genes

MDPI

Article

Tumor Androgen Receptor Protein Level Is Positively Associated with a Better Overall Survival in Melanoma Patients

Nupur Singh [1], Jude Khatib [2], Chi-Yang Chiu [3], Jianjian Lin [3], Tejesh Surender Patel [2] and Feng Liu-Smith [2,3,*]

[1] College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38103, USA
[2] Department of Dermatology, College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38103, USA
[3] Department of Preventive Medicine, College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38103, USA
* Correspondence: fliusmit@uthsc.edu

Abstract: Androgen receptor (AR) is expressed in numerous tissues and serves important biologic functions in skin, prostate, immune, cardiovascular, and neural systems, alongside sexual development. Several studies have associated AR expression and patient survival in various cancers, yet there are limited studies examining the relationship between AR expression and cutaneous melanoma. This study used genomics and proteomics data from The Cancer Proteome Atlas (TCPA) and The Cancer Genome Atlas (TCGA), with 470 cutaneous melanoma patient data points. Cox regression analyses evaluated the association between AR protein level with overall survival and revealed that a higher level of AR protein was positively associated with a better overall survival (OS) ($p = 0.003$). When stratified by sex, the AR association with OS was only significant for both sexes. The multivariate Cox models with justifications of sex, age of diagnosis, stage of disease, and Breslow depth of the tumor confirmed the AR-OS association in all patients. However, the significance of AR was lost when ulceration was included in the model. When stratified by sex, the multivariate Cox models indicated significant role of AR in OS of female patients but not in males. AR-associated genes were identified and enrichment analysis revealed shared and distinct gene network in male and female patients. Furthermore, AR was found significantly associated with OS in RAS mutant subtypes of melanoma but not in BRAF, NF1, or triple-wild type subtypes of melanoma. Our study may provide insight into the well-known female survival advantage in melanoma patients.

Keywords: melanoma; androgen receptor; protein expression; melanoma survival; cancer

Citation: Singh, N.; Khatib, J.; Chiu, C.-Y.; Lin, J.; Patel, T.S.; Liu-Smith, F. Tumor Androgen Receptor Protein Level Is Positively Associated with a Better Overall Survival in Melanoma Patients. *Genes* **2023**, *14*, 345. https://doi.org/10.3390/genes14020345

Academic Editor: Paolo Cinelli

Received: 10 December 2022
Revised: 19 January 2023
Accepted: 25 January 2023
Published: 28 January 2023

1. Introduction

Melanoma incidence continues to increase worldwide; in the US, it has increased by 320% since 1975 [1]. Research on how sex hormones and their receptors impact melanoma have not resulted in solid conclusions. Androgen receptor (AR), for example, was recently reported to exhibit effects of promoting cell proliferation, melanoma metastasis, and drug resistance in melanoma cells and mouse models [2–4]. While molecular studies and mouse models have provided much interesting information, we are interested in investigating whether AR was differentially expressed in melanoma tumors from men and women, and whether the tumor AR levels are associated with patient overall survival (OS). This study shall shed insight into a long-observed phenomena, i.e., the female survival advantage of melanoma patients [5,6].

As a male sex hormone receptor, the gene *AR* is located on the X chromosome [7]. Two androgenic hormones that are able to bind to AR include testosterone (T), and its metabolite dihydrotestosterone (DHT), and they are active in human skin in endocrine and paracrine manner [8–10]. AR and these hormones exert their genomic effects via induction

of transcriptional activities, and non-genomics activity through signal transduction, both of which are best studied in human prostate cancer [11–13].

Sex differences in cancer incidence have also been documented in several cancers, [14]. For instance, higher incidence rates of lung, liver, stomach, esophageal, and bladder malignancies alongside cutaneous melanoma are found in males compared to females [15–18]. Aside from lifestyle, the characterization of the molecular differences in cancer between male and female malignancies highlights the sex-based variations of gene expression on a molecular level [19]. Nonetheless, there remains a lack of complete understanding of what role AR signaling plays in most hormone-independent cancers alongside cutaneous melanoma.

Current literature has explored possible pathways into AR's effects, both harmful and protective, on cutaneous melanoma development. One proposed mechanism explains how AR and the protein Early Growth Response 1 (EGR1) increase melanoma proliferation through coordinated transcriptional regulation of several growth-regulatory genes, including the repression of EGR1-mediated transcriptional activation of p21Waf1/Cip1, a known tumor suppressor gene [20]. Another mechanism suggesting melanoma progression includes altering the miRNA-539-3p/USP13 signaling to reduce de-ubiquitination of MITF protein, increasing MITF degradation, and allowing further invasion [4]. Other mechanisms of AR's role in cancer risks have been proposed to provide a potential protective effect, specifically cancer-associated fibroblast (CAF) activation. Decreased AR expression in primary human dermal fibroblasts (HDFs) derived from multiple individuals led to early steps of CAF activation. The discovered mechanism includes the development of a complex in which AR combines with CSL/RBP-Jκ to normally repress the transcription of key CAF effector gene [21].

The conflicting findings of AR's protective or harmful role in melanoma progression shows the complexity of several convergent mechanisms likely implicated in melanoma's AR dependency. In this paper, we use The Cancer Genome Atlas (TCGA) and The Cancer Proteome Atlas (TCPA) to evaluate the relationship between *AR* gene and AR protein expression in human cutaneous melanoma, and their association with OS in patients. Genomic network was further explored in an attempt to understand the sex-differentiated roles of AR in patient overall survival.

2. Materials and Methods

2.1. The Source of Data

The data source used for all analyses (TCGA-SKCM) were obtained from The Cancer Genome Atlas (TCGA), with mRNA sequencing (RNA-Seq) data downloaded from Broad Firehose GDAC (http://gdac.broadinstitute.org/, accessed on 14 June 2022), and proteomics data from The Cancer Proteome Atlas (TCPA) (https://tcpaportal.org/, accessed on 14 June 2022). The RNA-Seq data were retrieved as RSEM (RNA-Seq by Expectation-Maximization) and Z scores [22]. Patient ID, sex, age of diagnosis, follow-up time, and survival status were also downloaded from the Broad GDAC site. The database contained 480 tumors from 471 patients with cutaneous melanoma. Protein expression data were available for 355 tumors. If patient duplicates were encountered, the data for metastatic tumor was selected and the primary tumor data were discarded. Tumor stages are grouped into early (Stage I and Stage II) or late stage (Stage III and Stage VI) or used as denoted in the dataset as Stage 0–4.

2.2. Statistical Methods

All statistics were analyzed using Stata 17. Linear regression was used to examine the association of mRNA and protein levels in tumor samples. AR levels (mRNA or protein) were compared between sex by Student *t*-test and/or rank-sum test. Cox regression analyses were performed to evaluate the association between AR protein level with overall survival. The AR-high and AR-low groups were defined by the median of AR protein level (−0.718). The regression model was further stratified by sex or adjusted to age, tumor stage

(early and late), Breslow depth, and ulceration status of the tumors. Sex was also used as an adjusting co-variable in the overall model. The overall survival was defined as the period from date of diagnosis until death from any cause. Significance levels are set at 0.05 (two-sided) for all analysis.

2.3. Gene Network Analysis

AR-co-expressed genes (based on RNA-Seq) were extracted from the cBioportal website (https://www.cbioportal.org/, accessed on 14 June 2022) using sex-stratified patient information. The AR-co-expressed genes were processed using adjusted q values of 0.05, followed by cutoff value of Spearman's coefficient of 0.3 [23]. A set of genes that are uniquely associated with AR in male and female tumors were identified and then subjected to a functional enrichment analysis using g:profiler web-based analysis tools (https://biit.cs.ut.ee/gprofiler/, accessed on 14 June 2022).

3. Results

3.1. The Sex Difference of AR Gene Expression at mRNA and Protein Level

The TCGA SKCM dataset was downloaded from the Broad Institute Firehose website. The baseline characterizations of patients are listed in Table 1. The protein quantification data are available for 353 patients and the mRNA data are available for all 471 patients. The mRNA level of AR was compared between tumors from male and female sources using log transformed RSEM. A total of 21 female tumors and 34 male tumors did not show detectable levels of mRNA (RSEM = 0), but they were also included in the analysis. Student *t*-test showed no sex difference in mean of log transformed RSEM values ($p = 0.10$). However, when the protein levels were used for sex comparison, tumors from females (N = 144) showed a significant lower level of AR protein than those from males (N = 208) ($p = 0.0099$) (Table 2).

Table 1. Baseline characterization of patients.

	Female	Male	Missing *	Total
Number of patients	180	290	1 (sex)	471
Number of tumors	183	296	1 (sex)	480
Tumors with available AR RPPA data	144	208	1 (sex)	353
Tumors with available AR mRNA data	180	289	1 (mRNA)	471
Number of primary tumors	45	64	0	109
Number of metastatic tumors	138	232	1 (sex)	371
Age at diagnosis (years)	58.5 ± 1.2	58.0 ± 0.9	9 (age)	58.2 ± 0.7
Stage of disease				
stage 0	2	5		7
stage 1	25	52		77
stage 2	61	93		154
stage 3	67	104		171
stage 4	8	15		23
missing	17	21	1 (sex)	39
total	180	290		471
Stage of disease **				
early	88	150		238
late	75	119		194
missing	17	21	1 (sex)	39
Ulceration				
no	57	89		146
yes	67	100		167
missing	56	101		158

Table 1. *Cont.*

	Female	Male	Missing *	Total
Breslow depth				
<1.0 mm	20	36		56
1.0–2.0 mm	27	53		80
2.0–4.0 mm	33	44		77
>4.0 mm	60	83		143
missing	40	74	1 (sex)	115

* missing means the number of patients missing the corresponding data, e.g., first row, 1 (sex) means 1 patient missing sex information. ** stages 0–2 are defined as early stage, while stages 3–4 are late stage.

Table 2. The sex difference in AR gene expression.

	mRNA			Protein		
Sex	Female	Male	Total	Female	Male	Total
N	180	289	469	144	208	352
Mean	−0.077	−0.053	−0.062	−0.744	−0.665	−0.691
Std.err.	0.065	0.048	0.039	0.023	0.019	0.015
Median	−0.347	−0.288	−0.312	−0.762	−0.695	−0.718
95% CI	−0.205 0.051	−0.148 0.041	−0.138 0.013	−0.789 −0.698	−0.703 −0.627	−0.727 −0.668
p value (sex difference)	0.77			**0.0099**		
mRNA vs. protein (linear regression)	Female: coefficient: 0.10 ± 0.03, $p = 0.003$ Male: coefficient: 0.11 ± 0.02, $p < 0.0001$ All: coefficient: 0.11 ± 0.017, $p < 0.0001$					

We then investigated whether tumor mRNA and protein levels of AR are positively associated. In fact, a linear regression model between AR protein and log-transformed RSEM data showed significant positive association of AR at mRNA and protein level ($p < 0.0001$ for all samples together, $p = 0.003$ for females and $p < 0.0001$ for males) (Table 2, Figure S1).

3.2. Tumor AR Protein Levels Are Positively Associated with Patient Overall Survival

The sex difference in survival is well known for melanoma. In order to examine whether AR plays a role in such sex difference, melanoma patients are grouped by their tumor AR protein levels. "AR-high" group of patients have tumor AR levels greater than median AR (−0.718) for the entire cohort, while "AR-low" group of patients have tumor AR levels lower than the median AR. Our initial Kaplan–Meier survival analysis suggested that higher AR levels were associated with better OS (Figure 1), and this result seemed true for all patients (log rank test $p = 0.0025$), for male patients ($p = 0.046$), or for female patients ($p = 0.0107$) (Figure 1a–c). Cox regression analysis (simple variate analysis) further revealed that higher AR levels were significantly associated with better OS in females ($p = 0.012$) or for all patients ($p = 0.003$), and the significance level was reduced to 0.047 (p value) in male patients (Table 3). Cox proportional assumption was tested based on the Schoenfeld residues, and a p value of 0.24 was returned, indicating Cox analysis was a proper method for survival analysis for this dataset, which is consistent with our previous report [24]. In this dataset, sex alone was not a significant determinant for overall survival (Cox regression HR = 1.14, $p = 0.39$) (Table A1).

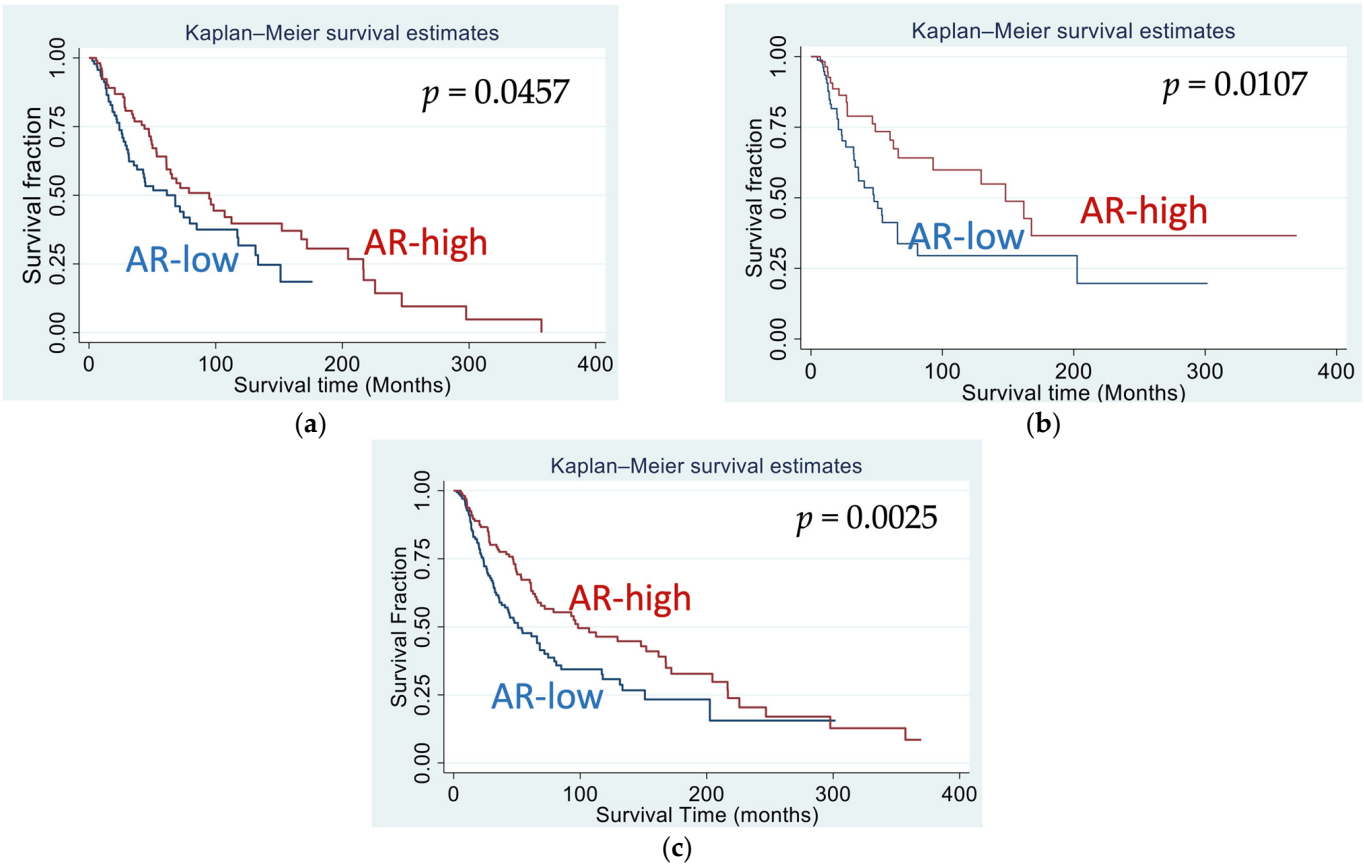

Figure 1. Kaplan–Meier survival curves for patients with high and low AR protein levels. (**a**) For both male and female patients; (**b**) for female patients; (**c**) for male patients (*p* values are derived from log rank test).

Table 3. AR is significantly associated with overall survival in melanoma patients.

Analysis	Patients/Model	HR	95% CI		*p* Value *	Variable(s) Included
Simple variate	Female	0.49	0.28	0.86	0.012	AR
	Male	0.66	0.44	0.99	0.047	AR
	All	0.61	0.44	0.84	0.003	AR
Multivariate	Model 1	0.65	0.47	0.90	0.009	AR, sex, age
	Model 2	0.67	0.48	0.95	0.025	AR, sex, age, stage (0–4)
	Model 3	0.68	0.48	0.96	0.029	AR, sex, age, stage (early, late)
	Model 4	0.80	0.54	1.20	0.29	AR, sex, age, stage (early, late), ulceration
	Model 5	0.59	0.40	0.87	0.008	AR, sex, age, stage (early, late), Breslow depth (4 category)

*, *p* value: for AR.

In the multivariate analysis, sex was used as a co-variable, and the AR association with overall survival was additionally adjusted by age of diagnosis, stage of disease (either stage 0 to stage 4 or early and late stages, as described in Section 2) (Table 3, Model 1–3). The association of AR with overall survival stayed significant after adjusting to these factors. When the presence of ulceration was added in the multivariate analysis, the association lost its significance (HR = 0.80, $p = 0.29$) (Table 3, Model 4). Interestingly, ulceration alone was significantly associated with overall survival (HR = 1.8, $p = 0.001$), and the significance remained after adjusting to age of diagnosis, stage of disease, and sex (HR = 1.46, $p = 0.04$).

These results may suggest an impact of AR on the ulceration status. However, tumors with or without ulceration did not show significant difference in the AR protein levels ($p = 0.23$ in Student *t*-test).

Another important prognostic factor for melanoma survival is Breslow depth. We grouped Breslow depth according to the AJCC TNM staging standards (1 = <1 mm; 2 = 1.01–2 mm; 3 = 2.01–4 mm; 4 = >4 mm) and included this variable in our multivariate COX analysis. The AR association with overall survival remained significant when the result was adjusted to Breslow depth, along with other factors (HR = 0.59, $p = 0.008$) (Table 3, Model 5).

3.3. The Sex Difference in the AR Association with OS

Table 4 shows that the AR protein level is not significantly associated with overall survival in men, even though the high AR is significantly associated with overall survival in women. Sex was then used as a stratification variable and the multivariate Cox models in male and female patients were analyzed separately, with age, stage, ulceration status, and Breslow depth as adjusting co-variables. AR levels were not associated with men's OS in any of the models, but they are associated with women's overall survival in all models except for Model 4 where ulceration was justified.

Table 4. AR protein level for survival between female and male sexes.

		Female				Male			
	Variables	**HR**	**[95% Conf. Interval]**		***p* Value**	**HR**	**[95% Conf. Interval]**		***p* Value**
Model 1	AR	0.51	0.29	0.90	**0.021**	0.70	0.47	1.06	0.092
	age	1.03	1.02	1.05	0	1.02	1.01	1.04	0.003
Model 2	AR	0.49	0.27	0.89	**0.02**	0.80	0.52	1.24	0.328
	age	1.03	1.01	1.05	0.001	1.02	1.00	1.03	0.028
	stage (0–4)	1.27	0.91	1.79	0.165	1.46	1.14	1.87	0.003
Model 3	AR	0.48	0.26	0.87	**0.016**	0.84	0.54	1.31	0.443
	age	1.03	1.01	1.05	0	1.02	1.00	1.03	0.017
	stage (early, late)	1.36	0.77	2.42	0.289	1.96	1.26	3.08	0.003
Model 4	AR	0.58	0.28	1.19	0.135	1.02	0.60	1.73	0.951
	age	1.03	1.01	1.05	0.009	1.02	1.00	1.04	0.106
	Stage (early, late)	1.50	0.77	2.91	0.23	2.16	1.26	3.71	0.005
	ulceration	1.28	0.64	2.58	0.489	1.60	0.91	2.81	0.1
Model 5	AR	0.49	0.25	0.96	**0.039**	0.65	0.39	1.09	0.102
	age	1.03	1.01	1.05	0.004	1.02	1.00	1.04	0.088
	Stage (early, late)	1.58	0.86	2.90	0.143	1.38	0.79	2.41	0.258
	Breslow Depth	1.27	0.92	1.74	0.149	1.71	1.28	2.28	0

Since testosterone levels are known to change with men's age, we also examined whether AR levels in tumors were different in older versus younger patients ($\leq$50 vs. >50 years). A Student *t*-test was used to evaluate the AR protein levels, and no difference in means was found ($p = 0.89$ for men and 0.13 for women).

3.4. The Differential AR Gene Network in Tumors from Men and Women

In order to understand how AR expression levels are associated with patient overall survival in women but not in men, the TGCA SKCM mRNA data were used to extract the AR co-expressed genes using the online tool from the cBioportal website. The entire genome was included and the co-expressed genes were identified using a cutoff q value of $q < 0.05$. 6413 genes from men and 3384 genes from women were retained for further comparison. When the Spearman's co-efficient for AR-association was set at $\rho > 0.34$, then 75 genes in women and 202 genes in men were retained for further comparison. Among these genes, 44 were unique for women, 171 were unique for men (Table A2), and 31 were shared by tumors from both sexes (Table A3, Figure S2). The 10 most significant genes for each sex are included in Table 5.

Table 5. Top 10 most significant sex-specific AR co-expressed genes in tumors.

Gene	Spearman's Coefficient	*p* Value	q Value	Sex	Approved Gene Name	HGNC ID	Location
KMT2A	0.42	4.3×10^{-9}	0.000025	F	lysine methyltransferase 2A	HGNC:7132	11q23.3
NECTIN3	0.41	8.8×10^{-9}	0.000025	F	nectin cell adhesion molecule 3	HGNC:17664	3q13.13
ROR1	0.41	1.4×10^{-8}	0.000029	F	receptor tyrosine kinase like orphan receptor 1	HGNC:10256	1p31.3
MACF1	0.41	1.6×10^{-8}	0.000029	F	microtubule actin crosslinking factor 1	HGNC:13664	1p34.3
CBL	0.39	5.5×10^{-8}	0.000065	F	Cbl proto-oncogene	HGNC:1541	11q23.3
AKAP2	0.39	7×10^{-8}	0.000078	F	A-kinase anchoring protein 2	HGNC:372	9q31.3
KERA	0.39	7.5×10^{-8}	0.00008	F	keratocan	HGNC:6309	12q21.33
PRDM10	0.39	8.3×10^{-8}	0.000082	F	PR/SET domain 10	HGNC:13995	11q24.3
MAML2	0.38	9.9×10^{-8}	0.00009	F	mastermind like transcriptional coactivator 2	HGNC:16259	11q21
ZFP91	0.38	1×10^{-7}	0.00009	F	ZFP91 zinc finger protein, atypical E3 ubiquitin ligase	HGNC:14983	11q12.1
SLIT2	0.49	7.6×10^{-19}	7.7×10^{-15}	M	slit guidance ligand 2	HGNC:11086	4p15.31
ITGA8	0.45	1.1×10^{-15}	4.5×10^{-12}	M	integrin subunit α 8	HGNC:6144	10p13
PREX2	0.45	1.1×10^{-15}	4.5×10^{-12}	M	phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 2	HGNC:22950	8q13.2
MARCHF8	0.43	1×10^{-14}	2.4×10^{-11}	M	membrane associated ring-CH-type finger 8	HGNC:23356	10q11.21-q11.22
RALGAPA2	0.43	1.3×10^{-14}	2.6×10^{-11}	M	Ral GTPase activating protein catalytic subunit α 2	HGNC:16207	20p11.23
ZDHHC15	0.43	2.5×10^{-14}	4.2×10^{-11}	M	zinc finger DHHC-type palmitoyltransferase 15	HGNC:20342	Xq13.3
IL6ST	0.42	4×10^{-14}	6×10^{-11}	M	interleukin 6 cytokine family signal transducer	HGNC:6021	5q11.2
PCSK5	0.42	8.5×10^{-14}	1×10^{-10}	M	proprotein convertase subtilisin/kexin type 5	HGNC:8747	9q21.13
MAN1A1	0.42	9.3×10^{-14}	1×10^{-10}	M	mannosidase α class 1A member 1	HGNC:6821	6q22.31
ASXL3	0.42	1.2×10^{-13}	1.3×10^{-10}	M	ASXL transcriptional regulator 3	HGNC:29357	18q12.1

The 44 and 171 genes identified in the female and male tumors, respectively, are subjected to enrichment analysis using an integrated web-based tool termed g:profiler (https://biit.cs.ut.ee/gprofiler/gost, accessed on 14 June 2022). Genes were ordered All significant enrichments for females and partial of that for males are listed in Table 6. For female tumors, AR is significantly associated with GO:MF (molecular function), GO:CC (cellular component), and TF (transcription factor) functions. For male tumors, AR is significantly associated with a wide range of functions, including 99 GO:BP (biological process), 19 GO:CC, 10 GO:MF, 8 TF, 1 Reactome (Neurophilin interactions with VEGF and VEGFR), and 4 WP (Wikipathways). Only the top three significant functions in each category are shown in the table.

The shared 31 genes in male and female tumors were used for the same profiling analysis, 18 enriched functions were identified, which are listed in Table A4.

Table 6. The sex-specific AR-associated enrichment of gene function.

Sex	Source	Term_Id	Adjusted_*p*_Value	Term_ Size	Query_ Size	Intersection_ Size	Term_Name
Female	GO:MF	GO:0042800	0.028832	18	14	2	histone methyltransferase activity (H3-K4 specific)
	GO:MF	GO:0106363	0.042024	2	1	1	protein-cysteine methyltransferase activity
	GO:CC	GO:0043296	0.009441	154	27	4	apical junction complex
	TF	TF:M09984_1	0.007033	5696	43	27	Factor: MAZ; motif: GGGGGAGGGGGNGRGRRRGNRG; match class: 1
	TF	TF:M12654_1	0.032391	44	3	2	Factor: PRDM15; motif: NYCCRNTCCRGGTTTTSC; match class: 1
	TF	TF:M09834_1	0.032799	2950	39	17	Factor: ZNF148; motif: NNNNNNCCNNCCCCTCCCCCACCCN; match class: 1
Male	GO:MF	GO:0046872	5.32×10^{-6}	4271	131	57	metal ion binding
	GO:MF	GO:0005509	5.9×10^{-6}	726	130	21	calcium ion binding
	GO:MF	GO:0043169	1.2×10^{-5}	4364	131	57	cation binding
	GO:BP	GO:0048731	1.65×10^{-11}	4369	163	78	system development
	GO:BP	GO:0048856	6.64×10^{-11}	5836	163	91	anatomical structure development
	GO:BP	GO:0007155	2.2×10^{-10}	1521	167	43	cell adhesion
	GO:CC	GO:0005887	3.25×10^{-10}	1649	156	41	integral component of plasma membrane
	GO:CC	GO:0031226	3.56×10^{-10}	1731	156	42	intrinsic component of plasma membrane
	GO:CC	GO:0071944	3.05×10^{-8}	6270	160	85	cell periphery
	REAC	REAC:R-HSA-194306	0.005273	4	15	2	Neurophilin interactions with VEGF and VEGFR
	WP	WP:WP4823	0.004028	44	11	3	Genes controlling nephrogenesis
	WP	WP:WP3943	0.004081	6	11	2	Robo4 and VEGF signaling pathways crosstalk
	WP	WP:WP5065	0.005193	5	15	2	SARS-CoV-2 altering angiogenesis via NRP1
	TF	TF:M00695_1	3.82×10^{-8}	7194	169	104	Factor: ETF; motif: GVGGMGG; match class: 1
	TF	TF:M12345_1	0.00052	1735	74	22	Factor: Zbtb37; motif: NYACCGCRNTCACCGCR; match class: 1
	TF	TF:M01199	0.002376	8683	169	105	Factor: RNF96; motif: BCCCGCRGCC

3.5. The Role of AR in Overall Survival in Four Melanoma Subtypes

The TCGA melanoma team classified this cohort of patients into four distinct subtypes with distinct somatic mutations in the tumors [25]. We obtained the classification information at patient level from their supplemental tables. A total of 316 patients were included in the analysis, but due to some tumors lacking AR protein data, only 230 patients were included in the survival analysis. Very interestingly, only in the RAS (mainly NRAS, but also including several mutants in KRAS and HRAS) mutants, did AR show significant association with overall survival (p = 0.013) (Table 7). The significance remained after adjusting to age of diagnosis and stage of disease (p = 0.047). When only sex is adjusted, the significant also remained (p = 0.022), but it was reduced to borderline (p = 0.057) when both sex and age are included.

Table 7. Role of AR in overall survival in four subtypes of melanoma.

	HR	[95% Conf. Interval]		*p* Value	N **
BRAF_Hotspot_Mutants	0.62	0.20	1.15	0.336	106
RAS_Hotspot_Mutants	0.44	0.23	0.84	**0.013** *	67
NF1_Any_Mutants	0.73	0.26	2.04	0.551	25
Triple_WT	0.97	0.39	2.45	0.950	32

* p value for RAS subtype = 0.047 after adjusting to age and stage of patients. ** N: number of patients in each subtype included in survival analysis.

4. Discussion

The findings of this study suggest that a higher level of tumor AR protein is positively associated with a better overall survival in cutaneous melanoma patients, which remains true after adjusting to age of diagnosis, stage of disease, sex of patients, and Breslow depth of the tumors. However, when patients are stratified by sex, the significant association was found only in female patients, but not in male patients, even though sex itself is not significantly associated with overall survival in this dataset. Additionally, when ulceration status is included in the model, the significance of AR association with OS was lost, suggesting that ulceration is still the most effective prognostic factor for melanoma OS. A statistical test of an interaction of AR with ulceration status revealed only borderline significance ($p = 0.10$, not shown in results). Nevertheless, our finding is significant, as this is one of the first studies to show an association of tumor AR level with overall survival in melanoma patients.

A previous report suggested an opposite role of AR in melanoma patient survival, i.e., higher AR was associated with worse survival [4]. That report did not specify melanoma subtype. The samples were collected in China, while the melanoma subtype in China is different than that in US—Chinese melanoma cases are mostly acral melanoma, which are distinct in oncogenic causes and pathological pathways than the US cases, which are mostly superficial spreading melanoma [26,27].

For melanoma, similar to many other cancer types, females in general show a survival advantage even after adjusting to many other prognostic factors. The underlying mechanism may be multi-fold, and we have been interested in the roles of sex hormones in such situations. Sex hormones and their receptors play critical roles in many pathophysiological conditions and impact many oncogenic pathways and cellular functions. AR was recently studied in melanoma cells, with a function of promoting proliferation, tumorigenesis, metastasis, and drug resistance [2–4], which is opposite to our findings.

The possible explanation may be directly linked to the androgen levels as the majority function of AR is linked to locally available testosterone and dihydrotestosterone. Therefore, in most cases, we must study the function of AR/T or AR/DHT together. It is particularly important to study the sex-specific impact, as men and women are distinctly different in the circulating T or DHT levels. Our study also showed a distinct gene network in tumors from male and female patients, further strengthening the importance of sex-specific investigation. Another possible reason is related to how to interpret the data. In one study, loss of AR led to more DNA damage [2], suggesting that AR played a protective role in genome integrity. When this occurs in normal melanocytes, one would expect AR serves as a tumor suppressor, as it was found in a subset of breast cancer [28]. This is also what our study suggests.

It is also noticeable that AR plays distinct functions in the male and female tumors, with shared functions in both sexes. The enriched functions are much broader in male tumors, indicating male-biased significance of AR. Since AR is involved in many more gene networks in males, the ability of these functions to maintain a relative cellular balance may be strengthened, which may help to explain why AR in men did not show a significant association with overall survival.

The weakness of this study is that we used only the TCGA data, with no replicating dataset. Therefore, this study requires further validation in a different patient cohort. As noted in one of our previous study [24], the patient sex did not show a significant association with OS, which is not the usual case for melanoma patients. That is the limitation of the patient cohort as well, and requires further replication.

5. Conclusions

The overall conclusion of this study is that tumor AR protein levels are associated with better OS in female patients, and not in male patients. We have also identified shared and distinct AR-associated gene networks in male and female tumors, which suggests AR exhibits common function in all tumors, and also exhibits distinct function in tumors from

male and female patients. This study is the first to include data from a large database source with over 350 data points from melanoma patients' tumors. Most of previous studies of AR in melanoma suggested that AR promoted tumor proliferation, metastasis, and drug resistance. Our study suggests that the role of AR should be considered in sex-specific manner, and in females, AR could be protective. Further investigation on these shared and distinct functions of AR in melanoma patients will help us to develop precise treatment strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes14020345/s1, Figure S1: The RPPA protein levels (Y axis, normally distributed) were plotted against log-transformed AR mRNA (RSEM readings). The red line is regression fitting line. Figure S2. Venn diagram of the shared and distinct AR-co-expressed genes in male and female tumors.

Author Contributions: Conceptualization, F.L.-S. and T.S.P.; methodology, F.L.-S. and C.-Y.C.; validation, C.-Y.C.; formal analysis, F.L.-S., J.L. and C.-Y.C.; investigation, N.S. and J.K. resources, F.L.-S.; data curation, F.L.-S.; writing—original draft preparation, N.S. and J.K.; writing—review and editing, F.L.-S. and T.S.P.; visualization, F.L.-S.; supervision, F.L.-S. and T.S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data used in this study are publicly available; the sites are listed in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Sex difference in overall survival.

	Variables	HR	95%	Conf.	*p* Value
Model 1	AR	0.65	0.47	0.90	0.009
	age	1.03	1.02	1.04	0
	sex	1.12	0.81	1.56	0.495
Model 2	AR	0.67	0.48	0.95	0.025
	age	1.02	1.01	1.04	0
	sex	0.95	0.67	1.34	0.763
	stage (0–4)	1.39	1.14	1.70	0.001
Model 3	AR	0.68	0.48	0.96	0.029
	age	1.03	1.01	1.04	0
	sex	0.94	0.66	1.33	0.733
	stage (early, late)	1.69	1.19	2.40	0.003
Model 4	AR	0.80	0.54	1.20	0.286
	age	1.02	1.01	1.04	0.001
	sex	0.88	0.58	1.32	0.536
	stage (early, late)	1.84	1.21	2.79	0.004
	ulceration	1.44	0.93	2.22	0.101
Model 5	AR	0.59	0.40	0.87	0.008
	age	1.02	1.01	1.04	0.001
	sex	0.90	0.61	1.34	0.615
	stage (early, late)	1.51	1.01	2.25	0.044
	Breslow depth	1.50	1.22	1.85	0

Table A2. Sex-specific AR co-expressed genes in tumors.

Gene	Spearman's Coefficient	*p* Value	q Value	Sex	Approved Gene Name	HGNC ID	Cytoband
USP47	0.35	1.50×10^{-6}	0.000403	F	ubiquitin specific peptidase 47	HGNC:20076	11p15.3
CTR9	0.32	8.70×10^{-6}	0.000864	F	CTR9 homolog, Paf1/RNA polymerase II complex component	HGNC:16850	11p15.4
THAP12	0.32	1.20×10^{-5}	0.001011	F	THAP domain containing 12	HGNC:9440	11q13.5
ZNF143	0.32	1.60×10^{-5}	0.001217	F	zinc finger protein 143	HGNC:12928	11p15.4
COPB1	0.31	1.70×10^{-5}	0.001233	F	COPI coat complex subunit β 1	HGNC:2231	11p15.2
ASB12	0.31	0.00002	0.001376	F	ankyrin repeat and SOCS box containing 12	HGNC:19763	Xq11.2
THAP7-AS1	−0.31	2.10×10^{-5}	0.001378	F	THAP7 antisense RNA 1	HGNC:41013	22q11.21
NR2F1-AS1	0.31	2.20×10^{-5}	0.001426	F	NR2F1 antisense RNA 1	HGNC:48622	5q15
MED17	0.31	2.70×10^{-5}	0.00163	F	mediator complex subunit 17	HGNC:2375	11q21
TOM1	−0.3	3.30×10^{-5}	0.001835	F	target of myb1 membrane trafficking protein	HGNC:11982	22q12.3
C11ORF95/ ZFTA	0.3	3.50×10^{-5}	0.001909	F	zinc finger translocation associated	HGNC:28449	11q13.1
HECTD2-AS1	−0.3	0.00004	0.002025	F	HECTD2 antisense RNA 1	HGNC:48679	10q23.32
NDUFB8	−0.3	4.20×10^{-5}	0.002074	F	NADH:ubiquinone oxidoreductase subunit B8	HGNC:7703	10q24.31
MARCH8/ MARCHF8	0.43	1.00×10^{-14}	2.40×10^{-11}	M	membrane associated ring-CH-type finger 8	HGNC:23356	10q11.21-q11.22
RNF152	0.39	3.80×10^{-12}	2.00×10^{-9}	M	ring finger protein 152	HGNC:26811	18q21.33
GDF10	0.38	2.00×10^{-11}	6.30×10^{-9}	M	growth differentiation factor 10	HGNC:4215	10q11.22
PLXNA4	0.37	4.90×10^{-11}	1.20×10^{-8}	M	plexin A4	HGNC:9102	7q32.3
ZNHIT2	−0.36	1.40×10^{-10}	2.50×10^{-8}	M	zinc finger HIT-type containing 2	HGNC:1177	11q13.1
ZC4H2	0.36	2.50×10^{-10}	4.00×10^{-8}	M	zinc finger C4H2-type containing	HGNC:24931	Xq11.2
NDST2	0.35	5.70×10^{-10}	7.50×10^{-8}	M	N-deacetylase and N-sulfotransferase 2	HGNC:7681	10q22.2
TCHH	0.35	6.90×10^{-10}	8.60×10^{-8}	M	trichohyalin	HGNC:11791	1q21.3
PCDHB6	0.35	9.70×10^{-10}	1.10×10^{-7}	M	protocadherin β 6	HGNC:8691	5q31.3
LBHD1	−0.35	1.10×10^{-9}	1.20×10^{-7}	M	LBH domain containing 1	HGNC:28351	11q12.3
SHISA6	0.35	1.10×10^{-9}	1.20×10^{-7}	M	shisa family member 6	HGNC:34491	17p12
LRRC55	0.35	1.20×10^{-9}	1.30×10^{-7}	M	leucine rich repeat containing 55	HGNC:32324	11q12.1
TIMM10	−0.35	1.20×10^{-9}	1.30×10^{-7}	M	translocase of inner mitochondrial membrane 10	HGNC:11814	11q12.1
PTPRD	0.34	2.30×10^{-9}	2.10×10^{-7}	M	protein tyrosine phosphatase receptor type D	HGNC:9668	9p24.1-p23
CDH23	0.34	2.70×10^{-9}	2.40×10^{-7}	M	cadherin related 23	HGNC:13733	10q22.1
CDH8	0.34	2.80×10^{-9}	2.40×10^{-7}	M	cadherin 8	HGNC:1767	16q21
DPYSL2	0.34	2.90×10^{-9}	2.50×10^{-7}	M	dihydropyrimidinase like 2	HGNC:3014	8p21.2
PCDHB18P	0.34	3.10×10^{-9}	2.60×10^{-7}	M	protocadherin β 18 pseudogene	HGNC:14548	5q31.3
CDC37	−0.34	3.20×10^{-9}	2.60×10^{-7}	M	cell division cycle 37, HSP90 cochaperone	HGNC:1735	19p13.2
SSTR1	0.34	3.40×10^{-9}	2.80×10^{-7}	M	somatostatin receptor 1	HGNC:11330	14q21.1
PCDHB10	0.34	3.70×10^{-9}	3.00×10^{-7}	M	protocadherin β 10	HGNC:8681	5q31.3
EDNRA	0.34	4.10×10^{-9}	3.30×10^{-7}	M	endothelin receptor type A	HGNC:3179	4q31.22-q31.23
EPB41L4A-DT	0.33	5.10×10^{-9}	3.80×10^{-7}	M	EPB41L4A divergent transcript	HGNC:25643	5q22.2
BACH2	0.33	5.80×10^{-9}	4.10×10^{-7}	M	BTB domain and CNC homolog 2	HGNC:14078	6q15
SEC24A	0.33	5.80×10^{-9}	4.10×10^{-7}	M	SEC24 homolog A, COPII coat complex component	HGNC:10703	5q31.1

Table A2. *Cont.*

Gene	Spearman's Coefficient	*p* Value	q Value	Sex	Approved Gene Name	HGNC ID	Cytoband
ZNF423	0.33	5.90×10^{-9}	4.10×10^{-7}	M	zinc finger protein 423	HGNC:16762	16q12.1
STAT3	0.33	7.00×10^{-9}	4.90×10^{-7}	M	signal transducer and activator of transcription 3	HGNC:11364	17q21.2
C12ORF45	−0.33	7.10×10^{-9}	4.90×10^{-7}	M	NOP protein chaperone 1	HGNC:28628	12q23.3
GALNT17	0.33	7.30×10^{-9}	5.00×10^{-7}	M	polypeptide N-acetylgalactosaminyltransferase 17	HGNC:16347	7q11.22
OLFML2B	0.33	7.50×10^{-9}	5.10×10^{-7}	M	olfactomedin like 2B	HGNC:24558	1q23.3
TMEM69	−0.33	8.10×10^{-9}	5.40×10^{-7}	M	transmembrane protein 69	HGNC:28035	1p34.1
MRPL16	−0.33	9.60×10^{-9}	6.20×10^{-7}	M	mitochondrial ribosomal protein L16	HGNC:14476	11q12.1
CYP4X1	0.33	1.00×10^{-8}	6.50×10^{-7}	M	cytochrome P450 family 4 subfamily X member 1	HGNC:20244	1p33|1
EPHB1	0.33	1.00×10^{-8}	6.40×10^{-7}	M	EPH receptor B1	HGNC:3392	3q22.2
METTL17	−0.33	1.00×10^{-8}	6.40×10^{-7}	M	methyltransferase like 17	HGNC:19280	14q11.2
SYT15	0.33	1.00×10^{-8}	6.50×10^{-7}	M	synaptotagmin 15	HGNC:17167	10q11.22
LMOD1	0.33	1.10×10^{-8}	6.70×10^{-7}	M	leiomodin 1	HGNC:6647	1q32.1
PCDHGA5	0.33	1.30×10^{-8}	7.60×10^{-7}	M	protocadherin γ subfamily A, 5	HGNC:8703	5q31.3
SUCNR1	0.33	1.30×10^{-8}	7.60×10^{-7}	M	succinate receptor 1	HGNC:4542	3q25.1
MTMR12	0.32	1.50×10^{-8}	8.50×10^{-7}	M	myotubularin related protein 12	HGNC:18191	5p13.3
FGF10	0.32	1.60×10^{-8}	9.20×10^{-7}	M	fibroblast growth factor 10	HGNC:3666	5p12
NLGN4Y	0.32	1.60×10^{-8}	9.00×10^{-7}	M	neuroligin 4 Y-linked	HGNC:15529	Yq11.221
PENK	0.32	1.60×10^{-8}	9.10×10^{-7}	M	proenkephalin	HGNC:8831	8q12.1
TMEM130	0.32	1.60×10^{-8}	9.20×10^{-7}	M	transmembrane protein 130	HGNC:25429	7q22.1
ZNF778	0.32	1.60×10^{-8}	9.10×10^{-7}	M	zinc finger protein 778	HGNC:26479	16q24.3
PBX1	0.32	1.80×10^{-8}	0.000001	M	PBX homeobox 1	HGNC:8632	1q23.3
COA4	−0.32	1.90×10^{-8}	1.10×10^{-6}	M	cytochrome c oxidase assembly factor 4 homolog	HGNC:24604	11q13.4
NPNT	0.32	1.90×10^{-8}	1.10×10^{-6}	M	nephronectin	HGNC:27405	4q24
GPR20	0.32	2.10×10^{-8}	1.10×10^{-6}	M	G protein-coupled receptor 20	HGNC:4475	8q24.3
TSPAN18	0.32	2.10×10^{-8}	1.10×10^{-6}	M	tetraspanin 18	HGNC:20660	11p11.2
SLITRK4	0.32	2.20×10^{-8}	1.20×10^{-6}	M	SLIT and NTRK like family member 4	HGNC:23502	Xq27.3
UQCR11	−0.32	2.30×10^{-8}	1.20×10^{-6}	M	ubiquinol-cytochrome c reductase, complex III subunit XI	HGNC:30862	19p13.3
NFATC3	0.32	2.50×10^{-8}	1.30×10^{-6}	M	nuclear factor of activated T cells 3	HGNC:7777	16q22.1
TRMT112	−0.32	2.50×10^{-8}	1.30×10^{-6}	M	tRNA methyltransferase activator subunit 11-2	HGNC:26940	11q13.1
EPHA7	0.32	3.50×10^{-8}	1.70×10^{-6}	M	EPH receptor A7	HGNC:3390	6q16.1
ATP8B1	0.32	3.60×10^{-8}	1.80×10^{-6}	M	ATPase phospholipid transporting 8B1	HGNC:3706	18q21.31
FLT4	0.32	3.60×10^{-8}	1.80×10^{-6}	M	fms related receptor tyrosine kinase 4	HGNC:3767	5q35.3
PTAFR	0.31	3.80×10^{-8}	1.90×10^{-6}	M	platelet activating factor receptor	HGNC:9582	1p35.3
MAGED4B	0.31	4.30×10^{-8}	0.000002	M	MAGE family member D4B	HGNC:22880	Xp11.22
NAP1L2	0.31	4.30×10^{-8}	0.000002	M	nucleosome assembly protein 1 like 2	HGNC:7638	Xq13.2
NEURL1B	0.31	4.70×10^{-8}	2.20×10^{-6}	M	neuralized E3 ubiquitin protein ligase 1B	HGNC:35422	5q35.1
TMEM132E	0.31	5.20×10^{-8}	2.40×10^{-6}	M	transmembrane protein 132E	HGNC:26991	17q12
MRPL21	−0.31	5.60×10^{-8}	2.50×10^{-6}	M	mitochondrial ribosomal protein L21	HGNC:14479	11q13.3
SAP30L	0.31	5.70×10^{-8}	2.50×10^{-6}	M	SAP30 like	HGNC:25663	5q33.2
ILDR2	0.31	6.40×10^{-8}	2.80×10^{-6}	M	immunoglobulin like domain containing receptor 2	HGNC:18131	1q24.1

Table A2. *Cont.*

Gene	Spearman's Coefficient	*p* Value	q Value	Sex	Approved Gene Name	HGNC ID	Cytoband
SLCO3A1	0.31	7.00×10^{-8}	0.000003	M	solute carrier organic anion transporter family member 3A1	HGNC:10952	15q26.1
LAMTOR5	−0.31	7.10×10^{-8}	0.000003	M	late endosomal/lysosomal adaptor, MAPK and MTOR activator 5	HGNC:17955	1p13.3
MMRN2	0.31	7.10×10^{-8}	0.000003	M	multimerin 2	HGNC:19888	10q23.2
ANK1	0.31	7.40×10^{-8}	3.10×10^{-6}	M	ankyrin 1	HGNC:492	8p11.21
UQCC3	−0.31	7.60×10^{-8}	3.20×10^{-6}	M	ubiquinol-cytochrome c reductase complex assembly factor 3	HGNC:34399	11q12.3
OTULIN	0.31	7.70×10^{-8}	3.20×10^{-6}	M	OTU deubiquitinase with linear linkage specificity	HGNC:25118	5p15.2
UNC5C	0.31	7.70×10^{-8}	3.20×10^{-6}	M	unc-5 netrin receptor C	HGNC:12569	4q22.3
SCN2B	0.31	7.90×10^{-8}	3.20×10^{-6}	M	sodium voltage-gated channel β subunit 2	HGNC:10589	11q23.3
CASTOR2	0.31	8.20×10^{-8}	3.30×10^{-6}	M	cytosolic arginine sensor for mTORC1 subunit 2	HGNC:37073	7q11.23
ARHGAP44	0.31	8.60×10^{-8}	3.50×10^{-6}	M	Rho GTPase activating protein 44	HGNC:29096	17p12
COX8A	−0.31	8.70×10^{-8}	3.50×10^{-6}	M	cytochrome c oxidase subunit 8A	HGNC:2294	11q13.1
FOXJ2	0.31	9.90×10^{-8}	3.90×10^{-6}	M	forkhead box J2	HGNC:24818	12p13.31
ATP1A2	0.31	1.00×10^{-7}	3.90×10^{-6}	M	ATPase Na+/K+ transporting subunit α 2	HGNC:800	1q23.2
ZBTB4	0.31	1.00×10^{-7}	0.000004	M	zinc finger and BTB domain containing 4	HGNC:23847	17p13.1
SEZ6L	0.3	1.20×10^{-7}	4.50×10^{-6}	M	seizure related 6 homolog like	HGNC:10763	22q12.1
SATB1	0.3	1.30×10^{-7}	4.90×10^{-6}	M	SATB homeobox 1	HGNC:10541	3p24.3
PCDHA9	0.3	1.40×10^{-7}	5.10×10^{-6}	M	protocadherin α 9	HGNC:8675	5q31.3
SCN3B	0.3	1.40×10^{-7}	4.90×10^{-6}	M	sodium voltage-gated channel β subunit 3	HGNC:20665	11q24.1
TMEM223	−0.3	1.40×10^{-7}	0.000005	M	transmembrane protein 223	HGNC:28464	11q12.3
ABCA8	0.3	1.50×10^{-7}	5.20×10^{-6}	M	ATP binding cassette subfamily A member 8	HGNC:38	17q24.2
PCDH12	0.3	1.50×10^{-7}	5.20×10^{-6}	M	protocadherin 12	HGNC:8657	5q31.3
ZNF436	0.3	1.50×10^{-7}	5.20×10^{-6}	M	zinc finger protein 436	HGNC:20814	1p36.12
RELN	0.3	1.60×10^{-7}	5.60×10^{-6}	M	reelin	HGNC:9957	7q22.1
PGM5	0.3	1.70×10^{-7}	5.80×10^{-6}	M	phosphoglucomutase 5	HGNC:8908	9q21.11
SLC25A22	−0.3	1.70×10^{-7}	5.70×10^{-6}	M	solute carrier family 25 member 22	HGNC:19954	11p15.5

Table A3. AR-co-expressed genes in tumors in both sexes.

Gene	Spearman's_ Men	p_Men	q_Men	Spearman_ Women	p_Women	q_Women	Approved Name	HGNC ID	Location
NHSL2	0.53	1.2×10^{-22}	2.4×10^{-18}	0.381265	1.3×10^{-7}	0.000108	NHS like 2	HGNC:33737	Xq13.1
ADAMTS12	0.48	6.7×10^{-18}	4.5×10^{-14}	0.41931	4.7×10^{-9}	0.000025	ADAM metallopeptidase with thrombospondin type 1 motif 12	HGNC:14605	5p13.3-p13.2
RUNX1T1	0.44	1.7×10^{-15}	5.8×10^{-12}	0.392157	5.2×10^{-8}	0.000065	RUNX1 partner transcriptional co-repressor 1	HGNC:1535	8q21.3
ZNF366	0.44	2.8×10^{-15}	8×10^{-12}	0.344886	2.1×10^{-6}	0.000458	zinc finger protein 366	HGNC:18316	5q13.1
FBN1	0.43	1.1×10^{-14}	2.4×10^{-11}	0.414372	7.3×10^{-9}	0.000025	fibrillin 1	HGNC:3603	15q21.1
LAMA2	0.43	2.4×10^{-14}	4.2×10^{-11}	0.370503	3.1×10^{-7}	0.000181	laminin subunit α 2	HGNC:6482	6q22.33
CDKL5	0.42	4.2×10^{-14}	6×10^{-11}	0.340764	2.9×10^{-6}	0.000514	cyclin dependent kinase like 5	HGNC:11411	Xp22.13

Table A3. *Cont.*

Gene	Spearman's_ Men	p_Men	q_Men	Spearman_ Women	p_Women	q_Women	Approved Name	HGNC ID	Location
MAN2A1	0.42	8.3×10^{-14}	1×10^{-10}	0.35174	1.3×10^{-6}	0.000378	mannosidase α class 2A member 1	HGNC:6824	5q21.3
LTBP2	0.42	8.5×10^{-14}	1×10^{-10}	0.352113	1.3×10^{-6}	0.000378	latent transforming growth factor β binding protein 2	HGNC:6715	14q24.3
TSHZ2	0.41	2.2×10^{-13}	2×10^{-10}	0.34521	2.1×10^{-6}	0.000458	teashirt zinc finger homeobox 2	HGNC:13010	20q13.2
PPM1L	0.41	3×10^{-13}	2.5×10^{-10}	0.349059	1.6×10^{-6}	0.000416	protein phosphatase, Mg2+/Mn2+ dependent 1L	HGNC:16381	3q25.33-q26.1
REST	0.41	3.9×10^{-13}	3×10^{-10}	0.359102	7.4×10^{-7}	0.000303	RE1 silencing transcription factor	HGNC:9966	4q12
SVEP1	0.39	2.9×10^{-12}	1.7×10^{-9}	0.415756	6.5×10^{-9}	0.000025	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	HGNC:15985	9q31.3
SON	0.39	6.4×10^{-12}	3×10^{-9}	0.343758	2.3×10^{-6}	0.00047	SON DNA and RNA binding protein	HGNC:11183	21q22.11
JCAD	0.39	8.3×10^{-12}	3.4×10^{-9}	0.34332	2.4×10^{-6}	0.000472	junctional cadherin 5 associated	HGNC:29283	10p11.23
SLIT3	0.39	8.6×10^{-12}	3.5×10^{-9}	0.341709	2.7×10^{-6}	0.000496	slit guidance ligand 3	HGNC:11087	5q34-q35.1
PEAK1	0.38	1.4×10^{-11}	5.1×10^{-9}	0.363921	5.1×10^{-7}	0.000249	pseudopodium enriched atypical kinase 1	HGNC:29431	15q24.3
TMEM200A	0.38	1.4×10^{-11}	5.1×10^{-9}	0.368847	3.5×10^{-7}	0.000194	transmembrane protein 200A	HGNC:21075	6q23.1
SLC12A6	0.38	1.7×10^{-11}	5.7×10^{-9}	0.372362	2.6×10^{-7}	0.000173	solute carrier family 12 member 6	HGNC:10914	15q14
PGR	0.38	2.2×10^{-11}	6.6×10^{-9}	0.352875	1.2×10^{-6}	0.000377	progesterone receptor	HGNC:8910	11q22.1
CCDC80	0.38	3.5×10^{-11}	9.1×10^{-9}	0.407269	1.4×10^{-8}	0.000029	coiled-coil domain containing 80	HGNC:30649	3q13.2
AKAP13	0.37	4.3×10^{-11}	1.1×10^{-8}	0.347455	1.8×10^{-6}	0.000437	A-kinase anchoring protein 13	HGNC:371	15q25.3
SELENOP	0.37	5.6×10^{-11}	1.3×10^{-8}	0.357733	8.2×10^{-7}	0.000311	selenoprotein P	HGNC:10751	5p12
KAT6A	0.36	2.6×10^{-10}	4×10^{-8}	0.344176	2.2×10^{-6}	0.000468	lysine acetyltransferase 6A	HGNC:13013	8p11.21
BICC1	0.36	2.6×10^{-10}	4.1×10^{-8}	0.403804	1.9×10^{-8}	0.000032	BicC family RNA binding protein 1	HGNC:19351	10q21.1
FHL1	0.36	4×10^{-10}	5.7×10^{-8}	0.359518	7.2×10^{-7}	0.000303	four and a half LIM domains 1	HGNC:3702	Xq26.3
SETD7	0.35	7.3×10^{-10}	8.9×10^{-8}	0.349784	1.5×10^{-6}	0.000403	SET domain containing 7, histone lysine methyltransferase	HGNC:30412	4q31.1
DCN	0.35	1×10^{-9}	1.2×10^{-7}	0.343684	2.3×10^{-6}	0.00047	decorin	HGNC:2705	12q21.33
OGN	0.35	1.3×10^{-9}	1.4×10^{-7}	0.355798	9.5×10^{-7}	0.000339	osteoglycin	HGNC:8126	9q22.31
PDGFRA	0.34	2×10^{-9}	1.9×10^{-7}	0.345107	2.1×10^{-6}	0.000458	platelet derived growth factor receptor α	HGNC:8803	4q12
TTBK2	0.34	2.2×10^{-9}	2.1×10^{-7}	0.348958	1.6×10^{-6}	0.000416	tau tubulin kinase 2	HGNC:19141	15q15.2

Table A4. Enriched function using AR-co-expressed genes in both sexes.

Source	Term_Name	Term_ID	Adjusted_ *p*_Value	Term_ Size	Query_ Size	Intersection_ Size	Intersections
GO:MF	extracellular matrix structural constituent	GO:0005201	0.001116	174	27	5	FBN1, LAMA2, OGN, LTBP2, DCN
GO:MF	glycosaminoglycan binding	GO:0005539	0.009894	244	30	5	FBN1, CCDC80, LTBP2, DCN, SLIT3
GO:MF	heparin binding	GO:0008201	0.043013	173	30	4	FBN1, CCDC80, LTBP2, SLIT3
GO:BP	cellular response to vascular endothelial growth factor stimulus	GO:0035924	0.002535	62	29	4	ADAMTS12, PDGFRA, DCN, JCAD

Table A4. *Cont.*

Source	Term_Name	Term_ID	Adjusted_ *p*_Value	Term_ Size	Query_ Size	Intersection_ Size	Intersections
GO:BP	anatomical structure morphogenesis	GO:0009653	0.002877	2722	31	15	ADAMTS12, SVEP1, FBN1, SLC12A6, LAMA2, PEAK1, FHL1, PGR, MAN2A1, AKAP13, PDGFRA, DCN, JCAD, SLIT3, CDKL5
GO:BP	circulatory system development	GO:0072359	0.002957	1109	30	10	SVEP1, FBN1, BICC1, SLC12A6, REST, AKAP13, PDGFRA, DCN, JCAD, SLIT3
GO:BP	developmental process	GO:0032502	0.006599	6424	31	22	ADAMTS12, SVEP1, FBN1, BICC1, RUNX1T1, NHSL2, SLC12A6, LAMA2, PEAK1, FHL1, REST, SELENOP, PGR, MAN2A1, TTBK2, AKAP13, PDGFRA, KAT6A, DCN, JCAD, SLIT3, CDKL5
GO:BP	regulation of cellular response to vascular endothelial growth factor stimulus	GO:1902547	0.006771	23	29	3	ADAMTS12, DCN, JCAD
GO:BP	system development	GO:0048731	0.009857	4369	31	18	ADAMTS12, SVEP1, FBN1, BICC1, SLC12A6, LAMA2, REST, SELENOP, PGR, MAN2A1, TTBK2, AKAP13, PDGFRA, KAT6A, DCN, JCAD, SLIT3, CDKL5
GO:BP	anatomical structure development	GO:0048856	0.033845	5836	31	20	ADAMTS12, SVEP1, FBN1, BICC1, SLC12A6, LAMA2, PEAK1, FHL1, REST, SELENOP, PGR, MAN2A1, TTBK2, AKAP13, PDGFRA, KAT6A, DCN, JCAD, SLIT3, CDKL5
GO:BP	multicellular organism development	GO:0007275	0.042139	4823	31	18	ADAMTS12, SVEP1, FBN1, BICC1, SLC12A6, LAMA2, REST, SELENOP, PGR, MAN2A1, TTBK2, AKAP13, PDGFRA, KAT6A, DCN, JCAD, SLIT3, CDKL5
GO:BP	vascular endothelial growth factor signaling pathway	GO:0038084	0.043128	42	29	3	PDGFRA, DCN, JCAD
GO:BP	cell adhesion	GO:0007155	0.044906	1521	4	4	ADAMTS12, SVEP1, FBN1, CCDC80
GO:CC	extracellular matrix	GO:0031012	0.000983	565	18	6	ADAMTS12, FBN1, CCDC80, LAMA2, OGN, LTBP2
GO:CC	external encapsulating structure	GO:0030312	0.000993	566	18	6	ADAMTS12, FBN1, CCDC80, LAMA2, OGN, LTBP2
GO:CC	basement membrane	GO:0005604	0.001726	99	9	3	FBN1, CCDC80, LAMA2
GO:CC	collagen-containing extracellular matrix	GO:0062023	0.002734	429	27	6	FBN1, CCDC80, LAMA2, OGN, LTBP2, DCN
HP	Microspherophakia	HP:0030961	0.045002	3	18	2	FBN1, LTBP2

References

1. Saginala, K.; Barsouk, A.; Aluru, J.S.; Rawla, P.; Barsouk, A. Epidemiology of Melanoma. *Med. Sci.* **2021**, *9*, 63. [CrossRef]
2. Ma, M.; Ghosh, S.; Tavernari, D.; Katarkar, A.; Clocchiatti, A.; Mazzeo, L.; Samarkina, A.; Epiney, J.; Yu, Y.R.; Ho, P.C.; et al. Sustained androgen receptor signaling is a determinant of melanoma cell growth potential and tumorigenesis. *J. Exp. Med.* **2021**, *218*, e20201137. [CrossRef] [PubMed]
3. Vellano, C.P.; White, M.G.; Andrews, M.C.; Chelvanambi, M.; Witt, R.G.; Daniele, J.R.; Titus, M.; McQuade, J.L.; Conforti, F.; Burton, E.M.; et al. Androgen receptor blockade promotes response to BRAF/MEK-targeted therapy. *Nature* **2022**, *606*, 797–803. [CrossRef] [PubMed]
4. Wang, Y.; Ou, Z.; Sun, Y.; Yeh, S.; Wang, X.; Long, J.; Chang, C. Androgen receptor promotes melanoma metastasis via altering the miRNA-539-3p/USP13/MITF/AXL signals. *Oncogene* **2017**, *36*, 1644–1654. [CrossRef] [PubMed]
5. Reintgen, D.S.; Paull, D.E.; Seigler, H.F.; Cox, E.B.; McCarty, K.S., Jr. Sex related survival differences in instances of melanoma. *Surg. Gynecol. Obstet.* **1984**, *159*, 367–372. [PubMed]
6. Micheli, A.; Ciampichini, R.; Oberaigner, W.; Ciccolallo, L.; de Vries, E.; Izarzugaza, I.; Zambon, P.; Gatta, G.; De Angelis, R.; Group, E.W. The advantage of women in cancer survival: An analysis of EUROCARE-4 data. *Eur. J. Cancer* **2009**, *45*, 1017–1027. [CrossRef]
7. Brown, C.J.; Goss, S.J.; Lubahn, D.B.; Joseph, D.R.; Wilson, E.M.; French, F.S.; Willard, H.F. Androgen receptor locus on the human X chromosome: Regional localization to Xq11-12 and description of a DNA polymorphism. *Am. J. Hum. Genet.* **1989**, *44*, 264–269.
8. Ju, Q.; Tao, T.; Hu, T.; Karadag, A.S.; Al-Khuzaei, S.; Chen, W. Sex hormones and acne. *Clin. Dermatol.* **2017**, *35*, 130–137. [CrossRef] [PubMed]
9. Bienenfeld, A.; Azarchi, S.; Lo Sicco, K.; Marchbein, S.; Shapiro, J.; Nagler, A.R. Androgens in women: Androgen-mediated skin disease and patient evaluation. *J. Am. Acad. Dermatol.* **2019**, *80*, 1497–1506. [CrossRef]
10. Slominski, A.; Zbytek, B.; Nikolakis, G.; Manna, P.R.; Skobowiat, C.; Zmijewski, M.; Li, W.; Janjetovic, Z.; Postlethwaite, A.; Zouboulis, C.C.; et al. Steroidogenesis in the skin: Implications for local immune functions. *J. Steroid. Biochem. Mol. Biol.* **2013**, *137*, 107–123. [CrossRef] [PubMed]

11. Palvimo, J.J.; Reinikainen, P.; Ikonen, T.; Kallio, P.J.; Moilanen, A.; Janne, O.A. Mutual transcriptional interference between RelA and androgen receptor. *J. Biol. Chem.* **1996**, *271*, 24151–24156. [CrossRef] [PubMed]
12. Sato, N.; Sadar, M.D.; Bruchovsky, N.; Saatcioglu, F.; Rennie, P.S.; Sato, S.; Lange, P.H.; Gleave, M.E. Androgenic induction of prostate-specific antigen gene is repressed by protein-protein interaction between the androgen receptor and AP-1/c-Jun in the human prostate cancer cell line LNCaP. *J. Biol. Chem.* **1997**, *272*, 17485–17494. [CrossRef]
13. Wilkenfeld, S.R.; Lin, C.; Frigo, D.E. Communication between genomic and non-genomic signaling events coordinate steroid hormone actions. *Steroids* **2018**, *133*, 2–7. [CrossRef]
14. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef]
15. Yang, L.; Zheng, R.; Wang, N.; Yuan, Y.; Liu, S.; Li, H.; Zhang, S.; Zeng, H.; Chen, W. Incidence and mortality of stomach cancer in China, 2014. *Chin. J. Cancer Res.* **2018**, *30*, 291–298. [CrossRef] [PubMed]
16. Uhlenhopp, D.J.; Then, E.O.; Sunkara, T.; Gaduputi, V. Epidemiology of esophageal cancer: Update in global trends, etiology and risk factors. *Clin. J. Gastroenterol.* **2020**, *13*, 1010–1021. [CrossRef] [PubMed]
17. Sayiner, M.; Golabi, P.; Younossi, Z.M. Disease Burden of Hepatocellular Carcinoma: A Global Perspective. *Dig. Dis. Sci.* **2019**, *64*, 910–917. [CrossRef]
18. Saginala, K.; Barsouk, A.; Aluru, J.S.; Rawla, P.; Padala, S.A.; Barsouk, A. Epidemiology of Bladder Cancer. *Med. Sci.* **2020**, *8*, 15. [CrossRef] [PubMed]
19. Yuan, Y.; Liu, L.; Chen, H.; Wang, Y.; Xu, Y.; Mao, H.; Li, J.; Mills, G.B.; Shu, Y.; Li, L.; et al. Comprehensive Characterization of Molecular Differences in Cancer between Male and Female Patients. *Cancer Cell* **2016**, *29*, 711–722. [CrossRef] [PubMed]
20. Schmidt, K.; Carroll, J.S.; Yee, E.; Thomas, D.D.; Wert-Lamas, L.; Neier, S.C.; Sheynkman, G.; Ritz, J.; Novina, C.D. The lncRNA SLNCR Recruits the Androgen Receptor to EGR1-Bound Genes in Melanoma and Inhibits Expression of Tumor Suppressor p21. *Cell Rep.* **2019**, *27*, 2493–2507.e4. [CrossRef] [PubMed]
21. Clocchiatti, A.; Ghosh, S.; Procopio, M.G.; Mazzeo, L.; Bordignon, P.; Ostano, P.; Goruppi, S.; Bottoni, G.; Katarkar, A.; Levesque, M.; et al. Androgen receptor functions as transcriptional repressor of cancer-associated fibroblast activation. *J. Clin. Investig.* **2018**, *128*, 5531–5548. [CrossRef] [PubMed]
22. Li, B.; Dewey, C.N. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* **2011**, *12*, 323. [CrossRef] [PubMed]
23. De Siqueira Santos, S.; Takahashi, D.Y.; Nakata, A.; Fujita, A. A comparative study of statistical methods used to identify dependencies between gene expression signals. *Brief. Bioinform.* **2014**, *15*, 906–918. [CrossRef] [PubMed]
24. Liu-Smith, F.; Lu, Y. Opposite Roles of BAP1 in Overall Survival of Uveal Melanoma and Cutaneous Melanoma. *J. Clin. Med.* **2020**, *9*, 411. [CrossRef] [PubMed]
25. Cancer Genome Atlas, N. Genomic Classification of Cutaneous Melanoma. *Cell* **2015**, *161*, 1681–1696. [CrossRef]
26. Guo, J.; Qin, S.; Liang, J.; Lin, T.; Si, L.; Chen, X.; Chi, Z.; Cui, C.; Du, N.; Fan, Y.; et al. Chinese Guidelines on the Diagnosis and Treatment of Melanoma (2015 Edition). *Ann. Transl. Med.* **2015**, *3*, 322. [CrossRef]
27. Hall, K.H.; Rapini, R.P. Acral Lentiginous Melanoma. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
28. Hickey, T.E.; Selth, L.A.; Chia, K.M.; Laven-Law, G.; Milioli, H.H.; Roden, D.; Jindal, S.; Hui, M.; Finlay-Schultz, J.; Ebrahimie, E.; et al. The androgen receptor is a tumor suppressor in estrogen receptor-positive breast cancer. *Nat. Med.* **2021**, *27*, 310–320. [CrossRef]