Review Articles

The Function of Vascular Endothelial Growth Factor in Cancer Metastasis

Kanjana Assawasuparerk*

Department of Pre-Clinical and Appiled Animal Science, Faculty of Veterinary Science, Mahidol University, Phutthamonthon, Nakonpathom, Thailand

***Corresponding author, E-mail address: kanjana.ass@mahidol.edu**

Abstract

Cancer metastasis is one of the leading causes of death in humans and animals diagnosed with cancer. It is a complex process that involves the spread of cancer cells from the primary cancer to another parts of the body through lymphatic and blood vessels. Cancer angiogenesis and lymphangiogenesis have been considered as essential processes in the cancer metastasis because the new formation blood and lymphatic vessels occur, thereby creating new route for cancer cells to metastasize. Vascular endothelial growth factors (VEGF) is a protein previously known for the role in promoting vasculogenesis and angiogenesis. Overexpression of VEGF is known to be implicated in a wide variety of disease processes, especially cancer metastatic process. At present, there are 7 members of the VEGF family, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF. Among all VEGF members, VEGF-A, VEGF-C and VEGF-D have been reported to be capable to promote cancer angiogenesis and lymphangiogenesis. Moreover, the molecular mechanism of VEGFs and their interaction with the VEGF receptors (VEGFRs) which involve in the processes of cancer metastasis has been extensively described in details. The previous research has shown that VEGF-A plays a major role in angiogenesis through the interaction with VEGFR1 and VEGFR2 receptors as well as with neuropilins presented on the endothelial cells. Following the stimulation of several signal transduction pathways, these interaction result in the endothelial cell proliferation and migration, by which subsequently leads to finally the neovascularization. Apart from the role of VEGF-A, in addition to the role of VEGF-A associated with angiogenesis, VEGF-C and VEGF-D have also been recently reported for their major roles in lymphagiogenesis through the interaction with VEGFR3 which is mostly present in lymphatic endothelial cells. Moreover, VEGF-C and VEGF-D are able to promote angiogenesis. Therefore, the inhibition of VEGF-A, VEGF-C, VEGF-D, and VEGFRs might be used in cancer treatment for the reduction in cancer metastasis.

Keywords: cancer metastasis, angiogenesis, lymhangiogenesis, vascular endothelial growth factor (VEGF)

การทำงานของ Vascular Endothelial Growth Factor ในการแพร่กระจายของมะเร็ง

กาญจนา อัศวศุภฤกษ์*

ภาควิชาปรีคลินิกและสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

***ผู้รับผิดชอบบทความ E-mail address: kanjana.ass@mahidol.edu**

บทคัดย่อ

การแพร่กระจายของมะเร็ง (cancer metastasis) เป็นหนึ่งในสาเหตุสำคัญของการเสียชีวิตในผู้ป่วยและสัตว์ที่เป็นมะเร็ง การแพร่กระจายเป็นกระบวนการที่ซับซ้อนที่เกี่ยวข้องกับการแพร่กระจายของเซลล์มะเร็งจากเนื้องอกปฐมภูมิโดยผ่านทางหลอดเลือดและหลอดน้ำเหลืองไปยังส่วนอื่นๆของร่างกาย การสร้างหลอดเลือด (angiogenesis) และหลอดน้ำเหลือง (lymphangiogenesis) ที่ไปเลี้ยงเซลล์มะเร็งอาจถือได้ว่าเป็นกระบวนการหลักในการแพร่กระจายของมะเร็ง เนื่องจากการสร้างหลอดเลือด และหลอดน้ำเหลืองขึ้นใหม่นี้สร้างทางผ่านสำหรับการแพร่กระจาย vascular endothelial growth factor (VEGF) คือโปรตีนที่ส่งเสริมการสร้างหลอดเลือดและหลอดน้ำเหลือง การแสดงออกของ VEGF ซึ่งจะส่งเสริมทำให้เกิดโรคต่างๆ รวมทั้งการแพร่กระจายของมะเร็ง ในปัจจุบันนี้มี VEGF ทั้งหมด 7 ชนิดดังนี้ VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F และ PIGF ขณะที่ VEGF-A, VEGF-C และ VEGF-D มีการรายงานว่าสามารถส่งเสริมให้เกิดการสร้างหลอดเลือดและหลอดน้ำเหลืองที่ไปเลี้ยงมะเร็ง อนึ่งกลไกในระดับโมเลกุลของ VEGF และการทำงานร่วมกับ VEGF receptors (VEGFRs) ในกระบวนการแพร่กระจายของมะเร็งจะมีการอธิบายในรายละเอียด VEGF-A ซึ่งมีบทบาทต่อการสร้างหลอดเลือด โดยทำงานร่วมกับ VEGFR1, VEGFR2 และ neuropilins ซึ่งเกิดขึ้นบนเซลล์เยื่อบุหลอดเลือดและหลอดน้ำเหลือง (endothelial cell) และกระตุ้นให้เกิดสัญญาณที่ส่งผลให้เกิดการเพิ่มจำนวน (proliferation) และการย้าย (migration) ของเซลล์เยื่อบุหลอดเลือด และสุดท้ายเกิดการสร้างหลอดเลือด VEGF-C และ VEGF-D ซึ่งมีบทบาทสำคัญต่อการสร้างหลอดน้ำเหลืองโดยการกระตุ้นผ่าน VEGFR-3 ซึ่งส่วนใหญ่เกิดขึ้นในเซลล์เยื่อบุหลอดน้ำเหลือง นอกจากนี้ VEGF-C และ VEGF-D สามารถทำให้เกิดการสร้างหลอดเลือดได้ ดังนั้นการยับยั้ง VEGF-A, VEGF-C และ VEGF-D และ VEGFRs สามารถนำมาใช้ในการรักษามะเร็งเพื่อลดการแพร่กระจายของมะเร็ง

คำสำคัญ : การแพร่กระจายของมะเร็ง, การสร้างหลอดเลือด, การสร้างหลอดน้ำเหลือง, แวสคิวละ เอ็นโดธีเลียม โกรธ แฟคเทอะ

Introduction

Cancer is the second cause of death worldwide (Li et al., 2000). It is a group of disease associated with abnormal cell division and uncontrolled cell growth. Cancer metastasis is regarded as the major cause of death in humans and animals with cancer. The spread of cancer cells from the primary tumor to distant organs via blood vessels and lymphatic and is a considerable characteristic of cancer metastasis (Li et al., 2000; Pepper, 2001). Cancer angiogenesis and lymphangiogenesis have been found to play a crucial roles in the growth, invasion and metastatic spread of cancer cells (Nakamura et al., 2003). Lymphangiogenesis enhance the metastasis by facilitating the spread of cancer cells through lymphatic vessels, resulting in the dissemination to regional lymph nodes and another distant organ. While, the angiogenesis may also trigger cancer growth at metastasis site (Hoeben et al., 2012).

Vascular endothelial growth factor (VEGF) members are the growth factors, which are important mediators contributing to enhanced angiogenesis and lymphangiogenesis in metastasis process (Xue et al., 2009). The VEGF family is composed of seven members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E (Orf virus VEGF), VEGF-F and placenta growth factor (PlGF) (McMahon, 2000; Olsson et al., 2006). There are three types of tyrosine kinases receptors for the VEGF family including VEGFR1, VEGFR2, VEGFR3, and two types of non-protein kinase receptors: neuropilin-1 (Nrp1) and neuropilin-2 (Nrp2) (Moreira et al., 2007). The binding of VEGF members to the cell-surface receptor tyrosine kinase in endothelial cells results in intratumoral angiogenesis and lymphangiogenesis, thereby leading to enhanced proliferation, migration and invasion of cancer cells (Dvorak, 2002). In addition, VEGF members are also able to induce vascular tortuosity and enhanced leakiness by which the process provides cancer cell invasion into the circulatory system and spread to distant parts of the body (Papetti and Herman, 2002). VEGF-A, VEGF-C and VEGF-D, the members of VEGF family of endothelial cell mitogens, are capable of promoting cancer angiogenesis and lymphangiogenesis (Davorak, 2002). In addition, VEGF members and VEGF signaling could be used as a therapeutic target for cancer metastatic restriction (Stacker et al., 2002; Hirakawa, 2005; Fisher et al., 2011).

The objectives of this review are as follows: 1) To provide the fundamental knowledge about VEGF members and their receptors in cancer metastasis. 2) To investigate the action of VEGF members and their receptors in angiogenesis and lymphangiogenesis in cancer metastatic process. 3) To discuss the possibility of VEGF members and other molecules involved in cancer metastasis as a therapeutic target in cancer treatment.

Cancer Metastatic Process

Cancer metastasis consists of multi-step processes in which malignant cells spread from the primary tumor to distant organs. The process can be determined by the terms invasion, distribution, implantation and development of cancer cells (Scanion and Murthy, 1991; Talmadge and Fidler, 2010). It is dependent on intrinsic and extrinsic properties of cancer cells. This process begins from the small primary tumor cells, which have characteristic non vascular. Individual tumor cells can attach with extracellular matrix (ECM), via integrins and N-cadherins. Integrins play a crucial role in the attachment of cancer cells to ECM. They are transmembrane receptors that bind to ECM molecules including

laminin, fibronectin, vitronectin and collagens, while N-cadherins are adhesion molecules that responsible for the interaction between cancer cells (homotypic adhesion) as well as for the attachment of cancer cells to ECM component (heterotypic adhesion)(Harlozinska, 2005).

Beside, N-cadherins can support cell motility and migration (Harlozinska, 2005). The primary tumor cells can undergo proliferation. When a tumor mass is to exceed 1-2 mm in diameter, the tumor must be established capillary network to supply oxygen and nutrient to the tumor cells. After that, cancerous cells could detach from the primary tumor which requires the loss of cell-cell adhesion by protease enzymes secreted from the tumor and/or host cells. These enzymes including matrix metalloproteinases (MMPs), serine proteinase, the system of plasminogen activators (uPA), cysteine proteinase and aspartate proteins. uPA and MMPs may disrupt cell adhesion molecules. Moreover, uPA and serine proteinase are able to degrade ECM components and the basement membrane around the tumor. uPA stimulates many growth factors and MMPs that additional contribute to degradation of ECM. When protease enzymes are secreted, it results in cancer cell invasion and intravasation (Woodhouse et al., 1997; Harlozinska, 2005). Next, cancerous cells invade into the circulatory system via the lymphatic system and blood circulation. Normally, cancer cells intravasate into a vein via lymphatic venous anastomoses directly into the vein wall. Cancer cells can spread to vasculature by both active and passive methods. Active intravasation refers to a process in which cancer cell actively moves toward and then into nearby blood vascular (Zetter, 1998). The first step is specific adhesion to capillary endothelial cells, followed by attachment to protein of the sub-endothelial basement membrane, for example laminin and types IV and V collagen. After that, the adhesion of the cancer cell to fibronectin, type I collagen and hyaluronan, which is required for the motion of cancerous cell into the sub-endothelial stroma and following growth at the secondary site of colonization (Harlozinska, 2005). The passive intravasation refers to a process in which cancer metastasizes through the passive shedding cancer cell into blood circulation (Zetter, 1998). Trauma of primary tumor will increase passive shedding cancer cell into blood circulation. Furthermore, cancer cells growing in a confined space have been exhibited to force against each other causing blood and lymphatic vascular to collapse, and then potentially forcing cancer cells into the vascular system. Even though cancer cells in the circulatory system are rapidly destroyed by interaction with lymphocyte, platelets, and other blood components, the cancer cells have still remained in the circulatory system and attached to the endothelial cells of blood and lymph vessels of distant (Fidler, 2003) organs. The cancer cells extravasate through the vessel wall and proliferate within the organ parenchyma leading to accomplish the metastasis process (Zetter, 1998)(Figure 1).

The role of angiogenesis and lymphangiogenesis in metastatic process

The metastatic process is concerned with the transport of malignant cells via the vascular system. This process may find blood vessels and lymphatic vessels participated in all steps including primary tumor growth, activation of local invasion, transportation of cancer cells and development of distant organs (Harlozinska, 2005). Within the confines of the primary tumor, nutrient is restricted by contention among actively proliferating cells and diffusion of metabolites which is hampered by

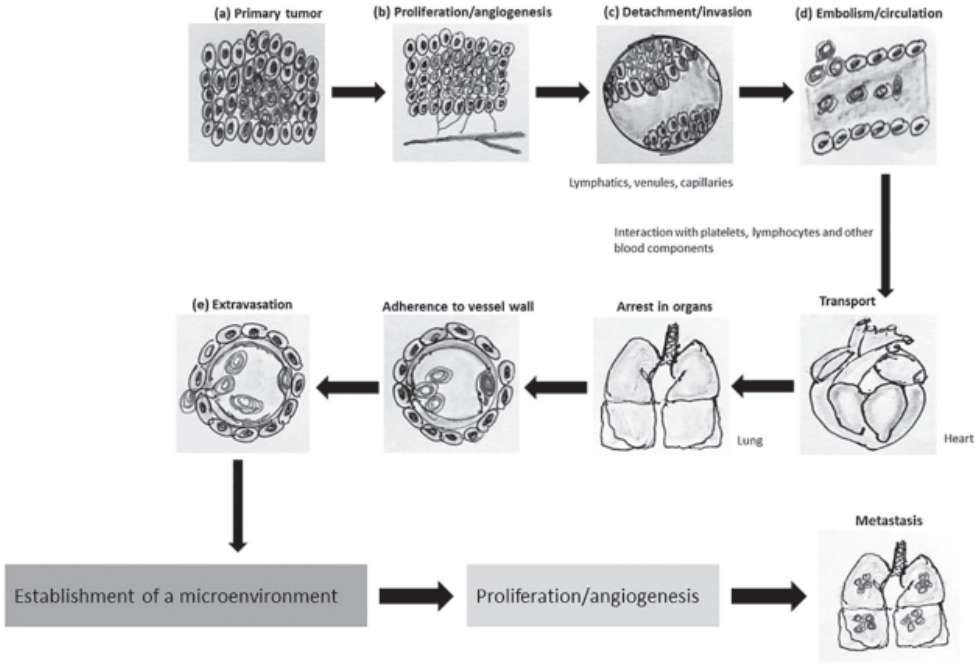

Figure 1. A diagram showing the mechanisms of metastasis. The tumor metastasis process is composed of cellular transformation and tumor growth (a), extensive vascularization (b), local invasion via the circulation (lymphatic, venules, or capillaries) (c), and detachment and embolization of tumor cells (d). After tumor cells have remained in circulation, they attach to the capillary endothelial basement membrane and extravasate through the vessel wall and proliferate within the distant organs result in the complete metastasis process (f).

elevated interstitial pressure. Thus, cancer cells induce the formation of new blood capillaries from preexisting vessels, and this provides cancer cells the capability to survive and propagate in an antagonistic environment. Cancer angiogenesis is the formation of new blood capillaries from preexisting vessels. It is a pathological condition that arises from abnormal deployment of normal angiogenesis. It is a significant mechanism for supply nutrient to cancer cells. Cancer angiogenesis is similar to normal angiogenesis. Cancer cells can induce the formation of blood vessels from the preexisting vasculature. Cancer cells are able to develop around an existing vessel. Recently, circulating endothelial precursors, angioblast-like cells draw from bone marrow have been suggested to conduce tumor-derived blood vessels. The cancer angiogenesis process begins from avascular primary tumor develops until inner region becomes hypoxic. Cancer cells may induce angiogenic factor expression for example vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and interleukin-8 (IL-8) promote tumor angiogenesis. Cancer cells induce angiopoietin-2 (Ang2) expression in the preexisting vessel. Ang2 move endothelial cells from stable, growth-arrested state to a plastic. Beside, cancer cells can induce FGF and VEGF. FGF can activate endothelial proliferation and stimulate cancer angiogenesis including synergism with VEGF. VEGF-induced hyperpermeability is to allow for local extravasation of proteases and matrix components from the blood stream. Afterwards, endothelial cells proliferate and migrate via remodeled matrix and leading to tube formation. Moreover, this process can find IL-8 that is not

well characterized in normal angiogenesis but has attracted attention in cancer angiogenesis. IL-8 is produced by macrophage. It promotes endothelial cell proliferation in cancer vasculature. Furthermore, IL-8 can activate the production of MMP-2, which degrades basement membranes and remodeled ECM for cell invasion and migration. Blood vessel of cancer has characteristics thin wall, torturous shape, lack of priests and different in diameter. Many fenestrae are found between endothelial cells. The vessel wall may consist of both cancers as well as endothelial cell (Papetti and Herman, 2002). Dissemination of cancer cells from the primary tumor to the lymphatic system via lymphatic vascular is one of the major routes for cancer metastasis (Duong et al., 2012). The lymphatic system plays a crucial in retaining tissue fluid homeostasis by draining lymph (protein-rich fluid) from the interstitial space back to the blood circulation (Mayby-Ei Haijami and Petrova, 2008). Cancer lymphangiogenesis is a process of adult neolymphangiogenesis during pathological conditions. In a tumor: lymphatic growth factors (VEGF-A, VEGF-C and VEGF-D) are secreted from malignant cell, inflammatory cell (e.g. Tumor-associated macrophages (TAMs)) and stromal cells. These growth factors activate the formation of neolymphatic, both in the intratumoral and peritumoral region, which accommodate the intravasation of malignant cells into lymphatic vasculature (Duong et al., 2012). Therefore, cancer vascularization is an essential process for the progression of cancer. Pathway in malignant cells spread associated with angiogenic and lymphatic, which are a major component of cancer metastasis (Pepper, 2001). Angiogenesis and lymphangiogenesis supply new vascular, which malignant cells can use to leave the primary tumor (Stacker et al., 2002). They may increase the entry of malignant cells into the circulation and leading to distant organs (Zetter, 1998).

Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) is growth factor promote cancer angiogenesis, lymphangiogenesis, invasion and migration (Dvorak, 2002). VEGF has a main function in tumor angiogenesis and lymphangiogenesis in metastasis process. Moreover, it can activate vessel growth, and intensify vascular permeability result in cancer cells to circulate from the primary mass to distant organs. Thus, VEGF is a major growth factor for cancer metastasis (Powers et al., 2000). The VEGF family composed of seven members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E (Orf virus VEGF), VEGF-F and placenta growth factor (PlGF) (McMahon, 2000; Olsson et al., 2006). There are three tyrosine kinases receptors for the VEGF family including VEGFR1, VEGFR2, VEGFR3 and two non-protein kinase receptors: neuropilin-1 (Nrp1) and neuropilin-2 (Nrp2). Binding sites of VEGF-A are identified on vascular endothelial cell corresponding to VEGFR1 and VEGFR2 while, VEGF-C and VEGF-D bind VEGFR3, which are identified in lymphatic endothelial cells (Moreira et al., 2007).

VEGF-A

VEGF-A is a major molecule in angiogenesis process. It is a homodimeric mitogenic glycoprotein that activates endothelial cell derived from arteries, veins and lymphatic (Moreira et al., 2007). In adulthood, the highest levels of VEGF-A are found in adrenal gland, heart, kidney and lung. However, gastric mucosa, liver, and spleen can find lower levels (Hoeben et al., 2004). It induces angiogenesis in physiological and pathological

conditions such as the normal development of embryonic vasculature, corpus luteum formation, wound healing and the abnormal angiogenesis in cancer (Roy et al., 2006; Moreira et al., 2007). At least seven homodimeric isoforms of VEGF-A polypeptides of distinct sizes are known to exist. The monomer compose of 121, 145, 148, 165, 183,189 and 206 amino acids (figure 2). The primitive VEGF-A transcript draws from a single VEGF-A gene, coding for eight exons. The amino acids are encoded by exons 1-5 and 8, which are preserved in all isoforms except VEGF-A 148. The VEGF- A isoforms vary by alternative splicing in exon 6 and 7, which encode two different heparin binding domains (Hoeben et al., 2004). The presence and absence of heparin binding domains affected on solubility and receptor binding. These domains encoded by exon 6 define binding to ECM. So, VEGF-A145, VEGF-A 189, VEGF-A 206 are bound to surface heparin-containing proteoglycans in ECM. Meanwhile, those deficient the domain is diffusible. VEGF-A 165 contains only one heparin-binding domain which encoded by exon 7 is moderately diffusible. The VEGF-A165 has appropriate characteristics of bioavailability and biological potency. While, VEGF-A121 absent the domains encoded by both exon 6 and 7, is very diffusible. However, VEGF-A165 and VEGF-A189 can be separated by plasmin to yield fragment containing the VEGF-A110 that is extremely diffusible. Therefore, lack of heparin binding domains due to alternative splicing in exon 6 and 7 or plasmin cleavage, leading to lack of mitogenic activity for vascular endothelial cells (Moreira, 2007). Furthermore, the lengthy forms of VEGF-A bind heparin domains

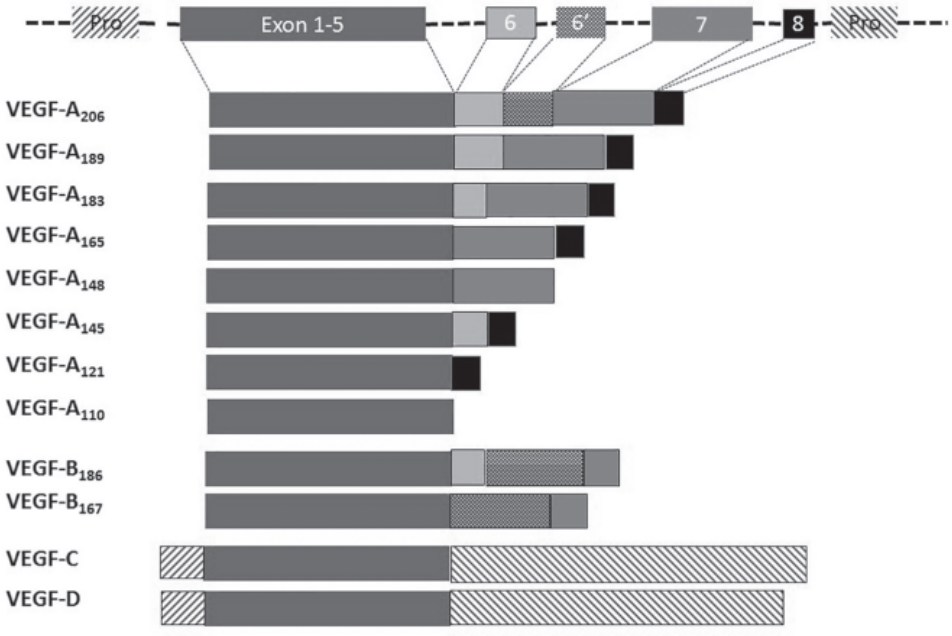

Figure 2. Structure of VEGF-A, VEGF-B, VEGF-C and VEGF-D. The VEGF-A comprises of eight exons that bring about to seven isoforms of VEGF-A121, 145, 148, 165, 183, 189 and 206 amino acid via distinct splicing. VEGF-A110 amino acid result from proteolytic cleavage which VEGFA-165 and VEGF-A189 are cleaved by plasmin. VEGF-B has two isoforms of VEGF-B167 and VEGF-B186 amino acids. VEGF-C and VEGF-D is proteolytically liberated from respective preparations. All VEGF members share an extremely preserved VEGF homology domain, which encoded by exons 1-5. (Encoded by exon 3 determine VEGFR1 binding site, Encoded by exon 4 determine VEGFR2 binding site, Encoded by exon 6, 7 determine heparin binding site and encoded by exon 8 determine neuropilin binding site)

with gradually higher affinity (Ferrara et al., 2003; Roy et al., 2006).

VEGF-A can bind with VEGFR1 and VEGFR2. Moreover, it binds to neuropilin-1 (Nrp-1) and neuropilin-2 (Nrp-2) (Kerbel, 2008). It increases vascular permeability by binding to VEGFR2 and afterward stimulating guanylyl cyclase and cGMP through the nitric oxide pathway. Increase of cGMP levels presumably increases vascular endothelial permeability by enhancing the vesiculo-vacuolar organelles, fenestrations and transcellular gaps. This growth factor mediated extravasation of fluid and plasma protein, together with fibrin might conduce to increased migration of endothelial cells in ECM. Besides, it also causes vasodilation by induction of eNOS and enhancing nitric oxide production. VEGF-A can support endothelial cell survival by inducing the expression Bcl-2 and A1 which is anti-apoptotic protein in the endothelial cells (Roy et al., 2006). VEGF-A may stimulate PI3K/Akt pathway leading to endothelial cell survival. Therefore, VEGF-A is inducer of vascular permeability and activator of endothelial cell proliferation and migration (Kerbel, 2008). In addition, it is a significant survival factor for the formation of new blood vessel. It is ability not only activating angiogenesis but also allowing the corresponding new blood vessels survival (Moreira et al., 2007; Kerbel, 2008).

VEGF-B

VEGF-B comprise of two forms that arise from alternative splicing of exon 6 creates VEGF-B167 and VEGF-B186. The VEGF-B167 is highly expressed in adult including skeletal muscles, myocardium and brown fat. Whereas, the VEGF-B186 is expressed lower levels and a limited number of tissues (Hoeben et al., 2004). VEGF-B binds to both VEGFR1 and Nrp-1. Moreover, it can form heterodimers with VEGF-A by VEGF-B167 binds heparin sulfate proteoglycans and is chiefly separated in the ECM, whereas VEGF-B186 is freely diffusible (Hoeben et al., 2004; Roy et al., 2006). The function of VEGF-B remain unclear. This growth factor seems to be required for angiogenesis in heart and muscle (Moreira et al., 2007; Sullivan and Brekken, 2010). In addition, VEGF-B mRNA can be found in several human cancers including benign thymoma, breast cancer, fibrosarcoma, non-Hodgkins lymphoma, primary melanoma and metastatic melanoma (Roskoski, 2007).

VEGF-C

VEGF-C is a growth factor for angiogenesis and lymphangiogenesis (Saaristo et al., 2000). The VEGF-C consists of seven exons (Hoeben et al., 2004). It is synthesized as a prepro-protein which undergoes proteolytic processing to create the mature form of VEGF-C by generation of two VEGF-C precursors from anti-parallel homodimer bind to the disulfide bonds stretching from each of the two C-terminal residues remains joined to the reverse N-terminus. The final step of processing, which befalls extracellular by protease cleave N-terminus and C-terminus to yield mature form of VEGF-C (figure 3). Mature form of VEGF-C is a homodimeric protein that it's high affinity for both VEGFR2 and VEGFR3. While, an unprocessed form of VEGF-C may bind to VEGFR3 (Joukov et al., 1977). This growth factor can induce mitogenesis, migration and survival of endothelial cells. VEGF-C is present in adult tissue including heart, ovary, placenta, skeletal muscle, small intestine and thyroid gland. It is lymphangiogenic growth factor. It is mediated by VEGFR3. It partakes in lymphangiogenesis during

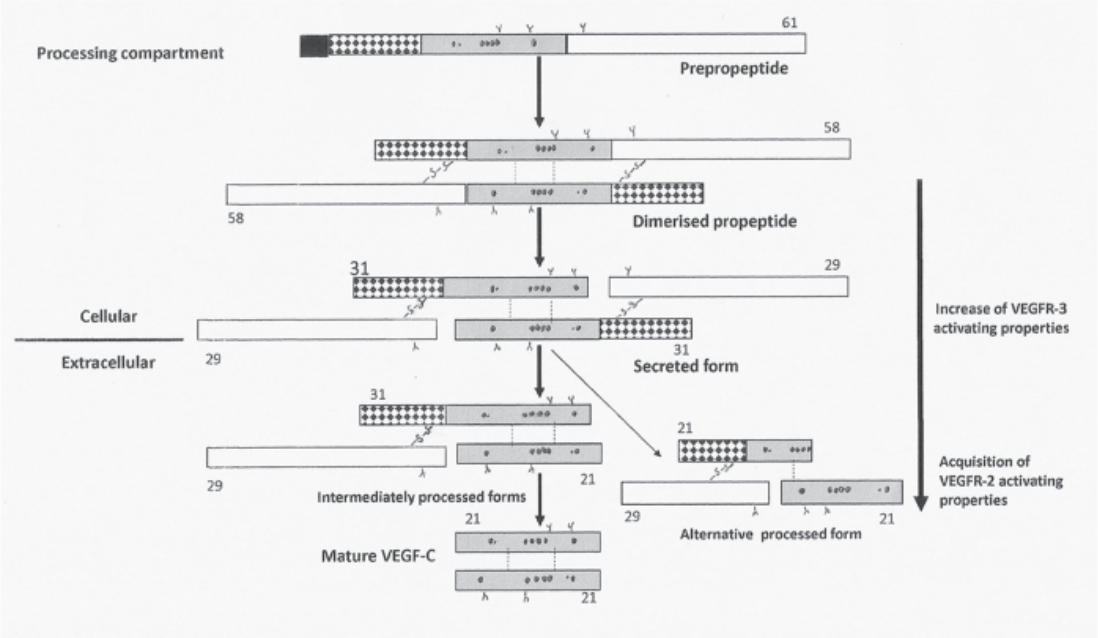

Figure 3. Model of proteolytic processing of VEGF-C. The regions of VEGF-C are marked as follows: black box is signal sequence, gray box is a VEGF homology domain, dotted box is N-terminal and white box is C- terminal propeptides. The VEGF-C is produced as two precursor from anti parallel homodimer bind to the disulfide bonds stretching from each of the two C-terminal residues remains joined to the reverse N-terminus. The final step of processing, which befalls extracellular by protease cleave N-terminus and C-terminus to yield mature form of VEGF-C. Mature form of VEGF-C is a homodimeric protein that it's high affinity for both VEGFR2 and VEGFR3.

embryogenesis and in the maintenance of differentiating lymphatic endothelial cells in adults. However, VEGF-C acts through VEGFR2 can induce blood vessel permeability. In addition, mature form of VEGF- C can bind to VEGFR2 and VEGFR3 result in blood vessel development (Roskoski, 2007).

VEGF-D

VEGF-D is a growth factor and same as VEGF-C. It is found in adult tissue, including heart, lung, skeletal muscle, colon, small intestine and vascular endothelium. It contains seven exons (Hoeben et al., 2004). This growth factor that undergoes a complex proteolytic process to create the mature form of VEGF-D. The precursor for VEGF-D contains N-terminus and C-terminus extensions that are cleaved to yield the mature form as same as VEGF-C. Whereas, unprocessed form of VEGF-D may bind to VEGFR3, which is significant in lymphangiogenesis leading to lymphatic vessel development. Mature form can bind to both VEGFR2 and VEGFR3 (Roy et al., 2006). VEGF-D can induce lymphatic vascular growth in adulthood, which response to pathological conditions. Furthermore, VEGF-D has been shown to have a role in cancer angiogenesis and lymphangiogenesis (Roskoski, 2007).

VEGF-E

VEGF-E was found in the genome of Orf virus (parapoxvirus) that infects goat, sheep and sometimes infect humans. Infection by a parapoxvirus causes proliferative skin lesions, which expanded capillary proliferation and dilation (Hoeben et al., 2004). It binds

specifically to VEGFR2 and Nrp-1 that are capable to activate endothelial cell mitogenesis and vascular permeability (Roy et al., 2006).

VEGF-F

VEGF-F was identified from snake venom. It binds selectively to VEGFR2. It contains a short C-terminal heparin binding region. Moreover, the C-terminal peptide of VEGF-F presents a specific block VEGF-A165 an activity both in vitro and in vivo (Roy et al., 2006).

Placenta growth factor (PlGF)

Placenta growth factor (PlGF) is a member of the VEGF family which was initially identified in placenta. However, it is known to be exhibited in lung and heart. It is homodimeric glycoprotein and is coded by seven exons (Roy et al., 2006). This growth factor contains of four isomers including PlGF-1, PlGF-2, PlGF-3 and PlGF-4. PlGF-1 and PlGF-3 are non-heparin binding diffusible isoforms, while PlGF-2 and PlGF-4 are heparin binding domains. These growth factors mediate effects via VEGFR1. Besides, PlGF-2 is capable to bind Nrp-1 and Nrp-2. Placenta growth factor acts through VGFR1 signaling support endothelial cell viability and angiogenesis. It has direct effects on endothelial cell, by inducing its own signaling and increasing VEGF-induced angiogenesis (Roy et al., 2006).

Vascular endothelial growth factor receptors (VEGFRs)

The activities of VEGF family are activated through the VEGF-specific tyrosine-kinase receptors including VEGFR1 (Flt-1, fms-like tyrosine kinase 1), VEGFR2 (KDR/Flk-1, fetal liver kinase-1) and VEGFR3 (Flt-4. fms-like tyrosine kinase-4). Each of receptor has seven extracellular immunoglobulin-like domains (Moreira et al., 2007).

VEGFR1 (Flt-1)

VEGFR1 is presented in endothelial cells as well as pericytes, placental trophoblast, osteoblast, macrophages, monocytes, renal mesengial cells and in some hematopoietic stem cell. It binds to VEGF-A, VEGF-B and PlGF. It has higher affinity for VEGF-A than VEGFR2. VEGFR1 has strength tyrosine kinase phosphorylation activity following activation by VEGF-A. VEGFR1 consists of six tyrosine residues in the C-terminus including tyrosines 1169, 1213, 1242, 1309, 1327 and 1333 have been identified as phosphorylation sites. Phosphorylation at tyrosine 1169 is implicated in the binding and stimulation of PLC? and result in the stimulation of MAP kinase signaling pathway (Roskoski, 2007). However, stimulation of VEGFR1 contributes to the increased presentation of urokinase type of plasminogen activator and plasminogen activator inhibitor-1 in endothelial cells leading to degrade ECM and cell cell migration. In addition, VEGFR1 stimulation at least by PlGF can support angiogenesis via intracellular cross talk with VEGFR2. Although, VEGF-A and PlGF can bind to VEGFR1 but they stimulate this receptor differently. Furthermore, VEGFR1 is concerned with monocyte chemotaxis and in the recruitment and survival of bone marrow derived progenitor cells. Expression of VEGFR1 is regulated by hypoxia, unlike that of VEGFR2 and VEGFR3 (Roy et al., 2006).

VEGFR2 (KDR/Flk-1)

VEGFR2 binds to lower molecular weight forms of VEGF-A (110-165 amino acid), VEGF-E, VEGF-F and mature forms of VEGF-C and VEGF-D. It is the dominant mediator of VEGF-A activated endothelial cell migration, proliferation, survival, and increased vascular permeability (Roskoski, 2007). Although, it has lower affinity for VEGF-A than VEGFR1. It presents an active protein-tyrosine kinase activity in response to its legend (Roy et al., 2006). VEGF-A induces the dimerization of VEGFR2 that result in autophosphorylation of the receptor and stimulation. This receptor consists of five tyrosine residues including tyrosine 951, 1054, 1059, 1175 and 1214. Tyrosine1175 and 1214 are two main phosphorylation sites on this receptor. Autophosphorylation of tyrosine residues 1054 and 1059 within the stimulation of VEGFR2 toward enhanced kinase activity. VEGFR2 phosphorylation at tyrosine 1175 contribute to PLCγ stimulation and protein kinase C (PKC) stimulation leading to cell proliferation. Moreover, Shb is phosphorylated and binds directly to tyrosine 1175 following VEGF-A activation through phosphatidylinositol 3-kinase (PI3K) leading to vascular permeability, cell migration and cell survival (Roskoski, 2007). VEGFR2 is the primitive receptor transmitting vascular endothelial growth factor signals in endothelial cells. The VEGFR2 signaling pathway is major effects of VEGF members (VEGF-A, VEGF-E, VEGF-F and mature form of VEGF-C and VEGF-D) including vasodilation, endothelial cell migration and proliferation (Kerbel, 2008). In addition, this receptor may be concerned with integrin-dependent migration of endothelial cells, as it forms a complicated with integrin aVβ3 on binding VEGF-A. Thus, VEGFR2 as known to mediated angiogenesis (Roskoski, 2007; Kerbel, 2008).

VEGFR3 (Flt-4)

VEGFR3 binds to VEGF-C and VEGF-D. It has characteristics proteolytic cleavage in the sixth immunoglobulin domain, which the two components of primitive chain remain linked by disulfide bonds (Joukov et al., 1997)(figure 3). This receptor consists of five tyrosine residues including tyrosine1230, 1231, 1265, 1337 and 1363 in the C-terminal of VEGFR3, which as autophosphorylation sites. Phosphorylation at tyrosine 1337 serve as the binding site for Shc and Grb2, which befall at the start the MAP kinase signaling pathway (Roskoski, 2007). It plays a major role in remodeling the primary capillary plexus during embryo. In the adult, VEGFR3 contributes to angiogenesis and lymphangiogenesis (Roy et al., 2006). Furthermore, VEGFR3 is upregulated on endothelial cell of cancer vasculature. VEGFR3 signaling pathway is important in the lymphangiogenesis process (Roy et al., 2006; Sullivan, 2010).

Neuropilins

Neuropilins are transmembrane non-protein-tyrosine kinase. The neuropilins consist of neuropilin-1 (Nrp-1) and neuropilin-2 (Nrp-2). They have a role in immunology and neuronal development. Besides, the neuropilins are involved in angiogenesis (Roy et al., 2006). Nrp-1 binds VEGF-A165, VEGF-B and PlGF. Nrp-2 binds VEGF-A165, VEGF-C and PlGF. Nrp-1 act as co-receptor increasing VEGF-A-VEGFR2 interaction, forming complicated with VEGFR1 (Roskoski, 2007). Therefore, neuropilin function as receptors for VEGF isoforms freely of VEGFR1, VEGFR2 and VEGFR3 (Roy et al., 2006; Roskoski, 2007).

Action of vascular endothelial growth factor in cancer metastasis process

Malignant tissues create various growth factors to induce angiogenesis and lymphangiogenesis, which is necessary for malignant cell growth, invasion and metastasis. VEGF is growth factor, which is known to mainly contribute to promote angiogenesis and lymphangiogenesis in cancer. This all together brings about to increase cancer growth rates, invasion and metastasis (Xue et al., 2009). Members of the VEGF family of endothelial cell mitogens, for example VEGF-A (VEGF or vascular permeability factor (VPF)), VEGF-C and VEGF-D cable to encourage cancer angiogenesis and lymphangiogenesis (Dvorak, 2002).

VEGF-A is a crucial angiogenic factor in tumor-induced angiogenesis (Catena et al., 2010). It has been considered to only support vascular formation. Recent studies from several laboratories find that VEGF-A can support lymphangiogenesis by it may assist lymphatic endothelial cell proliferation and migration. It's produced by malignant cells. It binds to VEGFR1 and VEGFR2 and neuropilin in endothelial cells and on some other cells (Hirakawa et al., 2005; Catena et al., 2010). Several researches demonstrated that VEGF-A overexpression in cancer xenotransplants promote cancer angiogenesis (Ferrara, 2002; Kuemmel et al., 2009). Has previously been reported that VEGF-A is significant for vascular formation at initial stages of cancer formation by transformed cells. Therefore, VEGF-A is an important promoter of cancer metastasis (Saito et al., 1998). In addition, found that VEGF-A can induce cancer growth by tumor-derived VEGF-A acts as an endocrine-like hormone to induce extramedullary hematopoiesis (Xue et al., 2009). Meanwhile, the report found that expression of VEGF-A within colorectal cancers (CRCs) was associated with lymphatic metastasis (Georage et al., 2001). Thus, VEGF-A is a major growth factor promote both angiogenesis and lymphangiogenesis which result in facilitating cancer metastasis.

VEGF-C is produced as pre-pro-protein that undergoes proteolytic processing to create many forms of the growth factors that vary in receptor binding properties (Roskoski, 2007). This growth factor can be divided two forms of growth factor that consists of unprocessed or partially processed and mature forms of VEGF-C (Moreira et al., 2007). It is presented in endothelial cells and cancer cells. It is significant roles in lymphangiogenesis and angiogenesis during embryonic and cancer. It binds to VEGVR2 and VEGFR3 by unprocessed VEGF-C is cable to bind VEGFR3 while, mature VEGF-C bind to both VEGFR2 and VEGFR3 (Roskoski, 2007). VEGFR2 and VEGFR3 are revealed notably on vascular endothelial cells, while VEGFR3 is expressed on lymphatic endothelial cells (LECs) (Ferrara, 2004; Chen et al., 2012). Current research, suggest that both receptors are found in cancer cells and contribute to cancer progression (Veikkola et al., 2000; Su et al., 2006; Su et al., 2008; Miettinen et al., 2012). VEGF-C signals through VEGFR2 can promote vascular endothelial cell migration, proliferation and survival which result in vascular formation. While, VEGF-C binds to VEGFR3 can encourage the growth of lymphatic vessel (Veikkola et al., 2000). Besides, VEGF-C can bind to more than one receptor. In cancer condition, VEGF-C can induce the activation of VEGFR3 homodimer and VEGFR2/VEGFR-3 heterodimer have different function. It binds to VEGFR2/VEGFR3 heterodimer may induce angiogenesis while, it binds to VEGFR3 associated with lymphangiogenesis (Nilsson et al., 2010; Chen

et al., 2012). In addition, studies have VEGFR3 signaling has an important associated with lymphatic formation and lymph node metastasis (Shi et al., 2008; Chien et al., 2009).

VEGF-D is synthesized as long precursor proteins that undergoes complex proteolytic processing and create mature form of VEGF-D. Unprocessed form of VEGF-D binds to VEGFR3, which is major in lymphangiogenesis. Meanwhile, mature form of VEGF-D trigger both VEGFR2 and VEGFR3 (Moreira et al., 2007; Roskoski, 2007). VEGF-D can be produced by cancer cells that will increase of both angiogenesis and growth rate (Stacker et al., 2002). This growth factor induces angiogenesis and lymphangiogenesis in cancer and concerned with lymphatic metastasis in human cancers (Jain and Padera, 2002). Moreover, it may encourage the metastatic spread of malignant cells through the lymphatic vasculature in a mouse model. A study of the importance of VEGF-D in cancer progression which found that VEGF-D can induce remodeling of collecting lymphatic vessel (CLV) for prepared to cancer dissemination. VEGF-D can induce both angiogenesis and lymphangiogenesis in cancer metastasis (Stacker et al., 2001).

Therefore, it could be summarized that the action of VEGF members in cancer metastasis can be divided two functions which have influenced angiogenesis and lymphangiogenesis in cancer metastasis.

Vascular endothelial growth factor involved in cancer metastasis as therapy targets in cancer

There are many evidences showing that VEGF members, specifically VEGF-A, VEGF-C and VEGF-D, and their receptors are involved in the angiogenesis and lymphangiogenesis in many cancers. There are evidences that neutralizing antibodies against VEGF-A and inhibiting VEGF-A receptors have efficiently put off vascular permeability, growth of cancer and reduce cancer size. This effect is mediated through suppression of angiogenesis (Dvorak, 2002; Ferrara, 2005). Besides, blocking of VEGF-A can prevent the spread of early-stage cancer by blockade cancer lymphangiogenesis and lymph node lymphangiogenesis. Moreover, the beginning of anti-VEGF-A treatment during the pre-surgery period, forthwith after the diagnosis of malignancy can reduce risk cancer metastasis (Hirakawa et al., 2005). Recently, blockade of VEGF-C/VEGF-D/VEGFR3 signaling pathways could prohibit the lymphatic metastatic spread (Stacker et al., 2002). There is evidence showing that VEGF-C inhibitor can protect the spread of early-stage cancer, by blockade lymphangiogenesis in malignancies and lymph nodes (Stacker et al., 2002; Hirakawa et al., 2007). Moreover, a monoclonal antibody against VEGFR3 that blockade the binding of VEGF-C result in inhibiting formation lymphatic vessel (Stacker et al., 2002). Therefore, apart from VEGF-A targeting, blocking of VEGF-C and VEGF-D should be considered as a target for treatment of cancers, since they involve in lymphagiogenesis. Moreover, it is also found that a neutralizing VEGF-D monoclonal antibody can prohibit interaction with VEGFR2 and VEGFR3 leading to blocking angiogenesis as well as lymphangiogenesis and metastatic spread. Therefore, an understanding of mechanisms concerned with the anti-cancer activity of vascular endothelial growth factors targeted treatment may improve recent cancer therapy.

Conclusions

VEGF family is a growth factor encourages vascular endothelial cell growth draw from artery, vein and lymphatic. In a cancer, vascular endothelial growth factors (VEGF-A, VEGF-C, and VEGF-D) are produced from cancer cells, inflammatory cells (tumor associated macrophages (TAMs)) and stromal cells. The cancer cell can produce VEGFs which act through their receptors in vascular endothelial cells in the paracrine manner and support vascular endothelial cell proliferation, differentiation and tube formation. On the other hand, VEGFs and their receptors can also function in the autocrine manner to increase cancer cell migration and invasion resulting in cancer metastasis. These factors, both VEGFs and their receptors, are known to chiefly conduce to support angiogenesis and lymphangiogenesis in cancer leading to increase cancer cell growth, invasion and metastasis. Therefore, together with the inhibition of VEGF-A, VEGF-C, VEGF-D, and their receptors could be used in cancer treatment to reduce cancer metastasis (Figure 4).

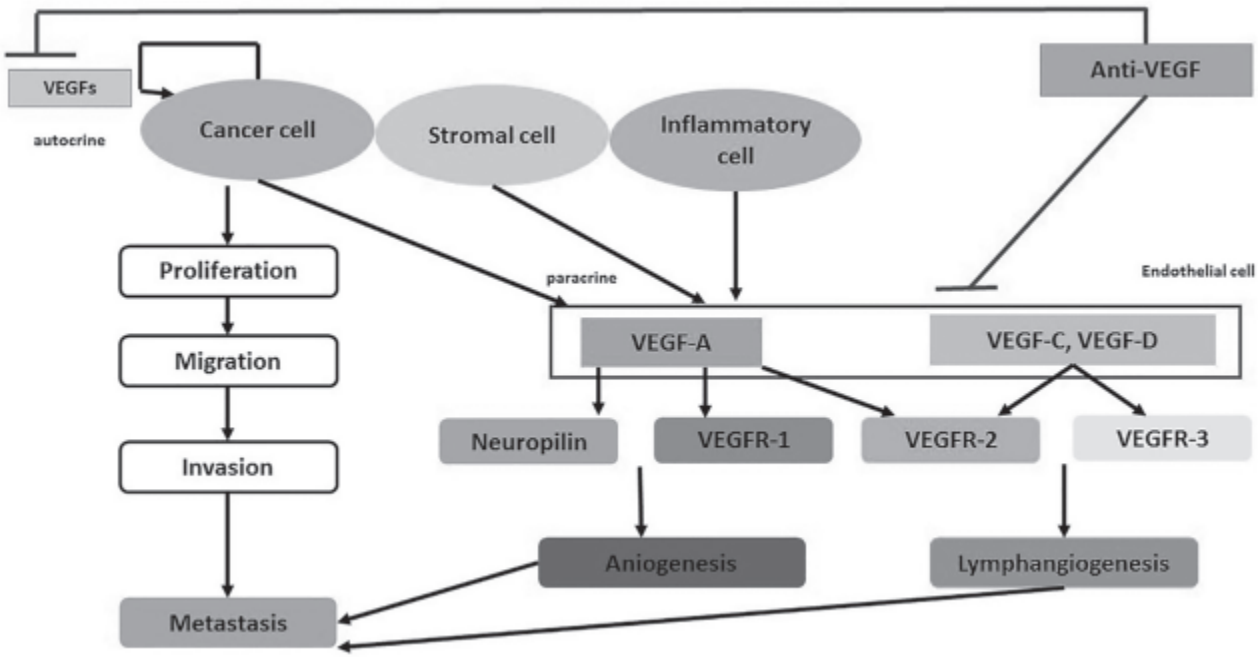

Figure 4. Diagram showing the molecular pathway of VEGF members and other molecules involved in angiogenesis and lymphagiogenesis in cancer metastasis. Cancer cells can produce VEGFs which act through their receptors in vascular endothelial cells in paracrine manner and support vascular endothelial cell proliferation, differentiation and tube formation. On the other hand, VEGFs also function in an autocrine manner to increase cancer cell migration and invasion resulting in cancer metastasis. VEGF-A, VEGF-C, and VEGF-D are produced from cancer cells, inflammatory cells (tumor associated macrophages (TAMs)) and stromal cells. VEGF-A is a major angiogenic factor. It binds to VEGFR1 and VEGFR2 and neuropilin in endothelial cells and on some other cells and stimulate the signal transduction pathway leading to angiogenesis. Mature form of VEGF-C and VEGF-D can bind to VEGFR2 and VEGFR3 and mediate angiogenesis. However, unprocessed form of VEGF-C and VEGF-D is capable to bind only VEGFR3 and triggers a signal transduction pathway leading to promote lymphangiogenesis.

References

Catena ML, Leyre L, Larrayos M, Molina E, Hermida J, Agorreta J, et al. VEGF121b and VEGF165b are weakly angiogenic isoforms of VEGF-A. Mol Cancer 2010; 9(320): 1-14.

Chen JC, Chang YW, Hong CC, Yu YH, Su JL. The role of the VEGF-C/VEGFRs axis in tumor progression and therapy. Int J Mol Sci 2012; 14(1): 88-107.

Chien MH, Ku CC, Johansson G, Chen MW, Haiao M, Su JL, et al. Vascular endothelial growth factor-c (VEGF-C) promotes angiogenesis by induction of COX-2 in leukemic cells via the VEGF-R3/JNK/AP-1 pathway. Carcinogenesis 2009; 30(12): 2005-9.

Duong T, Koopman P, Francois M. Tumor lymphangiogenesis as a potential therapeutic target. J Oncol 2012; 1-23.

Dvorak HF. Vascular permeability factor/ vascular endothelial growth factor a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 2002; 20(21): 4368-80.

Ferrara N, Gerber HP, Lecouter J. The biology of VEGF and its receptors. Nat Med 2003; 9(6): 669-76.

Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 2004; 9 (suppl 1): 2-10.

Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nat Rev Cancer 2002; 2(10): 795-803.

Ferrara N. VEGF as a therapeutic target in cancer. Oncology 2005; 69(suppl 3): 11-16.

Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003; 3(6): 453-8.

Fisher JC, Gander JW, Haley MJ, Hernandez SL, Huang J, Chang YJ, et al. Inhibition of cyclo-oxygenase 2 reduces tumor metastasis and inflammatory signaling during blockade of vascular endothelial growth factor. Vasc Cell 2011; 3(12): 1-12.

George ML, Tutton MG, Janssen F, Arnaout A, Abulafi AM, Eccles SA, et al. VEGF-A, VEGF-C, and VEGF-D in colon cancer progression. Neoplasia 2001; 3(5): 420-7.

Harlozinska A. Progress in molecular mechanisms of tumor metastasis and angiogenesis. Anticancer Res 2005; 25(5): 3327-33.

Hirakawa S, Brown LF, Kodama S, Paavoven K, Alitalo K, Detmar M. VEGF-C induced lymphangiogenesis in sentinel lymph nodes promotes tumor metastasis to distant sites. Blood 2007; 109(3): 1010-7.

Hirakawa S, Kodama S, Kunstfeld R, Kajiya K, Brown LF, Detmar M. VEGF-A induce tumor and sentinel lymphangiogenesis and promote lymphatic metastasis. J Exp Med 2005; 201(7): 1089-99.

Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruiin EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004; 56(4): 549-80.

Jain RK, Padera TP, Prevention and treatment of lymphatic metastasis by antilymphangiogenesis therapy. J Natl Cancer Inst 2002; 94(11): 785-86.

Joukov V, Sorsa T, Kumar V, Jeltsch M, Claesson-Welsh L, Cao Y, et al. Proteolytic processing regulates receptor specificity and activity of VEGF-C. EMBO J 1997; 16(13): 3898-391.

Kerbel RS. Tumor angiogenesis. N Engl J Med 2008; 358(19): 2039-49.

Kuemmel S, Thomas A, Landt S, Fuger A, Schmid P, Kriner M, et al. Circulating vascular endothelial growth factors and their soluble receptors in pre-invasive, invasive and recurrent cervical cancer. Anticancer Res 2009; 29(2): 641-5.

Li CY, Shan S, Cao Y, Dewhirst MW. Role of incipient angiogenesis in cancer metastasis. Cancer Metastasis Rev 2000; 19(1-2): 7-11.

Maby-El Haijami H, Petrova TV. Developmental and pathological lymphangiogenesis: from models to human disease. Histochem Cell Biol 2008; 130(6): 1063-78.

McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist. 2005; 5(suppl 1): 3-10.

Miettinen M, Rikala MS, Rys J, Lasota J, Wang ZF. Vascular endothelial growth factor receptor 2 as a marker for malignant vascular tumor and mesothelioma: an immunohistochemical study of

262 vascular endothelial and 1640 nonvascular tumors. Am. J. Surg. Pathol 2012; 36(4): 629-39.

Moreira IS, Fernandes PA, Ramos MJ. Vascular endothelial growth factor (VEGF) inhibition-a critical review. Anticancer Agents Med Chem 2007; 7(2): 223-45.

Nakamura Y, Yasuoka H, Tsuiimoto M, Yang Q, Lmabun S, Nakaharan M, et al. Prognostric significance of vascular endothelial growth factor D in breast carcinoma with long-term follow up. Clin Cancer Res 2003; 9(3): 716-21.

Nilsson I, Bhram F, Li X, Gualandi L, Koch S, Jarvius M, Soderberg O, et al. Vegf receptor 2/-3 heterodimers detected in situ by proximity ligation on angiogenic sprouts. EMBO J. 2010; 29(8): 1377-88.

Olsson AK, Dimberge A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling-in control of vascular function. Nat Rev Mol Cell Biol 2006; 7: 359-71.

Papetti M, Herman IM. Mechanisms of normal and tumor-derived angiogenesis. Am J Cell Physiol 2002; 282(5): C947-70.

Pepper MS. Lymphangiogenesis and tumor metastasis: myth or reality. Clin Cancer Res 2001; 7(3): 462-8.

Powers CJ, Mcleskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 2000; 7(3): 165-97.

Roskoski R Jr. Vascular endothelial growth factor (VEGF) signaling in tumor progression. Crit Rev Oncol Hematol 2007; 6(3): 179-213.

Roy H, Bhardwaj S, Ylä-Herttuala S. Biology of vascular endothelial growth factors. FEBS Lett 2006; 590(12): 2879-87.

Saaristo A, Karpanen T, Alitalo K. Mechanisms of angiogenesis and their use in the inhibition of tumor growth and metastasis. Oncogene 2000; 19(53): 6122-29.

Saito H, Tsujitani S, Ikeguchi M, Maeta M, Kaibara N. Relationship between the expression of vascular endothelial growth factor and the density of dendritic cells in gastric adenocarcinoma tissue. Br J Cancer 1998; 78(12):1573-7.

Scanion EF, Murthy S. Basic science overview the process of metastasis. CA-A Cancer J Clin 1991; 41(5): 301-5.

Shi X, Xi L, Weng D, Chen G, Song X, Wu P. Clinicopathological significance of VEGF-C, VEGFR-3 and cyclooxygenase-2 in early-stage cervical cancer. Int J Biomed Sci 2008; 4(1): 58-63.

Stacker SA, Achen MG, Jussila L, Baldwin ME, Alitalo K. Lymphangiogenesis and cancer metastasis. Nat Rev Cancer 2002; 2(8): 573-83.

Stacker SA, Baldwin ME, Achen MG. The role of tumor lymphangiogenesis in metastatic spread. FASEB J 2002; 16(9): 922-34.

Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Medicine 2001; 7(2): 186-91.

Su JL, Chen PS. Chien MH, Chen PB, Chen YH, Hsieh CY Cho CH, et al. The vegf-c/flt-4 axis promotes invasion and metastasis of cancer cells. Cancer cell 2006; 9(3): 209-23.

Su JL, Chen PS Chien MH, Chen PB, Chen YH, Lai CC, et al. Further evidence for expression and function of the VEGF-C/VEGFR-3 axis in cancer cells. Cancer cell 2008; 13(6): 557-60.

Sullivan LA, Brekken RA. The VEGF family in cancer and antibody-based strategies for their inhibition. MAbs 2010; 2(2): 165-67.

Talmadge JE, Fidler IJ. AACR centennial series: The biology of cancer metastasis: historical perspective. Cancer Res 2010; 70(14): 5649-69.

Veikkola T, Karkkainen M Claesson Welsh L, Alitalo K. Regulation of angiogenesis via vascular endothelial growth factor receptors. Cancer Res 2000; 60(2): 203-12.

Woodhouse EC, Chuaqui RF, Lioyya LA. General mechanisms of metastasis. Cancer 1997; 80(8 suppl): 1529-37

Xue Y, Chen F, Zhang D, Lim S, Cao Y. Tumor-derived VEGF modulates hematopoiesis. J Angiogenesis Res 2009; 1-9.

Zetter BR. Angiogenesis and tumor metastasis. Annu Rev Med 1998; 49: 407-24.