Comprehensive characterization of androgen receptor in male breast cancer

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BACKGROUND

- Male breast cancer (BC) accounts for less than 1% of new BC cases annually.
- Androgen receptor (AR), a member of steroid and nuclear receptor superfamily is emerging as an important factor in pathobiology of BC.
- While the estrogen receptor (ER) is well-studied in BC, the role of the AR is less understood, particularly in male patients.
- Here, we aimed to characterize the molecular and immunological features of *AR* gene expression in male BC.

METHODS

- 191 samples from male breast cancer patients were tested by NGS (592, NextSeq; WES, NovaSeq) and WTS (NovaSeq; Caris Life Sciences, Phoenix, AZ).
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB).
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Tumors with *AR*-high(H) and *AR*-low(L) RNA expression were classified as above or below the 50th percentile, respectively.
- Real world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates.
- Statistical significance was determined by chi-square and Mann-Whitney U test with *p*-values adjusted for multiple comparisons (*q*<.05).

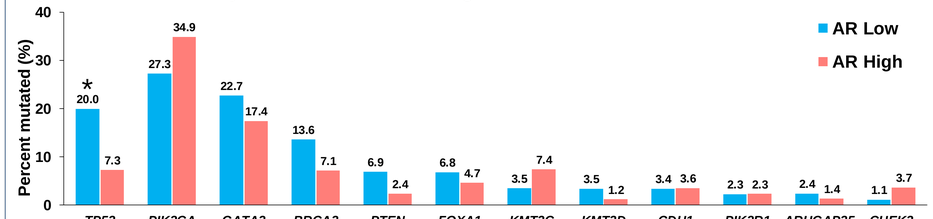
Table 1. BC cohort demographic characteristics

Variables	*AR*-low (50th percentile)	*AR*-high (50th percentile)
Count (N)	96	95
Median age (range)	64.5 (33->89)	69.5 (38->89)
Race (count, N)		
White	66.2% (51/77)	71.1% (54/76)
Black	22.1% (17/77)	18.4% (14/76)
Asian or Pacific Islander	5.2% (4/77)	9.2% (7/76)
Other	6.5% (5/77)	1.3% (1/76)
Ethnicity (count, N)		
Not Hispanic or Latino	93.2% (69/74)	90.9% (60/66)
Hispanic or Latino	6.8% (5/74)	9.1% (6/66)

Race and ethnicity data is self reported

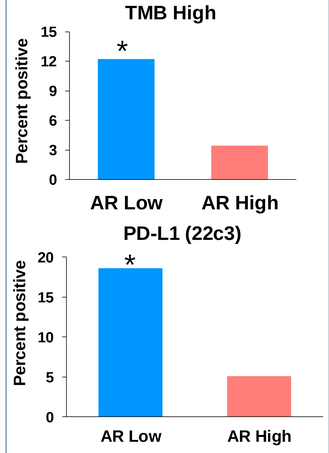
RESULTS

Figure 1. Mutation analysis of *AR*-low and *AR*-high male breast cancer



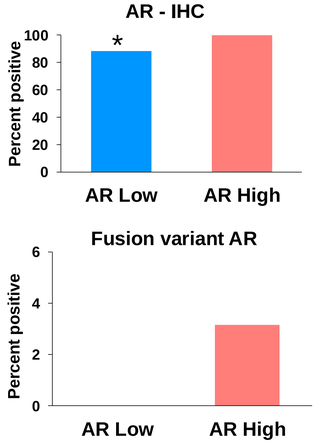
AR-H male BC had lower frequency of *TP53* mutations (20% *AR*-L vs 7% *AR*-H, p=0.02) compared to *AR*-L male BC tumors. *AR*-H had numerically higher frequency of *PIK3CA* (34.8% vs 27.2%) and *CHEK2* (3.7% vs 1.1%), but lower frequency of *BRCA2* (7.1% vs 13.6%) and *PTEN* (2.3% vs 6.9%) compared to *AR*-L, all p = 0.1-0.2. *p>0.05

Figure 2. TMB-high and PD-L1 positivity



AR-H male BC had lower frequency of TMB-high (3.45% vs 12.22%).
AR-H male BC had lower frequency and PD-L1 positivity (5.08% vs 18.57%). *p<0.05.

Figure 3. AR expression and fusion variant-AR



AR-H male BC had higher AR protein expression (100% vs 86.8%). *p<0.05.

AR-H male BC had numerically higher frequency of AR-fusion variant (3.2% vs 0%, p=0.08)

Figure 4. Immune cell infiltration

	AR-low	*AR*-high
B cell	5.26	5.93*
Mφ M1	2.83	2.67
Mφ M2	4.86	5.26
Neutrophil	2.66	2.77
NK cell	2.56	3.58*
Tregs	1.69	1.39*
DC	1.98	2.43*

Low Median%
High Median%

AR-H had higher infiltration of NK cells (3.58% vs 2.56%), dendritic cells (2.43% vs 1.98%), and B cells (5.93% vs 5.26%), all p<0.05. %). For monocytes, CD4 T cells and CD8 T cells median was 0 in both groups.

Figure 5. Differential gene expression analysis of *AR*-low and *AR*-high male breast cancer

Checkpoint gene	*AR*-low	*AR*-high
CD274	2.81	3.78*
FOXP3	1.67	2.51*
IDO1	0.82	0.96
HAVCR2	14.56	22.29*
LAG3	0.64	0.93*
PDCD1	0.26	0.33
PDCD1LG2	0.97	1.19
CTLA4	0.74	1.21

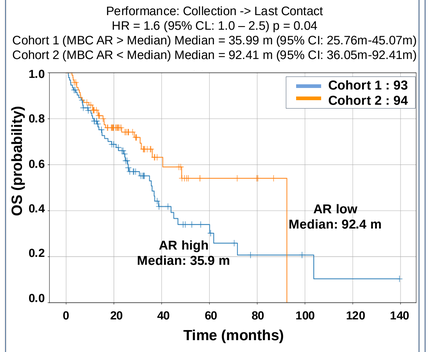
Stem cell gene	*AR*-low	*AR*-high
CD34	2.45	2.96*
CD44	223.47	370.9*
SOX2	0.23	0.28
NANOG	0.27	0.47
POU5F1	1.00	1.43*
KLF4	5.80	8.12*
ALDH1A1	5.39	7.47
ALDH2	14.51	19.6*

Drug efflux gene	*AR*-low	*AR*-high
ABCG2	1.20	1.35
FFAR4	0.24	0.35*
ABCC1	17.37	30.41*
ABCC2	0.69	0.87
ABCC3	9.67	15.18*
ABCB1	1.81	2.95*
ABCB5	0.23	0.30

Low TPM (Median)
High TPM (Median)

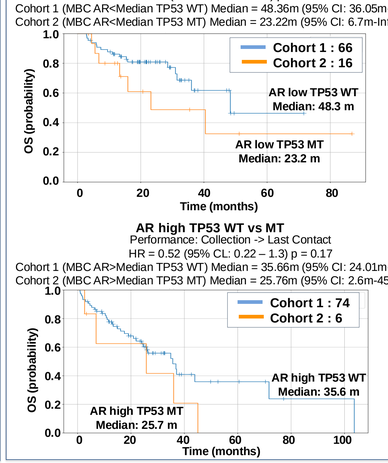
AR-H had higher expression of immune checkpoint genes (*CD274, FOXP3, HAVCR2, LAG3*; FC: 1.3-1.5), stem cell-related genes (*CD34, CD44, POU5F1, KLF4, ALDH2*; FC: 1.2-1.4) and drug efflux gene (*FFAR4, ABCC1, ABCC3, ABCB1*; FC: 1.4–1.7). *p<0.05.

Figure 6. *AR*-low vs *AR*-high and MBC patient overall survival (OS)



AR-H male BC had worse OS (mOS: 35.9 vs 92.4 month; HR 1.6, 95% CI 1.0-2.5, p = 0.04) compared to *AR*-L MBC.

Figure 7. *AR*-low vs *AR*-high and MBC survival in TP53 WT vs MT



AR-H with *TP53*-WT had numerically better survival (mOS: 35.6 vs 25.7 months, HR 0.52, 95% CI 0.20-1.35, p=0.17) compared to *TP53*-MT. Similarly, *AR*-L with *TP53*-WT had numerically better survival (mOS: 48.3 vs 23.2 months, HR 0.58, 95% CI 0.22-1.33, p=0.18) compared to *TP53*-MT.

CONCLUSIONS

Our analysis suggests a strong association between *AR* expression and *TP53* mutations, TMB-H, and PD-L1 positivity, immune cell infiltration, immune checkpoint and stem cell-related gene. Further exploration of specific alterations and immune-oncology markers associated with AR expression may help in clinical trial design for male patients with BC.