

Circulating exosomes may provide an alternate platform to monitor disease progression compared to circulating tumor cells

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Abstract

Background: Circulating Tumor Cells (CTCs) have been used to monitor disease progression in patients with different types of metastatic cancer. However, only 50% of metastatic breast, 57% of metastatic prostate cancer, and 18% of metastatic colon cancer blood specimens have adequate levels of CTCs for clinical laboratory analysis. Levels of exosomes, have recently been shown to correlate with tumor progression. **Methods:** Exosomes from 1 ml of plasma were isolated by ultracentrifugation. CD-81 antibodies were used to capture and measure the exosome level of breast cancer samples and healthy controls. CTCs were measured using the Cell Search CTC test protocol. Subsequently, EpCam positive exosomes were captured from metastatic breast (n=10), prostate (n=2), and colon cancer (n=3) samples, and compared to healthy controls (n=7). RNA was extracted from the EpCam positive exosomes and microRNA-21 (miR-21) expression was quantified by a qRT-PCR. **Results:** The preliminary study of breast cancer samples established 11 of the 14 samples (78.6%) had CD-81 specific exosome levels significantly above the level found in the 4 healthy samples (p=0.002). Only 7 of the 14 (50%) specimens analyzed had more than 5 CTCs, the clinical threshold for metastatic breast cancer. Three cancer samples had CD-81 measured exosome levels below the average of normal samples, one of these had >5 CTCs. miR-21 analysis of 15 additional metastatic cancer specimens, 5 of which had >5 CTCs, found miR-21 averaged 4.2×10^6 , 4.82×10^6 and 5.05×10^6 copies in the breast, prostate, and colon cancer samples respectively. Conversely, the plasma specimens from healthy donors collected in EDTA tubes averaged 1.8×10^4 copies of miR21.

Results

CD-81 Labeled Exosome Level vs CTCs in Plasma Derived Exosomes

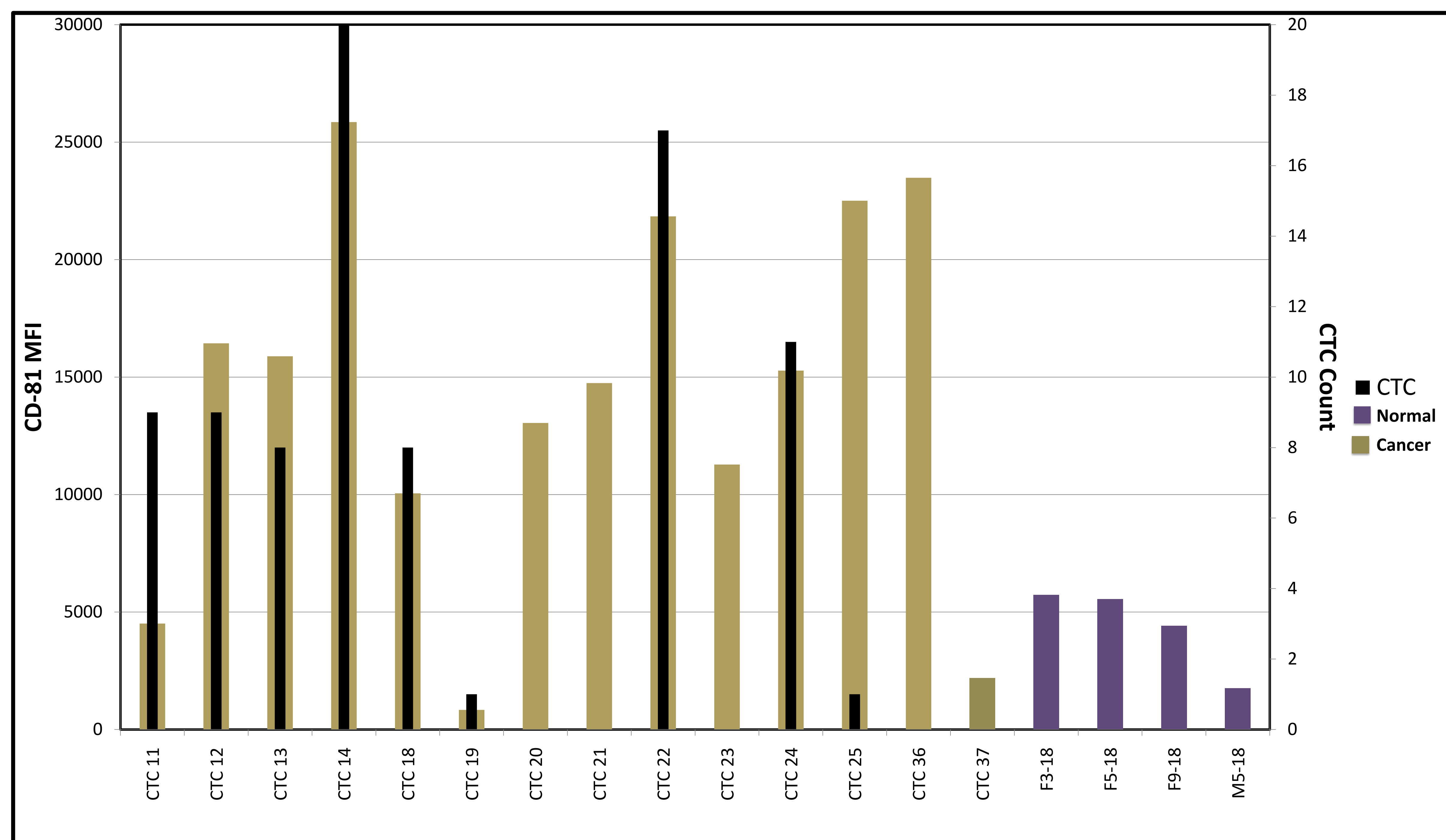


Figure 1: Exosomes collected from patient and normal plasma had exosomes levels measured with CD-81 and CTCs counted. Normal samples are purple and breast cancer samples are gold.

miR-21 Copy Number vs CTCs in EpCAM+ Plasma Derived Exosomes

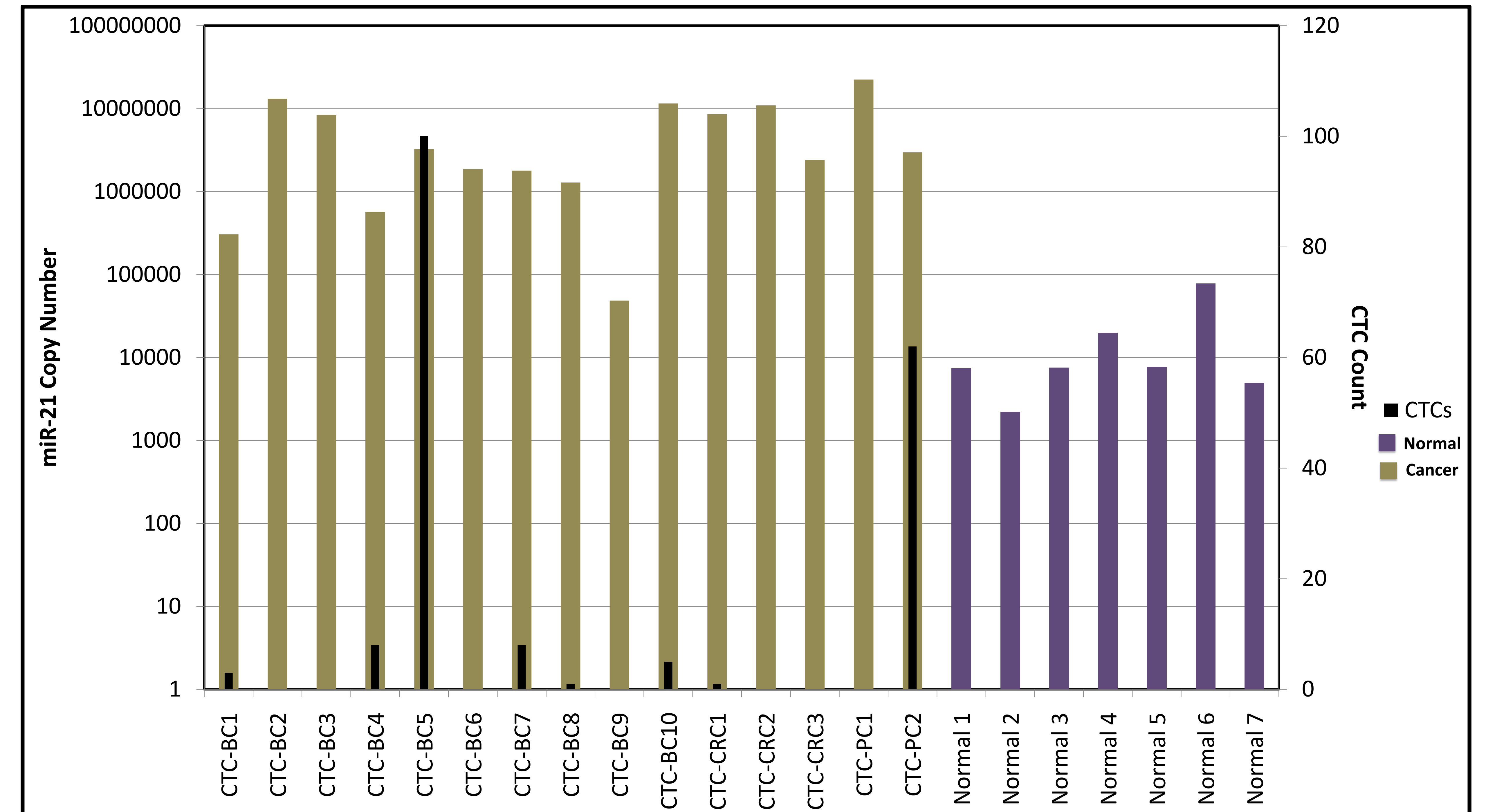


Figure 2: Copy number assessed by qRT-PCR of miR-21 from RNA extracted from EpCAM+ plasma derived exosomes. CTC counts were obtained from the same samples. Normal samples are purple and cancer samples are gold.

Exosomes

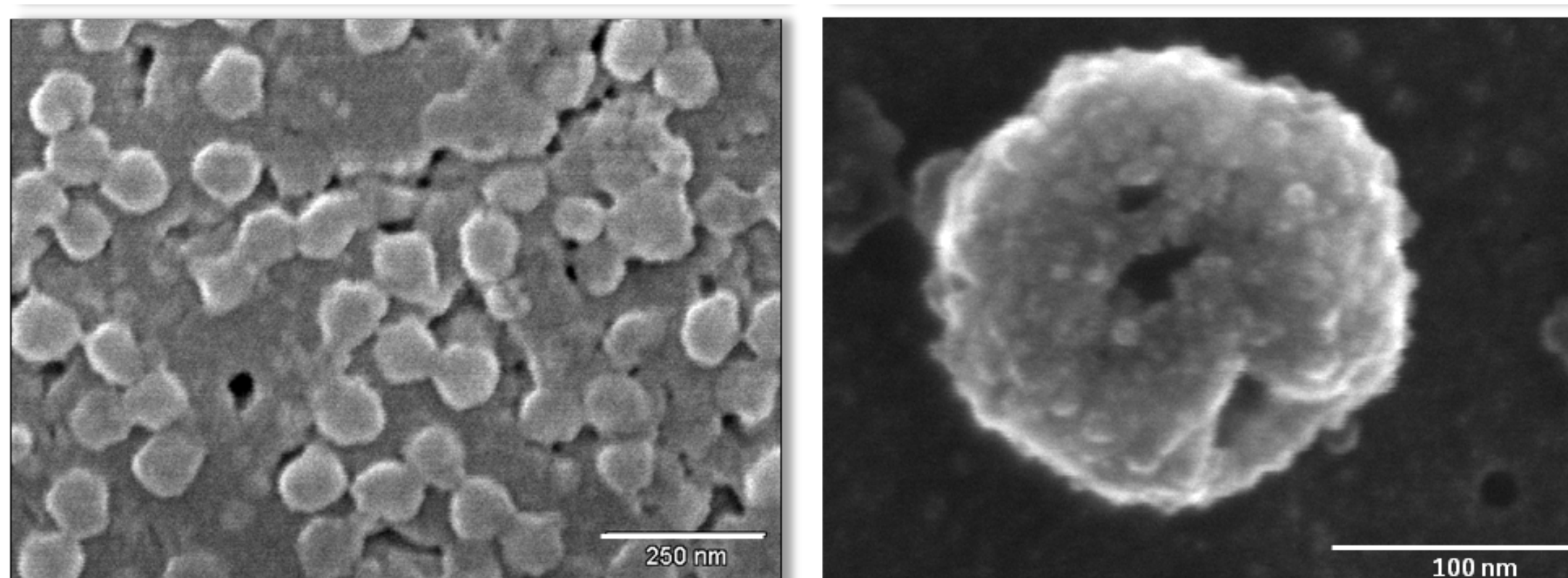


Figure 3. Scanning Electron Images (SEM).. (A) Vcap exosomes captured with Epcam. (B) Vcap exosome captured with Epcam. SEM was performed to visually inspect the size of exosomes being captured with our platform. Size distribution confirms that the majority of vesicles captured are within the 50-150 nm diameter size range defined for exosomes.

Figure 3 A

Figure 3 B

Conclusions

Conclusions: This study suggests that exosome analysis from plasma samples may offer a greater opportunity to monitor and track disease than CTC analysis, but it should be noted that further study will be necessary to establish clinical definitions of exosome load. Furthermore, tumor-derived exosomes provide the ability to characterize tumor of origin miR content, which presents opportunities for tumor-specific exosome-based biomarker analysis from a blood sample.