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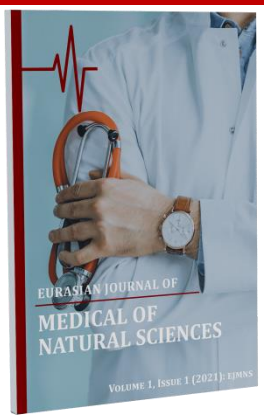

ENDOCRINE-ORIGINATED VASCULAR ENDOTHELIAL GROWTH FACTOR/PROKINETICIN-1 IN THE PROGRESSION OF CANCER AND ANGIOGENESIS WITHIN TUMORS

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ABSTRACT

Considerable evidence suggests that endocrine gland-derived vascular endothelial growth factor (EG-VEGF) is primarily expressed in endocrine glands and certain endocrine-dependent organs. Numerous studies indicate that EG-VEGF promotes angiogenesis and cell proliferation, despite its classification outside the VEGF family. While ample data exist regarding the involvement of this growth factor in normal developmental processes, conflicting findings have emerged regarding its role in pathological conditions, particularly malignant tumors. Therefore, our current paper aims to explore the function of EG-VEGF in both normal tissues and various malignant tumors, shedding light on its impact on angiogenic processes.

Introduction:

Over the past three decades, significant strides have been made in understanding the biological mechanisms underpinning cancer progression and metastasis. This period of intensive research has led to the discovery and characterization of numerous growth factors and their corresponding receptors, laying the foundation for targeted therapy approaches. Among the notable therapeutic agents to emerge from this endeavor are the humanized monoclonal antibodies trastuzumab and bevacizumab, which have seen widespread application in clinical practice.

Fifteen years ago, the discovery and characterization of endocrine gland-derived vascular endothelial growth factor (EG-VEGF) in the adrenal gland marked a significant milestone. Since then, accumulating evidence has suggested that EG-VEGF is primarily expressed in endocrine glands and select endocrine-dependent organs. Despite not belonging to the VEGF family, EG-VEGF has been shown to play a pivotal role in stimulating angiogenesis and cell proliferation. While several studies have elucidated its function in normal developmental processes, conflicting findings have emerged regarding its involvement in pathological conditions, particularly in malignant tumors.

In 2001, Li et al. isolated two peptides capable of inducing smooth muscle contraction in the gastrointestinal tract of rodents, which were named prokineticins. Concurrently, LeCouter et al. identified a novel factor exhibiting heightened expression in the placenta, ovary, testis, and adrenal gland, and observed its mitogenic effects on endothelial cells within endocrine glands. This factor, termed EG-VEGF, displayed sequence dissimilarity to VEGF but shared identity with prokineticin-1 or PROK-1. Notably, EG-VEGF expression was predominantly localized to endocrine glands and reproductive organs, while PROK-2 or Bv8 was associated with the nervous system.

The Structural Composition of EG-VEGF:

EG-VEGF, also recognized as prokineticin-1, belongs to a novel protein family. The inaugural member of this family, VPRA or venom protein A, was initially identified from the venom of the black mamba snake, alternatively known as "MIT-1". Notably, VPRA exhibits non-toxic properties. EG-VEGF shares a significant structural resemblance with VPRA, with a striking homology of approximately 80%. Consequently, EG-VEGF is regarded as the human counterpart of VPRA. This protein family encompasses additional members, such as Bv8, a secreted protein derived from the frog Bombina variegata, along with its mammalian analogs. Additionally, the family includes colipase, a digestive enzyme, and Dickkopf, a protein renowned for its role as an inhibitor of Wnt signaling pathways. The gene encoding EG-VEGF is situated on chromosome 1p21. Structurally, EG-VEGF bears resemblance to the Bv8 protein, with a homology proportion ranging from 70% to 76%, as well as to its murine and human counterparts.

Brief Overview of EG-VEGF: Expression and Functional Roles

Distinguishing between VEGF and EG-VEGF reveals distinct patterns of temporal and spatial expression. Notably, both VEGF and EG-VEGF are detected within the luteal body. While VEGF mRNA is identified during the initial stages of luteal body formation coinciding with capillary plexus development, EG-VEGF exhibits expression throughout the mid- and early-late luteal phases. These observations suggest a vital role for EG-VEGF in sustaining luteal body function.

Human placental development, particularly concerning blood vessel formation, is intricately linked to EG-VEGF secretion by the syncytiotrophoblast layer. Peak expression of EG-VEGF is noted between the 8th and 10th week of gestation in normal placental tissue. Moreover, EG-VEGF's regulation by hypoxia and its co-localization with VEGF suggest involvement in pre-eclampsia pathogenesis. Recent studies have provided insights into EG-VEGF's roles in both normal and pathological placental angiogenesis, as well as its dysregulation impacting trophoblast proliferation, decidual invasion, and potentially contributing to pregnancy loss.

In addition to their presence in adrenal carcinomas, both VEGF and EG-VEGF expression support the notion that complete angiogenesis inhibition in these tumors necessitates targeting both growth factors. Fenestrated endothelial cells in kidney and endocrine gland capillaries, crucial for their distinctive phenotype, are highly reliant on VEGF secretion. Studies by LeCouter et al. demonstrate that, in conjunction with VEGF, EG-VEGF contributes to the regulation of angiogenesis in the human ovary.

Recent investigations indicate that prokineticins and their receptors may exert influence on male reproduction, given their expression in male reproductive organs such as the testis and prostate. Likewise, extensive studies have scrutinized their presence in female reproductive organs, including the ovaries, uterus, and various tissues during pregnancy. Notably, efforts to elucidate the involvement of EG-VEGF in endometriosis pathogenesis have emerged.

Lee et al. demonstrated an upregulation of EG-VEGF, distinct from the well-established angiogenic factor VEGF, in ectopic endometriotic tissues. Moreover, they highlighted low or absent expression levels of EG-VEGF receptors (PROKR1 and PROKR2) in these tissues, suggesting a potential role for EG-VEGF as an endocrine/paracrine angiogenic factor stimulating new blood vessel formation in adjacent tissues. These findings hold promise for uncovering the underlying causes of endometriosis and may inform strategies for its prevention and treatment.

Additionally, recent studies have revealed detectable but low levels of EG-VEGF expression in normal human prostate tissue, contrasting with markedly increased expression in prostate carcinoma. Limited data are available regarding the expression of prokineticin-1 and prokineticin-2 in human cancers, although a study showcased EG-VEGF expression in colorectal cancer tumor cells expressing the β-isoform of the estrogenic receptor, suggesting hormonal influence. Elevated EG-VEGF expression has been linked to liver metastasis in colorectal cancer, indicating potential avenues for cancer-specific therapies. Prokineticins, expressed at higher levels in cancers compared to normal tissues, may hold promise as cancer-specific and tissue-specific targets, opening new vistas for therapeutic interventions.

Furthermore, Pasquali et al. reported a progressive increase in EG-VEGF protein expression during the evolution of prostate cancers, from low to high grade. These observations underscore the potential of prokineticins as prognostic biomarkers for prostate carcinoma progression.

The Mechanism of Action of EG-VEGF Molecule

EG-VEGF, also referred to as prokineticin-1, and prokineticin-2 exert their effects through two receptors, namely, PROKR1 and PROKR2. Upon receptor activation, intracellular calcium levels are stimulated, leading to phosphoinositide turnover and induction of mitogen-activated protein kinase (MAPK) signaling pathways. These pathways shed light on the actions of prokineticins, particularly on smooth muscle contraction and angiogenesis.

EG-VEGF initiates phosphorylation of mitogen-activated protein kinases, ERK1 and 2, as well as the Akt serine/threonine kinase of the phosphatidylinositol 3 kinase cell survival pathway. Evidence suggests EG-VEGF activation of both pathways in various types of endothelial cells in the human placenta, supporting the hypothesis that EG-VEGF acts as a growth factor stimulating proliferation and survival of endothelial cells in this context. Moreover, interaction between EG-VEGF and its receptors induces inositol phosphate mobilization and sequential phosphorylation of c-Src, ERK1, and epidermal growth factor receptor.

Additional pathways of EG-VEGF action may involve its proinflammatory function, manifested by time-dependent increases in the expression of IL-8 and COX-2. Numerous studies have elucidated EG-VEGF's involvement in tumor angiogenesis through specific organ-dependent pathways. For instance, in pancreatic cancer, EG-VEGF secreted by pancreatic islet

cells and stellate cells induces and sustains new blood vessel development, interacting with TGF-β1 and PDGF-A. In adrenal tumors, EG-VEGF interacts with steroidogenic factor 1 (SF-1), inducing nuclear expression of EG-VEGF, which correlates strongly with prognosis for patients with adrenal tumors.

Colon cancer has been extensively studied in relation to EG-VEGF expression, with reports indicating EG-VEGF strengthens cell invasion ability in colon cancer cell lines by acting on MMP-2, MMP-7, and MMP-9 via prokineticin receptor 2. EG-VEGF and its corresponding receptor PROKR2 have been identified as prognostic markers in colorectal carcinoma, suggesting a common pathway with VEGF. Notably, targeting both VEGF and EG-VEGF simultaneously enhances antitumor effects.

Limited data are available regarding EG-VEGF's mechanism of action in normal tissues, primarily focused on human reproduction. EG-VEGF's action peaks in midgestation ovaries, leading to increased ERK phosphorylation and elevated COX-2 expression, potentially influencing endometrial receptivity during implantation. High-quality oocytes obtained during in vitro fertilization stimulation exhibit increased vascularity due to high EG-VEGF levels in follicular fluid and serum, favoring enhanced clinical pregnancy rates and embryo maturation.

EG-VEGF Expression in Normal Tissues

EG-VEGF, detected in the ovary, placenta, testis, and adrenal glands, derives its name from its action on capillary endothelial cells within the endocrine glands. Extensive research has demonstrated EG-VEGF's ability to stimulate proliferation, chemotaxis, and survival of endothelial cells within steroidogenic tissues. As part of the prokineticin family alongside Bv8 or prokineticin-2, EG-VEGF exerts its effects via two receptors, PROKR1 and PROKR2.

During the first trimester of pregnancy, EG-VEGF and its receptors exhibit increased expression levels in the human placenta, particularly in the syncytiotrophoblast layer. Moreover, EG-VEGF is implicated in regulating trophoblast invasion. Expression levels of EG-VEGF and its receptors are elevated not only in normal physiological states but also in pathological conditions such as hypoxia, with plasma levels of EG-VEGF notably higher in pregnant women diagnosed with pre-eclampsia.

The placenta, renowned for possessing the highest vessel density in the human body, serves as a prominent focus in numerous studies exploring the proangiogenic actions of VEGF. Interestingly, VEGF elicits different effects on the two identified types of endothelial cells at this site: human placental microvascular endothelial cells (HPECs), found within the fetal capillaries of chorionic villi, and human umbilical vein macrovascular endothelial cells (HUVECs).

EG-VEGF-Induced Angiogenesis

Considerable attention has been devoted to investigating the proangiogenic effects of EG-VEGF.

Studies by LeCouter et al. did not reveal any proliferative impact of EG-VEGF on endothelial cells derived from the human umbilical vein. This finding was corroborated by Brouillet et al., confirming earlier observations by Ferrara et al.

It appears that EG-VEGF does not influence the proliferation and migration of endothelial cells from the human umbilical vein. However, EG-VEGF plays a crucial role in placental angiogenesis, notably through its selective action on human placental microvascular endothelial cells, which are part of the fetal capillaries within chorionic villi. In contrast, human

umbilical vein endothelial cells, often employed as a model in scientific research, represent macrovascular endothelial cells in contact with oxygenated blood. These two types of endothelial cells exhibit distinctions in gene expression, phenotype, and physiological characteristics.

The differential effects of EG-VEGF on microvascular and macrovascular endothelial cells within the placenta may be attributed to variations in intracellular Gαi1 and Gαi2 protein expression levels. Microvascular endothelial cells demonstrate higher expression of Gαi2 and lower expression of Gαi1 compared to macrovascular endothelial cells. This discrepancy aligns with previous findings reported by Masri et al. Additionally, Brouillet et al. demonstrated that Giα2-positive cells exhibit greater inhibition of adenylate cyclase compared to cells expressing predominantly Giα1.

Numerous studies have highlighted increased expression of EG-VEGF receptors, PROKR1 and PROKR2, in the placenta during the first trimester of pregnancy, consistent with findings by Brouillet et al. Notably, PROKR receptors are predominantly expressed in microvascular endothelial cells, which may elucidate EG-VEGF's effects on these cells. Investigations utilizing siRNA and blocking antibodies revealed that PROKR1 mediates EG-VEGF's angiogenic effects, while PROKR2 modulates cell permeability. Similar receptor actions have been observed in cardiomyocytes.

Despite their structural homology, PROKR1 and PROKR2 exhibit differential actions, potentially attributable to distinct signaling pathways and G protein recruitment, as demonstrated by Slessareva et al. Furthermore, EG-VEGF has been implicated in stimulating maternofetal exchanges by affecting the permeability of microvascular endothelial cells, which lack fenestrations and are interconnected by adherent and tight junctions.

Recent findings indicate that hepatic sinusoidal cells express PROKR2 exclusively, leading to ZO-1 internalization, a protein involved in junctional complexes and intercellular adhesions. This suggests EG-VEGF may regulate vascular permeability by modulating tight junction proteins.

Overall, the collective evidence supports the notion that EG-VEGF orchestrates angiogenesis during the first trimester of pregnancy and at term.

EG-VEGF's Role in Placental Vasculature Development

During term, human placentas undergo increased angiogenesis to accommodate heightened blood distribution. Scientific evidence indicates that EG-VEGF, akin to well-known growth factors such as VEGF and FGF-2, promotes vascular organization, permeability, and sprouting of placental microvascular endothelial cells.

Studies by Vural et al. demonstrate that EG-VEGF-induced angiogenesis in the ovary mirrors that induced by VEGF. Recent findings suggest that EG-VEGF, expressed and secreted by syncytiotrophoblasts, influences extravillous trophoblast cells, underscoring its significance in human placental angiogenesis.

Inhibition of EG-VEGF growth factor could enhance comprehension of its roles in both normal and tumor angiogenesis. Our research team endeavored to investigate the effects of anti-EG-VEGF antibody on the chick embryo chorioallantoic membrane, selected due to its similarity to the human placenta. Treatment with anti-EG-VEGF antibodies induced varied changes in the main vessels and capillaries of the chick embryo chorioallantoic membrane.

Notably, main vessels exhibited vasodilatation without structural compromise, while capillaries showed endothelial cell breakage and intimal discontinuities, leading to increased bleeding into the chorion. Additionally, anti-EG-VEGF antibody treatment reduced the proliferative potential of endothelial cells. These observations suggest a potential avian form of EG-VEGF, playing a significant role in chorioallantoic membrane and embryonic vasculature development.

Expression of EG-VEGF in Tumor Cells and its Impact on Patient Prognosis

Recent investigations have unveiled heightened expression of prokineticin-1 not only in various cancer types including colorectal, pancreatic, and prostatic carcinoma, but also in testicular carcinomas and neuroblastomas.

Of particular interest is the finding that mRNA EG-VEGF is absent in normal colorectal mucosa, yet present in all colorectal cancer cell lines. Additionally, the proliferation rate and intracellular signal transduction in colorectal cancer appear to be influenced by estradiol and the selective estrogen receptor modulator tamoxifen. Arai et al. have identified the expression of estrogen receptor β in colorectal cancer cell lines, thereby establishing a connection between colorectal cancer and hormonal influences.

Japanese researchers examined the correlation between EG-VEGF and colorectal cancer, detecting the expression of this growth factor in 5 out of 6 tumor cell lines.

EG-VEGF assumes a pivotal role in tumor angiogenesis, precipitating an exponential increase in tumor growth. As observed by Folkman, the formation of vascular networks during angiogenesis leads to an amplification of tumor volume. Kim et al. reported an active angiogenesis in tumors accompanied by a high proliferation rate.

EG-VEGF-stimulated angiogenesis fosters cell proliferation, with a high microvessel density correlating with an elevated proliferation index.

In an experimental endeavor by Japanese researchers, colon cancer cell lines were transfected with the gene expressing EG-VEGF. Upon in vivo examination, a noticeable escalation in cell proliferation rate was observed in subcutaneous implants and in the cecum implanted with cells from colorectal cell lines transfected with EG-VEGF. Dorsal air sac analysis and immunohistochemistry were employed to analyze the relationship between EG-VEGF and angiogenesis. Notably, colon cancer cell lines transfected with the EG-VEGF gene exhibited an increase in microvascular count.

EG-VEGF Evidently Promotes Tumor Cell Proliferation and Metastasis

The induction of metastases following the implantation of colon cancer cell lines transfected with EG-VEGF was systematically examined in the spleens of laboratory mice, resulting in observed metastases in the liver. Treatment of colorectal cancer cell lines overexpressing EG-VEGF with subcutaneously injected antisense EG-VEGF oligonucleotides into mice led to subsequent inhibition of angiogenesis and tumor growth.

Several other scholarly articles have investigated the role of EG-VEGF-induced pathological angiogenesis and tumor progression. Ovarian cancers are a significant cause of mortality in gynecological diseases, with numerous studies demonstrating VEGF overexpression in ovarian cancer. VEGF not only plays a pivotal role in angiogenesis but also in the neoplastic transformation of the ovarian surface epithelium.

In this context, efforts to impede VEGF action in advanced ovarian carcinomas appear warranted. However, some clinical trials utilizing anti-VEGF monoclonal antibodies did not yield anticipated benefits. The complexity of the angiogenic process may partially explain these unexpected outcomes, as angiogenesis is regulated by a multitude of growth factors such as PDGF and FGF, which promote endothelial cell proliferation and tumor growth.

The role of EG-VEGF in angiogenesis associated with ovarian carcinomas has received comparatively less attention. Researchers have endeavored to establish a correlation between EG-VEGF expression and prognosis in epithelial ovarian carcinomas.

The Contribution of EG-VEGF to Tumor Angiogenesis: Correlation with Microvascular Density

EG-VEGF, an organ-specific proangiogenic factor, is prominently expressed in tissues such as the ovary, testis, adrenal cortex, and notably, the placenta. While low levels of mRNA EG-VEGF have been identified in various organs including the colon, small intestine, liver, spleen, brain, thymus, and recently, in the anterior pituitary gland.

EG-VEGF expression has been examined across different cancer types such as colorectal cancer, ovarian carcinoma, and pancreatic adenocarcinoma. Its presence has also been noted in conditions like polycystic ovary syndrome. EG-VEGF's pivotal role in both normal and pathological angiogenesis has been increasingly recognized. Moreover, recent evidence supports its involvement in regulating tumor cell growth and survival.

Current data reinforces EG-VEGF's involvement in both physiological and pathological angiogenesis in the human ovary. Interestingly, while Zhang et al. did not detect EG-VEGF expression in ovarian cancer cell lines or cultured human ovarian surface epithelium, Bălu et al. observed positive EG-VEGF reactions in the epithelial cells of the ovarian surface. This suggests a potential for EG-VEGF overexpression in ovarian cancers originating from the surface epithelium.

Additionally, Fraser et al. highlight cyclic variations in EG-VEGF expression within the human luteal body. An increased percentage of ovarian tumors exhibit EG-VEGF positivity, displaying distinct expression patterns across various histopathologic types of ovarian carcinomas. The distribution of EG-VEGF-positive tumor cells within the tumor mass and stroma implies its potential implication in tumor progression and metastasis, a notion supported by similar findings in colorectal carcinoma.

Beyond EG-VEGF, other members of the heparin-binding growth factor family, such as heparin-binding EGF-like growth factor, are also expressed in ovarian cancer. These factors interact with EGFR, activating downstream signaling pathways associated with malignant phenotypes including metastasis and treatment resistance. Notably, advanced ovarian carcinomas exhibit EG-VEGF-positive cells, particularly at the tumor periphery, suggesting its potential involvement in determining the tumor's invasive behavior.

In contrast to these findings, Zhang et al. noted the presence of EG-VEGF expression in the early stages of ovarian carcinoma, whereas its expression decreased in advanced stages.

Another investigation conducted by Ngan et al. identified EG-VEGF expression in normal peri-implantation endometrial tissue specimens from women of reproductive age. However, mRNA EG-VEGF levels were infrequently detected in endometrial tissue specimens from postmenopausal women and those with endometrial carcinoma .

Italian researchers delved into the role of EG-VEGF in angiogenesis associated with prostate carcinoma. Angiogenesis is recognized as pivotal in the growth, invasion, and metastasis of prostate cancer, with prostate cells capable of synthesizing and secreting growth factors that orchestrate this process, including VEGF. Increased VEGF expression and microvascular density have been observed in most prostate cancers, indicating the significance of angiogenesis in their progression. Higher VEGF levels have been correlated with poorer prognoses.

A decline in EG-VEGF mRNA expression was noted in normal prostate epithelial cells, contrasting with its heightened expression in human prostatic carcinoma, correlating directly with the advancement of the Gleason score. EG-VEGF serves as a significant prognostic marker for prostate carcinoma progression.

Previous data indicate minimal EG-VEGF mRNA levels detected by Northern blot in the normal human prostate. Evaluation of EG-VEGF expression was conducted across normal prostate tissue, benign prostate hyperplasia, and prostate cancer cell lines. Normal human prostate tissue exhibits low levels of EG-VEGF, while its expression appears elevated in prostate carcinoma. Western blotting and immunohistochemistry revealed increased EG-VEGF mRNA and protein levels in prostate epithelial cells as the Gleason score advanced. PROKR1 and PROKR2 transcripts were identified in epithelial cell cultures of both normal and malignant prostate, suggesting EG-VEGF interaction with these receptors on prostatic epithelial cells. Pathological angiogenesis in prostate carcinoma has been associated with apoptosis regulation pathways, androgen receptor effects, as well as various cytokines and cell adhesion molecules, underscoring EG-VEGF's potential importance in this process. Prostate carcinoma specimens were studied using three established cell lines—PC3, DU-145, and LNCaP—as cell culture models.

Comparative studies on human prostate carcinomas, particularly those based on donor-matched pairs of normal and malignant primary cultures, are deemed highly informative. Sinisi et al. investigated EG-VEGF expression and its two receptors in primary cultures of normal (NPEC) and malignant (CPEC) epithelial prostate cells, along with EPN, a nontransformed human prostate epithelial cell line. Elevated EG-VEGF expression levels were observed in prostatic carcinoma specimens compared to normal prostatic tissue, suggesting EG-VEGF as a cancer-specific molecule. Conversely, decreased EG-VEGF mRNA expression was noted in normal epithelial prostate cells (NPEC) and the EPN cell line, with EG-VEGF protein appearing absent in normal prostate tissue. These findings could imply EG-VEGF inactivation in normal tissues or inhibition of its signaling pathway.

The progressive activation of EG-VEGF protein expression accompanies the acquisition of a malignant phenotype, likely due to its pivotal role in tumor angiogenesis. EG-VEGF prompts endothelial cell proliferation, migration, fenestration, and subsequently, tumor growth.

In the context of prostatic carcinoma, EG-VEGF emerges as a significant prognostic marker for disease progression. As prostate carcinomas transition from low-medium to high grade, a corresponding gradual increase in EG-VEGF protein levels is observed.

Literature underscores the crucial role of angiogenetic factors in testicular biology. Microvessel remodeling is documented not only during normal testicular development but also in pathological conditions like testicular cancer.

Given the rising incidence of testicular cancers, understanding the role of EG-VEGF in testicular cancer angiogenesis holds promise. Testicular carcinoma has surged to become the most prevalent malignancy in 25- to 35-year-old men.

VEGF expression is noted across various histopathological types of testicular cancer, particularly teratomas.

During fetal development, EG-VEGF triggers angiogenesis, vital for transporting testosterone synthesized in Leydig cells to target tissues. Additionally, angiogenesis facilitates the access of gonadotropins and regulatory proteins from the periphery to the testis.

A Danish research team endeavored to assess EG-VEGF mRNA and protein expression in normal fetal and adult testes, along with different cell types of testicular cancers. Using monoclonal antibodies against EG-VEGF protein, they found its expression in normal adult testes to be confined to Leydig cells. Immunohistochemical observations corroborated those from in situ hybridization methods. The restriction of EG-VEGF expression to Leydig cells contrasts with VEGF, detected in both Leydig and Sertoli cells. EG-VEGF expression was noted in Leydig cells of the human fetal testis starting from week 14 of gestation. Intriguingly, fetal production of STAR (steroidogenic acute regulatory protein), which regulates testosterone production by Leydig cells, also commences around week 14 of gestation. The synchronous onset of EG-VEGF expression at week 14 of gestation implies its potential significance in ensuring the normal endocrine function of the testis.

Research findings indicate the presence of a binding site for steroidogenic acute regulatory protein in the promoter region of the EG-VEGF gene, suggesting hormonal regulation of EG-VEGF expression. Multiple candidates are proposed for the hormonal regulation of EG-VEGF expression and secretion. Among these candidates, luteinizing hormone (LH) stands out as a prime candidate due to its modulation of genes involved in testosterone synthesis. However, recent evidence suggests that adrenocorticotropic hormone (ACTH) also stimulates testosterone synthesis in fetal testes.

EG-VEGF expression in the testis is confined to Leydig cells. Unlike VEGF, EG-VEGF assumes a pivotal role in the angiogenic process within Leydig cell tumors, thereby facilitating their growth.

Expression of EG-VEGF, both at the mRNA and protein levels, is exclusive to Leydig cell tumors. It is not detected in germinal cell tumors or Sertoli cell tumors. Furthermore, increased EG-VEGF expression is observed in samples associated with carcinoma in situ (CIS) compared to normal tissue. In situ hybridization and immunohistochemistry confirm EG-VEGF expression in Leydig cells surrounding tubules containing CIS.

Certain literature indicates a predominant expression of steroidogenic acute regulatory protein (STAR) in Leydig cell tumors. These tumors are known to produce and release testosterone under the regulation of luteinizing hormone (LH).

Data on the correlation between vascular endothelial growth factor (VEGF) and microvessel density (MVD) in testicular cancers are limited. VEGF expression is observed in both seminomas and nonseminoma germinal cell tumors. However, Leydig cell tumors exhibit a significantly higher microvessel count compared to seminomas, despite not showing remarkable VEGF expression.

There is minimal focus in the literature on the expression of EG-VEGF in the normal human pituitary gland, with only one scientific paper reporting its presence in pituitary adenomas.

Research conducted by Raica et al. demonstrated distinct expression patterns of EG-VEGF in the normal pituitary gland. Acidophilic cells in the anterior pituitary gland and chromophobe cells exhibited strong positive staining for EG-VEGF, while basophilic cells did not show EG-VEGF expression. The authors aimed to expand their investigation into the role of EG-VEGF in the development and progression of human pituitary adenomas.

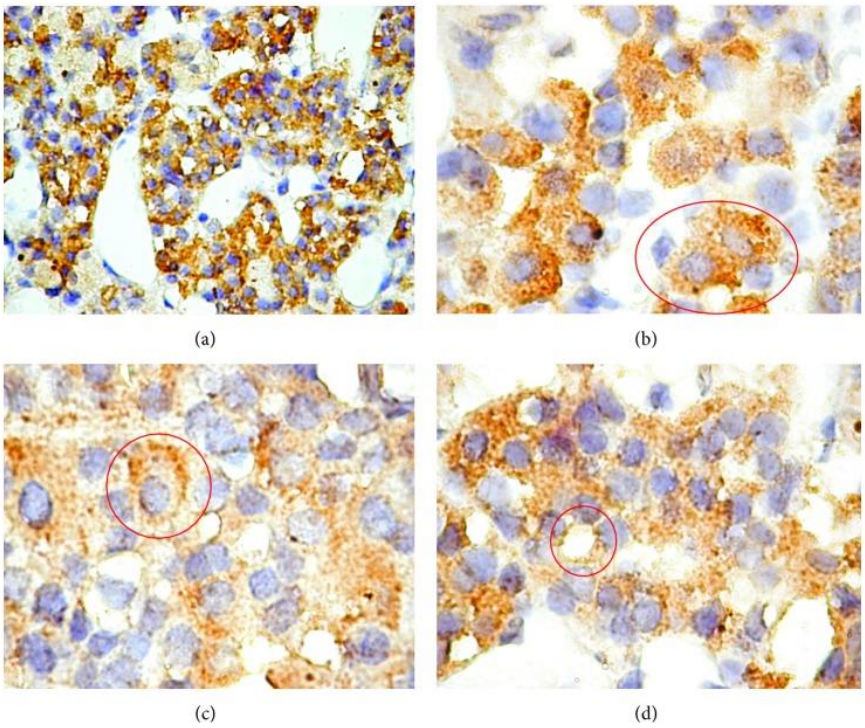

Figure 1

EG-VEGF expression in normal pituitary gland with a heterogeneous granular pattern (a). Acidophilic cells have a homogeneous (b) or heterogeneous (c) granular cytoplasmic expression of EG-VEGF, while chromophobe cells had a weak granular pattern of EG-VEGF expression (d).

Previous studies have indicated a decrease in EG-VEGF expression in human pituitary adenomas compared to normal pituitary tissue specimens. However, the findings of the aforementioned study contradict these previous results, revealing EG-VEGF overexpression in over 50% of pituitary adenoma cases analyzed. This overexpression was predominantly observed in adenomas exhibiting an acidophilic solid pattern or acidophilic/basophilic papillary pattern.

Compact pituitary adenomas with acidophilic cells displayed heightened EG-VEGF expression, which correlated with an upregulation of EGFR. Activation of EGFR by EG-VEGF inhibited apoptosis of adenoma cells, suggesting an antiapoptotic role for EG-VEGF in promoting tumor cell survival. This interaction between EG-VEGF and EGFR has been observed in placental changes associated with pregnancy loss, where EG-VEGF induces EGFR phosphorylation and activation.

The study titled "The expression and prognostic significance of EG-VEGF in pituitary adenomas" established, for the first time, a correlation between EG-VEGF and EGFR expression in pituitary adenomas, particularly in compact acidophilic pituitary adenomas. Notably, EG-VEGF expression in compact acidophilic pituitary adenomas appeared unaffected by hormonal profiles.

Papillary-type pituitary adenomas exhibited increased EG-VEGF expression, which correlated with elevated prolactin expression. Both EG-VEGF and prolactin were associated with enhanced proliferation rates of adenoma cells.

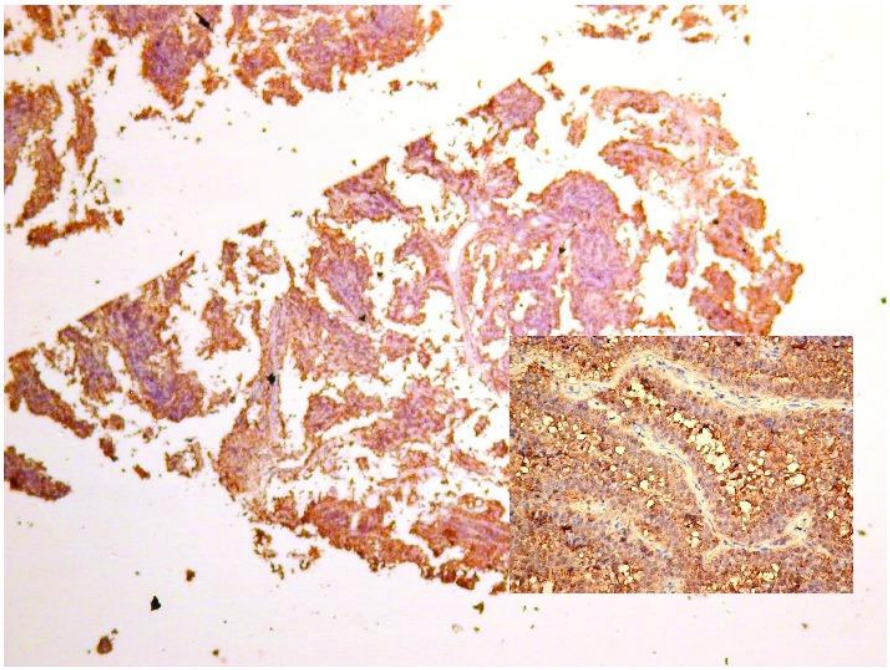

Figure 2

Pituitary adenoma with papillary morphology, with high expression of EG-VEGF.

The absence of a correlation between EG-VEGF and EGFR in papillary-type pituitary adenomas may stem from the distinct molecular profiles exhibited by different types of pituitary adenomas. In papillary-type adenomas, EG-VEGF expression was found to align with prolactin expression.

Literature reports have detailed the relationship between EG-VEGF and plasma prolactin levels, particularly in the context of hypogonadism diagnosis. Genetic mutations affecting prokineticins have been linked to reductions in plasma prolactin levels. Both EG-VEGF and prolactin are recognized as growth factors capable of promoting cell proliferation.

Pituitary adenomas characterized by acidophilic cells, most of which stained positive for prolactin, exhibited elevated EG-VEGF expression. Concurrently, they displayed increased proliferation rates, which significantly correlated with EG-VEGF expression. This suggests a synergistic effect between EG-VEGF and prolactin in driving tumor cell proliferation and tumor growth.

In contrast to the EG-VEGF-negative basophilic cells of the normal pituitary gland, basophilic cells in pituitary adenomas showed relatively heightened EG-VEGF expression,

particularly in those exhibiting a papillary growth pattern. Existing literature supports the involvement of EG-VEGF in determining papillary growth patterns in tumors originating from endocrine glands, such as the thyroid. Thyroid carcinomas with BRAF mutations, known for their aggressive behavior and propensity for lymph node metastases, often exhibit this papillary growth pattern.

Furthermore, in pituitary adenomas with basophilic cells, EG-VEGF overexpression was correlated with the expression of PDGF-A and, notably, PDGF-B.

Pituitary adenomas containing basophilic cells expressing EG-VEGF also exhibited positive staining for PDGF-A and PDGF-B. The association among these three growth factors was not only statistically significant but notably stronger for PDGF-B. PDGF-A and PDGF-B have been previously investigated and confirmed to participate in the pathogenesis of papillary-type thyroid carcinomas, which are typically linked with an unfavorable prognosis.

Consistent with prior research, a statistically significant relationship was identified between EG-VEGF overexpression and LH expression. Literature contains a scientific article detailing this correlation in pituitary adenomas, while other studies aimed at identifying biomarkers for fertility and implantation have also explored their association.

The significant correlation between EG-VEGF expression and the presence of GFAP and S100 protein implies a potential impact of this growth factor on follicular-stellate cells within both normal human pituitary glands and pituitary adenomas.

This statistically significant correlation suggests EG-VEGF may play a role in modulating the activity of follicular-stellate cells within pituitary adenomas, as well as in normal pituitary glands. However, currently available data do not confirm the synthesis of EG-VEGF in follicular-stellate cells from pituitary adenomas, nor do they provide information regarding the effects of EG-VEGF on these cells in either normal or adenomatous pituitary tissue.

Future Directions

For a considerable time, VEGF has been acknowledged as a pivotal factor in both physiological and pathological angiogenesis. Consequently, the consideration of inhibiting the VEGF pathway appears justified. However, therapies based on anti-VEGF antibodies are associated with adverse effects, including the induction of endothelial cell apoptosis and disruption of vascular function. The discovery of the EG-VEGF gene by LeCouter et al. has the potential to initiate a new phase in the search for tissue-specific antiangiogenic agents, potentially mitigating these systemic side effects.

The identification of EG-VEGF opens avenues for the development of angiogenesis inhibitors tailored to specific types of cancer. Recent findings strongly suggest the critical involvement of EG-VEGF in human reproductive pathology, particularly in processes related to implantation and infertility (see Table 1). Despite numerous studies exploring EG-VEGF-related pathways in carcinogenesis and angiogenesis, many malignant diseases require further investigation to elucidate the role of EG-VEGF in their pathogenesis (Table 1).

Table 1

Most recent papers reporting EG-VEGF involvement in human reproduction pathology and malignancies where EG-VEGF's role is less elucidated.

Authors	Year	Pathology	Brief overview of EG-VEGF role	Biomarker

Wang et al.	2016	Implantation, trophoblast invasion, and ciliogenesis	Interrelation between EG-VEGF, ERK1/2 activation, and intraflagellar transporter required for ciliogenesis IFT88	No
Morales et al.	2016	Breast cancer	EG-VEGF heterogeneity expression with no impact on diagnosis and prognosis in breast cancer	No
Jayasena et al.	2016	Miscarriage	Serum level of EG-VEGF failed to be associated with miscarriage	Not validated as serum biomarker
Li et al.	2010	Multiple myeloma	Multiple signaling pathway activation, Mcl1 upregulation, proliferation, and survival of multiple myeloma cells	No
Nakazawa et al.	2015	Sporadic colorectal cancer	Significantly higher in cases with serosal invasion, lymphatic invasion, venous invasion, lymph node metastasis, liver metastasis, hematogenous metastasis, and higher stage disease	Potential biomarker for worse prognosis, invasion, and metastasis
Li et al.	2006	Human hepatocellular carcinoma	Portal vein tumor thrombus formation promoted by angiogenesis via EG-VEGF	No

References:

1. Li M., Bullock C. M., Knauer D. J., Ehlert F. J., Zhou Q. Y. Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Molecular Pharmacology.* 2001;**59**(4):692–698. [PubMed] [Google Scholar]

2. Battersby S., Critchley H., Morgan K., Millar R., Jabbour H. Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle. *The Journal of Clinical Endocrinology and Metabolism.* 2004;**89**(5):2463–2469. doi: 10.1210/jc.2003-032012. [PubMed] [CrossRef] [Google Scholar]

3. Fraser H. M., Bell J., Wilson H., et al. Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. *The Journal of Clinical Endocrinology and Metabolism.* 2005;**90**(1):427–434. doi: 10.1210/jc.2004-0843. [PubMed] [CrossRef] [Google Scholar]

4. Hoffmann P., Feige J. J., Alfaidy N. Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy. *Endocrinology.* 2006;**147**(4):1675–1684. doi: 10.1210/en.2005-0912. [PubMed] [CrossRef] [Google Scholar]

5. Hoffmann P., Feige J. J., Alfaidy N. Placental expression of EG-VEGF and its receptors PKR1(prokineticin receptor-1) and PKR2 throughout mouse gestation. *Placenta.* 2007;**28**(10):1049–1058. doi: 10.1016/j.placenta.2007.03.008. [PubMed] [CrossRef] [Google Scholar]
6. Kisliouk T., Levy N., Hurwitz A., Meidan R. Presence and regulation of endocrine gland vascular endothelial growth factor/prokineticin-1 and its receptors in ovarian cells. *The Journal of Clinical Endocrinology and Metabolism.* 2003;**88**(8):3700–3707. doi: 10.1210/jc.2003-030492. [PubMed] [CrossRef] [Google Scholar]
7. Kisliouk T., Podlovni H., Meidan R. Unique expression and regulatory mechanisms of EG-VEGF/prokineticin-1 and its receptors in the corpus luteum. *Annals of Anatomy.* 2005;**187**(5–6):529–537. doi: 10.1016/j.aanat.2005.07.005. [PubMed] [CrossRef] [Google Scholar]
8. LeCouter J., Kowalski J., Foster J., et al. Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature.* 2001;**412**(6850):877–884. doi: 10.1038/35091000. [PubMed] [CrossRef] [Google Scholar]
9. Samson M., Peale F. V., Frantz G., Rioux-Leclercq N., De Meyts E. R., Ferrara N. Human endocrine gland-derived vascular endothelial growth factor: expression early in development and in Leydig cell tumor suggests roles in normal and pathological testis angiogenesis. *The Journal of Clinical Endocrinology and Metabolism.* 2004;**89**(8):4078–4088. doi: 10.1210/jc.2003-032024. [PubMed] [CrossRef] [Google Scholar]
10. Mollay C., Wechselberger C., Mignogna G., et al. Bv8, a small protein from frog skin and its homologue from snake venom induce hyperalgesia in rats. *European Journal of Pharmacology.* 1999;**374**(2):189–196. doi: 10.1016/S0014-2999(99)00229-0. [PubMed] [CrossRef] [Google Scholar]
11. Wechselberger C., Puglisi R., Engel E., Lepperdinger G., Boitani C., Kreil G. Bv8, a small protein from frog skin and its homologue from snake venom induce hyperalgesia in rats. *FEBS Letters.* 1999;**462**(1–2):177–181. doi: 10.1016/S0014-5793(99)01473-8. [PubMed] [CrossRef] [Google Scholar]
12. Alfaidy N., Hoffmann P., Boufettal H., et al. The multiple roles of EG-VEGF/PROK1 in normal and pathological placental angiogenesis. *BioMed Research International.* 2014;**2014**:p. 10. doi: 10.1155/2014/451906.451906 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
13. Ferrara N., Frantz G., LeCouter J., et al. Differential expression of the angiogenic factor genes VEGF and EG-VEGF in normal and polycistic human ovaries. *The American Journal of Pathology.* 2003;**162**(6):1881–1893. doi: 10.1016/S0002-9440(10)64322-2. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
14. Su M. T., Huang J. Y., Tsai H. L., Chen Y. C., Kuo P. L. A common variant of PROK1 (V67I) acts as a genetic modifier in early human pregnancy through down-regulation of gene expression. *International Journal of Molecular Sciences.* 2016;**17**(2):162. doi: 10.3390/ijms17020162. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
15. Su M. T., Lin S. H., Chen Y. C., Kuo P. L. Gene-gene interactions and gene polymorphisms of VEGFA and EG-VEGF gene systems in recurrent pregnancy loss. *Journal of Assisted Reproduction and Genetics.* 2014;**31**(6):699–705. doi: 10.1007/s10815-014-0223-2. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

16. LeCouter J., Lin R., Ferrara N. Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. *Nature Medicine.* 2002;**8**(9):913–917. doi: 10.1038/nm0902-913. [PubMed] [CrossRef] [Google Scholar]

17. Aravind L., Koonin E. V. A colipase fold in the carboxy-terminal domain of the Wnt antagonists–the Dickkopfs. *Current Biology.* 1998;**8**(14):477–478. doi: 10.1016/S0960-9822(98)70309-4. [PubMed] [CrossRef] [Google Scholar]

18. Glinka A., Wu W., Delius H., Monaghan A. P., Blumenstock C., Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature.* 1998;**391**(6665):357–362. doi: 10.1038/34848. [PubMed] [CrossRef] [Google Scholar]

19. Esser S., Wolburg K., Wolburg H., Breier R. G., Kurzchaliant T., Risau W. Vascular endothelial growth factor induces endothelial fenestrations in vitro. *The Journal of Cell Biology.* 1998;**140**(4):947–859. doi: 10.1083/jcb.140.4.947. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

20. LeCouter J., Kowalski J., Foster J., et al. Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature.* 2001;**412**(6850):877–884. doi: 10.1038/35091000. [PubMed] [CrossRef] [Google Scholar]