Peptide Lv augments intermediate-conductance calcium-dependent potassium channels (K\textsubscript{Ca}3.1) in endothelial cells to promote angiogenesis

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Abstract

Peptide Lv is a small endogenous secretory peptide that is expressed in various tissues and conserved across different species. Patients with diabetic retinopathy, an ocular disease with pathological angiogenesis, have upregulated peptide Lv in their retinas. The pro-angiogenic activity of peptide Lv is in part through promoting vascular endothelial cell (EC) proliferation, migration, and sprouting, but its molecular mechanism is not completely understood. This study aimed to decipher how peptide Lv promotes EC-dependent angiogenesis by using patch-clamp electrophysiological recordings, Western immunoblotting, quantitative PCR, and cell proliferation assays in cultured ECs. Endothelial cells treated with peptide Lv became significantly hyperpolarized, an essential step for EC activation. Treatment with peptide Lv augmented the expression and current densities of the intermediate-conductance calcium-dependent potassium (K\textsubscript{Ca}3.1) channels that contribute to EC hyperpolarization but did not augment other potassium channels. Blocking K\textsubscript{Ca}3.1 attenuated peptide Lv-elicited EC proliferation. These results indicate that peptide Lv-stimulated increases of functional K\textsubscript{Ca}3.1 in ECs contributes to EC activation and EC-dependent angiogenesis.

Introduction

Pathological angiogenesis/neovascularization manifests in various diseases including cancers [1], atherosclerosis [2], arthritis [3], and ocular diseases such as diabetic retinopathy (DR) and age-related macular degeneration [4–6]. Therapies targeting vascular endothelial growth factor (VEGF) or its receptors are widely used to combat these diseases by dampening neovascularization [7–9]. However, nearly 30% of patients do not respond or become resistant to anti-VEGF therapies [9–11]. Repetitive anti-VEGF injections are needed to block recurring neovascularization, which often leads to unwanted side effects [9–11]. One possible explanation for
the resistance to anti-VEGF treatments and recurring neovascularization is the involvement of other angiogenic factors that are insensitive to anti-VEGF agents [9–11]. Thus, finding VEGF-independent pro-angiogenic factors and understanding their molecular mechanisms become clinically imperative for the development of new therapeutics against pathological neovascularization.

We discovered a small endogenous peptide (~40 amino acids), peptide Lv, that is upregulated in the retinas of patients with early proliferative diabetic retinopathy (DR) as well as diabetic animals [12–14]. The gene encoding peptide Lv is in the V-set and transmembrane domain containing 4 gene (Vstm4; human gene ID: 196740, a.a. 55–94; mouse gene ID: 320736, a.a. 55–103; [12]). Its amino acid sequence is highly conserved (>90%) among humans, mice, rats, and chickens [12]. The mRNA of peptide Lv is expressed in various organs including the eye, heart, brain, liver, spleen, and lung [12] and cell types including retinal neurons, vascular endothelial cells (ECs), and cardiomyocytes [12, 13]. Functionally, peptide Lv elicits concentration-dependent vasodilation in porcine coronary and retinal arterioles [14], similar to how VEGF acts as a vasodilator [15]. Peptide Lv is also pro-angiogenic since it promotes developmental and pathological angiogenesis in vivo [14]. During development, chicken embryos treated with peptide Lv (in ovo) or postnatal mice injected with peptide Lv intraocularly have significant growth of microvasculature [14]. Mouse eyes with oxygen-induced retinopathy (OIR) have upregulated peptide Lv [14], which is similar to the upregulation of VEGF in these eyes [7, 8, 16, 17]. Intraocular injections with an antibody against peptide Lv, anti-Lv, dampen pathological neovascularization in mouse eyes with OIR or laser-induced choroidal neovascularization [14]. Furthermore, mice with a genetic deletion of peptide Lv (peptide Lv−/−) have significantly higher OIR-induced vaso-obliteration and lower OIR-neovascularization compared to the littermate controls (peptide Lv+/+; [14]). These data provide evidence that peptide Lv is an angiogenic factor that is involved in pathological neovascularization.

Upon further investigation, peptide Lv elicits vascular EC proliferation, migration, and sprouting, which are three fundamental properties in angiogenesis [14]. One of the initial steps for EC-dependent vasodilation or angiogenesis is the membrane hyperpolarization of ECs [18–22], and opening EC potassium (K+) channels is required for EC hyperpolarization [18–20, 23–27]. Several K+ channels expressed in ECs can mediate the outflow of K+, including intermediate conductance calcium-dependent K+ channels (IKCa/KCNN4/KCa3.1) [25, 26, 28], small conductance calcium-dependent K+ channels (sKCa/KCNN3/KCa2.3) [25, 26, 28], and ATP-sensitive K+ channels (KATP/Kir6.1) [19, 29–31].

Since it is likely that peptide Lv-elicited vasodilation and angiogenesis is in part through an EC-dependent process, we postulated that peptide Lv might elicit EC hyperpolarization through activating EC-expressed K+ channels. In this study, we combined patch-clamp electrophysiological recordings and molecular analyses to determine whether peptide Lv caused any changes in EC membrane potential, and which EC-expressed K+ channel(s) is involved and leads to peptide Lv-elicited angiogenesis.

**Materials and methods**

This study (agents and experimental activities) was approved by the Institutional Biosafety Committee (IBC) of Texas A&M University (IBC Permit: IBC 2020–104).

**Chemicals**

Peptide Lv was custom-made by Peptide 2.0 Inc (Chantilly, VA, USA). The murine amino acid sequence used to make peptide Lv is DSLLAVRWFFAPDGSQEALMVKMTKLRIQYYGNFSRTANQQRLLLE [12, 13]. Peptide Lv tested negative for endotoxin. Other
inhibitors and chemicals used in this study were: TRAM-34 (K\(_{Ca}\)3.1 inhibitor; #AAJ600 19-MB, Thermo Fisher Scientific, Waltham, MA, USA), DMH4 (VEGFR2 inhibitor; #4471, Tocris, Minneapolis, MN, USA), \(\beta\)-escin (#E1378, Sigma-Aldrich, St. Louis, MO, USA), and VEGF (#ab9571, Abcam, Waltham, MA, USA).

Cell cultures

Human umbilical vein endothelial cells (HUVECs; #200-05n, Cell Applications Inc, San Diego, CA, USA) and human retinal endothelial cells (HRECs; #ACBRI 181, Cell Systems, Kirkland, WA, USA) were cultured in EGM™-2 MV Microvascular Endothelial Cell Growth Medium (EGM; #CC-3202, Lonza, Walkersville, MD, USA) at 37°C and 5% CO\(_2\). For immuno blot and qPCR experiments, ECs were seeded onto 60 mm culture plates and grown to 100% confluency then treated with peptide Lv (500 ng/ml) or phosphate-buffered saline (PBS; vehicle control) for 4 hours. For patch-clamp experiments, ECs were seeded onto 12 mm acid-washed glass coverslips and placed in an incubator for 48 hours to allow cells to adhere. Cultures were then treated with peptide Lv (500 ng/ml) or PBS for 2, 3, and 4 hours prior to recordings. For cell proliferation assays, HUVECs were seeded onto 96-well plates in EGM and allowed to adhere overnight. Peptide Lv (500 ng/ml), VEGF (5 ng/ml), DMH4 (5 \(\mu\)M), and TRAM-34 (10 \(\mu\)M) were added to cells and continuously incubated for another 48 hours prior to proliferation assays.

Patch-clamp electrophysiology

Whole-cell patch-clamp recordings on cultured HUVECs were carried out using \(\beta\)-escin-based perforated patches [32–34]. The methods and parameters for recording endothelial membrane potentials (current-clamp) and the K\(_{Ca}\)3.1 current (voltage-clamp) are based on previous publications [35–37]. The external solution was (in mM): 160 NaCl, 4.5 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 10 HEPES, and 1 glucose at pH 7.5 adjusted with NaOH. The pipette solution was (in mM): 120 KCl, 1.75 MgCl\(_2\), 1 Na\(_2\)ATP, 10 EGTA, 4.1 CaCl\(_2\), and 10 HEPES at pH 7.2 adjusted with KOH. The free calcium concentration in the pipette solution was calculated to be 100 nM using an online calcium chelator calculator [38]. \(\beta\)-escin was freshly prepared as a 35 mM stock solution in water, kept on ice, and then added to the pipette solution to yield a final concentration of 35 \(\mu\)M. Before recordings, cells were first treated with peptide Lv (500 ng/ml) or PBS (vehicle; as controls) and maintained in the incubator for 0, 2, 3, or 4 hours. There was no statistical difference in amplitudes (either membrane potentials or currents) among the controls recorded, so their data were combined as a single control group. All recordings were performed at room temperature (23°C) using an A-M 2400 amplifier (A-M Systems Inc., Carlsborg, WA, USA). Signals were low-pass filtered at 1 kHz and digitized at 5 kHz with a Digidata 1500A interface (Axon Instruments/Molecular Devices, Union City, CA, USA) and pCLAMP 10.0 software (Molecular Devices). Electrode capacitance was compensated after gigaohm (G\(\Omega\)) seals were formed. The membrane capacitance, series resistance, and input resistance of the recorded ECs were measured by applying a +5 mV (100 ms) depolarizing voltage step from a holding potential of ~60 mV. Cells with an input resistance <1 G\(\Omega\) (smaller than 1 gigaohm) were discarded. The membrane capacitance reading was used as the value for whole cell capacitance (in pF). The outward currents (in pA) were elicited with a step-command from a holding potential at ~60 mV to 40 mV for 300 ms. From the same cell, the total outward current containing K\(_{Ca}\)3.1 was first recorded, followed by perfusion of 10 \(\mu\)M TRAM-34 (K\(_{Ca}\)3.1 inhibitor) to the recording chamber for 5 minutes, and then a second current elicited and recorded in the presence of TRAM-34. The K\(_{Ca}\)3.1 current from a single cell was isolated by a subtraction between the two recorded currents in the absence or
presence of TRAM-34. The current density (pA/pF) was obtained by dividing the $K_{Ca}^{3.1}$ current amplitude (measured at 200 ms; the tau point) by the whole cell capacitance. The membrane potentials were recorded under the current-clamp mode by injecting a 20 pA current for 750 ms.

**MTT assays**

The proliferation of HUVECs was determined using Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays (Thermo Fisher Scientific, Waltham, MA, USA) as we previously described [13, 14, 39]. HUVECs were seeded onto 24-well plates in EGM culture medium and allowed to adhere overnight. When cultures reached 60% confluency, peptide Lv (500 ng/ml), VEGF (5 ng/ml), DMH4 (5 μM), and TRAM-34 (10 μM) were added to cells and continuously incubated for another 48 hours. On the day of the assays, cells were incubated with the MTT solution (1.2 mM final concentration) for 4 hours at 37˚C, after which the solution was removed, and 10% sodium dodecyl sulfate was added to break the plasma membrane. The absorbance at 560 nm was measured using a microplate reader (Awareness Technology Inc., Palm City, FL, USA).

**Immunoblot analysis**

Cultured ECs were treated with PBS (vehicle control) or peptide Lv (500 ng/ml) for 4 hours then harvested and prepared for immunoblot analysis as we described previously [12, 13]. In brief, cells were lysed with a RIPA lysis buffer, and proteins were denatured by mixing with 2X Lamelli sample buffer and heating for 5 minutes at 95 ºC. Samples were separated using a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were incubated with the primary antibodies overnight at 4 ºC. The primary antibodies used were rabbit polyclonal anti-KCNN4, (K$_{Ca}^{3.1}$, #APC-0641:200; Alomone Labs, Jerusalem, Israel), rabbit polyclonal anti-KCNN3 (K$_{Ca}^{2.3}$, #APC-025; 1:200; Alomone Labs, Jerusalem, Israel), rabbit polyclonal anti-Kir6.1 (1:500; #NBP1-87710, Novus Biologicals, Littleton, CO, USA), and rabbit monoclonal β-actin (1:1000; #4970S, Cell Signaling Technologies, Danvers, MA, USA). Membranes were then washed with a TBS-tween solution followed by incubating with an anti-rabbit IgG HRP-linked antibody (1:1000; #7074S, Cell Signaling Technologies, Danvers, MA, USA) for 1 hour at room temperature (23 ºC). Bands were visualized using Super Signal West Pico or Femto chemiluminescent substrate (#34078 or #34096, Pierce Biotechnology Inc., Rockford, IL, USA). Membranes were scanned using an immunoblot scanner (LI-COR Biosciences, Lincoln, NE, USA). Band intensities were quantified using Image J (National Institutes of Health; NIH, Bethesda, MA, USA). The band intensities were first normalized to the internal control, β-actin, and subsequently, the relative changes were quantified according to the method described by Janes [40].

**Quantitative PCR (qPCR)**

qPCR was performed as described previously [12–14]. After the cells were collected, total ribonucleic acid (RNA) from each sample was prepared by using a commercially available purification kit (RNeasy kit; #74106, Qiagen, Germantown, MD, USA). From each sample, 500 ng of total RNA was used to quantify messenger (mRNA) by qPCR using a High-Capacity cDNA Reverse Transcription Kit (#4368814, Applied Biosystems, Grand Island, NY, USA), Taqman qPCR master mix (#4444556; Applied Biosystems, Grand Island, NY, USA), and SYBR green supermix ROX (#95055–500, QuantaBio, Beverly, MA, USA) with a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Primers used were purchased from Life Technologies (Carlsbad, CA, USA): Kcnn4 (Forward: 5’–ATCTCCAAGATGCACATGATCC–
3'; Reverse: 5'−TAGCCTGGTTCCTCCTCGTG−3'). **TRPV4** (Forward: 5'−CCAAGTACCCCGTGGTCTTCATC−3'; Reverse: 5'−AGGATGGTGGTGGCCCAC−3'); and **β-actin** (Forward: 5'−CAACGGCTCCGGCATGTGCAA−3'; Reverse 5'−GTACATGGCTGGGGTGTTGAAAGGTCTC−3').

For each experiment, a standard curve was generated with known quantities of RNAs loaded in serial dilutions (i.e., 2, 1, 1/2, 1/4, 1/8, 1/16, and 1/32). The cycle values, corresponding to the log values of the standard curve quantities, were used to generate a linear regression formula. The amplification efficiency of the qPCR reactions (90–100%) was calculated using the standard curve. The quantification of sample RNA was calculated by the \(2^{{(-\Delta\Delta Ct)}}\) method [41] using **β-actin** as the internal control.

**Statistical analysis**

All data are presented as mean ± standard error of the mean (SEM). Differences between two groups were analyzed using the student’s t-test. Differences between multiple groups were analyzed by one-way ANOVA and Tukey post hoc tests. The statistical software was Origin 8.6 (OriginLab, Northampton, MA, USA). Throughout, \(p<0.05\) was considered significant.

**Results**

**Peptide Lv hyperpolarizes the membrane potentials of ECs**

We previously showed that peptide Lv promotes vasodilation and angiogenesis [14]. Membrane hyperpolarization in ECs is an essential step in EC-dependent vasodilation and angiogenesis [18, 24, 42, 43], so we first tested whether peptide Lv could elicit EC hyperpolarization. Using whole-cell current-clamp recordings, we found that treatment with peptide Lv (500 ng/ml) for 3 or 4 hours in cultures elicited membrane hyperpolarization of HUVECs compared to the vehicle control (PBS; Fig 1). The average membrane potential for HUVECs without peptide Lv is \(-72.73 ± 0.71\) mV. After HUVECs were treated with peptide Lv for 3 or 4 hours, the average membrane potential was \(-78.88 ± 0.63\) mV and \(-79.61 ± 0.88\) mV, respectively. Thus, peptide Lv-elicited vasodilation and angiogenesis is in part through hyperpolarizing the EC plasma membrane.

**The intermediate-conductance calcium-dependent K⁺ channel (KCa3.1) is a major player in peptide Lv-elicited endothelial hyperpolarization**

Opening the endothelial K⁺ channels is required for EC hyperpolarization that leads to vasodilation [31, 44], so we next determined which K⁺ channels mediated peptide Lv-elicited EC hyperpolarization. Since peptide Lv elicited EC hyperpolarization after the cells were treated for 3 or 4 hours (Fig 1), we next tested which K⁺ channels were possibly upregulated by peptide Lv in cultured ECs. The HUVECs were first treated with peptide Lv (500 ng/ml) for 4 hours in cultures and subsequently harvested for qPCR or immunoblots to analyze various EC K⁺ channels that mediate EC hyperpolarization. Peptide Lv had no effect on the expression of the small-conductance calcium-dependent K⁺ channels (KCa2.3; Fig 2A), and it decreased the protein level of ATP-sensitive K⁺ channels (Kir6.1; Fig 2B). Since decreased expression of Kir6.1 reduces endothelial hyperpolarization [30], and peptide Lv caused a decrease of Kir6.1 in cultured ECs, we eliminated Kir6.1 as a potential player in peptide Lv-mediated EC hyperpolarization. We previously showed that peptide Lv augments L-type voltage-gated calcium channels in cardiomyocytes and photoreceptors [12, 13], so it is possible that peptide Lv may augment other calcium channels in ECs, such as the transient receptor potential cation channel 4 (TRPV4) that indirectly contributes to EC hyperpolarization, as calcium influx through
TRPV4 may allow calcium-dependent K⁺ channels to open [45, 46]. After cells were treated with peptide Lv, there was no change in the expression of TRPV4 (Fig 2C). However, we found that peptide Lv significantly increased the mRNA and protein expression of K⁺Ca₃.1 in HUVECs as well as in human retinal ECs (HRECs; Fig 3). These findings suggest that K⁺Ca₃.1 could be a key player in mediating endothelial hyperpolarization elicited by peptide Lv.

Peptide Lv augments K⁺Ca₃.1 current densities in ECs

As peptide Lv increased the mRNA and protein expression of K⁺Ca₃.1 in ECs, we next determined whether peptide Lv was able to increase the functional K⁺Ca₃.1. Cultured HUVECs were treated with PBS (vehicle control) or peptide Lv (500 ng/ml) for 2, 3, or 4 hours prior to whole-cell current-clamp recordings. TRAM-34 (10 μM; a K⁺Ca₃.1 inhibitor) was applied to the bath solution to isolate the K⁺Ca₃.1 current. HUVECs treated with peptide Lv (500 ng/ml) for 3 or 4 hours had significantly larger K⁺Ca₃.1 current densities compared to cells.
treated with a vehicle (Fig 4B). Thus, peptide Lv not only increased the mRNA and protein expression of \( K_{C_{a}3.1} \) but also augmented the \( K_{C_{a}3.1} \) activities in ECs. The augmentation of \( K_{C_{a}3.1} \) by peptide Lv positively correlated to peptide Lv-elicited EC hyperpolarization, indicating that \( K_{C_{a}3.1} \) was the \( K^+ \) channel that mediated peptide Lv-elicited EC hyperpolarization.

**Peptide Lv promotes endothelial proliferation through \( K_{C_{a}3.1} \)**

As we showed that peptide Lv facilitates angiogenesis in part through promoting EC proliferation [14], we tested whether blocking \( K_{C_{a}3.1} \) would inhibit peptide Lv-stimulated EC proliferation. Cultured HUVECs were treated with VEGF (5 ng/ml; positive control), peptide Lv (500
We previously showed that peptide Lv can bind to VEGFR2 and cause its activation through tyrosine phosphorylation [13], so it is not surprising that DMH4 reduced peptide Lv-elicited EC proliferation (Fig 5). Furthermore, blocking K\textsubscript{Ca}\textsubscript{3.1} (with TRAM-34) or both VEGFR2 and K\textsubscript{Ca}\textsubscript{3.1} (with DMH4+TRAM-34) significantly dampened peptide Lv-elicited EC proliferation. These data imply that peptide Lv-elicited angiogenesis is in part through K\textsubscript{Ca}\textsubscript{3.1}-dependent EC proliferation.

**Discussion**

In this study, we investigated a potential mechanism in the promotion of angiogenesis by peptide Lv. Hyperpolarization of ECs leads to EC activation and angiogenesis [18, 21, 22]. We previously showed that peptide Lv can bind to VEGFR2 and cause its activation through tyrosine phosphorylation [13], so it is not surprising that DMH4 reduced peptide Lv-elicited EC proliferation (Fig 5). Furthermore, blocking K\textsubscript{Ca}\textsubscript{3.1} (with TRAM-34) or both VEGFR2 and K\textsubscript{Ca}\textsubscript{3.1} (with DMH4+TRAM-34) significantly dampened peptide Lv-elicited EC proliferation. These data imply that peptide Lv-elicited angiogenesis is in part through K\textsubscript{Ca}\textsubscript{3.1}-dependent EC proliferation.
current density was obtained from the patch-clamp recording of a single EC (for n = 1), which is not a relative datum. Thus, peptide Lv-elicited increases in KCa3.1 protein expression (detected by Western blots) might not be directly reflected onto the recorded current densities (detected with patch-clamp recordings) and vice versa.

Regulation of vasomotion is critical in maintaining systemic blood flow, oxygen delivery, and the health of vessels and capillaries [47–49]. Naturally, vasodilation is a mechanism to

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**Fig 5. Inhibition of KCa3.1 dampens peptide Lv-elicited EC proliferation.** HUVECs were treated with peptide Lv (500 ng/ml), DMH4 (5 μM; a VEGFR2 inhibitor), TRAM-34 (10 μM, a KCa3.1 inhibitor), or a combination of the above for 4 hours. Treatment with VEGF (5 ng/ml) served as a positive control and PBS as the vehicle control. The light absorbance was measured at 560 nm (OD 560 nm) for the MTT proliferation assays. One-way ANOVA followed with Tukey post hoc tests were used for statistical analyses; n = 12–15 for each group; “*” denotes a significant difference from the vehicle control; “#” denotes a significant difference from the peptide Lv treated group; p<0.05.

[https://doi.org/10.1371/journal.pone.0276744.g005](https://doi.org/10.1371/journal.pone.0276744.g005)
widen blood vessels and enhance blood flow to supply oxygen and nutrients to local tissues [47, 48, 50, 51]. However, chronic vasodilation of existing vessels causes increased vascular permeability in downstream capillaries [42, 43, 52], stimulates angiogenesis [53, 54], and promotes pathological neovascularization in various diseases [55–60]. Increased blood flow causes elevated shear stress in downstream small vessels and capillaries, which facilitates pericyte recruitment and microvascular sprouting and growth [58–61]. For example, vasodilation in both retinal arterioles and venules are associated with pathological neovascularization observed in proliferative diabetic retinopathy [58, 62–64]. Vasodilators such as VEGF increase the incidence of early age-related macular degeneration by 70% [57]. Retinal blood flow is increased in the proliferative phase of OIR and retinopathy of prematurity [65–68] and in the progression of choroidal neovascularization [69, 70]. Hence, chronic vasodilation is associated with the development of pathological neovascularization.

Previously, we showed that peptide Lv elicits vasodilation in coronary and retinal arterioles in a concentration-dependent manner [14]. VEGF-elicited vasodilation through its receptor (VEGFR2) is nitric oxide (NO)-dependent [15]. In contrast to VEGF, peptide Lv-elicited vasodilation is not completely attenuated by L-N^G-Arginine methyl ester (L-NAME), a NO synthase inhibitor [14], suggesting that peptide Lv has an NO-independent pathway that mediates vasodilation and possible angiogenesis. In the present study, we showed a new route of peptide Lv’s action. Peptide Lv-caused EC hyperpolarization through augmentation of KCa3.1 can be an additional pathway in peptide Lv-elicited vasodilation and angiogenesis. Whether peptide Lv-elicited augmentation of KCa3.1 in ECs mediates the NO-independent vasodilation will need to be further examined in the future.

The angiogenic property of peptide Lv is in part through binding to VEGF-R2 [13], so the VEGFR2 antagonist DMH4 dampened peptide Lv-elicited EC proliferation. As peptide Lv also has VEGF/VEGFR2/NO-independent actions in vasodilation, it is possible that peptide Lv might contribute to the recurrent neovascularization and/or the resistance to anti-VEGF agents in patients. We demonstrated that peptide Lv is important in early photoreceptor development [12] and promotes cardiomyocyte function [13]. A recent study showed that peptide Lv plays a role in immune responses [71]. Macrophages treated with lipopolysaccharide (LPS) produce an increased inflammatory response that is dampened by treating the cells with peptide Lv [71]. In addition, bone-marrow derived macrophages isolated from mice with genetically knocked-out peptide Lv have a reduced inflammatory response compared to the macrophages isolated from wild-type mice [71]. As peptide Lv is a newly discovered small endogenous peptide that is expressed in multiple tissues and cell types, there may be more unknown functions and bioactivities of peptide Lv that are to be explored in the future.

**Supporting information**

**S1 Fig. The original Western immunoblot images.** The original Western immunoblot images included in Fig 2 is in S1 Fig.

(TIF)

**S2 Fig. The original Western immunoblot images.** The original Western immunoblot images included in Fig 3 is in S2 Fig.

(TIF)

**Author Contributions**

**Conceptualization:** Gladys Y. P. Ko.
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