Analytica Chimica Acta xxx (2017) 1–12

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer

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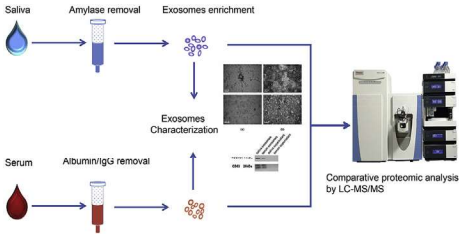
HIGHLIGHTS

- 319 and 994 exosomal proteins were identified from human saliva and serum, respectively.
- Around 80% of salivary exosomal proteins were shared with serum exosomes.
- 11 exosomal proteins were coincidently discovered in saliva and serum for the detection of lung cancer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form
9 June 2017
Accepted 10 June 2017
Available online xxx

Keywords:
Circulating exosomes
Saliva
Serum
Quantitative proteomics
Lung cancer detection

ABSTRACT

Circulating tumor exosomes harbor plenty of cancer biological information, which have emerged as promising targets for cancer early detection and diagnosis. Human serum and saliva are unique diagnostic body fluids, which contain numerous circulating exosomes. It is necessary to establish standardized isolation method and compare their proteome profiling for translational medicine. High abundant proteins in these body fluids were removed before exosomes isolation and obtained exosomes were further confirmed by morphology analysis and surface biomarker test. Label free quantification was applied to systematically compare the protein profiling in saliva and serum exosomes. 319 and 994 exosomal proteins were identified from saliva and serum by LC-MS/MS, respectively. To explore their utility for cancer proteomics, we systematically compared the proteome of saliva and serum exosomes from healthy subjects and lung cancer patients. In particular, 11 potential candidates were coincidently discovered in both body fluids of lung cancer patients. Our finding enforced the hypothesis that cancer related proteins were presented in saliva and serum exosomes, which promoted the unique features of exosomes in our body fluids. A circulating exosomes based body fluid test could be easily established for monitoring cancer once these candidates were validated.

1. Introduction

As a crucial member of extracellular vesicles, exosomes are released by almost all types of cells with a size range of 30 nm–100 nm and a lipid bilayer membrane surrounded. They

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http://dx.doi.org/10.1016/j.aca.2017.06.005
0003-2670/

contain proteins, nucleic acids, and lipids that are reflective of the original cells and may mediate specific cell-to-cell communications [1]. Despite their precise physiological role remains unknown, exosomes are detected so far in the microenvironment of most tumors, and emerging evidences suggest that they play an important role in tumorigenesis [2]. Interestingly, exosomes released by cells or organs could enter our circulatory system and be detected in blood [3], urine [4], saliva [5], and etc. Thus, circulating tumor exosomes provide a new biomarker source for future clinical applications. Moreover, circulating exosomes offer many advantages for bioanalysis. For instance, exosomes are membrane bodies which are more conservative and stable than those proteins and nucleic acids in body fluid. Besides, exosomes are microparticles and could be efficiently enriched, easier than low abundance biomolecules in body fluids. Therefore, circulating tumor exosomes could avoid the limitations of body fluid itself, and be noninvasive biomarkers source for liquid biopsy of cancer.

The routinely tested body fluid in clinic is blood, due to the fact that it contains rich biological information relevant to physiological and pathological processes. Meanwhile, saliva has emerged as a novel medium for cancer detection, which serves as a “mirror of the body” [6]. Both of them are important supporter of our biological system and serving as critical medium for the monitoring of our health. Lots of efforts have been put to develop proteomic biomarkers from these body fluids. However, only few of these tons of thousands candidates could reach the clinic, partially due to the huge dynamic range and high abundant protein issues [7]. Nevertheless, recent work revealed that these body fluids harbor plenty of exosomes, which are promising targets for cancer biomarker discovery [8,9].

In this context, the research community has hypothesized that exosomes originated from cancer cells will be transported to the extracellular microenvironment with cancer specific proteins. Meanwhile, based on the unique “messenger” capability, exosomes could therefore reflect the changes caused by systematic tumors, which could be found in saliva and serum. To date, circulating exosomes have been proved as important foretellers of systemic cancers. A good example is that serum exosomes have been assessed to explore diagnostic biomarkers for glioblastoma multiforme [10] and breast cancer [11]. Meanwhile, the potential of saliva exosomes for biomarker research has been explored in recent publications, especially for cancers [12,13]. Therefore, comprehensively analysis the exosomal proteome from saliva and serum could help us to discover their potential diagnostic values in future clinic.

Until now, frequently used exosomes isolation methods include classical ultra-centrifugation method and commercial kit extraction method. Ultra-centrifugation method could obtain highly pure exosomes which has been viewed as standard method for exosomes isolation [14]. However, it is time consuming and frequently cause sample loss and result in low recovery rate. Meanwhile, based on the affinity to exosomes membrane, commercial kit possesses the advantages of facile, quick, and easy to operate, which is suitable for implementation in the clinics. Even though, both methods are suffered from high abundant proteins interference [15]. Indeed, commercially depletion kit was routinely used to remove Albumin and IgG from human serum prior to exosomes isolation [16]. For the removal of high abundant protein in saliva, we have previously developed a method through combine starch based affinity chromatography with filter system to specifically remove salivary Amylase and prepare extracellular vesicles [17]. Furthermore, the removal of high abundance proteins from samples before exosomes enrichment could benefit downstream proteomic analysis.

In this study, our goal is to explore the similarities of exosomal proteome between serum and saliva and evaluate the possibility of circulating exosomes as diagnostic source for cancer. To this end, we isolated exosomes from saliva and serum and characterized them, thoroughly. Their proteins were extracted and analyzed. We then requisitioned the same lung cancer patients' saliva and serum samples to obtained exosomes, and compared their proteome comprehensively through utilizing label free quantification method. We further analyzed whether circulating exosomes of saliva and serum could be used to discriminate lung cancer patients from healthy subjects.

2. Materials and methods

2.1. Body fluid sample collection and preparation

Body fluid samples were collected according to approved protocols (IRB#M15017) by Institutional Review Board (IRB) of Shanghai Jiao Tong University. All subjects provided written informed consents. The methods were carried out in accordance with the approved guidelines. All experimental protocols were approved by Bio-X Ethics Committee of Shanghai Jiao Tong University.

Serum samples were obtained from healthy subjects and lung cancer patients. For serum collection, a total of 5 mL of venous blood was collected in BD Vacutainer blood collection tubes with silicone-coated interiors (BD Diagnostics, Franklin Lakes, NJ, USA) by a standard venipuncture method. The collected serum samples were stored at room temperature to allow for blood clotting and then centrifuged at 1500 g for 15 min at 4 °C in order to remove the fibrin clot and other cellular elements. The serum samples were then immediately aliquoted and frozen at −80 °C. Prior to exosomes isolation, IgG and Albumin in serum were depleted by the ProteoExtract® Albumin/IgG removal kit (Merck 122642) according to the manual.

Saliva samples were obtained from healthy subjects and lung cancer patients according to standard protocols [17]. The collected saliva-containing tubes were placed on ice, supplemented with EDTA-free Protease Inhibitor Cocktail (Roche Applied Science, Basel, Switzerland) and then centrifuged at 2600 g for 15 min at 4 °C. The resulting supernatant was then immediately aliquoted and frozen at −80 °C. Prior to exosomes isolation, Amylase in saliva was removed by the affinity chromatography column packed with starch (Sigma, CA, USA), as previously described [17].

2.2. Exosomes isolation and exosomal proteins extraction

Exosomes from 100 μL of pre-processed serum and saliva was isolated by PureEXO@ isolation kit (101Bio, CA, USA) according to the manufacturer's recommendations, respectively. Briefly, 100 μL PureExo beads were added to 100 μL serum or saliva. The mixture was incubated for 10 min at room temperature and the 30 kD filter was used to keep exosomes by PBS solution elution. For exosomal proteins extraction, 1:1 (v/v) RIPA buffer (Sigma, St. Louis, USA) was added to break the membrane structure and release exosomal proteins. Protease Inhibitor Cocktail was then added to the sample before storage at −80 °C.

2.3. 1D-SDS PAGE

Exosomal proteins were loaded into a 10% SDS-PAGE Gel (Invitrogen™, ThermoFisher, New York, USA) and were run at 120 V for 60 min in MOPS SDS running buffer. Pre-stained protein standard (Life technologies, Shanghai, China) was used to track protein migration. The resulting gels were stained with Fast Sliver Stain Kit (Beyotime, Beijing, China). Filter aided proteome preparation (FASP) tryptic digestion was carried out overnight at 37 °C using

Please cite this article in press as: Y. Sun, et al., Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer, Analytica Chimica Acta (2017), http://dx.doi.org/10.1016/j.aca.2017.06.005