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Differential protein expression and miR content of sorted subsets of circulating microvesicles from cancer patients and healthy controls

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Abstract

MicroRNAs (miRs) are small non-coding RNAs that are 20 to 25 nucleotides in length and regulate expression of entire families of genes. A major source of circulating miRs in cancer patients is believed to be circulating microvesicles (cMV) within biologic fluids such as blood. The transfer of these modifiers of RNA translation from diseased cells into the bloodstream can have broad impacts on disease detection, progression and/or prognosis. The goal of these studies was to determine whether there are differences in miR composition within different subpopulations of cMV based on surface protein composition.

Methods

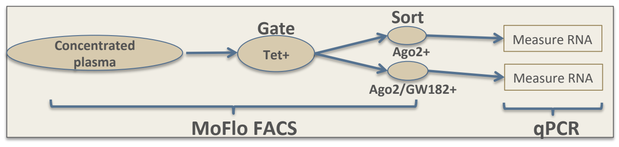
We used flow cytometry to phenotype and sort plasma-derived cMV from 22 individuals (3 breast cancer, 2 lung cancer, 10 prostate cancer, 1 bladder cancer and 6 non-cancer controls). 1) cMV were stained for proteins associated with membranes such as tetraspanins (BD Biosciences CD9, 63, 81), Ago2 (Abcam) and GW182 (Bethyl Laboratories); 2) cMV were stained for tetraspanins and Ago2. Both experiments were analyzed using a Beckman Coulter MoFlo XDP. For phenotypic analysis, events were gated on tetraspanin expression to distinguish cMV from nano-sized irrelevant debris, and co-expression of Ago2 and/or GW182 was determined. Quadrant-based sorting was performed for single, double and/or triple-positive events. miR content was determined using conventional Taqman probes (ABI) with the ABI 7900 thermal cycler on RNA extracted from sorted cMV or input plasma.

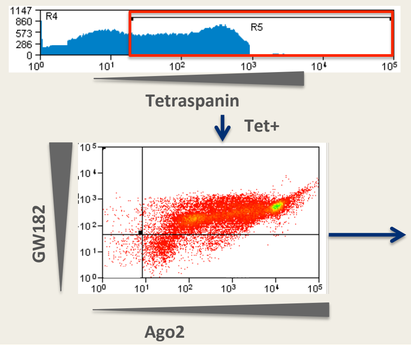
Results

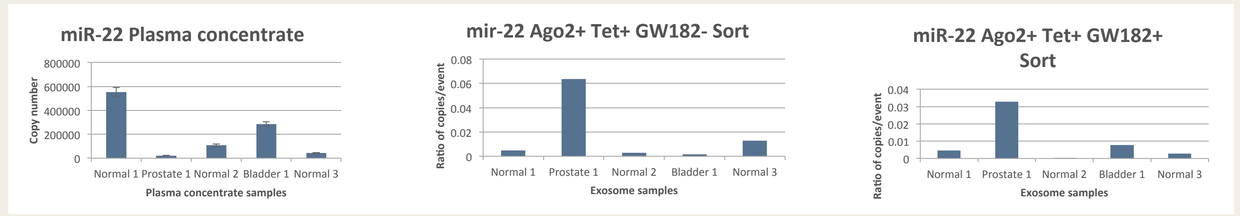
The results of these studies demonstrate that differing populations of cMV can be enriched using flow cytometry, and these enriched populations can have distinguishable miR profiles. Unfractionated cMV were not able to discriminate cancers from non-cancers using miRs-let-7a, -16, -22, -148a or -451 in this population of patients. When sorted, tetraspanin and Ago2 populations of cMV were compared, miR expression was generally 5-fold higher in prostate cancer patients than in healthy controls.

Conclusions

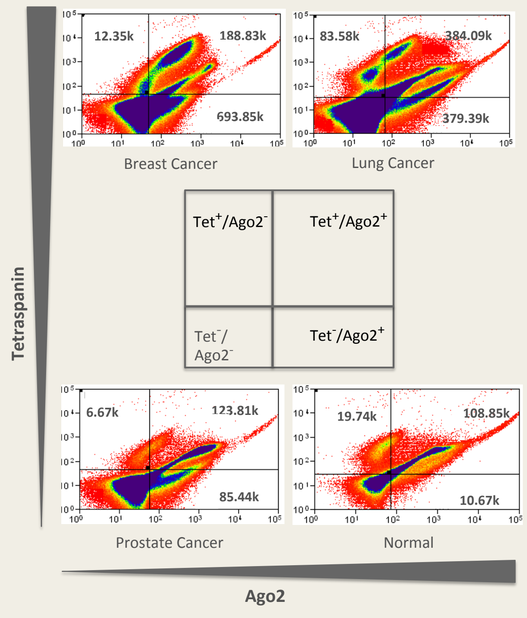
These studies demonstrate that cMV can be consistently phenotyped, analyzed and sorted using flow cytometry and that subpopulations of cMV contain unique miR profiles. These subsets are distinct from non-sorted samples, which can be useful in distinguishing cancer plasma from non-cancer plasma.

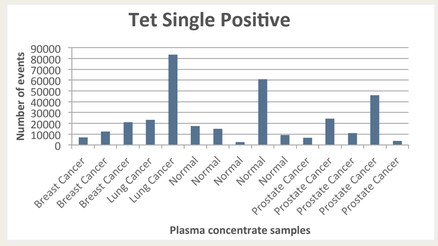


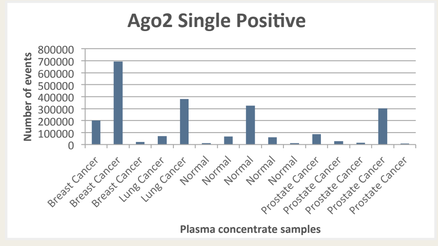


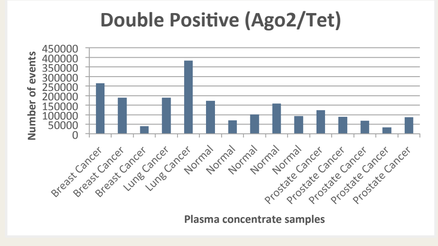


Experiment 1: Plasma concentrate from normal, prostate, and bladder cancer patients were sorted for Tetraspanin (Tet)+/Ago2+/GW182- or Tet+/ Ago2+/GW182+. RNA was extracted from concentrate and sorted populations and miRs were evaluated.

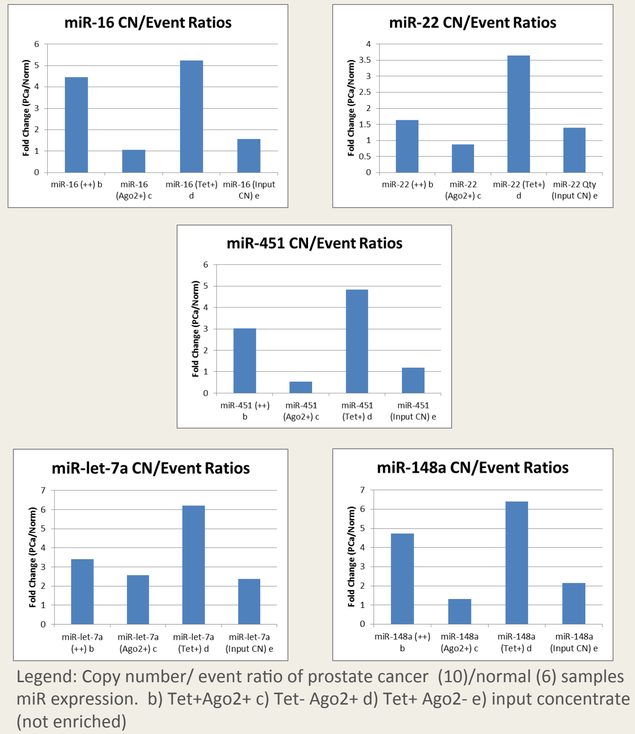








Experiment 2: Plasma concentrate from normal and cancer patients were sorted for Tet+/Ago2+, Tet+/Ago2-, Tet-/Ago+. RNA was extracted from concentrate and sorted populations and miRs were evaluated.



Legend: Copy number/ event ratio of prostate cancer (10)/normal (6) samples miR expression. b) Tet+Ago2+ c) Tet- Ago2+ d) Tet+ Ago2- e) input concentrate (not enriched)