EVix ELISA tools reveal differences in CD3, CD4, CD8 and MHC Class 2 positive extracellular vesicles (EVs) derived from the colon cancer cell lines SW480 and SW620.

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ABSTRACT

SW480 and SW620 are paired cell lines established from a primary tumor and its metastatic counterpart obtained from the same patient and have a shared genetic background. These cell lines are considered to be a useful model for the changes which may take place during progression of colon cancer. Thus, the SW480 and SW620 cell pairing provides insight into metastatic and malignant behavior of human colon cancer. We report that using EVix Elisa tools we can identify differences between extracellular vesicles (EVs) released by these respective cell lines. EVs from SW480 cells are distinct from EVs from SW620 cells. While both SW480 and SW620 have similar tetraspan expression patterns on their respective EV populations, a marked difference exists in immune associated molecules. We have found that EVs from SW480 cells can be captured with antibodies against CD3, CD4, CD8, MHC Class 1 and 2 and TCR CB1. These captured EVs were detected by anti-tetraspan (CD9, CD63, CD81) antibodies. In contrast EVs from SW620 cells were not captured using the same antibodies against CD3, CD8, TCR CB1 and MHC Class 2. EVs from SW620 were positive for CD4 and MHC Class 1 antigens similar to EVs from SW480. Comparable amounts of starting EVs from the SW480 and SW620 cell lines were evaluated as determined by Cholera Toxin B capture and human tetraspan (CD9, CD63, CD81) detection. The presence of immune associated molecules in EVs isolated from human SW480 and SW620 colon cancer cell lines is of interest. The differences in the EV populations positive or negative for immune associated molecules suggest differences in respective tumor cell interaction with the host immune system.

INTRODUCTION

EVix ELISA kits capture extra cellular vesicles (EVs) via monoclonal antibodies immobilized onto microwell plates. Detection of EVs captured onto the microwell is done after washing steps to remove non bound EVs. The capture step selects antigen specific EVs. Anti-tetraspanin antibodies can be used to both capture and detect EVs. Non-tetraspanin cell surface molecules can also be targeted to capture EVs. The captured EV population can then be probed for the presence of other EV surface molecules.

Evaluation of EVs obtained from cultures of colon cancer cell lines, SW480 and SW620 was carried out to determine if differences between the EVs obtained from the two cultured lines could be detected. Dilutions of SW480 and SW620 EV preps was conducted on Cholera Toxin B capture plates using tetraspanin detection antibodies. The cell lines revealed differences in antigen specific captured EV populations detected with anti-tetraspanin antibodies. Using three tetraspanin detection antibodies simultaneously (Trifecta Detect) on antigen specific captured EVs showed differences in EVs from other sources including a mixed lymphocyte reaction, HEK 293 cells and normal human urine. Comparison of EV populations from SW480 and SW620 cells revealed differences in immune associated molecules. While almost equivalent amounts of Class I positive EVs were captured for the cell lines, there was marked differences in CD8, TCR-B1, CD24, CD37, CD4, CD10, CD63, Class II MHC, CD40 and CD8 captured EV populations when detected using anti-tetraspanin antibodies. Attempts to block EV capture by excess soluble capture antibody added simultaneously with EVs revealed specific blocking of capture for SW620 EVs positive signals. In contrast blocking of SW480 EVs capture with soluble antibody revealed signals which were not as efficiently blocked as others. There was some blocking for all capture antibodies except for CD8 capture.

SW480 and SW620 derived EV preps were also run on a size exclusion chromatography column. The complexity of the EV populations revealed tetraspanin positive EVs present in both the void and included volume. Other antigen capture detect combinations revealed population differences in retention times.

MATERIALS AND METHODS

Serum free culture supernatants were concentrated 50X across 300 kD ultrafiltration membranes (Biomax Ultrafiltration Discs EMD Millipore Corporation, Billerica MA) in stirred cell concentrators under gravity flow. Samples were than dia-filtered three times, by adding 10X volume of tris-glycine buffer to the concentrated samples, and re-concentrating to starting volume. No tetraspanin binding activity was seen in 300 MWCO flow through, suggesting the majority of tetraspanin containing EVs were retained by the 300 MWCO membrane.

Concentrated EV samples were run on a Sephacryl S500HR (Sigma Life Sciences) 40-20,000 kDA (dextrans) 2.5 cm diameter column (40 ml bed volume). 1ml sample volumes were applied to the column and 1 ml fractions were collected. Column fractions were assayed with EVix ELISA kits.

EVix ELISA kits (InCepix, St. Paul, MN) were used to capture and detect EVs. Capture and detect antibodies are indicated in the figure legends.

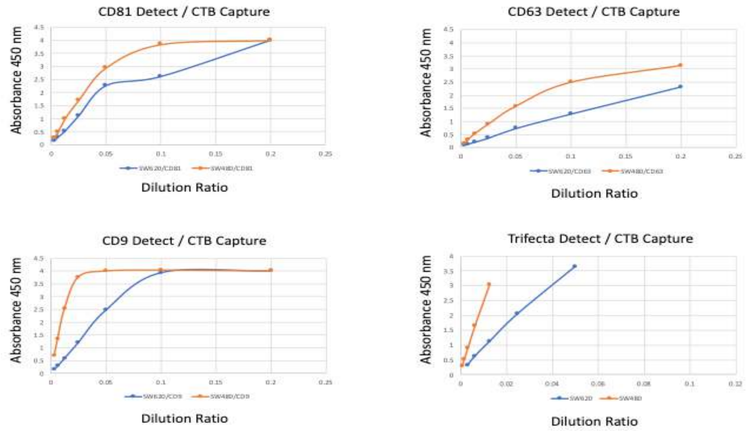

Figure One: Dilution curves of SW480 and SW620 EV preps. Capture plates were coated with Cholera Toxin B subunit which binds GAM-1 ganglioside. Detection was either CD9, CD63, CD81 individually, or all three detection antibodies added simultaneously (Trifecta). SW480 EVs appear to have higher tetraspanin content than SW620 EVs.

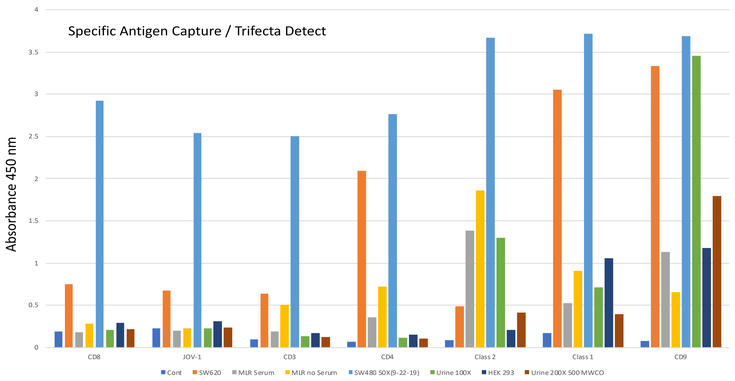

Figure Two: Trifecta detection of specific antigen captured EVs from different sources. SW480, SW620, HEK293 cell culture supernatants serum free 50X. Mixed lymphocyte reaction supernatants with or without serum were not concentrated. Urine 100X (300 kD MWCO Concentrate). Urine 200X of 500 MWCO flow through. Capture antibody on horizontal below bars.

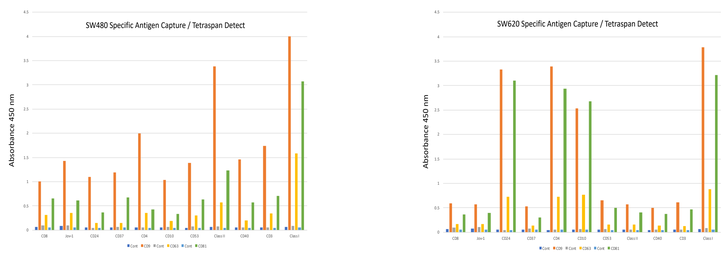

Figure Three: Specific antigen capture of SW480 and SW620 EVs detected with individual anti-tetraspanin antibodies. Capture antigens are labeled on bottom of bars. Control signal is non-specific antibody binding in absence of EV sample.

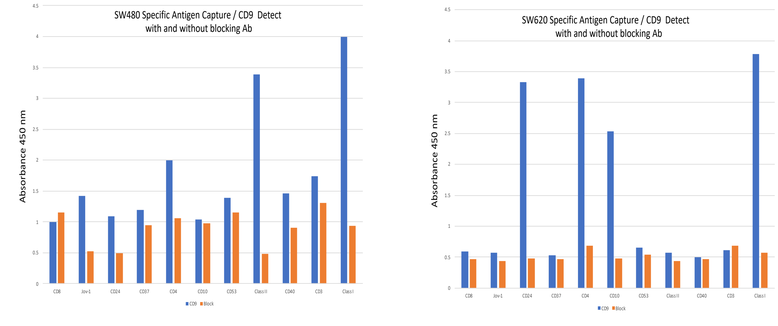

Figure Four: SW480 and SW620 EV preps captured in absence or presence (Blocked) of soluble matched capture antibody. Detection antibody was anti tetraspanin CD9 for both conditions. Blocking soluble antibodies were present during overnight capture. Blocking antibodies were used at 20X concentrations. Both preparations had similar amounts of Class I captured EVs detected by anti CD9. Blocking was nearly complete. In contrast, TCR B1 (Jov-!), CD3, and Class II MHC signals were present in SW480 preps but not in SW620. CD10 signal was greater in SW620 compared to SW480. While the CD8 capture signal was higher in SW480 than SW620, soluble antibody did not inhibit the capture of EVs in these wells.

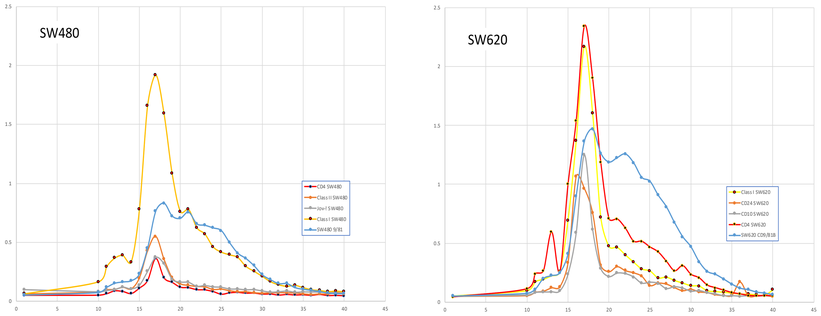

Figure Five: Size Exclusion Chromatography (SEC) of 50X SW480 and SW620 EV preps. Column bed was Sephacryl S500 HR (40,000 to 20,000,000 MW). A 1 ml volume was applied to column and 1 ml fractions collected. Capture and detect antibody pairs are shown in legend. Tetraspanin capture (CD9) was detected with Tetraspanin detect (CD81) shown for both preps. The values of the tetraspanin capture curves (CD9/CD81B) are reduced by dilution. The specific antigens (Class I MHC, CD4, Class II MHC, Jov-1, CD10, and CD24) were detected with anti-CD9 tetraspanin antibody. Tetraspanin binding was observed across the different retained fractions. Tetraspanin antigenicity is present in the void volume as well as the included volume. The overall tetraspanin capture and detect curve shapes appear similar for the SW480 and SW620 preparations.

CONCLUSIONS

1. EVs collected in vitro from SW480 and SW620 colon cancer cells show differences in antigenicity.
2. Tetraspanin density appears to be different in EVs obtained from SW480 when compared to SW620 cells. SW480 EVs showed steeper CD9 dilution curves compared to SW620 suggesting that antigen availability was greater for SW480 EVs.
3. Immune associated antigens are present on EVs prepared from both SW480 and SW620 cell cultures. These antigens include T cell antigens, CD4, CD3, TCR-B1 and possibly CD8. Additionally, there are differences in the CD10, Class II MHC, CD24 antigenicity in EVs obtained from the two cell lines.
4. There are diverse EV populations in the preparations examined, both regarding antigenicity and size, based on behavior on SEC.
5. EVix Dual antigen capture/detect ELISA kits are useful in phenotyping EV populations and can be used during isolation or characterization of EVs via SEC.