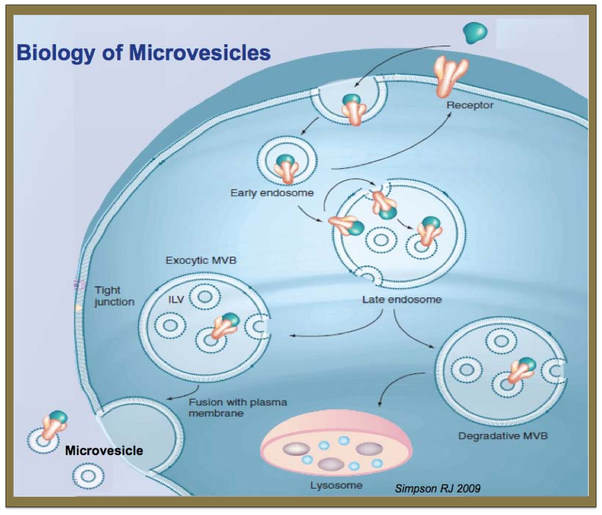
A sensitive exosome-based biosignature for the diagnosis of prostate cancer

Christine Kuslich, Traci L. Pawlowski, Ta Deng, Teresa Tinder, Joon Kim, Jeff Kimbrough, David Spetzler Caris Life Sciences, Phoenix, AZ

Abstract

Background: The screening and diagnosis of prostate cancer (PCa) would be significantly improved by identifying biomarkers that are not only highly specific and sensitive but also surveyed easily from the blood or urine. Exosomes are endosome-derived vesicles between 40-100 nm in diameter that are secreted by many cell types including prostate epithelial cells in both normal and neoplastic states. The quantity and molecular composition of exosomes shed from cancer cells differs considerably from those shed by normal cells. This study explored whether plasma-derived exosomes can be utilized as a robust blood-based biosignature for PCa and other disease states. **Methods**: A novel multiplexed platform for quantifying and profiling exosomes from plasma was used to develop an exosome-derived biosignature comprised of seven surface membrane protein biomarkers. Antibodies used to capture and detect these targeted biomarkers are specific to membrane proteins for: exosomes generally (CD9, CD81, and CD63), exosomes from prostate epithelial cells (PSMA and PCSA), and tumor-associated exosomes (EpCam and B7H3). A training set comprised of 34 PCa patients and 49 age-range matched men from the general population was used to generate an exosome-specific PCa biosignature. The training set results were subsequently confirmed with a blinded pilot validation comprised of 42 PCa and 35 age-range matched men. **Results**: The blood-based exosome PCa assay correctly identified PCa patients in the training set with a sensitivity of 83% and specificity of 95%, AUC = 0.881. Furthermore, the assay distinguished between PCa and BPH samples (n=15), with a sensitivity and specificity of 83% and 85%, respectively, with an AUC = 0.844. The blinded validation cohort confirmed the sensitivity and specificity of the assay. **Conclusion**: This preliminary study demonstrates the ability of an exosome-associated biosignature to distinguish PCa from both unaffected and BPH samples. Exosome profiling could provide a powerful tool to monitor PCa progression and therapeutic response from a blood sample.

Background



Exosomes are endosome-derived vesicles between 40-100 nm in diameter with a unique cup shape morphology that are secreted by most cell types (*1*). These vesicles are formed intracellularly by invagination followed by fusion with the multivesicular body (MVB). The MVB ultimately merges with the plasma membrane, leading to exocytosis of the exosomes with membrane protein composition indicative of their cell of origin (*1*). Exosomes can be distinguished from other microparticles and microvesicles by the presence of a characteristic protein composition and their physical morphology. As a result of their endosomal origin, all exosomes contain proteins involved in membrane transport and fusion (such as Rab GTPases, Annexins, flotillin), in MVB biogenesis (such as Alix and TSG101), in processes requiring heat shock proteins (hsc70 and 90), integrins and tetraspanins (such as CD63, CD9, CD81 and CD82) (*1*).

Results

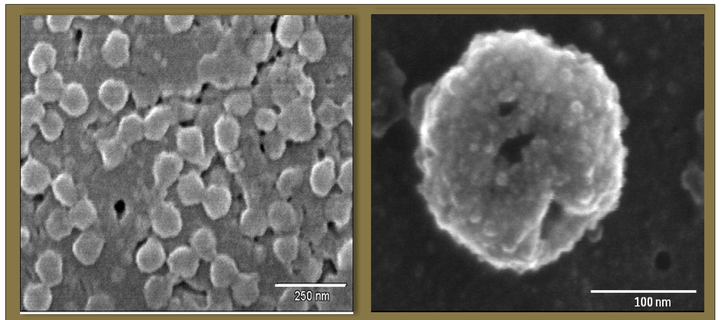


Figure 1. Scanning Electron Images (SEM).. (A) Vcap exosomes captured with Epcam. (B) Vcap exosome captured with Epcam. SEM was performed to visually inspect the size of exosomes being captured with our platform. Size distribution confirms that the majority of vesicles captured are within the 50-150 nm diameter size range defined for exosomes.

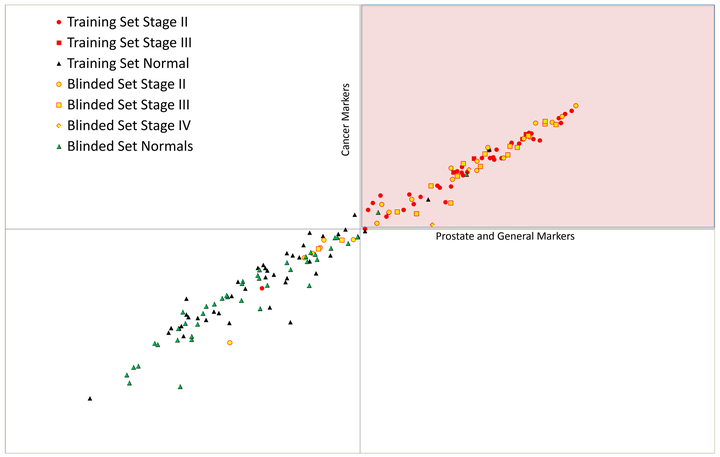
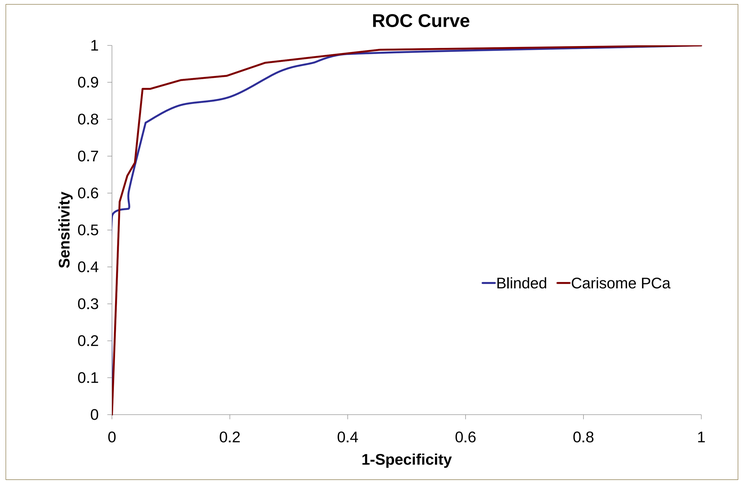


Figure 2. Scatter plot of the data showing the prostate and general markers vs. the cancer markers. Data points that fall in the upper right hand quadrant are considered positive for the test, while all others are negative.



	Training + Blinded	PCa Blinded
AUC	0.938	0.917

Figure 3. Receiver-operator curves for the exosome Pca test, and PSA (light blue) (21). The blinded set was composed of 77 samples, 42 Pca and 35 normal. The combined set (Training + Blinded cohorts) was composed of 160 samples, 76 Pca and 84 normal. The assay correctly identified the Pca samples with **81% sensitivity and 95% specificity in the blinded validation set**.

Methods

- **Frozen plasma samples from 59 stage II and III prostate cancer patients and 61 age-range matched normal men were obtained and divided into a training set (29 biopsy-confirmed prostate cancer and 31 self-stated age-range matched normals) and validation set (30 prostate cancer and 30 normals).**
- **For each patient sample the exosomes were isolated from the plasma by a novel method of exosome separation.**
- **Overall exosome levels were determined via fluorescent signal using a combination of seven antibodies. A fluorescence threshold was established for each antibody.**
- **The thresholds for each antibody were developed using the training set and tested with the blinded validation set. Samples were required to score "above threshold" for all antibodies to be considered positive for cancer (red quadrant in figure 2).**

Conclusions

- **A training set cohort (N=83) comprised of stage II and stage III PCa plasma samples was used to define an assay threshold that could correctly differentiate the biopsy-confirmed PCa plasma samples from age-range matched self-defined "normal" plasma samples.**
- **The blinded study (N=77) correctly identified prostate cancer samples with 81% sensitivity and 95% specificity, with an AUC 91.7%.**
- **The entire cohort of 160 samples performed at a sensitivity of 89% and a specificity of 95%, with an AUC of 93.8%.**
- **Further improvements to this assay have been completed and a large clinical validation cohort is being tested currently based on these improvements.**
- **The exosome-based diagnostic platform offers a new opportunity for a method of detection that is minimally-invasive, blood-based and has the type of analytical performance that is greatly needed for early reliable identification of prostate cancer.**

References

1.C. Thery, L. Zitvogel, S. Amigorena, *Nat Rev Immunol* **2**, 569 (Aug, 2002).
2.R. M. Johnstone, M. Adam, J. R. Hammond, L. Orr, C. Turbide, *J Biol Chem* **262**, 9412 (Jul 5, 1987).
3.B. T. Pan, R. M. Johnstone, *Cell* **33**, 967 (Jul, 1983).
4.B. T. Pan, K. Teng, C. Wu, M. Adam, R. M. Johnstone, *J Cell Biol* **101**, 942 (Sep, 1985).
5.C. Thery, M. Ostrowski, E. Segura, *Nat Rev Immunol* **9**, 581 (Aug, 2009).
6.K. Al-Nedawi *et al.*, *Nat Cell Biol* **10**, 619 (May, 2008).
7.M. Bebawy *et al.*, *Leukemia* **23**, 1643 (Sep, 2009).
8.D. D. Taylor, C. Gercel-Taylor, *Gynecol Oncol* **110**, 13 (Jul, 2008).
9.G. Rabinowits, C. Gercel-Taylor, J. M. Day, D. D. Taylor, G. H. Kloecker, *Clin Lung Cancer* **10**, 42 (Jan, 2009).
10.J. Skog *et al.*, *Nat Cell Biol* **10**, 1470 (Dec, 2008).
11.G. L. Andriole *et al.*, *N Engl J Med* **360**, 1310 (Mar 26, 2009).
12.K. Lin, R. Lipsitz, T. Miller, S. Janakiraman, *Ann Intern Med* **149**, 192 (Aug 5, 2008).
13.P. C. Walsh, *J Urol* **170**, 312 (Jul, 2003).
14.R. Harris, K. N. Lohr, *Ann Intern Med* **137**, 917 (Dec 3, 2002).
15.M. S. Litwin, D. J. Pasta, J. Yu, M. L. Stoddard, S. C. Flanders, *J Urol* **164**, 1973 (Dec, 2000).
16.E. Johansson *et al.*, *Eur Urol* **55**, 422 (Feb, 2009).
17.P. C. Walsh, *J Urol* **169**, 1588 (Apr, 2003).
18.G. Steineck *et al.*, *N Engl J Med* **347**, 790 (Sep 12, 2002).
19.F. J. Fowler, Jr. *et al.*, *J Gen Intern Med* **21**, 715 (Jul, 2006).
20.J. B. Rietbergen, A. E. Kruger, R. Kranse, F. H. Schroder, *Urology* **49**, 875 (Jun, 1997).
21.Thompson IM et al. / JAMA. 2005;294:66-70.