MiRNA Profiling: Mouse Blastocyst Extracellular Microvesicles Inhibit Melanoma Cell Invasion

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Research

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Abstract

Background: Microvesicles (MVs) mediate the transmission of information between cells through the miRNAs, proteins, lipids and mRNAs carried by MVs, which inhibit/promote the function of receptor cells. This study confirmed that blastocyst MVs inhibited the invasion of melanoma cells and showed a complex miRNA pedigree. Functional miRNAs were found, and the mechanism of inhibiting invasiveness was analyzed based on the miRNA lineage characteristics of mouse blastocyst MVs, combined with the miRNA bioinformatics information of miRBase, microRNA.org, NCBI and other databases.

Methods: MVs derived from D3.5 mouse blastocysts were isolated and the miRNA gene expression of MV was detected by fluorescence quantitative polymerase chain reaction (qPCR). The dynamic changes in tumor cell invasiveness in the co-culture system of blastocyst MVs and melanoma B16-F10 cells were monitored in real time. The MV miRNAs expression profile of blastocyst was identified using the qPCR array. Bioinformatics tools were used to analyze the characteristics of miRNA pedigree in blastocyst MV to screen functional miRNAs, which reduce tumor cell invasiveness and to predict functional miRNA target genes. The target genes were analyzed by KEGG and GO pathway enrichment analyses using David and FunRich software.

Results: The average diameter of round MVs extracted from mouse blastocysts was 196.4 ±7.8 nm. When B16-F10 melanoma cells were co-cultured with mouse blastocyst MVs, no significant difference in invasive ability was found between the two groups before 72 h, but increased significantly after 72 h ($P < 0.05, P < 0.01$). A total of 336 miRNAs were analyzed in mouse blastocyst MVs. Further analysis showed that 224 miRNAs were associated with invasion. Also, 32 invasion-related miRNAs with the strongest differential expression were verified by real-time quantitative polymerase chain reaction (qPCR). The GO analysis of the target genes showed that the 32 functional miRNAs were mainly related to 8 biological processes, including DNA binding, sequence-specific DNA binding, metal ion binding, chromatin binding, nucleotide binding, sequence specificity, DNA binding transcription factor activity and ubiquitin-protein ligase activity. The KEGG pathway analysis showed that the 32 functional miRNAs were mainly related to the PI3K-Akt signaling pathway.

Conclusion: Blastocyst MVs carrying miRNAs regulated the decrease in the invasive ability of melanoma cells through the PI3K-Akt signaling pathway.

Background

Extracellular microvesicles (MVs) are microbubbles composed of a boundary membrane and inclusions. They originate from the formation of buds caused by extroverted membrane protuberances or external signal stimuli in the process of apoptosis. The buds are finally separated from the membrane, forming vesicles[1]. In this process, some bioactive molecules, such as proteins, lipids, mRNAs and miRNAs, are selectively wrapped in the cavity of MVs or embedded in the membrane, thus mediating the transmission of information between cells[2]. The maternal-fetal cell dialog during embryo implantation is also related

to MVs secreted by the cells. The extracellular MVs produced by the embryo and endometrium are released into the uterine cavity together to mediate communication among the embryo, endometrial epithelium, and stroma, and transmit the message from the pre-implantation embryo to the interstitial layer and beyond[3]. Embryo implantation is also considered as an immune inflammatory response. Giacomini[4]and other related tests also proved that the exosomes from pre-implantation embryos carried molecules, such as nonclassical major histocompatibility complex (MHC) I molecules, that regulated the local endometrial immune system and maternal immune system and increased endometrial receptivity.

Great similarities exist between the process of embryo implantation and tumor invasion in pathophysiological activity, expression of cell invasion-related genes, apoptosis, immune escape and so on. Cell invasion and migration are important in embryonic development, dynamic balance, pathology and tumor evolution[5]. Compared with the uncontrolled invasion of malignant tumor cells, the process of blastocyst implantation into the maternal endometrium is strictly regulated. Herrler[6] confirmed that a close balance between activation and inactivation of T cells at the implantation site in the process of embryo implantation and trophoblast invasion controlled the initiation of full trophoblast invasion and limited this invasion to a range that could be borne by the maternal system, thus ensuring the healthy growth of the embryo. In other words, certain factors inhibited invasiveness after the high invasion of trophoblast cells. A previous study found that mouse embryos invaded the tumor and changed the fine morphology of the tumor and its invasiveness. The blastocyst fine cell surface showed higher MMP-9 and FAK activities during the invasion process[7]. However, the addition of MMP-9 inhibitors did not affect the invasion of embryos on ovarian cancer cells[8]. Therefore, intercellular chemical communication might not be the only independent mechanism of this phenomenon, thus pointing toward microvesicular communication between blastocyst cells and tumor cells. Mouse embryonic cells might regulate tumor cell invasiveness through extracellular MV transmission.

MicroRNAs (miRNAs) are non-coding single-stranded RNAs composed of 20–25 nucleotides[9]. MiRNAs can be transmitted horizontally between cells through MV communication. Valadi et al.[10]confirmed that MVs carried miRNAs into the receptor cell, which affected the function of the receptor cell. MiRNAs participated in the regulation of many signal transduction pathways and were vital in embryonic development, organogenesis, stem cell differentiation, cell proliferation, apoptosis, angiogenesis, tumorigenesis and immune response[11]. MiR-16[12], miR-34c[13] and miR-146a[14] regulated the invasiveness of tumor cells. Studies confirmed that placenta-specific miR-517A and miR-21 were continuously released from trophoblast cells into maternal circulation through trophoblast-derived exosomes and targeted maternal endothelium during pregnancy, which was conducive to embryo implantation[15]. Specific C19MC miRNAs[16] secreted by human trophoblast cells existed in exosomes or plasma and were important in placental-maternal communication, which guided maternal adaptation to pregnancy.

In this study, 3.5-day-old mouse blastocysts were used as the source of MVs, and MVs were isolated by differential and density gradient centrifugation. RTCA xCELigence DP cell function analyzer was used to

detect the invasiveness of tumor cells in the co-culture system of mouse blastocyst MVs and B16-F10 melanoma cells. Further, the morphology of MV was examined, and the miRNAs contained in MVs were identified using the qPCR array. The miRNAs that might inhibit tumor cell invasion and their mechanism were predicted through the bioinformatics analysis of these miRNAs and their target genes. The present study confirmed the existence of MVs in mouse blastocysts, suggesting that the inhibitory effect of mouse blastocysts on tumor cell invasion might be regulated by miRNA carried by MVs through the PI3K-Akt signaling pathway.

Methods

Animals and blastocyst acquisition

The animal experiments complied with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The BALB/c mice aged 6–8 weeks were maintained at a temperature of 25 °C-27 °C and humidity of 40–50% with a continuous supply of chow and water. Female mice received 10 U PMSG intraperitoneally, followed by 10 U hCG within 48. After the hCG injection, the female mice were immediately placed in cages with male mice (1:1) for copulation. The presence of a vaginal plug was set on the first day of pregnancy (d1). The blastocysts were collected on day 3.5 of pregnancy by flushing each uterine horn with 1% BSA.The blastocysts developed to the mid-late stage were collected under a stereomicroscope. After adding the red blood cell lysate for 15 min, the blastocysts were washed three times with PBS and a trace amount of hydrochloric acid (pH 2.5) was applied to remove the zona pellucida. The blastocysts were digested with an appropriate amount of trypsin and dispersed at 37 °C for 30 min. Then, 10% treated MV-depleted FBS was added (FBS was centrifuged overnight at 100,000 *g* at 4 °C. The supernatant after centrifugation was filtered using a 0.22-μm filter and stored at 4 °C).

MV purification

MVs were prepared from the supernatant of mouse blastocysts. Mouse embryo suspension was harvested and centrifuged at 300 *g* for 10 min to eliminate the precipitate and at 2000 *g* for 10 min, followed by filtration through a 5.0-μm filter. The supernatant after centrifugation was ultracentrifuged at 50,000 *g* for 70 min, and the precipitate was collected. Then, the supernatant was pelleted by ultracentrifugation at 100,000 *g* for 70 min, and the pellet was collected. The mixture of pellets obtained by centrifugation at 50,000 *g* and 100,000 *g* was ultracentrifuged at 100,000 *g* for 70 min using the density gradient. All ultracentrifugation steps were performed at 4 °C. The pellets were resuspended in PBS and used for the following experiment.

Scanning electron microscopy

For scanning electron microscopy, isolated MV pellets were fixed using 1.5% glutaraldehyde at 4 °C overnight. Then, 3-μL samples were taken, dried on a clean silicon wafer, and observed under a low vacuum (100 Pa) using an FEI Nova NanoSEM 450 ultra-high-resolution scanning electron microscope.

Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) was conducted using NanoSight Nanoparticle Tracking Analyzer (model NS300, Malvern, UK) to determine the vesicle size distributions. The MVs were dissolved in PBS and vortexed for 1 min to maintain even distribution of MVs.

RNA extraction and real-time PCR

The resulting precipitated solution was extracted with total RNA using the TRizol method and digested with DNase to eliminate DNA contamination. The miRNA expression profile in mouse blastocyst MVs was detected using a 384-well miRNA qPCR array. Real-time qPCR reactions were detected using the ViiA 7 by the real-time qPCR analysis system (Thermo Fisher Scientific-CN). The total RNA was isolated using an RNA extraction kit (Quanto Bio). TaqMan-based qPCR used the 7900HT fast real-time PCR system (Applied Biosystems). EC4 and EC5 were used as external controls for miRNAs in the real-time qPCR analyses. The gene expression was estimated using the cycle threshold (CT value, CT < 35).

Bioinformatics analysis

The miRBase, microRNA.org, NCBI, and other databases were searched for miRNAs that reduced the invasiveness of tumor cells, and the search results were screened. The 11 miRNA target gene prediction software included DIANA-microT, Micro Inspector, miRanda, Mir Target2, mi Target, NB miR, TarPic Tar, PITA, RNA22, RNAhybrid and TargetScan. These were used to predict the expression of the 26 miRNA target genes. Two kinds of software, David and FunRich, were used to perform the GO enrichment analysis of target genes. David was used to perform KEGG pathway enrichment analysis on the aforementioned target genes.

Cell culture

Mouse melanoma B16-F10 cells (purchased from Shanghai Cell Bank, Chinese Academy of Sciences) were maintained in RPMI 1640 medium (Hyclone, USA) containing 10% fetal bovine serum, 50×10^3 U/L penicillin and 50 mg/L streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

Laser confocal microscope

Under the condition of avoiding light, B16-F10 cells were co-cultured with PKH26-stained blastocyst MVs in a laser confocal dish for 0, 24, 48 and 72 h, and the co-culture system was incubated in an incubator containing 5% CO_2 with 90% saturated humidity at 37℃. Under the condition of avoiding light, the process of MVs uptake of blastocysts by B16-F10 cells at different time points was observed under a laser confocal microscope.

xCELLigence living cell analysis system

The cells were made into an 8×10^5 cells/mL cell suspension, and then the suspension was mixed with MV precipitate to form a co-culture system. The invasion force of tumor cells was measured using RTCA xCELLigence DP fine cell work and energy analyzer in real time and dynamic state. The rate of cell

invasion was monitored using the xCELLigence System CIM-plate in real time. The detection frequency was once every 15 min, and the cell index was recorded continuously for 11 h.

Statistical analysis

The experimental data was calculated using GraphPad Prism 5.0 software. Statistically significant differences between the two groups were evaluated with Prism 5.0 using the LSD *t* test. A P value < 0.05 indicated a statistically significant difference; a P value > 0.05 indicated no statistically significant difference.

Results

Isolation of mouse blastocyst MVs

Mouse blastocyst MVs were collected by differential centrifugation at 50,000 *g* and 100,000 *g* combined with density gradient centrifugation at 100,000 *g* (Fig. 1A). NTA showed that the average particle size of the MVs was 196.4 ± 7.8 nm (Fig. 1B). Scanning electron microscopy showed that the MVs were about 100–500 nm in size and had a round shape (Fig. 1C).

Co-culture of MVs and melanoma cells and real-time monitoring of melanoma cell invasion by RTCA

B16-F10 cells were co-cultured with the mouse blastocyst MVs. Cell index curves for the control (red) and co-culture (green) groups gradually diverged over time (Fig. 2A). Before the 36-h time point, the cell index was higher in the co-culture group than in the control group, and was reversed after 36 h. At the time points of 12, 24, 36, 48, 60, 72, 84, 96, and 108 h, the invasive ability of the two groups had no significant differences before the 72-h time point (P > 0.05). In the time period between 72 h and 84 h, the invasive ability was significantly lower in the co-culture group than in the control group, with significant differences (P < 0.05). At the 96-h and 108-h time points, the difference was significant (P < 0.01). These results suggested that the invasiveness of the tumor cells was significantly lower in the co-culture group than in the control group.

The uptake of blastocyst MVs by B16-F10 cells in a co-culture system was observed using a laser confocal microscope. As shown in Fig. 2K, PKH26-labeled blastocyst MVs began to accumulate in the cytoplasm of B16-F10 cells co-cultured with MVs, without PKH26 labeling for 24 h. After co-culture for 48 and 72 h, the number of PKH26-labeled blastocyst MVs significantly increased, and a large number of PKH26-labeled blastocyst MVs, gathered around the cytoplasm and nucleus of B16-F10 cells, indicating that B16-F10 cell uptake of blastocyst MVs positively correlated with time.

Identification of invasion-related miRNAs based on bioinformatics

The 336 miRNAs in MVs derived from mouse pre-implantation blastocysts were identified by the qPCR array. In the miRNAs of MVs, the sequence information of 195 miRNAs was the same as that of human homologous miRNAs, while the difference in 80 miRNAs and human homologous miRNAs was only of 1–2 nucleotides (score > 90). This part of miRNAs accounted for about 81.85% of the total miRNAs in MVs (Fig. 3A). In addition, 30 miRNAs had no resemblance to any human miRNAs, including six miRNAs (MMU-miR-688, MMU-miR-714, MMU-miR-680, MMU-miR-690, MMU-miR-712-3p, and MMU-miR-453) endemic to mice; no similar miRNAs were found in any other species (Fig. 3A). As shown in Fig. 3B, no significant difference was noted in miRNA distribution among the three kinds of MVs separated at different centrifugal speeds. Also, no significant difference was found in the relative expression of the first nine kinds of miRNAs, and their Ct values were between 9 and 1.9.

A total of 224 miRNAs were related to the invasion in MVs, accounting for 43.83% of the total miRNAs related to invasion (Fig. 4A). Further, 175 miRNAs were invasion-suppressed miRNAs; 79 and 30 miRNAs could promote invasion, of which 30 miRNAs were related to invasion at the same time (Fig. 4B and 4C). Although the miRNAs that inhibited invasion were dominant in terms of quantity, some miRNAs, such as miR-346 and miR-21, promoting invasion were expressed at a high level in MVs.

The literature and database were searched for 65 miRNAs that inhibited tumor invasiveness (Supplementary Table 1). Compared with the results of mouse blastocyst MV qPCR array, 32 miRNAs had invasive inhibition potential in mouse blastocyst MVs. The differences were observed in the following miRNAs: miR-143, miR-144, miR-146a, miR-148a, miR-149, miR-150, miR-15b, miR-16, miR-195, miR-200a, miR-206, miR-218, miR-222, miR-22, miR-29a, miR-29a*, miR-30b, miR-375, miR-381, miR-409-3p, miR-338-3p, miR-34c, miR-320, miR-154, miR-106b, miR-182, miR-200b, miR-130b, miR-9, and miR-125b-3p (Supplementary Table 2). The function of miRNAs in blastocyst MVs was analyzed by GO and KEGG pathway enrichment analyses (Fig. 5). The GO enrichment analysis showed that the target genes of miRNAs in MVs were associated with eight biological processes. (Supplementary Table 3). The KEGG pathway analysis showed that the target genes were enriched in the following pathways: PI3K–Akt signaling pathway, ubiquitin-mediated proteolysis, site adhesion, and tumor pathway (Supplementary Table 4).

Among all signal transduction pathways, the PI3K–Akt signal transduction pathway was related to most miRNAs in MVs. As shown in Fig. 6, most miRNAs targeted VEGFA, VEGFR, SRC, PI3K, Akt, eNOS and RAC1 to inhibit signal amplification, resulting in the limitation of cell migration and invasion mediated by the PI3K-Akt signaling pathway.

Discussion

Cell invasion and migration are important in embryonic development, dynamic balance, pathology, and tumor evolution[4]. The process of blastocyst implantation into maternal endometrium is strictly regulated compared with the uncontrolled invasion of malignant tumor cells. The implantation process involves extensive crosstalk communication between trophoblast cells and endometrium through the embryo and

endometrial-derived factors that regulate trophoblast cell invasion and migration[17]. Herrler[6] confirmed that a close balance between activation and inactivation of T cells at the implantation site during embryo implantation and trophoblast invasion controlled the beginning of full trophoblast invasion and limited this invasion to a range that could be borne by the maternal system, thus ensuring the healthy growth of embryos. In other words, some factors inhibited invasiveness after the high invasion of trophoblast cells. In the present study, RTCA xCELLigence DP cell function analyzer was used to dynamically compare the cell index of B16-F10 melanoma cells with that of B16-F10 melanoma cells in the co-culture and control groups. The invasiveness of B16-F10 cells was significantly lower in the co-culture group than in the control group, suggesting that blastocyst might inhibit tumor invasiveness by secreting MVs.

The function of MVs depends on their source cells[18]. They regulate the function of target cells by transporting bioactive molecules from source cells[19]. They are also expressed as a medium for cell–cell communication, transmitting information through proteins, specific mRNAs, miRNAs, and so forth[20]. Some studies demonstrated a variety of roles of miRNAs in embryo–maternal signal transduction[15–16]. Especially, miRNAs were related to the regulation of trophoblast invasion in embryo implantation. For example, trophoblast-derived exosomes carry miR-517A and miR-21 to target maternal intima during pregnancy so as to promote embryo implantation. MV can transport miRNAs and proteins, especially affecting the function of the immune and vascular systems[21–22]. For example, MVs in uterine fluid promote endometrial–embryonic signal transduction by transporting miR-200C, miR-17, and miR-106a[23].

The results showed that mouse blastocyst MVs contained 336 different miRNAs. The database search revealed 511 miRNAs related to the invasion. Most of the studies on the inhibition or promotion of cell invasion by miRNAs were highly similar to studies on human homologous miRNAs in sequence, but only few studies reported on mouse miRNAs. The database with 224 miRNAs related to the invasion in mouse blastocyst MVs was analyzed to evaluate the effect of mouse miRNAs on invasiveness. MiRNAs that inhibited invasion had absolute superiority in terms of quantity and relative expression. In addition, some miRNAs promoted or inhibited cell invasion at the same time. In addition, literature and database were used to screen 65 miRNAs related to tumor invasion, of which 32 miRNAs were also included in mouse blastocyst MVs, such as miR-34c, miR-409-3p, miR-143, miR-144, miR-146a, and miR-148a. Li et al.[24] confirmed that miR-34c was overexpressed in endometrial carcinoma HEC-1-B cells, and E2F3 protein was used as a target to inhibit proliferation, migration, and invasion, and induce cell cycle arrest and apoptosis. Sachdeva et al. confirmed that miR-145 inhibited the invasiveness of breast cancer cells BT-549, MDA-MB-231, and LM2-442 by targeting the *MUC1* gene[25]. Wang et al. also confirmed that the tumor suppressor gene miR-1224 inhibited the proliferation and invasion of osteosarcoma cells by targeting B7-H3[26].

MiRNA is a highly conserved noncoding RNA that can form a hairpin-like endogenous transcription precursor pri-miRNA. MiRNA pairs with the 3'-UTR base of the target mRNA and silences the gene at the posttranscriptional level by inhibiting the translation of mRNA or directly degrading mRNA. Further, 32

miRNAs were related to the inhibition of cell invasiveness in 3.5-day mouse blastocyst MVs. The functional miRNA target gene GO analysis showed that the aforementioned miRNAs were mainly related to sequence-specific DNA binding, metal ion binding, chromatin binding, nucleotide binding, sequence specificity, DNA transcription factor activity, ubiquitin-protein ligase activity, and other biological processes. The KEGG pathway analysis showed that these miRNA target genes were rich in a variety of tumor-related signaling pathways, including PI3K–Akt, mTOR, and FoxO. The PI3K–Akt signaling pathway belongs to the family of casein kinase receptor transduction pathways. Akt is a protein kinase and an important target gene of PI3K. Studies showed that this pathway was involved in tumor metastasis and invasion[27]. Mouse blastocyst MVs may downregulate the expression of downstream target proteins through the PI3K–Akt signaling pathway, thus inhibiting the invasiveness of melanoma B16-F10 cells. MiRNAs can recognize target mRNAs by base complementary pairing and cause target mRNA degradation or translation inhibition. The decreased invasiveness of melanoma cells after MV uptake may also be related to the role of miRNAs in MVs, which is largely regulated by the PI3K–Akt signaling pathway.

Conclusions

To sum up, this study confirmed the existence of MVs in mouse pre-implantation blastocysts. It showed that the inhibitory effect of MVs on the invasiveness of B16-F10 melanoma cells might be related to the functional miRNAs in MVs and was mediated through the PI3K–Akt signaling pathway to regulate the invasive ability of melanoma cells.

Abbreviations

MVs/MV:Extracellular microvesicle /microvesicle.

D3.5:3.5 days pregnant.

NCBI: National Center for Biotechnology Information

qPCR: Quantitative real-time PCR

MMPs: Matrix metalloproteinases

FAK : Focal adhesion kinase

PI3K: Phosphatidylinositol 3 kinase

AKT: Protein kinase B

PBS:Phosphate buffered saline

FBS :Fetal bovine saline

RTCA/RTCA xCELLigence DP: Real Time Cellular Analysis

mRNA: Message RNA

PMSG: Pregnant mare serum gonadotropin

hCG:Human chorionic gonadotrophin

FEI Nova NanoSEM 450: Scanning electron microscope

NIH: National Institutes of Health

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

RPMI: Roswell Park Memorial Institute

BSA: Bovine serum albumin

VEGFA: Vascular endothelial growth factor A

VEGFR: Vascular endothelial growth factor receptor

SRC: Protein tyrosine kinase

eNOS: Endothelial nitric oxide synthase

Rac1:Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein)

MUC1:Mucin-1

UTR: Untranslated region

FOXO: Forkhead box

LSD：Least significance difference

Declarations

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Authors' contributions

LQF and QL conceived and designed the study; RXD and QL performed the experiments with the assistance of YTW and XYB for in work. RXD and QL analyzed data; RXD and LQF wrote the paper, and all the other authors were involved in writing the manuscript. All authors edited and approved the final draft of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

All studies were performed according to Chongqing Medical University Medical Research Ethics Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

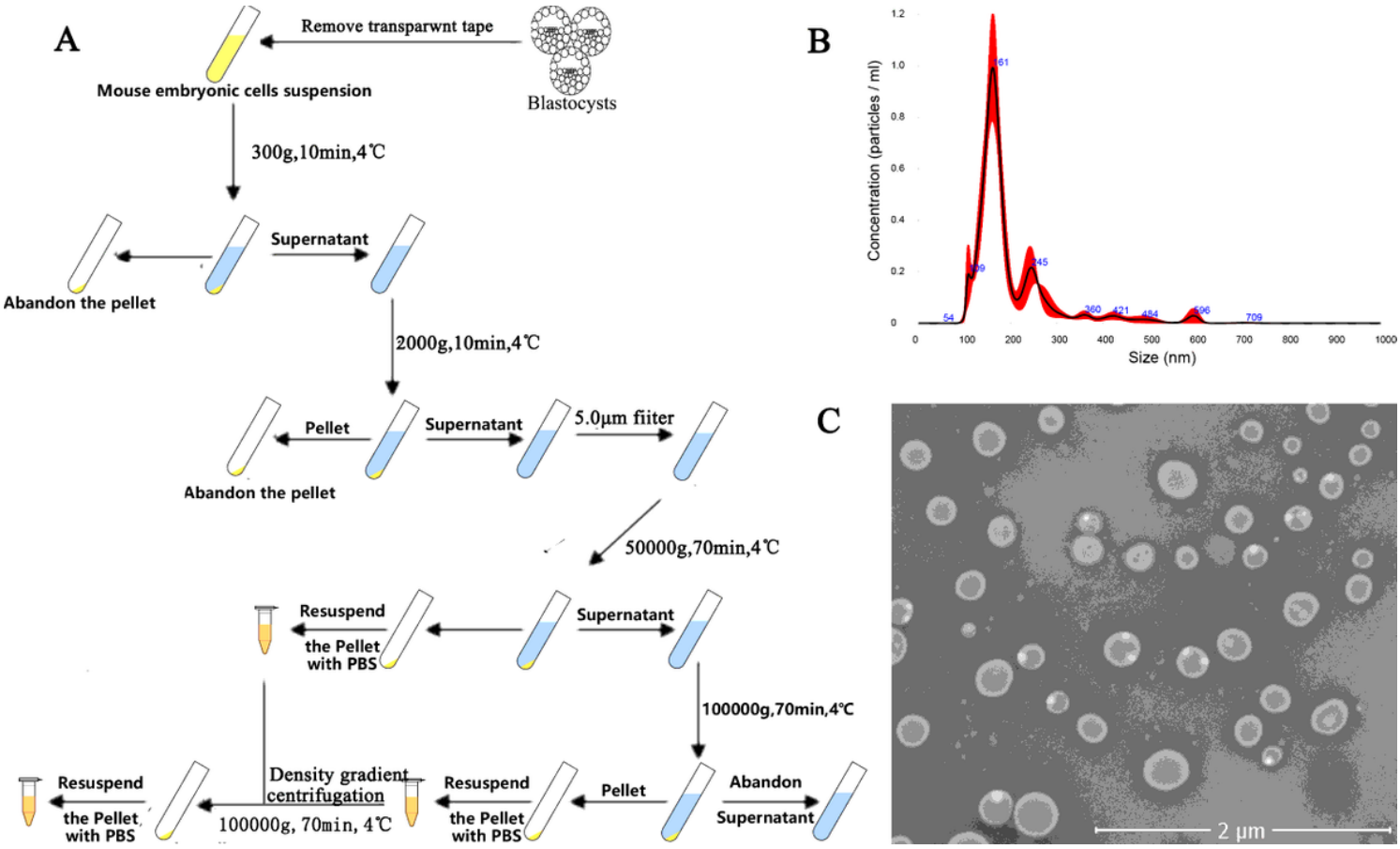


Figure 1

Microvesicles (MV) from mouse blastocysts. (A) Ultracentrifugation of mouse blastocyst to obtain MVs. (B) Results of nanoparticle tracking analysis of MVs. (C) Scanning electron microscopy image of MVs isolated from mouse blastocysts. Scale bar, 2 μm.

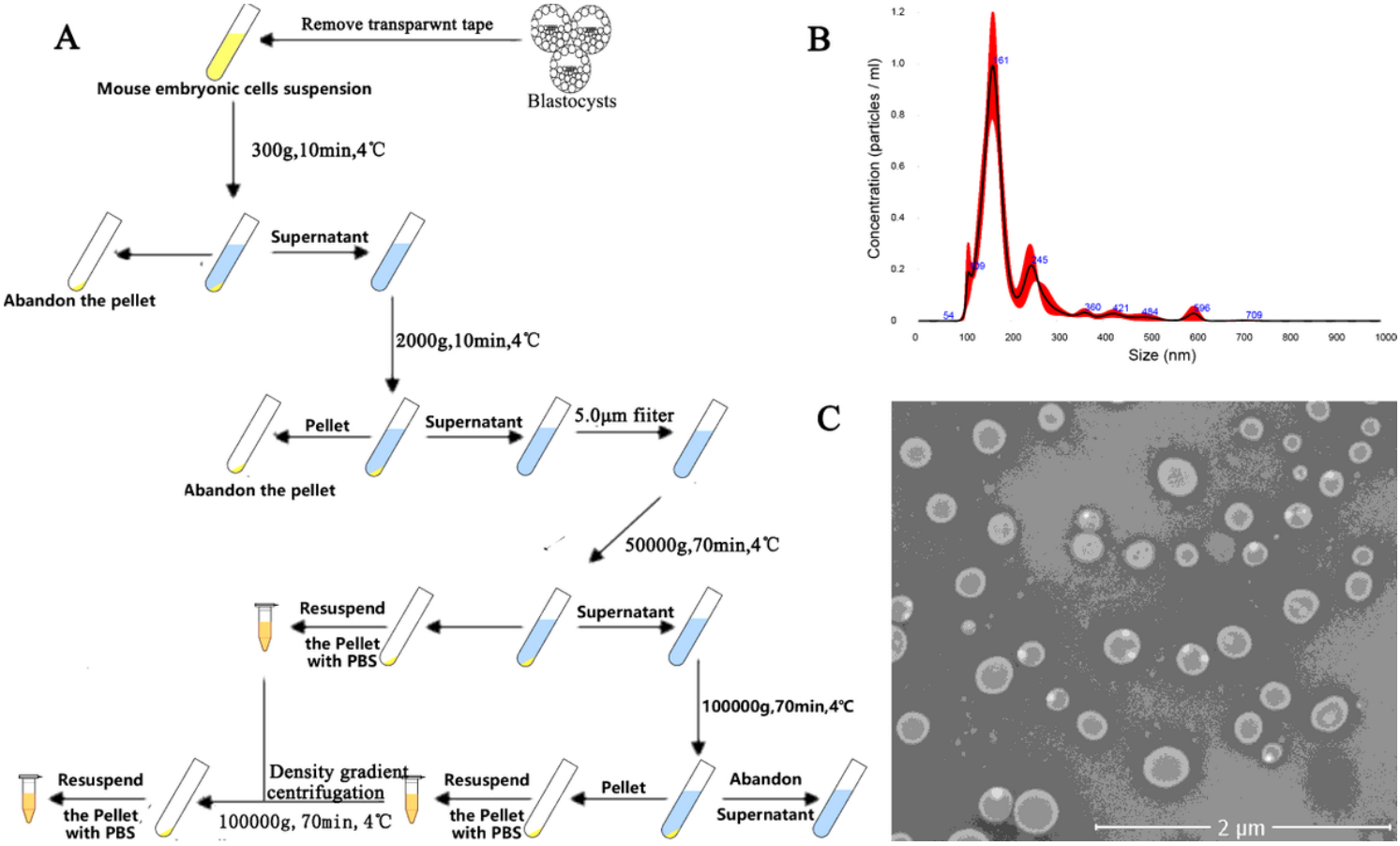


Figure 1

Microvesicles (MV) from mouse blastocysts. (A) Ultracentrifugation of mouse blastocyst to obtain MVs. (B) Results of nanoparticle tracking analysis of MVs. (C) Scanning electron microscopy image of MVs isolated from mouse blastocysts. Scale bar, 2 μm.

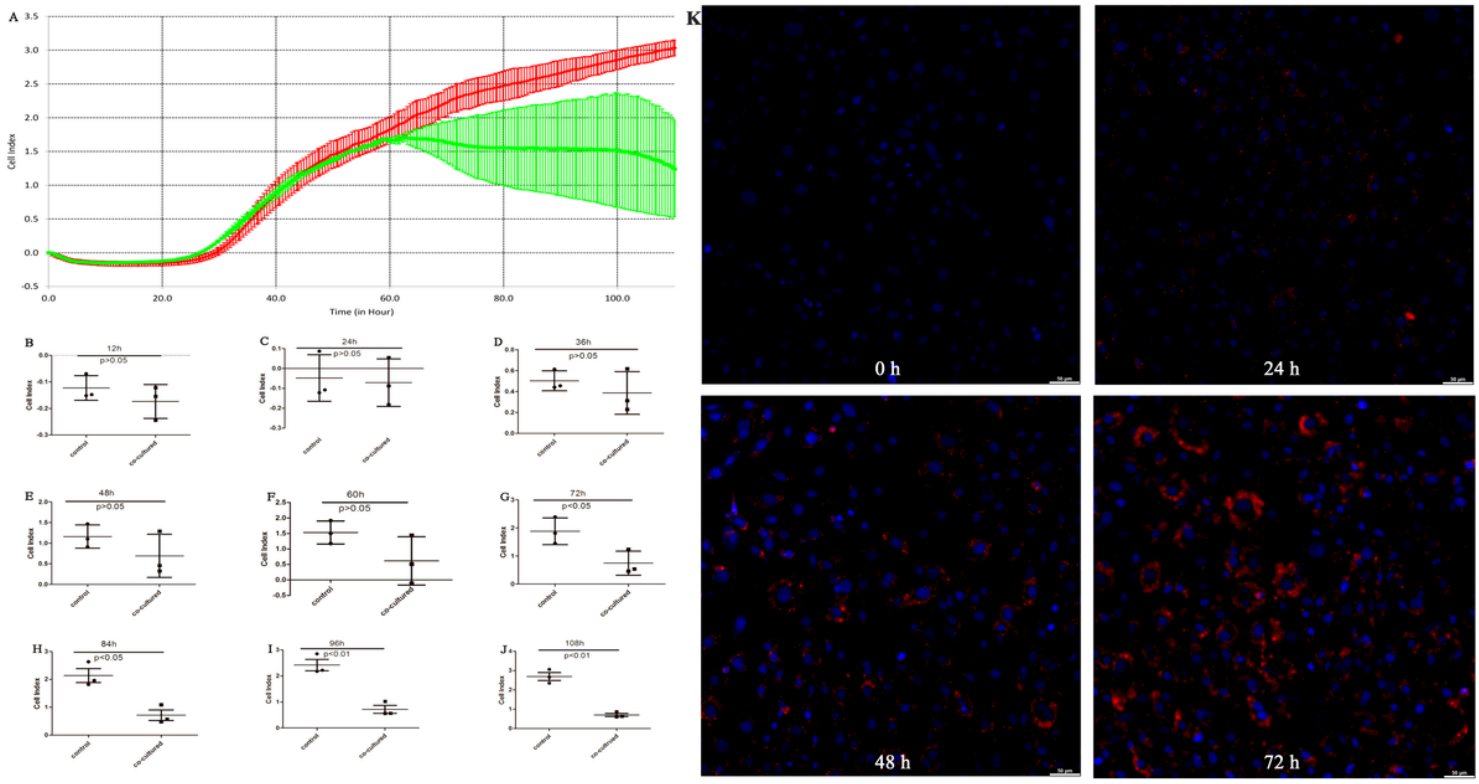
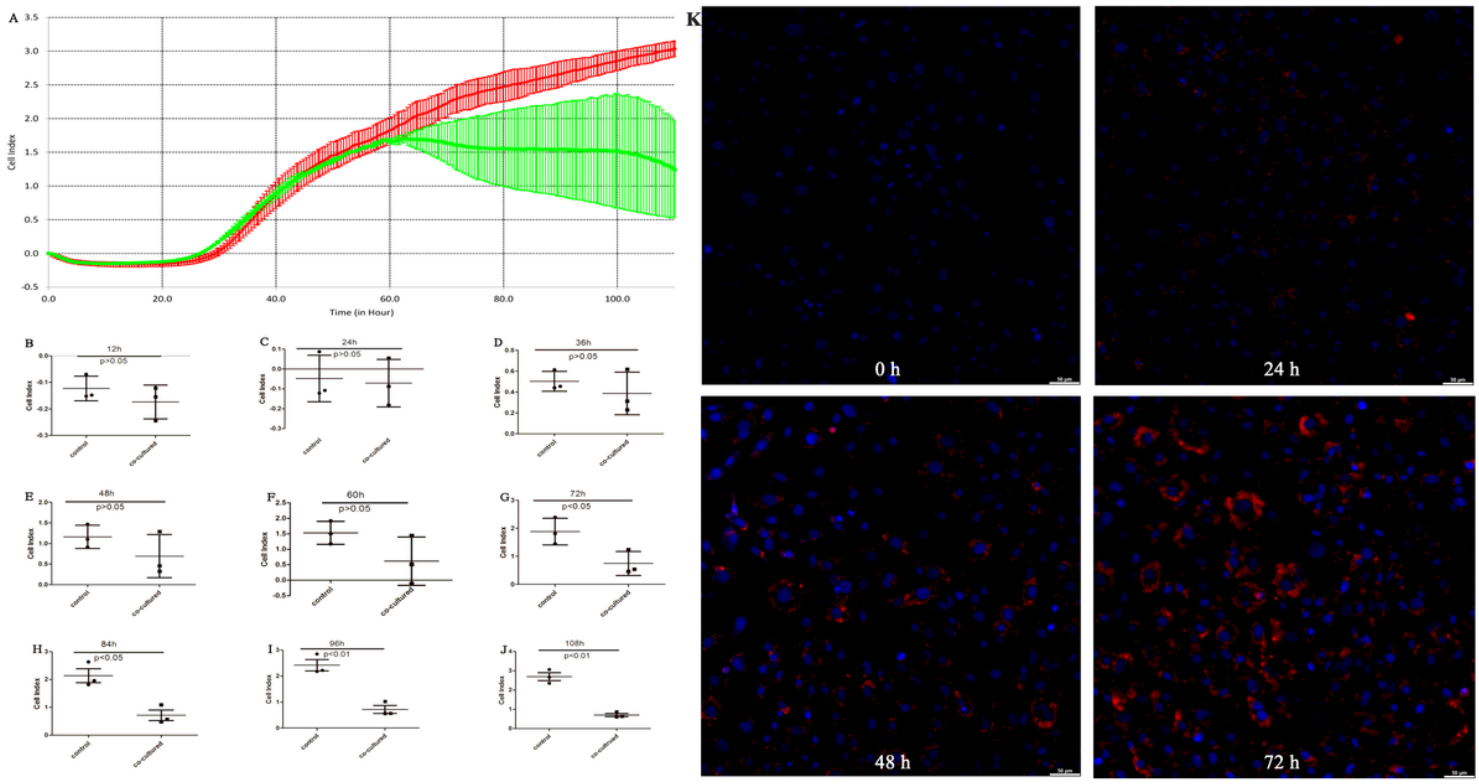


Figure 2

RTCA xCELLigence DP was used to monitor the change in the invasiveness of melanoma B16-F10 cells co-cultured with mouse blastocyst MVs within 110 h in real time. (A) B16-F10 cells co-cultured with mouse blastocyst MVs (green curve) and B16-F10 cells (red curve). (B–J) Co-cultured for 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, and 108 h; the comparison of cell index of mouse blastocyst MVs in the co-culture and control groups. (K) B16-F10 cell uptake of blastocyst MVs.



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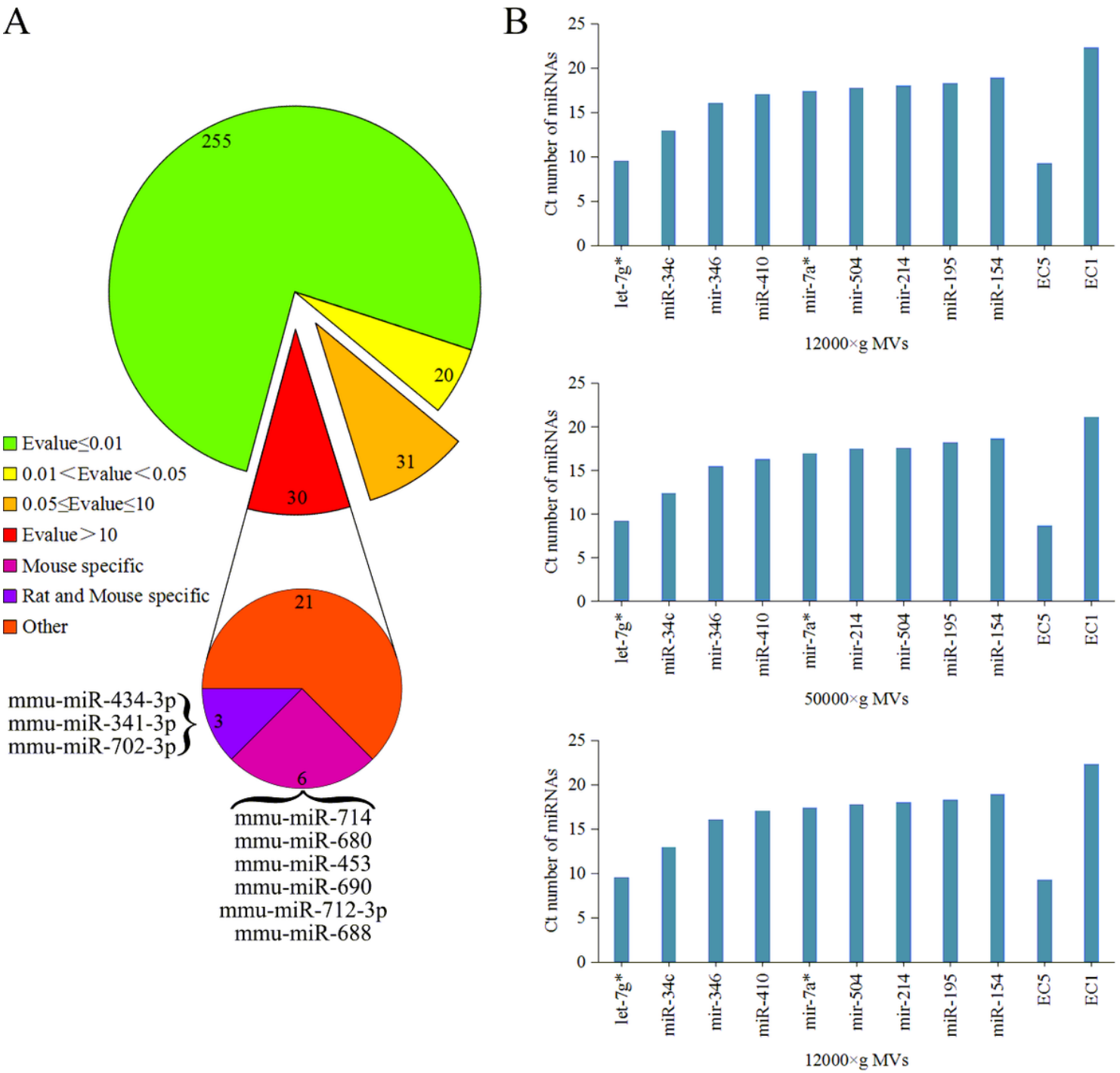
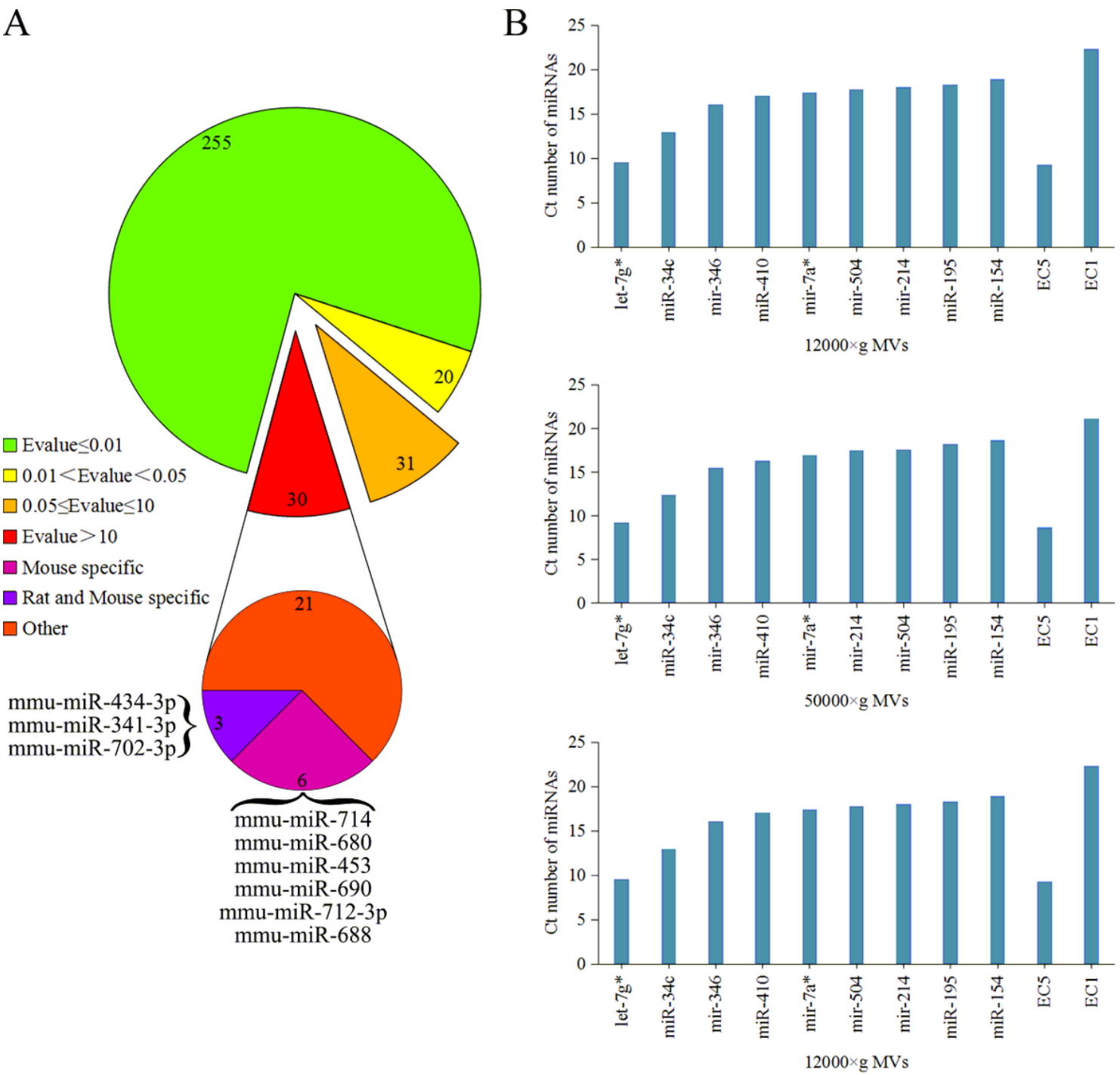


Figure 3

MiRNAs in MVs derived from pre-implantation blastocysts. (A) Pie chart comparing miRNA in MVs and human homologous miRNAs. (B) Real-time PCR results for miRNAs in MVs. EC1 and EC5 were used as external controls for qPCR.



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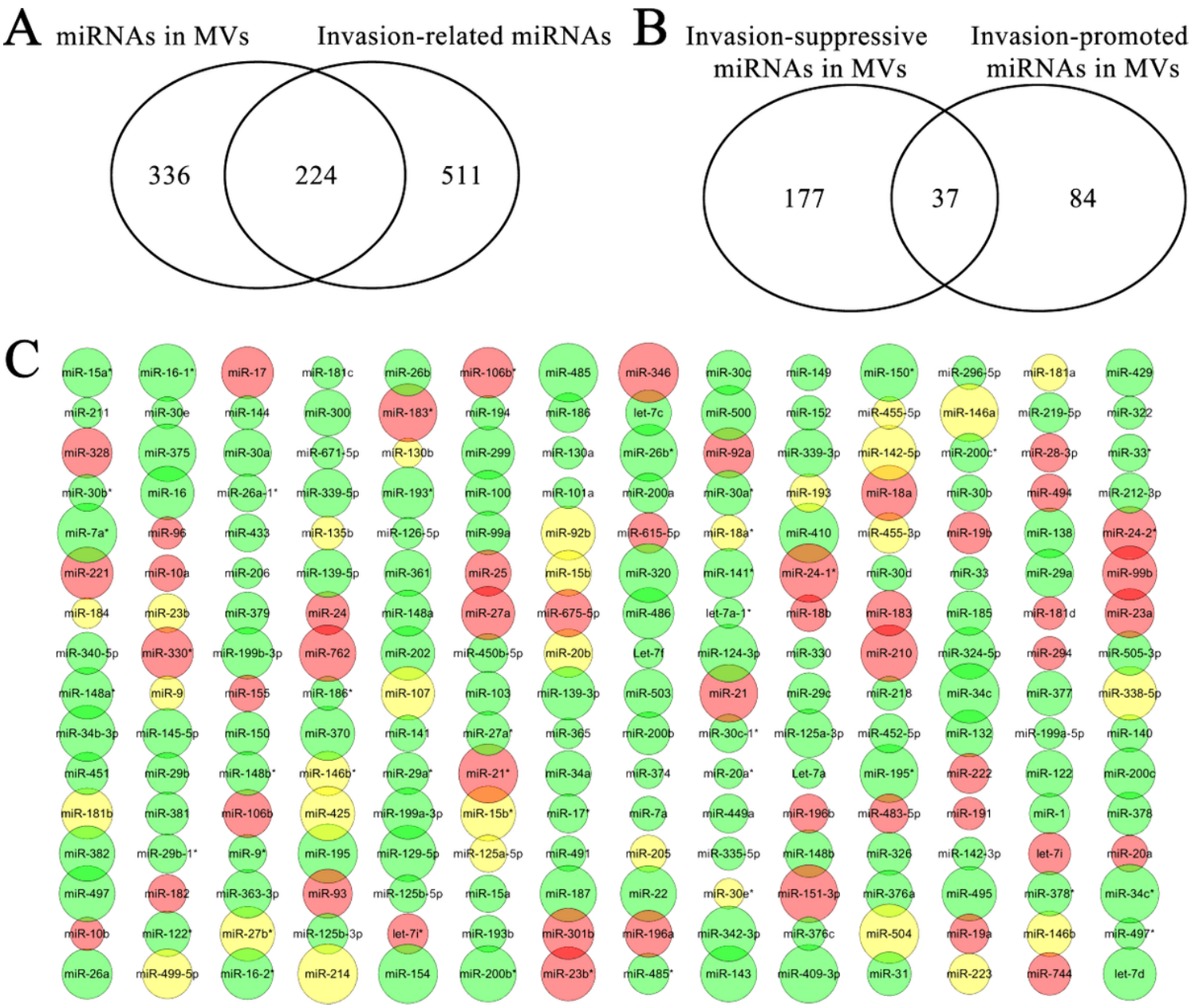
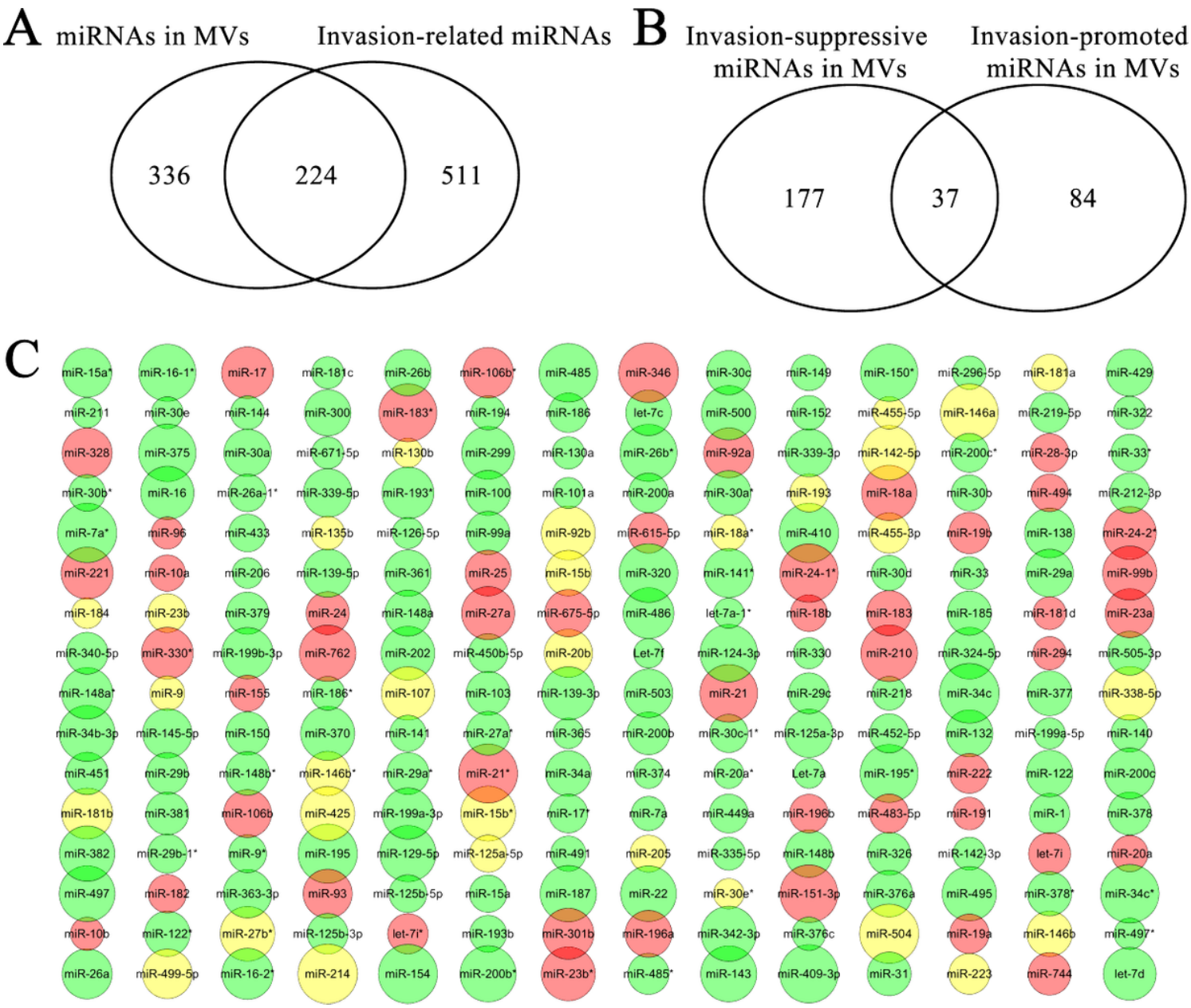


Figure 4

Invasion-related miRNAs in MVs derived from pre-implantation blastocysts. (A) Venn diagram comparing miRNA expression in MVs and invasion-related miRNAs. (B) Venn diagram comparing invasion-suppressive miRNAs and invasion-promoted miRNAs in MVs. (C) Relative expression of invasion-related miRNAs in MVs and its effects on invasion. Green circles represent invasion-suppressive miRNAs, red circles represent invasion-promoted miRNAs, and yellow circles represent miRNAs that simultaneously promoted or inhibited invasion. The size of the circle represents the relative expression of the miRNA in MVs, and the higher the expression, the larger the circle.



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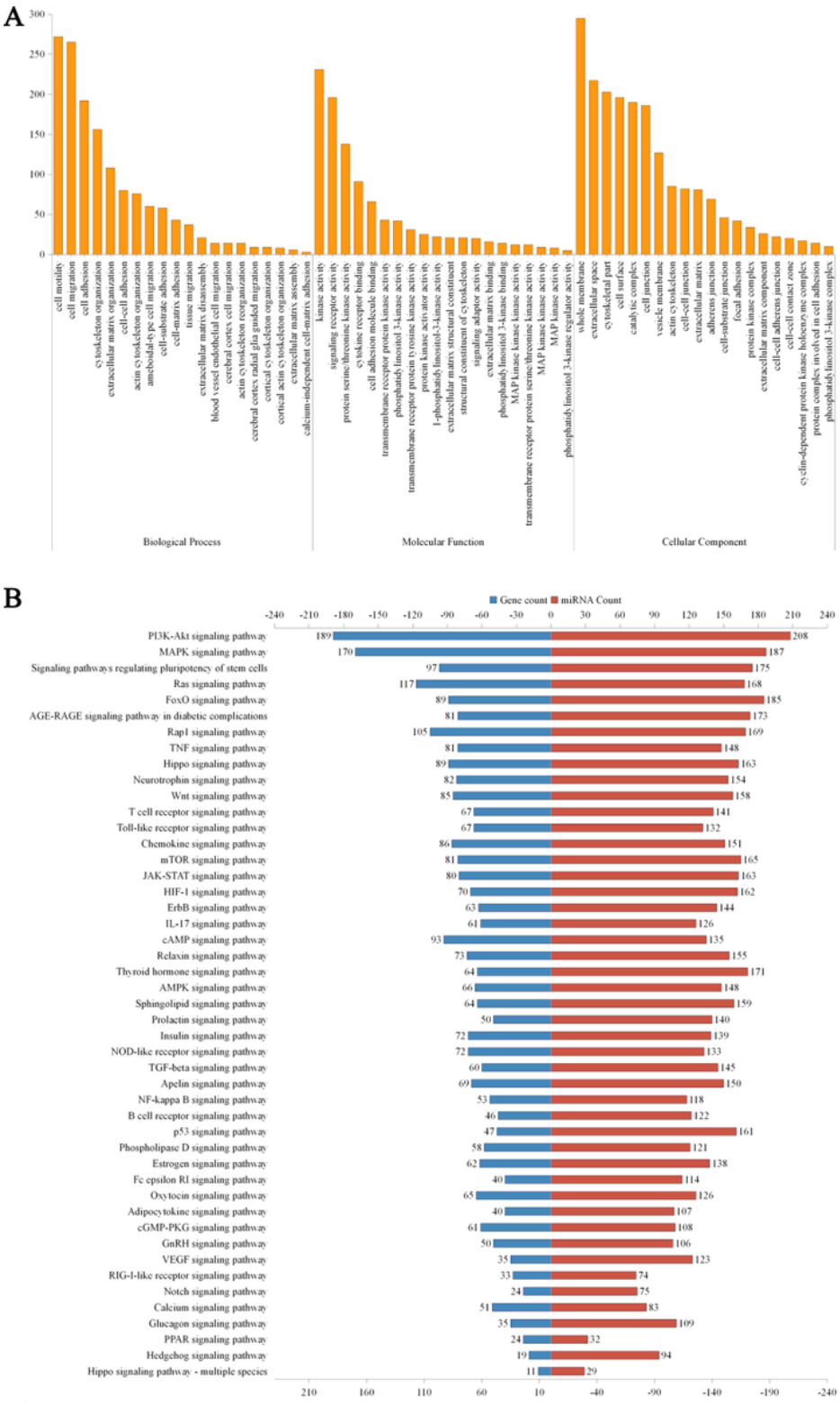
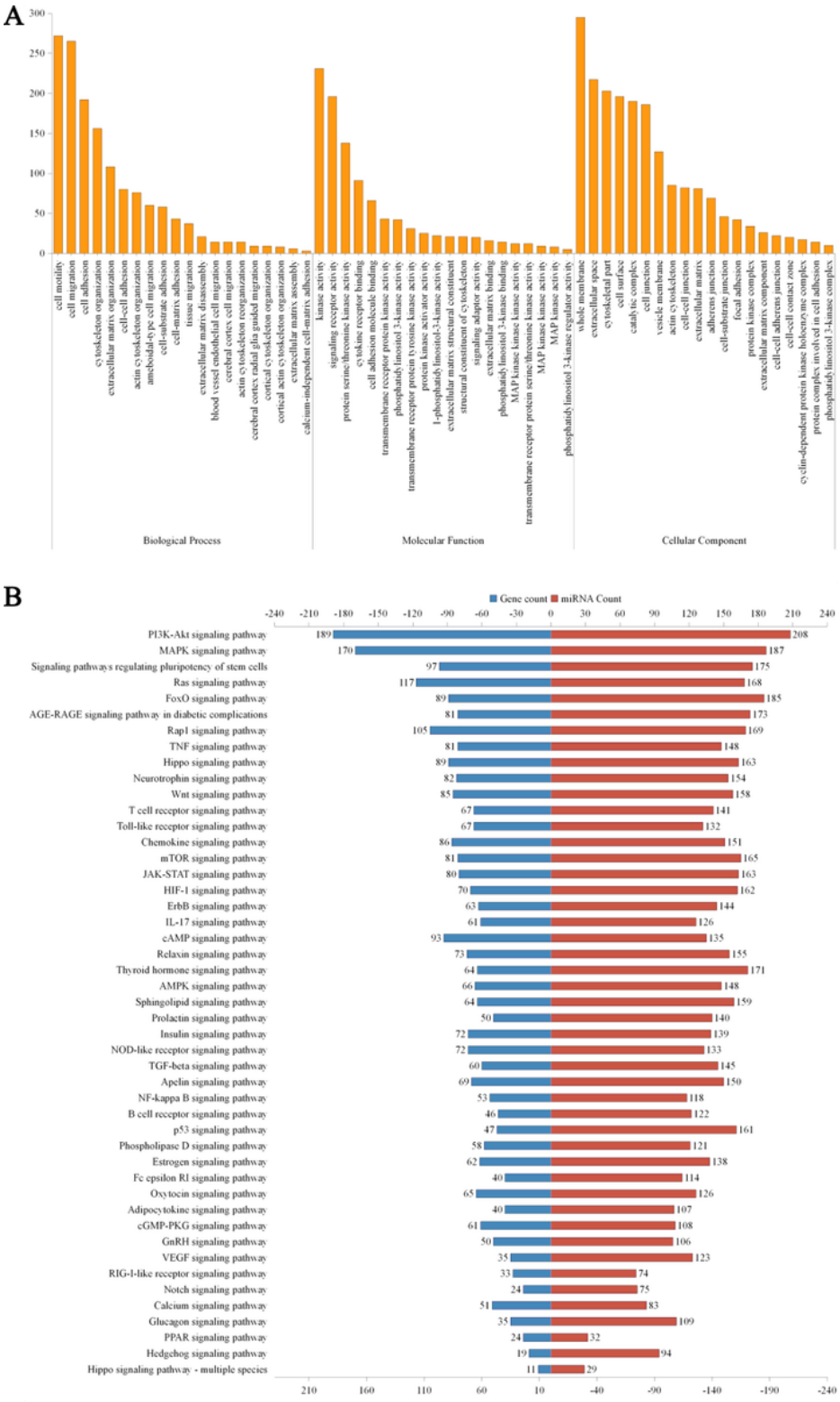


Figure 5

GO and KEGG pathway enrichment analyses of miRNAs in MVs derived from pre-implantation blastocysts. (A) GO analysis of miRNAs in MV target genes. (B) KEGG pathway analysis of miRNAs in MVs and their target genes.



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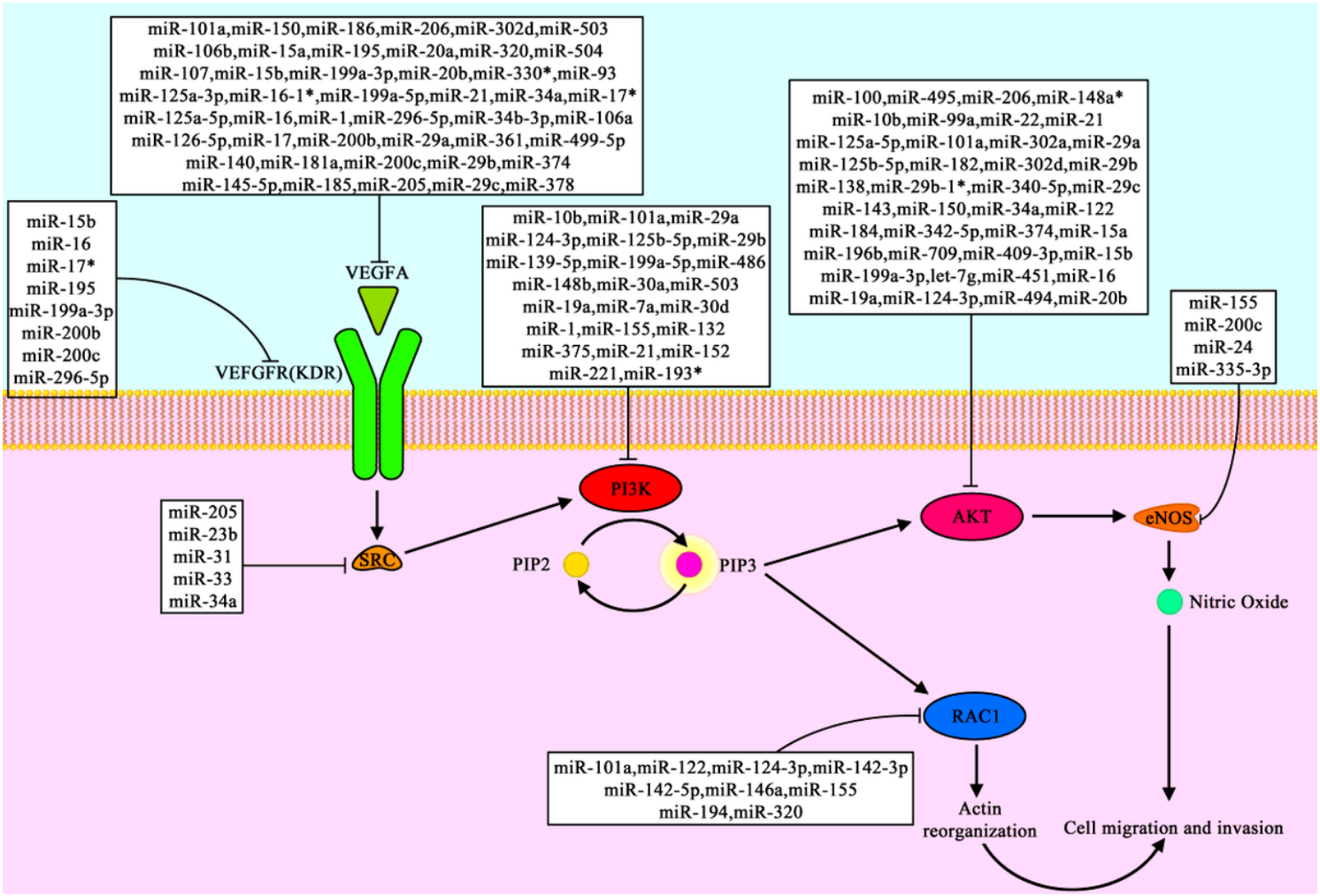
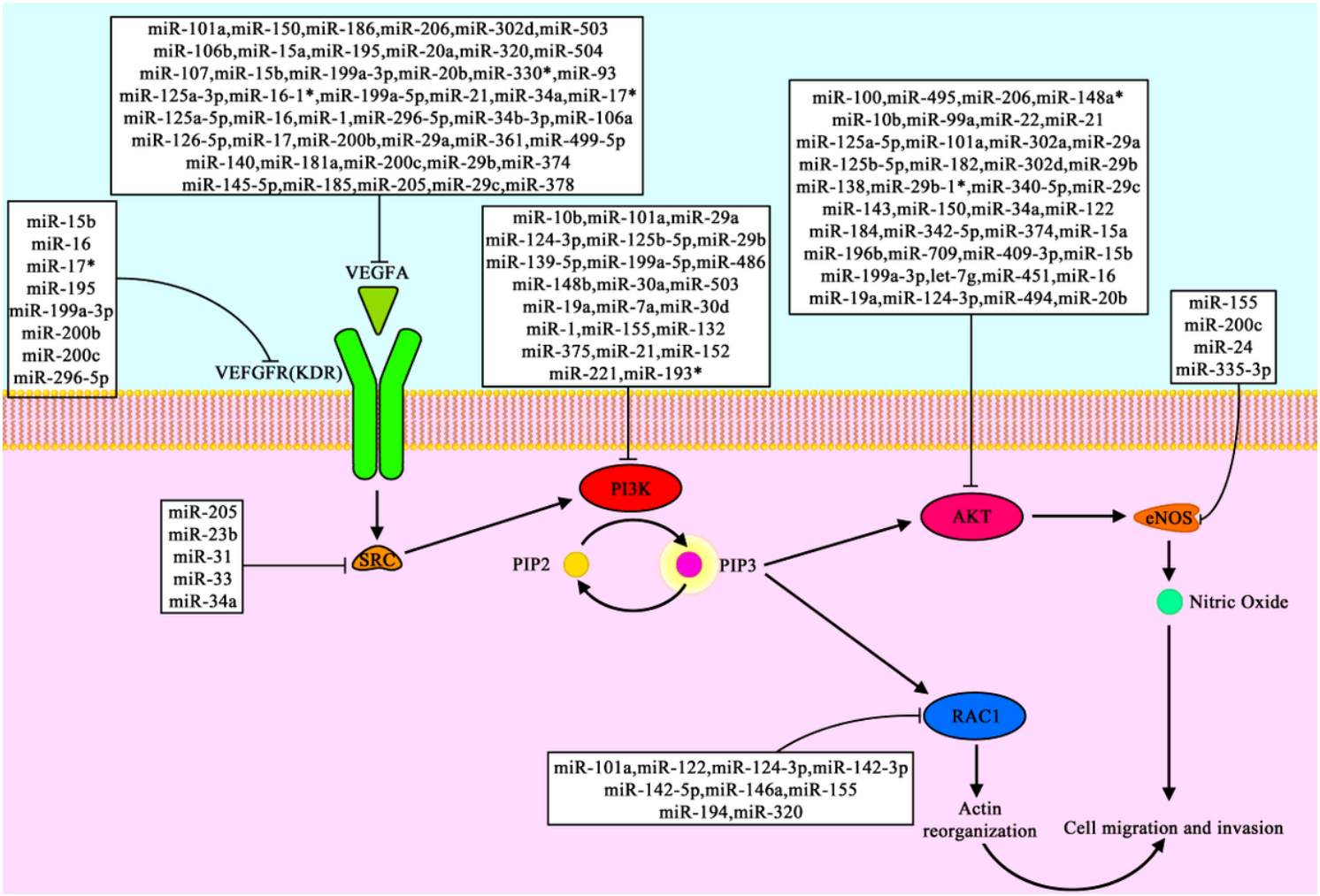


Figure 6

Relationship of miRNAs in MVs and PI3K–Akt signaling pathway.



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Supplementary Files

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- SupplementaryTables.docx
- SupplementaryTables.docx