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Original Research

Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers

Tore Skotland [a,b], Kim Ekroos [c], Dimple Kauhanen [c], Helena Simolin [c], Therese Seierstad [d], Viktor Berge [e], Kirsten Sandvig [a,b,f], Alicia Llorente [a,b,*]

[a] *Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital-The Norwegian Radium Hospital, 0379 Oslo, Norway*
[b] *Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, 0379 Oslo, Norway*
[c] *Zora Biosciences Oy, 02150 Espoo, Finland*
[d] *Division of Radiology and Nuclear Medicine, Oslo University Hospital, 0379 Oslo, Norway*
[e] *Department of Urology, Oslo University Hospital, 0586 Oslo, Norway*
[f] *Department of Biosciences, University of Oslo, 0316 Oslo, Norway*

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Abstract ***Background:*** Exosomes have recently appeared as a novel source of noninvasive cancer biomarkers, since these nanovesicles contain molecules from cancer cells and can be detected in biofluids. We have here investigated the potential use of lipids in urinary exosomes as prostate cancer biomarkers.

Methods: A high-throughput mass spectrometry quantitative lipidomic analysis was performed to reveal the lipid composition of urinary exosomes in prostate cancer patients and healthy controls.

Results: Control samples were first analysed to characterise the lipidome of urinary exosomes and test the reproducibility of the method. In total, 107 lipid species were quantified in urinary exosomes. Several differences, for example, in cholesterol and phosphatidylcholine, were found between urinary exosomes and exosomes derived from cell lines, thus showing the importance of *in vivo* studies for biomarker analysis. The 36 most abundant lipid species in urinary exosomes were then quantified in 15 prostate cancer patients and 13 healthy controls. Interestingly, the levels of nine lipids species were found to be significantly different when the two groups were compared. The highest significance was shown for phosphatidylserine (PS) 18:1/18:1 and lactosylceramide (d18:1/16:0), the latter also showed the highest patient-to-control ratio. Furthermore, combinations of these lipid species and PS 18:0-18:2 distinguished the

* *Corresponding author*: Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital-The Norwegian Radium Hospital, 0379 Oslo, Norway.
E-mail address: Alicia.Martinez.Llorente@rr-research.no (A. Llorente).

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two groups with 93% sensitivity and 100% specificity. Finally, in agreement with the reported dysregulation of sphingolipid metabolism in cancer cells, alteration in specific sphingolipid lipid classes were observed.
Conclusion: This study shows for the first time the potential use of exosomal lipid species in urine as prostate cancer biomarkers.

1. Introduction

Prostate cancer is a global health problem. It represents 12% of all cancer cases worldwide, and it is the second most commonly diagnosed cancer in men [1]. Many patients face problems with undertreatment or overtreatment due to the lack of optimal tools to help in clinical decisions [2]. Combinations of existing clinical markers such as Gleason score, tumour stage and prostate-specific antigen (PSA) have been used to indicate whether a patient will benefit from treatment but are not optimal in situations where patients have to be followed up over time due to the side-effects of biopsies. In these situations, analysis of cancer-derived parameters in biofluids, such as blood or urine samples, would be more convenient.

Cells release different types of extracellular vesicles to the extracellular environment (EVs), such as exosomes, vesicles of 30–150 nm diameter released by cells after fusion of multivesicular bodies with the plasma membrane [3,4]. Exosomes have recently appeared as a novel source of non-invasive biomarkers for several diseases, including cancer [5,6]. This is because exosomes released by tumour cells contain tumour-related molecules that can be analysed in biological fluids such as blood, urine, seminal fluid, and breast milk [7,8]. Several exosomal molecules including proteins [9,10], lipids [11], mRNAs [5] and microRNAs [12–14] have been identified as potential prostate cancer biomarkers.

Urine has an advantage for prostate cancer diagnosis, since its composition directly reflects changes in the functioning of the urogenital system. Urinary exosomes, first discovered in 2004 [15], originate from cells of the organs involved in reproduction and urine excretion. It is not clear to which extent prostate cells contribute to the exosome population in urine. However, the fact that several prostate-specific molecules, such as prostatic acid phosphatase, prostate transglutaminase and prostate-specific membrane antigen have been detected in urinary exosomes [5,10,16–18], indicates that urinary exosomes originate from prostate cells to some extent.

Lipid metabolism is often disturbed in cancer cells, and this is expected to be reflected in a different lipid composition in normal versus cancer cells [19]. The exploration of lipids as cancer biomarkers has just begun, and it is expected to rapidly increase in the near future due to recent technological advances in mass spectrometry (MS), allowing precise characterisations of lipidomes [11,20–22]. Interestingly, changes in lipids such as cholesterol (CHOL) [23,24], sphingolipids [25] and phosphoinositides [26] have been associated with prostate cancer. Several differences in phospholipid species have been associated with cancer phenotype, metastatic potential and cell morphology in mammary cells and breast cancer cells [27]. In addition, profiling of phospholipid species has recently been used to study the effect of D-allose on prostate cancer cell lines [28]. Furthermore, it has recently been shown that prostate cancer patients have higher plasma lipid concentrations of all lipid classes except phosphatidic acid, and that the plasma concentration of individual lipid species within the same lipid class varied to a large extent [29]. Moreover, 35 lipid species in plasma [29] and 10 lipid species in urine [30] have been identified as potential prostate cancer biomarkers.

We have recently characterised the lipidome of exosomes released by the metastatic prostate cancer cell line PC-3 [11]. In order to further investigate the use of exosomes as a source of lipid prostate cancer biomarkers, we have herein performed a quantitative molecular lipidomic analysis of urinary exosomes from 15 prostate cancer patients and 13 healthy controls. We identified the levels of nine individual exosomal lipid species to be significantly different between the two groups. The potential implications of these results for the use of exosomal lipids as biomarkers for prostate cancer are discussed.

2. Materials and methods

2.1. Urine samples

Urine samples were collected from healthy controls and from prostate cancer patients 1–7 days before prostatectomy as previously described [31]. The cohort description used to compare the control and the prostate cancer group is presented in Supplementary Table S1. The pH in urine samples and the presence of leucocytes, nitrites, proteins, glucose, ketones and blood were analysed with a Combur-Test strip where seven parameters were measured in an Urysys 1100 urine analyser (Roche Diagnostics, Basel, Switzerland). The level of leucocytes (Leu) was classified as 25 Leu/μl: +, 100 or more Leu/μl: ++, 500 Leu/μl: +++. The level of erythrocytes (Ery)