AWARD NUMBER: W81XWH-14-1-0453

TITLE: Genetic Variations in SLCO Transporter Genes Contributing to Racial Disparity in Aggressiveness of Prostate Cancer

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REPORT DATE: June 2019

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
June 2019	Final	15 Sep 2014 - 14 Mar 2019

4. TITLE AND SUBTITLE
Genetic Variations in SLCO Transporter Genes Contributing to Racial Disparity in Aggressiveness of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0453

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The proposed studies are expected to (1) identify genetic variations in the genes of androgen transporters that are associated with the racial differences in prostate cancer aggressiveness; (2) identify key androgen transporters of which the expression and/or the alteration of expression in cancer relative to benign prostate tissue are associated with racial differences in prostate cancer aggressiveness. Progress in the reporting period includes: 1) Completion of genotyping for all 11 SLCO members using PCaP DNA samples; 2) Finish data processing and preliminary data analyses for genotyping, and identified SNPs that may be associated with prostate cancer characteristics; 3) Continued RNAScope analysis of SLCO transporter in prostate cancer tissue sections and discover unique cell type-specific expression of a SLCO transporter; 4) Further delineation of androgen uptake mechanism on molecular levels.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	37	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Compared to European American (EA) men, African American (AA) men suffer higher incidence of, and greater mortality rate from prostate cancer. Results of multiple studies indicate that prostate cancer in AA men may progress faster than prostate cancer in EA men, and thereby becomes more aggressive. This study is focused specifically on identification of genetic/biological culprits that cause more aggressive types of prostate cancer in AA men. In particular, the proposed studies are focused on the question of how differences in transporter-mediated androgen uptake may contribute to the more aggressive type of prostate cancer in AA versus EA. The proposed studies are expected to (1) identify genetic variations in the genes of androgen transporters that are associated with the racial differences in prostate cancer aggressiveness; (2) identify key androgen transporters of which the expression and/or the alteration of expression in cancer relative to benign prostate tissue are associated with racial differences in prostate cancer aggressiveness.

2. KEYWORDS

Prostate cancer, health disparity, androgen, transporter, genetic variation.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1 (months 1-18): DNA samples as well as relevant clinical and epidemiological data will be requested for 2258 cases (1130 AA and 1128 EA) from the North Carolina-Louisiana Prostate Cancer Project (PCaP). A total of 952 SNPs along with a panel of 50 ancestry informative markers (AIMs) will be used for genotyping of 11 SLCO transporters. Genotyping will be performed via the GoldenGate Assay by Illumina Bead Station System in the Genomics Core Facility at Roswell Park Cancer Institute (RPCI).

Specific Aim 2 (months 7-30) is to examine in situ expression profiles of SLCO transporters in prostate tissue and investigate associations of the expression profiles with prostate cancer aggressiveness in AA and EA. Expression of SLCO transporters at transcriptional levels will be examined first in tissue microarrays (TMAs) constructed from prostate cancer and distant benign tissues of 92 AA and 92 EA patients from the Pathology Resource Network (PRN) at Roswell Park Cancer Institute (RPCI). The predominantly expressed SLCO transporters in AA or EA, and the transporters with expression significantly altered in cancer relative to benign tissues, will be selected and expression at protein levels will be examined using immunohistochemistry (IHC) on TMAs requested from the PCaP. The data on expression will be combined with the data on disease characteristics from the PCaP to investigate associations of the expression profiles with prostate cancer aggressiveness in AA and EA.

Specific Aim 3 (months 25-36) will characterize functions of candidate SLCO transporters in androgen uptake and evaluate the biological effects on AR signaling in human prostate cancer cell lines. Based on the findings from Aim 1 and Aim 2, candidate SLCO transporters will include the transporters that are predominant in either AA or EA, show significantly altered expression between tumor and benign tissue, or harbor genetic variants that are significantly associated with prostate cancer aggressiveness. Relevant cell models will be constructed using over-expression or siRNA knock-down for functional analysis.

What was accomplished under these goals?

Aim 1. The work proposed for Aim 1 has been completed successfully.

Genotyping. To comprehensively examine single nucleotide polymorphisms (SNPs) in genes of all 11 SLCO transporters, two types of SNPs were selected: tag SNPs selected for African American (AA) and

European American (EA) population separately based on Hapmap data with minor allele frequency of at least 0.05, and potential functional SNPs selected from the literature. To control for potential bias due to population admixture, a panel of 128 ancestry informative markers (AIMs) were included. A custom 1,152-OPA was assembled and the breakdown number of SNPs for each gene was presented in Table 1. A total of 2159 participants with sufficient DNA samples and clinical data were identified and requested from PCaP. The samples were randomly plated onto 24 96-well plates along with 96 duplicates and one set of in-house trio samples for quality control purpose. Genotyping was performed using an Illumina GoldenGate assay at the Genomics Core Facility at Roswell Park Comprehensive Cancer Center (RPCCC).

Table 1. The number of SNPs tested for each gene

Gene	# of SNPs
AIM	128
other	8
SLCO1A2	99
SLCO1B1	102
SLCO1B3	51
SLCO1C1	63
SLCO2A1	79
SLCO2B1	63
SLCO3A1	300
SLCO4A1	40
SLCO4C1	44
SLCO5A1	134
SLCO6A1	41
Total	1152

Results of 44 participants were excluded from the analysis due to withdrawal of consent (2%). Results of another 65 participants (3%) were further removed during the quality control due to call rate <90% (59), abnormal heterozygosity (1), and unintended relatedness (2). A total of 2050 individuals (993 AA and 1057 EA) were included in the final analysis with an average call rate above 95%. Out of 1152 SNPs, 107 (9.3%) were removed from either AA or EA analysis due to call rate <90% (106) and violation of Hardy-Weinberg Equilibrium (1), resulting in 1045 SNPs in the final analysis with an average call rate above 95%.

Table 2 summarizes the descriptive characteristics of the study population by self-reported race. Comparing with EA men, AA men tended to be diagnosed at younger ages and were more likely to have prostate cancer with high aggressiveness. There was no difference in other tumor characteristics including primary and sum Gleason Grade as well as clinical stage. AA and EA men had similar rate of family history of prostate cancer, showing the majority (approximately 75%) had no family history. The self-reported race status was supported by the distribution of ancestry proportions with the minimum of Asian ancestry in the study population. Therefore, either African or European ancestry proportion was adjusted in race-specific analysis but Asian ancestry was no longer considered. To note, the ancestry component information was requested from PCaP. Our own panel of AIMs generated similar estimates and further confirmed the validity of ancestry analysis.

Table 2. Descriptive and tumor characteristics by race

	African American (N=993)		European American (N=1057)		P_value*
	Mean	SD	Mean	SD	
Age at diagnosis, yrs	61.9	7.8	64.2	7.9	<0.001
European ancestry	0.08	0.15	0.97	0.07	<0.0001
African ancestry	0.90	0.16	0.01	0.04	<0.0001
Asian ancestry	0.02	0.05	0.02	0.05	0.687
	N	%	N	%	
Study site					0.416
North Carolina	436	43.9%	483	45.7%	
Louisiana	557	56.1%	574	54.3%	
1st Degree family history of prostate cancer					0.245
No	740	74.5%	811	76.7%	
Yes	253	25.5%	246	23.3%	
Primary Gleason Grade					0.082
<4	770	80.0%	858	83.0%	
≥4	193	20.0%	176	17.0%	
Gleason Grade Sum					0.147
<8	861	86.9%	936	89.0%	
≥8	130	13.1%	116	11.0%	
Stage					0.825
T1/2	946	98.1%	1019	98.3%	
T3/4	18	1.9%	18	1.7%	
Aggressiveness					0.0007
Low/Intermediate	750	79.0%	872	84.9%	
High	199	21.0%	155	15.1%	

* Chi square test was used for categorical variables, and student t-test was used for continuous variables if normally distributed, otherwise Kruskal-Wallis test was used.

Association of SNPs with cancer characteristics. To examine associations of genetic variations in SLCO transporters with prostate cancer characteristics, Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression model adjusting for age, study site, family history of prostate cancer and African ancestry proportion. A co-dominant (genotypic) model was primarily assumed, then a dominant model was included with consideration of small number of homozygotes for most of tested SNPs. The analysis was performed in AA and EA separately with the following four cancer characteristic outcomes: 1. prostate cancer aggressiveness (high versus intermediate/low), which is defined using three variables described as following: (i) high aggressiveness (Gleason sum ≥ 8 or PSA > 20 ng/mL or Gleason sum ≥ 7 and clinical stage T3–T4), (ii) low aggressiveness (Gleason sum < 7 and clinical stage T1–T2 and PSA < 10 ng/mL), and (iii) intermediate aggressiveness (all other cases); 2. primary Gleason Grade (≥4 versus <4); 3. sum Gleason Grade (≥8 versus <8); and 4. clinical stage (T3/4 versus T1/2). Three P values are presented: P_trend, for genetic dose response by coding genotypes as 0, 1, 2 on the basis of the number of variant alleles; P_adj, P_trend after FDR correction for multiple comparisons; and P_interaction, Wald test of the product term between race and genotype for the differences in associations between AA and EA men. All analyses were performed using R and/or SAS 9.4 (Cary, NC). Only SNPs with corrected P_trend below the level of 0.05 were presented in Table 2. Primary Gleason grade was not included in Table 2, since no SNPs were significantly associated with primary Gleason grade.

As shown in Table 3, all significant SNPs belong to two genes, SLCO2A1 and SLCO5A1. SNPs in SLCO2A1 were associated with reduced tumor aggressiveness as well as tumor sum Gleason grade; however, the associations were primary observed in AA population, but not EA population. In contrast, SNPs in SLCO5A1 were positively associated with high clinical stage, and the associations were primarily found in EA population, but not AA population. For certain SNPs, such as rs9917636 and rs3811662 in SLCO2A1 and rs16919172 in SLCO5A1, the observed associations were significantly different between AA and EA population, showing P value for interaction <0.05. Further investigation found that the four significant SNPs in SLCO5A1 (rs16919172, rs4370538,

Table 3. Differential associations of single-nucleotide polymorphisms in SLCO transporters with prostate cancer characteristics between African American and European American men in the PCaP study

Gene	SNP	Chr	Genotype	European American # high vs low	European American OR (95% CI)[a]	European American P_trend[b]	European American P_adj[c]	African American # high vs low	African American OR (95% CI)[a]	African American P_trend[b]	African American P_adj[c]	P_interaction[d]
Aggressiveness High vs. Low/Intermediate												
SLCO2A1	rs9917636	3	AA	33/231	1.00	0.896		62/150	1.00	<0.001	0.03	0.004
			AG	88/417	1.52 (0.98-2.36)			93/367	0.59 (0.41-0.86)			
			GG	34/222	1.04 (0.62-1.74)			44/233	0.45 (0.29-0.71)			
			AG/GG vs. AA	122/639	1.35 (0.89-2.05)			137/600	0.54 (0.38-0.77)			
SLCO2A1	rs3811662	3	GG	150/861	1.00	0.021		160/519	1.00	0.001	0.039	0.004
			GA	5/10	3.77 (1.22-11.64)			38/210	0.58 (0.39-0.86)			
			AA	0/0				1/20	0.16 (0.02-1.2)			
			GA/AA vs. GG					39/230	0.54 (0.37-0.8)			
Sum Gleason Grade ≥8 vs. <8												
SLCO2A1	rs3811662	3	GG	114/922	1.00	0.574		108/603	1.00	0.001	0.049	0.218
			GA	2/13	1.55 (0.33-7.23)			22/235	0.52 (0.32-0.84)			
			AA	0/0				0/22				
			GA/AA vs. GG					22/257	0.47 (0.29-0.77)			
SLCO2A1	rs9874493	3	AA	84/671	1.00	0.967		94/518	1.00	0.001	0.049	0.352
			AG	30/243	1.04 (0.66-1.62)			36/294	0.66 (0.44-1)			
			GG	2/22	0.88 (0.2-3.85)			0/48				
			AG/GG vs. AA	32/265	1.02 (0.66-1.59)			36/342	0.56 (0.37-0.85)			
Stage 3/4 vs. 1/2												
SLCO5A1	rs16919172	8	AA	11/914	1.00	<0.001	0.039	15/729	1.00	0.604		0.028
			AG	7/101	6.24 (2.32-16.8)			3/199	0.78 (0.22-2.75)			
			GG	0/3				0/12				
			AG/GG vs. AA	7/104	5.98 (2.23-16.08)			3/211	0.74 (0.21-2.64)			
SLCO5A1	rs4370538	8	AA	10/901	1.00	<0.001	0.035	6/468	1.00	0.101		0.25
			AG	8/113	6.95 (2.64-18.27)			10/414	2.22 (0.77-6.36)			
			GG	0/3				2/64	2.85 (0.54-14.94)			
			AG/GG vs. AA	8/116	6.67 (2.54-17.52)			12/478	2.31 (0.83-6.39)			
SLCO5A1	rs4377973	8	GG	11/908	1.00	0.001	0.039	6/447	1.00	0.331		0.383
			GC	7/108	5.93 (2.21-15.95)			11/422	2.28 (0.81-6.44)			
			CC	0/3				1/77	1.08 (0.13-9.32)			
			GC/CC vs. GG	7/111	5.68 (2.12-15.26)			12/499	2.09 (0.75-5.77)			
SLCO5A1	rs10096246	8	AA	11/911	1.00	0.001	0.039	10/572	1.00	0.526		0.078
			AG	7/105	6.04 (2.24-16.26)			7/336	1.29 (0.48-3.47)			
			GG	0/3				1/37	1.69 (0.2-14.02)			
			AG/GG vs. AA	7/108	5.79 (2.15-15.57)			8/373	1.33 (0.51-3.45)			

[a] ORs and 95% CIs were estimated from co-dominant and dominant models adjusting for age at diagnosis, state (NC or LA), 1st degree family history of prostate cancer (yes/no), African ancestry component.

[b] P_trend was estimated for genetic dose response by coding genotypes as 0, 1, 2 according to the number of variant alleles.

[c] P_adj was generated from P-trend after correction for multiple comparison by FDR. Only SNPs with P_adj less than 0.05 either from AA or EA population were presented.

rs4377973, rs10096246) are in high linkage disequilibrium (LD) in EA population but not AA population based on 1000 genome data, which explains the similar associations across the SNPs. However, all identified SNPs in both SLCO2A1 and SLCO5A1 are located in introns and are not in LD with any potential functional SNPs or coding SNPs, rendering further functional analysis difficult. An exome sequencing project focusing on SLCO2A1 and SLCO5A1 is under development to further delineate the signals and identify functional genetic variants. Overall, two conclusions can be drawn from the results. First, among all 11 SLCO family members, SLCO2A1 and SLCO5A1 may play an important role in prostate cancer development. This conclusion is further supported by our findings from RT-PCR examination of SLCO expression in human prostate malignant and benign tissues, showing dominant expression of SLCO2A1 and SLCO5A1 in the prostate. Second, the role of SLCO2A1 and SLCO5A1 in prostate cancer development differs by race, showing race-specific associations between genetic variations and aggressive cancer characteristics.

Table 4. Risk allele frequency among African American (AA) and European American (EA) men.

Gene name	SNP	chr	Risk allele	Frequency_AA	Frequency_EA	P value*
SLCO2A1	rs3811662	3	G (risk allele in AA)	**0.85**	1	1.4156E-64
SLCO2A1	rs9874493	3	A (risk allele in AA)	**0.78**	0.85	1.2065E-06
SLCO2A1	rs9917636	3	A (risk allele in AA)	**0.47**	0.50	0.07559565
SLCO5A1	rs10096246	8	G (risk allele in EA)	0.22	**0.06**	3.73E-49
SLCO5A1	rs16919172	8	G (risk allele in EA)	0.12	**0.06**	5.8094E-12
SLCO5A1	rs4370538	8	G (risk allele in EA)	0.29	**0.06**	4.1155E-79
SLCO5A1	rs4377973	8	C (risk allele in EA)	0.30	**0.06**	8.2061E-90

* P values were calculated by Chisq test of genotype distribution between AA and EA.

Risk allele or genotype as well as its frequency for identified SNPs in SLCO2A1 and 5A1 were presented in Table 1 for AA and EA separately. Except for rs9917636 in SLCO2A1, all other SNPs were significantly different in distribution of genotypes between AA and EA. More importantly, the frequency of risk-allele/genotype in AA (averagely 0.52) was substantially higher than those in EA (0.06), which is in line with the high proportion of prostate cancer diagnosed in AA with aggressive phenotypes.

Three SNPs in SLCO2A1 were significantly associated with aggressive phenotype of prostate cancer in AA, with the common homozygote as the risk-genotypes. When combining all three SNPs by counting the number of risk-genotypes and using AA men with no presence of risk-genotype (0) as the reference, odd ratios (ORs) increased with the number of risk genotypes, showing 1.61 (95%CI, 0.90-2.89) with one, 1.96 (95%CI, 1.16-3.30) with two, and 3.49 (95%CI, 1.87-6.53) with three risk-genotypes. This association was only observed in AA, not in EA. For SLCO5A1, four SNPs were found significantly associated with advanced stage of prostate cancer in EA only, with the minor allele as the risk-alleles. Since these four SNPs are in high linkage disequilibrium (LD) in EA, there were no sufficient numbers to access dose-dependent relationship in EA. Regardless, a strong association was found among EA men with at least one risk-allele in comparison to the reference group with on risk-allele in any of the four SNPs

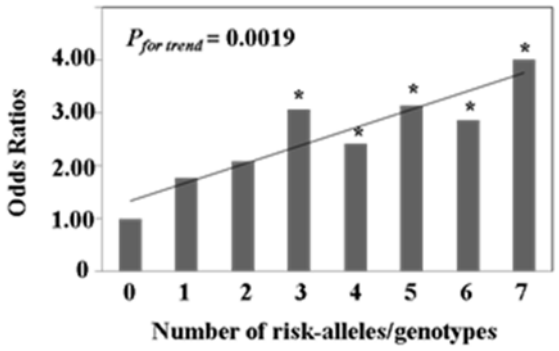

Fig. 1. Increase of odds of aggressive phenotype of prostate cancer with the number of risk allele/genotype in SLCO2A1 and 5A1 among AA.

(OR=6.59, 95%CI=2.51-17.3). The risk-alleles in SLCO5A1 are much common in AA (averagely 0.23) than in EA (0.06), and interestingly, a significant association with aggressive phenotype of prostate cancer appeared among AA men with the presence of at least three risk-alleles when combining all four SNPs (OR=1.61, 95%CI=1.05-2.49) in comparison to AA men with no risk-alleles. Indeed, a dose-dependent relationship was found in AA men when combing risk alleles/genotypes in both SLCO2A1 and 5A1 as shown in Figure 1. A 14% increase of odds having aggressive phenotype of prostate cancer was observed with the increase of per risk allele or genotype in SLCO2A1 and 5A1 among AA men (OR=1.14, 95%CI=1.05-1.24). In summary, high frequency of genetic variants in SLCO2A1 and 5A1 that are associated with aggressiveness of prostate cancer in AA population may help explain disproportionally high risk of aggressive prostate cancer and high mortality rate from prostate cancer in AA men. Further biological investigation of SLCO2A1 and 5A1 is warranted.

A portion of the work of Aim 1 has been summarized and communicated with the PCaP team as the initiation to prepare a manuscript. The PCaP team reviewed our summary of results and provided very good suggestions. As a result, an abstract was reviewed and approved by the PCaP Committee for submission to the American Urological Association (AUA) 2018 Annual Meeting in San Francisco. This abstract was accepted for presentation in a moderated poster section (Abstract # MP21-16), and the abstract was published (J. Urol., Vol. 199, No. 4S, Supplement, Saturday, May 19, 2018, e269).[1]

Aim 2. The association of expression of SLCO2A1 and SLCO5A1 with disease characteristics of prostate cancer in EA and AA. Based on the findings in Aim 1, SLCO2A1 and SLCO5A1 were selected to assess the expression at the protein levels in benign and prostate cancer tissues from AA and EA, and to determine the association of expression levels with disease characteristics. The methods for IHC was optimized and the scoring was conducted by the Pathology Network Shared Resource (PNSR) using algorithms developed specific for the IHC of SLCO2A1 and SLCO5A1. This approach was used to replace originally proposed manual scoring by 3 independent scorers to avoid human error, and to increase accuracy and reproducibility. Representative IHC images are presented to show the positive cells in contrast to the negative staining by control non-immuned IgG in the cells in benign and malignant prostate tissue (Fig. 2).

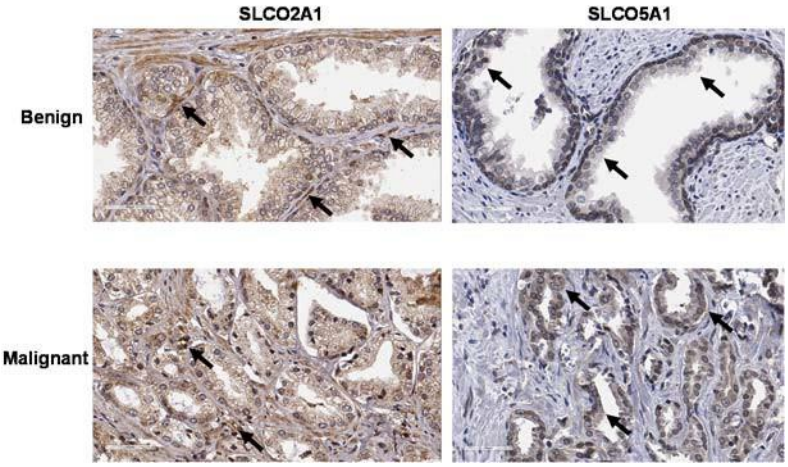

Fig. 2. IHC staining of SLCO2A1 and 5A1 in benign and malignant prostate tissue.

Expression of SLCO5A1 in benign and malignant prostate tissue differed between AA and EA. Expression of SLCO5A1 and SLCO2A1 was evaluated using sections of the RPCCC 92 AA/92 EA TMA (Fig. 3). Expression of SLCO5A1was higher in both the benign and malignant tissue from EA compared to the benign and malignant tissue from AA. There was no

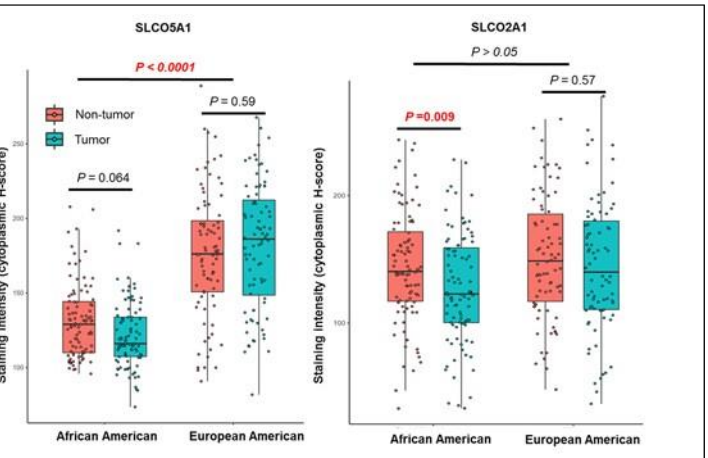

Fig. 3. Expression of SLCO5A1 and SLCO2A1 in prostate cancer and benign tissue specimens from EA and AA.

difference in expression of SLCO5A1 between benign and malignant tissue in either AA population or EA population. SLCO2A1 expression was consistent between types of tissue and between the populations.

Protein levels SLCO5A1 are associated with cancer characteristics in EA. Expression levels of SLCO5A1 and SLCO2A1 on the 92 AA/92 EA TMA were analyzed for association with disease characteristics (Table-5).

Table 5. Expressin of SLCO5A1 and SLCO2A1 in African American (AA) and European American (EA) prostate cancer patients

	SLCO5A1						SLCO2A1					
	AA			EA			AA			EA		
	N	H-Scores	P	N	H-Scores	P	N	H-Scores	P	N	H-Scores	P
Primary gleason grade												
Low (<4)	71	116 (74-183)	0.748	69	182 (82-261)	0.037	69	124 (36-228)	0.392	67	151 (34-278)	0.492
High (>=4)	24	127 (85-192)		16	210 (119-268)		23	118 (33-226)		16	119 (54-240)	
Sum gleason grade												
Low (<8)	82	116 (74-183)	0.439	78	183 (82-261)	0.0466	80	123 (36-228)	0.333	76	142 (34-278)	0.55
High (>=8)	13	124 (96-192)		7	216 (130-268)		12	121 (33-226)		7	118 (103-230)	
Pre-surgical PSA												
Low (< median)	47	115 (74-160)	0.374	41	182 (111-224)	0.1264	44	121 (36-204)	0.413	42	135 (34-244)	0.548
High (>= median)	47	118 (85-1912)		45	189 (82-268)		47	124 (33-228)		42	134 (37-278)	
Age at surgery*												
<50	16	118 (93-183)	0.486	4	172 (166-194)	0.123	15	134 (36-228)	0.341	4	53 (46-183)	0.153
50-59	1	135		41	176 (82-241)		46	120 (37-182)		7	186 (57-278)	
60-69	47	115 (73-118)		34	192 (111-261)		31	123 (33-226)		41	137 (34-240)	
>=70	31	118 (85-183)		7	210 (156-268)		0			33	143 (61-251)	
Pathological T stage												
T2	64	116 (74-183)	0.828	60	181 (82-254)	0.0678	62	132 (36-228)	0.275	59	146 (34-278)	0.87
T3	26	118 (85-192)		22	199 (118-268)		25	109 (33-207)		22	137 (61-251)	
T4	5	116 (87-183)		4	205 (157-222)		5	123 (63-176)		4	126 (99-197)	
Biochemical recurrence												
No	84	116 (74-192)	0.34	69	185 (82-268)	0.6733	83	122 (33-228)	0.132	68	142 (37-278)	0.589
Yes	9	124 (107-146)		16	184 (119-261)		7	145 (104-207)		16	134 (34-251)	
Biochemial failure												
No	63	116 (74-183)	0.418	57	182 (82-254)	0.0212	62	124 (33-228)	0.724	56	149 (37-278)	0.913
Yes	30	116 (87-192)		28	208 (118-268)		28	119 (37-207)		28	134 (34-251)	
Radical prostectomy failure												
No	56	116 (74-183)	0.543	55	182 (82-254)	0.0303	56	123 (33-228)	0.838	54	139 (37-278)	0.797
Yes	37	116 (87-192)		30	203 (118-268)		34	122 (37-207)		30	135 (34-251)	

* Age at surgery was significantly correlated with expression of SLCO5A1 (r=0.24) and 2A1 (r=0.26) in EA but not in AA (r=0.03 and 0.04, respectively).

Higher levels of SLCO5A1 was associated with higher Gleason grades, biochemical failure, and radical prostatectomy failure. Intriguingly, the association was only found in EA but not AA, despite higher expression of SLCO5A1 in EA than AA. Additionally, age at surgery was significantly associated with expression of SLCO5A1 in EA but not AA. Expression of SLCO2A1 was not associated with any tested disease characteristic in either population, despite of the SNPs that were associated with disease characteristics in AA. To note, SLCO2A1 is expressed in both epithelial cells and endothelial cells, and our analysis was focused on expression in epithelial cells. It is of interest to examine whether levels of SLCO2A1 expressed in endothelial cells are associated with disease characteristics.

Based on the results in the 92 AA/92 EA, sections of a TMA set from PCaP were requested for IHC staining of SLCO5A1. The IHC has been finished in Dr. Wu's laboratory. The marking, examining, and automated scoring using the algorithm developed by PNSR is undergoing and is expected to be completed in July of 2019.

Aim 3. Characterize functions of candidate SLCO transporters in androgen uptake and evaluate the biological effects on AR signaling in human prostate cancer cell lines. We identified a selective and active androgen uptake mechanism that is new. The preliminary findings were first presented at the IMPACT meeting 2016 in a poster and an oral presentation.[2] We have summarized our key findings in the Aim and developed a

manuscript, which was submitted to Molecular and Cellular Endocrinology. After revision during EWOF, the manuscript has been accepted and is now published.[3] The key findings of the published results are are summarized following, the full manuscript is provided in Appendix.

Uptake of ^{3}H-T by LAPC-4 and VCaP cells was evaluated over a time course of 60 minutes (Fig. 4A). Cells were treated with 1nM ^{3}H-T. LAPC-4 and VCaP cells showed similar uptake patterns. The ^{3}H-T accumulation was nearly linear over the time course. Therefore, 20 minutes treatment duration was used for dynamic experiments for sufficient scintillation count readings and the shortest treatment duration that allowed the performance of experimental procedures. The dynamic curves of the uptake were similar between the cell lines and followed a typical transporter-mediated uptake pattern (Fig. 4B). The uptake reached plateau between 5 and 10 nM ^{3}H-T. Nonlinear regression was performed using the function provided in the GrapgPad Prism 7 software to calculate maximum specific binding (Bmax) and equilibrium binding constant (Kd). VCaP showed higher Bmax and Kd compared to LAPC-4.

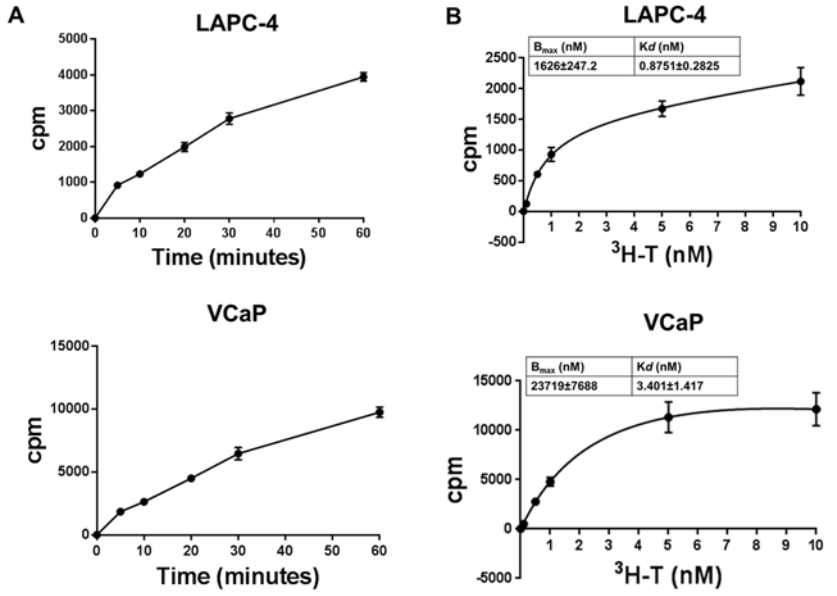

Fig. 4. ^{3}H-T-uptake by LAPC-4 and VCaP cells. (A) Uptake over a time course of 30 min; (B) Kinetic analysis of uptake at 20 min using multiple concentrations of ^{3}H-T.

T uptake was evaluated in the presence of non-radioactive T, non-radioactive DHEA, or non-radioactive DHEAS to determine whether the DHEA and DHEAS competed with T for the same uptake mechanism. EpiT is an epimer of T, differing from T only in the configuration at the hydroxyl-bearing carbon, C-17. T/EpiT ratio in plasma of adult males is 1:1. EpiT was included in the experiment to determine whether T uptake mechanism differentiated T from its epimer. LAPC-4 and VCaP cells were treated with 1nM of ^{3}H-T in the presence of 1, 5, 10, 100 or 300 nM of non-radioactive T, DHEA, DHEAS, or epiT (Fig. 5). Uptake of ^{3}H-T was blocked by T and epiT. On the contrary, non-radioactive DHEA and DHEAS did not block ^{3}H-T uptake. The data indicated that the T uptake mechanism selectively excluded adrenal androgens DHEA and DHEAS, but did not differentiate T from epiT.

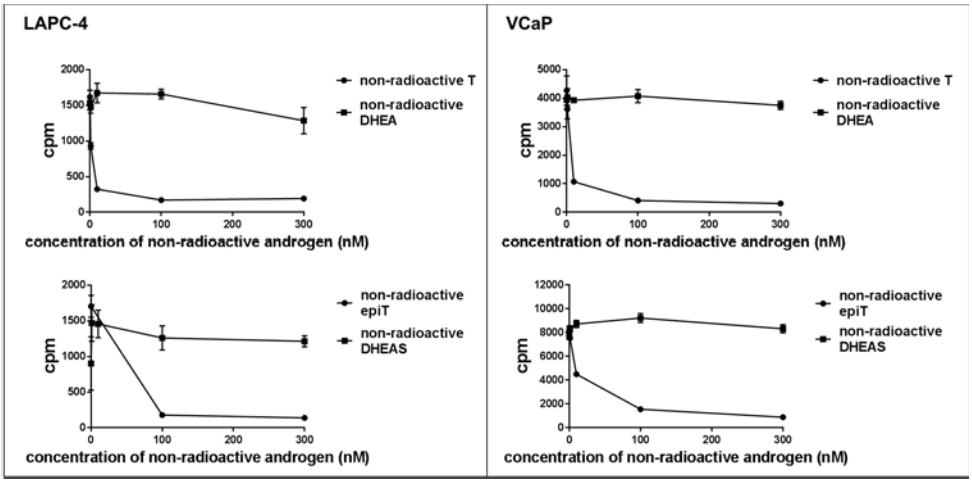

Fig. 5. ^{3}H-T uptake was blocked by T or epiT, but not by DHEA or DHEAS.

^{3}H-T uptake by LAPC-4 and VCaP cells were fully blocked by 300 nM DHT, progesterone, or E2 (Fig. 6). The results indicated that the T uptake mechanism also recognized progesterone and E2.

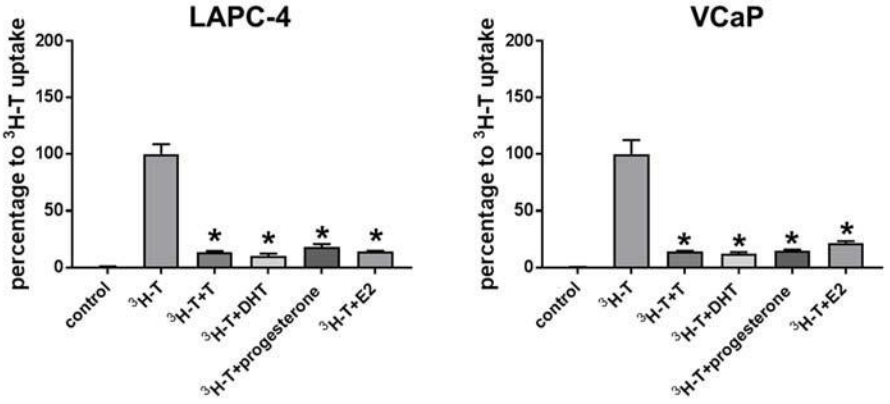

Fig. 6. DHT, progesterone, and E2 blocked ^{3}H-T uptake. Results were presented as percentage of the intracellular ^{3}H-T taken up in the absence of non-radioactive androgen. * significantly different from ^{3}H-T alone value ($p < 0.05$).

V-ATPase is known to drive transmembrane transporter-mediated uptake. Cells were treated with a V-ATPase-specific inhibitor, bafilomycin A1 for 24 hr, and treated with 1 nM ^{3}H-T in the presence of bafilomycin A1 to examine effect on T uptake (Fig. 7A). Bafilomycin A1 treatment modestly reduced T uptake by LAPC-4 and VCaP cells. The data indicated that V-ATPase or similar ATP-driven mechanism might contribute to the uptake of T. Progesterone fully blocked T uptake, suggesting shared uptake mechanism for T and progesterone. The progesterone receptor membrane component 1 (PGRMC1) mediates transmembrane transport of progesterone (Thomas, 2008,Meyer, Schmid, Scriba et al., 1996,Kimura, Nakayama, Konishi et al., 2012,Cahill, 2007). Cells were treated with AG-205, a PGRMC1 inhibitor (Will, Liu and Peluso, 2017,Guo, Zhang, Wang et al., 2016), to determine whether PGRMC1 was involved in T uptake and cell viability (Fig. 7B and C). Cells were treated with multiple doses of AG-205 for 24 hr, then treated with 1 nM ^{3}H-T to assess T uptake. MTT assay was performed using the identical culture condition in parallel with the uptake experiment to determine effect of AG-205 on cell viability. Treatment with AG-205 reduced ^{3}H-T uptake by LAPC-4 cells and reduced cell viability. However, reduction in T uptake was greater than reduction in cell viability (Fig. 7B). Treatment with AG-205 reduced ^{3}H-T uptake by VCaP cells, but did not reduce cell viability (Fig. 7C). The data suggested that PGRMC1 or similar mechanism might contribute to T uptake, although the mechanism may not be a primary one.

SLCO5A1 is selected for further testing the role in cell growth or AR signaling due to its predominant expression in benign and malignant prostate tissue. Stable clones that over-express the transporters are the optimal choice for these tasks. This is because transiently transfected cells are not suitable for long-term experiments such as growth, and inconsistent transfection efficiency may cause inconsistent results that are further worsened by double transfection involved in luciferase-based promoter-reporter assay for AR activity. This effort was initiated during EWOF based on the findings from Aim 1 and 2. We already obtained the over-expressing plasmids for the SLCO transporters used puromycin resistance as a selection marker and also established puromycin sensitivities of human prostate cancer cell lines used for the proposed work. We are in the process of establishing stable clones for the biological studies.

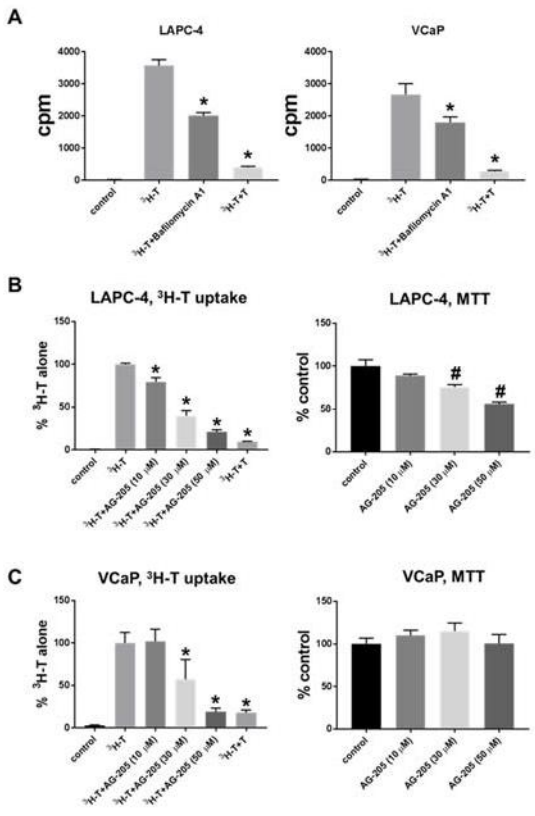

Fig. 7. Uptake of ^{3}H-T was reduced when cells were treated with bafilomycin A (10 nM) or AG-205. (A) Pretreatment with bafilomycin A reduced ^{3}H-T uptake by LAPC-4 and VCaP cells. (B) Pretreatment with AG-205 reduced ^{3}H-T uptake by LAPC-4 cells and cell viability of LAPC-4 cells. (C) Pretreatment with AG-205 reduced ^{3}H-T uptake by VCaP cells, but not viability of VCaP cells. Effect on ^{3}H-T uptake was presented in percentage of ^{3}H-T in cells that were treated with ^{3}H-T alone. Effect on viability was presented in percentage of MTT reading of controls. * significantly different from ^{3}H-T uptake value ($p < 0.05$). # significantly different from control value ($p < 0.05$).

SLCO5A1, 2A1 and 2B1 were also transiently over-expressed in a human prostate cancer cell line LAPC-4. SLCO2B1 was included in the experiment because of its similar cell type specificity shared with SLCO2A1. The transfected cells were tested for the uptake of ^{3}H-DHEAS. Non-radioactive DHEAS, or so-called cold DHEAS, was added in 300-fold excessive amount for competition with ^{3}H-DHEAS. Transporter-specific uptake of DHEAS would be indicated by the reduction of the radioactive in the cell lysate in the presence of cold DHEAS (Figure 8). The reduction occurred in the SLCO5A1 over-expressing cells. This preliminary finding indicated that DHEAS may be a substrate for SLCO5A1, but not for SLCO2A1. This observation echoes the different expression pattern of SLCO2A1 and 5A1 in the prostate (Aim 2) and the race-specific associations of genetic variants in SLCO2A1 and 5A1 with prostate cancer characteristics (Aim1). Currently this finding is being verified in other cell lines. If the finding is confirmed, then SLCO5A1 may indeed play a very important role in intracellular T/DHT production in cancer cells, because our other data showed that DHEAS is a substrate for intratumoral T/DHT production (data not shown).

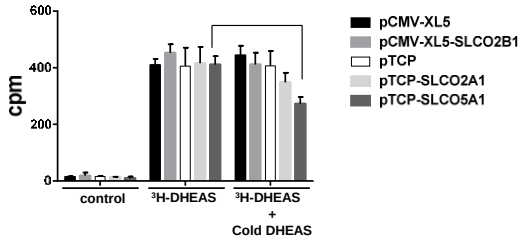

Fig 8. SLCO5A1 over-expressing cells demonstrated transporter-mediated uptake of DHEAS.

What opportunities for training and professional development has the project provided?

One of the biggest gain for Dr. Wu is to witness the evolving progression of the analysis of the genotyping data, led by Dr. Tang and Dr. Zhu. As a laboratory-based basic science researcher by training, Dr. Wu would

not have had the opportunity to learn from the two co-investigators on the sophisticated statistical models that were applied to the different data and different study questions. This was the first time for Dr. Wu to interact closely with a molecular epidemiologists and a biostatistician on large-scale data analysis. The experience is invaluable. A lesson that Dr. Wu learned from the interaction with Dr. Mohler and Dr. Azabdaftari was on the complicated issues revolving around the use of TMA, and how important the expertise of a pathologist is to the research using TMA. Dr. Azabdaftari would inspect each core to verify its pathological characteristic, and to exclude unreliable staining caused by misclassifications of the core, or by missing cancer cells in a cancer core. This process refreshed Dr. Wu's previously over simplified view that only relies on the clinical pathological reports for core classification. Another gain for Dr. Wu is the establishment of the ability for IHC staining in his own laboratory. During Year 3 and the EWOF, a couple of unexpected events occur that negatively impacted the progress of IHC study using the TMAs. A biggest difficulty was caused by the departure Dr. Elena Pop who was in charge of all the IHC studies. Dr. Wu and Dr. Tang had to recruit new hands to finish the manual scoring of the previously stained sections, and to work with their lab staff to establish all the skills and protocols to continue the studies. In order to improve the quality and duplicability of the results, and also to address the shortage of manual scorers, they worked with the PNSR staff to develop and implement automated scoring using algorithms. Dr. Wu's laboratory is now a skilled lab that is able to independently conduct IHC studies. In addition, the high-quality final results led to the new findings in the EWOF.

How were the results disseminated to communities of interest?

1. Preliminary findings were presented in IMPaCT 2016, in the format of a poster and a selected oral presentation.

2. Findings in Aim 3 on an active and selective steroid transport system has been published.

   Parsons TK, Pratt RN, Tang L, and Wu Y: An active and selective molecular mechanism mediating the uptake of sex steroids by prostate cancer cells. Mol Cell Endocrinol. 2018 Dec 5; 477:121-131.

3. Findings in Aim 1 have been presented in a moderated poster section (Abstract # MP21-16) in the American Urological Association (AUA) 2018 Annual Meeting in San Francisco.

   Tang L, Zhu Q, Bensen J, Taylor J, Smith G, Pop E, Azabdaftari G, Mohler J, and Wu Y: Associations of genetic polymorphisms in SLCO transporters with clinical aggressiveness of prostate cancer in the North Carolina-Louisiana prostate cancer project. J. Urol., Vol. 199, No. 4S, Supplement, Saturday, May 19, 2018, e269.

What do you plan to do during the next reporting period to accomplish the goals?

We have successfully completed most of the proposed research. We are in the process of summarizing new findings made in the EWOF for report of results in a new manuscript. Dr. Wu and Dr. Tang are also in the process to complete minor experiments for confirmations and follow-ups that are necessary to strengthen the manuscript in development.

4. IMPACT

What was the impact on the development of the principle discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGEs/PROBLEMS

Changes in approach and reasons for change.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.

A couple of unexpected events delayed the projects. One challenge to the completion of the project was the sudden departure of Dr. Elena Pop (co-I). Dr. Pop was in charge of all the IHC and TMA studies. Her sudden departure caused significant delay of the project. We had to train lab staff to take over IHC staining, and to examine and mark the stained sections in order to collaborate with Dr. Gissou Azabdaftari (pathologist, co-I) to verify cancer cells labeled for scoring. Visual scoring was also delayed because of relocated manpower for the increased work load to compensate and regain the proper expertise. To better address the situation and to obtain more objective data on IHC staining, we collaborated with Roswell Park PNSR to develop algorisms tailored for automatic scoring of SLCO2A1 and SLCO5A1 staining.

Changes that had a significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Yue Wu, Ph.D. (2 cal months) – PD/PI

Li Tang, Ph.D. (1 cal month) – Co PD/PI

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. Updated active other supports of Dr. Yue Wu (PI), Dr. James Mohler (co-I), Dr. Gissou Azabdaftari (co-I), and Dr. Qianqian Zhu (co-I) are presented as follows. Nothing to report for Dr. Li Tang (co-PI) , Dr. Elena Pop (co-I) and Dr. John Wilton (co-I).

Changes in active support

Wu, Y.

New
Title: Prostate-Specific Androgen Transporters are the Missing Target for Complete ADT (Smith)
Time Commitments: 2.40 calendar months
Supporting Agency: NIH/NCI (R01CA193829) Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Neeraja Sathyamoorthy, ns61@nih.gov
Performance Period: 12/1/18-11/30/20
Level of Funding: $1,961,530
Brief description of project's goals: The experimental goals of this project are to determine if dehydroepiandrosterone-sulfate (DHEA-S) represents an endogenous substrate for intracrine dihydrotestosterone (DHT) production and AR-activation by prostate epithelial cells in both the presence and absence of circulating testosterone (ADT), if intracrine metabolism of DHEA-S?DHT is mediated through a steroid 5a-reductase 1 (SRD5A1)- or a SRD5A2-dependent mechanism, and if prostate endothelial cell-specific mechanisms of DHEA-S uptake, transport or efflux represent prostate endothelial cell-specific therapeutic targets to make ADT more effective.
List of specific aims:
Aim 1. Determine inter-patient variability in up-take and metabolism of circulating T and DHEA-S, expression profiles of genes associated with androgen uptake/metabolism in human pECs and CaP/pEpi cells, and the short-term effect of T-deprivation on these processes.
Aim 2. Define the molecular mechanisms that mediate uptake, trans-cellular transport and efflux of circulating androgens in human pECs and pEpi cells, and confirmed in pECs with CaP.
Aim 3. Determine whether interdiction of adrenal androgen usage by pEC and/or CaP/pEpi has the potential to enhance the effect of T-deprivation (ADT).
Overlap: None

Nothing to report for Tang, L.

Mohler, J.L.

Active to Completed
Title: Cancer and Leukemia Group B: RPCI/SUNYAB (PI – Levine)
Time Commitments: 0.60 calendar months
Supporting Agency: NIH/NCI
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Brian Iglesias, Grants Management Officer
Email: brian.iglesias@nih.gov Phone: 240-276-6278
Performance Period: 04/15/2013 – 02/28/2019
Level of Funding: $112,486
Brief description of project's goals: The major goal of this project is to advance our understanding of malignant diseases and, thereby, improve our ability to treat people affected with them. The rapid accumulation of clinical data and experience through cooperative research expedites progress in cancer therapy.
Overlap: None

New
Title: Network Lead Academic Participating Site Grant from the Roswell Park Cancer Inst. (UG1CA233191)
Time Commitments: 0.60 calendar months
Supporting Agency: NIH/NCI

Name and address of the Funding Agency's Grants Officer: Margaret M. Mooney, mooneym@mail.nih.gov
Performance Period: 3/06/2019-2/28/2025
Level of funding: $2,763,882
Brief description of project's goals: Roswell Park has had a long tradition of contributing to the national cooperative groups over many decades. Roswell Park is committed to the many strengths inherent to cooperative group research: therapeutic advances; a better understanding of the biology of cancer; cancer prevention; means to improve the quality of life of cancer patients; piloting of new drugs and radiology and radiation and surgical techniques; establishing the relevance of new cellular and molecular advances to the predictive, prognostic, and therapeutic approaches to patients; and the advancement of patient advocacy.
Overlap: None

Not Previously Reported
Title: A Small-Molecule Inhibitor of the Terminal Steps for Intracrine Androgen Synthesis in Advanced Prostate Cancer (Mohler)
Time Commitments: .975 calendar months
Supporting Agency: NCI-1R21CA205108-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Nicole Franklin, Grants Management Specialist, National Cancer Institute, 9609 Medical Center Drive, West Tower, Room 2W556, Bethesda, MD 20892 (regular mail), Phone: 240-276-5210, Email: nicole.franklin@nih.gov
Performance Period: 04/10/2016-03/31/2020 (NCE)
Level of Funding: $ 416,398
Brief description of project's goals: This research seeks to explore if a small-molecule inhibitor of the catalytic site shared by the five 3α−oxidoreductases will decrease T and DHT metabolism through the frontdoor and backdoor pathways.
List of specific aims:
1. Identify a candidate inhibitor against the catalytic site shared by the five 3α−oxidoreductases
2. Synthesize and test re-designed candidate inhibitors and conduct PK/PD and toxicity studies to produce a lead compound inhibitor of the five 3α−oxidoreductases
3. Determine whether the inhibitor of the 3α−oxidoreductases decreases tissue T and DHT levels and impairs CRPC growth
Overlap: None

Title: The NF-kappaB-androgen Receptor Axis Drives Failure of Medical Therapy in Human Benign Prostatic Hyperplasia (Matusik)
Time Commitments: 0.30 calendar months
Supporting Agency: NIH/NIDDK Melissa Haney, Manager, Dept of Urologic Surgery, Vanderbilt University. 615-322-3172, Melissa.haney@vanderbilt.edu
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: not assigned
Performance Period: 09/16/2016 – 07/31/2021
Level of funding: $151,840 (sub)
Brief description of project's goals: N B and AR signaling controls the failed response to 5ARIs in BPH.
List of specific aims:
1. Determine cross-talk between NF B and AR signaling to regulate failure of edical therapy
2. Determine the SRD5A isoforms contribution during resistance to medical therapy
3. Determine if failure of medical therapy is driven by NF B and/or AR-V7 in BPH patients. New insight into how BPH patients fail 5α-reductase inhibitors holds the promise to identify pathways to apply novel approaches to medical therapy in the treatment of BPH.
Overlap: None

Title: Genetic and Epigenetic Prostate Cancer Related alterations in early onset disease in African American Men (Woloszynska-Read)
Time Commitments: 1.20 calendar months

Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Department of Defense, USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014/ LYMOR BARNHARD
Performance Period: 04/01/2017-03/31/2020
Level of funding: $1,242,951
Brief description of project's goals: Proposed research aims to identify molecular alterations that distinguish aggressive forms of early onset prostate cancer commonly found in Afrinca American men will contribute to the development of African American tumor (epi)genetic signature(s) and ultimately will lead to personalized medicine strategies for this group of patients.
List of specific aims:
1. Determine the relative frequency of genetic lesions found in PCa in AAs and EAs.
2. Determine novel, clinically relevant methylomic and transcriptomic differences in PCa from AAs and EAsObtain and link vital status data and cause of death in PCaP research subjects
Overlap: None

Title: Racial differences in financial impact of prostate cancer treatment and outcome (Mohler)
Time Commitments: 1.8 calendar months
Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Not assigned
Performance Period: 07/01/2017-06/31/2020
Level of funding: $445,328
Brief description of project's goals: Recurrence of advanced CaP during androgen deprivation therapy leads to a variety of new FDA-approved treatments, which may include immunotherapy, androgen metabolism inhibitors, small molecule anti-androgens, radio-pharmaceuticals, and docetaxel, cabazitaxel or cisplatin, all of which can extend survival but cause side effects and are expensive. Complexities of insurance coverage and Medicare reimbursement, a trend toward increasing co-pays for covered medications and differences in availability of financial assistance from pharmaceutical companies for new agents makes challenging the anticipation of the amount of financial burden posed by advanced CaP. If cured of localized CaP, costs may result from treatment of side effects, such as incontinence and impotence. CaP has been reported to produce the highest level of financial distress among 7 common cancers studied. Patients and their family members have suffered loss of their home, had to quit or decrease job hours or intensity, or forego expensive treatments shown to prolong life. AAs compared to Caucasian Americans (CAs) have been reported to benefit from higher religiosity and "caregiverness" but suffer from lower socioeconomic reserve and medical sophistication. The central hypothesis is that the financial impact of CaP treatment and oncologic outcome differs between AAs and CAs newly diagnosed with CaP.
List of specific aims:
1. Locate and contact PCaP research subjects to update CaP status, CaP treatments received and comorbidities, repeat the QoL assessments performed at baseline and follow-up, and administer new surveys on financial burdens and stress and caregiver QoL and support
2. Locate and contact PCaP research subjects' treating physicians to update treatments received and oncologic outcome data
3. Obtain and link vital status data and cause of death in PCaP research subjects
4. Examine the role financial burden and stress have on CaP survival and QoL and whether this relationship was modified by race.
Overlap: None

Title: Cholesterol Lowering Intervention for Prostate Cancer Active Surveillance/Jr. Faculty Award to Alliance NCORP Research Base – Pilot Project (Kim/Mohler - PIs)
Time Commitments: 0.60 calendar months
Supporting Agency: Cedars/NCI

Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Subcontract with Cedars Sinai. Cedars-Sinai Medical Center, Attention: Margaret Jenkins, Administrative Program Coordinator Department of Surgery, Research Division, 8635 W. 3rd Street, Suite 973W, Los Angeles, CA 90048 margaret.jenkins@cshs.org
Performance Period: 04/01/2015 – 07/31/2019
Level of funding: $93,955 (sub contract)
Brief description of project's goals: The proposed research tests the hypothesis that intensive cholesterol lowering will decrease the growth rate of benign and malignant prostate epithelium. The proposed research could provide the data necessary to justify a phase III clinical trial to address one of the major problems in urologic oncology how to prevent the progression of low risk prostate cancer to provide men higher levels of confidence for selection of active surveillance.
Overlap: None

Title: Understanding the Relative Contributions of and Critical Enzymes for the 3 Pathways for Intracrine Metabolism of Testicular Androgens in Advanced Prostate Cancer (Mohler/Watt/Bies)
Time Commitments: 1.65 calendar months
Supporting Agency: DOD
Performance Period: 09/30/16 - 09/29/19
Level of Funding: $ 660,315
Brief description of project's goals: This research seeks a better understanding of intracrine androgen metabolism acutely in response to initiation of issuing in vivo models and sophisticated PK/PD modeling.
List of specific aims:
1. Determine the relative use of the 3 pathways for intracrine androgen metabolism in vitro, in vivo and in clinical specimens
2. Identify the principal androgen metabolism enzymes (ie. 3a-oxidoreductases) responsible for primary backdoor DHT synthesis from androstanediol
3. Determine the requirements for SRD5A1-3 in the frontdoor pathway of DHT synthesis from T and its precursors and of SRD5A1 and HSD17B3 in the secondary backdoor pathway of DHT synthesis from androstanedione.
Overlap: None

Title: Qualifying Multi-Transcript Signatures for Active Surveillance of Prostate Cancer (Kim)
Time Commitments: 0.6 calendar months
Supporting Agency: NIH/NCI
Performance Period: 09/02/14 - 08/31/19
Level of Funding: $45,112 (annual direct for sub)
Brief description of project's goals: The consortium is well-positioned to rapidly translate and promote validated signatures since all assays will be performed in a CLIA-approved laboratories, and study investigators have leadership positions in cooperative groups, national professional societies, and national guidelines committees.List of specific aims:
1. to validate biomarkers in prostate needle biopsies predictive of adverse pathology and progression in men considering active surveillance
2. to test the effects of African-American ethnicity on the biomarker signatures.
Overlap: None

Azabdaftari, G.

Not previously reported
Title: Differentiation Therapy Targeting Prostate Cancer Stem Cells in Advanced Prostate Cancer
Time Commitment: 0.6 Calendar Months (PI, Huss)
Supporting Agency: Roswell Park Alliance Foundation
Name of Funding Agency's Grants Officer: Judith Epstein, Judith.Epstein@RoswellPark.org

Performance Period: 04/07/2017-09/30/2019
Funding: $50,000
Brief description of project's goals: The major goal of this award is to test if differentiation therapy can eliminate the cancer stem cell population in advanced prostate cancer to increase the effectiveness of androgen deprivation therapy.
Specific Aims: See goals above.
Role: Co-Investigator (Pathologist)
Overlap: None

Title: Bladder Cancer Stem Cells and Gender Disparity
Time Commitment: 0.6 Calendar Months (PI, Huss)
Supporting Agency: Roswell Park Alliance Foundation
Name of Funding Agency's Grants Officer: Judith Epstein, Judith.Epstein@RoswellPark.org
Performance Period: 10/30/2017-10/29/2019
Funding: $50,000
Brief description of project's goals: The major goal of this award is to test if hormone signaling contributes to gender disparity observed in the incidence and aggressiveness of bladder cancer.
Specific Aims: See goals above.
Role: Co-Investigator (Pathologist)
Overlap: None

Title: Genetic and Epigenetic Prostate Cancer Related alterations in early onset disease in African American Men (Woloszynska-Read)
Time Commitments: 0.6 calendar months
Supporting Agency: DoD
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Department of Defense, USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014/ LYMOR BARNHARD
Performance Period: 04/01/2017-03/31/2020
Level of funding: $1,242,951
Brief description of project's goals: Proposed research aims to identify molecular alterations that distinguish aggressive forms of early onset prostate cancer commonly found in Africa American men will contribute to the development of African American tumor (epi)genetic signature(s) and ultimately will lead to personalized medicine strategies for this group of patients.
List of specific aims:
3. Determine the relative frequency of genetic lesions found in PCa in AAs and EAs.
4. Determine novel, clinically relevant methylomic and transcriptomic differences in PCa from AAs and EAsObtain and link vital status data and cause of death in PCaP research subjects
Overlap: None

Title: DSRG GU-Decoding the Molecular and Cellular Landscape of Meta Small Renal Tumor
Time Commitment: 0.6 Calendar Months (PI, Kauffman)
Supporting Agency: Roswell Park Alliance Foundation
Name of Funding Agency's Grants Officer: Judith Epstein, Judith.Epstein@RoswellPark.org
Performance Period: 11/03/2017-9/30/2019
Funding: $50,000
Brief description of project's goals: **This project seeks to** characterize the landscape of molecular and immunologic alterations defining metastatic small renal tumor patients**.**
Specific Aims: See goals above.
Role: Co-Investigator (Pathologist)
Overlap: None

Active to Completed
Title: Decoding the molecular and cellular landscape of the metastatic small renal tumor
Time Commitments: 0.6 calendar months
Supporting Agency: Roswell Park Alliance Foundation
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Judith Epstein, Director Grants & Foundation Office, Elm & Carlton Streets, Research Studies Center Room 234, Buffalo, NY 14203, Judith.Epstein@RoswellPark.org
Performance Period: 11/03/2017-11/02/2018
Level of Funding: $50,000
Brief description of project's goals: To characterize the landscape of molecular and immunologic alterations defining metastatic small renal tumor patients. Identification of alterations differentiating metastatic and nonmetastatic small renal tumors would have immediate opportunity for investigator-initiated clinical trials. Biomarkers for small renal tumor metastasis discovered in this study could be validated prospectively in clinical trials among these new patients and those returning active surveillance patients. Targeted therapies could be offered to those patients with small renal tumors to allow patients to avoid resection; or to supplement resection in the neoadjuvant or adjuvant setting. This proposal will generate several resources available to the RPCI research community. Secondly, the whole genome sequencing and RNA-seq experiments proposed will provide valuable genome-wide mutational and expressional data, which can be queried with future markers of interest.
List of specific aims:
1. Survey the DNA mutational landscape and identify those mutations specific to metastatic small renal tumors relative to nonmetatatic small renal tumors.
2. Identify gene expression alterations at the RNA and protein levels specific to metastatic small renal tumors relative to nonmetastatic small renal tumors.
3. Identify immune cell profiles in patient blood specific to metastatic small renal tumors relative to nonmetastatic small renal tumors.
Overlap: None

Zhu, Q.

New
Title: Somatic Mutations and their Etiological Determinants for Breast Cancer in African American Women (R01CA228156)
Time Commitment: 0.6 calendar (Yao)
Supporting Agency: NIH/NCI
Grants Officer: Damali Martin, martinda@mail.nih.gov
Performance Period: 2/1/19-1/31/24
Level of Funding: $6,562,433
Brief Description of Project's Goals: This study will investigate etiological links of tumor mutations with genetic and environmental factors by leveraging the available rich epidemiologic and genotype data resources.
Specific Aims: The proposed work will greatly advance the field of breast cancer research by characterizing tumor mutational landscape in AA populations and determining whether cancer biology at the somatic mutation level differs by ancestral population. The findings may have translational significance by revealing cancer causation and providing new targets and motivations for cancer preventive initiatives.
Overlap: NONE

Title: Immuno-Oncology Translational Network: Data Management and Resource-Sharing Center at RPCI (1 U24 CA232979-01)
Time Commitments: 1.20 calendar (PI-Hutson/Liu/Morgan/Odunsi)
Supporting Agency: NCI
Grants Officer: Funmi Elesinmogun, elesinmf@mail.nih.gov

Performance Period: 9/30/18-6/30/23
Level of Funding: $6,276,836
Brief Description of Project's Goals: The goal of our Data Management and Resource-Sharing Center (DMRC) application is to serve as an administrative and analytic hub for translational studies in the NCI-supported Immuno-Oncology Translation Network (IOTN).
List of Specific Aims:
1. Provide a centralized administrative infrastructure to coordinate the IOTN activities
2. Promote the IOTN and engage in the interaction with the broader scientific community
3. Provide multidisciplinary analytic expertise to support the IOTN collaborative research
4. Develop improved data integration methods to enhance the IOTN research capacity

Overlap: NONE

Title: Immuno-Oncology Translational Network: Data Management and Resource-Sharing Center High Tech Match Grant
Time Commitments: 1.20 calendar (PI-Hutson/Liu/Morgan/Odunsi)
Supporting Agency: NYSTAR
Grants Officer: Funmi Elesinmogun, elesinmf@mail.nih.gov
Performance Period: 9/30/18-6/30/23
Level of Funding: $388,016 (annual direct)
Brief Description of Project's Goals: The goal of our Data Management and Resource-Sharing Center (DMRC) application is to serve as an administrative and analytic hub for translational studies in the NCI-supported Immuno-Oncology Translation Network (IOTN).
List of Specific Aims:
1. Provide a centralized administrative infrastructure to coordinate the IOTN activities
2. Promote the IOTN and engage in the interaction with the broader scientific community
3. Provide multidisciplinary analytic expertise to support the IOTN collaborative research
4. Develop improved data integration methods to enhance the IOTN research capacity

Overlap: NONE

Title: Impact of HIV, Oral Microbiome and Mycobiome on Oral HPV (R56DE028156)
Time Commitments: 0.60 calendar
Supporting Agency: NIH
Grants Officer: Neeraja Sathyamoorthy, ns61r@nih.gov
Performance Period: 9/18/18 - 9/17/19
Level of Funding: $422,533 (annual total)
Brief Description of Project's Goals: The overall goal of this application is to understand the role and mechanisms of functioning of KLF9-TXNRD2 axis in melanomagenesis and characterize TXNRD2 inhibitor auranofin as a potential anti-melanoma agent.
List of Specific Aims: The proposed study will investigate the natural history of oral HPV in HIV-positive individuals, and assess the risk effects of oral bacterial infections (microbiome) and fungal infections (mycobiome) on oral HPV persistence, an intermediate biomarker HPV-associated oral papilloma and oropharyngeal cancer risks.
Overlap: NONE

Not Previously Reported
Title: Infrastructure for Pathways, A Prospective Study of Breast Cancer Survivorship (5 U01 CA195565)
Time Commitments: 1.2 calendar(PI-Ambrosone)
Supporting Agency: NIH
Grants Officer: Joanne W Elena, wattersj@mail.nih.gov
Performance Period: 06/01/16-05/31/21
Level of Funding: $1,597,828

Brief Description of Project's Goals: In this infrastructure application, we plan to enhance the Pathways Study resource by continuing to follow study participants and documenting outcomes; enhancing biospecimen resources with a follow-up blood sample and tumor biobank; and by adding new data from KPNC medical records, genome-wide assays, and neighborhood characteristics. These activities will make the Pathways Study an outstanding and unique resource for research on breast cancer survivorship and prognosis.
List of Specific Aims: This grant will support the infrastructure of the Pathways study, a prospective cohort of breast cancer patients. It will support collection of data biospecimens and tumor tissues, and follow-up of participants for cancer outcomes.
Overlap: NONE

Title: Genetic underpinnings of ethnic disparities in bone toxicities between Hispanic and non-Hispanic children treated for acute Lymphoblastic leukemia (1 R03 CA223730)
Time Commitments: 0.60 calendar (PI-Yao)
Supporting Agency: NCI
Grants Officer: Kelly Filipski, Kelly.filipski@nih.gov
Performance Period: 12/01/17-11/30/19
Level of Funding: $174,100
Brief Description of Project's Goals: This R03 grant seeks to perform a novel pharmacogenomic study based on 05-001 and its successor trial DFCI 11-001 to identify genetic underpinnings of ethnic disparities in bone toxicities. We plan to first test global genetic ancestry with bone toxicities, followed by a bivariate genome-wide association study (GWAS) to jointly analyze osteonecrosis and fracture as two related traits.
List of Specific Aims:
1. Determine whether the composition of genetic ancestry in Hispanic children is an underlying cause for the ethnic disparities in therapy-related bone toxicities, namely osteonecrosis and fracture, in children with ALL in the DFCI 05-001 and 11-001 trials.
2. a). Investigate single variants and polygenic risk scores from previous GWAS of bone-related phenotypes with bone toxicities in children treated for ALL.
b). Identify genetic loci associated with therapy-related bone toxicities by performing bivariate GWAS analyses with directional alignment and meta-analysis in the DFCI 05-001 and 11-001 trials
c). Investigate whether genetic variants and polygenic scores significant in 2a and 2b explain disparities in bone toxicities between Hispanic and non-Hispanic children with ALL.
Overlap: NONE

Title: b-catenin in vaccine-induced anti-tumor CD8 cell immunity (5 R01 CA198105)
Time Commitments: 0.30 calendar (PI-Jiang)
Supporting Agency: NCI
Grants Officer: Anthony Welch; Office: (301) 846-5691
Performance Period: 07/01/15-06/30/20
Level of Funding: $2,007,280
Brief Description of Project's Goals: The long-term goal is to develop strategies to block tumor-induced immunosuppression to augment CD8+ T cell immunity and improve cancer vaccine efficacy. The objectives in this application is to elucidate the underlying mechanisms of how tumors inhibit cross-priming through b-catenin in DCs, and validate blocking b-catenin signaling as a novel strategy to improve cancer vaccine efficacy.
List of Specific Aims:
1. To determine whether activation of ß-catenin in DCs suppresses anti- tumor CD8+ T cell immunity under diverse cancer vaccinations.
2. To elucidate the molecular mechanisms of how tumors inhibit cross-priming through ß-catenin in DCs.
3. To determine whether blocking ß-catenin pharmacologically improves cancer vaccine efficacy.
Overlap: NONE

Title: The Role of TAZ in Breast Cancer Initiation and Progression (1 R01 CA207504-02)
Time Commitments: 0.60 calendar (PI-Zhang)
Supporting Agency: NIH
Grants Officer: Elizabeth G Snyderwine, Elizabeth_snyderwine@nih.gov
Performance Period: 07/01/17-06/30/22
Level of Funding: $2,002,312
Brief Description of Project's Goals: Our long-term goal is to understand the mechanisms of TNBC relapse and thus help to improve the survival of breast cancer patients. Our overall objective here, which is the next step in pursuit of that goal, is to determine how TAZ activation induces TNBC tumor progression and metastasis. Our central hypothesis is that both TAZ-dependent cell cycle activation and expansion of transformed mammary stem cell (Ma-SC) populations are required for TAZ-initiated breast tumorigenesis. Our hypothesis has been formulated on the basis
List of Specific Aims:
1. Identify the critical downstream targets that are required for TAZ-initiated oncogene addiction.
2. Determine how TAZ induces the formation of heterogeneous mammary tumors using a unique TAZ transgenic mouse model.
3. Determine the role of TAZ in breast tumor heterogeneity and its impact on tumor metastasis.
Overlap: NONE

Active to Completed

Title: Epidemiology of Breast Cancer Subtypes in African American Women: A Consortium (5 P01 CA151135-03)
Time Commitments: 1.20 calendar (PI- Ambrosone/Palmer/Millkan)
Supporting Agency: NCI
Grants Officer: Elizabeth Gillanders; lgilland@mail.nih.gov
Performance Period: 8/1/11-7/31/16
Level of Funding: $2,976,051
Brief description of project's goals: In this Program Project, we will pool data and samples from the Carolina Breast Cancer Study (CBCS), the Black Women's Health Study (BWHS), the Women's Circle of Health Study (WCHS) and the Multi-ethnic Cohort (MEC) and continue to accrue cases for a final sample size of more than 5500 cases and 5500 controls.
List of specific aims:
1. Genetic loci identified in recent GWAS findings, using fine-mapping to identify potential causal alleles.
2. Pregnancy history and lactation, and potential modification by genetic variants in related pathways.
3. Body size, early life and adult physical activity, and gene/environment interactions.
4. Risk factors that may have been adaptive in Africa to endemic infectious disease (robust immune response) and intense sunlight (high skin pigmentation), but that in later life and western society may result in hyper-inflammatory milieu and vitamin D deficiency.
Overlap: NONE

Title: Regulation of lactosaminyl glycan biosynthesis in hematopoietic cells (5 P01 HL107146-07)
Time Commitments: 0.60 calendar (PI- Lau)
Supporting Agency: NIH
Grants Officer: Rita Sarkar, sarkarr@nhlbi.nih.gov
Performance Period: 07/01/15-5/31/19
Level of Funding: $618,700
Brief Description of Project's Goals: Goal is to elucidate hematopoietic stem and progenitor cell surface glycan structures and identify the key glycan-modifying enzymatic activities occupying their biosynthetic checkpoints.
List of Specific Aims:
1. To characterize the glycosylation changes in an in vitro system of HSPC differentiation using a multitiered approach.

2. To characterize lactosaminyl glycosylation changes in early HSPC development.
3. To assess the contribution of the extrinsic pathway in HSPC glycan formation.
4. To identify the roles of glycans in HSPC biology and hematopoietic repopulation.
5. To develop systems biology-based modeling framework to describe HSPC glycan biosynthesis.
Overlap: NONE

Nothing to report for Pop, E.

Nothing to report for Wilton, J. H.

What other organizations were involved as partners?

Nothing to report.

Bibliography

1. Tang L, Zhu Q, Bensen J, Taylor J, Smith G, Pop E, Azabdaftari G, Mohler J, and Wu Y: Associations of genetic polymorphisms in SLCO transporters with clinical aggressiveness of prostate cancer in the North Carolina-Louisiana prostate cancer project. J. Urol., Vol. 199, No. 4S, Supplement, Saturday, May 19, 2018, e269.
2. Yue Wu, James Mohler, and Li Tang: A Selective and Active Mechanism for Androgen Uptake by Prostate Cancer Cells. Poster and oral presentation, IMPACT Meeting, Aug, 2016.
3. Parsons TK, Pratt RN, Tang L, and Wu Y: An active and selective molecular mechanism mediating the uptake of sex steroids by prostate cancer cells. Mol Cell Endocrinol. 477:121-131, 2018.

Appendix I. Publication.

Molecular and Cellular Endocrinology 477 (2018) 121–131

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

An active and selective molecular mechanism mediating the uptake of sex steroids by prostate cancer cells

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ARTICLE INFO

Keywords:
Sex steroids
Testosterone
Androgen
Uptake
Prostate cancer

ABSTRACT

Steroid hormones play important roles in normal physiological functions and diseases. Sex steroids hormones are important in the biology and treatment of sex hormone-related cancer such as prostate cancer and breast cancer. Cells may take up steroids using multiple mechanisms. The conventionally accepted hypothesis that steroids cross cell membrane through passive diffusion has not been tested rigorously. Experimental data suggested that cells may take up sex steroid using an active uptake mechanism. ^{3}H-testosterone uptake by prostate cancer cells showed typical transporter-mediated uptake kinetic. Cells retained testosterone taken up from the medium. The uptake of testosterone was selective for certain steroid hormones but not others. Data also indicated that the active and selective uptake mechanism resided in cholesterol-rich membrane domains, and may involve ATP and membrane transporters. In summary, the present study provided strong evidence to support the existence of an active and selective molecular mechanism for sex steroid uptake.

1. Introduction

Steroid hormones regulate numerous functions that include differentiation, development, metabolism, reproduction, and immune response, and also play important roles in diseases (Simons, 2008). Steroid hormones include androgens, estrogens, progestins, glucocorticoids, mineralocorticoids. Androgens, estrogens and progestins are also called sex steroids, of which the most common ones are progesterone, testosterone (T) and dihydrotestosterone (DHT), and estradiol (E2), respectively. Sex steroids are important to the biology and treatment of sex hormone related cancer (Groner and Brown, 2017). Targeting the androgen signaling and the estrogen signaling has been a main modality of treatment for prostate cancer and breast cancer, respectively (Dai et al., 2017; Stuchbery et al., 2017; Snaterse et al., 2017; Castrellon, 2017; Africander and Storbeck, 2017; Jordan and Brodie, 2007). Sex steroids also are involved in ovary and endometrial cancer, and non-sex hormone-related cancer such as colon cancer (Chuffa et al., 2017; Kamal et al., 2016; Plaza-Parrochia et al., 2017; Jeon et al., 2016; Gharwan et al., 2015; Diep et al., 2015; Lin et al., 2011; Barzi et al., 2013). Adrenal glands produce androgens that are named adrenal androgens. Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) are the predominant adrenal androgens in the circulation (Rainey and Carr, 2004; Rainey et al., 2002). DHEA and DHEAS are substrates for T and DHT production through the steroidogenesis pathway (Labrie et al., 1998). Adrenal androgens have multiple functions of their own that are independent of AR (Traish et al., 2011; Maninger et al., 2009).

Most of sex steroids in the plasma are bound with proteins, mainly with sex hormone-binding globulin (SHBG) (Hammond, 2011; Joseph, 1994; Mendel, 1992; Rosner et al., 2010). The steroids bind with respective nuclear receptors to regulate expression of genes upon entering the cells. Progesterone, T/DHT, and E2 bind with progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), respectively (Grimm et al., 2016; Bain et al., 2007; Hilton et al., 2017; Heemers and Tindall, 2007; Heinlein and Chang, 2002; Wilson, 2009). The ligand-bound receptors translocate to the nucleus to regulate expression of sex hormone-targeted genes. A wealth of knowledge has been accumulated with regard to the production, metabolism, and circulation of sex steroids, as well as nuclear receptor-mediated biological functions and cell signaling pathways. No nuclear receptors have been identified for the adrenal androgens.

Cells may take up steroid using multiple mechanisms, which include passive diffusion and more selective or more active, transporter-mediated mechanisms. The conventional belief is that sex steroids cross the bi-phospholipid layer of the cell membrane through passive diffusion due to their hydrophobic property (Oren et al., 2004). However, cellular transport of other hydrophobic molecules including fatty acids and cholesterol is mediated by mechanisms other than passive diffusion (Klaassen and Aleksunes, 2010; Ikonen, 2006; Altmann et al., 2004).

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https://doi.org/10.1016/j.mce.2018.06.009
Received 8 February 2018; Received in revised form 31 May 2018; Accepted 16 June 2018
Available online 18 June 2018

Abbreviations

AR	androgen receptor
ATCC	American Type Culture Collection
cpm	counts per minute
CS-FBS	charcoal-stripped fetal bovine serum
DHEA	dehydroepiandrosterone
DHEAS	DHEA sulfate
DHT	dihydrotestosterone
E2	estradiol
ER	estrogen receptor
epiT	epitestosterone
FBS	fetal bovine serum
OATP	organic anion-transporting polypeptide
OST	organic solute and steroid transporter
PBS	phosphate buffered saline
PR	progesterone receptor
PGRMC1	progesterone receptor membrane component 1
RLU	relative luminescent unit
RLB	reporter lysis buffer
SHBG	sex hormone-binding globulin
SLCO	solute carrier organic anion
T	testosterone

There have been reports that uptake of steroids may be mediated by molecular mechanisms, although the contribution of these proposed mechanisms to the uptake of sex steroids remains unknown. T, DHEAS, or estrogens are known to bind with cell membrane-bound, G-protein coupled receptors including Gnα11, ZIP9, and GPER-1 to augment non-classical or non-genotropic functions of the steroids (Shihan et al., 2014; Bulldan et al., 2016; Shihan et al., 2013; Carmeci et al., 1997; Funakoshi et al., 2006; Shihan et al., 2015). Membrane receptor-mediated endocytosis also may be involved in the internalization of sex steroids, although the role of endocytosis in nuclear receptor-mediated functions of the steroids is not clear (Lin and Scanlan, 2005; Hammes et al., 2005; Porto et al., 1995). The organic solute and steroid transporter (OST) proteins OSTα-OSTβ was proposed to be a newly identified putative steroid transporter (Ballatori, 2005). The organic anion-transporting polypeptide (OATP) superfamily member solute carrier organic anion (SLCO) family member SLCO1B3 was reported as a T transporter (Hamada et al., 2008). More data are needed to describe accurately the uptake of sex steroids by cells in order to understand fully the transport and tissue distribution that may affect the function of the steroids.

It was found that prostate cancer cells were able to accumulate intracellular T at 20–50-fold of that was added in the culture medium (Wu et al., 2013), which indicated that the cells acquired exogenous T using an active uptake mechanism. In the present study, the uptake of T was examined using a ^{3}H-T as substrate using prostate cancer cell lines. Progesterone, E2, epitestosterone (epiT), DHT, DHEA and DHEAS were evaluated for competition for the uptake of ^{3}H-T. Activation of AR was compared between T and the steroids that blocked T uptake. Potential molecular mechanisms were investigated using specific chemical inhibitors. An active and selective uptake mechanism was described.

2. Materials and methods

2.1. Reagents

T, epi-T, DHT, DHEA and DHEAS were purchased from Steraloids (Newport, RI). Progesterone, E2, (2-hydroxypropyl)-β-cyclodextrin (2HP-β-CD), AG-205, and bafilomycin A1 were purchased from Sigma-Aldrich (St. Louis, MO). 1,2,6,7-^{3}H(N) T (^{3}H-T), 1,2,6,7-^{3}H(N) progesterone (^{3}H-progesterone) and 6,7-^{3}H(N) E2 (^{3}H-E2) were purchased from PerkinElmer (Waltham, MA). Specific activity of the ^{3}H-T, ^{3}H-progesterone, and ^{3}H-E2 were 83.4, 96.6, and 60.0 mCi/μmol, respectively. The concentration of ^{3}H-T, ^{3}H-progesterone, and ^{3}H-E2 were 12, 10.4, and 16.7 μM respectively, based on the specific radioactivity 1 μCi/μl provided by the manufacturer.

2.2. Cell culture

Human prostate cancer VCaP cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA). Human prostate cancer LAPC-4 cell line was established by Dr. Charles Sawyers group (Klein et al., 1997). VCaP and LAPC-4 cell lines express wild type AR (Sobel and Sadar, 2005a,b). Cell lines were propagated in medium supplemented with 10% fetal bovine serum (FBS) (Atlantic Biologicals, Atlanta, GA). VCaP cells were maintained in DMEM medium (Thermo Fisher Scientific, Waltham, MA). LAPC-4 cells were maintained in RPMI1640 medium (Thermo Fisher Scientific). All media contained 2 mM L-glutamine (Corning Life Sciences, Tewksbury, MA), 100 U/ml penicillin and 100 μg/ml streptomycin (Corning Life Sciences). For LAPC-4 cells, tissue culture vessels were coated with 1.7 μg/ml poly-D-lysine (Sigma-Aldrich) in H_2O at 0.076 ml/cm^2, at room temperature for 15 min, and followed by aspiration of the coating reagent and overnight air dry. Phenol red-free version of each medium was used for pre-culture and treatments. Cells were incubated at 37 °C, in an atmosphere with 95% air and 5% CO_2.

2.3. Uptake of ^{3}H-T

Cells were seeded at 5 × 10^4 per well in 0.5 ml medium supplemented with 10% FBS on 24-well plates, and cultured for 3 days or 7 days for LAPC-4 or VCaP, respectively. Cell culture medium was replaced with medium supplemented with 10% charcoal-stripped (CS-FBS) to remove androgens (Fiandalo et al., 2017). Cells were cultured for 1 day before treatment. Cells were treated in 0.25 ml of treatment medium. Cells were rinsed 4 times with 0.25 ml phosphate buffered saline (PBS). Cells in each well were lysed in 0.125 ml lysis buffer (2% SDS, 10% glycerol, 10 mM Tris-HCl, pH6.8) and incubated at 37 °C for 30 min. At the end of lysis, 0.5 ml of SOLVABLE (PerkinElmer) was added to each well. The content was mixed thoroughly using gentle swirling, and transferred into a 25 ml scintillation vial that contained 5 ml Ultra Gold Scintillation fluid (PerkinElmer). Radioactivity of the lysate was measured on a scintillation counter. Each experiment was set up in 4 replicates. Two types of controls were included in every experiment. The background control cells was not treated with ^{3}H-T nor T to control the background radiation readings. The non-specific binding controls cells were treated with ^{3}H-T in the presence of T that was 300-fold of the concentration of ^{3}H-T. Radioactivity of the non-specific binding controls, counts per minute (cpm), were subtracted from the cpm reading of each treatment for ^{3}H-T-specific total radioactivity.

2.4. Release of intracellular ^{3}H-T

Cells were seeded on 6-well plate at 4 × 10^5 cells per well in 2 ml medium supplemented with 10% FBS and cultured for 3 days or 7 days for LAPC-4 or VCaP, respectively. Cells were cultured for 1 day in 2 ml phenol red-free medium with 10% CS-FBS, and treated with 1 nM ^{3}H-T for 20 min. Cells were rinsed 3 times with phenol red-free medium with 10% CS-FBS. One set of treated cells were lysed in 0.2 ml lysis buffer, mixed with 0.5 ml SOLVABLE and 5 ml Ultra Gold Scintillation fluid, and analyzed on the scintillation counter for total radioactivity. The total activity of the cell culture was used as the baseline total cellular ^{3}H-T. The remaining sets of cells were cultured in 2 ml fresh phenol red-

free medium with 10% CS-FBS, and 0.2 ml medium was sampled for each time point at 0, 5, 10, and 30 min. Cell cultures were rinsed 3 times with phenol red-free medium with 10% CS-FBS, lysed in 0.2 ml lysis buffer, mixed with 0.5 ml SOLVABLE and 10 ml Ultra Gold Scintillation fluid, and analyzed on the scintillation counter for total radioactivity. The total activity of the cell culture was used as the total cellular ^{3}H-T at 30 min. Each 0.2 ml sampled medium was mixed with 0.5 ml SOLVABLE and 10 ml Ultra Gold Scintillation fluid, and analyzed on a scintillation counter. The radioactivity of each medium sample was adjusted for change in volumes of the culture medium caused by sampling for calculation of the total radioactivity released by the whole cell culture into the medium.

2.5. ARE-luciferase assay

ARE-luciferase was performed as described to evaluate the AR stimulatory effect of androgens (Wu et al., 2011a). Cells were seeded in 6-well plate at 3×10^5 per well and incubated for 3 days and 7 days for LAPC-4 and VCaP cells, respectively. Cells were transfected with the ARE-luciferase promoter reporter plasmid using Lipofectamine 2000 (Thermo Fisher Scientific) in Opti-MEM medium (Thermo Fisher Scientific) overnight following the manufacturer's instruction. Transfected cells in all wells were trypsinized using trypsin-EDTA, washed once with medium and combined to be plated in 24-well plates at 1.5×10^5 cells per well in cultural medium supplemented with 10% CS-FBS. Cells were cultured for 1 day, and treated for 1 day. Cells were rinsed 3 times with PBS. Cell lysate was harvested using 100 µl 1X reporter lysis buffer (RLB) (Promega, Madison, WI) in each well. The plate containing lysates was shaken on a plate shaker at 250 rpm for 10 min, and frozen at −80 °C. The frozen lysate was thawed at room temperature and transferred to a clean Eppendorf tube, centrifuged at 10,000×*g* for 10 min. A portion of the supernatant was used for protein determination using BCA protein assay (Thermo Fisher Scientific) and protein concentration standards diluted in 1X RLB to obtain the total amount of proteins (mg) in the lysate used for luciferase assay. Luciferase activity of the supernatant was measured using the Luciferase Assay System reagent (Promega). Luminescence was measured using a luminescence plate reader. The luciferase activity was expressed as relative luminescent unit (RLU) per µg proteins.

2.6. MTT assay

MTT assay was used to evaluate cell viability and as an indicator for total cell number (Li et al., 2007). Experiments for MTT assay were set up in 24-well plates. Medium was removed at the end of treatment, and 0.5 ml freshly prepared treatment medium that contained 0.1% (w/v) MTT was added in each well. Cells were cultured at 37 °C for 2 h, and 0.5 ml solublization solution (20% SDS in 0.02 M HCl in H_2O) was added to the cell culture and swirled to mix thoroughly. The mixed content in the plates was incubated at 37 °C overnight. The content was mix again by vortex. A hundred µl sample was transferred in to a well on a 96-well plate, and read at 570 nm. Experiments were set up in 4 replicates, and each sample was analyzed 3 times for luciferase activity.

2.7. Statistical analysis

The Student t-test was used to determine statistical differences between variable values, and $p < 0.05$ was considered significant.

3. Results

3.1. Uptake of ^{3}H-T by prostate cancer cells

Uptake of ^{3}H-T by LAPC-4 and VCaP cells was evaluated over a time course of 60 min (Fig. 1A). Cells were treated with 1 nM ^{3}H-T. LAPC-4 and VCaP cells showed similar uptake patterns. The ^{3}H-T accumulation was nearly linear over the time course. Therefore, 20 min treatment duration was used for dynamic experiments for sufficient scintillation count readings and the shortest treatment duration that allowed the performance of experimental procedures. The dynamic curves of the uptake were similar between the cell lines and followed a typical transporter-mediated uptake pattern (Fig. 1B). The uptake reached plateau between 5 and 10 nM ^{3}H-T. Nonlinear regression was performed using the function provided in the GrapgPad Prism 7 software to calculate maximum specific binding (Bmax) and equilibrium binding constant (Kd). VCaP showed higher Bmax and Kd compared to LAPC-4.

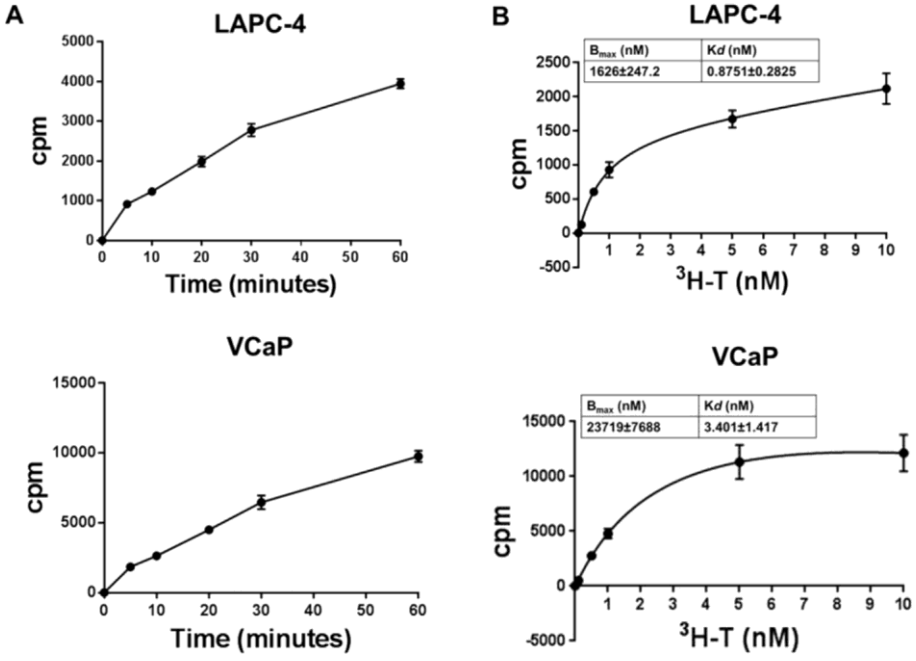

Fig. 1. ^{3}H-T-uptake by LAPC-4 and VCaP cells. (A) Uptake over a time course of 30 min; (B) Kinetic analysis of uptake at 20 min using multiple concentrations of ^{3}H-T.

3.2. Prostate cancer cells retained ^{3}H-T

Release of intracellular ^{3}H-T into the medium was only nominal compared to the uptake of ^{3}H-T from the medium (Fig. 2). Intracellular ^{3}H-T established in 20 min treatment remained almost unchanged after 30 min in the absence of ^{3}H-T in the medium. The data indicated that prostate cancer cells retained ^{3}H-T that was taken up from the medium. Cross-membrane ^{3}H-T gradient was not sufficient to drive the permeation of ^{3}H-T.

3.3. T-uptake mechanism selectively excluded adrenal androgens

T uptake was evaluated in the presence of non-radioactive T, non-radioactive DHEA, or non-radioactive DHEAS to determine whether the DHEA and DHEAS competed with T for the same uptake mechanism. EpiT is an epimer of T, differing from T only in the configuration at the hydroxyl-bearing carbon, C-17. T/EpiT ratio in plasma of adult males is 1:1 (Starka, 2003; Nuck and Lucky, 1987; Aguilera et al., 2002; Bellemare et al., 2005). EpiT was included in the experiment to determine whether T uptake mechanism differentiated T from its epimer. LAPC-4 and VCaP cells were treated with 1 nM of ^{3}H-T in the presence of 1, 5, 10, 100 or 300 nM of non-radioactive T, DHEA, DHEAS, or epiT (Fig. 3). Uptake of ^{3}H-T was blocked by T and epiT. On the contrary, non-radioactive DHEA and DHEAS did not block ^{3}H-T uptake. The data indicated that the T uptake mechanism selectively excluded adrenal androgens DHEA and DHEAS, but did not differentiate T from epiT.

3.4. T uptake mechanism showed selectivity for progesterone and E2

^{3}H-T uptake by LAPC-4 and VCaP cells were fully blocked by 300 nM DHT, progesterone, or E2 (Fig. 4). The results indicated that the T uptake mechanism also recognized progesterone and E2.

3.5. Compatibility of a steroid to the T uptake mechanism did not coincide with its ability to activate AR

ARE-luciferase promoter activity assay was performed to determine the ability to activate AR of sex steroids that competed with T for the uptake mechanism (Fig. 5). T at 1 nM activated AR. Progesterone or E2 at 300 nM did not activate or only modestly activated AR in spite of their ability of fully blocking T uptake at the concentration. The results suggested that blocking of T uptake by progesterone and E2 was not attributable to blocking AR-T binding. T uptake and AR-T binding differed in mechanisms for recognizing AR.

3.6. Cholesterol flushing by 2HP-β-CD impaired the T uptake mechanism

Various transmembrane transporters and receptors reside in cholesterol-rich membrane structures such as the lipid raft (Orlowski et al., 2007; Helms and Zurzolo, 2004; Herrera et al., 2011; Klappe et al., 2009; Irwin et al., 2011; Cuddy et al., 2014; Sano et al., 2014; Goma et al., 2014; Szilagyi et al., 2017). Cells were treated with a cholesterol flush agent, 2HP-β-CD (Christian et al., 1997; Wu et al., 2011b; Song et al., 2014; Gaspar et al., 2017) to determine whether cholesterol-rich membrane domains played a role in uptake of T (Fig. 6). Treatment with 2HP-β-CD impaired T uptake in both cell lines. The results indicated that the T uptake mechanism may reside in cholesterol-rich niches on the cell membrane.

3.7. Inhibition of V-ATPase and PGRMC1 modestly reduced T uptake

V-ATPase is known to drive transmembrane transporter-mediated uptake (Chene, 2002; Huss and Wieczorek, 2009; Martinez-Zaguilan et al., 1993). Cells were treated with a V-ATPase-specific inhibitor, bafilomycin A1 (Bowman et al., 1988; Shi et al., 2013; Manabe et al., 1993; Yoshimori et al., 1991) for 24 h, and treated with 1 nM ^{3}H-T in the presence of bafilomycin A1 to examine effect on T uptake (Fig. 7A). Bafilomycin A1 treatment modestly reduced T uptake by LAPC-4 and VCaP cells. The data indicated that V-ATPase or similar ATP-driven mechanism might contribute to the uptake of T. Progesterone fully blocked T uptake, suggesting shared uptake mechanism for T and progesterone. The progesterone receptor membrane component 1 (PGRMC1) mediates transmembrane transport of progesterone (Thomas, 2008; Meyer et al., 1996; Kimura et al., 2012; Cahill, 2007). Cells were treated with AG-205, a PGRMC1 inhibitor (Will et al., 2017; Guo et al., 2016), to determine whether PGRMC1 was involved in T uptake and cell viability (Fig. 7B and C). Cells were treated with multiple doses of AG-205 for 24 h, then treated with 1 nM ^{3}H-T to assess T uptake. MTT assay was performed using the identical culture condition in parallel with the uptake experiment to determine effect of AG-205 on cell viability. Treatment with AG-205 reduced ^{3}H-T uptake by LAPC-4 cells and reduced cell viability. However, reduction in T uptake was greater than reduction in cell viability (Fig. 7B). Treatment with AG-205 reduced ^{3}H-T uptake by VCaP cells, but did not reduce cell viability (Fig. 7C). The data suggested that PGRMC1 or similar mechanism might contribute to T uptake, although the mechanism may not be a primary one.

3.8. Uptake of progesterone and E2 was different from uptake of T

Uptake of 3H-progesterone or ^{3}H-E2 was examined using LAPC-4 and VCaP cells (Fig. 8). Excessive amount of non-radioactive (cold) progesterone or E2 only modestly reduced accumulation of ^{3}H-

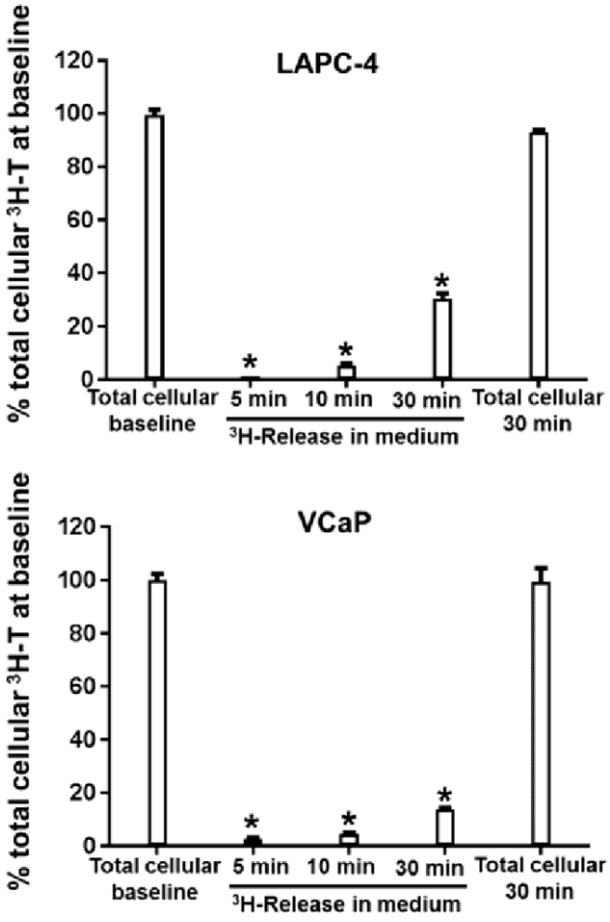

Fig. 2. Release of intracellular ^{3}H-T by LAPC-4 and VCaP cells. Results were presented as percentage of the base-line intracellular ^{3}H-T, which was taken up by cells in 20 min. * significantly different from total cellular baseline value ($p < 0.05$).

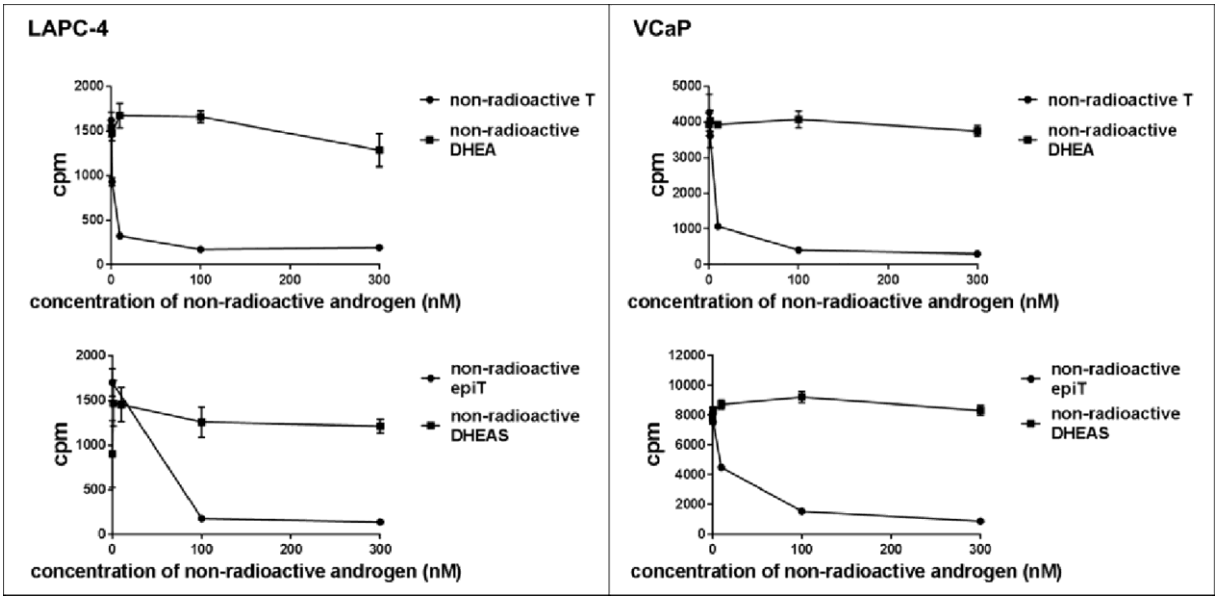

Fig. 3. ^{3}H-T uptake was blocked by T or epiT, but not by DHEA or DHEAS.

progesterone or ^{3}H-E2, respectively. These results were different from the effect of excess amount of non-radioactive T on ^{3}H-T uptake, which showed > 90% reduction of ^{3}H-T uptake. The results indicated that a non-specific, presumably passive diffusion-mediated uptake mechanism was dominant compared to a more specific, transporter-mediated mechanism. Uptake of progesterone and E2 was reduced by 2HP-β-CD in both cell lines, indicating that the uptake mechanisms resided predominantly in cholesterol-rich cell membrane domains as the uptake mechanism for T. AG-205 and bafilomycin A1 reduced the uptake of progesterone and E2 in LAPC-4 modestly, which was similar to the effects of these reagents on T uptake (Fig. 8A). However, AG-205 and bafilomycin A1 had limited or no effect on progesterone or E2 uptake in VCaP cells. Cellular context seemed to play a role in the sensitivity of progesterone and E2 uptake to the reagents.

4. Discussion

Previously, accumulation of intracellular T in prostate cancer cells were determined using LC-MS-MS (Wu et al., 2013). The cells were able to accumulate intracellular T in a range of 25–50 nM, which was much higher than the 1 nM T in the cultural medium that was used to treat the cells. The results indicated the existence of an active uptake mechanism for androgen. The existence of mechanisms in cellular membrane for androgen uptake has been proposed 4 decades ago using human and dog prostate tissue (Giorgi, 1976; Giorgi et al., 1973). However, these studies did not draw attentions from the research community. Passive diffusion remained a widely accepted but un-tested mechanism that underlies the membrane-crossing of steroids. Putative pathways for free diffusion of steroid hormones across biomembranes were proposed based on the combination of a continuum-solvent model and the calculation of the free energy of interaction steroid hormones with lipid membranes (Oren et al., 2004). However, the authors noted major limitations of the modeling system including the lack of proteins in the lipid membrane model, the structureless representation of the lipid layer, and the neglect of effects due to changes in the conformations of cholesterol and steroids upon interaction with the membrane. The inability of DHEA and DHEAS to block 3H-T uptake that was reported in the present study also indicated an uptake mechanism that was more selective than the passive diffusion model, at least for the uptake of testosterone by the prostate cancer cell lines.

In the present study, ^{3}H-T was used to determine the kinetic of T uptake by prostate cancer cells. LAPC-4 and VCaP cells showed typical transporter-mediated uptake kinetics. VCaP cells showed greater Bmax and Kd than LAPC-4. The reason for this difference is not clear. Previous results from a LC-MS-MS analysis of intracellular androgens in LAPC-4 and VCaP showed that nearly 1/3 of T taken up by LAPC-4 was reduced

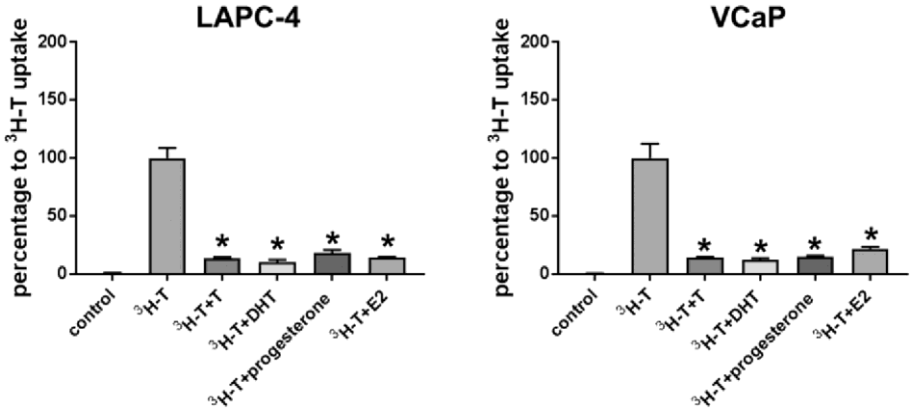

Fig. 4. DHT, progesterone, and E2 blocked ^{3}H-T uptake. Results were presented as percentage of the intracellular ^{3}H-T taken up in the absence of non-radioactive androgen. * significantly different from ^{3}H-T alone value ($p < 0.05$).

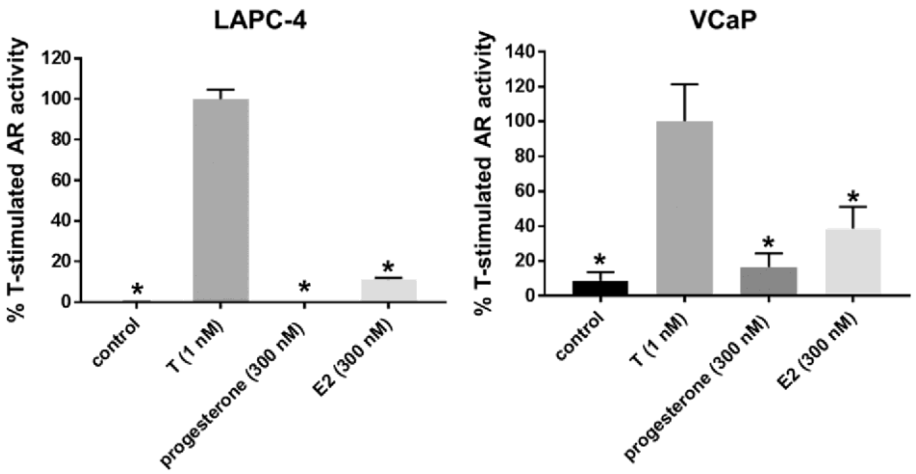

Fig. 5. Progesterone or E2 activation of AR at 300 nM was modest compared to T activation of AR at 1 nM. Results were expressed as percentage of the ARE luciferase activity (RLU/μg protein) that was stimulated by 1 nM T. * significantly different from T (1 nM)-stimulated AR activity ($p < 0.05$).

to DHT, whereas VCaP cells did not reduce T to DHT. The other androgens in T-treated LAPC-4 and VCaP cells were similar and nominal (Wu et al., 2013). A majority of the ^{3}H-T that was taken up by the cells remained in its original form over the course of the experiments. Since DHT was a more preferred ligand for AR than T, T to DHT conversion should increase Bmax and Kd in LAPC-4 if AR was the main contributor to retention of radioactivity from the ^{3}H-T that was taken up. Therefore, neither T metabolism to other metabolites nor binding with AR seemed to contribute to the difference in the Bmax and Kd. It is known that steroid binding globulins are involved in steroid uptake in cells, including prostate cancer cells (Caldwell and Jirikowski, 2014; Nakhla et al., 1999). ^{3}H-T was added as a free, un-bound reagent into the medium for the uptake experiments. Since the experiments were performed primarily in medium supplemented with 10% CS-FBS, binding of ^{3}H-T with proteins in the CS-FBS such as albumin may contribute to the uptake of ^{3}H-T. The kinetic curves of ^{3}H-T uptake in serum-free medium remained similar as the kinetic curves of ^{3}H-T uptake in the presence of 10% CS-FBS (Fig. S1, Supplementary Data). The Bmax was increased in LAPC-4 cells but decreased in VCaP cells, whereas, K*d* was increased in LAPC-4 cells but decreased in VCaP cells. The changes may due to the response of cells to serum-free condition. Nevertheless, the overall kinetic of the uptake followed that observed in the presence of CS-FBS. Therefore, the uptake mechanism remained effective in the absence of proteins in the serum.

^{3}H-T release data argued against the hypothesis that sex steroids cross the cellular membrane through passive diffusion. The baseline intracellular ^{3}H-T that was established by a 20 min treatment remained almost unchanged after 30 min in the absence of ^{3}H-T in the medium. A passive diffusion model would expect the intracellular ^{3}H-T at 30 min would be reduced significantly due to diffusion of the intracellular ^{3}H-T into the medium, which was in a volume that greatly exceeded the total cellular volume. Other factors that may contribute to the high capacity of intracellular ^{3}H-T retention may include the binding of 3H-T with carrier proteins and the incorporation of ^{3}H-T into the subcellular membrane systems such as the endoplasmic reticulum or the lysosomes that are involved in steroid metabolism.

The uptake mechanism showed selectivity for T and DHT, and excluded DHEA and DHEAS. This was evidenced by the ability of full competition of DHT and epiT for T uptake, and no competitive effect of DHEAS and DHEA for T uptake. The reason for this specificity is unknown. However, the preference of the androgen uptake mechanism is significant given the structural and physical similarities shared between T and DHEA. In male humans, T and DHT are primarily produced in the testis, and therefore are referred to as testicular androgens. DHEAS and DHEA are produced in the adrenal glands, and are also referred to as adrenal androgens. In addition to the difference in the origination of the two classes of androgens, difference is biological functions is well known. The only function of the testicular androgens is to bind with and activate the AR to regulate expression of genes, which are primarily driving genes for the functions, differentiation and growth of prostate epithelial cells. On the other hand, the adrenal androgens are known to modulate a wide range of functions of their own and independent of AR (Traish et al., 2011; Maninger et al., 2009; Wolf and Kirschbaum, 1999). It is not known whether the selectivity of the T uptake

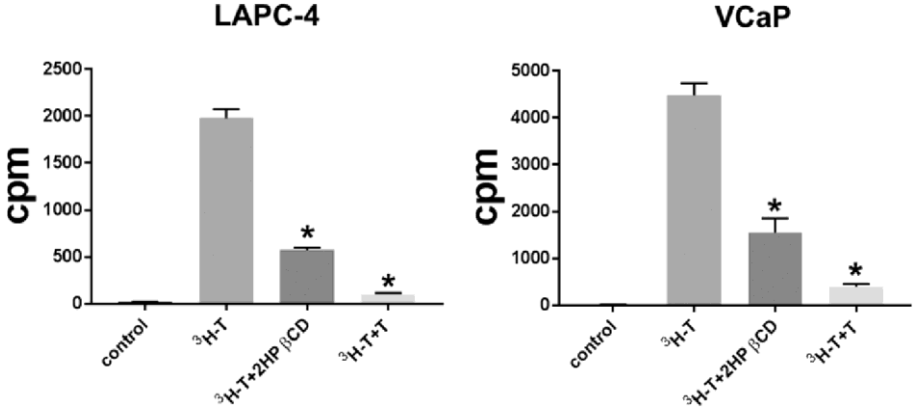

Fig. 6. Treatment with cholesterol flusher 2HP-β-CD reduced ^{3}H-T uptake. LAPC-4 and VCaP cells were pre-treated with 72 μl/ml 2HP-β-CD for 3 h, and treated with 1 nM ^{3}H-T in the presence of 2HP-β-CD. * significantly different from ^{3}H-T uptake value ($p < 0.05$).

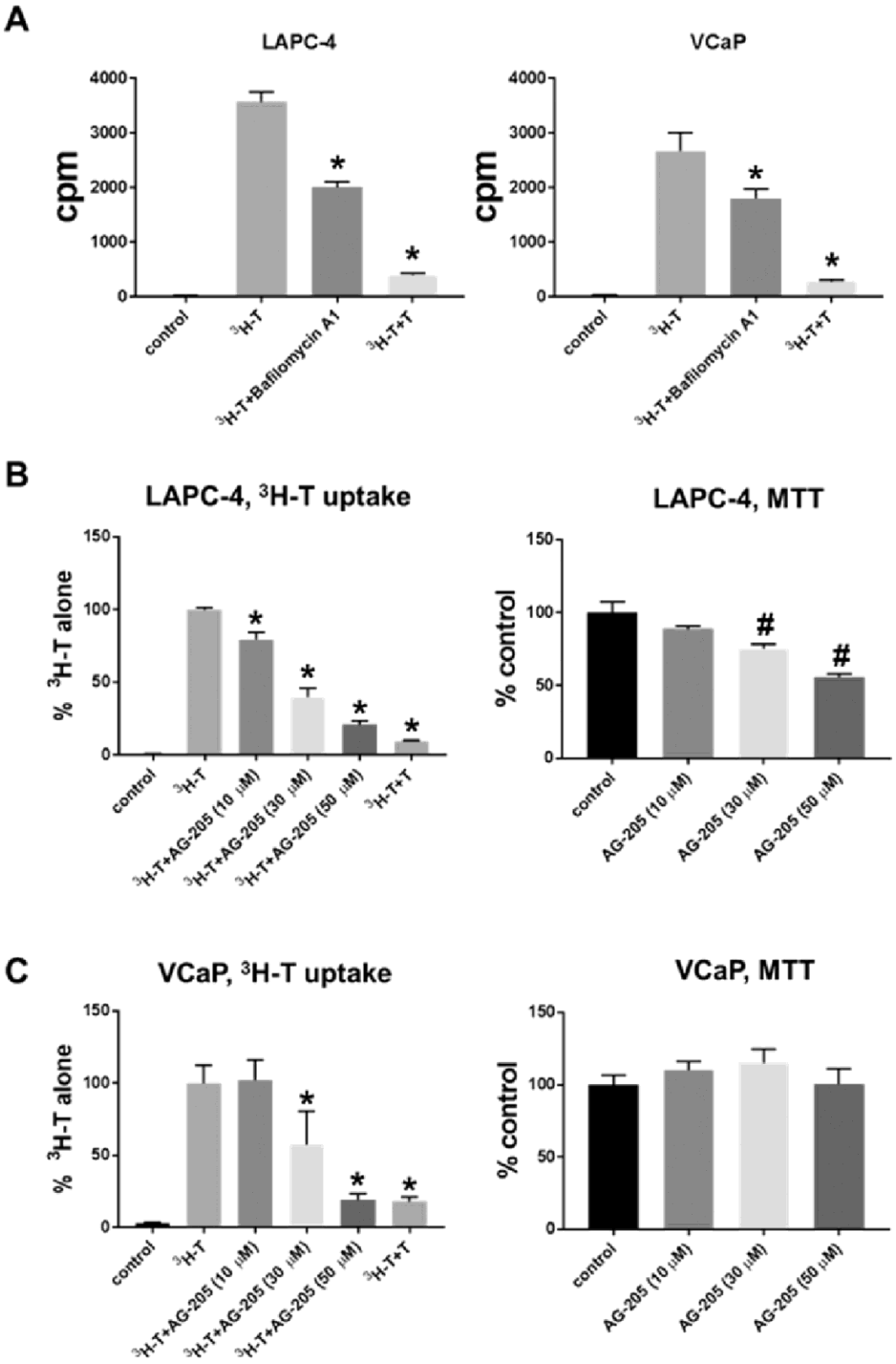

Fig. 7. Uptake of ^{3}H-T was reduced when cells were treated with bafilomycin A (10 nM) or AG-205. (A) Pretreatment with bafilomycin A reduced ^{3}H-T uptake by LAPC-4 and VCaP cells. (B) Pretreatment with AG-205 reduced ^{3}H-T uptake by LAPC-4 cells and cell viability of LAPC-4 cells. (C) Pretreatment with AG-205 reduced ^{3}H-T uptake by VCaP cells, but not viability of VCaP cells. Effect on ^{3}H-T uptake was presented in percentage of ^{3}H-T in cells that were treated with ^{3}H-T alone. Effect on viability was presented in percentage of MTT reading of controls. * significantly different from ^{3}H-T uptake value ($p < 0.05$). # significantly different from control value ($p < 0.05$).

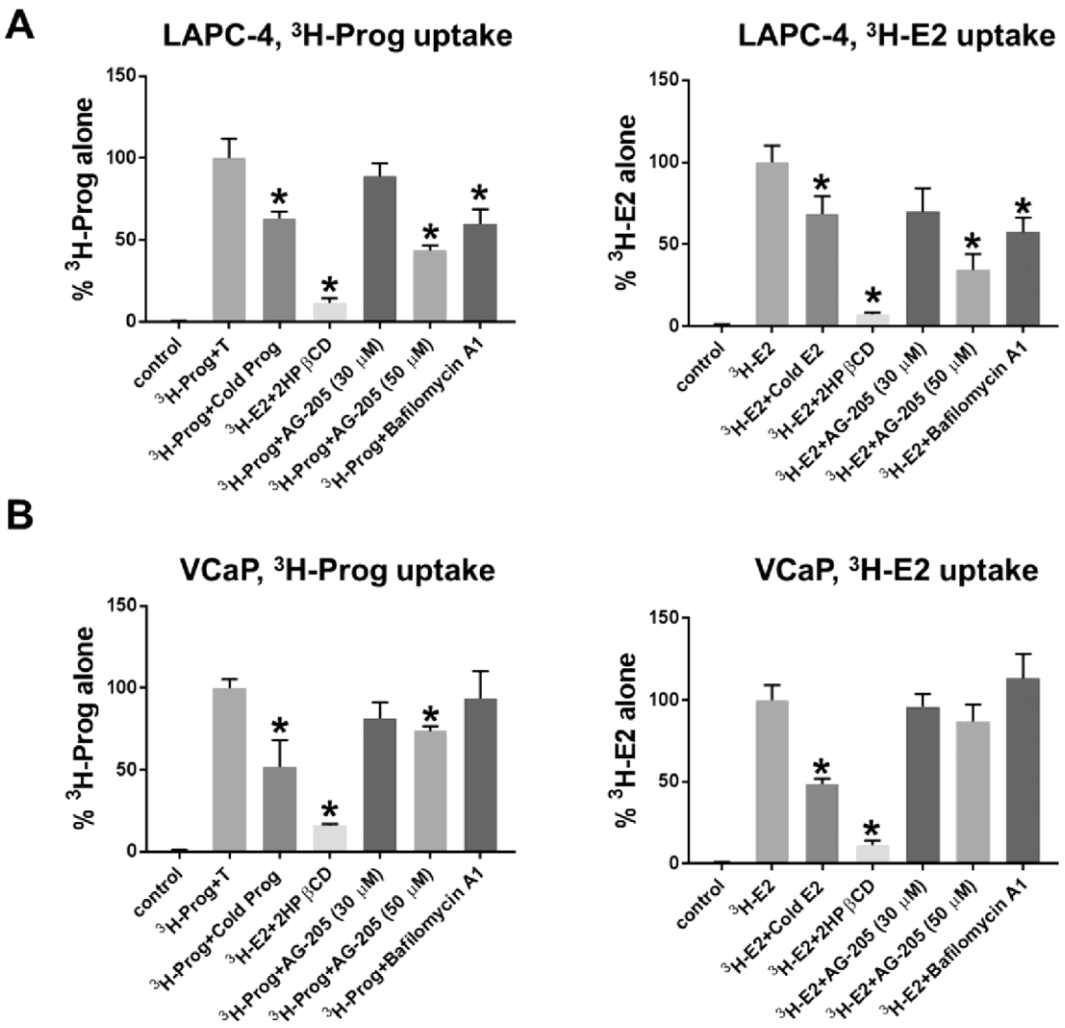

Fig. 8. Uptake of progesterone and E2 was mediated by both specific, transporter-mediated mechanism and non-specific mechanism. Data were presented as percentage to intracellular 3H-progesterone (Prog) or 3H-E2 in cells treated with 3H-progesterone or 3H-E2 alone. Cold prog or cold E2 was 300 nM non-radioactive progesterone or E2. * indicated significant differences compared to cells treated with 3H-progesterone or 3H-E2 alone ($p < 0.05$).

mechanism reflects the difference in the functions among different classes of androgens. DHEAS is the predominant circulating sulfated steroid in both male and female humans, and is a major contributor to tissue production of T/DHT or estrogens in benign and malignant breast and prostate tissue (Labrie et al., 1998; Labrie et al., 2005). Although the key findings of the present study focused on T uptake primarily, mechanisms for uptake of sulfated steroids such as DHEAS and other conjugated steroids should also be studied more closely.

The T uptake mechanism is shared by estrogen such as E2, and by another major sex steroid, progesterone. In contrast to the adrenal androgens, these sex steroids could fully block the uptake of the T uptake. This finding is in agreement with the similarity in the action mechanisms of testicular androgens and estrogens in the reproductive functions, tissue and organs in male and females. Whether the shared uptake mechanism resulted from the evolution of mammal reproduction is up for debate. On the other hand, the uptake mechanism for progesterone and E2 was not the same as the uptake mechanism for T, which was indicated by the differences in the effects of respective non-radioactive progesterone or E2, 2HP-β-CD AG-205 and bafilomycin. A transporter-mediated, specific uptake mechanism was predominant for T uptake, whereas, both transporter-mediated, specific uptake mechanism and non-specific, presumably passive diffusion, contributed to progesterone or E2 uptake comparably. It is likely that although the T uptake mechanism was compatible to progesterone and E2, the uptake of progesterone and E2 utilized separate but slightly similar mechanisms.

The prostate cancer cells lacked active uptake mechanism for adrenal androgens. Neither DHEAS nor DHEA showed an active uptake kinetic (data not shown). However, it is notable that the experimental concentrations of the adrenal androgens, especially DHEAS, are far below the circulating concentrations in adult humans. Due to the limit of the concentration of the commercially available ^{3}H-DHEAS and ^{3}H-DHEA, the highest experimental concentrations were at or lower than 20 nM, whereas, physiological concentrations of DHEAS is 3.5–10 μM (Rainey and Carr, 2004). Therefore, the results could not rule out the possibility that there exists a low affinity but high threshold uptake mechanism for the adrenal androgens. Indeed, there are reports that SLCO transporters were able to uptake DHEAS with km values in the μM range (Roth et al., 2012; Kullak-Ublick et al., 1998; Obaidat et al., 2012). One additional intriguing finding was that ASD only partially blocked uptake of T. Although also considered as a minor adrenal androgen with circulating concentration in nM range, ASD is a metabolite of DHEA and is further metabolized for T or DHT production (Luu-The et al., 2008). Therefore, the partially blockade of T uptake by ASD may reflect the unique position of ASD as an intermediate between adrenal androgens and testicular androgens.

The molecular mechanism underlying the active uptake of T by prostate cancer cells was not identified. The reduction of T uptake by

the treatment with 2HP-β-CD indicated that the molecular mechanism maybe reside in the cholesterol-rich membrane structures such as the lipid raft, which is in line with the lipophilic properties of sex steroids. Further support for this hypothesis comes from lipid raft-localized receptor and transporter for vitamin D receptor and cholesterol, which are both lipophilic (Ikonen, 2006; Huhtakangas et al., 2004; Huff et al., 2006; Zhao and Simpson, 2010). T uptake was slightly reduced when cells were treated with bafilomycin A1, a V-ATPase inhibitor. V-ATPase is an important energy provider to a variety of membrane-embedded transporters (Huss and Wieczorek, 2009). The slight inhibitory effect of the V-ATPase inhibitor is not sufficient to indicate the T uptake mechanism is driven by V-ATPase, but nevertheless implicated the involvement of certain ATPase in the uptake process. The PGRMC1 mediates transmembrane transport of progesterone, although whether this mechanism is the primary progesterone uptake mechanism is not clear (Thomas, 2008; Meyer et al., 1996; Kimura et al., 2012; Cahill, 2007). In spite of the complete block of T uptake by progesterone, T uptake was only marginally reduced when cells were treated with the PGRMC1 inhibitor AG-205 (Will et al., 2017; Guo et al., 2016). MTT data showed cytotoxic effect of AG-205 on the tested cells. However, the reduction in T uptake could not be fully explained by the cytotoxicity. The results suggest that PGRMC1 may not be a primary uptake mechanism for progesterone, but the T uptake mechanism may share certain degrees of similarity with the PGRMC1 system.

The binding between the AR and T may also contribute to the accumulation of intracellular ^{3}H-T, since T is an AR ligand. However, AR-T binding could only explained a nominal portion of the intracellular T accumulation. An average volume of a mammalian cell, a HeLa cell for example, is 2000 μm^3 (Puck et al., 1956; Cohen and Studzinski, 1967; Fujioka et al., 2006; Zhao et al., 2008). The estimated number of proteins in a mammalian cell is 10^{10}, with cell volume at 4000 μm^3 (Milo, 2013). The lowest documented intracellular accumulation of T was 20 nM (Wu et al., 2013), which is translated to 4.8×10^7 AR molecules per cell, or nearly 1/500 of the total protein numbers in a cell, assuming the binding ratio is 1:1. This number is unrealistically large for a transcription factor, and a lot more than reported AR per cell, which is below 2000 (Colvard et al., 1989). The calculated Kd and Bmax values of AR uptake were different between VCaP and LAPC-4. The both cell lines have only wild type AR, and the expression levels of AR in the cell lines were comparable. If the uptake had been caused by AR-T binding, it would be expected that the km and Vmax values were similar.

The AR-null cell lines PC-3 and DU145 did not actively take up T, which was evidenced by the lack of T-specific uptake kinetic compared to VCaP and LACP-4 (data not shown). This observation raised a question with regard to the transform of prostate cancer cells from AR-positive to AR-null. It is not clear whether the loss of AR follow the loss of the uptake mechanism or vice versa.

SLCO1B3 was reported as a T transporter (Hamada et al., 2008). OSTα-OSTβ also was proposed to be a steroid transporter (Ballatori, 2005). It is not clear how an organic anion transporter (SLCO1B3) or an organic cation transporter (OSTα-OSTβ) is able to mediate the transport of uncharged steroids such as T, and how much these transporters may contribute to the overall steroid uptake. DHEAS is a known substrate for the SLCO transporters (Roth et al., 2012; Kullak-Ublick et al., 1998; Obaidat et al., 2012). If the SLCO transporters are the major T uptake mechanism, one would assume that DHEAS would block T uptake. However, DHEAS had no effect on T uptake. The molecular mechanism for steroid uptake remains elusive and requests further investigation.

In summary, our data demonstrated the existence of an active and selective uptake mechanism for uptake of sex steroids T, DHT, E2 and progesterone. Although the underlying molecular mechanism remains elusive, the findings nevertheless bear significance in the action of sex steroids in human hormonal physiology and sex hormone related diseases. The multiple mechanisms involving transporters or cholesterol-rich cell membrane domains for progesterone, E2, T/DHT, and DHEAS/DHEA are proposed (Fig. 9). The implication of the active uptake mechanism is that the distribution, abundance, and functional genetic variations of the involved molecules may affect the sensitivity and tissue or organ specificity to the sex hormones. Anomalies in the uptake mechanism may contribute to hormone-associated disease. Take prostate cancer or breast cancer for example, overly active uptake mechanisms may render cancer cells the ability to acquire more androgen or estrogen for growth, or evade anti-hormone therapies such as androgen deprivation therapy by taking excessive amount of sex steroids. Another note is that although a selective and active transporter-mediated uptake mechanism, especially for T uptake, existed in the cell models used in this study, passive diffusion may remain as a way of entry of steroids into cells. The relative contributions of these different uptake mechanisms may differ among cell models and among different steroids. Passive diffusion may be difficult to block due to the lack of

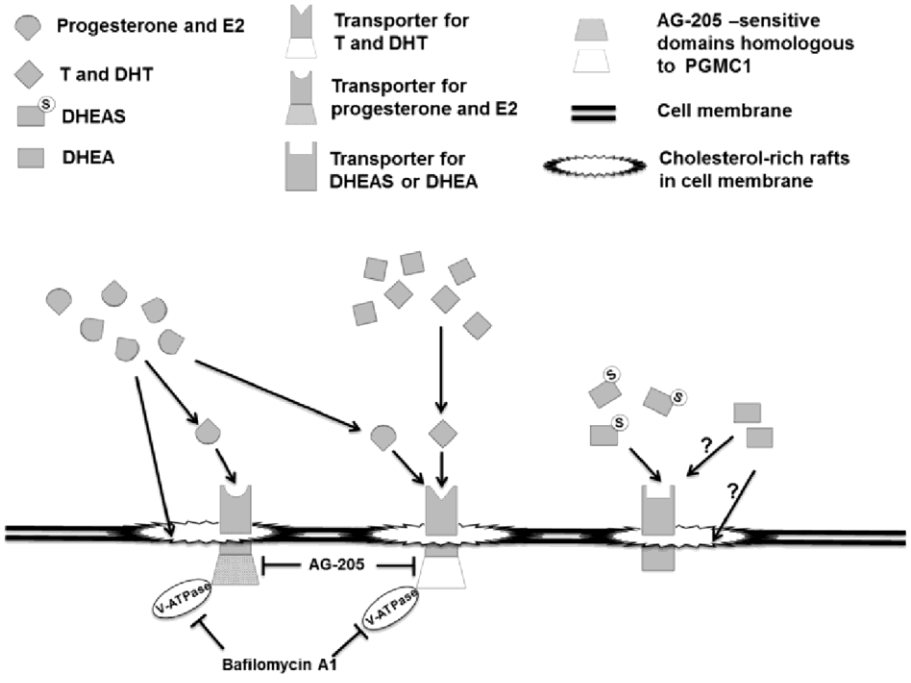

Fig. 9. Steroid-specific transport systems on cell membrane are proposed. Uptake of testicular androgens T and DHT is mediated by transporters that are located in cholesterol-rich domains in the cell membrane. T/DHT transporters also recognize progesterone and E2, but not DHEAS and DHEA. Therefore, uptake of T is blocked by DHT, progesterone and E2, but not by DHEAS and DHEA. Uptake of progesterone and E2 is mediated by specific transporters and facilitated by other mechanisms, presumably passive diffusion, which reside in cholesterol-rich cell membrane domains. T/DHT uptake system and progesterone/E2 uptake system share AG-205-sensitive regulatory motifs that is homologous to PGRMC1, and the uptake is driven partly by V-ATPase. Adrenal androgen DHEAS and DHEA rely on other mechanisms for uptake. SLCO transporters may contribute to DHEAS uptake. It is not clear whether DHEA uptake is mediated by transporters or facilitated by other mechanisms such as passive diffusion, and whether these mechanisms are located in the cholesterol-rich domains in cell membrane.

specific carriers. On the other hand, transporter-mediated uptake may be subjected to blockers. However, the efficiency and off-target effects of the blockade would depend on the spectrum, or substrate-specificity of the transporters and their respective blockers.

Acknowledgement

This work was supported by a Department of Defense grant W81XWH-14-1-0453 (to Y.W.), two awards from the Roswell Park Alliance Foundation (to Y.W.), and National Cancer Institute grant P30CA16056 involving the use of Roswell Park Comprehensive Cancer Center's Shared Resources.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.mce.2018.06.009.

References

Africander, D., Storbeck, K.H., 2018. Steroid metabolism in breast cancer: where are we and what are we missing? Mol. Cell. Endocrinol. 466, 86–97.
Aguilera, R., Hatton, C.K., Catlin, D.H., 2002. Detection of epitestosterone doping by isotope ratio mass spectrometry. Clin. Chem. 48, 629–636.
Altmann, S.W., Davis Jr., H.R., Zhu, L.J., Yao, X., Hoos, L.M., Tetzloff, G., Iyer, S.P., Maguire, M., Golovko, A., Zeng, M., Wang, L., Murgolo, N., Graziano, M.P., 2004. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Science 303, 1201–1204.
Bain, D.L., Heneghan, A.F., Connaghan-Jones, K.D., Miura, M.T., 2007. Nuclear receptor structure: implications for function. Annu. Rev. Physiol. 69, 201–220.
Ballatori, N., 2005. Biology of a novel organic solute and steroid transporter, OSTalpha-OSTbeta. Exp. Biol. Med. 230, 689–698.
Barzi, A., Lenz, A.M., Labonte, M.J., Lenz, H.J., 2013. Molecular pathways: estrogen pathway in colorectal cancer. Clin. Canc. Res. 19, 5842–5848.
Bellemare, V., Faucher, F., Breton, R., Luu-The, V., 2005. Characterization of 17alpha-hydroxysteroid dehydrogenase activity (17alpha-HSD) and its involvement in the biosynthesis of epitestosterone. BMC Biochem. 6, 12.
Bowman, E.J., Siebers, A., Altendorf, K., 1988. Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. U. S. A. 85, 7972–7976.
Bulldan, A., Dietze, R., Shihan, M., Scheiner-Bobis, G., 2016. Non-classical testosterone signaling mediated through ZIP9 stimulates claudin expression and tight junction formation in Sertoli cells. Cell. Signal. 28, 1075–1085.
Cahill, M.A., 2007. Progesterone receptor membrane component 1: an integrative review. J. Steroid Biochem. Mol. Biol. 105, 16–36.
Caldwell, J.D., Jirikowski, G.F., 2014. Sex hormone binding globulin and corticosteroid binding globulin as major effectors of steroid action. Steroids 81, 13–16.
Carmeci, C., Thompson, D.A., Ring, H.Z., Francke, U., Weigel, R.J., 1997. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. Genomics 45, 607–617.
Castrellon, A.B., 2017. Novel strategies to improve the endocrine therapy of breast cancer. Onco Rev. 11, 323.
Chene, P., 2002. ATPases as drug targets: learning from their structure. Nat. Rev. Drug Discov. 1, 665–673.
Christian, A.E., Haynes, M.P., Phillips, M.C., Rothblat, G.H., 1997. Use of cyclodextrins for manipulating cellular cholesterol content. J. Lipid Res. 38, 2264–2272.
Chuffa, L.G., Lupi-Junior, L.A., Costa, A.B., Amorim, J.P., Seiva, F.R., 2017. The role of sex hormones and steroid receptors on female reproductive cancers. Steroids 118, 93–108.
Cohen, L.S., Studzinski, G.P., 1967. Correlation between cell enlargement and nucleic acid and protein content of HeLa cells in unbalanced growth produced by inhibitors of DNA synthesis. J. Cell. Physiol. 69, 331–339.
Colvard, D.S., Eriksen, E.F., Keeting, P.E., Wilson, E.M., Lubahn, D.B., French, F.S., Riggs, B.L., Spelsberg, T.C., 1989. Identification of androgen receptors in normal human osteoblast-like cells. Proc. Natl. Acad. Sci. U. S. A. 86, 854–857.
Cuddy, L.K., Winick-Ng, W., Rylett, R.J., 2014. Regulation of the high-affinity choline transporter activity and trafficking by its association with cholesterol-rich lipid rafts. J. Neurochem. 128, 725–740.
Dai, C., Heemers, H., Sharifi, N., 2017. Androgen signaling in prostate cancer. Cold Spring Harb. Perspect. Med. 7.
Diep, C.H., Daniel, A.R., Mauro, L.J., Knutson, T.P., Lange, C.A., 2015. Progesterone action in breast, uterine, and ovarian cancers. J. Mol. Endocrinol. 54, R31–R53.
Fiandalo, M.V., Wilton, J.H., Mantione, K.M., Wrzosek, C., Attwood, K.M., Wu, Y., Mohler, J.L., 2017. Serum-free complete medium, an alternative medium to mimic androgen deprivation in human prostate cancer cell line models. Prostate.
Fujioka, A., Terai, K., Itoh, R.E., Aoki, K., Nakamura, T., Kuroda, S., Nishida, E., Matsuda, M., 2006. Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes. J. Biol. Chem. 281, 8917–8926.
Funakoshi, T., Yanai, A., Shinoda, K., Kawano, M.M., Mizukami, Y., 2006. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. Biochem. Biophys. Res. Commun. 346, 904–910.
Gaspar, J., Mathieu, J., Alvarez, P., 2017. 2-Hydroxypropyl-beta-cyclodextrin (HPbetaCD) reduces age-related lipofuscin accumulation through a cholesterol-associated pathway. Sci. Rep. 7, 2197.
Gharwan, H., Bunch, K.P., Annunziata, C.M., 2015. The role of reproductive hormones in epithelial ovarian carcinogenesis. Endocr. Relat. Canc. 22, R339–R363.
Giorgi, E.P., 1976. Studies on androgen transport into canine prostate in vitro. J. Endocrinol. 68, 109–119.
Giorgi, E.P., Shirley, I.M., Grant, J.K., Stewart, J.C., 1973. Androgen dynamics in vitro in the human prostate gland. Effect of cyproterone and cyproterone acetate. Biochem. J. 132, 465–474.
Goma, A., Mir, R., Martinez-Soler, F., Tortosa, A., Vidal, A., Condom, E., Perez-Tomas, R., Gimenez-Bonafe, P., 2014. Multidrug resistance protein 1 localization in lipid raft domains and prostasomes in prostate cancer cell lines. OncoTargets Ther. 7, 2215–2225.
Grimm, S.L., Hartig, S.M., Edwards, D.P., 2016. Progesterone receptor signaling mechanisms. J. Mol. Biol. 428, 3831–3849.
Groner, A.C., Brown, M., 2017. Role of steroid receptor and coregulator mutations in hormone-dependent cancers. J. Clin. Invest. 127, 1126–1135.
Guo, M., Zhang, C., Wang, Y., Feng, L., Wang, Z., Niu, W., Du, X., Tang, W., Li, Y., Wang, C., Chen, Z., 2016. Progesterone receptor membrane component 1 mediates progesterone-induced suppression of oocyte meiotic prophase I and primordial folliculogenesis. Sci. Rep. 6, 36869.
Hamada, A., Sissung, T., Price, D.K., Danesi, R., Chau, C.H., Sharifi, N., Venzon, D., Maeda, K., Nagao, K., Sparreboom, A., Mitsuya, H., Dahut, W.L., Figg, W.D., 2008. Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. Clin. Canc. Res. 14, 3312–3318.
Hammes, A., Andreassen, T.K., Spoelgen, R., Raila, J., Hubner, N., Schulz, H., Metzger, J., Schweigert, F.J., Luppa, P.B., Nykjaer, A., Willnow, T.E., 2005. Role of endocytosis in cellular uptake of sex steroids. Cell 122, 751–762.
Hammond, G.L., 2011. Diverse roles for sex hormone-binding globulin in reproduction. Biol. Reprod. 85, 431–441.
Heemers, H.V., Tindall, D.J., 2007. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr. Rev. 28, 778–808.
Heinlein, C.A., Chang, C., 2002. Androgen receptor (AR) coregulators: an overview. Endocr. Rev. 23, 175–200.
Helms, J.B., Zurzolo, C., 2004. Lipids as targeting signals: lipid rafts and intracellular trafficking. Traffic 5, 247–254.
Herrera, J.L., Diaz, M., Hernandez-Fernaud, J.R., Salido, E., Alonso, R., Fernandez, C., Morales, A., Marin, R., 2011. Voltage-dependent anion channel as a resident protein of lipid rafts: post-transductional regulation by estrogens and involvement in neuronal preservation against Alzheimer's disease. J. Neurochem. 116, 820–827.
Hilton, H.N., Clarke, C.L., Graham, J.D., 2017. Estrogen and progesterone signalling in the normal breast and its implications for cancer development. Mol. Cell. Endocrinol.
Huff, M.W., Pollex, R.L., Hegele, R.A., 2006. NPC1L1: evolution from pharmacological target to physiological sterol transporter. Arterioscler. Thromb. Vasc. Biol. 26, 2433–2438.
Huhtakangas, J.A., Olivera, C.J., Bishop, J.E., Zanello, L.P., Norman, A.W., 2004. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha,25(OH)2-vitamin D3 in vivo and in vitro. Mol. Endocrinol. 18, 2660–2671.
Huss, M., Wieczorek, H., 2009. Inhibitors of V-ATPases: old and new players. J. Exp. Biol. 212, 341–346.
Ikonen, E., 2006. Mechanisms for cellular cholesterol transport: defects and human disease. Physiol. Rev. 86, 1237–1261.
Irwin, M.E., Mueller, K.L., Bohin, N., Ge, Y., Boerner, J.L., 2011. Lipid raft localization of EGFR alters the response of cancer cells to the EGFR tyrosine kinase inhibitor gefitinib. J. Cell. Physiol. 226, 2316–2328.
Jeon, S.Y., Hwang, K.A., Choi, K.C., 2016. Effect of steroid hormones, estrogen and progesterone, on epithelial mesenchymal transition in ovarian cancer development. J. Steroid Biochem. Mol. Biol. 158, 1–8.
Jordan, V.C., Brodie, A.M., 2007. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. Steroids 72, 7–25.
Joseph, D.R., 1994. Structure, function, and regulation of androgen-binding protein/sex hormone-binding globulin. Vitam. Horm. 49, 197–280.
Kamal, A., Tempest, N., Parkes, C., Alnafakh, R., Makrydima, S., Adishesh, M., Hapangama, D.K., 2016. Hormones and endometrial carcinogenesis. Horm. Mol. Biol. Clin. Invest. 25, 129–148.
Kimura, I., Nakayama, Y., Konishi, M., Terasawa, K., Ohta, M., Itoh, N., Fujimoto, M., 2012. Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins. Curr. Protein Pept. Sci. 13, 687–696.
Klaassen, C.D., Aleksunes, L.M., 2010. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol. Rev. 62, 1–96.
Klappe, K., Hummel, I., Hoekstra, D., Kok, J.W., 2009. Lipid dependence of ABC transporter localization and function. Chem. Phys. Lipids 161, 57–64.
Klein, K.A., Reiter, R.E., Redula, J., Moradi, H., Zhu, X.L., Brothman, A.R., Lamb, D.J., Marcelli, M., Belldegrun, A., Witte, O.N., Sawyers, C.L., 1997. Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat. Med. 3, 402–408.
Kullak-Ublick, G.A., Fisch, T., Oswald, M., Hagenbuch, B., Meier, P.J., Beuers, U., Paumgartner, G., 1998. Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain. FEBS Lett. 424, 173–176.
Labrie, F., Belanger, A., Luu-The, V., Labrie, C., Simard, J., Cusan, L., Gomez, J.L., Candas, B., 1998. DHEA and the intracrine formation of androgens and estrogens in

peripheral target tissues: its role during aging. Steroids 63, 322–328.
Labrie, F., Luu-The, V., Belanger, A., Lin, S.X., Simard, J., Pelletier, G., Labrie, C., 2005. Is dehydroepiandrosterone a hormone? J. Endocrinol. 187, 169–196.
Li, S., Zhou, Y., Wang, R., Zhang, H., Dong, Y., Ip, C., 2007. Selenium sensitizes MCF-7 breast cancer cells to doxorubicin-induced apoptosis through modulation of phospho-Akt and its downstream substrates. Mol. Canc. Therapeut. 6, 1031–1038.
Lin, B.C., Scanlan, T.S., 2005. Few things in life are "free": cellular uptake of steroid hormones by an active transport mechanism. Mol. Interv. 5, 338–340.
Lin, J.H., Manson, J.E., Kraft, P., Cochrane, B.B., Gunter, M.J., Chlebowski, R.T., Zhang, S.M., 2011. Estrogen and progesterone-related gene variants and colorectal cancer risk in women. BMC Med. Genet. 12, 78.
Luu-The, V., Belanger, A., Labrie, F., 2008. Androgen biosynthetic pathways in the human prostate. Best Pract. Res. Clin. Endocrinol. Metabol. 22, 207–221.
Manabe, T., Yoshimori, T., Henomatsu, N., Tashiro, Y., 1993. Inhibitors of vacuolar-type H(+)-ATPase suppresses proliferation of cultured cells. J. Cell. Physiol. 157, 445–452.
Maninger, N., Wolkowitz, O.M., Reus, V.I., Epel, E.S., Mellon, S.H., 2009. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front. Neuroendocrinol. 30, 65–91.
Martinez-Zaguilan, R., Lynch, R.M., Martinez, G.M., Gillies, R.J., 1993. Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells. Am. J. Physiol. 265, C1015–C1029.
Mendel, C.M., 1992. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. J. Androl. 13, 107–116.
Meyer, C., Schmid, R., Scriba, P.C., Wehling, M., 1996. Purification and partial sequencing of high-affinity progesterone-binding site(s) from porcine liver membranes. Eur. J. Biochem. 239, 726–731.
Milo, R., 2013. What is the total number of protein molecules per cell volume? A call to rethink some published values. Bioessays 35, 1050–1055.
Nakhla, A.M., Leonard, J., Hryb, D.J., Rosner, W., 1999. Sex hormone-binding globulin receptor signal transduction proceeds via a G protein. Steroids 64, 213–216.
Nuck, B.A., Lucky, A.W., 1987. Epitestosterone: a potential new antiandrogen. J. Invest. Dermatol. 89, 209–211.
Obaidat, A., Roth, M., Hagenbuch, B., 2012. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. Annu. Rev. Pharmacol. Toxicol. 52, 135–151.
Oren, I., Fleishman, S.J., Kessel, A., Ben-Tal, N., 2004. Free diffusion of steroid hormones across biomembranes: a simplex search with implicit solvent model calculations. Biophys. J. 87, 768–779.
Orlowski, S., Comera, C., Terce, F., Collet, X., 2007. Lipid rafts: dream or reality for cholesterol transporters? Eur. Biophys. J. 36, 869–885.
Plaza-Parrochia, F., Romero, C., Valladares, L., Vega, M., 2017. Endometrium and steroids, a pathologic overview. Steroids 126, 85–91.
Porto, C.S., Lazari, M.F., Abreu, L.C., Bardin, C.W., Gunsalus, G.L., 1995. Receptors for androgen-binding proteins: internalization and intracellular signalling. J. Steroid Biochem. Mol. Biol. 53, 561–565.
Puck, T.T., Marcus, P.I., Cieciura, S.J., 1956. Clonal growth of mammalian cells in vitro; growth characteristics of colonies from single HeLa cells with and without a feeder layer. J. Exp. Med. 103, 273–283.
Rainey, W.E., Carr, B.R., 2004. Adrenal androgens in human physiology. Semin. Reprod. Med. 22, 279–280.
Rainey, W.E., Carr, B.R., Sasano, H., Suzuki, T., Mason, J.I., 2002. Dissecting human adrenal androgen production. Trends Endocrinol. Metabol. 13, 234–239.
Rosner, W., Hryb, D.J., Kahn, S.M., Nakhla, A.M., Romas, N.A., 2010. Interactions of sex hormone-binding globulin with target cells. Mol. Cell. Endocrinol. 316, 79–85.
Roth, M., Obaidat, A., Hagenbuch, B., 2012. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br. J. Pharmacol. 165, 1260–1287.
Sano, O., Ito, S., Kato, R., Shimizu, Y., Kobayashi, A., Kimura, Y., Kioka, N., Hanada, K., Ueda, K., Matsuo, M., 2014. ABCA1, ABCG1, and ABCG4 are distributed to distinct membrane meso-domains and disturb detergent-resistant domains on the plasma membrane. PLoS One 9, e109886.
Shi, Y., Han, J.J., Tennakoon, J.B., Mehta, F.F., Merchant, F.A., Burns, A.R., Howe, M.K., McDonnell, D.P., Frigo, D.E., 2013. Androgens promote prostate cancer cell growth through induction of autophagy. Mol. Endocrinol. 27, 280–295.
Shihan, M., Kirch, U., Scheiner-Bobis, G., 2013. Dehydroepiandrosterone sulfate mediates activation of transcription factors CREB and ATF-1 via a Galpha11-coupled receptor in the spermatogenic cell line GC-2. Biochim. Biophys. Acta 1833, 3064–3075.
Shihan, M., Bulldan, A., Scheiner-Bobis, G., 2014. Non-classical testosterone signaling is mediated by a G-protein-coupled receptor interacting with Gnalpha11. Biochim. Biophys. Acta 1843, 1172–1181.
Shihan, M., Chan, K.H., Konrad, L., Scheiner-Bobis, G., 2015. Non-classical testosterone signaling in spermatogenic GC-2 cells is mediated through ZIP9 interacting with Gnalpha11. Cell. Signal. 27, 2077–2086.
Simons Jr., S.S., 2008. What goes on behind closed doors: physiological versus pharmacological steroid hormone actions. Bioessays 30, 744–756.
Snaterse, G., Visser, J.A., Arlt, W., Hofland, J., 2017. Circulating steroid hormone variations throughout different stages of prostate cancer. Endocr. Relat. Canc. 24, R403–R420.
Sobel, R.E., Sadar, M.D., 2005a. Cell lines used in prostate cancer research: a compendium of old and new lines–part 2. J. Urol. 173, 360–372.
Sobel, R.E., Sadar, M.D., 2005b. Cell lines used in prostate cancer research: a compendium of old and new lines–part 1. J. Urol. 173, 342–359.
Song, W., Wang, F., Lotfi, P., Sardiello, M., Segatori, L., 2014. 2-Hydroxypropyl-beta-cyclodextrin promotes transcription factor EB-mediated activation of autophagy: implications for therapy. J. Biol. Chem. 289, 10211–10222.
Starka, L., 2003. Epitestosterone. J. Steroid Biochem. Mol. Biol. 87, 27–34.
Stuchbery, R., McCoy, P.J., Hovens, C.M., Corcoran, N.M., 2017. Androgen synthesis in prostate cancer: do all roads lead to Rome? Nat. Rev. Urol. 14, 49–58.
Szilagyi, J.T., Vetrano, A.M., Laskin, J.D., Aleksunes, L.M., 2017. Localization of the placental BCRP/ABCG2 transporter to lipid rafts: role for cholesterol in mediating efflux activity. Placenta 55, 29–36.
Thomas, P., 2008. Characteristics of membrane progestin receptor alpha (mPRalpha) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progestin actions. Front. Neuroendocrinol. 29, 292–312.
Traish, A.M., Kang, H.P., Saad, F., Guay, A.T., 2011. Dehydroepiandrosterone (DHEA)–a precursor steroid or an active hormone in human physiology. J. Sex. Med. 8, 2960–2982 quiz 2983.
Will, E.A., Liu, X., Peluso, J.J., 2017. AG 205, a progesterone receptor membrane component 1 antagonist, ablates progesterone's ability to block oxidative stress-induced apoptosis of human granulosa/luteal cells. Biol. Reprod. 96, 843–854.
Wilson, E.M., 2009. Functional motifs of the androgen receptor. In: Tindall, D.M., J., L. (Eds.), Androgen Action in Prostate Cancer. Springer, pp. 241–267.
Wolf, O.T., Kirschbaum, C., 1999. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. Brain Res Brain Res Rev. 30, 264–288.
Wu, Y., Chhipa, R.R., Zhang, H., Ip, C., 2011a. The antiandrogenic effect of finasteride against a mutant androgen receptor. Canc. Biol. Ther. 11, 902–909.
Wu, S.N., Yeh, C.C., Huang, H.C., Yang, W.H., 2011b. Cholesterol depletion with (2-hydroxypropyl)- beta-cyclodextrin modifies the gating of membrane electroporation-induced inward current in pituitary tumor GH3 cells: experimental and analytical studies. Cell. Physiol. Biochem. 28, 959–968.
Wu, Y., Godoy, A., Azzouni, F., Wilton, J.H., Ip, C., Mohler, J.L., 2013. Prostate cancer cells differ in testosterone accumulation, dihydrotestosterone conversion, and androgen receptor signaling response to steroid 5 alpha-reductase inhibitors. Prostate 73, 1470–1482.
Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M., Tashiro, Y., 1991. Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. J. Biol. Chem. 266, 17707–17712.
Zhao, G., Simpson, R.U., 2010. Membrane localization, Caveolin-3 association and rapid actions of vitamin D receptor in cardiac myocytes. Steroids 75, 555–559.
Zhao, L., Kroenke, C.D., Song, J., Piwnica-Worms, D., Ackerman, J.J., Neil, J.J., 2008. Intracellular water-specific MR of microbead-adherent cells: the HeLa cell intracellular water exchange lifetime. NMR Biomed. 21, 159–164.