Malays J Pathol 2023; 45(2) : 195 – 204

ORIGINAL ARTICLE

Increased tube formation and up-regulation of FGFR3 mRNA expression in microvascular endothelial cell by exosomes derived from SW480-7

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Abstract

Introduction: Extracellular vesicles (exosome-like vesicles) are small membrane vesicles ranging from 20-200nm in size that are released by various cells into the extracellular space. These extracellular vesicles play a major role in cell-to-cell communication and contain materials, such as proteins, mRNAs, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). The effect of exosomes derived from an invasive colon cancer cell line on angiogenesis is unclear. Hence, the aim of this study is to investigate the effect of exosomes derived from an invasive colon cancer cell line on angiogenesis of endothelial cells. *Materials and Methods:* In the present study, the exosomes from the cell culture supernatants of an invasive colon cancer cell line SW480-7 were characterised. The effect on tube formation and expression of angiogenic genes in a microvascular endothelial cell, telomerase-immortalised microvascular endothelial cell (TIME) was examined after co-cultured with exosomes secreted from SW480-7. *Results:* Zetasizer result showed average diameter of exosomes derived from SW480-7 was 246.2 nm and morphological analysis showed the size of majority of exosomes were less than 200 nm. Results showed that exosomes derived from SW480-7 increased tube formation and up-regulated FGFR3 mRNA expression in TIME. *Conclusion:* Our findings suggest that exosomes derived from SW480-7 increased tube formation and up-regulated expression of FGFR3 mRNA in TIME.

Keywords: Exosomes, endothelial cell, SW480-7, colorectal cancer, FGFR3

INTRODUCTION

Exosomes are small membrane vesicles ranging in size between 20-150nm. Exosomes contain protein, lipid and significant amounts of nucleic acid such as miRNA, mRNA as well as lncRNA.[1] The numerous proteins and bioactive lipids in exosomes differ in composition depending on the types and states of donor cells.[2] Exosomes are not cellular debris. Increasing number of studies demonstrated that exosomes play critical roles in mediating cell to cell communication.[3] The role of exosomes has been extensively studies such as their role of exosome play in immunological responses in the tumour microenvironment, production of cancer associated fibroblasts, angiogenesis and metastasis.[1,3,4]

Angiogenesis is a process in which new blood vessels are formed[5] and is characterised by a number of cellular events including endothelial cell migration, invasion and differentiation into capillaries. Tumour growth and metastasis depend on angiogenesis.[6] Oxygen and nutrients are delivered via blood vessels to every part of the body, and also nourish malignant tumours.[7] Recently, numerous studies demonstrated that cancer-derived exosomes promote angiogenesis.[3] For example, exosomes secreted from melanoma cells promote metastasis and angiogenesis of endothelial cells.[8] Besides that, exosomes derived from chronic myelogenous leukaemia cells were reported to enhance migration and tube formation by endothelia cells.[9] Another study has also shown that exosomes derived from glioblastoma stimulate endothelial cell proliferation.[10,11] Colorectal cancer (CRC) is the third most common cancer diagnosed and second most in term of mortality.[12,13] In CRC, macrovesicles derived from SW480 (human adenocarcinoma cells) was reported containing cell cycle-related mRNA that promote proliferation of endothelial

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cells.[2] Together, these studies suggests the role of cancer-derived exosomes in angiogenesis and tumour progression.

Many studies have revealed the role of tumour-derived exosomes in the angiogenesis of endothelial cells. However, the effect of exosomes derived from invasive subpopulation of CRC has yet to be elucidated. Therefore, the aim of the present study was to investigate the effect of exosomes derived from invasive colon cancer cell line in tube formation as well as the level of mRNA expression in endothelial cells after treatment with exosomes. An invasive subpopulation of the colon cancer cell line SW480, namely SW480-7, which was previously-established via seven sequential passages of cells through the Matrigel-coated transwells was used in this study. The endothelial cells namely telomerase-immortalised microvascular endothelial cell (TIME) was used to examine the angiogenic effect of tumour-derived exosomes on tube formation of endothelial cells. This immortalised microvascular endothelial cells retain the characteristics of the original cell line.[14,15]

MATERIALS AND METHODS

Cell line and cell cultures

TIME was cultured in EBM-2 (Lonza, USA) supplemented with hEGF, hydrocortisone, FBS, VEGF, hFGF-B, R3-IGF-1, ascorbic acid and 12.5 mg/ml of blasticidine S at 37 °C and 5 % CO_2. An invasive subpopulation of colon cancer cell lines, SW480 namely SW480-7 was established *via* 7 sequential passages through matrigel-coated transwells.[16,17] SW480-7 was cultured in RPMI 1640 medium supplemented with 10 % FBS 100 U/ml penicillin, 100 ug/ml streptomycin, and 2 g/l sodium bicarbonate (Gibco, Invitrogen Corp., Carlsbad, CA). TIME and SW480-7 were grown in 25 cm^2 flask. The percentage of cell confluence was observed and the absence of bacterial and fungal contaminants was confirmed using an inverted phase contrast microscope. Once the cells reached 80-90% confluence, sub-culturing was performed.

Isolation of exosomes derived from supernatant of SW480-7

SW480-7 cell line was grown in RPMI 1640 medium supplemented with 10 % FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 g/l sodium bicarbonate. Once the cells grew and reached approximately 75% subconfluency, old medium was replaced with RPMI 1640 medium supplemented with 10% exosome-depleted FBS (cat. no. A2720801, Thermo Fisher Scientific), 100 U/ml penicillin, 100 ug/ml streptomycin, and 2 g/l sodium bicarbonate for 48 hours. The culture supernatant was then collected in a 15 ml conical tube and then centrifuged for 15 min at 3000 x *g* at 4 °C to remove cellular fragments and cell debris. The upper layer of supernatant was then transferred to a new 50 ml conical tube. Exosomes produced by SW480-7 in cell culture supernatants were isolated using exoEasy Maxi Kit (cat. no. 76064, Qiagen) according to the manufacturer's protocol. Purified exosomes were stored in 1.5 ml protein low binding tube (cat. no. 0030108.116, Eppendorf) at -20 °C for short term storage, and for longer term storage, the stock was kept at -80 °C.

Quantification of exosomal protein by BCA protein assay kit

The exosomal protein concentration was quantified by using Micro BCA Protein assay kit (cat. no. 23227, Thermo Fisher Scientific) according to manufacturer's instructions. Eluates that containing exosomes were lysed by mixing with 2X RIPA (radioimmunoprecipitation assay) lysis buffer and then lysates were quantified using the BCA Protein assay. Samples and standards were mixed with working reagent and incubated 30 minutes at 37°C. Absorbance was measured at 570 nm. The concentrations of the exosomes were quantified using BSA as standard. The working concentrations for experiment were freshly prepared by dilution with cell media.

Reconstitution of standard exosomes

Exosomes derived from SW480 cell culture supernatant (catalog no: HBM, PEC SW480 100/4) purchased from Hansa Biomed was used as a reference standard in characterisation of size distribution and TEM analysis. This exosome standard was reconstituted by adding deionised water, 100 μl for lyophilised standard 100 μg, to get a final concentration of 1 μg/μl. Reconstituted exosomes were transferred into a low protein binding tube and stored at -20°C for 1 month or at -80 °C for up to six months.

Size distribution analysis of exosomes by Zetasizer

The size distribution of exosome samples was analysed using a zetasizer nano S instrument (Malvern Instrument, UK). Two µl of exosomes standard from SW480 cell culture supernatants (0.10 μg/μl) was diluted with 1 ml distilled

water in a low volume disposable sizing cuvette. Twenty μl of exosomes (143 μg/ml) derived from SW480-7 were also diluted in 1 ml of ddH_2O.The size distribution was measured by the instrument as cumulant (z-average) size and polydispersity index (PDI), respectively.

Transmission electron microscope (TEM)
The morphology of exosomes derived from SW480-7 cell culture supernatants and purchased SW480 exosomes were viewed by a transmission electron microscope. One drop (10 μl) of the exosomes from elution buffer were loaded onto a carbon-coated copper grid (200 mesh and coated by formvar carbon film) for 5 minutes and the excess fluid was absorbed by clean filter paper. The grid containing exosomes was incubated with a drop of 1 % uranyl acetate at room temperature for 3-5 minutes and excess uranyl acetate was absorbed by clean filter paper. Then, the copper grid was washed twice with pre-filtered sterile distilled water and kept overnight in a 1.5 ml centrifuge tube. Finally, the images of exosomes were viewed and captured under a Hitachi 7100 electron microscope.

Endothelial tube formation assay
Cell seeding and pre-treatment
A total of ten thousand TIME cells in 600 µl of complete growth medium per well supplemented with 2% of exosome-depleted FBS were seeded into a 12-well plate and incubated overnight at 37°C and 5% CO_2. The cells were treated with various concentration of exosomes.

Preparation of growth factor-reduced BD matrigel matrix
BD Matrigel was thawed overnight at 4°C. Then, a vial containing BD Matrigel was swirled to ensure that the material was evenly dispersed. Equal volume of serum-free RPMI was mixed with Matrigel to make 1:1 dilution from the original concentration. The mixture was then vortexed and centrifuged at 151 x *g* for 3 times to ensure even mixing. The Matrigel was kept at 4°C for several days before used.

Coating of µ-slide angiogenesis with growth factor-reduced BD matrigel
BD Matrigel was thawed as recommended. Using a low retention and pre-cooled pipette, it was mixed to homogeneity. The μ-slide angiogenesis was kept on ice and 10 μl of Matrigel (10 mg/ml) was added to each well. Then, the μ-slide was incubated at 37°C and 5 % CO_2 for 30-60 minutes to allow polymerisation.

Cell harvesting and seeding in µ-slide angiogenesis
Pre-treated cells with or without exosomes were harvested and seeded into an angiogenesis μ-slide. Medium in a 12-well plate was transferred into a 1.5 ml tube while 500 μl of filtered phosphate buffered saline (PBS) was added into the well to wash away the cells. Then, 150 μl of trypsin, 0.25 % (Gibco, USA) was added. The cells were re-suspended in old medium and placed back into a 1.5 ml tube. The cells were pelleted down at room temperature by centrifugation at 405 x *g* (Eppendorf Centrifuge 5804) for 5 minutes. The supernatant was removed without disturbing the pellet until only 100 μl was remained in the tube. Cell number was determined by mixing 10 μl of cell suspension and 10 μl of trypan blue trypan blue, 0.4% (w/v) (Sigma, USA). The cells in 50 μl of complete growth medium contained desired inhibitor with various concentrations were prepared and seeded into a μ-slide angiogenesis. The tube formation was then monitored every 4 hours.

Quantification of capillary-like tube formation
Capillary-like tube formation was visualised using Olympus BX51 microscope and images were captured at 40X magnification. Tube formation was quantified by the total branching length and total segment length using the Image J software (Tube formation analysis by ImageJ software (National Institues of Health (NIH), USA) are available in Appendix). Data was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test when comparing every mean to a control mean whereas ANOVA followed by Tukey-Kramer multiple comparison test is performed when comparing every mean with every other mean. All data are presented as mean ± standard deviation (SD). $P < 0.05$ is considered statistically significant.

Gene expression profiling with qPCR
Cells seeding and treatment
TIME cells seeded at a density of 40 X 10^4 cells per ml were cultured in 35 mm X 10 mm culture petri dish (BD Falcon, USA) containing 2 ml of complete growth medium supplemented with 2 % exosomes-depleted FBS. The cells were incubated overnight at 37°C, 5 % CO_2. The cells were then treated with exosomes for 20 hours. Cells treated with Buffer XE, which was used to elute exosomes during isolation, served as the vehicle control.

Table 1: Genomic DNA elimination mix

Component	Amount
RNA	25 ng- 5 µg
Buffer GE	2 µl
RNase-free water	Variable
Total volume	**10 µl**

RNA extraction

Purification of total RNA from TIME cells was conducted using RNeasy mini kit (cat. no. 74104; Qiagen) according to manufacturer's protocol. Buffer RLT (350 µl) containing 0.01 % of β-mercaptoethanol was added into the culture dish to lyse the cells directly. The sample was transferred into a 1.5 ml tube and 1 volume of 70 % ethanol was added to the lysate. Briefly, the components were mixed by pipetting up and down. The lysate was then transferred to an RNeasy mini spin column in a 2 ml collection tube and centrifuged for 15 second at ≥ 8000 x *g*. To remove residual DNA, extracted RNA was treated with DNase I by using On-column DNase digestion protocols. To wash the column, Buffer RW 1 was added to the RNeasy column and centrifuged for 15 seconds at ≥ 8000 x *g*. The flow-through was discarded from the 2 ml collection tube. To prepare DNase I incubation mix (80 µl), 10 µl of DNase I was mix with 70 µl Buffer RDD. This component was added directly into RNeasy column membrane and placed on the bench top for 15 minutes. After incubation, Buffer RW1 (350 µl) was added to RNeasy column and centrifuged at ≥ 8000 x *g* for 15 seconds. The column was washed once with 700 µl of buffer RWT, and then twice with 500 µl of buffer RPE centrifuged for 2 minutes at ≥8000 x *g*. The collection tube and flow-through were discarded and the column was placed into a new 2 ml collection tube. To remove residual of ethanol trapped in membrane, the lid of RNeasy spin column was opened and centrifuged at full speed for 1 minute. Fifty microliter of RNase-free water was added directly to the spin column membrane and eluted RNA was stored at -80°C.

Reverse transcription

Reverse transcription (RT) was conducted with RT² first strand kit (cat. no. 330404, Qiagen) according to the manufacturer's protocol. All reagents from kit were thawed at room temperature. Briefly, the contents were centrifuged to bring down the liquid to bottom. Genomic DNA (gDNA) elimination mix was made according to Table 1 in a 0.2 ml PCR tube.

The mixture was then mixed, incubated at 42°C for 5 minutes and then the tubes were placed on ice. Reverse transcription mix was prepared according to Table 2. Ten microliter of reverse transcription mix was added into each tube containing ten microliter genomic DNA elimination mix. The samples were then incubated at 42°C for exactly 15 minutes and immediately stopped the reaction at 95° C for 5 minutes. Ninety one microliter of RNA-free water was added to the 0.2 ml PCR tube containing 20 µl mixtures to make a final volume of 111 µl. The diluted cDNA was stored at -20°C.

RT² Angiogenesis PCR Array

The effect of SW480-7 derived exosomes on the expression of angiogenic genes in TIME was conducted with a RT² Angiogenesis PCR array (cat. no.330231, Qiagen). RT² SYBR green mastermix was thawed at room temperature and vortexed gently. The mastermix was then centrifuged to bring the contents to the bottom of tube. The PCR components mix was prepared as shown in Table 3 in a loading reservoir. Twenty-five μl components mix was added in to each

Table 2: Reverse transcription mix

Component	Volume for 1 reaction
5 X Buffer BC3	4µl
Control P2	1 µl
RE3 reverse transcriptase mix	2 µl
RNase-free water	3 µl
Total volume	**10 µl**

Table 3: PCR array components mix

Array format	96-well format A
2 X RT² SYBR Green Mastermix	1350 µl
CDNA synthesis reaction	102 µl
Nuclease-free water	1248 µl
Total volume	**2700 µl**

well of the RT² lncRNA PCR array by using an 8-channel pipettor and only 6 tips were used. The RT² lncRNA PCR array was sealed tightly with optical thin-wall 8-cap strips (Formats A) and centrifuged at room temperature. The plate was placed in the real-time cycler (Eppendorf, Wesseling-Berzdorf, Germany) and the PCR cycling program was set (Table 4).

RESULTS

Characterization of exosomes isolated from SW480-7

Exosome isolated from SW480-7 were characterised by zetasizer, transmission electron microscope (TEM) and Western Blot analysis. The commercial purchased exosomes from SW480 cell culture supernatants were used as a reference standard. The particle size distribution of exosomes purified from cell culture supernatants of SW480-7 were determined by using Zetasizer. The average diameter was 246.2 nm (Figure. 1B) (ranged from 122.4-615.1 nm). To characterise the morphology of exosomes, the exosomes derived from SW480-7 were examined using a Hitachi 7100 electron microscope. TEM result shows that exosomes were smaller than 200 nm (Figure. 1D). We further characterised the exosomes derived from SW480-7 by Western Blot analysis and the result was reported in previous study. Western Blot analysis confirmed that exosomal marker protein, namely ALIX (data not shown) was expressed in the exosome secreted from SW480-7 and whole cell lysate from SW480-7.[18] Altogether, our results demonstrated that the exosomes from cell culture supernatant of SW480-7 was successfully isolated. The particle size distribution of the commercial purchased exosome derived from SW480 cell culture supernatants was 130.4 nm (Figure. 1A) (ranged from 68.06-295.3 nm) and TEM result shows the exosomes also smaller than 200nm (Figure. 1C).

Effect of exosomes derived from SW480-7 on tube formation by TIME

To investigate the effect of exosomes derived from SW480-7, the exosomes released were purified using EXOeasy Maxi Kit and the angiogenic effect was assessed using the tube formation assay by telomerase-immortalised microvascular human endothelial cells. In this experiment, TIME cells treated with 5% of exosome-depleted FBS acted as the positive control while TIME cells treated with 2 % exosome-depleted FBS was the negative control as shown in Figure 2.

As shown in Figure 3, treatment of TIME cells with the combination of 2% -exosome-depleted FBS and 2 μg/ml of SW480-7-derived exosomes resulted in approximately a 1.49-fold increase in segment length and 1.18-fold in branching length as compared to control. Treatment of TIME with 5% exosome-depleted FBS shown 1.56-fold increase in segment length and 1.20-fold in branching length. This trend was similar to the TIME that were treated with the combination of 2% -exosome-depleted FBS and 2 μg/ml of SW480-7-derived exosomes. Statistical analysis showed significant differences ($P<0.05$) in segment length of TIME cells treated with 5% of exosome-depleted FBS versus 2% exosome-depleted FBS medium. Similarly, TIME cells treated with 2 μg/ml of SW480-7 also showed significant differences in segment

Table 4: PCR cycling conditions

Cycles	Duration	Temperature
1	10 min	95 °C
40	15 s	95 °C
	1 min	60 °C

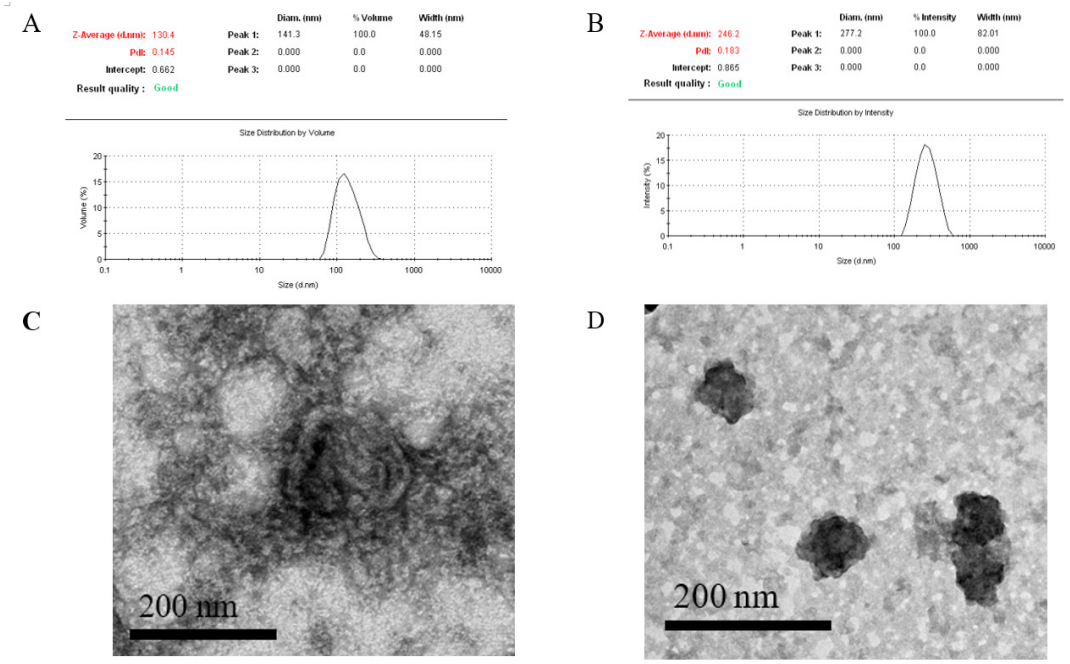

FIG. 1: Size distribution of exosomes. **A** Particle size distribution of standard exosomes from SW480 cell culture supernatant. **B** Particle size distribution of exosome-like vesicles derived from SW480-7 (invasive) cell culture supernatants. Microscopic analysis of morphology of exosomes derived from **C** SW480 (commercially available exosomes) and **D** SW480-7 cell culture supernatants using transmission electron microscopy.

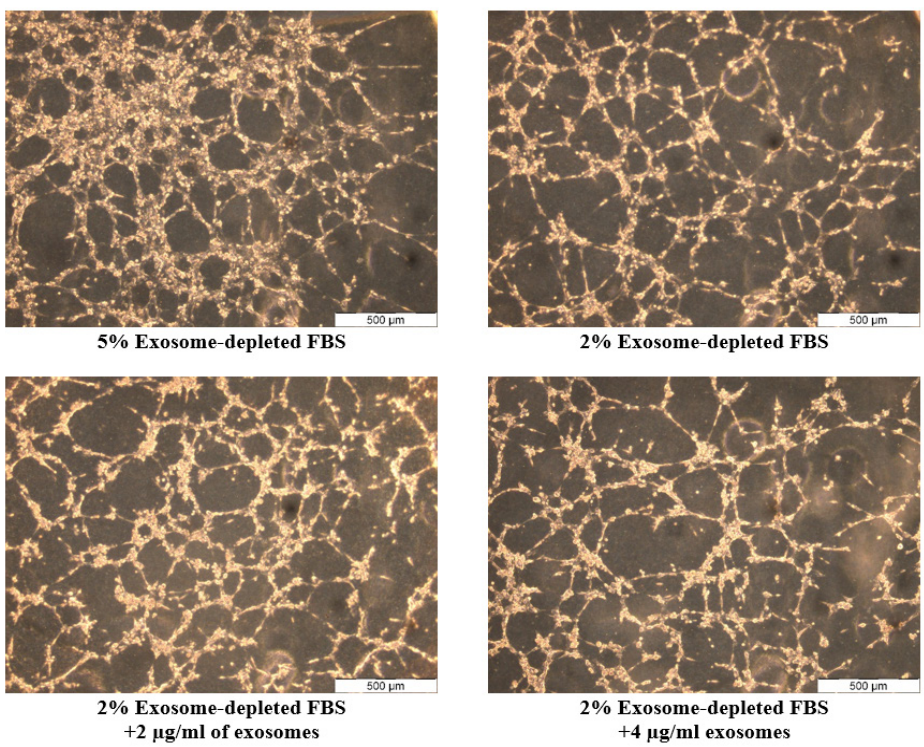

FIG. 2: Effect of exosomes derived from SW480-7 on capillary tube formation in cultured telomerase immortalized microvascular endothelial cells (TIME). TIME cells were grown on Matrigel-coated µ-slide angiogenesis for 4 h in the presence (2 μg/ml, and 4 μg/ml) or absence of SW480-7-derived exosomes. Tube formation was visualised with an Olympus BX51 microscope and representative fields at X40 magnification are shown.

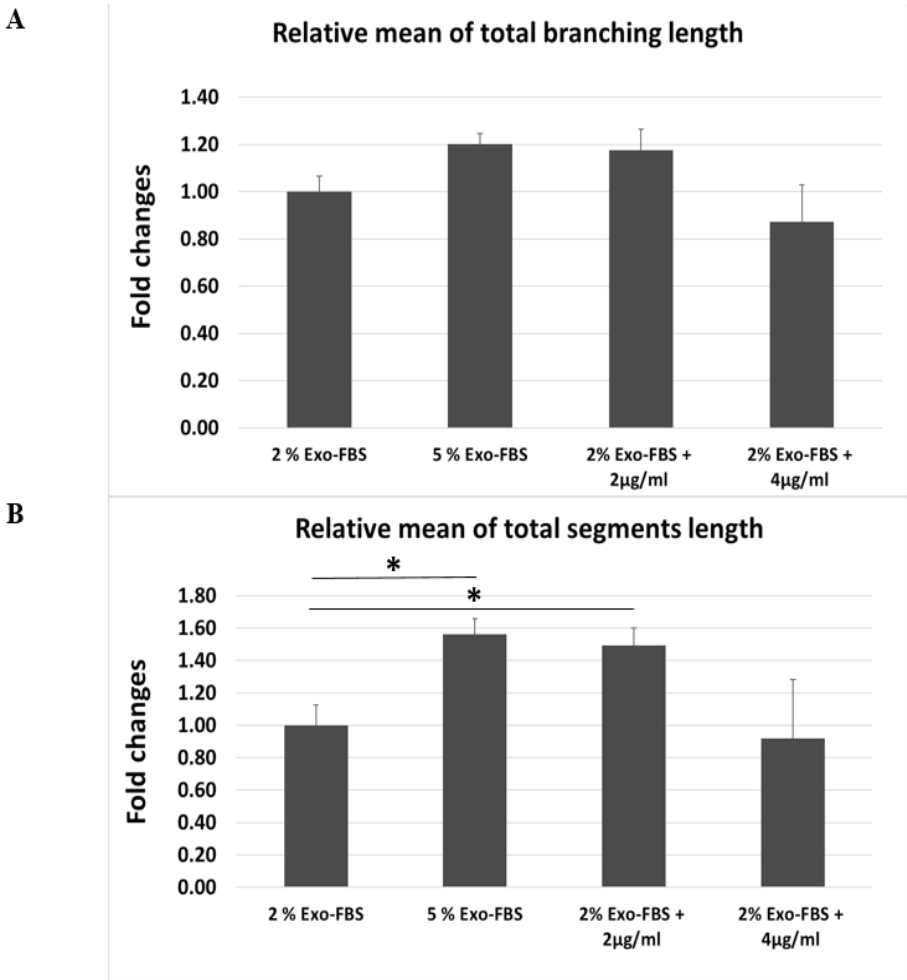

FIG. 3: Quantification of capillary-like tube formation by TIME. Tube formation was quantified by **A** the total branching length and **B** total segments length using Image J software. One-way analysis of variance (ANOVA) was used to compare the mean of each treatment. Each column represents mean ± SD of three independent experiments compared with control (n = 3). In this study, * indicates P< 0.05. Exo-FBS represents exosome-depleted FBS and 2 and 4 μg/ml represents the concentration of exosomes derived from SW480-7 cell line.

length as compared with TIME cells treated with 2% exosome depleted FBS. However, there was no significant difference in branching length. Unexpectedly, treatment of TIME with higher concentrations of exosomes (4 μg/ml) showed no effect in branching length and segment length as compared to control (P>0.05) in both segment length and branching length.

SW480-7-derived exosomes up-regulate FGFR3 mRNA expression in TIME

To analyse the angiogenic effects of exosomes derived from SW480-7 on TIME, Human Angiogenesis RT^2 Profiler PCR Array was used to profile the expression of 84 key genes involved in modulating the biological processes of angiogenesis. Differentially expressed genes were selected for validation by individual qPCR assay. Based on the screening result from the overall expression profile, higher expression of FGFR3 was found in TIME cells treated with exosomes and there is no effect on TIME treated with Buffer XE. While the other 83 genes were either not detected or were detected at a very low rate. The expression level of FGFR3 mRNA in TIME cells treated with 2 μg/ml of SW480-7-Exo increased by 2-fold (P<0.05) and 1.64-fold (P<0.05) as compared with untreated control cells and vehicle-treated cells, respectively. There was no difference in FGFR3 mRNA expression between untreated cells and vehicle-treated cells. Taken together, our data demonstrated

that SW480-7-derived exosomes up-regulated the mRNA level of FGFR3 in TIME.

DISCUSSION

Vesicles derived from cells were first defined from studies in 1983. The term 'exosomes' was coined for these vesicles four years later and these particles were described as the diposing machinery of cell debris and unnecessary proteins.[19] Recently, an increasing number of studies has indicated that exosomes participate in much more complex functions such as promoting angiogenesis, remodeling the microenvironment and promoting tumour growth.[20] In the present study, exoEasy Maxi Kit uses a membrane-based affinity binding step or spin column-based method to isolate exosomes from cell culture supernatants. To date, no specific exosomal protein marker has been identified to verify exosomes from specific a cell type. Hence, combination of characterisation methods is required to identify the efficacy of the current isolation method. The morphology of exosomes isolated were determined by TEM, vesicles size distribution by laser diffraction and exosomal protein marker by Western Blotting.

The size distribution of the isolated exosomes was measured by laser diffraction instrument. Results showed that exosomes purified from SW 480-7 cells had an average diameter of 246.2nm while the average diameter for exosomes from SW480 cell culture supernatants was 130.4 nm. The differences in exosome sizes from these two samples may be due to different isolation techniques. Another reason for the larger vesicles could be likely due to clumping of vesicles.[21] To further justify and characterise the exosomes extracted, the morphology of exosomes was viewed under TEM. TEM not only provides a view of the morphology of exosomes but also provides an overview of the level of contamination of the sample such as cell debris and apoptotic bodies. A recent study showed that the exosomes purified by the spin column method had less granular background as compared with the ultracentrifuge method.[22] In the present study, TEM results showed that our

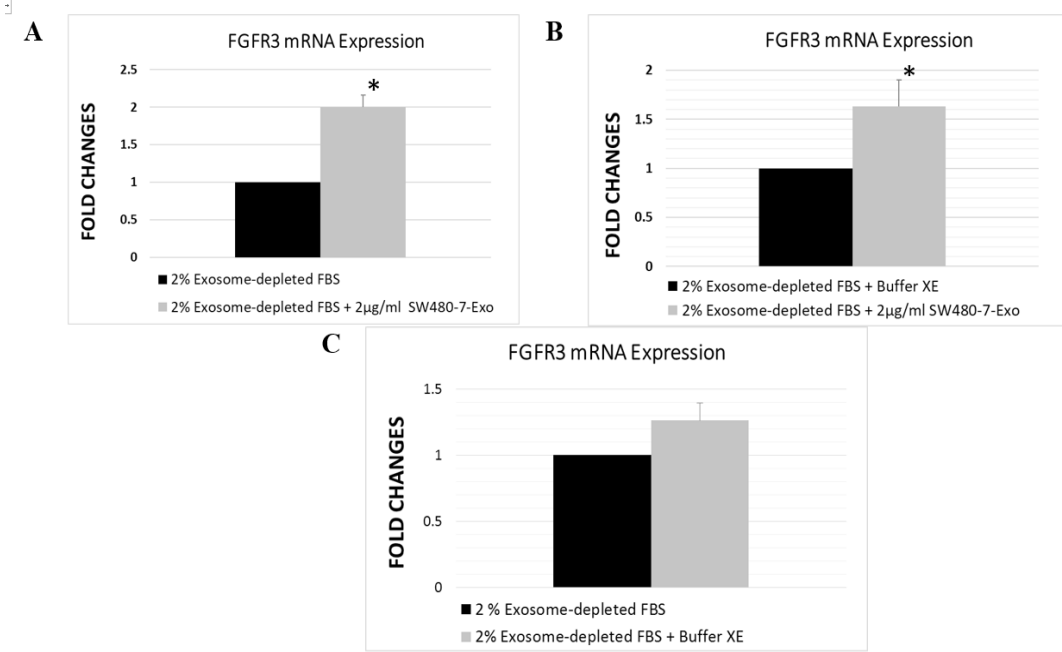

FIG. 4: FGFR3 mRNA expression in TIME cell line was analysed by qRT-PCR. TIME cell line was cultured in medium supplemented with 2% of exosome-depleted FBS. **A.** Comparison of FGFR3 mRNA expression in untreated control cells and cells treated with 2 μg/ml of exosomes derived from cell culture supernatants of SW480-7 (SW480-7-Exo). **B.** Comparison of FGFR3 mRNA expression in (Buffer XE) control and cells treated with 2 μg/ml of SW480-7-Exo. **C.** Comparison of FGFR3 mRNA expression in untreated control cells and cells treated with vehicle (Buffer XE). Each column represents mean ± SD of three independent experiments compared with control (n = 3). Data were analyzed with GraphPad Prism version 6 by one sample t-test. In this study, * indicates $P < 0.05$.

exosomes are in the estimated size range and no cell debris or apoptotic bodies were found.

Exosomes are small membranous vesicles produced by cells through biogenesis.[23] Exosomes are cell type-specific and carry genetic and proteomic materials that are similar to their origin of cell.[1] In recent years, multiple studies show that exosomes are involved in regulation of gene expression, physiological and pathological processes, including angiogenesis. Tumour cells are actively secrete exosomes that rich in angiogenic protein that can be uptaken by endothelial cells and increase vascular development by modulating the angiogenic function of endothelial cells.[24,25] Once normal endothelial cells uptake and internalized tumour-derived exosomes, angiogenic signaling pathway will be activated and promote angiogenesis.[26,27] The role and potency of exosomes derived from invasive CRC cell lines in angiogenesis are not completely revealed and poorly understood. Hence, the angiogenesis functional study for role of exosomes derived from invasive CRC is important. In this regard, here, we present evidence showing that co-culturing of exosomes derived from invasive SW480-7 cell lines induced tube formation of TIME. We next examined the mRNA expression level in endothelial cells after incubation with the exosomes. We found that exosomes derived from SW480-7 cell lines up-regulated angiogenesis related gene FGFR3 after co-culturing. A recent study has reported that higher expression level of FRFR3 in melanoma tissues promotes melanoma growth, metastasis and EMT behaviour, likely by affecting the phosphorylation levels of ERK, AKT, and EGFR (28). However, in present study, the specific underlying molecular mechanism up-regulated of FGFR3 mRNA expression in TIME by exosomes derived from SW480-7 is unknown. Further experiments need to be conducted to elucidate the possible mechanisms underlying this network.

CONCLUSION

In summary, we have successfully isolated and characterised the exosomes from invasive colon cancer cell lines, namely SW480-7. The present study has demonstrated that exosomes derived from SW480-7 increased tube formation and up-regulated expression of FGFR3 mRNA in TIME. Our results suggest that exosomes derived from this invasive colon cancer cell line contain angiogenic factors that can increase the vascular development.

Acknowledgements: This work was financially supported by grants from Ministry of Education, Malaysia (Fundamental Research Grant Scheme (FRGS/1/2015/SKK08/UPM/02/5)).

Authors' contribution: CTN designed, conducted the experiments, analyzed the data and wrote the manuscript. WK supervised and analyzed the data. MFJ and NM supervised. HFS conceptualized, designed, supervised, analysed data and edited the main manuscript text. All authors read and approved the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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