Review Article

The potential role of Ral-interacting protein 76 and vascular endothelial growth factor on angiogenesis in the tumor and ovarian corpus luteum microenvironment

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Abstract: Tumors and the ovarian corpus luteum have complex mechanisms in the growth microenvironment. Angiogenesis is especially important for demonstrating the molecular mechanism of dynamic cellular function in tumors and corpus luteum. Angiogenesis in tumors and corpus luteum seems to have a similar function, and Ral-interacting protein 76 (RLIP76) and vascular endothelial growth factor (VEGF) are expressed in the tissues of tumors and ovarian corpus luteum. RLIP76 is a potential factor with VEGF in the tumor and corpus luteum angiogenesis. RLIP76 regulates a small GTPase (R-Ras) in cell survival, spreading, and migration. VEGF activates angiogenic functions in tumor and endothelial cells. Hypoxia-inducible factor-1 (HIF-1) is important in tumor growth, tumor angiogenesis, and corpus luteum. VEGF and HIF-1 regulate the angiogenic function of RLIP76, and RLIP76 controls vascular growth in endothelial and tumor cells. RLIP76, R-Ras, VEGF, and HIF-1 may be useful in the research of corpus luteum and cancer therapy and the study of mechanisms of tumor and corpus luteum angiogenesis. This review will help to elucidate the roles of RLIP76 and VEGF in tumor and corpus luteum angiogenesis, tumorigenesis, and the specific regulation of RLIP76 and VEGF. Thus, we reviewed the potential role of RLIP76 and VEGF in the angiogenesis of the tumor and corpus luteum in the tumor and ovarian microenvironment.

Keywords: Ral-interacting protein 76; vascular endothelial growth factor (VEGF); tumor; corpus luteum

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Introduction

The ovarian corpus luteum is the female reproductive organ and rapid growth and regression with many structural and functional mechanisms (1). During the formation and regression of the corpus luteum, this temporary endocrine gland undergoes physiological events, including angiogenesis and apoptosis (2,3). Also, the corpus luteum microenvironment complex signaling pathways, including Ral-interacting protein 76 (RLIP76), small GTPase (R-Ras), vascular endothelial growth factor (VEGF), and hypoxia-inducible factor-1 (HIF-1). Recently, we found that RLIP76 and R-Ras expressed in the luteal endothelial and luteal cells. RLIP76 and R-Ras protein are also expressed in humans' ovaries and tumor blood vessels (4-6). The process of corpus luteum and the tumor is similar in tumorigenesis and ovarian corpus luteum formation, such as angiogenesis events.

Tumor tissue is an abnormal solid mass whose growth depends on vascular density (7). The tumor vasculature is regulated by tumor growth and therapeutic intervention for cancer (8,9). Studies on tumor angiogenesis are important to understand the molecular mechanisms for inhibiting tumor growth. Tumor angiogenesis is essential for invading tumor growth and is an important factor in regulating cancer progression. The inhibition of angiogenesis in tumors is a novel cancer therapeutic approach to novel cancer therapy. Tumor cells secrete angiogenic factors during tumor growth,

Figure 1 The schematic diagram of the RLIP76 domain. RLIP76 comprises 655 amino acids, AP2 binds on the N-terminal domain, and POB1 is the C-terminal domain. Amino acids 69-74 and 418-425 are the ATP binding sites. RLIP76 has a Rho/Rac GAP and Ral binding domain and an ARNO binding site (binds R-Ras). RLIP76, Ral-interacting protein; AP2, activating protein 2; ATP, adenosine triphosphate; ARNO, Arf nucleotide site opener; Rac, Rho family of GTPase; R-Ras, GTPase related-Ras; POB1, partner of RalBP1.

and the factor supports vascular growth. Also, endothelial and tumor cells have various cell functions, such as proliferation, migration, and spreading. To study the angiogenesis in tumorigenesis, tumor angiogenesis may be an important key in the mechanism of tumor inhibition and cancer therapy associated with RLIP76, R-Ras, VEGF, and HIF-1.

RLIP76, also known as RalBP1, is a multifunctional protein for treating tumors, such as for the regression of melanoma, breast and lung cancer, and inhibition of carcinoma (10,11). RLIP76 is overexpressed in melanomas, lung, and ovarian cancers, indicating that RLIP76 can regulate tumor growth and stimulate vasculature development for tumor progression (12). Recently, we have reported that RLIP76 regulated the neovascularization of tumors using knockout mice and wild-type mice (10). Tumorigenesis (xenografted tumors) and tumor angiogenesis (xenografted Matrigel plugs) in RLIP76 knockout mice were attenuated, suggesting that RLIP76 was required for cancer progression and survival via vascular growth and tumor angiogenesis. Also, RLIP76 regulates cell survival, spreading, and migration by activated R-Ras, suggesting that small GTPase regulates cell morphology and motility. Moreover, the corpus luteum has a similar microenvironment, which is angiogenesis for cell growth. Angiogenesis of tumors and the corpus luteum is essential for growing tissues and cells. The proteins of RLIP76 and VEGF are expressed in the corpus luteum as tumors, suggesting that the tumor and corpus luteum have analogous microenvironments. In this article, we will describe the roles of RLIP76/R-Ras and VEGF via HIF-1 in the angiogenesis and microenvironment of the corpus luteum and tumor and the specific regulation in the ovarian corpus luteum and tumors.

The microenvironment in the tumor

RLIP76 is a multifunctional and potential modular protein in mammals, which plays an important role in tumor cells, cell signaling, and xenobiotic defense mechanisms (13,14). RLIP76 is composed of 655 amino acids and contains a GTPase activating protein domain (RhoGAP domain), a Ral-binding domain (RalBD), and ATP-binding sites, and interacts with Ras-related protein (R-Ras, *Figure 1*). The protein is a guanosine triphosphate (GTP)-dependent interaction partner with RalA. It binds directly to RalB. Ral protein, a signal transducer, activates guanosine diphosphate and binds to guanosine triphosphate. Also, RLIP76 is a Ral effector that interacts with Ral protein and activates cell functions, such as cell migration and spreading (15,16).

RLIP76 regulates tumorigenesis, cell spreading, migration, mitosis, endocytosis, and apoptosis (17,18). Briefly, RLIP76 inhibits apoptosis and promotes the proliferation of human malignant glioma via activation of Rac1-Jun NH_2 kinase (JNK). This suggests that RLIP76 is required for tumorigenesis and suppression of apoptosis. In cell motility, RLIP76 is a mediator in the effect of R-Ras on cellular spreading and migration through the activation of Arf6 GTPase via binding to RLIP76/R-Ras. Additionally, RLIP76 leads to the loss of mitochondrial fission through cyclin B-cyclin-dependent kinase 1 (CDK1) kinase, a mitotic cyclin-dependent kinase, and activation by phosphorylation of dynamin-related protein 1 (DRP1) during mitosis (17). RLIP76 is involved in endocytosis by binding to activating protein 2 (AP2) on the N-terminal region, partner of RalBP1 (POB1) on the C-terminal and associated Eps homology domain protein, RalBP1-associated Eps domain-containing protein 1 (Reps1) (19,20). In endothelial cells (ECs), RLIP76 regulates the intracellular levels of 4-hydroxy-t-2,3-nonenal (HNE) and glutathione (13). It also activates the C-JNK signaling pathway in ECs, suggesting that RLIP76 leads to cell differentiation and cellular signaling pathways in apoptosis (21,22).

The RLIP76 is required for cell spreading and migration with R-Ras activation. We studied RLIP76 in the migration and spreading of ECs and tumor cells, which is regulated by the R-Ras-regulated pathway, such as a small GTPase R-Ras effector (23,24). The adapter function of RLIP76

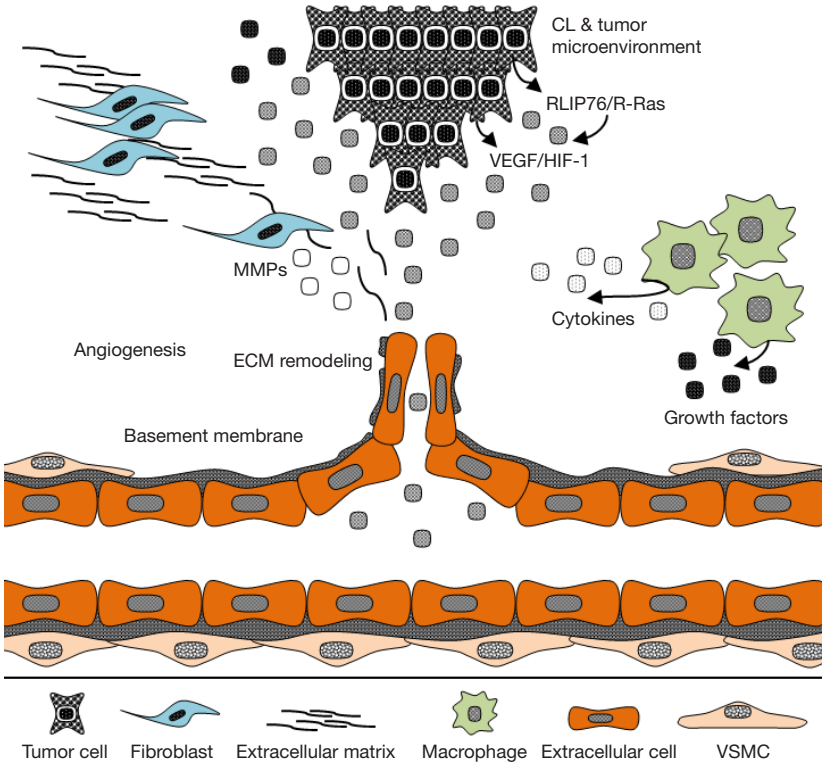

Figure 2 RLIP76, R-Ras, VEGF, and HIF-1 in ovarian corpus luteum and tumor microenvironment. Corpus luteum and tumors have hypoxic conditions that affect corpus luteum and tumor growth and tumor angiogenesis. Luteal and tumor cells secrete VEGF by HIF-1-mediated regulation of RLIP76, and cytokines and growth factors are regulated in the corpus luteum and tumor angiogenesis. MMPs and ECM remodeling are also required for corpus luteum, tumor growth, and neovascular formation. RLIP76, Ral-interacting protein; R-Ras, Rho family of GTPase; VEGF, vascular endothelial growth factor; HIF-1, hypoxia-inducible factor-1; MMPs, matrix metalloproteinases; ECM, extracellular matrix; VSMC, vascular smooth muscle cell.

also induces a small GTPase guanine exchange factor (ARNO). Awasthi *et al.* reported that R-Ras is necessary to recycle endosomes to spread and migrate cancer cells. Thus, RLIP76 is important in cell spreading by mediating the Ral protein (24).

Moreover, Goldfinger *et al.* reported that R-Ras-bound RLIP76 regulates cell spreading and migration via the Arf6-activated Rac1 pathway (25). Arf6 is inactivated by the blocked RLIP76, and the negative control of R-Ras inhibited the Arf6 activation. Also, the mutated Arf6 blocked Rac1 localization and formation in the cells. These results suggest that the activation and presence of RLIP76 may be associated with Arf6/Racl interaction. However, some studies reported that RLIP76 is not bound to multiple Ras families, such as H-Ras, but not RalA in mammalian cells (26-28). It seems likely that RalA is not directly regulated in the RLIP76/Ras pathway of cell spreading. Indeed, R-Ras directly binds to RLIP76 and stimulates R-Ras in cell spreading, suggesting that RLIP76 interacts with R-Ras and specific mediators.

RLIP76 and VEGF in the tumor

In angiogenesis, RLIP76 has potential roles in the function of ECs in the microvasculature. RLIP76 regulates tumorigenesis and metastasis by regulating endothelial cell migration and proliferation and stimulating tumor cells' activation and differentiation. Also, the vasculature in solid tumors is very complex and has many branched capillaries during angiogenesis (10,22). Overall, RLIP76 plays a role in molecular and physiological functions and processes in tumor angiogenesis (*Figure 2*).

Recently, we reported a novel physiological role of RLIP76 in angiogenesis and tumorigenesis in mice (10). During the growth of carcinoma, we tested the vascular quantification of carcinoma in wild-type and RLIP76

knockout mice using X-ray micro-computed tomography in a 3D system (performed using SKYScan software and analyzed by CTAn 3D program). We found that the growth of tumor vasculature in RLIP76 knockout mice was suppressed. Briefly, the volumes, density, and tortuosity of vasculature were diminished in both types of mice. Analysis in wild-type and knockout mice is important to determine the role of RLIP76 in tumor angiogenesis, mean vessel density, specific surface areas, and vessel tortuosity in angiogenesis. RLIP76 is a main factor that grows vasculature and affects tumor angiogenesis. Additionally, the vascular network can understand the tumor's structure and growth by measuring the microvascular branch, suggesting that vessels in tumors are unstable and irregular during tumor growth.

The molecular mechanism of RLIP76 in corpus luteum and tumor angiogenesis is still unknown. Tumor growth has various microenvironment conditions, such as low oxygen, cytokines, growth factors, matrix metalloproteinases (MMPs) and extracellular matrix (ECM) remodeling, ECs, fibroblasts, and macrophages (29,30). We recently found that RLIP76 regulates VEGF, an angiogenic growth factor, in the tumor cell function as it stimulates VEGF expression in melanoma and carcinoma cells, suggesting VEGF production by RLIP76 from tumor cells could be regulated in tumor angiogenesis (31). Additionally, RLIP76 regulates the transcriptional activity of HIF-1 and phosphoinositide 3-kinase (PI3K) in tumors. RLIP76 is required for HIF-1 activation in melanoma and carcinoma tumors. Moreover, RLIP76 is an important protein in PI3K activation since HIF-1 could activate the PI3K pathways, as we have previously shown that the inhibition of RLIP76 by short hairpin ribonucleic acid (shRNA) suppressed PI3K activity downstream of PI3K. Thus, we suggest RLIP76 and VEGF are required for PI3K activity in tumor angiogenesis.

The microenvironment in the ovarian corpus luteum

The morphological and physiological characteristics of the ovarian corpus luteum are dynamically changed during the estrous cycle, such as progesterone production, vessel distribution, and weight (2). The Corpus luteum in the ovulated stage is generated from granulosa cells, theca cells, ECs, and other pericytes. Normally, granulosa and theca cells differentiate into luteal steroidogenic cells by luteinizing hormone. At the same time, proliferation is actively increased (32,33). In practice, the corpus luteum development in cows proceeded 8 days after ovulation in cows. The weight of the corpus luteum increases three or four times when the development of the corpus luteum is finished (34). Accordingly, nutrients, oxygen, hormones, and growth factors are sufficiently supported for the differentiation and growth of luteal steroidogenic cells through the vascular development system, suggesting the formation of vessels is necessary for completing the development of the corpus luteum in the luteal phase (33).

Luteal steroidogenic cells are classified into large and small luteal steroidogenic according to cell size, and large luteal steroidogenic cells originate from granulosa cells. In contrast, thecal cells differentiate into small luteal steroidogenic cells (33). Luteal steroidogenic cells produce progesterone from cholesterol via enzymes, such as steroidogenic acute regulatory protein (StAR), cholesterol side chain cleavage (P450scc), and 3β-Hydroxysteroid dehydrogenase (3β-HSD). Synthesized progesterone in the corpus luteum moves on the blood vessels, which regulates the development of the endometrium for embryo implantation and pregnancy maintenance (35). Additionally, we reported that steroidogenic synthesis and angiogenetic factors were selectively expressed in luteal cell types of the bovine corpus luteum (36). Thus, we strongly suggest that the formation and maintenance of vessels during the development of the corpus luteum is important for transporting the synthesized progesterone.

Prostaglandin F2 alpha induces corpus luteum regression (luteolysis), which affects the loss of progesterone production and activation of apoptotic signals in luteal cells (36-38). During luteolysis, cytokines, nitric oxide, leukotriene C4, and endothelin-1 increase in the corpus luteum microenvironment (39). These factors lead to apoptosis in luteal steroidogenic cells and ECs (37). Additionally, we found that the connection of cell adhesions in bovine luteal steroidogenic cells is decreased by prostaglandin F2 alpha (36). The corpus luteum comprises heterogeneous cells, and cell adhesion between ECs and luteal steroidogenic cells is the main function for maintaining the corpus luteum structure (34). Therefore, we suggest understanding adhesions between ECs and luteal steroidogenic cells by prostaglandin F2 alpha, which is very important for anti-angiogenesis using hormone therapy in reproductive biomedical fields.

In addition, progesterone and oxytocin, including progesterone receptor (PR) and oxytocin receptor (OR), play essential roles in maintaining the corpus luteum in the ovary (40). During pregnancy in the mammalian ovary,

the balance of progesterone is necessary for developing endometrium and fetus. Also, progesterone concentration increases during the formation of the corpus luteum, but the production of progesterone decreases during the regression of the corpus luteum. The dynamic phenomenon of progesterone may be essential in the function mechanism of ovarian corpus luteum, such as prostaglandin F2 alpha. Oxytocin is a nonapeptide hormone released from the posterior pituitary gland (41). Oxytocin stimulates uterine contractions via G-protein coupled receptors in the female reproductive system. Interestingly, Shirasuna *et al.* reported that the concentration of oxytocin enhanced significantly with endothelin 1 and prostaglandin F2 alpha during luteolysis in the bovine corpus luteum (42). Endometrium prostaglandin F2 alpha stimulates ovarian oxytocin, and the stimulated oxytocin also elevates the prostaglandin F2 alpha in the corpus luteum. Actually, prostaglandin F2 alpha directly induces a low progesterone concentration in the ovarian corpus luteum, and oxytocin directly induces an increase in progesterone secretion. Therefore, we suggest that prostaglandin F2 alpha and oxytocin may interact with progesterone during luteolysis in the ovarian corpus luteum. Overall, reproductive hormones play a dynamic role in the formation and regression of the corpus luteum in the mammalian ovary. Thus, prostaglandin F2 alpha and oxytocin will be triggered with progesterone to process the functional luteolysis in the ovarian corpus luteum.

RLIP76 and VEGF in the ovarian corpus luteum

Angiogenesis of the corpus luteum has a dynamic microenvironment in which blood flow conditions, low oxygen conditions, changed cell types, dependent hormone concentration, and specific hormones. Normally, angiogenesis regulates ECs, MMPs, ECM, and basement membrane (BM). Angiogenesis also forms novel vessels during tissue development with cell spreading, migration, proliferation, and differentiation. We explained above that the corpus luteum is similar to normal angiogenesis functions. The corpus luteum especially depends on steroid hormones, such as estrogen, progesterone, and prostaglandin F2 alpha. The corpus luteum has luteal cells, ECs, and other cells that form the corpus luteum, a very complex tissue (43). We reported cell types of corpus luteum isolated from bovine corpus luteum, such as luteal steroidogenic cells, luteal theca cells, and luteal ECs (44). Granulosa and theca cells changed to luteal cells during corpus luteum formation, and luteal ECs came to vascular (43,44). Thus, we suggest that angiogenesis function in the corpus luteum is very important for finding novel functions in the ovarian corpus luteum.

VEGF is a major angiogenesis promoter and is an important factor in the physiology of ovarian corpus luteum. We found angiogenic receptors expressed in corpus luteum tissues during estrous cycles (45). VEGF receptor 2 (VEGFR2) and angiopoietin 1 and 2 receptors (Tie2) are important for growing ECs and vascular formation. Both angiogenic receptor proteins are expressed in the early and middle estrous cycle but not the late estrous cycle. We suggest that VEGF and angiopoietin lead to the formation of noel vascular in the ovarian corpus luteum. Although the specific angiogenic proteins depend on other hormones, the mechanisms of corpus luteum formation are interesting.

The corpus luteum has formation and regression of tissues during estrus cycles since the corpus luteum is a temporary endocrine organ (2). Regression of the corpus luteum is also important, such as the formation of the corpus luteum. At the end of the formation, activation of angiogenic proteins is decreased to prepare luteolysis. Hormone secretion, such as progesterone and prostaglandin F2 alpha, is also changed. Progesterone comes to luteal cells and regulates cell proliferation, migration, and spreading (46,47). Prostaglandin F2 alpha is increased during corpus luteum regression and comes to uterus and ovarian luteal cells. Thus, we are continually studying the novel mechanism of the formation and regression of the corpus luteum. Knowing the interaction of ovarian hormones and angiogenic proteins is very important in the angiogenesis of the ovarian corpus luteum.

The ovarian corpus luteum has RLIP76 and VEGF proteins. RLIP76 has many molecular biology functions and physiological functions in various cellular. In the signaling pathway, RLIP76 plays an essential role in the Ras protein activator, binding protein partner of endocytosis regulation and cytoskeleton, transport function in the cell stress response, and biochemical property in the biological membrane (13,19,48,49). Recently, we found mRNA and protein of RLIP76 in the bovine corpus luteum during estrous cycles (43). To determine whether RLIP76 in the corpus luteum is associated with increased angiogenic VEGF expression and activation, we experimented with mRNA and protein expression. In our studies, RLIP76 mRNA was expressed at high levels in the early stage of the bovine corpus luteum, suggesting RLIP76 leads to the generation of the corpus luteum in the bovine. However, the mechanism of the RLIP76 function is still

unclear in mammals. Awasthi *et al.* reported that RLIP76 overexpressed in ovarian carcinoma and melanoma cells (50). RLIP76 is also regulated in PI3K/AKT and Erk signaling pathways and VEGF/HIF-1 in hypoxia conditions (18,31). H-Ras, a GTPase protein, increases the VEGF expression in primary murine ECs (51). Arbiser *et al.* demonstrated that H-Ras leads to enhanced VEGF levels, and elevated expression of VEGF under hypoxia conditions, a known VEGF stimulator (51). HIF-1 is also overexpressed in low oxygen conditions (hypoxia). Kim *et al.* also reported that H-Ras was expressed in the early and middle stages of the bovine corpus luteum (45). Moreover, H-Ras acts on the formation of tumor tissues by binding to G-protein receptors. H-Ras also enhances VEGF expression and increases the activity of the matrix metalloproteinase-2, 9 (MMP-2, 9) involved in cell proliferation, migration, and angiogenesis. Therefore, since H-Ras is expected to play a new functional role in the ovarian corpus luteum, the mechanism needs to be clarified through additional cell experiments. In addition, we may need to measure the mRNA and protein expression of H-Ras and RLIP76 in the tissue and cell of the ovarian corpus luteum by estrous cycle to determine whether the factors involved in tumorigenesis were expressed in corpus luteum tissue. Moreover, the mRNA and protein of the VEGF levels were highly expressed in the early and middle corpus luteum in cows, and VEGFR2 was also expressed at the highest level in the early corpus luteum. These results suggest that RLIP76 and VEGF play an important role in the angiogenesis of the bovine corpus luteum.

VEGFR 2 and Tie 2 are the main angiogenesis-related receptors, both are higher in bovine corpus luteum tissues in the early and middle stages than in the later stage. Angiopoietin family, including Angiopoietin 1 and 2 (Ang 1 and Ang 2), competitively binds to the endothelial cell-specific receptor, Tie 2. Tie 2 is involved in the stabilization and reconstitution of blood vessels (52). Ang 1 is also an angiogenic factor that signals pathways via the EC-specific Tie 2 receptor tyrosine kinase. Ang 1 binds Tie 2 and enhances the tyrosine phosphorylation of Tie 2 in ECs. Also, Tie 2 mRNA and protein were found to be expressed at the highest levels in the corpus luteum of buffalo (53). Also, Hayashi *et al.* and Sugino *et al.* reported that the Ang 1/Tie 2 signal system was developed in the bovine corpus luteum (54,55). Ang 1 and Tie 2 mRNA were expressed in theca interna of bovine during mature follicles, follicular development, and follicular atresia. The ratio of Ang 2/Ang 1 mRNA was increased in the early stage, and Tie 2 mRNA was also elevated. Ang 1 for vascularization is activated by Tie 2 in ECs, but Ang 2 is the antagonist for Ang 1. In fact, Ang-2 can induce the regression of vascular with Ang 1 inhibition. Thus, VEGF, Ang 1, Ang 2, and Tie 2 play important roles in angiogenesis and ovarian corpus luteum. The balance of both Ang 1 and Ang 2 may be key for structural and functional luteolysis of the corpus luteum. However, whether VEGF, Ang 1/Tie 2, and Ang 2 regulate luteolysis and vascular regression in the ovarian corpus luteum is unclear. Moreover, the concentration of estradiol and progesterone was enhanced in Ang 1-treated cells. It means that the life of the ovarian corpus luteum is also dependent on reproductive hormones, such as estrogen, progesterone, prostaglandin F2alpha, and oxytocin. The signaling pathway of the ovarian corpus luteum has a complex maze. As the described results, we suggest that the Ang 1/Tie 2 system is important in the VEGF-regulated signaling pathway in the ovarian corpus luteum.

VEGF receptors, VEGFR 1 (Flt 1, fms-related tyrosine kinase 1) and VEGFR 2 (KDR, kinase insert domain receptor), are needed for the activation of the VEGF function (56). Both receptors of VEGF play a central role in angiogenesis, including the migration and proliferation of ECs. VEGFR 2 has a strong positive signal in angiogenesis, such as VEGF/VEGFR 2. On the other hand, VEGFR 1 has an anti-angiogenesis function in the embryogenesis of mice. Thus, we focused on the VEGF/VEGFR 2 signaling pathway in the ovarian corpus luteum. In fact, VEGF binds both VEGFR 1 and VEGFR 2 in mammals, such as VEGFR 1 for cell migration in pathological angiogenesis, and VEGFR 2 for cell proliferation in ECs. Takahashi *et al.* reported that protein kinase C (PKC)/mitogen-activated protein kinase (MAPK) pathway activation with VEGFR 2 is necessary for DNA synthesis in ECs (57). Gecaj *et al.* found that the level of VEGFR 2 was higher during the early estrous cycle in the bovine corpus luteum (58). Kim *et al.* showed that vascular receptors are strongly expressed in the early and middle phases of the corpus luteum in bovine (45). VEGF and its receptor signal systems are well documented in the ovarian corpus luteum. However, the reproductive hormones-related VEGF signaling pathway is still unclear in luteal ECs. Since ovarian corpus luteum depends on estrogen, progesterone, prostaglandin F2alpha, and oxytocin, the research groups may need more studies about the function of reproductive hormones with VEGF-regulated signals to understand the angiogenesis and apoptosis mechanisms. Nevertheless, these demonstrated findings that the VEGF/VEGF2 signaling pathway is

essential for angiogenesis and the therapy of ovarian cancer in the ovarian corpus luteum.

Conclusions

RLIP76 regulates tumor growth and vascular formation in tumor angiogenesis and controls VEGF protein expression in tumor cells. RLIP76 can influence VEGF induced by HIF-1 activation under hypoxic conditions. We suggest that RLIP76/R-Ras regulates VEGF via HIF-1alpha activation, stimulating tumor angiogenesis and enhancing tumor growth. Moreover, angiogenesis in the ovarian corpus luteum is essential for luteal formation and maintenance during the luteal phase. Thus, RLIP76 and VEGF may be required in angiogenesis mechanisms to activate the ovarian tumor's and corpus luteum's physiological functions in the bovine. In the future, research groups need to study reproductive hormones-regulated molecular processes and signaling pathways with RLIP76 and VEGF in the ovarian corpus luteum, ovarian cancer, and tumor, since the reproductive endocrine system is important in the ovary. Moreover, the RLIP/Ras family and VEGF/HIF-1 are involved in the angiogenesis of tumors and ovarian corpus luteum in humans and animals. These results will be useful for knowing novel hormone functions and angiogenesis mechanisms of ovarian cancer, tumors, and corpus luteum in humans and animals.

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Footnote

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Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-770/coif). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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