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Re: Circulating Extracellular Vesicles in Human Disease
Shah R, Patel T, Freedman JE

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Experts' summary:
In their paper, Shah et al provided a sort of brief introduction concerning the existence and role of extracellular vesicles (EVs), with dedicated attention to those translational and clinical studies eventually useful to suggest their potential role in human diseases. EVs are defined in the paper as membrane-bound organelles that are extruded from tissues—tons and of several types—and contain different types of molecular cargo, thus including a variety of proteins, lipids, and nucleic acids that may be specific for the cell of origin and its function. Originally, EVs have been subdivided according to their size and biogenesis, with *exosomes* having a diameter of <150 nm and *ectosomes*, otherwise defined as *microvesicles*, with a diameter up to 1000 nm. The article critically focuses on the importance of the role of EVs in intercellular signaling, which is of increasing interest due to their potential as noninvasive biomarkers in both disease detection and disease prognosis, with partial attention even to the aspect of therapy, both in oncology and non-oncology fields.

Experts' comments:
In multicellular organisms, cells evolved in numerous ways to communicate and the production of EVs represents an efficient way which cells use to deliver key messages. Therefore, EVs have been lyrically compared to Hermes, the wing-shod messenger of the Olympians, who has served as a link between two worlds, transferring messages from the gods to mankind [1]. EVs are hugely involved in several physiological processes, including inflammatory and immune responses, development, reproduction and pregnancy, tissue repair, and blood coagulation. Likewise, EVs are also implicated in pathological events, such as tumor development and progression, immune response deregulation, and the establishment of a pre-metastatic niche [2]. Notably, EVs secreted by transformed cells resemble the significant variations that occur within the disease [3]. Therefore, EVs can be exploited as potential biomarkers in biological fluids. By definition, a reliable and specific biomarker should help and promote an early diagnosis, monitor the disease progression, and predict the response to a specific treatment, thus supporting the selection of the most appropriate therapeutic option. This approach leads to patient stratification and paves the way to some forms of precision medicine. In this context, EVs would offer a novel perspective in the search for accurate cancer biomarkers by providing a protected reservoir of circulating molecules. The identification of EVs typical cargoes, holding biomarkers of otherwise undetectable amounts, can be eventually translated into clinically relevant information. Thereof, a continuous implementation in terms of EVs isolation will shape the potential future pipelines. For instance, relevant to the urological fields, is the quite recent findings that a small group of miRNAs extracted from EpCAM-positive exosomes allowed the differentiation between clear-cell renal carcinoma and healthy controls [4,5]. Why is urology such a lucky field? Because we may consider at least two unique biofluids: urine and semen. Indeed, the great advantage of monitoring urological tumors is the possibility to exploit urine samples, which are easy to retrieve and are actually enriched in cancer cells-derived molecules due to the direct contact with the tumor itself. That noninvasive diagnostic method is well accepted by patients, thereby improving their adherence to the surveillance programs. This is not only true for the most intuitive condition of bladder cancers [6] but a diagnostic potential has also been identified in urinary exosomes purified from patients with prostate cancer [7,8]. Even of greater translational interest, a study has been also conducted to validate the performance of a novel urine exosome gene expression assay to discriminate a benign disease toward clinically significant prostate cancers in men with a prostate-specific antigen value between 2 and 10 ng/ml on initial biopsy. Why is this clinically important? The test may improve the identification of patients with high-grade disease while avoiding unnecessary biopsies more efficiently than existing risk calculators and standard clinical data [9]. The field for semen analysis is then extremely open and innovative.

Even if EVs were mostly considered as a biomarker source, they received renewed attention over the past few years when they were used for the first time as a drug delivery system [10]. Indeed, their biological compatibility, the ability to overcome biological barriers and to localize at tumor sites, while simultaneously protecting a therapeutic cargo and increasing its circulation time, make them suitable carriers for therapeutic interventions. Various cargoes such as cytotoxic drugs (doxorubicin and paclitaxel) or RNAs (miRNA and siRNA) have been inserted in EVs; shuttled molecules maintain their biological activity and are capable of modulating and reprogramming recipient cells. The targeting behavior of EVs can be tuned via genetic engineering of producing cells or direct chemical addition of specific ligands

to the surface [11]. In the urological field, only very limited examples of EVs-based treatment are available, all of those being in vitro models, thus indicating that there is plenty of room for improvement. A few examples include: (1) siRNA-loaded EVs achieved successful knockdown of PLK-1 mRNA and protein in a bladder cancer cell line [12], and (2) EV-mediated drug delivery has been demonstrated to enhance the cytotoxicity of the antimitotic cancer therapeutic paclitaxel in autologous prostate cancer cells [13].

Overall, the road is still very long; however, a few promising advances clearly show that that path is likely to be fruitful. In that scenario, we may see two roads and two velocities: on one hand, a clinical translation in its embryonic stage for EV-based therapeutic interventions, with a great potential in terms of anticancer therapy, thus confirming Shah et al when they wrote that we are beginning to recognize EVs as potential drug delivery vectors in a whole new class of therapeutic agents; on the other hand, a faster path for potential biomarkers discovery, thanks to the liquid tissues availability in institutional biobanks (thus including urine and semen). However, translational science has to climb quite high mountains since only a few biomarkers achieved high sensitivity and negative predicting values. Prior to clinical adoption, an external validation in a prospective observational setting is certainly compulsory, but only the future will tell us whether an effective role could be found even in the urological field (preferably if broadly considered, from non-oncological to oncological disorders).

Conflicts of interest: The authors have nothing to disclose.

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Re: Paternal Lifestyle Factors in Relation to Semen Quality and In Vitro Reproductive Outcomes

Borges E Jr, Braga DPAF, Provenza RR, Figueira RCS, Iaconelli A Jr, Setti AS

Andrologia 2018;50:e13090

Experts' summary:

Borges and colleagues analyzed the effect of paternal lifestyle habits on semen quality and on intracytoplasmic sperm injection (ICSI) outcomes. Semen from 965 Brazilian men was meticulously assessed, of whom 233 received an estimation of their ICSI outcomes. While sperm count and DNA fragmentation were negatively influenced by cigarette smoking and alcohol consumption, other semen parameters such as semen volume, concentration, total sperm count, and total motile sperm count were negatively influenced only by cigarette smoking. Furthermore, smoking and drinking negatively influenced fertilization and blastocyst formation rates.

Experts' comments:

The theory of the early origin of diseases considers that