Improved isolation method to increase the purity of salivary exosomes for biomarker discovery in pancreatic cancer

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Abstract

Improved isolation method to increase the purity of salivary exosomes for biomarker discovery in pancreatic cancer

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Background. Pancreatic cancer (PC) is one of the most aggressive malignancies, with a poor prognosis and a five-year survival rate below 10% due to late diagnosis and limited treatment options. Nowadays, no specific diagnostic markers have been identified. Exosomes represent a complex mode of intercellular communication in health and disease including cancer1. Recently, salivary exosomes emerged as very promising source of non-invasive biomarkers for diagnostic purpose in PC2. Differential Ultracentrifugation remains the prevailing method for isolating exosomes from fluids. This method is cost-effective, has a low risk of contamination from separation reagents, and allows the collection of a large number of exosomes. However, exosomes isolated by this method commonly have lots of impurities and the yield is also relatively low. Objective. Application of a simple, rapid and cheap filtering device (Patent Number 102023000023502) for the isolation of salivary exosomes in order to investigate the potentiality in PC biomarker discovery. Methods. Saliva samples were collected from seven PC patients and four healthy individuals, then exosomes were isolated using the filtering device. Exosome characterisation and quality control were conducted by transmission electron microscopy, dynamic light scattering, western blotting and total protein determination. Moreover, a proteomic MS-based analysis was performed to fully characterize the protein content according to MISEV2018 guidelines. Results. The filtering device provided exosome samples with reduced contamination, as evidenced by transmission electron microscopy and dynamic light scattering analyses. The proteomic analysis identified the CD9 tetraspanin protein that is recommended as exosome biomarker, and the dipeptidyl peptidase IV (DPP IV/CD26) that has been shown to be predominantly associated with the salivary exosome's membrane. Moreover, we identified 24 proteins often found in exosomes according to the ExoCarta database. The WikiPathways platform was used for a complete functional analysis showing significant protein enrichment in PC subtype, counting 6 proteins (Cornifin-B; Protein S100-A2; Serpin B3; Trefoil factor 3; Lymphocyte antigen 6D; Small proline-rich protein 3). Conclusion. This pilot study not only demonstrates the effectiveness of our filtering device for salivary exosomes isolation, but also offers a source for future diagnostic applications in PC.

References

1. Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, Tao Y, et al. Exosomes: key players in cancer and potential therapeutic strategy. Signal Transduction and Targeted Therapy, 2020, doi: 10.1038/s41392-020-00261-0.

2. Machida T, Tomofuji T, Maruyama T, Yoneda T, Ekuni D, Azuma T, et al. miR−1246 and miR−4644 in salivary exosome as potential biomarkers for pancreatobiliary tract cancer. Oncology Reports, 2016, doi: 10.3892/or.2016.5021.

3. I. Helwa, J. Cai, M. D. Drewry, A. Zimmerman, M. B. Dinkins, M. L. Khaled, M. Seremwe, W. M. Dismuke, E. Bieberich, W. D. Stamer, M. W. Hamrick and Y. Liu, PLoS One, 2017, 12, e0170628

Do you have any conflicts of interest?

No, I do not have a conflict of interest.