Editorial Commentary

How do androgens affect prostate cancer extracellular vesicles?

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Comment on: Martens-Uzunova ES, Kusuma GD, Crucitta S, *et al.* Androgens alter the heterogeneity of small extracellular vesicles and the small RNA cargo in prostate cancer. J Extracell Vesicles 2021;10:e12136.

Received: 30 November 2021; Accepted: 16 December 2021; Published: 25 January 2022.

doi: 10.21037/exrna-21-29

View this article at: https://dx.doi.org/10.21037/exrna-21-29

In their study published in *Journal of Extracellular Vesicles*, Martens-Uzunova *et al.* investigate the effect of dihydrotestosterone (DHT) on small extracellular vesicles (S-EV) (1). Androgens are central to the treatment of prostate cancer, yet it remains relatively unclear what their effect, if any, on EV characteristics are. DHT was selected for study as an androgen with potent effects on the androgen receptor. The focus on S-EV likely relates in part to the technology available to measure the heterogeneity of these EVs (2). A priori, one would expect that androgens, which promote broad intracellular growth programs in prostate cancer cells, would alter in some way either the contents, quantity, or characteristics of prostate cancer cell S-EVs.

To examine this question, Martens-Uzunova *et al.* use the widely used LNCaP human prostate cancer cell line and the derivative C4-2B model. Their work is further supported by several studies of patient plasma and prostate tissue. Light scattering techniques estimated the size distribution of S-EV, with the authors observing differences between plasma derived from patients with benign prostate hyperplasia, localised prostate cancer and advanced prostate cancer. Selective criteria ensured significant clinical differences between the patient cohorts. Differences in size distribution for the tetraspanins were observed, with the differences more pronounced among patients with prostate specific antigen levels over 20 ng/mL in the advanced prostate cancer group. Whether these size differences are related to host circulating factors or circulating tumoral factors remains unknown with these data; differences in CD41a platelet-derived S-EVs suggest that a host immune component could be at play. However, it was CD9, CD63 and CD81 capture where the greatest differences in single S-EV phenotype was often significant. Together, the results suggest heterogeneity in S-EV profiles between patient clinical cohorts, though explications are elusive given the descriptive nature of the detailed data generated.

The same detailed characterization of S-EV using prostate cell lines LNCaP, C4-2B or PC3 did not generally demonstrate significant differences in distribution of tetraspanins and S-EV size. Using the highly androgen response LNCaP, differences in individual S-EV were observed following DHT stimulation of LNCaP cells. Overall, S-EVs positive for prostate-specific membrane antigen (PSMA) did not change with DHT or treatment with the potent androgen receptor antagonist enzalutamide, though consistent changes were observed in select CD9/CD63 subpopulations. To suggest that these changes are induced by changes in the parenteral cells, Martens-Uzunova *et al.* measure how CD9 and CD63 expression intensity on the surface of LNCaP cells are induced by DHT.

Next, the authors undertake detailed characterisation of small RNAs in S-EV from LNCaP cells. Here again, many of the described differences are in subcategories of RNA library analyses. From the 286 miRNAs expressed at relatively higher levels in DHT-treated LNCaP S-EVs, the authors highlight and verify by polymerase chain reaction (PCR) expression that higher levels of miR19a-3p are induced by DHT expression. Notably, from the greater than 4-fold expression seen on RNA-seq, a 1.86-fold increase using sensitive locked PCR was the only validated significant difference. Following bioinformatic characterization of different small RNA types from their sequencing data, it is ultimately the miRNA levels which the authors choose to investigate further using microarray data from prostate cancer patients and controls with long clinical follow-up. A comparison of miR19a-3p and miR361-5p

expression from previously published cohorts indicated that miR19a-3p level were higher in prostate cancer samples collected by transurethral resection of the prostate (TURP) compared to benign TURP controls. However, in another cohort of radical prostatectomy patients, no difference was seen. No significant difference was seen for miR361-5p was seen in either clinical cohort. Taken together, these data are at best suggestive that specific RNA changes in prostate S-EV may be induced by DHT.

New technology can open the door for more detailed exploration of cancer biology. The improvement in single EV capture technology permits an increasingly detailed description of the incredible complexity which exists at the molecular level. Caveats exist to interpreting the results presented in the article by Martens-Uzunova *et al.*, as multiple analyses are presented without adjustment for repeated hypothesis testing and no information on false discovery rates (or equivalent) for comparisons of small RNA expression. Moreover, the precision of the assay techniques used, absolute quantities and normalization factors need to be weighed in the interpretation, particularly when describing small quantities. With the results similar between LNCaP and C4-2B cells, whether the results in these cell lines are generalizable to patient samples remains unknown. Further, demonstrating differences between small patient cohorts as was performed in this study is not equivalent to detecting androgen-induced differences in patients. The authors should nonetheless be commended for focusing on clinical samples; understanding the large heterogeneity which exists in patient samples is important to ultimately have an impact on patient care (3). Further research is needed to explore the interesting and highly complex topic of how circulating hormones and androgen deprivation therapy may alter the phenotype of both prostate cancer cell and patient endogenous EVs.

Overall, Martens-Uzunova *et al.* provide new insights into the changes in S-EV from LNCaP cells which may occur as a result of androgen exposure. They highlight the marvelous level of detail which exists at the level of S-EV in prostate cancer cells. As critical to prostate cancer progression, the effects of androgens remain central to prostate cancer treatment. Further studies may help define the development and function of prostate EVs, but it remains crucial that studies carefully target and integrate clinical data from prostate cancer disease states where the utility of diagnostic or therapeutic EVs is most pertinent.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *ExRNA*. The article has undergone external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://exrna.amegroups.com/article/view/10.21037/exrna-21-29/coif). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/exrna-21-29
Cite this article as: Toren P. How do androgens affect prostate cancer extracellular vesicles? ExRNA 2022;4:1.