Enriching for Rare Subpopulations of Circulating Microvesicles by the Depletion of Endothelial- and Leukocyte-Derived Microvesicles

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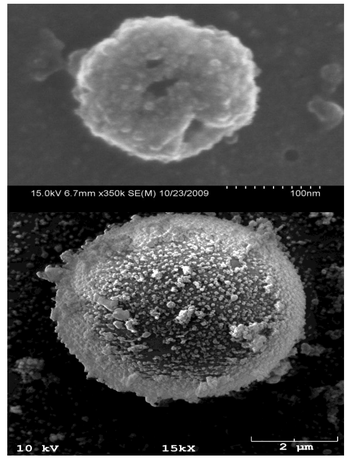
Abstract

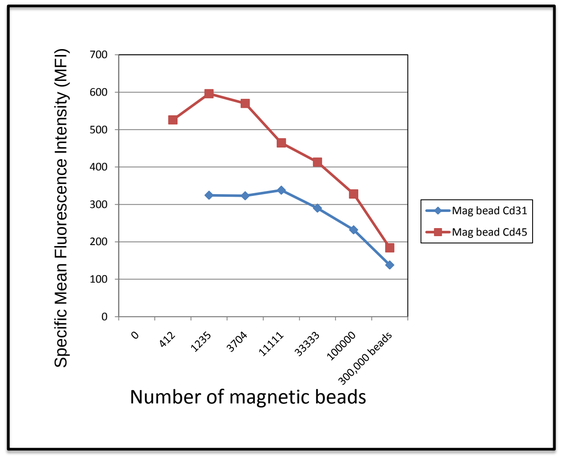
Endothelial- and leukocyte-derived circulating microvesicles (cMV) represent a majority of the cMV present in the blood. The purpose of this study was to determine if depletion of these more common cMV populations is possible, allowing for the enrichment and analysis of the remaining subpopulations of cMV. Circulating microvesicles were isolated from the plasma of breast cancer patients. Magnetic beads conjugated to antibodies specific for either CD31 or CD45 were used to deplete the isolated endothelial- and leukocyte-derived cMV. The remaining cMV population was characterized with a multiplexed immunoassay against a panel of 20 different antigens. In addition to the expected depletion of the biomarker used to remove the targeted cMV in the remaining cMV population, a concomitant depletion of associated biomarkers was observed. In the case of the cMV population in which CD31 had been used to remove endothelial-derived cMV, for instance, there was a significant concomitant depletion of DLL4, a highly associated endothelial marker. This was not the case with general epithelial microvesicle markers, such as CD9, which were still very much present in the cMV population remaining after depletion. These findings suggest a potential method for a more complete characterization of biomarker profiles associated with cMV derived from disease-associated cells and provide the foundation for a novel cMV-based strategy for disease detection through a non-invasive blood test.

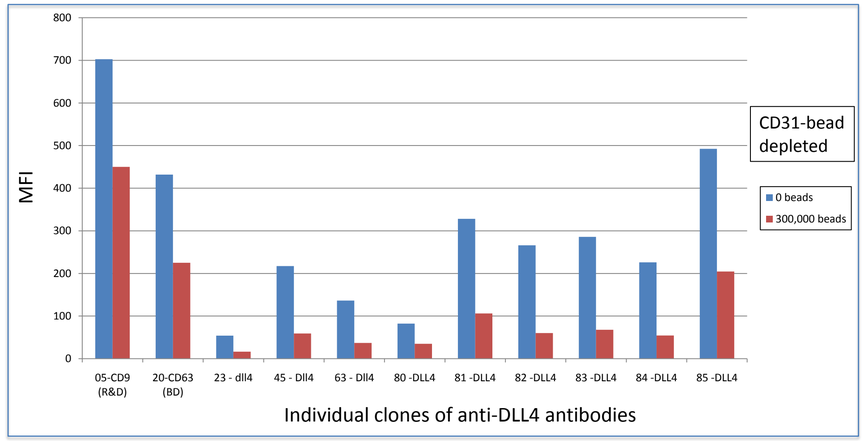
Methods

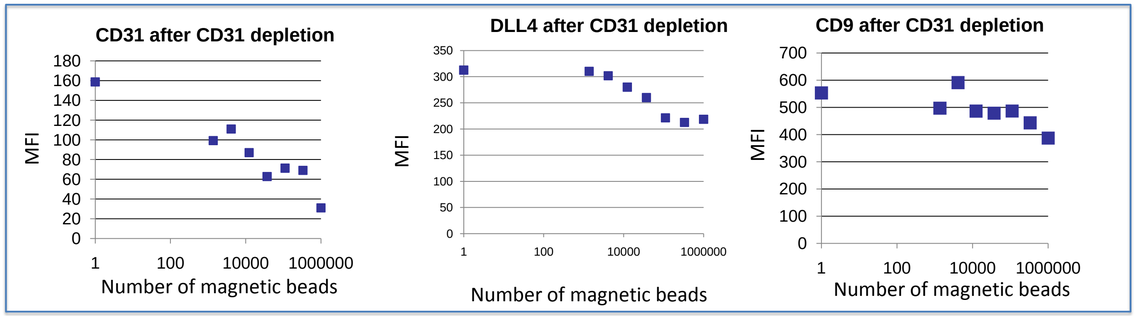
Exosomes are endosome-derived vesicles between 40-100 nm in diameter that are secreted by most cell types. These vesicles are formed intracellularly by invagination followed by fusion with the multivesicular body(MVB). The MVB ultimately merges with the plasma membrane, leading to exocytosis with membrane protein composition indicatve of their cell of origin. Exosomes can be distinguished from other microparticles by the presence of a characteristic protein composition and their physical morphology. As a result of their endosomal origin, all exosomes contain membrane proteins including tetraspanins (CD63, CD9, CD81 and CD82).

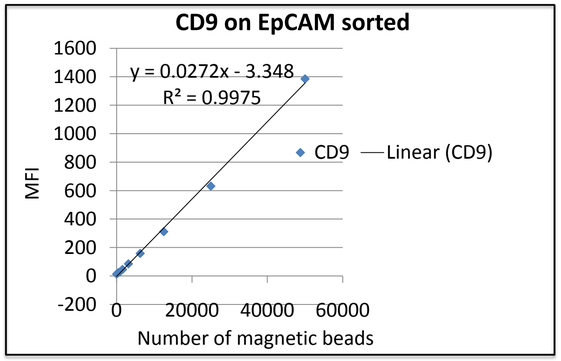
Normal plasma was spiked with tissue culture derived exosomes from the VCaP cell lines and processed using concentrating filters according to our normal processing. Then increasing numbers of magnetic beads coated with either CD31, or CD45 antibodies were incubated with 100ul normal plasma + 100ng/ml of purified VCaP exosomes for one hour at RT. The beads were then separated from the samples using a magnet and both the supernatant and the magnetic beads were run on Luminex. The supernatant were run with the following bead panel and the magnetic beads were put into tetraspanin detector for Luminex detection in order to confirm the binding of EpCAM and B7H3 specific cMVs to the respective beads.

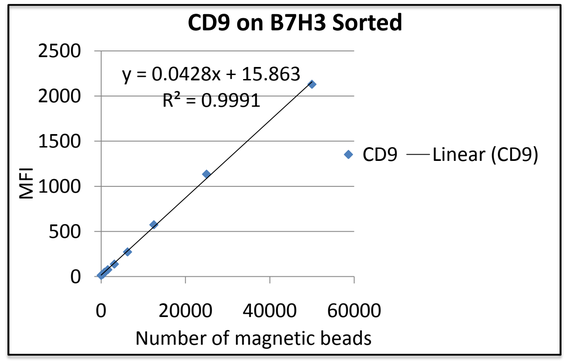












Results

Luminex analysis of the supernatant from the magnetic bead collection demonstrated that the depletion of CD31 was specific with the highest number of beads resulting in only background Mean Fluorescence Intensity (MFI). Additionally, DLL4 is a marker associated with angiogenesis on endothelial cells. With the depletion of endothelial –derived cMVs (defined as CD31+) the level of DLL4 staining declined, but not to background levels. This indicates that not all endothelial cell derived cMVs express DLL4 in normal plasma. The CD31 depleted cMVs were also evaluated for detection using nine different clones of anti-DLL4 antibodies. MFIs from all clones decreased compared with non-depleted specimens. There were significant differences between clones with regard to both the amount of fluorescence in the undepleted specimens and the decrease after CD31 depletion. Finally, studies were performed to confirm that magnetic antibody-conjugated beads bound their specific cMVs in a linear fashion. For these studies the tetraspanin CD9 was evaluated on increasing numbers of cMV-coated beads. The expression of CD9 increased linearly with increasing beads with R^2 values of 0.999 and 0.998.

Conclusions

The studies described here demonstrate that binding of cMVs to antibody-conjugated beads is specific for the antibody and linear with regard to number of beads used. Additionally, just as with other biologic assays involving antibodies such as ELISAs, IHC or flow cytometry there are differences in assay performance related to binding properties of the Abs used. Nine clones of anti-DLL4 were compared with differing result indicating important differences in binding of these different Abs.