The Effect of WKYMVm Peptide on Promoting mBMSCs Secrete Exosomes to Make M2 Macrophages Polarization Through FPR2 Pathway

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Abstract

Background: When the multicystic vesicles (precursor of exosomes) are formed in cells, there are two results. One is to be decomposed by lysosomes and the other one is to become exosomes which are transported out through transmembrane. On the other hand, M2 macrophages can promote the formation of local vascularization and provide necessary support for the repair of bone defects. In order to provide a new idea for the treatment of bone defects, the purpose of our study is to investigate the effect of WKYMVm peptide on the secretion of exosomes from murine bone marrow-derived MSCs （mBMSCs） and the effect of exosomes on the polarization of M2 macrophages.

Methods: WKYMVm peptide was used to activate the Formyl Peptide Receptor 2 (FPR2) pathway in mBMSCs. At first, we used CCK-8 kit to detect the cytotoxic effect of WKYMVm Peptide on mBMSCs. Secondly, we used western blotting and Quantitative real-time Polymerase Chain Reaction （Qrt-PCR） to detect the expression of Interferon Stimulated Gene 15 （ISG15） and Transcription Factor EB （TFEB） in mBMSCs. Thirdly, we used exosomes extraction kit and western blotting to detect the content of exosomes secreted by mBMSCs. Finally, we used the immunofluorescence and western blotting to count the number of M2 macrophages polarization.

Results: We firstly found that WKYMVm peptide had no toxic effect on mBMSCs at the concentration of 1μmol/L. Secondly, we found that when the FPR2 pathway was activated by WKYMVm peptide in mBMSCs, the expression of ISG15 and TFEB were decreased, leaded to the secretion of exosomes increased. Finally, we proved that the exosomes secreted by mBMSCs could promote the polarization of M2 macrophages. Moreover, all these effects can be blocked by WRWWWW (WRW4) peptide, an inhibitor of FPR2 pathway.

Conclusion: Our findings demonstrated the potential value of WKYMVm peptide in promoting the secretion of exosomes by mBMSCs and eventually leading to M2 macrophages polarization. We believed that our study could provide a research basis for the clinical treatment of bone defects.

1. Introduction

Many diseases can lead to bone defects or poor bone formation, including fractures, bone tumors, osteoporosis and so on[1,2]. At present, studies have shown that the function of M2 macrophages is to promote T helper 2 cell (Th2) immune response and participate in tissue remodeling, anti-inflammatory response, fibrosis formation, tumor development and other pathological processes[3,4,5]. M2 macrophages can down regulate immune response by secreting inhibitory cytokines Interleukin–10 (IL–10) or Transforming Growth Factor-β （TGF-β） and promote local vascularization, thus provide a choice for the treatment of bone defects[6,7]. Many local or systemic factors, such as inflammation, can affect the occurrence and development of vascularization. Related literatures show that in the process of vascularization, M2 macrophages polarization can participate in the immunoregulation of Mesenchymal Stem Cells (MSCs) and function regulation of osteoblasts[8]. As we know, vascularization is a complex

process, which requires many cytokines to participate in it, and M2 macrophages can promote the occurrence of angiogenesis[9]. According to the relevant research background, we propose the following hypotheses. The first point, there is an important inflammatory related ubiquitin like protein ISG15, When the expression of ISG15 is increased, it can decompose multicystic vesicles by lysosomes[10,11,12]. In order to increase the secretion of exosomes, we assume that we can reduce the expression of ISG15 by activating the FPR2 pathway in mBMSCs. The second point, When the expression of TFEB is increased, the lysosome activity is increased[13]. Therefore, we assume that we can activate FPR2 pathway to decrease the expression of TFEB, which can reduce lysosome activity and increase exosome secretion. If the activation of FPR2 pathway does promote the secretion of exosomes by mBMSCs, we propose the final hypothesis that when the number of exosomes secreted by mBMSCs is increased, M2 macrophages polarization will be promoted.

In recent years, promoting local vascularization is considered to be a new way to solve bone defects[14]. Macrophages, as a group of cells with plasticity and pluripotency, show obvious functional differences under the influence of different microenvironments in vivo and in vitro[15]. According to the different activated state and function, macrophages can differentiate into M1 macrophages and M2 macrophages[16]. M2 macrophages can promote local vascularization by secreting cytokines and regulating immune response[9].

There are three common isoforms of FPRs: FPR1, FPR2 and FPR3[17]. FPRs can exist in a variety of cells, such as neutrophils, monocytes/macrophages, which can secrete a variety of cytokines and participate in a variety of cell behaviors[18]. Among these isoforms, according to our hypotheses, activation of FPR2 pathway can promote the secretion of exosomes by inhibiting ISG15 expression and decreasing the expression of TFEB.

From the above rationale, we have been assumed that the activation of FPR2 pathway in mBMSCs can reduce the decomposition of intracellular multicystic vesicles (precursor of exosomes) and increase the secretion of exosomes. It also has been pointed out that there are abundant miRNA–146 in the exosomes secreted by MSCs, which can lead to M2 macrophages polarization[19]. In this study, we explored the mechanism of promoting exosomes secretion and M2 macrophages polarization.

2. Materials And Methods

2.1 Materials and reagents

The murine bone marrow-derived MSCs (mBMSCs) were purchased from Wuhan Fine Biotech company. The murine bone marrow-derived macrophage cell line RAW264.7 cells were purchased from American Type Culture Collection (ATCC). According to our team's previous research results[20], we choose WKYMVm peptide as the activator of FPR2 pathway. The WKYMVm peptide with purity more than 95% was synthesized by GL Biochem company and dissolved in acetonitrile. However, WRW4 peptide, an inhibitor of FPR2 pathway, with purity more than 95% was also synthesized by GL Biochem and dissolved in acetonitrile[20]. F–12 medium, Dulbecco's Modified Eagle's Medium (DMEM) and 0.25% trypsin were

purchased from Hyclone company. Fetal Bovine Serum (FBS) without exosomes was purchased from Lonsera company. DMSO was purchased from Sigma-Aldrich company. Penicillin and streptomycin were purchased from Beyotime Biotechology company. Phosphate Buffered Saline (PBS) solution, 4% paraformaldehyde, Triton X–100 solution and fluorescent mounting media were purchased from Solarbio company. PCR prime and Trizol buffer were purchased from Invitrogen company. RNA reverse transcription kit and Fluorescence quantitative PCR kit were purchased from Takara company. RIPA dissociation solution, PMSF, WB suit and sealing solution were purchased from Bioss company. All kinds of primary antibody and secondary antibody were purchased from Abcam company. Exosomes extraction kit was purchased from QIAGEN company. In addition, cell counterkit–8 assay (CCK–8) was purchased from Beyotime Biotechology company.

2.2 Cell culture

mBMSCs were cultured in a T25 culture flask filled with 90% F–12 medium and 10% FBS supplemented with penicillin (100 units/mL) and streptomycin (100 mg/mL) at incubation environment of 37.0℃ and 5% CO_2. RAW 264.7 cells were also cultured in a T25 culture flask containing 90% of DMEM medium and 10% FBS supplemented with penicillin (100 units/mL) and streptomycin (100 mg/mL) in an incubator set of 37.8℃ and 5% CO_2. 24 hours later, the non-adherent cells were removed. After reaching confluence, the adherent cells would continue to be cultured. The whole incubation process remained unchanged in 37.0℃ and 5% CO_2 environment for mBMSCs and 37.8℃ and 5% CO_2 environment for RAW 264.7 cells. All operation procedures were strictly in accordance with the operation manual proposed by Army Medical University.

2.3 Cytotoxicity assay

In this study, we used WKYMVm peptide as an activator for FPR2 pathway, so it was necessary to detect the toxicity of WKYMVm peptide. We used CCK–8 to detect the toxicity of WKYMVm peptide on mBMSCs. According to previous studies[20], we choose 1μmol/L as the concentration of WKYMVm peptide and 10μmol/L as the concentration of WRW4 peptide. mBMSCs were seeded in 96-well plates with a density of $5*10^3$ per well, and after one night of culture, the mBMSCs were tested with WKYMVm peptide and observed for 24, 48 and 72 hours. Next, we added CCK–8 solution (100μl/well) into the 96-well plates and leave it in the dark for another one hour. Finally, we detected and recorded the absorbance at 450nm with a microplate reader and compared with the control group without WKYMVm peptide.

2.4 Western blotting

To detect the protein expression, western blotting was used. β-Actin served as an internal control. According to the experimental design, mBMSCs, exosomes or M2 macrophages were dissolved in a cell lysate containing PMSF. The proteins were transferred to PVDF membrane by electrophoresis. The PVDF membrane was placed in the sealing liquid and shaken at low speed for 1 hour. Next, the sealed PVDF membrane was putted into the antibody box of the primary antibody and placed in the environment of 4℃ overnight. Then the PVDF membrane was washed with TBST for three times, after that PVDF

membrane was putted into the antibody box containing the secondary antibody and incubated for 2 hours at room temperature. We also washed PVDF membrane three times with TBST. At last, we used the Chemi Doc XRS Imaging System (Bio-Rad) to capture images of protein bands and analyzed the image with Image J software.

2.5 RNA isolation and Qrt-PCR

To investigate gene expression, RNA isolation and Qrt-PCR were used. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal control. mBMSCs were lysed with Trizol buffer. Complementary DNA was synthesized from 1 μg total RNA by reverse transcription kit, and the gene expression was detected by Qrt-PCR.

2.6 Exosomes extraction

To collect exosomes secreted by mBMSCs, exosomes extraction kit was used. In the continuous centrifugation stage, the first centrifugation was performed at 300 g for 5 minutes to remove cells. The second centrifugation was performed at 1200 g for 10 minutes and the third centrifugation was performed at 10000 g for 30 minutes to remove cellular debris. The exosomes were obtained from the supernatant according to the instructions of the exosomes extraction kit.

2.7 Immunofluorescence

In order to count the polarized number of M2 macrophages, immunofluorescence was used. The cells were fixed with 4% paraformaldehyde for 20 minutes. Next, the cells were permeated with 0.3% Triton X–100 solution for 15 minutes. Then cells were covered with 5% BSA at 37℃ for 30 minutes. After the BSA was removed, we added the primary antibody and antibody diluents in the ratio of 1:200 into the plates overnight at 4℃. The next day, we removed the primary antibody at room temperature. Then we added the secondary antibody and antibody diluents in the ratio of 1:400 into the plates. We incubated the cells at 37℃ for 30 minutes. Then we removed the secondary antibody. The cells were incubated with DAPI solution at room temperature for 5 minutes. Finally, we added the fluorescent mounting media into the plates and observed the cells under the fluorescence microscope. The quantification of M2 macrophages polarization was performed with Image J.

2.8 Preparation of WKYMVm peptide-conditioned medium and WKYMVm peptide plus WRW4 peptide-conditioned medium

One day after mBMSCs were stimulated by WKYMVm peptide or WKYMVm peptide plus WRW4 peptide, the supernatant was collected by centrifugation at 3000 rpm for 10 minutes. The collected supernatant was used as conditioned medium for growing RAW 264.7 cells in follow up study. In this study, it was called WKYMVm peptide-conditioned medium or WKYMVm peptide plus WRW4 peptide-conditioned medium.

2.9 ISG15 expression assay

mBMSCs were seeded in 6-well plates with a density of $1*10^6$ per well. After one night of culture, WKYMVm peptide and WKYMVm peptide plus WRW4 peptide were added into different plates. After 24 hours, we used western blotting and Qrt-PCR (Forward primer: TTTCCTGGTGTCCGTGA - Reverse primer: TCTGGGCAATCTGCTTCT) to detect the ISG15 expression. The ISG15 expression of WKYMVm peptide group was compared with the control group without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide group.

2.10 TFEB expression assay

mBMSCs were seeded in 6-well plates with a density of $1*10^6$ per well, WKYMVm peptide and WKYMVm peptide plus WRW4 peptide were added into different plates the next day. After 24 hours, we used western blotting and Qrt-PCR (Forward primer: AAGAACAGGGGTGAGGCA—Reverse primer: CCCAGGCTCAGGAGAGG) to detect the TFEB expression. The TFEB expression of WKYMVm peptide group was compared with the control group without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide group.

2.11 Exosomes content assay

It had been reported that the exosomes secreted by MSCs containing abundant miRNA–146, which could promote the polarization of M2 macrophages[19]. mBMSCs were seeded in 6-well plates with a density of $1*10^6$ per well. After one night of culture, WKYMVm peptide and WKYMVm peptide plus WRW4 peptide were added into different plates. After 24 hours, we centrifuged the cell culture medium continuously. After that, we used the exosomes extraction kit to collect exosomes. Then the exosomes were observed under the transmission electron microscopy (JEM–1400PLUS, Japan) and analyzed by western blotting (Marker: CD9, CD63). At last, we compared the exosomes content of WKYMVm group with the control group without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide group.

2.12 Assay of M2 macrophages polarization promoted by exosomes secreted by mBMSCs

In addition, we also needed to count the polarized number of M2 macrophages. We cultured RAW 264.7 cells in the WKYMVm peptide-conditioned medium, the medium without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide-conditioned medium. After 24 hours, we used the immunofluorescence (Marker: CD206) and western blotting (Marker: CD206, Arg–1) to count the polarized number of M2 macrophages. At first, RAW 264.7 cells were seeded in 96-well plates with a density of $5*10^3$ per well for immunofluorescence and 6-well plates with a density of $1*10^6$ per well for western blotting. The next day the WKYMVm peptide-conditioned medium, the medium without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide-conditioned medium were removed. Then we washed the plates with PBS twice. After immunofluorescence and western blotting were completed, the polarized number of M2 macrophages in the WKYMVm peptide-conditioned medium group were compared with the medium without WKYMVm peptide group and the WKYMVm peptide plus WRW4 peptide-conditioned medium group.

2.13 Statistical analysis

All the data came from three independent researchers. All data expressed in the form of mean ± standard deviation (SD). Student's t test was used to compare the differences between the two groups. All statistical analysis was performed by SPSS 22.0 software, and the value of P lower than 0.05 was statistically significant. (*P<0.05, **P<0.01, ***P<0.001)

3. Results

3.1 WKYMVm peptide mediated cytotoxicity in mBMSCs

mBMSCs (Figure 1A) were incubated with WKYMVm peptide for 24, 48 and 72 hours. Next, we used CCK–8 to detect whether the proliferation and cell viability of mBMSCs were changed. We found that WKYMVm peptide did not affect the proliferation and viability of mBMSCs (Figure 1B) compared with the control group without WKYMVm peptide.

3.2 WKYMVm peptide activated the FPR2 pathway of mBMSCs, resulted in the decreasing of ISG15 expression

According to our previous study, WKYMVm peptide could activate the FPR2 pathway in MSCs[20]. In this study, we found that when the FPR2 pathway was activated, the expression of ISG15 in mBMSCs was decreased significantly compared with the control group without WKYMVm peptide (Figure 2A,2B). At the same time, WRW4 peptide, an inhibitor of FPR2 pathway, could block the decreasing effect of ISG15 expression induced by WKYMVm (Figure 2A,2B).

3.3 The activation of FPR2 pathway by WKYMVm peptide could decrease the expression of TFEB

TFEB, an intracellular transcription promoter, is also an important factor in activating lysosome activity. By detecting the expression of TFEB in mBMSCs, we found that compared with the control group without WKYMVm peptide, the TFEB expression of WKYMVm peptide group was decreased (Figure 3A,3B). At the same time, WRW4 peptide, an inhibitor of FPR2 pathway, could block the decreasing effect of TFEB expression induced by WKYMVm (Figure 3A,3B).

3.4 The activation of FPR2 pathway by WKYMVm peptide could promote mBMSCs secrete exosomes

In this study, we detected the content of exosomes secreted by mBMSCs and compared with the control group without WKYMVm peptide. The morphology was shown in Figure 4A. We found that the content of exsosomes secreted by mBMSCs was increased with the WKYMVm peptide (Figure 4B,4C). At the same time, WRW4 peptide, an inhibitor of FPR2 pathway, could block the increasing effect of exosomes secretion induced by WKYMVm (Figure 4B,4C).

3.5 The exosomes secreted by mBMSCs could promote the polarization of M2 macrophages

We cultured RAW 264.7 cells (Figure 5A) in the WKYMVm peptide-conditioned medium, the medium without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide-conditioned medium. We found that the WKYMVm peptide-conditioned medium could lead to the promotion of M2 macrophages polarization compared with the medium without WKYMVm peptide (Figure 5B,5C,5D,5E,5F,5G). At the same time, WRW4 peptide, an inhibitor of FPR2 pathway, could block the increasing effect of M2 macrophages polarization induced by WKYMVm peptide-conditioned medium (Figure 5B,5C,5D,5E,5F,5G).

4. Discussion

Exosomes, originally discovered in the 1980s, are vesicles secreted by cells, with a diameter of about 30–100nm, which can participate in a variety of cell activities[21]. Bone derived exosomes can be secreted by a variety of cells, including osteocytes, osteoblasts, osteoclasts and mesenchymal stem cells (MSCs)[22]. A lot of literatures have shown that bone derived exosomes could regulate the behavior of other cells by regulating cell communication and transporting cellular active substances[23]. Furthermore, exosomes containing many biological molecules, such as proteins, enzymes and miRNAs, which have important physiological or pathological effects[24]. On the other hand, according to our previous studies, WKYMVm peptide, as a powerful activator of FPR2 pathway, can activate the FPR2 pathway in MSCs[20]. However, when the FPR2 pathway of MSCs is activated, what kind of changes will take place, and what impact on the secreted exosomes of MSCs, and what effects of secreted exosomes on the differentiation of macrophages are rarely reported. According to the current research results, we know that M2 macrophages can promote local vascularization, and local vascularization provides a solution for repairing bone defects[8,9]. Therefore, in this study, we mainly studied that when FPR2 pathway of mBMSCs was activated by WKYMVm peptide, a series of changes could promote the secretion of exosomes, and the exosomes secreted by mBMSCs could ultimately promote M2 macrophages polarization.

At present, many scholars have pointed out that M2 macrophages can promote local vascularization, and the increasing of local vascularization is the basis of bone defects repair[8,9]. Our studies found that mBMSCs could increase the secretion of exosomes through a series of reactions by activating the FPR2 pathway, and the exosomes were rich in miRNA–146, which was the key to promote the polarization of M2 macrophages[19]. First of all, WKYMVm peptide can activate the FPR2 pathway of mBMSCs and decrease the expression of inflammatory related ubiquitin like protein ISG15. We confirmed the above hypothesis by measuring the expression of ISG15 in mBMSCs and comparing with the control group without WKYMVm peptide. Moreover, the decreasing expression of ISG15 induced by the activation of FPR2 pathway by WKYMVm peptide could be blocked by WRW4 peptide. Secondly, activation of FPR2 pathway can lead to the expression of TFEB in mBMSCs reduced, and more exosomes released. Similarly, we confirmed the above hypothesis by measuring the expression of TFEB and comparing with the control group without WKYMVm peptide. We also found that the decreasing expression of TFEB induced by the activation of FPR2 pathway by WKYMVm peptide could be blocked by WRW4 peptide.

There are three stages in the secretion of exosomes. Stage one: the inner membrane of endosomes form luminal vesicle. Stage two: when the content of luminal vesicles reaches a certain number, they begin to gather together and fuse into multicystic vesicles. Stage three: multicystic vesicles, also known as precursor of exosomes, can either be fused with lysosomes and be decomposed or can be fused with plasma membrane and be released out of cell[25,26]. Our study mainly increased exosomes secretion by regulating the third stage, that was, changing lysosome activity. We compared the content of exsosomes secreted by mBMSCs in WKYMVm peptide group with the control group without WKYMVm peptide and confirmed that the activation of FPR2 pathway could promote the secretion of exosomes in mBMSCs. At the same time, the increasing content of exsosomes secreted by mBMSCs induced by the activation of FPR2 pathway by WKYMVm peptide could be blocked by WRW4 peptide. Finally, we investigated the effect of exosomes secreted by mBMSCs on the polarization of M2 macrophages. According to the previous theory, the exosomes secreted by MSCs containing abundant miRNA–146, which can promote the polarization of M2 macrophages[19]. In our study, we cultured RAW 264.7 cells in the WKYMVm peptide-conditioned medium, the medium without WKYMVm peptide and the WKYMVm peptide plus WRW4 peptide-conditioned medium. Then we counted the polarized number of M2 macrophages in above three groups. We found that the secreted exosomes of mBMSCs did promote the polarization of M2 macrophages. In the meantime, the increasing polarized number of M2 macrophages induced by WKYMVm peptide-conditioned medium could also be blocked by WRW4 peptide.

Promoting local vascularization is the premise of the treatment of bone defects, and M2 macrophages have a clear role in promoting local vascularization[27]. It has been reported that even if MSCs are removed, the polarization of M2 macrophages can continue for a period of time[28,29], indicated that the exosomes secreted by MSCs play a very important role in the polarization of M2 macrophages.

In the inflammatory environment, the expression of many inflammatory related proteins will increase[30,31]. For example, Lipoproteins （LPS） can be involved in a variety of inflammatory reactions[32,33]. We found that when the FPR2 pathway of mBMSCs was activated, the effect was opposite to the LPS-induced response. In LPS-induced inflammatory response, IL–1β, Tumor Necrosis Factor-α （TNF-α）, IL–6 and other classic inflammatory factors are released in large quantities, such proinflammatory factors can activate multiple inflammatory related pathways, including Nuclear Transcription Factor-κB (NF-κB), Mitogen-activated Protein Kinase (MAPK) and Signal Transducer and Activator of Transcription (STAT) signaling pathways[31,32,33]. Thus resulted in the increasing of ubiquitin like protein ISG15 expression and then affecting the secretion of exosomes by MSCs[34,35]. Interestingly, STAT3 pathway, a subtype of STAT pathway, plays an important role in LPS-induced inflammatory response[36,37]. According the previous studies, IL–6 can combine with glycoprotein 130 (gp130) and transform into Membrane-bound IL–6 Receptor (Mil–6R), which can activate STAT3 pathway[38]. Further studies show that CD9 can prevent STAT3 pathway from being degraded by ubiquitin dependent lysosomes andcan stabilize gp130[39]. Therefore, we believe that in inflammatory environment, when CD9 expression is blocked, IL–6/gp130/STAT3 pathway will be affected, eventually lead to the decreasing of ISG15 expression. So, we suggest that WKYMVm peptide can activate the FPR2 pathway on mBMSCs and inhibit the binding of IL–6 with gp130 to reduce the expression of CD9, which

can achieve the positive regulation of exosomes secretion. However, the activation of FPR2 pathway in MSCs has anti-inflammatory effect, which can resist LPS-induced inflammatory response, thus resultedin decreasing ubiquitin like protein ISG15 expression and positively regulating the exosomes secretion by MSCs[40,41,42]. The mechanism of exosomes secreted by mBMSCs and M2 macrophages polarization stimulated by exosomes was shown in Figure 6.

5. Conclusions

In conclusion, our study proved that after the activation of FPR2 pathway in mBMSCs, the secretion of exosomes could be promoted by regulating the expression of ISG15 and TFEB. In addition, our study also confirmed that the exosomes secreted by mBMSCs could promote the polarization of M2 macrophages. Due to M2 polarization has the function of promoting local vascularization[43,44], our study provides a new idea for the treatment of bone defects, and also makes the regulation of secretion of MSCs exosomes become the direction of future research.

However, our study still has some limitations. First of all, our studies focused on MSCs of mice bone marrow. Although it have been confirmed that the activation of FPR2 pathway can promote the secretion of exosomes and eventually lead to polarization of M2 macrophages, further research is needed to prove whether human MSCs are also regulated by this mechanism. Secondly, the mechanism of local vascularization after M2 macrophages polarization still needs further study. Finally, it is necessary to establish relevant animal models to clarify the detailed mechanism of local vascularization promoting bone defects repair through animal experiments.

Abbreviations

MSCs: Mesenchymal Stem Cells

mBMSC: Murine bone marrow-derived MSCs

FPR: Formyl Peptide Receptors

Qrt-PCR: Quantitative real-time Polymerase Chain Reaction

ISG15: Interferon Stimulated Gene 15

TFEB: Transcription Factor EB

Th2: T helper 2 cell

IL: Interleukin

TGF: Transforming Growth Factor

PBS: Phosphate Buffered Saline

FBS: Fetal Bovine Serum

TNF: Tumor Necrosis Factor

LPS: Lipoproteins

NF-κB: Nuclear Transcription Factor κ

MAPK: Mitogen-activated Protein Kinase

STAT: Signal Transducer and Activator of Transcription

gp130: glycoprotein 130

Mil–6R: Membrane-bound IL–6 Receptor

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Authors' contributions

Wenbo Zhao and Junxian Hu completed the experiments; Wenbo Zhao and Junxian Hu analysed the data; Wenbo Zhao wrote the paper; Wenbo Zhao and Qingyi He designed the study; Wenbo Zhao and Qingyi He performed final manuscript.

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References

1. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioact Mater. 2017;2(4):224-247.
2. Yu X, Tang X, Gohil SV, et al. Biomaterials for bone regenerative engineering. Adv Healthcare Mater. 2015;4(9):1268-1285.
3. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol. 2014;14(6):392-404.
4. Gharun K, Senges J, Seidl M, et al. Mycobacteria exploit nitric oxide induced transformation of macrophages into permissive giant cells. Embo Rep. 2017;18(12):2144-2159.
5. Lampiasi N, Russo R, Zito F. The alternative faces of macrophage generate osteoclasts. Biomed Res Int. 2016;9089610.
6. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol. 2007;8(6):464-478.
7. Bian S, Zhang L, Duan L, et al. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. J Mol Med. 2014;92(4):387-397.
8. Chellaiah MA, Ma T. Membrane localization of membrane type 1 matrix metalloproteinase by CD44 regulates the activation of Pro-matrix metalloproteinase 9 in osteoclasts. BioMed Res Int. 2013;302392.
9. Fang S, Li Y, Chen P. Osteogenic effect of bone marrow mesenchymal stem cell derived exosomes on steroid induced osteonecrosis of the femoral head. Drug Design Dev Ther. 2019;13:45-55.
10. Bjørge IM, Kim SY, Mano JF, et al. Extracellular vesicles, exosomes and shedding vesicles in regenerative medicine: a new paradigm for tissue repair. Biomater Sci UK. 2017;6(1):60-78.
11. Bliss SA, Sinha G, Sandiford OA, et al. Mesenchymal stem cell derived exosomes simulate cycling quiescence and early breast cancer dormancy in bone marrow. Cancer Res. 2016;76(19):5832-5844.
12. Furuta T, Miyaki S, Ishitobi H, et al. Mesenchymal stem cell derived exosomes promote fracture healing in a mouse model. Stem Cells Transl Med. 2016;5(12):1620-1630.
13. Ebrahimi S, Hosseini M, Shahidsales S, et al. Targeting the Akt/PI3K signaling pathway as a potential therapeutic strategy for the treatment of pancreatic cancer. Curr Med Chem. 2017;24(13):1321-1331.
14. Li L, Lu H, Zhao Y, et al. Functionalized cell free scaffolds for bone defect repair inspired by self healing of bone fractures: A review and new perspectives. Mater Sci Eng C Mater Biol Appl. 2019;98:1241-1251.
15. Rotival M, Ko JH, Srivastava PK, et al. Integrating phosphoproteome and transcriptome reveals new determinants of macrophage multinucleation. Mol Cell Proteomics. 2015;14(3):484-498.

16. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;496(7446):445-455.
17. Cattaneo F, Parisi M, Ammendola R. WKYMVm induced cross talk between FPR2 and HGF receptor in human prostate epithelial cell line PNT1A. FEBS Lett. 2013;587(10):1536-1542.
18. Heo SC, Kwon YW, Jang IH, et al. WKYMV induced activation of formyl peptide receptor 2 stimulates ischemic neovasculogenesis by promoting homing of endothelial colony forming cells. Stem Cells. 2014;32(3):779-790.
19. Barrera RJ, Lavoie JR, Maganti HB, et al. Micro RNA profiling of exosomes from marrow derived mesenchaymal stromal cells in patients with acute myeloid leukemia: implications in leukemogenesis. Stem Cell Rev. 2017;13:817-825.
20. Juxian Hu, Xianghe Li, Yueqi Chen, et al. The protective effect of WKYMVm peptide on inflammatory ostolysis through regulating NF-κB and CD9/gp130/STAT3 signalling pathway. J Cell Mol Med. 2020;24(2):1893-1905.
21. RM Johnstone, M Adam, JR Hammond, et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem. 1987;262(19):9412-9420.
22. Janas T, Janas MM, Sapon K, et al. Mechanism of RNA loading into exosomes. FEBE Lett. 2015;589(13):1391-1398.
23. Deng L, Wang Y, Peng Y, et al. Ostoblast derived microvesicles: a novel mechanism for communication between osteoblasts and osteoclasts. Bone. 2015;79:37-42.
24. Valadi H, Ekastrom K, Bossios A, et al. Exosome mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654-659.
25. Coumans FAW, Brisson AR, Buzas EL, et al. Methodological guidelines to study extracellular vesicles. Circ Res. 2017;120(10):1632-1648.
26. García-Silva S, Benito-Martín A, Sánchez-Redondo S, et al. Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and mutation. J Exp Med. 2019;216(5):1061-1070.
27. Tang D, Chen S, Hou D, et al. Regulation of macrophage polarization and promotion of endothelialization NO generating and PEG-YIGSR modified vasculargraft. Mater Sci Eng C Mater Biol Appl. 2018;84:1-11.
28. Grigoriadis AE, Wang ZQ, Cecchini MG, et al. c-FOS: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. Science. 1994;266(5184):443-448.
29. Cui Y, Luan J, Li H, et al. Exosomes derived from mineralizing osteoblasts promote ST2 cell osteogenic differentiation by alteration of microRNA expression. FEBS Lett. 2016;590(1):185-192.
30. Park-Min KH, Lim E, Lee MJ, et al. Inhibition of osteoclastogenesis and inflammatory bone resoption by targeting BET proteins and epigenetic regulation. Nat Commun. 2014;5:5418.

31. Dai J, Escara WJ, Keller JK, et al. Primary prostate cancer educates bone stroma through exosomal pyruvate kinase M2 to promote bone metastasis. J Exp Med. 2019;216(12):2883-2899.
32. Bilen MA, Pan T, Lee Y, et al. Proteomics profiling of exosomes from primary mouse osteoblasts under proliferation versus mineralization conditions and characterization of their uptake into prostate cancer cells. J Proteome Res. 2017;16(8):2709-2728.
33. Xing L, Bushnell TP, Carlson L, et al. NF-κB p50 and p52 expression is not required for RANK expression osteoclast progenitor formation but is essential for RANK and cytokine mediated osteoclastogenesis. J Bone Miner Res. 2002;17(7):1200-1210.
34. Yuhao Liu, Chao Wang, Gang Wang, et al. Loureirin B suppresses RANK induced osteoclastogenesis and ovariectomized via attenuating NFATc1 and ROS activities. Theranostics. 2019;9(16):4648-4662.
35. Takayanagi H, Kim S, Koga T, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell. 2002;3(6):889-901.
36. Lam J, Takeshita S, Barker JE, et al. TNF alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest. 2000;106(12):1481-1488.
37. Park MY, Kim HS, Lee M, et al. FAM19A5, a brain specific chemokine, inhibits RANKL induced osteoclast formation through formyl peptide receptor 2. Sci Rep. 2017;7(1):15575.
38. IA Schepetkin, AI Khlebnikov, MP Giovannoni, et al. Development of small molecule non-peptide formay peptide receptor (FPR) ligands and molecular modeling of their recognition. Curr Med Chem. 2014;21(13):1478-1504.
39. Dalia M, Clara IR, Arantza I. Immunomodulatory effects of MSCs in bone healing. Int J Mol Sci. 2019;20(21):5467.
40. Sobacchi C, Schulz A, Coxon FP, et al. Osteopetrosis: genetics, treatment and new insights into osteoclast function. Nat Rev Endocrinol. 2013;9:522-536.
41. Bourdonnay E, Zaslona Z, Penke LR, et al. Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling. J Exp Med. 2015;212(5):729-742.
42. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006;98(5):1076-1084.
43. Tian T, Zhang T, Lin Y, et al. Vascularizaiton in craniofacial bone tissue engineering. J Dent Res. 2018;97(9):969-976.
44. Anjali PK, Saravana KR, Ralf HA. Couping of angiogenesis and osteogenesis by a specific vessel subtype in bone. Nature. 2014;507(7492):323-328.

Figures

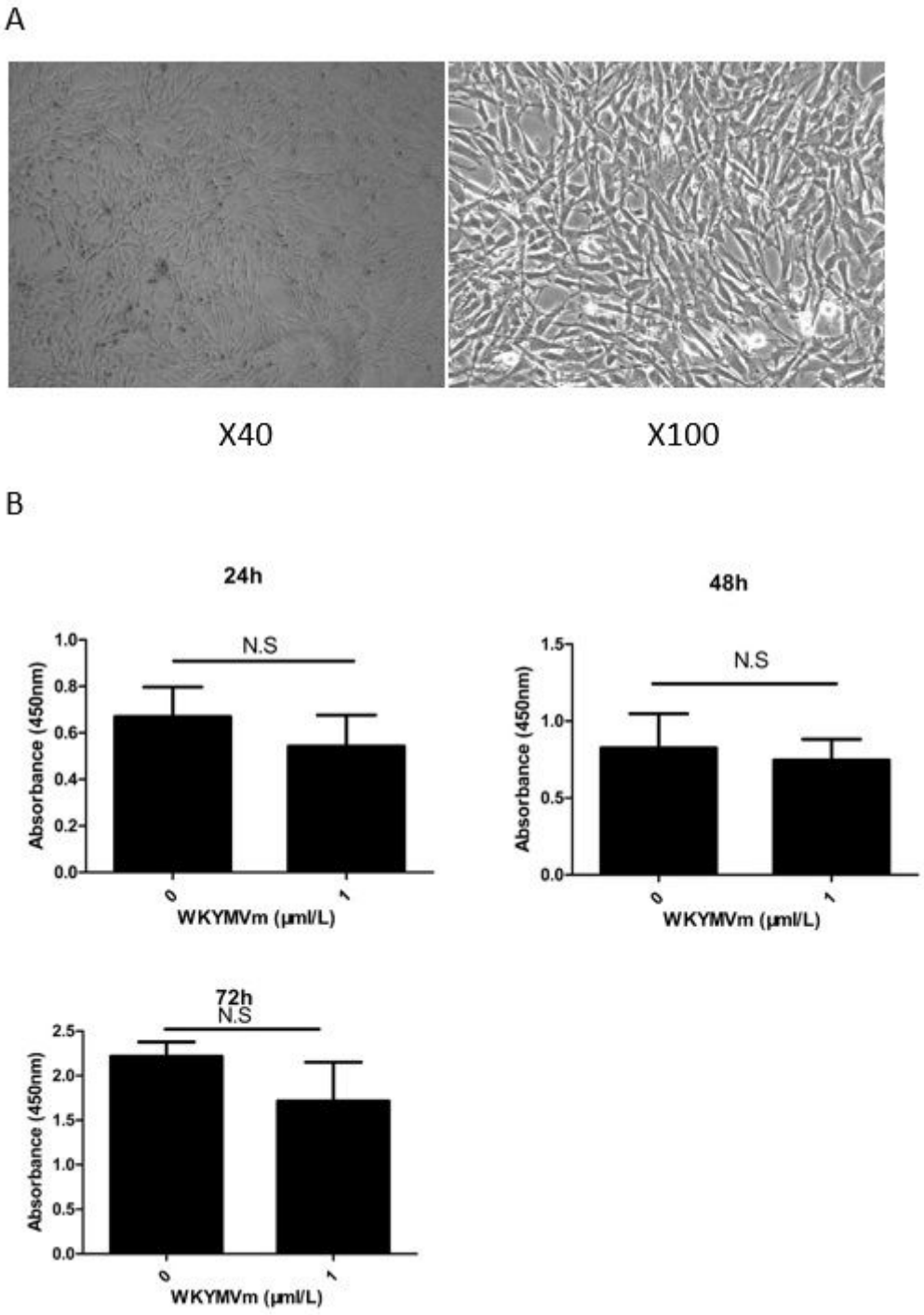

Figure 1

A: Morphology of mBMSCs under optical microscope. B: Effects of WKYMVm peptide on 24h, 48h and 72h toxicity of mBMSCs.

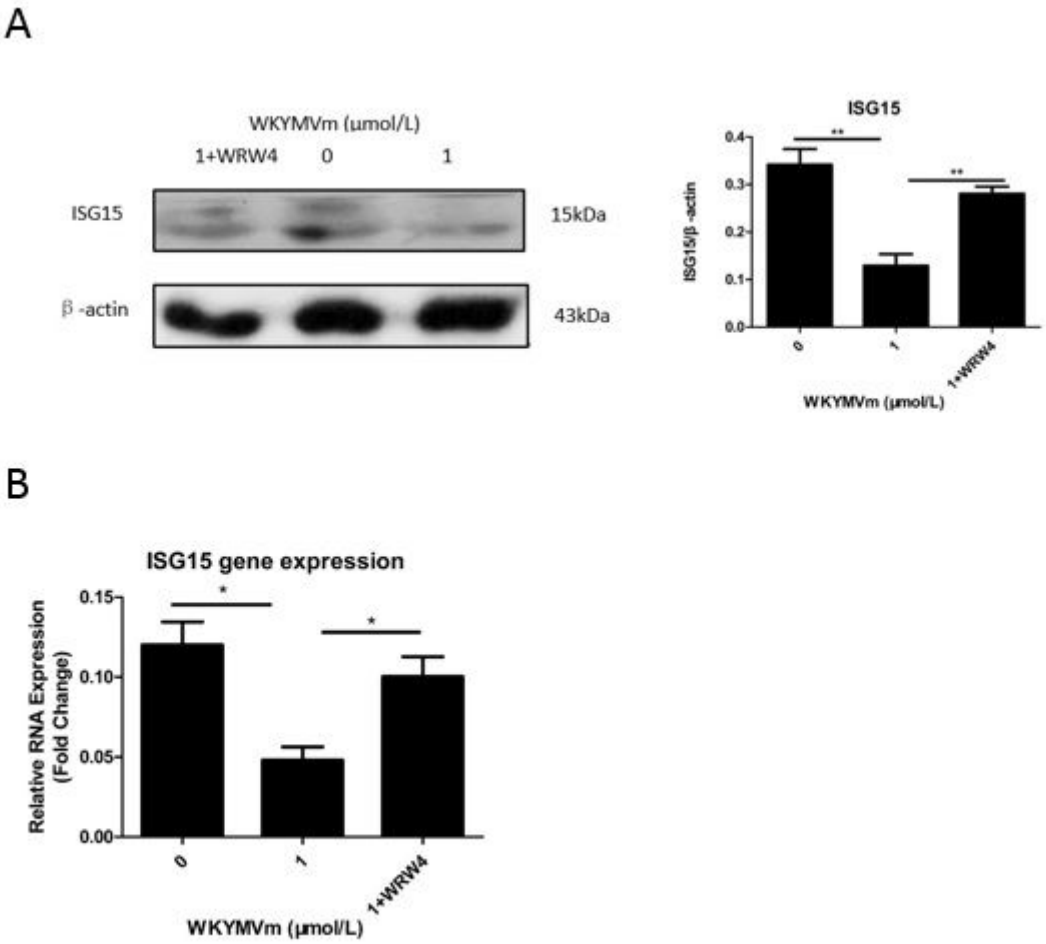

Figure 2

A: Effects of WKYMVm peptide on the ISG15 protein expression. B: Effects of WKYMVm peptide on the ISG15 gene expression.

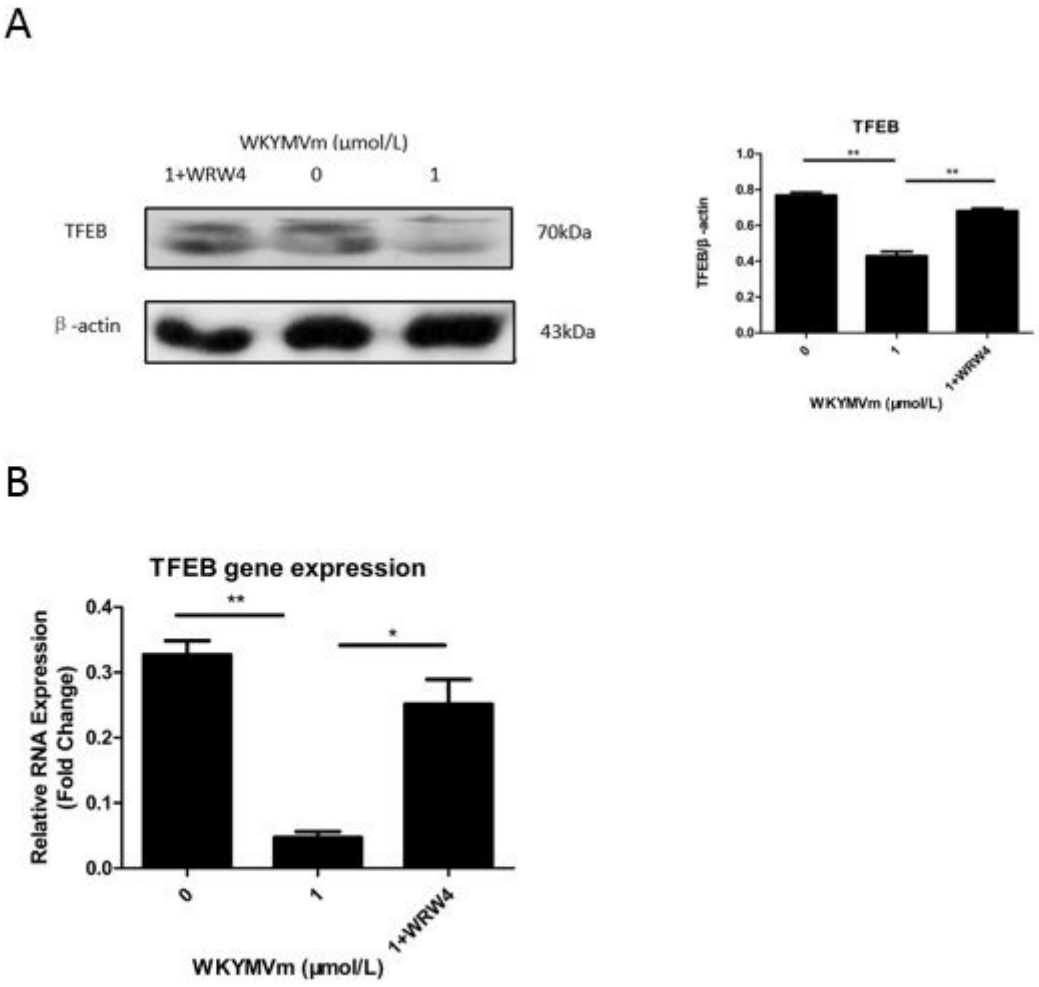

Figure 3

A: Effects of WKYMVm peptide on the TFEB protein expression. B: Effects of WKYMVm peptide on the TFEB gene expression.

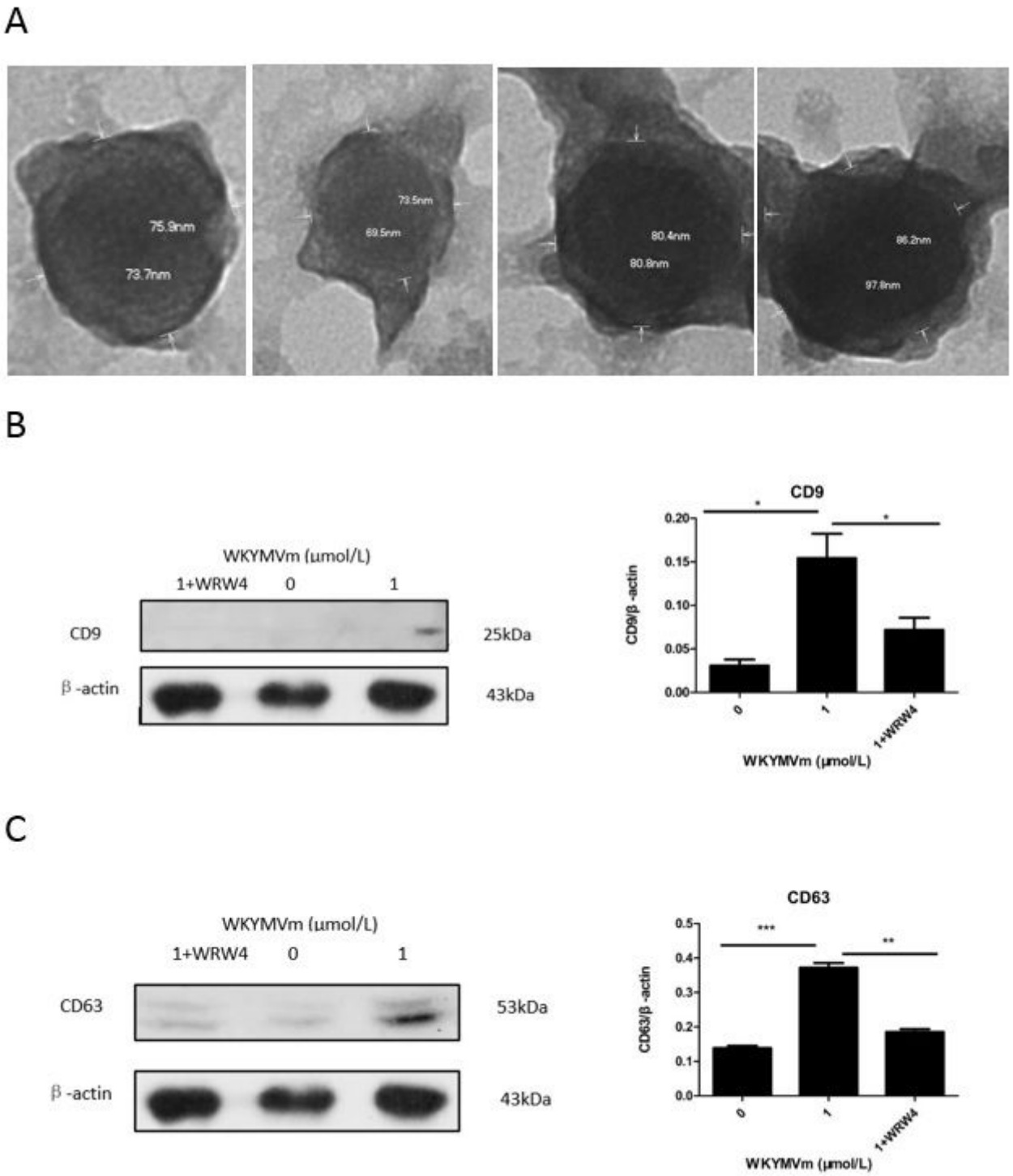

Figure 4

A: Morphology of exsomes secreted by mBMSCs under the transmission electron microscopy. B: Effects of WKYMVm peptide on the content of exsosomes secreted by mBMSCs (Marker: CD9). C: Effects of WKYMVm peptide on the content of exsosomes secreted by mBMSCs (Marker: CD63).

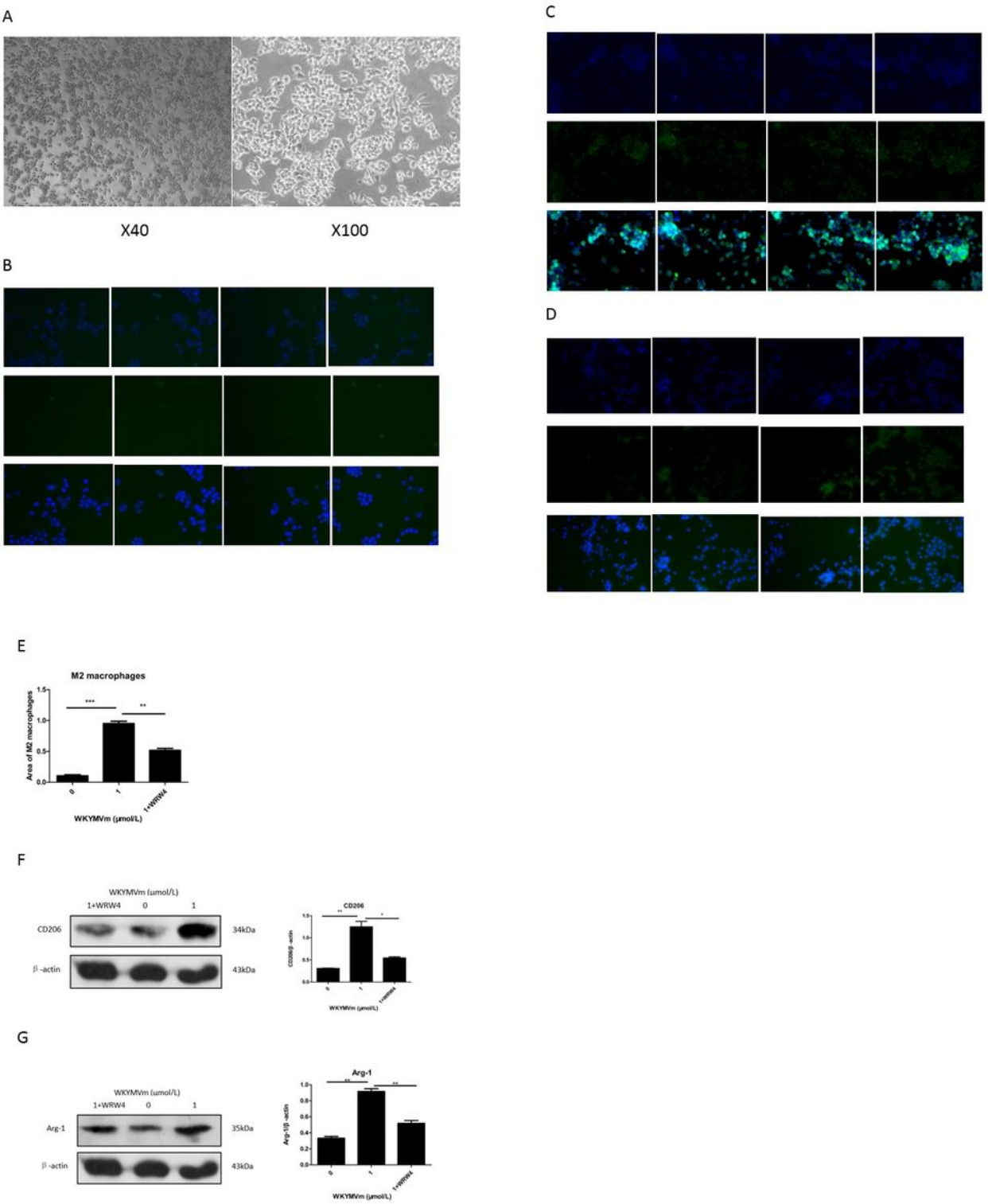

Figure 5

A: Morphology of RAW 264.7 cells under optical microscope. B: DAPI (blue), CD206 (green) and merge (blue/green) were observed by immunofluorescence microscopy in the control group without WKYMVm peptide. C: DAPI (blue), CD206 (green) and merge (blue/green) were observed by immunofluorescence microscopy in the WKYMVm peptide group. D: DAPI (blue), CD206 (green) and merge (blue/green) were observed by immunofluorescence microscopy in the WKYMVm peptide plus WRW4 peptide group. E:

Quantification of M2 macrophages polarization by immunofluorescence (Marker: CD206). F: Quantification of M2 macrophages polarization by western blotting (Marker: CD206). G: Quantification of M2 macrophages polarization by western blotting (Marker: Arg-1)

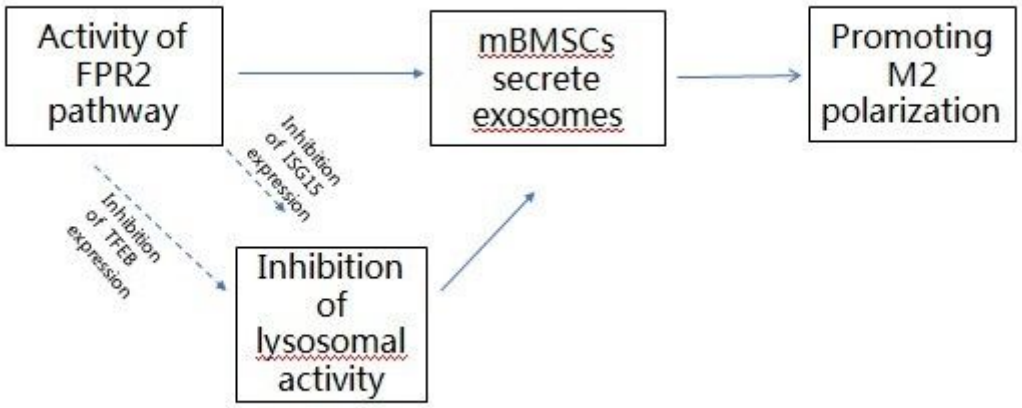

Figure 6

Schematic diagram of exosomes secreted by mBMSCs and M2 macrophages polarization stimulated by exosomes (Solid line represents activation; Dotted line represents inhibition).

Supplementary Files

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- Supplementary.docx