Abstract

The primary aim of the present study is to isolate and analyze urinary exosomes in order to investigate their potential as molecular markers in oncology. This is particularly significant for a group of cancers known for their high risk of recurrence, including bladder cancer, which is the most common type of cancer affecting the urological system. However, thus far, no reliable marker has been identified to differentiate between patients with bladder cancer (BC), those with benign hematuria (HEM), and healthy individuals (CTRL). Markers reported in the literature often yield high false-positive results, largely due to the frequent occurrence of hematuria even in benign cases. Urine serves as an appropriate diagnostic source for BC due to its non-invasive collection method and direct contact with urological tumor cells. Additionally, urine contains extracellular vesicles known as exosomes, which possess a composition similar to that of the cells from which they originate. These exosomes are rich in proteins, making them suitable for proteomic analysis that can potentially facilitate biomarker discovery and establish a non-invasive diagnostic tool for various diseases, including BC. In our research, we focused on optimizing a protocol for isolating urinary exosomes using ultrafiltration and size exclusion chromatography, with the aim of distinguishing BC patients based on proteomic analysis. Prior to subjecting the samples to mass spectrometry (MS), we employed paramagnetic microparticles for sample preparation. Exosome characterization was carried out using western blot, nanoparticle tracking analysis, and scanning electron microscopy. Proteomic analysis of the urinary exosome samples was conducted through label-free LC-MS/MS. Bioinformatics tools were utilized to investigate the altered proteins, with a specific focus on BC. The study results demonstrated significant differences in the identified exosomal proteins between the BC group and the control group. Our findings revealed the most pronounced expression changes in FSCN1 and apolipoproteins when comparing BC and CTRL groups, while HEXB, AKR1C2, and XRCC5 proteins displayed significant alterations when comparing BC patients with either healthy subjects or HEM patients. Multivariate sPLS-DA analysis identified sets of biomarkers capable of distinguishing BC patients from healthy individuals or patients with benign hematuria. Proteomic profiling of exosomes has the potential to contribute to biomarker discovery and enhance the differential diagnosis of BC.

Key words: isolation of exosomes from urine, proteomic analysis, diagnosis, bladder cancer