A THESIS FOR THE DEGREE OF MASTER

Effects of Cyclooxygenase-2 in Canine Melanoma-Derived Extracellular Vesicles on Tumor Microenvironment In Vitro

개 흑색종 유래 세포외 소포체 내 Cyclooxygenase-2의 종양 미세환경에 대한 in vitro 효과

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이 논문을 수의학 석사 학위논문으로 제출함

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Abstract

Effects of Cyclooxygenase-2 in Canine Melanoma-Derived Extracellular Vesicles on Tumor Microenvironment In Vitro

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Tumor cell-derived extracellular vesicles (TEVs) promote tumor growth and metastasis; thus, they have drawn the attention of researchers. TEVs regulate the tumor microenvironment by facilitating crosstalk between immune and stromal cells. Macrophages are one of the key components involved in malignant behavior in melanomas. Generally, when activated, macrophages polarize into M1 (pro-inflammatory) or M2 (anti-inflammatory, pro-tumor) phenotypes. However, the role of canine melanoma-derived EVs in macrophage polarization is elusive. In this study, we aimed to analyze the pro- and anti-inflammatory cytokines that are common markers for M1 or M2 macrophages in vitro. The analysis was performed under coculture conditions of canine melanoma-derived (LMeC) EVs with canine macrophages (DH82). We used quantitative reverse transcription polymerase chain reaction, western blotting, and immunofluorescence. The results revealed that

canine melanoma-derived EVs polarize M1 macrophages (inducible nitric oxide synthase, tumor necrosis factor α) into M2 macrophages (cluster of differentiation (CD)206, interleukin-10). Furthermore, by blocking COX-2 expression, it was confirmed that COX-2 present within canine melanoma-derived EV (MEV) is a key factor for these immunomodulatory effects. Also, we found that MEVs regulate macrophages, inducing the release of angiogenetic cytokines (vascular endothelial growth factor, transforming growth factor β). Collectively, these results suggest that MEVs perform an immunomodulatory function on macrophages, which support tumor progression. This results enhance understanding of the mechanism of MEVs in TME, and further provide a basis for the development of EV-targeting cancer therapy.

Keywords: Dog, Melanoma, Macrophage, Endothelial cell, Tumor microenvironment

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1. Introduction

In several cases, it is not enough to simply recognize and remove cancer-related antigens in order to treat the disease. In most cases, the survival and removal of cancer cells are affected by various surrounding cells (stromal cells, inflammatory cells, etc.) and their complex environment, which includes blood vessels and extracellular matrix (Baghba et al., 2020). On considering this microenvironment, there has been a gradual increase in the level of importance attached to the development of anticancer drugs and strategies for their use (Pitt et al., 2016).

Extracellular vesicles (EVs) are cell-derived membranous vesicles, ranging in size from 15 nm to several microns in diameter (Tai et al., 2019). EVs mimic the functions of the cell of origin by transferring bioactive cargoes (deoxyribonucleic acid [DNA], ribonucleic acid [RNA], proteins, and lipids) to the adjacent environment (Yanez-Mo et al., 2015). Likewise, tumor-derived EVs (TEVs) can promote tumor-supporting processes, such as immunosuppression, invasion, angiogenesis, and metastasis, by modulating diverse immune components in the tumor microenvironment (TME). Specifically, TEVs suppress natural killer cells and cytotoxic cluster of differentiation (CD)8+ lymphocytes, induce myeloid-derived suppressor cells and M2-like macrophages, and stimulate regulatory T cell expansion (Ma et al., 2021; Tai et al., 2019).

In addition, macrophages are the key component of the TME. Once differentiated, they are classified into two general polarized types (M1 and M2)

based on their function. 1) The M1 or pro-inflammatory phenotype exhibits enhanced phagocytosis and produces pro-inflammatory cytokines, whereas 2) the M2 or anti-inflammatory phenotype resolves inflammation and promotes angiogenesis and tissue repair (De Palma and Lewis, 2013; Reed et al., 2021). Tumor-associated macrophages (TAMs) are predominantly polarized to an M2-like phenotype, and this feature explains their ability to promote tumor growth and metastasis (Mantovani et al., 2002). Based on recent studies, TAMs serve as major metastasis promoters by releasing growth factors and various immunosuppressing proteins (Lin et al., 2019).

Malignant melanoma, the most aggressive tumor type in humans and dogs, is characterized by frequent relapse and metastases. In a recent study, melanoma-derived EVs (MEVs) were found to mediate immunosuppression and to support tumor growth via pro-angiogenetic functions (Hood et al., 2009). Additionally, a recent investigation in humans detected cyclooxygenase-2 (COX-2) overexpression in oral and cutaneous melanomas and demonstrated that COX-2 expression correlates with malignancy and might be a marker of poor prognosis (Silveira et al., 2021). Similarly, in canine melanoma, COX-2 expression was significantly greater in highly malignant cutaneous and oral melanomas (Pires et al., 2010). However, there have been few studies on the TME-regulating key factors present in the extracellular secretion from melanomas. Research on this aspect is necessary for the future development of personalized anticancer drugs through TME-related research.

In this study, we aimed to investigate the effects of canine MEVs on macrophage polarization and endothelial cells in the TME. The purpose of this

study was to elucidate the major factors within MEVs, which affect the TME, through the analysis of pro- and anti-inflammatory cytokines and angiogenesis-promoting factors.

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2. Materials and Methods

2.1. Cell culture

The following cells were cultured in this study: LMeC, a canine melanoma cell line (Korean Cell Line Bank, Seoul, Korea); DH82, a canine macrophage-like cell line (Korean Cell Line Bank, Seoul, Korea); and endothelial cells with characterization established in previous experiments (An et al., 2021). LMeCs and endothelial cells were cultured in Roswell Park Memorial Institute-1640 medium (RMPI; PAN Biotech, Aidenbach, Germany) with 10% heat-inactivated fetal bovine serum (FBS; PAN Biotech, Aidenbach, Germany) and 1% penicillin-streptomycin (P/S; PAN Biotech, Aidenbach, Germany) at 37°C in a 5% CO2 atmosphere. DH82 cells were cultured in Dulbecco's modified Eagle's medium (PAN Biotech, Aidenbach, Germany) with 10% FBS and 1% P/S at 37°C in a 5% CO2 atmosphere. The culture medium was replaced every 2–3 days, and cells were sub-cultured on reaching 70–80% confluency.

2.2. Small interfering RNA(siRNA) transfection of LMeCs

When the confluency of LMeCs reached approximately 40%, they were transfected with COX-2 siRNA or control siRNA (sc-29279 and sc-37007, respectively; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 48 h using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA), according to the

manufacturers' instructions. COX-2 knockdown was confirmed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and western blotting.

2.3. Isolation of canine melanoma cell line EVs

LMeCs were cultured for 48 h in RMPI with 5% exosome-depleted FBS (Thermo Fischer Scientific, San Jose, CA, USA) and 1% P/S (PAN Biotech). The media from each cultured LMeC sample were collected and centrifuged at 100 × g for 5 min in order to remove cells and cell debris. Each supernatant was transferred to a fresh tube, and an appropriate volume of ExoQuick-CG Exosome Precipitation Solution (System Biosciences, Palo Alto, CA, USA) was added. EVs were extracted according to the manufacturer's instructions. The total protein concentrations of EVs were measured by bicinchoninic acid (BCA) assays.

2.4. Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (D-PlusTM CCK Cell Viability Assay Kit; Dong-In Biotech, Seoul, Korea) in order to determine whether Lipofectamine or GW4869 had any influence on canine melanoma cell growth. The cells were seeded at a density of 3.3 x 104 cells/well in a 96-well plate. At 24 or 48 h after transfection or treatment with GW4869 (10 μM or 20 μM), a CCK-8 assay was conducted.

2.5. RNA extraction, complementary deoxyribonucleic acid (cDNA)

synthesis, and real-time quantitative PCR

RNA was extracted using the Easy-Blue Total RNA Extraction kit (Intron Biotechnology, Sungnam, Korea). In each sample, the total RNA concentration was measured at an absorbance of 260 nm using a NanoPhotometer (IMPLEN, Munich, Germany). Cell Script All-in-One 5x 1st cDNA Strand Synthesis Master Mix (Cell Safe, Seoul, Korea) was used to synthesize cDNA, and the samples were analyzed using AMPIGENE qPCR Green Mix Hi-ROX with SYBR Green dye (Enzo Life Sciences, Farmingdale, NY, USA) and forward and reverse primers (Bionics, Seoul, Korea). The expression levels of each gene were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers used in this study were GAPDH, COX-2, interleukin 10 (IL-10), inducible nitric oxide synthase (iNOS), CD206, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF) of dogs, and the sequences are shown in Table 1.

2.6. Coculture of LMeCs with DH82 cells

DH82 cells were seeded in six-well plates at a density of 5 × 10 5 cells/well and were incubated overnight. After adherence to the plates was confirmed, lipopolysaccharide (LPS) (200 ng/ml) and/or GW4869 (10 μM) were added for 48 h. Next, the medium was removed, the cells were washed three times with Dulbecco's phosphate-buffered saline (DPBS), and the control medium was introduced. Using 0.4-μm pore size inserts, LMeCs were plated onto the

macrophage cells at a density of 2 × 105 cells/well and ratio of 5:2. All cells were incubated for 48 h and then harvested for RNA extraction.

2.7. Western blot analysis

Total proteins from cells were extracted using the PRO-PREP Protein Extraction Kit (iNtRON Biotechnology, Seongnam, Korea) and were measured using the Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). The total protein content in each 20-μg sample was analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with antibodies against CD81 and COX-2.

2.8. Statistical analysis

GraphPad Prism (version 6.01) software (GraphPad Software, La Jolla, CA, USA) was used to perform the statistical analysis. Student's t-test and a one-way analysis of variance (ANOVA) were used to analyze the data, followed by the Bonferroni multiple comparison test. The data are presented as mean ± standard deviation (SD). Differences with a P value of < 0.05 were considered statistically significant.

3. Results

3.1. Immunomodulatory effects of MEVs

To verify the innoxiousness of our experimental conditions with 10 and 20 μM of GW4869 for 24 h and 48 h, a CCK-8 assay was performed; however, no significant difference was found between the control and experimental groups (Figure 1A). With the drug, GW4869 (exosome inhibitor), there was a significant reduction in the level of EV proteins produced by the melanoma cells (Figure 1B). To determine the effects of melanoma exosomes on M1 or M2 macrophage polarity, a cytokine RT-qPCR was conducted. The cytokine markers used in this study were as follows: TNF-α and iNOS for M1 macrophages or IL-10 and CD206 for M2 macrophages. LPS-treated DH82 canine macrophages cocultured with melanoma cells exhibited reduced levels of the messenger ribonucleic acid (mRNA) of TNF-α, a pro-inflammatory cytokine, and of iNOS, a marker of the M1 phenotype. By contrast, the levels of IL-10, an anti-inflammatory cytokine, and of CD206, a marker of the M2 phenotype, were significantly increased when the LPS-treated DH82 cells were cocultured with melanoma cells. These results were reversed following the pretreatment with GW4869 (Figure 1C).

3.2. Identification of COX-2-depleted MEVs

There was no significant difference between the control and experimental

groups in the results of the CCK-8 analysis performed to confirm any effects of RNA transfection on the growth of melanoma cells for 24 h and 48 h (Figure 2A). COX-2 expression in naïve melanoma was reduced in the COX-2 siRNA-transfected melanoma cells, whereas no significant difference was found in control siRNA-transfected groups (Figure 2B). The tendency of this result was the same when protein levels were analyzed (Figure 2C).

3.3. COX-2 within MEVs: a major factor in M1 to M2 macrophage polarization in vitro

After observing the increasing tendency of M1 to M2 macrophage polarization in the cocultures with melanoma cells, we hypothesized that COX-2 was the key factor in MEVs. LPS-stimulated DH82 cells exhibited increasing mRNA levels of M1 phenotype cytokines (iNOS, TNF-α). After the treatment of MEVs, M1 phenotype cytokine (iNOS, TNF-α) levels were decreased, but M2 phenotype cytokine (CD206, IL-10) levels were increased. These results were reversed in the COX-2-depleted EV groups (Figure 3A). CD11c (M1 macrophage surface marker) and CD206 (M2 macrophage surface marker) were analyzed through immunofluorescence. LPS treatment increased the levels of CD11c+ macrophages. Following MEV treatment, the LPS-primed macrophages showed an increase in CD206 expression and a decrease in CD11c expression. The inhibition of COX-2 in CD206+ macrophages resulted in polarization to an M1 phenotype (Figure 3B).

3.4. Effects of MEV COX-2 on mRNA expression of angiogenetic factors in macrophages

To evaluate the angiogenetic aspect of MEVs, the levels of VEGF, an angiogenesis-stimulating factor, in macrophages were measured. There was a greater increase in the levels of VEGF mRNA in the EV-treated groups compared to in the EV-untreated groups. COX-2-depleted EV-treated groups showed a significant decrease in VEGF expression (Figure 4).

3.5. Effects of MEV COX-2 on angiogenetic activities in endothelial cells

Considering that COX-2 originating from MEVs increased the VEGF levels in LPS-primed macrophages, a subsequent coculture was performed with endothelial cells in order to evaluate whether it affects angiogenetic factors in conditions more similar to the TME. The mRNA levels of VEGF and TGF-β were significantly higher in endothelial cells cocultured with MEV-treated DH82 than in those cocultured with MEV-untreated DH82. After COX-2 knockdown in MEVs, the levels of these cytokines decreased and were similar to those in the LPS-primed M1 DH82 groups (Figure 5).

4. Discussion

In its totality, the TME is composed of complex and diverse elements, such as the cells surrounding cancer cells, extracellular matrix, growth hormones, and signaling substances (Khalaf et al., 2021). In 1889, Dr. Stephen Paget presented the “seed and soil” theory and announced that the TME plays an important role as it acts as “soil” for the growth of “seeds” called cancer cells; this suggests that the TME plays an essential role in the development and progression of cancer. Therefore, as a strategy to overcome the limitation of existing anticancer therapies, which are not able to prevent cancer progression and metastasis on their own through directly attacking cancer cells, attention is being paid to developing new therapies that target the TME (Langley and Fidler, 2011; Ribatti et al., 2006).

Through this study, we not only confirmed the effects of canine MEVs on the TME, but also studied the role of key factors in the EVs in the TME.

The interaction between tumor cells and the stromal environment contributes to the phenotypic polarization of TAMs (Liu et al., 2021). TAMs are known to be associated with tumor growth and metastasis in various tumors. TAMs are generally accepted to be very similar to M2 macrophages. Tumor cells interact with macrophages, and as a result, most macrophages are converted to M2 phenotypes and are likely to exhibit immunosuppressive potential. Therefore, targeting TAMs and their associated molecules might be a strategy for cancer immunotherapy (Tan et al., 2021; Zhang et al., 2020).

The evaluation of markers commonly used to detect M1 (iNOS, TNF-α)

and M2 (CD206, IL-10) macrophages demonstrated that canine MEVs induce M1 to M2 polarization. The levels of inflammatory cytokines increased in the culture media of LPS-stimulated M1 macrophages, and they were downregulated when cocultured with melanoma cells compared with the upregulation in the levels of anti-inflammatory cytokines. Previous studies have shown that M2 polarization in macrophages results in chemotherapy resistance and tumor progression (Zhang et al., 2020); these results along with the results of our study suggest that MEVs can be used as the target of new anticancer drugs.

Further, the mRNA expression of several angiogenetic cytokines (VEGF, TGF-β) was increased when canine endothelial cells were cocultured with MEVs. These pro-angiogenetic cytokines, which promote tumor migration and proliferation and the angiogenesis of vascular endothelial cells, have been targeted to suppress tumor growth and metastasis (Zhang et al., 2018; Zirlik and Duyster, 2018). Increasing evidence suggests that targeting TAMs complements anti-angiogenetic therapy during oncotherapy (Zhang et al., 2020).

In addition, as a result of blocking COX-2 expression, it was confirmed that COX-2 present within canine MEVs is a key factor that regulates the TME.

Previous studies have demonstrated that TEVs play an important role in creating a TME that is advantageous for tumor progression. They regulate immune cells to allow tumor cells to escape from immune surveillance and prepare a metastatic niche for tumor cells (Umansky et al., 2017). These features were targeted in recent studies on immunotherapy (Fleming et al., 2018). TEVs can modulate the TME in order to escape the action of the immune system; this is possible because of their abilities to suppress T cell activation and to drive tumor

progression through the polarization of M2-like macrophages (Ma et al., 2021). This study is the first to investigate the possibility that MEVs could be considered as major targets for tumor growth inhibition and TME regulation, with particular focus on immunomodulation against macrophages and angiogenesis (Fleming et al., 2019). Due to the immunosuppressive function of TEVs, they have become a novel target of chemotherapy (Ma et al., 2020).

The release of COX-2, an enzyme that converts arachidonic acid into prostaglandin endoperoxide, is induced in monocytes, endothelial cells, and tumor cells by cytokines, tumor promoters, etc. (Fosslien, 2000). As it is closely related to cell proliferation, immune regulation, angiogenesis, and metastasis, its abundance in tumor cells is strongly associated with malignancy in many cancers, such as lung, colon, breast, and ovarian cancers (Misra and Sharma, 2014). In a recent study, COX-2 expression was found to be high in TAMs in advanced melanoma, which sheds light on its potential to be used as a marker for melanoma progression (Bianchini et al., 2007). To the best of our knowledge, there are currently no reports on COX-2 expression in TAMs in canine malignant melanoma. This study examined the influence of COX-2 in canine MEVs on macrophage polarization.

This study demonstrated the *in vitro* anti-inflammatory effects of MEVs; therefore, *in vivo* experiments are required. Although we created conditions similar to the TME through transwell culture plates, other immune or stromal cells have to be assessed together, as there are numerous cell populations in the TME. Nevertheless, this is the first study to elucidate the tumor-friendly environmental regulators and angiogenetic activity of canine MEVs. Moreover, we found that COX-2 within canine MEVs plays a major role in macrophage polarization; this

finding highlights an important mechanism of MEV pathophysiology and serves as a basis for the development of TAM-based anticancer therapy through TAM reprogramming.

5. Conclusion

This study revealed that canine melanoma extracellular vesicles induce M1 to M2 macrophage polarization, and COX-2 present in MEVs is a major factor that regulates the TME. These results indicate that MEVs contribute to creating tumor-friendly environment and that EVs and COX-2 are factors that can be targeted by new anticancer drugs. However, further study is essential to investigate MEVs' role in tumor progression *in vivo* and clinical relevance of targeting TAM for suppressing tumor growth and metastasis in canine melanoma.

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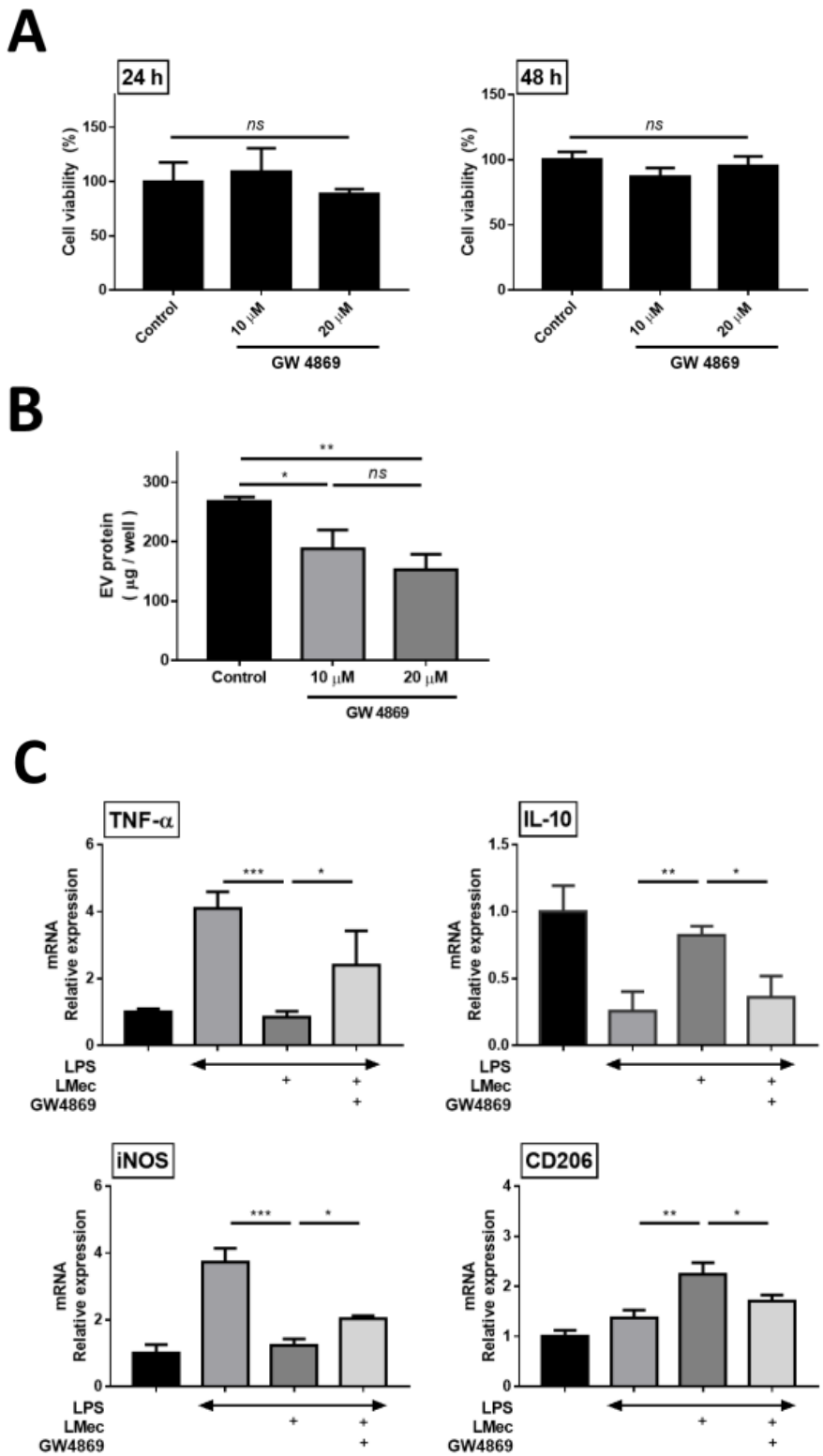
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iNOS
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GW4869

Figure 1. Expression of M1/M2 cytokines by canine macrophages (DH82) co-cultured with canine melanoma cell line (LMeC) according to GW4869 treatment. (**a**) Cell viability assay using CCK-8 assays. Canine melanoma cell line (LMeC) were incubated for 24 or 48hr and in two different dilutions of GW4869 (10 and 20 uM). (**b**) LMeC-derived EVs protein concentration under GW4869 treatment by BCA assays. (**c**) mRNA expression levels of common cytokines of M1/M2 macrophages co-cultured with LMeCs with or without GW4869 treatment using qRT-PCR. The results are shown as the mean ± SD (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, as analyzed by one-way ANOVA).

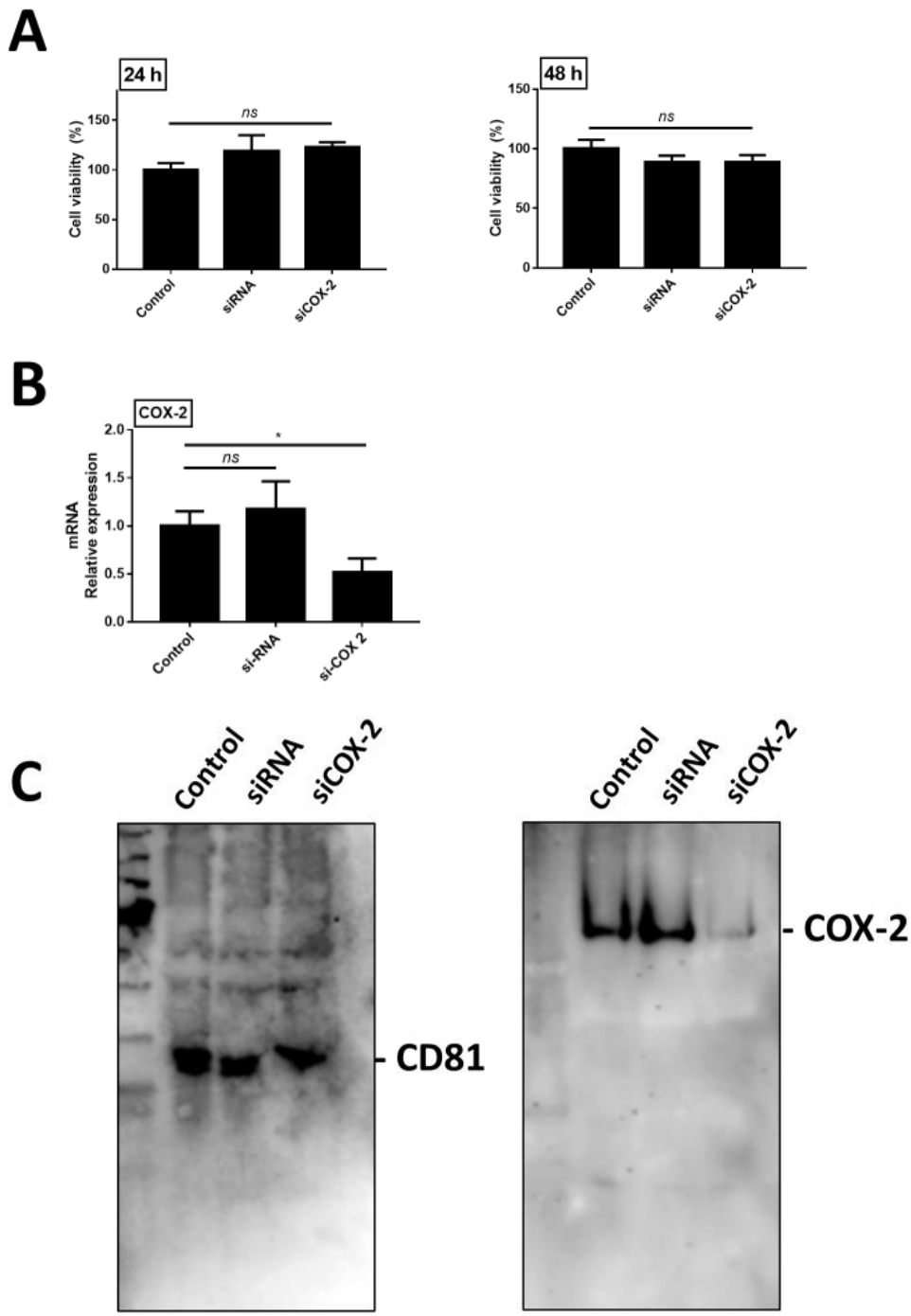
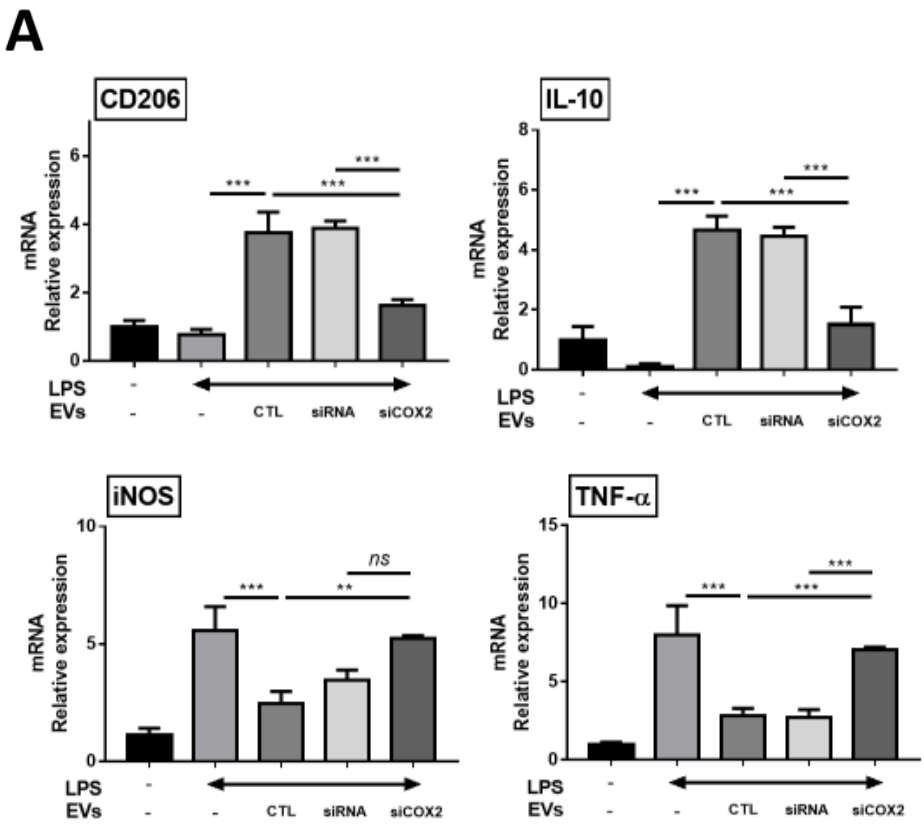


Figure 2. Identification of COX-2-depleted MEVs. (**a**) Cell viability analysis of LMeCs incubated for 24 and 48 hr after transfected with COX-2 siRNA (siCOX-2) or scrambled siRNA(siRNA), or naïve EVs, using CCK-8 assays. COX-2

expression in LMeCs after 48 hr of transfection with siCOX-2 or siRNA, or naïve EVs were determined by qRT-PCR(**b**) and western blot (**c**). The results are shown as the mean ± SD (*$p < 0.05$, as analyzed by one-way ANOVA).


A
CD206
IL-10
iNOS
TNF-α
mRNA
Relative expression
LPS
EVs
CTL
siRNA
siCOX2
***
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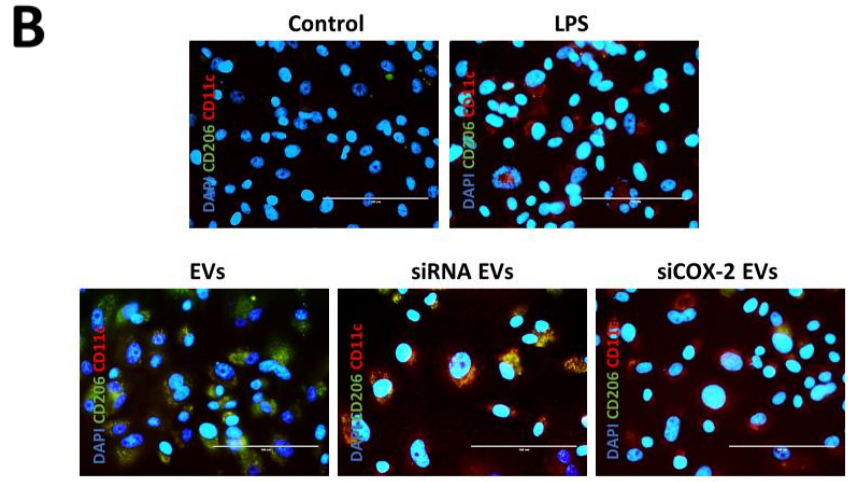
ns


B
Control
LPS
EVs
siRNA EVs
siCOX-2 EVs
DAPI CD206 CD11c

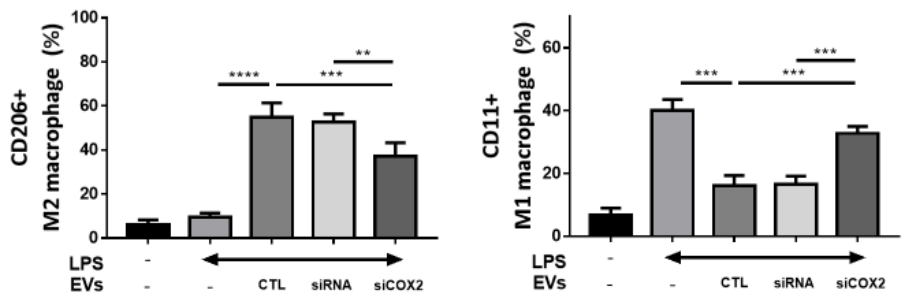


Figure 3. Effects of COX-2 in MEVs in macrophage polarization *in vitro*. LPS-stimulated DH82 were co-cultured for 48hr with MEVs, extracted after transfection with siCOX-2 or siRNA, or naïve EVs (CTL). (**a**) mRNA Expression of M1/M2 cytokines using qRT-PCR. (**b**) The M1 and M2 population were evaluated by measuring CD11c+ (red) and CD206+ (green) cells, respectively, by immunofluorescence and flow cytometry. The results are shown as the mean ± SD (**$p < 0.01$, and ***$p < 0.001$, as analyzed by one-way ANOVA).

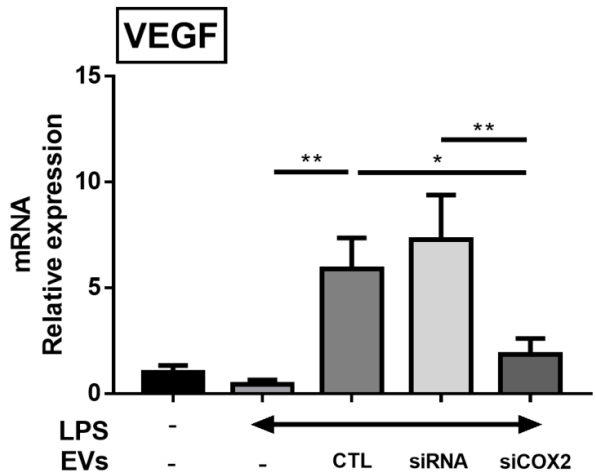


Figure 4. Effects of COX-2 in MEVs in mRNA expression levels of vascular endothelial growth factor (VEGF) measured by using qRT-PCR. LPS-stimulated DH82 were co-cultured for 48hr with MEVs, extracted after transfection with siCOX-2 or siRNA, or naïve EVs (CTL). The results are shown as the mean ± SD (*$p < 0.05$ and **$p < 0.01$, as analyzed by one-way ANOVA).

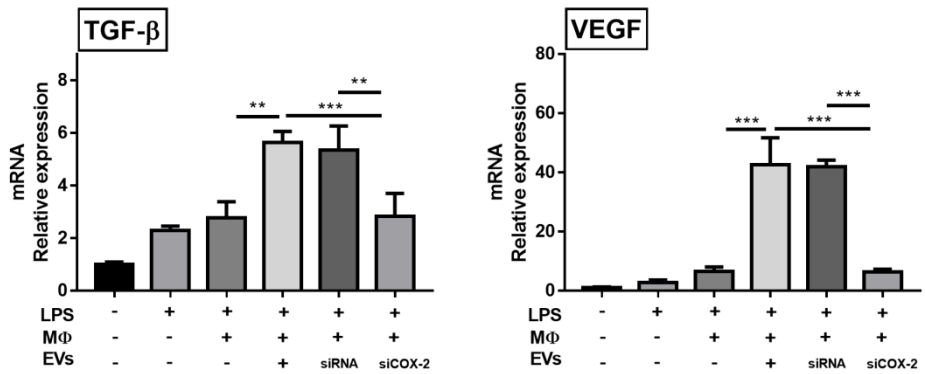


Figure 5. Effects of COX-2 in MEVs in mRNA expression levels of angiogenetic cytokines in endothelial cells after 48hr of co-culture with DH82 and MEVs measured by using qRT-PCR. The results are shown as the mean ± SD (**$p < 0.01$, and ***$p < 0.001$, as analyzed by one-way ANOVA).

Table 1. Sequences of PCR primers used in this study.

Gene	Forward (5'-3')	Reverse (5'-3')	Reference
ciNOS	AAATTATGTCCTGTCCCCT TTCTAC	TTTAAGTTGAATCTTTTTCC TGTGG	(Manning et al., 2010)
cIL-10	ATTTCTGCCCTGTGAGAAT AAGAG	TGTAGTTGATGAAGATGTC AAGCTA	(Yi et al., 2014)
cGAPDH	TTAACTCTGGCAAAGTGGA TATTGT	GAATCATACTGGAACATGT ACACCA	(Yi et al., 2014)
cTNF-α	TCATCTTCTCGAACCCCAA G	ACCCATCTGACGGCACTAT C	(Yi et al., 2014)
cCD206	GGAAATATGTAAACAGGAA TGATGC	TCCATCCAAATAAACTTTT TATCCA	(Manning et al., 2010)
cCOX-2	GCCTTACCCAGTTTGTGGA A	AGCCTAAAGCGTTTGCGAT A	(Yang et al., 2021)
cTGF-β	GGAAAACACCAACAAAAT CTATGAG	GCTATATTTCTGGTACAGCT CCACA	(Park et al., 2020)
cVEGF	GAATGCAGACCAAAGAAA GATAGAG	GATCTTGTACAAACAAATG CTTTCTC	(Kim et al., 2019)

7. 국문초록

개 흑색종 유래 세포외 소포체 내 Cyclooxygenase-2의 종양 미세환경에 대한 in vitro 효과

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종양 세포 유래 세포외 소포체(TEVs; Tumor cell-derived extracellular vesicles)는 종양 성장과 전이를 촉진하여 새로운 항암치료 전략으로 주목받고 있다. TEVs는 면역 세포와 기저세포 사이의 대화를 촉진함으로써 종양 미세 환경을 조절한다. 한편, 대식세포는 흑색종에서 악성도에 관여하는 핵심 요소 중 하나이다. 일반적으로 활성화되면 대식세포는 M1(전염증) 또는 M2(항염증, 전종양) 표현형으로 분극된다.

그러나 대식세포 분극에서 개 흑색종(LMeC) 유래 세포외 소포체의 역할에 대한 연구는 드물게 이루어지고 있다. 본 연구에서는 in vitro 상에서 M1 또는 M2 대식세포에 대한 일반적인 지표인 전염증 및 항염증성 사이토카인을 분석하였다. 이는 개 흑색종 유래 세포외 소포체(MEVs)와 개 대식세포를 합배양한 조건에서 수행하였고, 분석에는 RT-qPCR, western blot, 면역 형광법을 사용했다. 그 결과 MEVs는 M1 대식세포(iNOS, TNF-α)를 M2 대식세포(CD206, IL-10)로 분극시킨다는 것을 확인하였다. Cyclooxygenase-2를 차단하였을 때의 결과를 비교하여 이러한 분극 효과에 COX-2가 주요 인자임을 밝혀냈다. 또한, MEVs가 대식세포를 조절하여 혈관생성 사이토카인(VEGF, TGF-β)의 방출을 유도한다는 것을 발견했다. 종합하면, MEVs가 면역 조절 기능을 수행하여 종양친화적 환경을 조성하는 데 기여하며, 이러한 결과는 개 흑색종에서 종양미세환경 수준에서의 기전을 밝히고, 나아가 세포외 소포체를 표적으로 하는 항암제의 개발에 폭넓은 이해를 제공할 것으로 사료된다.

주요어 : 개, 흑색종, 대식세포, 내피세포, 종양미세환경

학번 : 2020-29642