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Antitumor Activity of Rivoceranib Against Canine Mammary Gland Tumor Cell Lines

JEONG-HA LEE[1], QIANG LI[1], JU-HYUN AN[1], HYUNG-KYU CHAE[1], JIN-WOO CHOI[2], BUM-JIN KIM[3], WOO-JIN SONG[1] and HWA-YOUNG YOUN[1]

[1]*Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea;*
[2]*HLB LifeScience Co., Ltd., Seongnam, Republic of Korea;*
[3]*LSK BioPartners, Inc. (dba LSK BioPharma), Salt Lake City, UT, U.S.A.*

Abstract. *Background/Aim: Canine mammary gland tumors (CMGTs) are the most common tumors in female dogs. Rivoceranib (also known as apatinib) is a novel anti-angiogenic tyrosine kinase inhibitor that selectively binds to vascular endothelial growth factor receptor-2 (VEGFR2). The aim of this study was to disclose the antitumor effects of rivoceranib on CMGT cell lines. Materials and Methods: The direct effects of rivoceranib on CMGT cells in vitro were analyzed by cell proliferation and migration assays. Cell-cycle distribution and apoptotic ratio were analyzed by flow cytometry. Expression levels of phosphorylated VEGFR2 were evaluated by western blot analysis. Results: Rivoceranib treatment significantly reduced the proliferation and migration of CMGT cells in a dose-dependent manner. Flow cytometry results revealed significant increases in G_0/G_1 phase arrest and apoptosis proportional to the drug concentration used. Rivoceranib reduced the level of phosphorylated VEGFR2. Conclusion: We confirm that rivoceranib exerts antitumor effects on CMGT cells by inhibiting biological functions.*

Canine mammary gland tumors (CMGTs) are the most common neoplasms in female dogs (1, 2). Over 95% of CMGTs are of epithelial origin, half of which are diagnosed as malignant (3, 4), and approximately 50% of malignant CMGTs have the potential to undergo metastasis. Adjuvant chemotherapy is recommended for patients with high risk of metastasis or recurrence (1, 2, 5). However, very few studies have demonstrated the efficacy of adjuvant chemotherapy in patients with CMGT (1), highlighting the need for an effective chemotherapy agent for these patients.

As vascular endothelial growth factor (VEGF) and its receptors are crucial in carcinogenesis (6-8), agents targeting VEGF may prove useful for metastatic neoplasms (9). The combination of anti-angiogenic drugs and conventional chemotherapy was proved to exert therapeutic effects against malignant tumors (10). VEGF family members mediate angiogenesis, and VEGF receptor-2 (VEGFR2) is a major receptor (11, 12). As VEGF-mediated angiogenesis is rare and occurs during wound healing and the female reproductive cycle, targeting VEGF is deemed to exert minimum effects on normal physiological procedures (13).

Rivoceranib (also known as apatinib; distributed by HLB Life Science Co., Ltd) is a small tyrosine kinase inhibitor targeting VEGFR2 (14). It is approved in China as a third-line anti-angiogenic drug for advanced metastatic gastric cancer (15). Few phase II studies have demonstrated the effects of rivoceranib on human breast cancer (16, 17). However, there have been no reports on the direct effects of rivoceranib on canine mammary cancer cell lines.

In the present study, we explored the direct effects of rivoceranib on the proliferation, migration, cell-cycle distribution, and apoptosis of CMGT cells. Furthermore, we measured the protein levels of cyclin D1 and phosphorylated VEGFR2 in rivoceranib-treated CMGT cells. Our findings provide experimental evidence on the application of rivoceranib as an antitumor agent in clinical practice.

Materials and Methods

Cell cultures. Canine malignant mammary gland tumor cell lines (CIP) were obtained from the Department of Veterinary Clinicopathology,

Correspondence to: Hwa-Young Youn, Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea. Tel: +82 28801266, Fax: +82 28731213, e-mail: hyyoun@snu.ac.kr and Woo-Jin Song, Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea. Tel: +82 28801266, Fax: +82 28731213, e-mail: woojin1988@snu.ac.kr

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Seoul National University (SNU). CIP cells were derived from both primary (CIPp) and metastatic tumors (CIPm). The CIPp and CIPm cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% solution of 10,000 units/ml penicillin and 100 μg/ml streptomycin (PAN Biotech, Aidenbach, Germany) at 37˚C in a humidified chamber containing 5% CO_2. The media were replenished every 3 days. The cells were subcultured upon reaching 90% confluency.

Antitumor agents. Powder-type rivoceranib (known as apatinib) gifted by HLB Life Science Co. Ltd. (Seongnam, Republic of Korea) was dissolved in dimethyl sulfoxide (Duchefa Biochemie, Haarlem, the Netherlands) at a concentration of 40 mM. The solution was stored at −20˚C.

Isolation of canine peripheral blood mononuclear cells (cPBMCs). All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of SNU (protocol no. SNU-190517-2), and all protocols were in accordance with approved guidelines. Blood samples were obtained from healthy canine donors (n=3, Maltese, 3-11 years old, a female and two males) with the consent of the owner. The donors had been regularly presented for medical check-up at the Seoul National University Veterinary Medicine Teaching Hospital. The blood samples were diluted with an equal volume of Dulbecco's phosphate-buffered saline (DPBS; Pan Biotech) and layered over Ficoll-Paque solution (GE Healthcare Life Science, Chicago, IL, USA) in conical tubes. After centrifugation at 450 × *g* for 30 min with low brake, the buffy coat layer was aspirated with a pipette. RBC lysis buffer (Sigma-Aldrich) was added to the aspirated cells, which were then incubated for 5 min at 20˚C. The cells were washed with DPBS and centrifuged at 450 × *g* for 10 min. After removal of the supernatant, cPBMCs were resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% solution of 10,000 units/ml penicillin and 100 μg/ml streptomycin.

Cell viability assay. CIPp and CIPm cells were seeded in 96-well plates at 1,000 cells/well and incubated overnight. The cells were then treated with rivoceranib at 0, 3.125, 6.25, 12.5, 25, 50, and 100 μM or 0.5% dimethyl sulfoxide without rivoceranib for 24, 48, and 72 h. Following incubation, 10 μl Cell Counting Kit (CCK) solution (D-Plus™ CCK cell viability assay kit; Dong-in Biotech, Seoul, Republic of Korea) was added and the cells were incubated in the dark at 37˚C for 1 h. The absorbance at 450 nm wavelength was determined with a spectrophotometer (Epoch Microplate spectrophotometer; BioTek Instruments, Winooski, VT, USA).

cPBMCs were seeded in 96-well plates at a density of 1×10^5 cells/well. Two-third of cells were stimulated with 25 μg/ml concanavalin A (Sigma-Aldrich) or 1 μg/ml lipopolysaccharide (Sigma-Aldrich) and one-third of cells were left in an unstimulated state. All cells were subsequently treated with different concentrations of rivoceranib. After incubation for 24, 48, and 72 h, the cells were treated with 10 μl CCK solution (D-Plus™ CCK Cell Viability Assay kit; Dong-in Biotech) for 1 h and the absorbance at 450 nm was determined with a spectrophotometer (BioTek Instruments).

Wound-healing assay. CIPp and CIPm cells were seeded in 12-well plates at a density of 1×10^5 cells/well. After reaching 100% confluency, the cells were treated with 2 μg/ml of mitomycin (Enzo Life Science, Farmingdale, NY, USA) for 2 h. The cell monolayer was manually scratched with a 1 ml pipette tip and floating debris was removed with two washes of PBS. The adherent cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and 1% solution of 10,000 units/ml penicillin and 100 μg/ml streptomycin in the presence or absence of rivoceranib (12.5 or 25 μM) for 24 h. Wound healing was estimated by measuring the width of the wound area under a microscope (ULWCD 0.30; Olympus, Tokyo, Japan) at 100× magnification. The width was imaged at 0, 12, and 24 h using a TCapture program (Tucsen Photonics, Fuzhou, China). The width of the wound was calculated as the percentage relative migration width as follows: [(Relative width at 0 h – relative width at 12 h or 24 h)/relative width at 0 h] ×100.

Cell-cycle analysis. CIPp and CIPm cells were seeded in six-well plates at a density of 1×10^5 cells/well and incubated at 37˚C overnight. The cells were treated with different concentrations of rivoceranib (0, 12.5, or 25 μM) for 48 h. Following incubation, the cells were harvested, washed with PBS, and centrifuged at 300 × *g* for 5 min. The cells were fixed with pre-chilled 70% ethanol at −20˚C for 2 h. After fixation, the cells were washed with PBS and centrifuged at 300 × *g* for 5 min. The cells were stained with 0.5 mL of propidium iodide (PI)/RNase staining buffer (BD Pharmingen, San Diego, CA, USA) and incubated at 20˚C for 15 min in the dark. The cell-cycle distribution was analyzed within 1 h using a flow cytometer (FACs Aria II; BD Bioscience, San Jose, CA, USA).

Apoptosis analysis. CIPp and CIPm cells were seeded in six-well plates at a density of 1×10^5 cells/well at 37˚C overnight. The cells were treated with different concentrations of rivoceranib (0, 12.5, and 25 μM) for 48 h. After treatment, the cells were harvested, washed twice with cold PBS, and centrifuged at 300 × *g* for 5 min. After washing, Annexin V Apoptosis Detection Kit I (BD Pharmingen) was used according to the manufacturer's instructions. The cells were resuspended in a 1× binding buffer at a of ~1×10^6 cells/ml, and 100 μl of the solution (~1×10^5 cells) was transferred to a 15 ml conical tube. To stain cells, 5 μl of fluorescein isothiocyanate (FITC) annexin V and 5 μl of PI were used. The cells were incubated for 15 min at 20˚C in the dark and the apoptosis rate was analyzed within 1 h using a flow cytometer (BD Bioscience).

Western blot assay. CIPp and CIPm cells were treated with 0, 12.5, and 25 μM of rivoceranib for 48 h. Total protein was extracted from cells using PRO-PREP Protein Extraction Solution (Intron Biotechnology, Seong-nam, GG, Republic of Korea). The protein concentration was quantified using the Bio-Rad DC Protein assay kit (Bio-Rad, Hercules, CA, USA). For cyclin D1 expression analysis, 20 μg protein samples were loaded and separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For pVEGFR2 analysis, 50 μg protein samples were separated with 8% SDS-PAGE. The proteins were transferred onto polyvinylidene difluoride membranes (0.45 μM; Merck Millipore, Burlington, MA, USA), and then blocked with 5% skim milk diluted in Tris-buffered saline containing 0.1% Tween-20 for 1 h at 20˚C. The membranes were probed with antibodies against cyclin D1 (1:1000; Novus Biologicals, Centennial, CO, USA), phosphorylated (p)VEGFR2 (1:250; Cell Signaling Technology, Danvers, MA, USA), and β-actin (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA) at 4˚C overnight. Following incubation, the membranes were washed and treated with either goat anti-mouse horseradish peroxidase-labeled

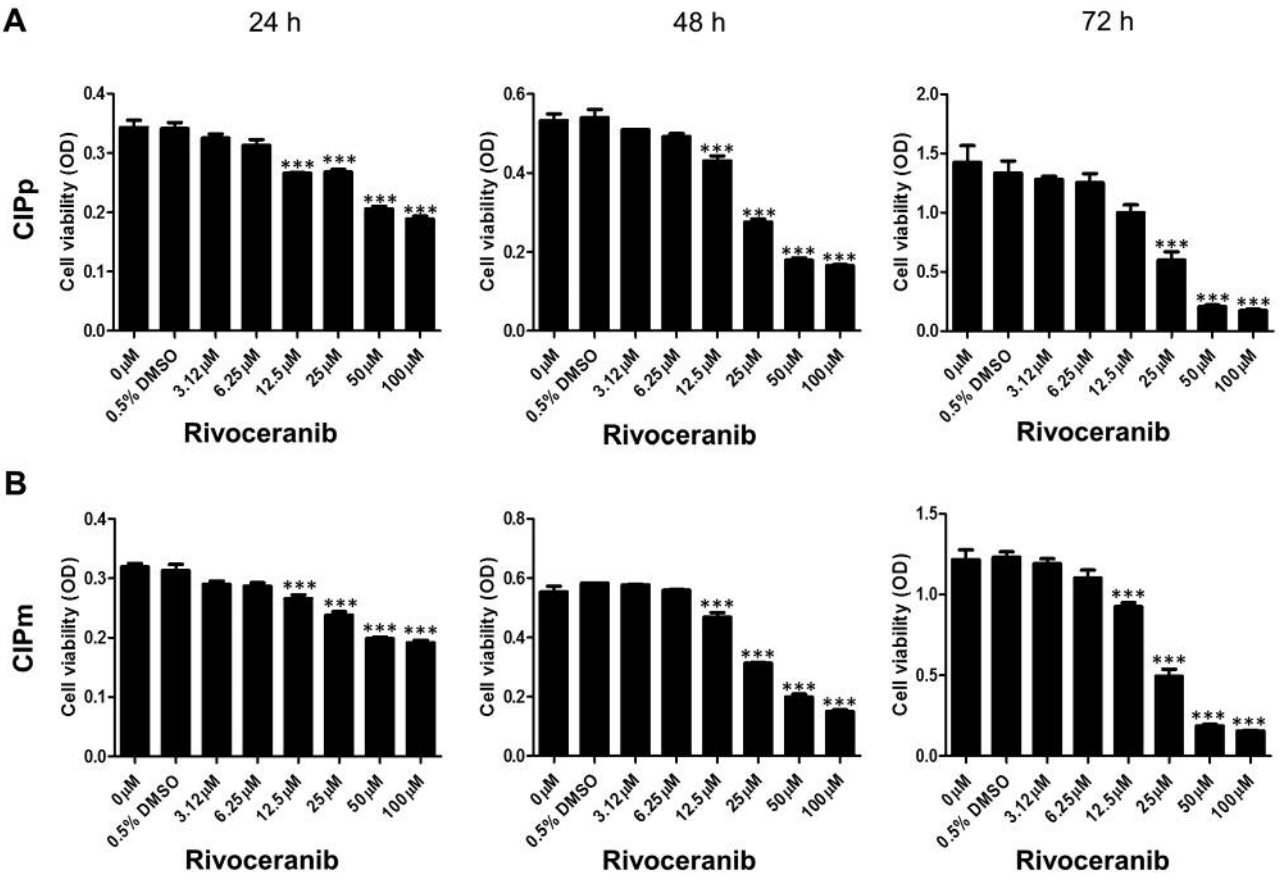

Figure 1. *Rivoceranib inhibited proliferation of CIP canine mammary gland tumor cells in a dose-dependent manner. A: Viability assay of primary CIP (CIPp) cells treated with different concentrations of rivoceranib for 24, 48, and 72 h. B: Viability assay of metastatic CIP (CIPm) cells treated with different concentrations of rivoceranib for 24, 48, and 72 h. All experiments were performed in triplicates. The data represent mean±standard deviation (n=3). ***Significantly different at p<0.001 versus control group (0.5% dimethyl sulfoxide).*

secondary antibody (Bethyl Laboratories, Montgomery, TX, USA) or goat anti-rabbit horseradish peroxidase-labeled secondary antibody (Enzo Lifesciences) for 1 h. Immunoreactive bands were detected using ImageQuant Las4000mini (GE Healthcare Life Sciences, Chicago, IL, USA).

Statistical analysis. Each experiment was performed at least thrice. GraphPad Prism (version 5) software (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. The data were analyzed using the Student's *t*-test and one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. The results are presented as the mean value±standard deviation (SD). Differences with a value of $p<0.05$ were considered statistically significant.

Results

Rivoceranib inhibited the proliferation of CMGT cell lines. To assess the cytotoxic effects of rivoceranib, CIPp and CIPm cells were treated with different concentrations (0-100 μM) of the drug for 24, 48, and 72 h. The viability of both CIPp and CIPm cells decreased in response to treatment with rivoceranib in a dose-dependent manner (Figure 1). Rivoceranib significantly reduced the viability of CIPp cells at 12.5, 25, 50 and 100 μM after 24 and 48 h as compared with the control group (Figure 1A; $p<0.001$). The viability of CIPp cells was significantly reduced at 25, 50 and 100 μM after 72 h (Figure 1A; $p<0.001$). Rivoceranib also significantly reduced the viability of CIPm cells at concentrations of 12.5 μM and above at all time points compared to the control group (Figure 1B; $p<0.001$). However, no significant difference was observed in the viability of the cells treated with rivoceranib at concentrations below 6.25 μM for different time points. Thus, rivoceranib was found to inhibit the proliferation of CMGT cells in a dose-dependent manner.

Rivoceranib exerted no cytotoxic effects on cPBMCs. To determine the cytological effects of rivoceranib on cPBMCs,

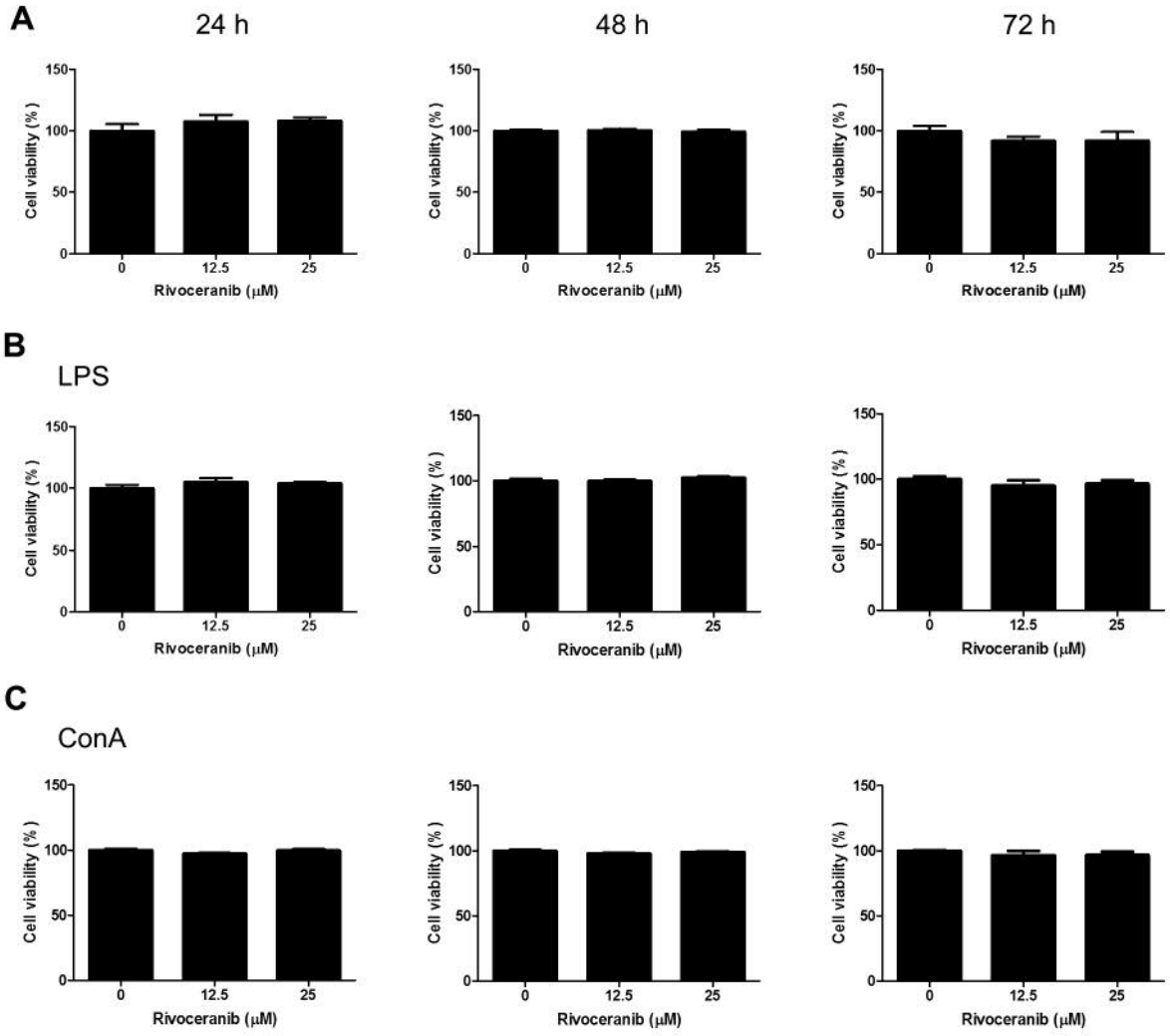

Figure 2. *Rivoceranib exerted no significant cytotoxicity on normal canine peripheral blood mononuclear cells (PBMCs). A: Viability assay of normal canine PBMCs treated with 0, 12.5, and 25 μM rivoceranib for 24, 48, and 72 h. B: Viability assay of normal canine PBMCs stimulated with 1 μg/ml lipopolysaccharide (LPS). C: Viability assay of normal canine PBMCs stimulated with 25 μg/ml concanavalin A (ConA). All experiments were performed in triplicates. The data represent the mean±standard deviation (n=3).*

the cells were treated with different concentrations (0, 12.5, and 25 μM) of rivoceranib for 24, 48, and 72 h. Cell viability was not significantly different following treatment with 0, 12.5, and 25 μM rivoceranib for different times (Figure 2A). We used concanavalin A or lipopolysaccharide to stimulate cPBMCs before rivoceranib treatment. The viability of the stimulated cells was not significantly different at all the evaluated time points (Figure 2B and C). Thus, rivoceranib had no significant cytotoxic effects on normal immune cells.

Rivoceranib inhibited the migration of CMGT cell lines. We estimated the effects of rivoceranib on cell migration using the wound-healing assay. Rivoceranib disrupted wound closure in CIPp and CIPm cells (Figure 3A). As shown in Figure 3B, rivoceranib significantly inhibited the migration of both CIPp and CIPm cells in a dose-dependent manner ($p<0.001$). These results confirm the ability of rivoceranib to suppress the migration of CIPp and CIPm cells.

Rivoceranib induced cell-cycle arrest in CMGT cell lines. The cell-cycle distribution of CIPp and CIPm cells after rivoceranib treatment for 48 h was determined using flow cytometry. As shown in Figure 4A, naïve CIPp and CIPm cells were mostly found in the G_0/G_1 phase (66.33%±0.21% and 66.09%±0.32%, respectively), and similarly distributed between the S phase (14.09%±0.16% and 14.82%±1.07%,

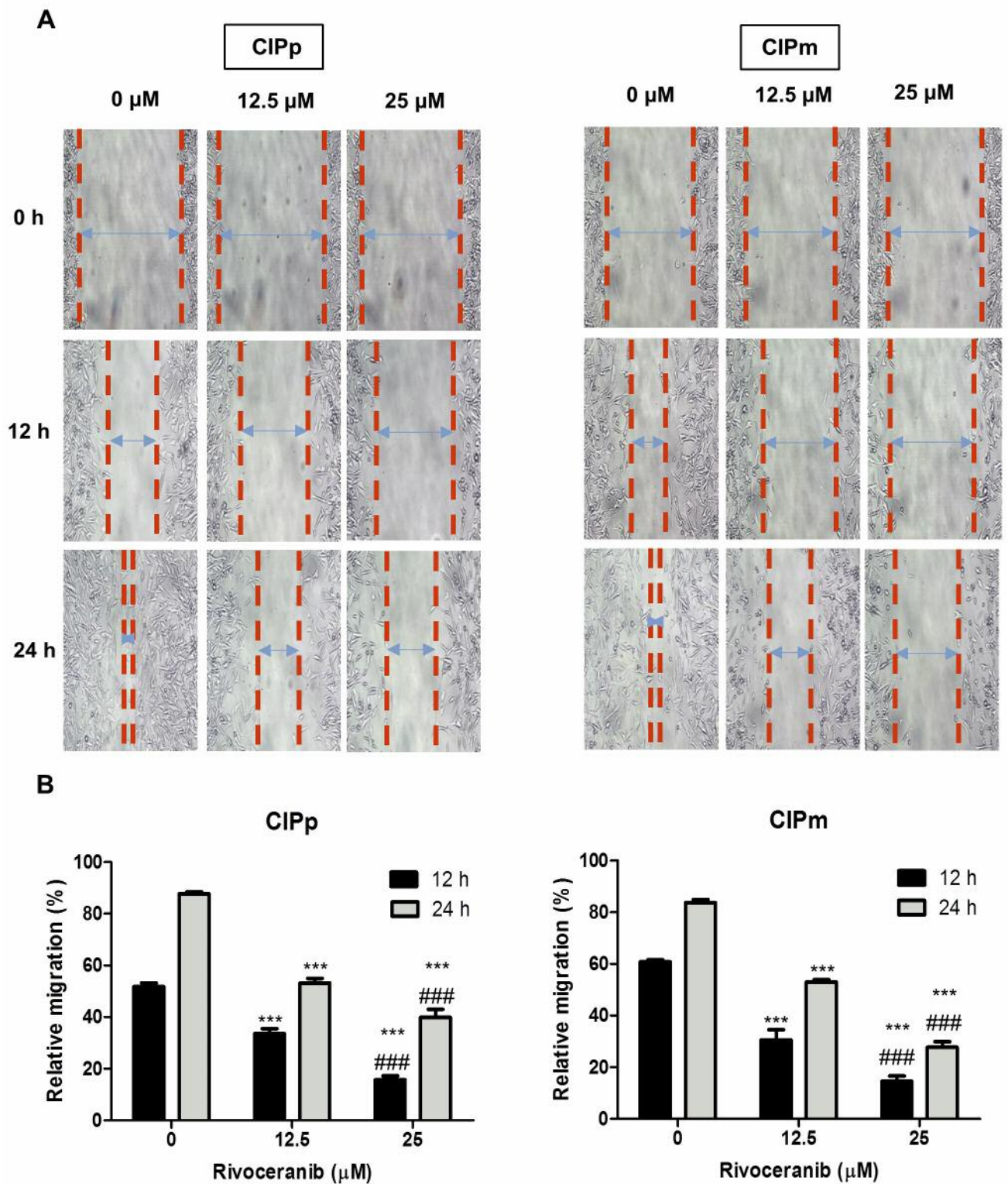

Figure 3. *Rivoceranib inhibited the migration of CIP canine mammary gland tumor cells. A: The migratory capacity of primary (CIPp) and metastatic (CIPm) CIP cells was measured after treatment with rivoceranib (0, 12.5, and 25 μM) for 48 h using scratch wound-healing assays. B: The relative migration is shown via histograms. All experiments were performed in triplicates. The data represent the mean±standard deviation (n=3). Significantly different at: ***p<0.001 versus control group. ###p<0.001 in cells treated with 25 μM versus those treated with 12.5 μM.*

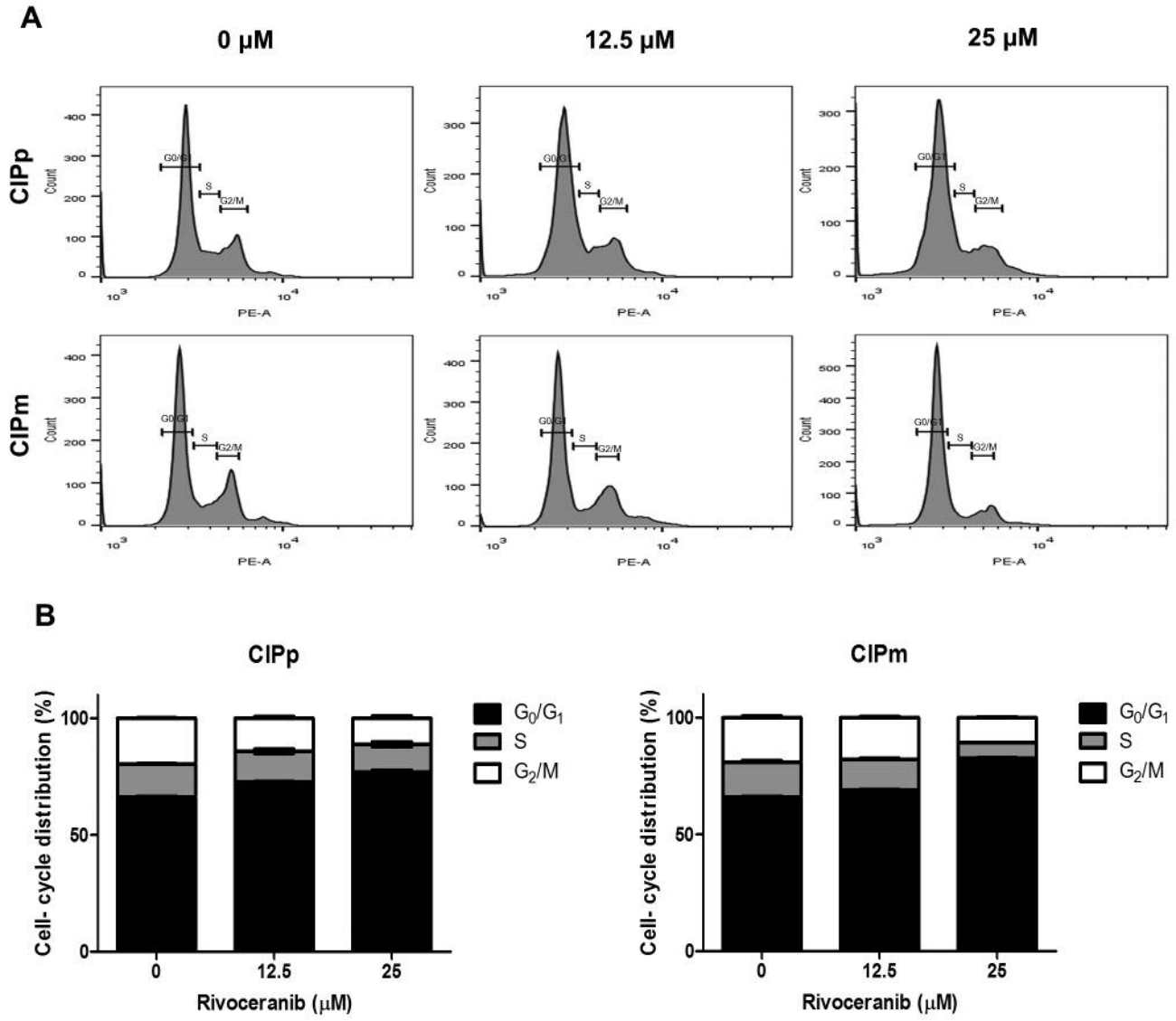

Figure 4. *Rivoceranib induced G_0/G_1 phase arrest of CIP canine mammary gland tumor cells in a dose-dependent manner. A: The cell-cycle distribution of primary (CIPp) and metastatic (CIPm) CIP cells treated with rivoceranib (0, 12.5, and 25 μM) for 48 h as detected using flow cytometry. B: Quantification of data shown in A. The percentages of cells in G_0/G_1, S, and G_2/M phases are shown. All experiments were performed in triplicates. The data represent the mean±standard deviation (n=3).*

respectively), and G_2/M phase (19.58%±0.09% and 19.09%±0.99%, respectively). After treatment with 12.5 μM rivoceranib, 72.76%±0.17% and 69.11%±0.18% of CIPp and CIPm cells, respectively, were detected in the G_0/G_1 phase, 13.09%±0.74% and 12.97%±0.64% in the S phase, and 19.58%±0.58% and 17.92%±0.58% in the G_2/M phase. Treatment with 25 μM rivoceranib led to 76.96%±0.56% and 82.62%±0.29% of CIPp and CIPm cells, respectively, were found to be in the G_0/G_1 phase, 11.85%±0.84% and 6.58%±0.08% in the S phase, and 11.19%±0.75% and 10.80%±0.28% in the G_2/M phase. The ratio of the cells in the G_0/G_1 phase after rivoceranib treatment significantly increased in a dose-dependent manner (Figure 4B; $p<0.001$). Thus, rivoceranib induced G_0/G_1 phase arrest in a dose-dependent manner.

Rivoceranib promoted apoptosis of CMGT cell lines. We assessed the apoptotic ratio of CIPp and CIPm cells after incubation with rivoceranib for 48 h using flow cytometry (Figure 5A). The apoptotic ratio significantly increased for

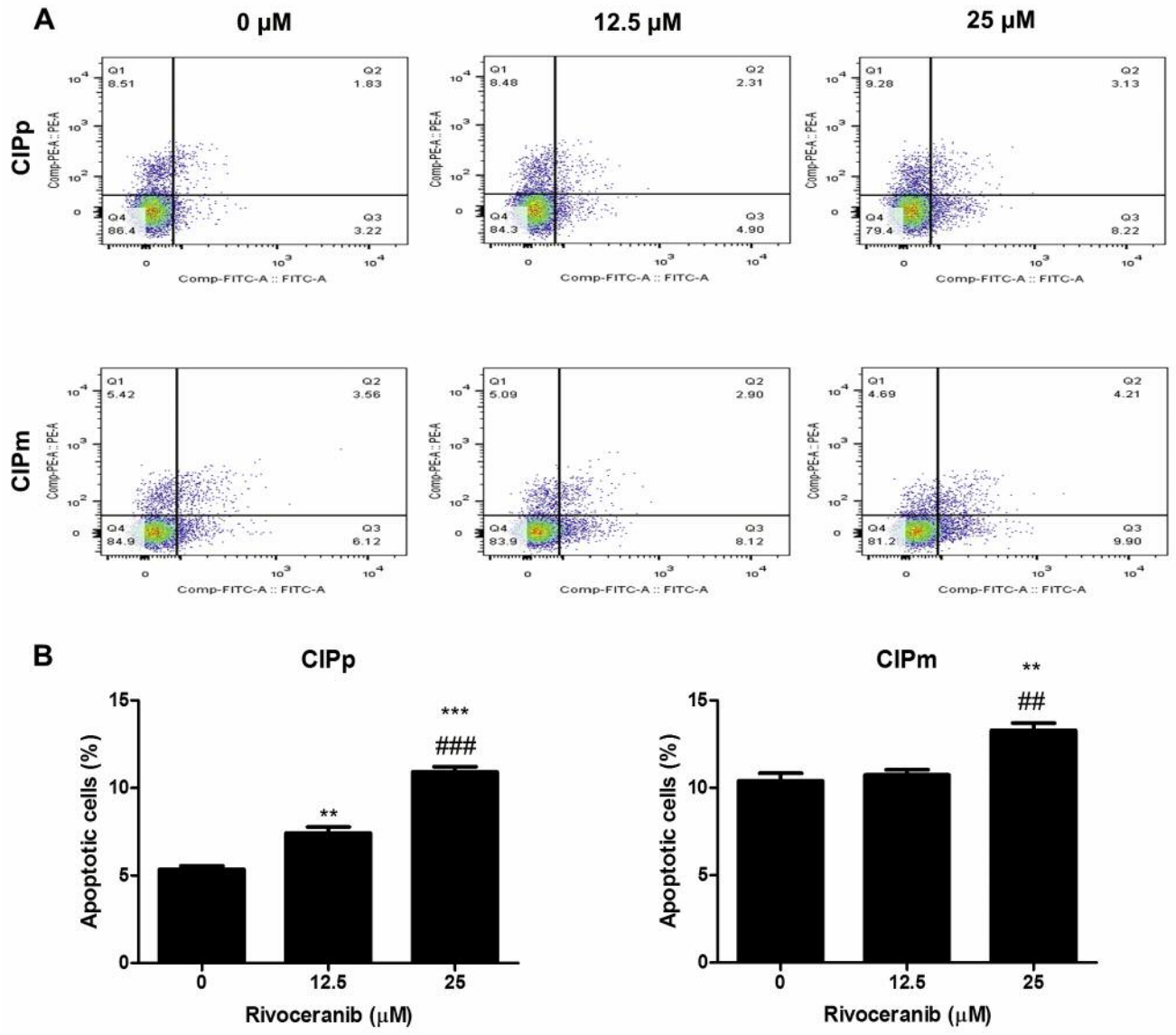

Figure 5. *Rivoceranib promoted apoptosis of primary (CIPp) and metastatic (CIPm) CIP canine mammary gland tumor cells. A: The apoptotic ratio was determined using flow cytometry. CIPp and CIPm cells were treated with rivoceranib (0, 12.5, and 25 μM) for 48 h. B. Quantification of data shown in A. The percentage of apoptosis was detected using annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining. Early apoptotic cells stained as annexin V^+/PI^-. Late apoptotic cells were stained as annexin V^+/PI^+. All experiments were performed in triplicates. The columns represent the mean±standard deviation (n=3). Significantly different at: $^{**}p<0.01$ and $^{***}p<0.001$ versus control group; $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ in cells treated with 25 μM versus those treated with 12.5 μM.*

CIPp cells treated with 12.5 μM $p<0.01$) and 25 μM ($p<0.001$) of rivoceranib, while that of CIPm cells significantly increased at 25 μM ($p<0.01$; Figure 5B). These results demonstrate that rivoceranib induced apoptosis in CMGTs.

Rivoceranib down-regulated the expression of cyclin D1 and pVEGFR2 in CMGT cell lines. To investigate the underlying mechanism, we measured the effect of rivoceranib on the protein level of cyclin D1. As shown in Figure 6A, the expression level of cyclin D1, a G_0/G_1 phase-related protein, significantly decreased in both CIPp and CIPm cells after rivoceranib treatment in a dose-dependent manner (Figure 6B; $p<0.001$). Thus, rivoceranib induce G_0/G_1 phase arrest.

We measured the effect of rivoceranib on the protein level of pVEGFR2. As shown in Figure 7A, the expression level of pVEGFR2, an angiogenesis-related protein, was significantly

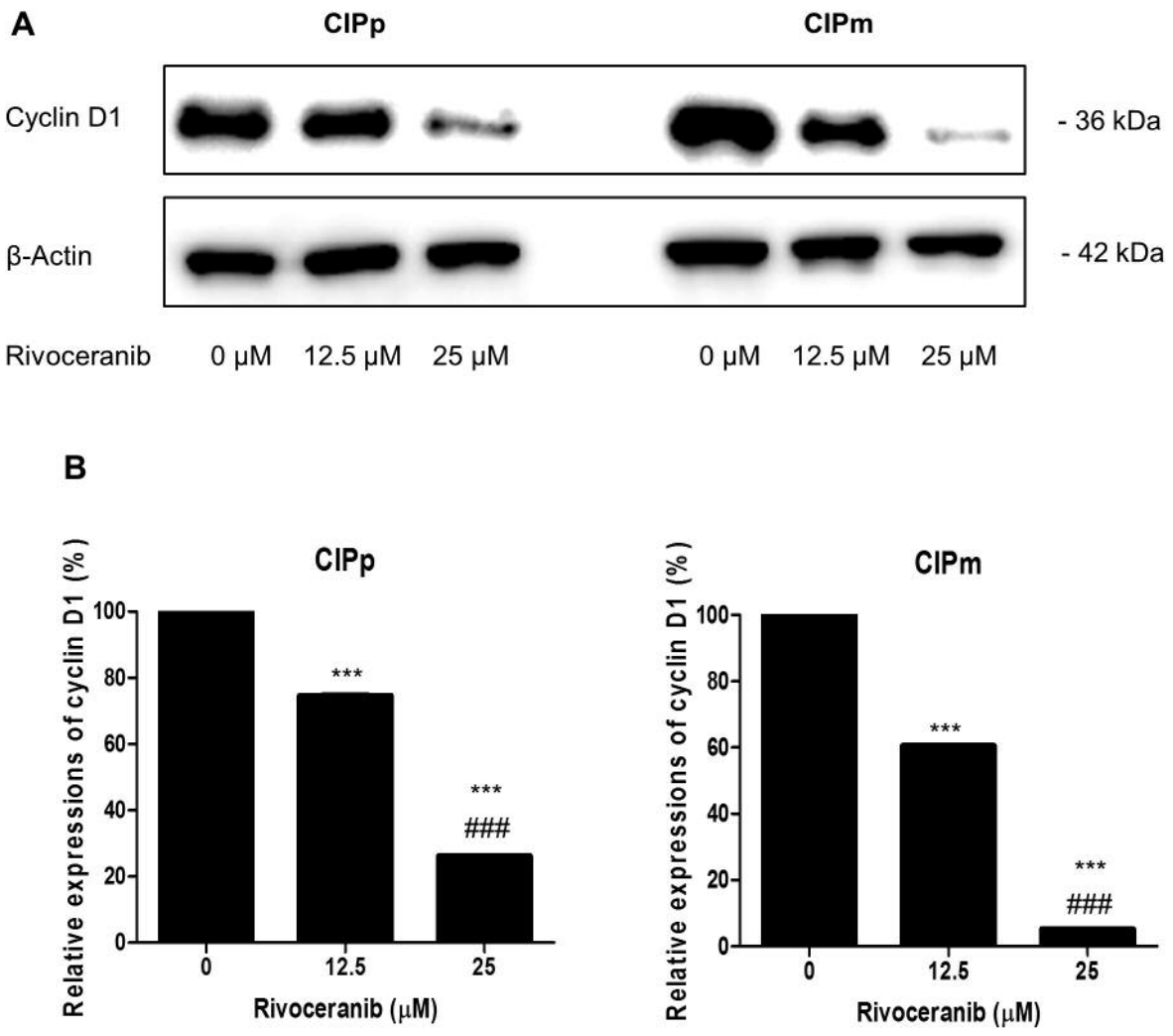

Figure 6. *Rivoceranib inhibited the expression of cyclin D1 in CIP canine mammary gland tumor cells in a dose-dependent manner. A: Primary (CIPp) and metastatic (CIPm) CIP cells were treated with rivoceranib (0, 12.5, and 25 μM) for 48 h. The expression of cyclin D1 was measured with western blotting. Beta-actin was used as a loading control. B: Quantification of data shown in A. Relative expression of cyclin D1 in rivoceranib-treated and control cells is shown. All experiments were performed in triplicates. The column represents the mean±standard deviation (n=3). Significantly different at: ***p<0.001 versus the control group. ###p<0.001 in cells treated with 25 μM versus those treated with 12.5 μM.*

reduced in CIPp and CIPm cells in a dose-dependent manner by rivoceranib (Figure 7B; $p<0.001$). However, the intensity of pVEGFR2 was stronger in CIPp than in CIPm cells. These data indicate that rivoceranib treatment down-regulated the expression of pVEGFR2 in CMGTs.

Discussion

In the present study, rivoceranib was found to significantly inhibit the proliferation and migration of CMGT cells in a dose-dependent manner. However, we failed to note any significant effect on the viability of normal cPBMCs or immune cells. We found that the cytotoxic effects of rivoceranib were attributed to an increase in the apoptosis rate and G_0/G_1 phase arrest. The level of the G_0/G_1 phase-related protein cyclin D1 was reduced in a dose-dependent manner, consistent with the result of G_0/G_1 phase arrest. Furthermore, we found that the expression of pVEGFR2, a crucial signal transducer in angiogenesis pathway (13), was significantly reduced in a rivoceranib concentration-dependent manner. In addition, CIPp cells showed more prominent down-regulation of pVEGFR2 expression than did CIPm cells. Together these findings are consistent with the clinical implications of rivoceranib, suggestive of its

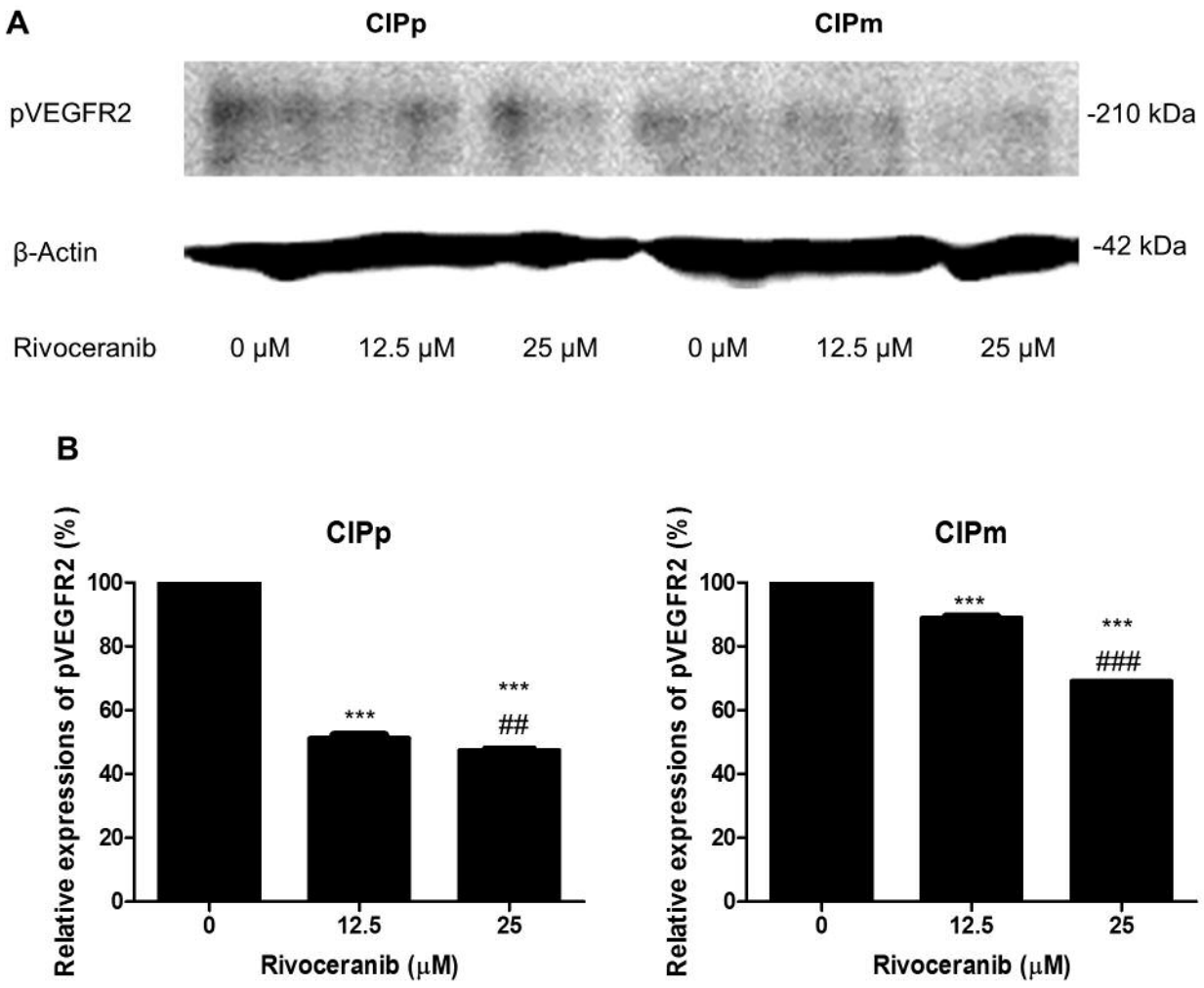

Figure 7. *Rivoceranib inhibited the expression of phosphorylated vascular endothelial growth factor receptor-2 (pVEGFR2) in CIP canine mammary gland tumor cells in a dose-dependent manner. A: Primary (CIPp) and metastatic (CIPm) CIP cells were treated with rivoceranib (0, 12.5, and 25 μM) for 48 h. The expression of pVEGFR2 was measured with western blotting. Beta-actin was used as a loading control. B: Quantification of data shown in A. Relative expression of pVEGFR2 in rivoceranib-treated and control cells is shown. All experiments were performed in triplicates. The data represent mean±standard deviation (n=3). Significantly different at: ***p<0.001 versus the control group; ##p<0.01 and ###p<0.001 in cells treated with 25 μM versus those treated with 12.5 μM.*

application as an efficient antitumor agent for the adjuvant chemotherapy of patients with CMGT.

CMGTs are the most prevalent tumors in female dogs (1, 2). Approximately 50% of these tumors are malignant, and half of the malignant forms metastasize (18, 19). Surgery alone is insufficient for the treatment for CMGTs at risk of recurrence or metastasis (5). Therefore, adjuvant treatment is necessary for patients at high risk (19, 20). Current adjuvant chemotherapy includes doxorubicin, carboplatin, 5-fluorouracil, and cyclophosphamide (1, 21). However, it is difficult to demonstrate clinical efficacy of adjuvant chemotherapy without any anti-angiogenic therapy (22).

The concept of using anti-angiogenesis as an antitumor treatment regimen was introduced by Judah Folkman who revealed angiogenesis to be the key factor in tumor growth (23). Tumors obtain nutrients from the blood supply; hence, tumor cells secrete various pro-angiogenic growth factors in order to survive (9, 24). Among the pro-angiogenic growth factors, VEGF is a key factor involved in developmental angiogenesis (24-27). VEGF was reported to be overexpressed in approximately 30-60% of solid tumors (28) and inhibition of VEGF expression was shown to reduce tumor growth in an animal experiment (29-31). VEGF binds to two receptors, VEGFR1 and VEGFR2 (32). Of the two

receptors, VEGFR2 is the key mediator of VEGF (33). All approved anti-angiogenic drugs are known to affect the binding of VEGF to VEGFR2 or to activate intracellular VEGFR2 (33).

Rivoceranib (also known as apatinib or YN968D1) is a novel selective VEGFR2 tyrosine kinase inhibitor with a 10-fold stronger binding capacity than sorafenib (14). In human gastric cancer, rivoceranib was shown to be a possible third-line antitumor agent in a phase III clinical trial (15). A few studies have reported the promising therapeutic effects of rivoceranib on various human tumor types in phase II clinical trials (16, 34-37). In murine xenograft models such as of human lung, colon, stomach and nasal cancer, tumor growth was inhibited by rivoceranib alone or in combination with other conventional chemotherapy agents (14, 38, 39). As rivoceranib exerts indirect anti-angiogenesis antitumor effects, the studies on rivoceranib have mostly reported its indirect effects (14, 40, 41). However, recent studies have confirmed the direct antitumor activity of rivoceranib in various human tumor cell lines (42-46). Rivoceranib directly inhibited the proliferation and migration (44-46) and enhanced apoptosis and cell-cycle arrest of human tumor cell lines (45-47). These results are consistent with our canine data. In the present study, we confirmed that pVEGFR2 expression was significantly down-regulated in rivoceranib-treated CMGTs.

The present study has a few limitations. As we conducted experiments without recombinant VEGF except for the small quantity of VEGF in the serum-containing media and used CMGT cells alone without endothelial cells, the protein expression of pVEGFR2 was weak. Studies have revealed higher levels of circulating VEGF in dogs with malignant tumors than in healthy dogs (48-53). Secondly, further research including *in vivo* experiments and clinical trials are warranted to confirm our findings.

In conclusion, the present study demonstrates the inhibitory effects of rivoceranib on the biological functions of CMGTs *in vitro*. To the best of our knowledge, this study revealed the direct inhibitory effects of rivoceranib on CMGT cell lines for the first time. These findings provide the basis to support the potential clinical application of rivoceranib as an adjuvant chemotherapy for patients with CMGT.

Conflicts of interest

The Authors have no conflicts of interest to report in relation to this study

Authors' Contributions

Jeong-Ha Lee and Qiang Li designed the study and performed experiments. Jeong-Ha Lee analyzed the data, and wrote and revised the article. Jin-Woo Choi and Bum-Jin Kim provided the rivoceranib. Woo-Jin Song assisted in analysis of data and helped in revision of the article. Ju-Hyun An, Hyung-Kyu Chae, Woo-Jin Song and Hwa-Young Youn reviewed the article. Woo-Jin Song and Hwa-Young Youn supervised the work. All Authors read and approved the final article.

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