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Screening of Exosomal MicroRNAs From Colorectal Cancer Cells

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Title: Screening of Exosomal MicroRNAs from Colorectal Cancer Cells

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ABSTRACT

Background: Cells release extracellular membrane vesicles including microvesicles known as exosomes. Exosomes contain microRNAs (miRNAs) however the full range within colorectal cancer cell secreted exosomes is unknown.

Objective: To identify the full range of exosome encapsulated miRNAs secreted from 2 colorectal cancer cell lines and to investigate engineering of exosomes over-expressing miRNAs.

Methods: Exosomes were isolated from HCT-116 and HT-29 cell lines. RNA was extracted from exosomes and microRNA array performed. Cells were engineered to express miR-379(HCT-116-379) or a non-targeting control (HCT-116-NTC) and functional effects were determined. Exosomes secreted by engineered cells were transferred to recipient cells and the impact examined.

Results: Microvesicles 40-100nm in size secreted by cell lines were visualised and confirmed to express exosomal protein CD63. HT-29 exosomes contained 409 miRNAs, HCT-116 exosomes contained 393, and 338 were common to exosomes from both cell lines. Selected targets were validated. HCT-116-379 cells showed decreased proliferation (12-15% decrease, $p < 0.001$) and decreased migration (32-86% decrease, $p < 0.001$) compared to controls. HCT-116-379 exosomes were enriched for miR-379. Confocal microscopy visualised transfer of HCT-116-379 exosomes to recipient cells.

Conclusions: Colorectal cancer cells secrete a large number of miRNAs within exosomes. miR-379 decreases cell proliferation and migration, and miR-379 enriched exosomes can be engineered.

Key words: exosomes, microRNAs, colorectal cancer

Abbreviations: HCT-116-379; HCT-116 colorectal cancer cells transduced with a miR-379 over-expresing lentiviral vector, HCT-116-NTC; HCT-116 colorectal cancer cells transduced with a non-targeting control lentiviral vector, HCT-116-WT; non transduced, wild-type HCT-116 colorectal cancer cells, CRC – Colorectal Cancer, PBS – phosphate buffered saline

INTRODUCTION

Cancer cells are capable of promoting pro-tumorigenic features such as angiogenesis through paracrine signalling which can potentially promote metastases [1]. In addition to release of soluble proteins, multiple cell types including colorectal cancer cells have been shown to release a variety of extracellular membrane vesicles including microvesicles known as exosomes. Exosomes are lipid vesicles with a diameter of 40-100nm which are highly stable, facilitating collection from various body fluids as well as cell culture media [2]. They are known to contain lipids, DNA and RNA and have recently been recognised as vehicles capable of facilitating intercellular communication [3]. Emerging evidence indicates that exosomes may play a role in a range of biological processes through transfer of their contents from donor cells to recipient cells [4]. In cancer, exosomes also play a significant role with evidence showing a pro-tumorigenic effect associated with the transfer of protein content [5]. One of the most exciting aspects of exosomes involvement in cancer is that they have recently been found to contain miRNAs [6].

miRNAs are a class of small non-coding RNA molecules 19-25 nucleotides in length. miRNAs regulate gene expression at a post transcriptional level leading to the inhibition of mRNA translation or mRNA degradation [7]. Functional studies have shown miRNAs to participate in almost every cellular process including apoptosis, proliferation and differentiation [7, 8]. Some miRNAs are dysregulated in the tissues and circulation of CRC patients making them promising as biomarkers [8, 9]. Recent studies, however, have reported a number of miRNAs believed to be released by colorectal tumours as circulating markers of disease are expressed by red blood cells and may be present in the plasma and serum secondary to haemolysis [10]. Tumour derived exosomal miRNAs may provide a more specific miRNA signature advancing the potential of early detection and individualised treatments [11, 12].

Recent studies have shown exosomes are capable of delivering chemotherapeutic agents in vitro and can deliver anti-tumour miRNAs to breast cancer cells and prostate cancer cells when injected systemically in vivo or directly into tumours [13-15]. Identification of potent tumour suppressor miRNAs in CRC is useful for development of therapeutics in this regard. miR-379 has previously been identified as a tumour suppressor in breast cancer [16]. A genome wide analysis of miRNAs imprinted on the 14q32.31 locus including miR-379 reveals downregulation of these miRNAs in human cancers indicating this entire miRNA cluster is a locus of tumour-suppressors [17]. These data, taken in conjunction with previous studies showing compelling evidence that miR-379 is a tumour suppressor in breast cancer, indicate miR-379 is an ideal candidate for further investigation in CRC. This study aimed to firstly identify the full range of miRNAs packaged into exosomes and secreted by two CRC cell lines with different phenotypical characteristics. The effect of miR-379 in CRC cells and its potential to be transferred to recipient cells in exosomes was also examined.

METHODS

Cell Lines and Culture Conditions

HCT-116 and HT-29 CRC cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cell lines were last authenticated in May 2014 using DNA fingerprinting. Cells were cultured in McCoys-5A media supplemented with 10% fetal bovine serum (FBS) and 100U/ml penicillin/ 100 µg streptomycin (P/S). Cells were incubated at 37^0C and 5% CO_2.

Isolation of CRC Cell Secreted Exosomes

To prepare exosome-free media, exosomes were removed from FBS. FBS was centrifuged at 300 x g for 10 minutes followed by 2000 x g for 10 minutes to remove cellular debris. The supernatant was then passed through a 0.2µm sterile filter. Exosomes were separated from the supernatant by ultracentrifugation at 110,000 x g at 4°C for 70 minutes. After ultracentrifugation the exosome free FBS supernatant was collected.

For exosome isolation, 2 x 10^6 cells were seeded into T-175 flasks containing 17 mLs of pre-prepared exosome free culture medium and cultured for 48 hours. A process of differential centrifugation, passage through a microfilter and ultra-centrifugation was performed to isolate exosomes [6]. In detail, following 48 hours incubation the media containing exosomes secreted by cells was harvested. The cells remaining in the T-175 cm2 flask were then trypsinised, counted and stored. A falcon containing cell conditioned media with exosomes was placed into a centrifuge and spun at 300 x g for 10 minutes at 4 ^{o}C. The media was then carefully collected from the falcon using a pipette leaving 1-2 mL remaining at the bottom which contains cellular debris. Collected media was then transferred to a new falcon. This underwent centrifugation at 2000 x g for 10 minutes at 4 ^{o}C. It was carefully collected from the falcon using a pipette leaving 1-2 mL remaining at the bottom, passed through a 0.2 µm

sterile filter and collected in a new 50 mL falcon. A primary fixative was added at this point prior to ultracentrifugation to maintain exosome morphology if exosomes were being isolated for visualisation with Transmission Electron Microscopy (TEM). The media containing exosomes was then transferred to an ultracentrifugation tube taking care to avoid any bubble formation. A Sorvall 100SE ultracentrifuge was used with a Ti70 fixed angle rotor. The following settings are used on the ultracentrifuge: spinning time was set for 1 hour and 10 minutes, temperature was set at 4 ^{o}C, acceleration was set at 9, deceleration at 1 and speed was set at 100,000 x g. The ultracentrifugation tubes were carefully removed from the rotor following the spin and the supernatant collected from the tubes using a disposable pipette. The supernatant represents the cell conditioned media from which exosomes have been removed. The exosome pellet was collected by pipetting 60 µL of PBS up and down the area marked where the exosome pellet forms in the ultracentrifugation tube. Alternatively if exosomes were harvested for Western Blot analysis, protein lysis buffer was used to re-suspend the exosome pellet.

Transmission Electron Microscopy

HCT-116 and HT-29 exosomes were isolated as previously described. A primary fixative (2% Glutaraldehyde, 2% Paraformaldehyde in a 0.1M Sodium Cacodylate/HCL buffer at a pH of 7.2) was added to exosome containing media prior to the final ultracentrifugation step. Exosomes were re-suspended in 50µL of PBS and treated with a secondary fixative (1% Osmium Tetroxide). The resultant exosome pellet was subsequently dehydrated with graded alcohol and then incubated in resin in a 65°C oven. The resin block was trimmed using an ultramicrotome to sections of 80-100nm thickness (Reichert-Jung Ultracut E). Slices were loaded onto a copper grid and stained with 1.5% aqueous uranyl acetate followed by lead citrate. Viewing was performed with a Hitcachi H7000 Transmission Electron Microscope.

Western Blotting

HCT-116 and HT-29 secreted exosomes were collected as previously described. After ultracentrifugation, exosomes were re-suspended in Triton-X lysis buffer [150mM NaCl, 20 mM HEPES, 2 mM EDTA, 1% Triton-X100, 2mM Sodium Orthovanadate, 10mM Sodium Fluoride, 10ul/mL Protease inhibitor cocktail (Fisher Scientific)] and frozen at -20°C. Protein content was determined using the Micro BCA™ Protein Assay Kit (Thermo Scientific). Protein (100μg) was reduced in DTT (0.5 M) for 10 minutes at 70°C and samples run on a 4-15% gradient pre-cast Mini-PROTEAN® TGX™ Gels (Bio-Rad) for 60 minutes at 100V. Protein molecular weight standards (20-220 kDa) were run simultaneously on each gel. Electroblotting was performed for 60 minutes at 100V to transfer protein samples to a nitrocellulose membrane. Blots were blocked in 5% milk in TBS-T for 1 hour, and probed with an antibody targeting CD63 (1:1,000; Abcam) overnight and washed in TBS-T. Horseradish peroxidase labelled goat anti-rabbit (1:3,000; Abcam) secondary antibody was then added to the membranes for 1.5 hours. Following washing steps, SuperSignal West Dura Chemiluminescent substrate (Thermo Scientific) was applied to the membranes for 5 minutes. Images were captured using a Syngene G-Box and GeneSnap software.

RNA extraction and microRNA array

RNA was extracted from exosomes using the *mir*Vana™ miRNA isolation kit following manufacturer's instructions. The Nanodrop™ Spectrophotometer (Thermo Scientific) was used to assess RNA present. Samples extracted from HCT-116 and HT-29 exosomes were then delivered to Exiqon Services, Denmark. The quality of the total RNA was verified by an Agilent 2100 Bioanalyzer profile. All RNA from sample and artificial reference was labeled with Hy3™ and Hy5™ fluorescent label, respectively, using the miRCURY LNA™ microRNA Hi-Power Labelling Kit, Hy3™/Hy5™ (Exiqon, Denmark). The Hy3™-labeled samples and a Hy5™-labelled reference RNA sample were mixed pair-wise and hybridized

to the miRCURY LNA™ microRNA Array 7th Gen (Exiqon, Denmark), which contains capture probes targeting all human microRNAs registered in miRBASE 18.0 [18]. The hybridization was performed according to the miRCURY LNA™ microRNA Array Instruction manual. The miRCURY LNA™ microRNA Array slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc., USA) and image analysis was carried out using the ImaGene 9.0 software (BioDiscovery, Inc., USA). The quantified signals were background corrected [19] and normalized using quantile normalization method. Technical quality assessment was performed based on results from spike in controls in the array. miRNAs above a certain threshold were considered present. The background threshold was calculated for each individual microarray slide as 1.2 times the 25th percentile of the overall signal intensity of the slide. Expression levels were then calculated on a scale from 0-10 and those with levels less than 4 were deemed undetectable.

Target validation using RQ-PCR

Exosomal miRNAs of interest were reverse transcribed using the MultiScribe™-based High-Capacity cDNA Archive Kit (dNTP 100mM, RT Buffer 10x, RNase Inhibitor 20U/µl, Stem loop primer 50nM, MultiScribe RT 50U/µl) (Applied Biosystems) and miRNA-specific primers. The miRNAs were quantified by real-time PCR using TaqMan microRNA kits (Life Technologies) and the ABI 79000 Fast real-time PCR system (Applied Biosystems) These reactions were carried out in a final volume of 10 µl comprising of 0.7 µl cDNA, 5 µl TaqMan® Universal PCR fast Master Mix (2x), 0.5 µl TaqMan® primer-probe mix (0.2µM), Forward primer (1.5µM), and Reverse Primer (0.7µM) (Applied Biosystems). For target validation the average cycle threshold at which the selected miRNA appeared is given as the mean cycle threshold (CT) over 3 separate exosome isolates +/- standard deviation.

Cell transduction

HCT-116 cells were transduced with a miR-379 (mature sequence: UGGUAGACUAUGGAACGUAGG; 50nM, HCT-116-379) or a non-specific targeting control miRNA (non-target control, HCT-116-NTC) mimic (SMARTchoice shMIMIC Lentiviral microRNAs). Lentiviral vectors contained a red fluorescent protein and Puromycin resistance element. Transductions were performed using polybrene at a final concentration of 8μg/mL. Puromycin selection was commenced 48 hours after transduction at a concentration of 8μg/mL. To confirm adequate transduction efficiency cells were fixed in 4% paraformaldehyde, nuclei were counterstained with DAPI and visualised using a fluorescence microscope (Olympus BX60, analaSIS® software - Ridom GmbH, Münster, Germany) to confirm the presence of red fluorescent protein. Confirmation of stable transduction was performed with RQ-PCR at 1 and 2 weeks. miR-16 was used as the endogenous control. miR-379 expression was calculated in miR-379 over-expressing versus NTC cells. For every sample, reactions were performed in triplicate for the miRNA of interest and the endogenous control miRNA. The relative quantity of mRNA and miRNA expression was calculated using the comparative cycle threshold (ΔΔCt). The geometric mean of the Ct value was used to normalise the data and the sample with the lowest expression level was applied as a calibrator. Following confirmation of transduction, exosomes secreted by HCT-116-379 cells and HCT-116-NTC were harvested as previously described and miR-379 levels were assessed using RQ-PCR to determine if miR-379 over-expression was observed in HCT-116-379 exosomes establishing engineering of miRNA enriched exosomes.

Proliferation Assays

To assess the effect of miR-379 on cell proliferation, cells were seeded into a 96 well plate at a density of 1 x 10^3 cells per well. HCT-116-379 and HCT-116-NTC cells were seeded and incubated for 24, 48 or 72 hours. At each of these time-points cell proliferation was measured using a CellTiter 96® AQ_{ueous}Non-Radioactive Cell proliferation Assay (Promega). To

establish any functional effect exerted by exosomes secreted by transduced cells exosomes were isolated from HCT-116-379, HCT-116-NTC and HCT-116-WT cells as previously described. HCT-116-WT cells were seeded onto a 96 well plate at a density of 1 x 10^3 per well and received media containing exosomes from HCT-116-379, HCT-116-NTC or HCT-116-WT cells. Proliferation was measured at 48 hours. Each experiment was performed in triplicate with results expressed against a negative control (cells in exosome depleted media) as mean +/- SEM. P values were calculated using ANOVA.

Migration Assays

To assess the effect of miR-379 on cell migration, Transwell® Permeable Supports (Corning Inc, Sigma–Aldrich) with 8.0 μm pores were used to track migration of HCT-116-379 and HCT-116-NTC cells in response to a chemoattractant (10% FBS). 7.5 x 10^4 cells were seeded onto the membranes in serum free media. Migration in response to the same serum free medium was employed as a negative control. Migrated cells were stained using haematoxylin and counted in five fields of view per membrane using an Olympus B×60 microscope and image analysis software. Each experiment was repeated in triplicate, with results expressed against the negative control as Mean +/- SEM. P values were calculated using Students T test (two tailed).

Confocal microscopy

Exosomes were isolated from transduced cells expressing a red fluorescent protein. Recipient WT cells without a red fluorescent protein were seeded onto glass coverslips at a density of 4 x 10^5 per coverslip. Isolated exosomes were added to coverslips and incubated in a humidification box for 4 hours. Following incubation cells on coverslips were fixed with 4% Paraformaldehyde. DNA in cell nuclei was then stained using DAPI (Invitrogen) and the slides mounted using slowfade gold anti-fade reagent (Invitrogen). Immunofluorescent Z-

stack images (0.1 μm steps) were captured using an Olympus IX81 Microscope fitted with an Andor Revolution Confocal system (Andor, Belfast, Northern Ireland), 60× oil immersion objective lens and an EMCCD Andor iXonEM + camera.

RESULTS

Exosome characterisation

Microvesicles with a spherical morphology ranging in size between 40 and 100 nm consistent with exosomes were found to be secreted by both cell lines and successfully visualised with TEM (Figure 1A). Western Blot for the exosome-associated protein CD63 revealed a characteristic band between 50-60 kDa in size (Figure 1B).

Identification of exosomal microRNA content

Expression analysis revealed the number of miRNAs present in the exosomes of both CRC cell lines to be within the expected range for human samples. 409 miRNAs were detected above threshold in HT-29 derived exosomes. 393 miRNAs were detected in HCT116 exosomes, with 338 miRNAs common to exosomes from both HCT-116 and HT-29 cells. The 20 most highly expressed miRNAs in HCT-116 cell secreted exosomes and HT-29 cell secreted exosomes were identified (Table 1). The miRNAs which were found to have the highest difference in expression levels between the exosomes secreted by either HCT-116 or HT-29 cell lines were also identified (Table 2).

Validation of selected array data by qRT-PCR

Selected miRNAs (miR-10b, miR-143 and miR-149-5p) were validated in three separate exosome isolates from both HCT-116 and HT-29 cell secreted exosomes. miR-10b, miR143 and miR-149-5p were consistently detectable in exosome extracts from both cell lines. miR-10b (HCT-116 exosomes 36.25 +/- 0.84 CT, HT-29 exosomes 35 +/- 0.3 CT) , miR-143

(HCT-116 exosomes 36.42 +/- 2.42 CT, HT-29 exosomes 35.98 +/- 2.32 CT) and miR-149-5p (HCT-116 exosomes 35.41 +/- 1.85 CT, HT-29 exosomes 37.94 +/- 0.34 CT).

miR-379 over-expression in exosomes and functional impact

HCT-116 cells were transduced with a miR-379 and NTC-mimic as described, and the successfully transduced cells were visualized using fluorescence microscopy (Figure 2A). A significant elevation in miR-379 expression in HCT-116-379 cells (2.20 $\log_{10}$ RQ increase relative to HCT-116-NTC) was confirmed at 1 and 2 weeks following transduction (Figure 2B). Proliferation at 24, 48 and 72 hours was determined in HCT-116-379 cells compared to NTC cells. A significant decrease in proliferation was observed in HCT-116-379 cells compared to HCT-116-NTC cells (12-15% decrease, ANOVA $p < 0.001$, Figure 3A). A significant decrease of up to 86% was observed in HCT-116-379 cell migration towards a chemo-attractant (10% FBS) when compared to HCT-116-NTC cells (32-86% decrease, $p < 0.001$, Figure 3B). Exosomes secreted by HCT-116-379 cells contained detectable levels of miR-379 while those secreted by WT and NTC cells did not.

Transfer of exosomes between cell populations and functional effects

Exosomes with a red fluorescing protein were isolated from miR-379 over-expressing cells. Recipient WT cell nuclei were stained blue with DAPI. Exosomes isolated from HCT-116-379 cells were incubated with HCT-116-WT cells and confocal microscopy visualised exosomes clustering around HCT-116-WT cells suggesting uptake of miR-379 enriched exosomes by HCT-116-WT cells (Figure 4A). Proliferation of HCT-116-WT cells in response to -379, -NTC and -WT secreted exosomes was measured at 48 hours. A trend towards decreased proliferation of WT cells in response to miR-379 exosomes compared to NTC and WT exosomes was observed, although this was not significant (Figure 4B).

DISCUSSION

Dysregulated circulating miRNAs have been identified as promising biomarkers in CRC in several studies but contradicting evidence exists regarding their reproducibility [9]. Patient factors, medium from which miRNA is extracted (whole blood/serum/plasma), and differing collection and storage have led to heterogeneous data. Exosomal miRNAs may provide tumour specific biomarkers negating these concerns. The profiling of miRNAs secreted within exosomes by both HCT-116 and HT-29 CRC cells has identified a large number of miRNAs. Overall, when non-human spike-in miRNAs were excluded, array data revealed 409 miRNAs detected above threshold in HT-29 derived exosomes. 393 miRNAs were detected in HCT-116 exosomes, with 338 miRNAs common to exosomes from both HCT-116 and HT-29 cells. Analysis of miRNA expression levels in both CRC cell secreted exosomes show areas with distinct differences. The differences between the miRNA profiles of exosomes secreted by these two distinct CRC cell lines may represent differences in growth pattern and phenotype [20].

Previous studies have identified selected miRNAs present in exosomes secreted by CRC cells in vitro [11]. A number of miRNAs have also been identified in exosomes isolated from the circulation of CRC patients [12]. This is the first study to describe the full range of miRNAs present in both HCT-116 and HT-29 CRC cell secreted exosomes, demonstrating a large range of miRNAs some of which have well described roles in cancer pathogenesis, and others which have only recently been discovered. Among the most highly expressed miRNAs in both CRC cell line exosomes are several which have previously described roles in gastrointestinal cancers (miR-10b-3p, let7b-5p, miR-378c, miR-145-5p) [21-24]. In addition, included in the most highly expressed are some miRNAs with little or no available data regarding their association with disease states, associated gene targets or functional roles. These miRNAs may represent new targets as biomarkers worthy of further investigation.

miR-10b was found to be among the most highly expressed miRNAs in both CRC cell line exosomes and was validated in multiple exosome samples. High levels of miR-10b have been associated with a higher incidence of lymphatic invasion and poor prognosis in CRC tissues [25]. Interestingly, exosome-mediated transfer of miR-10b to recipient non-invasive breast cancer cell lines has been shown to promote cell invasion in breast cancer [26]. miR-143 and miR-149-5p were also validated. These miRNAs were not among the most highly expressed but the fact that they are detectable in multiple exosome isolated from both CRC cell lines indicates validity of array data.

miR-379 down-regulates several genes involved in the TGF-β signalling pathway and a large proportion of CRCs are known to display abnormalities in TGF-β pathway factors [27,28]. A significant reduction in colorectal cancer cell proliferation in response to over-expression of miR-379 was observed. Cells over expressing miR-379 were found to proliferate between 12-15% slower compared to controls. A marked reduction in migration of CRC cells in response to over-expression of miR-379 was observed ranging as high as an 86%. This effect is interesting as miR-379 has a documented effect on cyclin B1. Cyclin B1 in turn has a documented inhibitory effect on E-cadherin leading to induction of CRC cell migration and invasion which is contrary to the effect observed in this study and worthy of further investigation [28]. Exosomes which were secreted from miR-379 over-expressing cells were found to contain detectable levels of miR-379 compared to exosomes secreted by HCT-116-NTC and HCT-116-WT cells. In addition, miR-379 was not detected through miRNA array performed on HCT-116-WT exosomes. Incubation of HCT-116-WT cells in media containing exosomes enriched in miR-379 produced an observable decrease in proliferation however it was not significant. As the effect of miR-379 over-expression on migration was very significant, ranging from 32-86%, further investigation of the effect of miR-379 enriched exosomes on cell migration may yield a more pronounced effect.

The data presented identifies a wide range of miRNAs present in CRC cell secreted exosomes which may provide useful targets as biomarkers for further investigation. miR-379 displays tumour suppressor properties in CRC cells and its potential to be delivered to recipient cells warrants further investigation.

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Figure and Table Legends

Figure 1

Title: Characterisation of exosomes isolated from HCT-116 and HT-29 colorectal cancer cell lines.

A) Transmission electron microscopy images of exosomes from HCT-116 and HT-29 CRC cells (bar = 500nm)

B) Western Blot for exosome associated protein CD63 performed with protein extracted from both HCT-116 and HT-29 CRC cell lines.

Figure 2

Title: Confirmation of transduction forcing miR-379 over-expression in HCT-116-379 CRC cells

A) Visualisation of transduced cells with fluorescence microscopy. (i) - Cell nuclei stained blue with DAPI. (ii) – Red Fluorescent Protein of successfully transduced cells

B) Elevated miR-379 expression in HCT-116-379 cells 1 and 2 weeks following transduction

Figure 3

Title: Analysis of functional effects of miR-379 over-expression

A) Cell proliferation following transduction resulting in a decrease in proliferation (range 12-15%) in the presence of miR-379 over-expression ($p<0.001$)

B) Cell migration following transduction resulting in a decrease in migration (range 32 86% decrease) in the presence of miR-379 over-expression ($p<0.001$)

Figure 4

Title: Exosome transfer and functional effect on recipient cells

A) Confocal microscopy image of exosomes isolated from miR-379 over expressing cells (red due to RFP labelling) clustering around WT cells (blue DAPI stained nuclei).

B) Functional effects of exosomes isolated from HCT-116-WT, HCT-116-NTC and HCT-116-379 cells on recipient WT cells.

Table 1

Title: The 20 most highly expressed miRNAs in HCT-116 and HT-29 cell secreted exosomes. (Complete dataset available at www.microvesicles.org. Experiment ID – Vesiclepedia_557) [29]

Table 2

Title: The 10 most differentially expressed miRNAs contained within exosomes secreted by both cell lines (the miRNAs with the most significant difference in expression in one cell line compared to another)

Table 1

Most highly expressed microRNAs in HCT-116 exosomes	Most highly expressed microRNAs in HT-29 exosomes
hsa-miR-3679-3p	hsa-miR-642b-5p
hsa-miR-4279	hsa-miR-10b-3p
hsa-miR-642b-5p	hsa-miR-3679-3p
hsa-miR-4454	hsa-miR-4279
hsa-miR-10b-3p	hsa-miR-943
hsa-miR-624-5p	hsa-miR-624-5p
hsa-miR-943	hsa-miR-1248
hsa-miR-4723-3p	hsa-miR-145-5p
hsa-miR-1249	hsa-miR-1249
hsa-miR-378c	hsa-miR-4723-3p
hsa-miR-1248	hsa-let-7b-5p
hsa-miR-718	hsa-miR-378c
hsa-let-7b-5p	hsa-miR-365a/b-3p
hsa-miR-1246	hsa-miR-30d-3p
hsa-miR-4274	hsa-miR-4274
hsa-miR-1207-3p	hsa-miR-718
hsa-miR-4728-3p	hsa-miR-548k
hsa-miR-365a/b-3p	hsa-miR-361-5p
hsa-miR-30d-3p	hsa-miR-1207-3p
hsa-miR-3679-3p	hsa-miR-4311

Table 2

HCT-116 cell secreted exosomes	HT-29 cell secreted exosomes
Differentially expressed microRNAs	**Differentially expressed microRNAs**
miR-4497	miR-1250-5p
miR-377-5p	miR-511-5p
miR-3960	miR-1224-3p
miR-670-5p	miR-29c-5p
miR-4787-5p	miR-3144-3p
miR-1246	miR-513c-5p
miR-5100	miR-25-3p
miR-4454	miR-192-3p
miR-1260b	miR-1225-5p
miR-4708-3p	miR-1304-5p

Figure 1

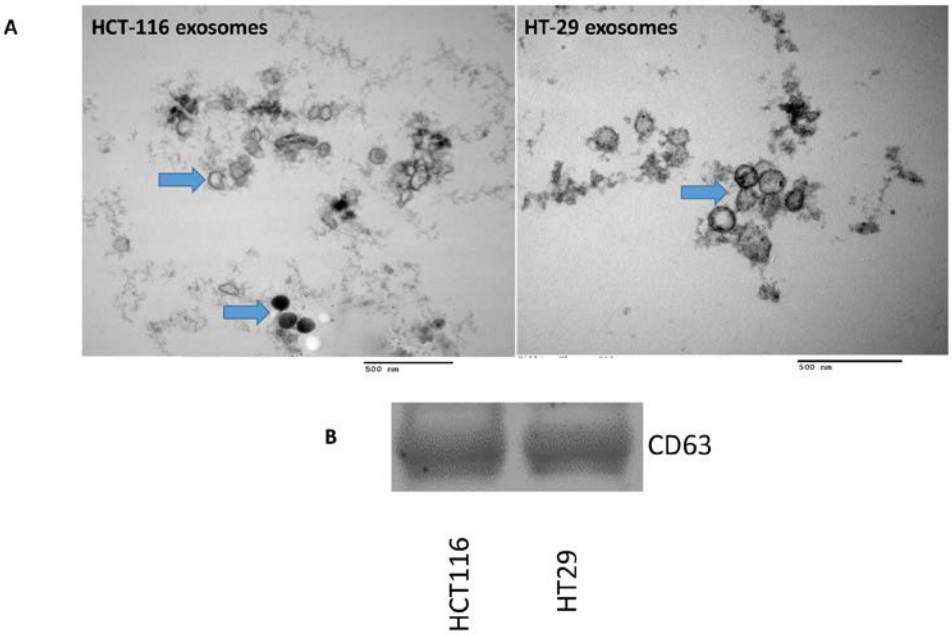

A
HCT-116 exosomes
HT-29 exosomes
500 nm
500 nm
B
CD63
HCT116
HT29

Figure 2

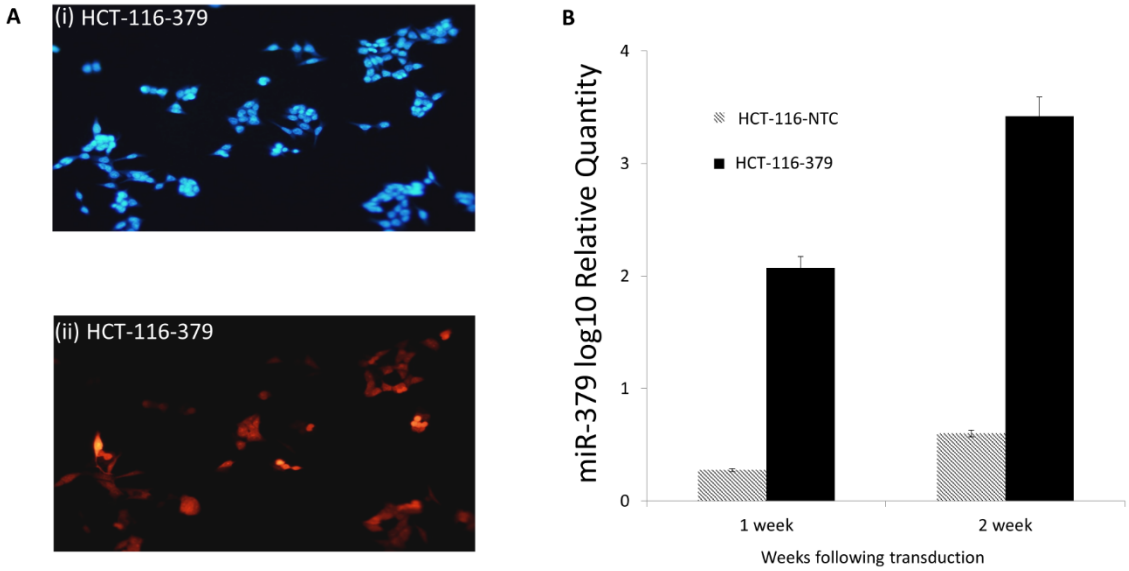

A
(i) HCT-116-379
(ii) HCT-116-379
B
miR-379 log10 Relative Quantity
4
3
2
1
0
HCT-116-NTC
HCT-116-379
1 week
2 week
Weeks following transduction

Figure 3

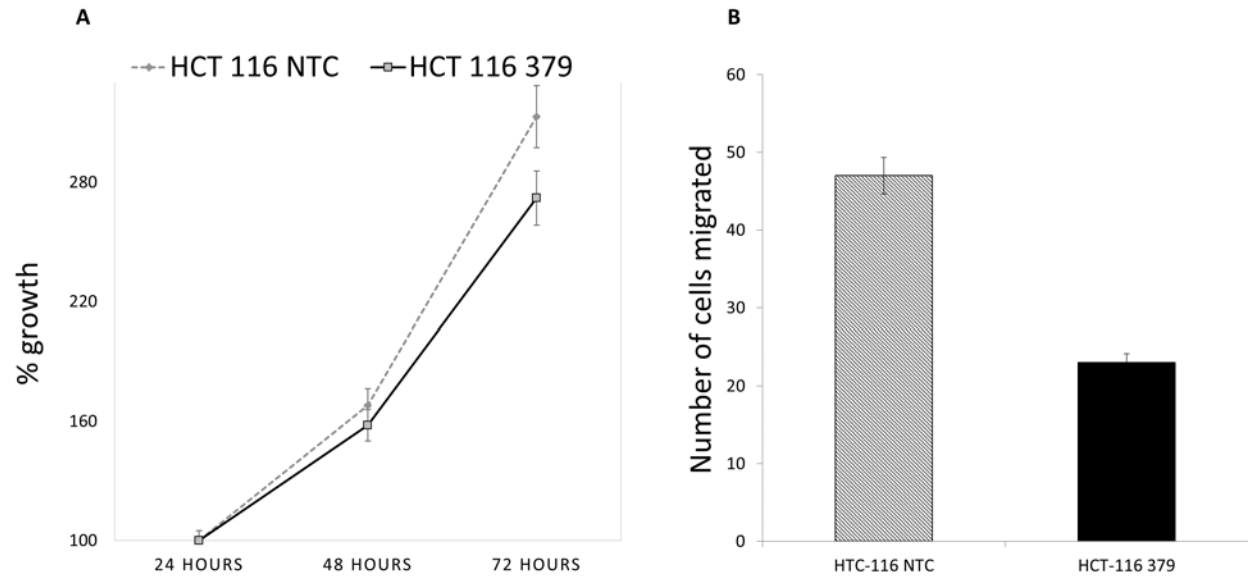
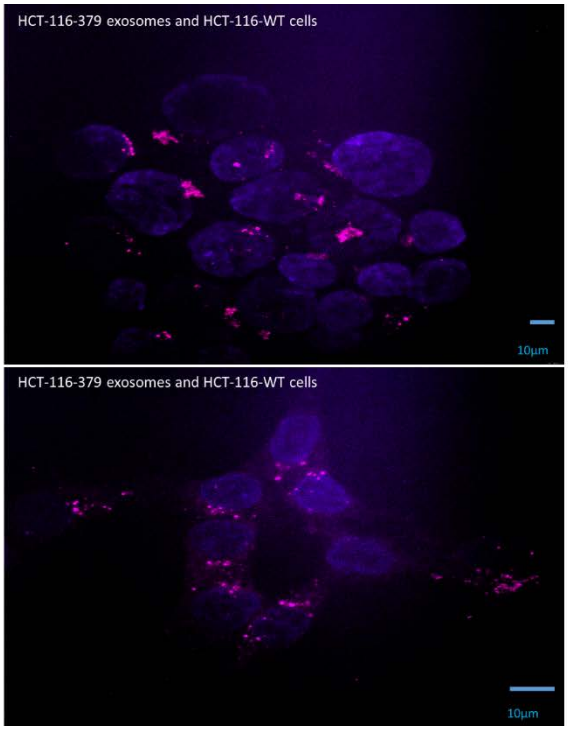
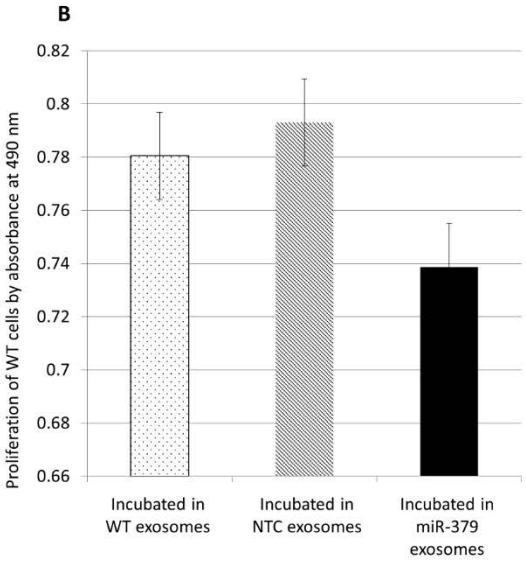

A
HCT 116 NTC
HCT 116 379
% growth
280
220
160
100
24 HOURS
48 HOURS
72 HOURS
B
Number of cells migrated
60
50
40
30
20
10
0
HTC-116 NTC
HCT-116 379

Figure 4

A


HCT-116-379 exosomes and HCT-116-WT cells
10µm
HCT-116-379 exosomes and HCT-116-WT cells
10µm

B


0.82
0.8
0.78
0.76
0.74
0.72
0.7
0.68
0.66
Proliferation of WT cells by absorbance at 490 nm
Incubated in WT exosomes
Incubated in NTC exosomes
Incubated in miR-379 exosomes