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# Plasma exosomal AKR1C3 mRNA expression is a predictive and prognostic biomarker in metastatic castration-resistant prostate cancer patients

## Introduction

Aldo-keto reductase family 1 member C3 (AKR1C3) is important in prostate cancer progression, being a potential biomarker in metastatic castration-resistant prostate cancer (mCRPC). Previous explorations of AKR1C3 are mainly based on tissue samples. This study investigates using plasma-based liquid biopsy to validate the prognostic and predictive value of AKR1C3 in mCRPC patients.

## Materials and Methods

We prospectively recruited 62 mCRPC patients. All patients received repeated prostate biopsies at the time of mCRPC diagnosis, and immunohistochemistry (IHC) staining was used to detect protein expression of AKR1C3 in the tissues. We took their blood simultaneously and performed digital droplet polymerase chain reaction (ddPCR) to measure expression levels of AKR1C3 in the exosomes. The detected plasma and tissue AKR1C3 expression levels were analyzed for patients' overall survival (OS) and progression-free survival under first-line abiraterone use (ABI-PFS).

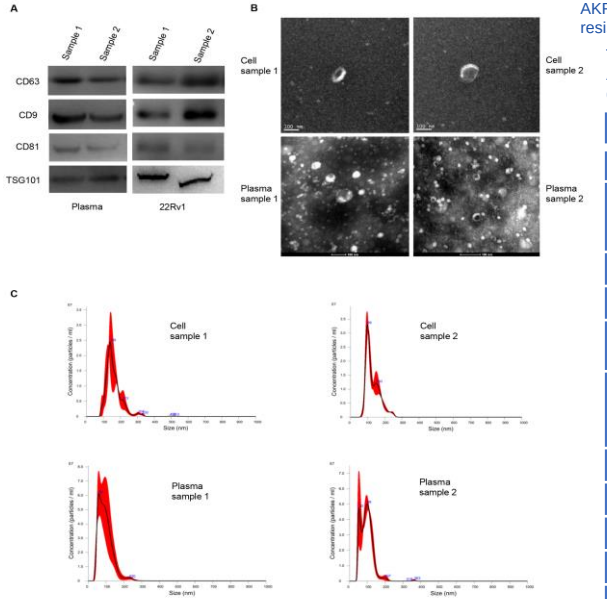


**Figure 1.** Characterization of exosomes. Four selected samples were random representation of our patient's plasma and cell culture supernatant. A. immunoblots for commonly used exosome-expressed protein markers: tetraspanins (CD63, CD81), TSPANs (CD9), and cytosolic proteins (TSG101). B. scanning electron microscopy images of extracted exosomes. C. Nanoparticle tracking analysis for particle diameter.

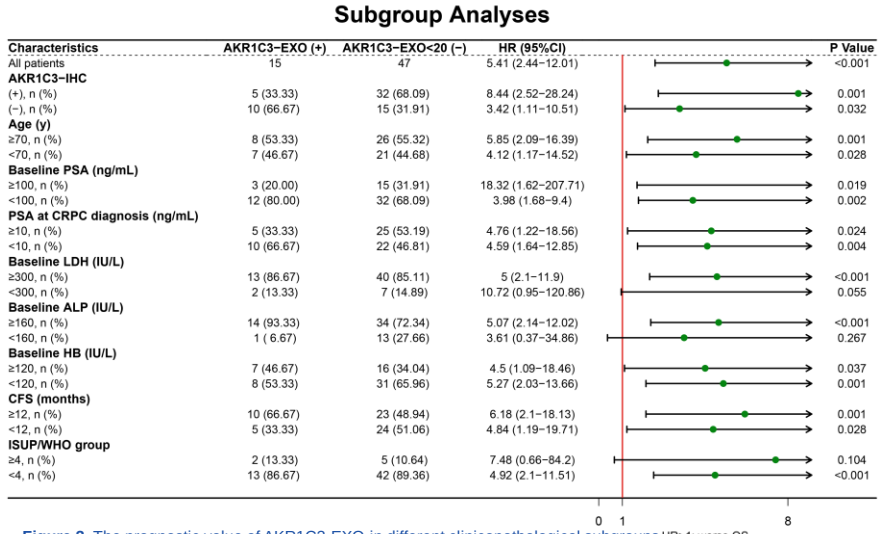


**Figure 3.** The prognostic value of AKR1C3-EXO in different clinicopathological subgroups HR>1: worse OS
AKR1C3-EXO: plasma exosomal AKR1C3 expression (positive: ≥20 copies/20 μL; negative: <20 copies/20 μL); PSA: prostate-specific antigen; CRPC: castration-resistant prostate cancer; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; HB: hemoglobin; CFS: castration-resistance free survival; HR: hazard ratio.

## Results

All other baseline characteristics were balanced between the two groups. 15/62 (24.2%) and 25/62 (40.3%) patients showed AKR1C3-EXO positive (≥20 copies/20 μL) and AKR1C3-IHC positive, respectively. Positive AKR1C3-EXO expression was associated with decreased patients' survival [ABI-PFS: 3.9 vs. 10.1 months, P<0.001; OS: 16.2 vs. 32.5 months, P<0.001]. AKR1C3-IHC positivity was also correlated with ABI-PFS and OS (P=0.010, P=0.016). In patients with worse baseline blood tests (including higher alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) level and lower hemoglobin (HB) level), and lower ISUP/WHO group (<4), their OS was significantly worse when showing AKR1C3-EXO positive.

**Table. Univariate and multivariate survival analyses of AKR1C3-EXO and other clinicopathological factors in predicting OS and ABI-PFS.**
AKR1C3-EXO: plasma exosomal AKR1C3 expression; PSA: prostate-specific antigen; CRPC: castration-resistant prostate cancer; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; HB: hemoglobin; CFS: castration-resistance free survival; HR: hazard ratio.

| | OS | | | | ABI-PFS | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate analyses | | Multivariate analyses | | Univariate analyses | | Multivariate analyses | |
| | HR (lower limit, upper limit) | P | HR (lower limit, upper limit) | P | HR (lower limit, upper limit) | P | HR (lower limit, upper limit) | P |
| AKR1C3-EXO (+) | 5.41 (2.44-12.01) | <0.001 | 4.51 (1.97-10.36) | <0.001 | 3.81 (1.69-8.58) | 0.001 | 3.67 (1.62, 8.29) | 0.002 |
| AKR1C3-IHC (+) | 2.16 (1.14-4.1) | 0.019 | 1.62 (0.82, 3.20) | 0.169 | 2.5 (1.28-4.86) | 0.007 | 2.27 (1.03, 4.99) | 0.042 |
| Baseline PSA>100 ng/mL | 1.14 (0.57-2.25) | 0.712 | - | - | 1.29 (0.62-2.66) | 0.498 | - | - |
| PSA at CRPC diagnosis>10 ng/mL | 1.15 (0.61-2.18) | 0.664 | - | - | 0.75 (0.39-1.45) | 0.389 | - | - |
| LDH>300 IU/L | 0.55 (0.19-1.56) | 0.263 | - | - | 0.45 (0.14-1.49) | 0.191 | - | - |
| ALP>160 IU/L | 0.67 (0.32-1.43) | 0.303 | - | - | 0.63 (0.3-1.35) | 0.235 | - | - |
| HB>120 g/L | 0.9 (0.45-1.78) | 0.764 | - | - | 0.79 (0.4-1.58) | 0.508 | - | - |
| CFS>12 months | 0.61 (0.31-1.19) | 0.147 | - | - | 0.55 (0.28-1.08) | 0.083 | 0.863 (0.383, 1.944) | 0.722 |
| ISUP/WHO group>4 | 1.25 (0.38-4.07) | 0.714 | - | - | 1.38 (0.42-4.53) | 0.594 | - | - |
| Age>70 | 0.75 (0.39-1.43) | 0.383 | - | - | 1.02 (0.53-1.95) | 0.952 | - | - |

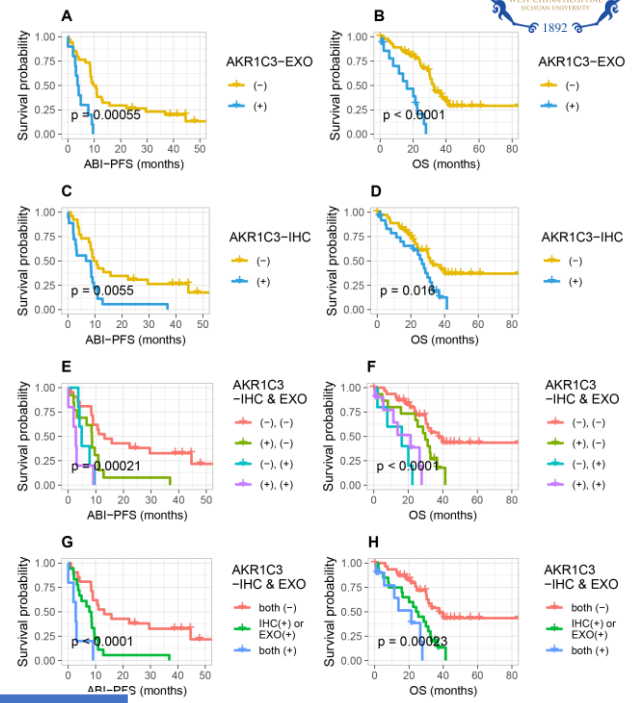


**Figure 2.** Kaplan-Meier curves. A, B. AKR1C3-EXO expression in predicting ABI-PFS and OS. C, D. AKR1C3-IHC expression in predicting ABI-PFS and OS. E, F, G, H. combined expression of AKR1C3-EXO and AKR1C3-IHC in predicting ABI-PFS and OS. AKR1C3-EXO: plasma exosomal AKR1C3 expression (positive: ≥20 copies/20 μL; negative: <20 copies/20 μL); AKR1C3-IHC: AKR1C3 IHC staining in repeat prostate biopsies; ABI-PFS: progression-free survival under first-line abiraterone (the time from abiraterone treatment to progression); OS: overall survival (CRPC diagnosis to all-cause death).

## Conclusion

AKR1C3-EXO is associated with patient prognosis regarding OS and ABI-PFS and can be used as a biomarker in mCRPC.

The authors declare no conflict of interests.


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