Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Melanoma Res. Author manuscript; available in PMC 2022 October 01.

Published in final edited form as:

Melanoma Res. 2021 October 01; 31(5): 472–475. doi:10.1097/CMR.0000000000000762.

Proteomic profiles of melanoma cell-derived exosomes in plasma: discovery of potential biomarkers of melanoma progression

Sujan Kumar Mondal[1], **Theresa L. Whiteside**[1,2]

[1]Department of Pathology, University of Pittsburgh School of Medicine and UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA

[2]Departments of Immunology and Otolaryngology, University of Pittsburgh School of Medicine and UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA

Abstract

Cancer liquid biopsy encompassing circulating tumor cells (CTC), circulating tumor DNA (ctDNA) and/or tumor-derived exosomes (TEX) emerges as a novel approach to early detection, non-invasive monitoring of responses to therapy and predicting patient survival. TEX are a key component of liquid biopsy, because they mimic tumor cells in their proteomic and genetic content. Two recent proteomic analyses of TEX released into plasma by melanoma cells confirms the potential of TEX as diagnostic and prognostic markers in melanoma.

Keywords

Melanoma; Exosomes; Proteomics; Liquid biopsy; Biomarkers

Cancer is the second leading cause of death worldwide. Although early detection of cancer and novel therapies indicate a significant improvement in the 5-years survival rates of most malignancies [1], late-stage cancers do not respond to treatments and have poor survival [2,3]. Therefore, early detection of cancer and selection of effective therapies are critical for improving cancer outcomes. To this end, a search for biomarkers for cancer detection and for biomarkers of response to therapy has been intense [4,5]. So far, only a few promising biomarkers useful for cancer screening or prognosis have emerged, and among them, tumor-derived exosomes or TEX have become the major focus of attention [6–8].

Exosomes are a subset of extracellular vesicles (EVs) produced by all cells and present in all body fluids. They are nano-sized (50 to 150nm) membrane vesicles that carry proteins, nucleic acids, and lipids [9]. They originate from the late endosomes and multivesicular bodies (MVBs), and their molecular/genetic cargo reflects that of the parent cells. Exosomes found in body fluids of cancer patients are a mix of vesicles derived from tumor cells (so called TEX) and from non-malignant cells (non-TEX). It is the TEX carrying an

Correspondence to: Theresa L. Whiteside, PhD, UPMC Hillman Cancer Center, UPCI Research Pavilion, Suite 1.27, 5117 Centre Avenue, Pittsburgh, PA 15213, Phone: (412) 624-0096, Fax: (412) 624-0264, whitesidetl@upmc.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

excess of immunosuppressive proteins and few immunostimulatory proteins in melanoma that largely contribute to tumor-induced immune suppression and promote tumor immune escape [10]. During cancer development and progression, the molecular content of TEX changes, undergoing alterations that mimic those in the tumor. Consequently, the TEX cargo is expected to provide insights into molecular/genetic events that accompany cancer activity, progression, and response to therapy [11]. In addition, TEX are known to mediate tumor-induced immune suppression in cancer [12–14], so that the analysis of molecular/genetic contents of TEX offers an opportunity for utilizing TEX as a measure of existing immune suppression or its changes following immunotherapy.

While this rationale for considering TEX as potential cancer biomarkers has been the basis for several recent studies [8,12,13], the validation of the concept requires a convincing demonstration that exosomes mimic the molecular, genetic and functional profiles of the tumor cell from which they originate. This demonstration was recently provided by Hoshino et al [15]. Using proteomics, the investigators provided a “proof of principle” for the role of plasma-derived exosomes which they named “extracellular vesicles and particles” (EVPs), as cancer biomarkers. The investigators performed high resolution mass spectrometry (HRMS) of EVPs derived from 426 human cancer and non-cancer samples, which included matched tumor explants (TT) and tumor-adjacent (AT) or tumor-distant (DT) non-malignant tissue and plasma EVP samples. Analysis of this large collection of samples required a machine-learning approach and led to definition of: (i) 13 proteins shared by >50% of EVPs that qualified as reliable pan-EVP markers; (ii) a panel of cancer-specific EVP proteins that could ultimately be used to distinguish between individuals with or without cancer. Thus, a panel of 11proteins (overexpressed in >50% of all EVP specimens) discriminated lung and pancreatic cancer from non-cancer EVPs obtained from tumor tissues or paired plasma; (iii) a panel of distinct tumor-type specific proteins that enabled discrimination of tumor types regardless of disease stage. Additional confirmatory analysis of EVPs from 77 patients across 16 different tumor types and of 43 EVP specimens from individuals without cancer confirmed the presence of the common tumor-associated protein signature only in cancer tissue- or plasma-derived EVPs with sensitivity of 100% and specificity of 92%. Overall, proteomic studies performed by Hoshino et al showed that EVPs from paired tumor tissues or plasma of cancer patients carry the same panel of proteins, and thus EVPs in cancer plasma can be reliably used as liquid tumor biopsy for cancer detection and discrimination of different cancer types.

Another recent study, in which the co-authors of this commentary took part, has taken a different approach to biomarker discovery in patients with metastatic melanoma. Instead of searching for a unique identifiable signatures of tumor-derived EVPs among *total EPVs* isolated from cancer patients’ plasma, as did Hoshino et al., Pietrowska’s et al [16] strategy was to first isolate *TEX* from plasma of patients with melanoma. To this end, we used immunocapture to separate melanoma cell-derived exosomes (MTEX), from non-malignant cell derived exosomes, non-MTEX, and using HRMS, search for molecular signatures discriminating the two subsets. We also searched for a distinct TEX signature that would correlate with melanoma progression and outcome. The strategy is based on immune capture of TEX by antibodies specific for antigens carried on the TEX surface and absent from the surface of non-MTEX. Biotinylated antibodies specific for the epitope to chondroitin

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

sulfate peptidoglycan-4 (CSPG4) expressed only on melanoma cells and pericytes but not on any other human cells [17,18] and carried by MTEX were used for immunocapture of MTEX on streptavidin-labeled beads [19]. Non-MTEX were not immunocaptured by anti- CSPG4 mAb and were also placed on beads by capture with biotinylated anti-CD63 mAb. Detection of antigens carried by MTEX and non-MTEX was performed by on-bead flow cytometry. MTEX were positive for CSPG4 and melanoma-associated antigens (MAA), while non-MTEX were negative, confirming that the separation of these exosome subsets was successful. MTEX were enriched in immunoinhibitory proteins and suppressed functions of primary human immune cells, while non-MTEX were immunostimulatory [10]. The isolation of plasma exosomes from plasma by size exclusion chromatography (SEC) followed by immunocapture of MTEX were critical steps for removal of most abundant plasma proteins, reducing outer exosome “corona” of contaminating proteins.

The HRMS analysis of paired MTEX and non-MTEX obtained from plasma of 15 melanoma patients identified 573 protein species of which 423 were shared and 73 were only upregulated in MTEX. Among the latter group, 16 proteins were selected as the “MTEX discriminating” panel based on their consistently significant upregulation in 8/15 MTEX samples examined and their known involvement in cancer progression. The reactome pathway analysis showed that 12/16 proteins overexpressed in MTEX were linked to the functional pathways enriched in MTEX, including signal transduction, disease activity or immune reactivity. These data showed that a paired comparison of MTEX and non-MTEX by HRMS identified the signature of 16 proteins, all functionally associated with cancer progression, that were upregulated only in MTEX and discriminated MTEX from non-MTEX.

All of the 15 randomly selected patients with metastatic melanoma evaluated in this study were previously treated with oncologic therapies. Among them, 7 patients had no evident disease (NED) and 8 had progressive disease (PD) at the time of phlebotomy for exosome isolation from plasma. This provided an opportunity to search for proteins that could discriminate PD patients from those with NED. The MTEX protein content of these two patient groups was compared using the acquired HRMS data. Many proteins (n=75) were elevated in MTEX of patients with PD relative to the MTEX of NED patients, and 12/75 proteins were significantly and consistently elevated. In addition, 8 proteins were lower in abundance in MTEX of PD than NED patients. The molecular signature of ALIX (PDCD6IP) and the four proteins whose abundance strongly correlated with ALIX (profilin-1, HSP90, tubulin and β-tubulin-1) was significantly overexpressed in MTEX of patients with PD and emerged as a potentially significant prognostic biomarker in metastatic melanoma. Remarkably, ALIX, a multifunctional protein also known as programmed cell death 6-interacting protein, was the best discriminating protein ($p<0.0003$) of the twelve proteins upregulated in MTEX of patients with PD. The data suggested that a comparison of ALIX alone in MTEX was sufficient to discriminate between melanoma patients with PD vs NED. Finally, among 8 proteins decreased in abundance in MTEX of PD patients, Contactin 1 (CNTN1) was found to be highly up-regulated in some patients with NED/SD and was not detectable in paired MTEX of PD patients. Thus, this study identified a MTEX signature that reflected melanoma progression and discriminated patients with NED from those with PD after oncological therapy.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The two papers discussed above, both using proteomic analysis of exosomes in a search for potential cancer biomarkers, represent two different experimental approaches to this search. The first is a comprehensive, broadly envisioned comparative HRMS analysis of proteomes in paired EVP specimens obtained from tumor cells, adjacent and distant non-malignant cells and plasma. It largely aims at the confirmation of the principle that plasma contains exosomes originating from and molecularly faithful to the parental tumor. Further, unique molecular signatures of plasma vesicles discriminate cancer from non-cancer and distinguish different tumor types from one another. Thus, plasma exosomes can serve as a *bona fide* liquid tumor biopsy, providing tumor-relevant information with remarkable sensitivity and specificity. In this study only few melanoma specimens were included, and the conclusions, while in principle applicable to all solid tumor types, do not specifically address melanoma. The second study was based on the notion that the content of tumor-derived exosomes (TEX) in plasma is distinct from that of non-malignant exosomes, and that interrogating TEX rather than all plasma exosomes is likely to be the more informative as a liquid tumor biopsy Taking advantage of immune capture for isolation of TEX from plasma, this study used HRMS to identify protein profiles that distinguish TEX from non-malignant cell-derived exosomes in plasma of melanoma patients. Further, this study showed that the TEX protein profile in plasma has prognostic significance, as it can distinguish melanoma patients with progressive disease from those who are disease free after oncologic therapy.

The comparison of these two proteomics-based studies for their usefulness as a *melanoma* liquid biopsy is not possible, except for emphasizing that both were successful in illustrating the power of proteomics in defining tumor type-specific protein profiles that exosomes carry. The remarkable feature of both proteomic studies is that the cohorts of patients donating paired tissues and plasma for exosome HRMS were relatively small for biomarker discovery studies. Nevertheless, proteomic-based comparisons detected highly significant differences in molecular profiles of MTEX and non-MTEX or of MTEX from patients with progressive vs non-progressive melanoma after oncologic therapy. In the Hoshino's study, similarity of tumor-derived and plasma-derived EVPs was documented using limited numbers of human tissue specimens, which are difficult to procure. While the proteomics of total plasma exosomes a la Hoshino et al may be experimentally less demanding than the proteomics of isolated TEX as in Pietrowska's study, it appears that for future biomarker studies targeted proteomics represents a highly promising and more practical venue for biomarker discovery.

The overall conclusion supported by the data from both studies is that proteomics provides a highly sensitive platform for molecular profiling of exosomes and that such profiling might have a predictive value for disease presence, progression and outcome. Melanoma, like other cancers, is highly diverse genetically, molecularly and clinically. The capability offered by proteomics to relate or link exosome protein profiles with different disease manifestations, including resistance or sensitivity to therapies, for example, represent a potential paradigm shift in diagnosis, prognosis and evaluations of responses to therapy in melanoma. Also, proteomics of exosomes offers more than a mere path to biomarker discovery. The provocative finding reported in the Pietrowska's study that only ALIX (PDCD6IP) in MTEX and four other proteins correlating in abundance with ALIX, might be sufficient for discrimination of melanoma patients responding or not to oncologic therapies provides a clue that exosome cargo components are functionally important and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

may influence disease progression. Thus, exosome proteomics may lead to discovery of melanoma antigens that are not only pan exosome markers, like ALIX, but are involved in apoptosis and many cellular interactions that influence disease progression. In this respect, ALIX otherwise known as ALG2 interacting protein X, is a multifunctional protein reported to mediate apoptosis and a variety of other cellular functions [20].

As of today, it appears that exosomes in cancer plasma have qualified as significant components of liquid tumor biopsy in melanoma or other cancers. Together with CTC and ctDNA, plasma exosomes should be monitored in future studies to solidify their role as diagnostic or prognostic biomarkers but also as regulatory elements in cancer progression and therapeutic outcome.

References:

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin2018; 68:7–30. [PubMed: 29313949]
2. Prigerson HG, Bao Y, Shah MA, Paulk ME, LeBlanc TW, Schneider BJ, et al.Chemotherapy Use, Performance Status, and Quality of Life at the End of Life. JAMA Oncol2015; 1:778–784. [PubMed: 26203912]
3. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al.T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature2017; 545:60–65. [PubMed: 28397821]
4. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer2019; 19:133–150. [PubMed: 30755690]
5. Goossens N, Nakagawa S, Sun X, Hoshida Y. Cancer biomarker discovery and validation. Transl Cancer Res2015; 4:256–269. [PubMed: 26213686]
6. Moller A, Lobb RJ. The evolving translational potential of small extracellular vesicles in cancer. Nat Rev Cancer2020; 20:697–709. [PubMed: 32958932]
7. Mathew M, Zade M, Mezghani N, Patel R, Wang Y, Momen-Heravi F. Extracellular Vesicles as Biomarkers in Cancer Immunotherapy. Cancers (Basel)2020; 12.
8. Whiteside TL. The potential of tumor-derived exosomes for noninvasive cancer monitoring: an update. Expert Rev Mol Diagn2018; 18:1029–1040. [PubMed: 30406709]
9. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol2013; 200:373–383. [PubMed: 23420871]
10. Sharma P, Diergaarde B, Ferrone S, Kirkwood JM, Whiteside TL. Melanoma cell-derived exosomes in plasma of melanoma patients suppress functions of immune effector cells. Sci Rep2020; 10:92. [PubMed: 31919420]
11. Kucharzewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringner M, et al.Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proc Natl Acad Sci U S A2013; 110:7312–7317. [PubMed: 23589885]
12. Theodoraki MN, Yerneni SS, Hoffmann TK, Gooding WE, Whiteside TL. Clinical Significance of PD-L1(+) Exosomes in Plasma of Head and Neck Cancer Patients. Clin Cancer Res2018; 24:896–905. [PubMed: 29233903]
13. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al.Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature2018; 560:382–386. [PubMed: 30089911]
14. Whiteside TL. Exosomes and tumor-mediated immune suppression. J Clin Invest2016; 126:1216–1223. [PubMed: 26927673]
15. Hoshino A, Kim HS, Bojmar L, Gyan KE, Cioffi M, Hernandez J, et al.Extracellular Vesicle and Particle Biomarkers Define Multiple Human Cancers. Cell2020; 182:1044–1061 e1018. [PubMed: 32795414]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

16. Pietrowska M, Zebrowska A, Gawin M, Marczak L, Sharma P, Mondal S, et al.Proteomic profile of melanoma cell-derived small extracellular vesicles in patients' plasma: a potential correlate of melanoma progression. J Extracell Vesicles2021; 10:e12063. [PubMed: 33613873]
17. Ferrone S, Whiteside TL. Targeting CSPG4 for isolation of melanoma cell-derived exosomes from body fluids. HNO2020; 68:100–105.
18. Campoli M, Ferrone S, Wang X. Functional and clinical relevance of chondroitin sulfate proteoglycan 4. Adv Cancer Res2010; 109:73–121. [PubMed: 21070915]
19. Sharma P, Ludwig S, Muller L, Hong CS, Kirkwood JM, Ferrone S, et al.Immunoaffinity-based isolation of melanoma cell-derived exosomes from plasma of patients with melanoma. J Extracell Vesicles2018; 7:1435138. [PubMed: 29511460]
20. Monypenny J, Milewicz H, Flores-Borja F, Weitsman G, cheung A, et al.ALIX regulates tumor-mediated immunesuppression by by controlling EGFR activity and PD-L1 presentation. Cell Reports2018; 24:630–641. [PubMed: 30021161]