgens and asked me, as he's asked many others, "If this is true, what does it mean?" The obvious implication of the finding that castration-recurrent prostate cancer is producing its own testicular androgens is that medications were needed to prevent intracrine metabolism of T and especially DHT. Our original finding of intracrine metabolism of testicular androgens led to the repurposing of an antihypertensive, abiraterone acetate, by Gerhardt Attard, MD, PhD and Johann de Bono, MB, ChB, PhD [10].

Were we prescient or had we just failed to read the literature, as was suggested by John Isaacs, PhD (**Table 2**)? Of course, Dr. Isaacs was correct and I was unaware of the work by Jack Geller, MD who had shown in 1979 that DHT levels were 4.47 nM after castration in men who had or had not received DES but 12.4 nM in men who'd received 1 mg of DES (which is now known to be an inadequate dose in most men) [11]. Dr. Geller's paper even suggested that adrenal androgens were the source of recurrence of prostate cancer during androgen deprivation therapy.

So how did it take until 2004 to reappreciate intracrine metabolism of testicular androgens? I think we took a detour when Ferdinand Labrie, MD, PhD published in 1989 a seminal work that led to the adoption of combined androgen blockade [12]. He measured DHT levels that were much higher that anything we've ever measured in an indeterminate number of androgen-stimulated prostate cancer samples. He showed that DHT remained at very high levels at various times after orchiectomy. The levels of DHT became undetectable in (only) 4 patients when flutamide was added to orchiectomy and DHT levels were measured 2 months later. Although the sensitivity of his assay was not indicated, it may be in the 1 nM range, in which case his assay was just at what we now know to be the levels of DHT produced in castration-recurrent prostate cancer. Our mass spectrometry findings were confirmed 3 years later by the Seattle group when they measured tissue T and DHT levels in castration-recurrent prostate cancer obtained from bone metastases in 8 patients [13]. T and DHT levels were lower than what we measured, which could be due to small sample size, tissue degradation or use of different mass spectrometry methodology. Several groups have now confirmed using mass spectrometry that castration-recurrent prostate cancer produces levels of T and DHT that are sufficient for androgen receptor transactivation.

Targeted interruption of intracrine androgen metabolism

The race is on to characterize more precisely intracrine androgen metabolism of testicular

androgens so that more effective therapies can be developed that truly deprive castration-recurrent prostate cancer of testicular androgens. Intracrine metabolism of testicular androgens appears to be an organ-specific characteristic of the prostate. Benign or malignant prostate cell lines, but not non-prostatic cell lines, can metabolize T or DHT from adrenal androgens [5]. The Seattle group has shown that adrenal androgens may be used as substrate for testicular androgens or, when adrenal androgens are unavailable, testicular androgens can be metabolized from cholesterol [13-17]. Hence, intracrine metabolism must be better understood and interrupted by targeting one or more of the three pathways for intracrine metabolism of testicular androgens. Our group is focused on the terminal steps of the primary backdoor pathway [18], whereas the Sharifi group is focused on the secondary backdoor pathway [19]. Other groups have targeted individual androgen metabolism enzymes, such as 17βHSD6 by the Harvard group [20]. The redundancy in the pathways for intracrine metabolism of testicular androgens suggests more sophisticated and perhaps combinations of attacks must be made to impair effectively production of testicular androgens by prostatic tissue and especially castration-recurrent prostate cancer. An alternative approach is to compete more effectively for the T or DHT produced by intracrine metabolism using anti-androgens. The evolution of anti-androgens has produced more effective treatment from flutamide [12] to bicalutamide [21] to enzalutamide [22] to apalutamide [23] and to even newer approaches that link an anti-androgen with another agent, such as inhibition of apoptosis protein [24].

The development of androgen metabolism synthesis inhibitors and more effective anti-androgens raises the possibility that we may now be seeing castration-recurrent prostate cancers that more closely represent “androgen-independent” prostate cancer (the neuroendocrine or small cell phenotype) for which new and different therapies will be necessary that do not target the androgen receptor. Until such time that these therapies are developed, it behooves the field to continue to target the androgen receptor as effectively as possible. Success will require better agents to prevent intracrine metabolism of testicular androgens or more effective anti-androgens to deprive the androgen receptor of its preferred ligands, even when present at reduced levels.

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Address correspondence to: Dr. James L Mohler, Department of Urology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA; University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA. Tel: (716) 845-8433; Fax: (716) 845-3300; E-mail: James.Mohler@RoswellPark.org

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