Lung cancer is the most common malignity in the world and the frequency of its occurrence gradually increases. Development of metastasis is one of the most life-threatening conditions for the patients with cancer. Tumour growth and metastasis events are dependent upon angiogenesis. New blood vessels embedded within the tumor provide a gate for tumour cells. Thus, tumor cells enter the blood circulation and metastasis to distant organs such as lung or bone. Prevention of tumour blood circulation by blocking these angiogenic factors has brought new approaches to fight with cancer. VEGF-A is mainly responsible for angiogenesis and increase in vascular permeability, also known as vascular permeability factor. The VEGF and its receptors are important in both regulation of neovascularization and pathological angiogenesis. VEGF receptor family consists of three members: VEGF-R1 (Flt-1), VEGF-R2 (KDR/Flk-1) and VEGF-R3 (Flt-4) which belong to receptor tyrosine kinase super family. VEGF-A binds strongly to VEGF-R1, it does not bind to VEGF-R3. But, VEGF-R-3 binds only VEGF-C and VEGF-D. VEGF and its receptors are expressed in cancer cells, in both NSCLC and SCLC. Many studies reported that VEGF-R1, receptor of both VEGF and PIGF, can be expressed in many cancer types including NSCLC. As a conclusion suppression the VEGF-R1 signaling can be an important target for treating cancer metastasis.

Key words: VEGF, receptors, cancer

Carcinogenesis

Lung cancer is the most common malignity in the world and the frequency of its occurrence gradually increases. Beside it is the most frequent cause of death in men in the USA, it has also become the most commonly seen cancer in women by overtaking breast cancer in recent years.1

Cigarette is one of the major etiological factors for lung cancer. The association of smoking with the occurrence of lung cancer has been demonstrated by both epidemiologic studies and animal experiments. The other factors that lead to lung cancer are occupational factors, radiation, history of previous lung infections, genetic factors (proto-oncogenes and mutations in tumour suppressor genes).

The most urgent questions when cancer first diagnosed are where the primary localization of disease is, whether regional lymph nodes are involved and if there is any metastasis to distant organs.

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Despite early diagnosis, developments in surgical techniques and general patient care, use of effective local and systemic adjuvant therapies, most of the deaths caused by cancer still occur due to metastasis refractory to treatment. Once metastasis is developed, treatment of the most patients becomes extremely difficult, because the complete eradication of metastasis in different organs and the different region of the same organ by using surgery, radiotherapy and/or chemotherapy is almost impossible and creates the most important issue of current cancer treatment approaches. Surgical treatment is recommended in the early stage of the disease. However disease expands even in half of the patients that had completed resection. In the last 20 years, death rates poorly changed despite radiotherapy, chemotherapy and developments in surgical treatments.\(^2\)

Non-fatal genetic damages of the cells, such as mutations, form the basis of carcinogenesis. Genetic damages caused by chemical substances, environmental agents like radiation and inherited mutations are the major causes of the cancer development.

According to the histological classification schemes of World Health Organization,\(^3\) generally there are 4 types of lung cancers: small cell carcinoma, squamous cell (epidermoid) carcinoma, adenocarcinoma and large cell carcinoma. In terms of therapeutic approaches, lung cancer is separated into two groups depending: Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC).

NSCLC forms 75% of lung cancers and 28% of all cancer-related deaths. Also, there is no known marker for the detection of NSCLC in asymptomatic cases.

At present, the most reliable determining factor for the outcome of lung cancer treatment is classification made according to the Tumour-Node-Metastasis (TNM classification). However, since sometimes prognosis of patients in the same stage can be different, new prognostic factors and risk factor have been investigated in recent years.\(^2\)

### Pathogenesis of Metastasis

Development of metastasis is one of the most life-threatening conditions for the patients with cancer. When neoplasia becomes invasive, it spreads rapidly by vascular and lymphatic ways. Invasion and metastasis impair normal organ functions by destruction and/or local pressure. Therefore, it is important to detect metastasis in a patient with cancer. Patients with metastatic disease are generally defeated by cancer generally due to spread of disease and, less frequently, side effects of anti-cancer drugs.\(^4\)

Necessary steps for the formation of metastasis are similar to each other.\(^5\) Metastasis formation can be reviewed in 2 main phases:

1. Extracellular matrix invasion
   - Separation of tumour cells from each other (loosening)
2. Attachment to matrix components (attachment)
3. Migration of tumour cells (migration)
   - Vascular spread via angiogenesis and settling in the secondary focus.

### Angiogenesis

Two types of systems are present in new vessel formation.

1. Vasculogenesis
2. Angiogenesis

In the early periods of embryogenesis, blood islands are formed by the gathering of endothelial
cells of mesoderm origin, termed as angioblasts, and progenitor hematopoietic cells. This primordial vascular net formation which develops as a result of differentiation of angioblast, is called vasculogenesis. Vasculogenesis is responsible for the constitution of the major blood vessels during embryogenesis. On the other hand, angiogenesis is the process of formation of new blood vessels from pre-existing vessel net. It takes place in many events like embryonic development, wound healing, female reproduction cycle (ovulation, menstruation, placental growth), bone development and reconstruction.7

Angiogenesis is regulated by a sensitive balance between angiogenesis activating factors, which include vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factors (aFGF, bFGF), angiopoietin 1, tumor necrosis factor (TNF), hepatocyte growth factor (HGF) like proteins, and angiogenesis inhibiting factors such as VIII collagen, thrombospondin, fibronectine, α, β, γ interferon in humans.8,9 Basic angiogenic and antiangiogenic factors are summarized in Table II.10,11

Impaired angiogenesis results in many pathologic events.12 For example, it causes cartilage damage in arthritis by attacking of new capillaries to the joint.13 Ocular neovascularization which generally occurs during diabetes, is the most common cause of blindness.14 Abnormal angiogenesis is one of the most important pathologic event during the progress of cancer.15 Cancer tissue needs the support of new vessels to grow more after reaching a couple of mm in size. Therefore angiogenesis is essential for tumor growth and tumor invasion.16,17

Angiogenesis consists of several steps:18
1. Release of proteases from active endothelial cells
2. Destroying basal membrane that surrounds present vessel net
3. Migration of endothelial cells to interstitial area
4. Endothelial cell proliferation
5. Lumen formation
6. Formation of new basal membrane with the help of pericytes.
7. Fusion of newly formed vessels.
8. Initiation of blood flow

Solid tumours directly stimulate endothelial cell growth, and also, produce many soluble factors that stimulate host cells, such as macrophages, to release some cytokines which induce the proliferation and migration of endothelial cells.19-21 Tumour growth and metastasis events are dependent upon angiogenesis. When tumor cells begin conversion into angiogenic phenotype, transformation occurs, and neovascularization stimulating factors such as TNF, VEGF, TGF-β and bFGF are produced. These factors are also present in the matrix and can be mobilized via tumour cells. Tumour has to stimulate formation of new capillaries constantly to be able to grow. New blood vessels embedded within the tumor provide a gate for tumour cells. Thus, tumor cells enter the blood circulation and metastasis to distant organs such as lung or bone.18

### Table 2

<table>
<thead>
<tr>
<th>Angiogenic factors</th>
<th>Anti-angiogenic factors</th>
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<td>Transforming growth factor α and β (TGFα and β)</td>
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<td>Scatter factor (hepatocyte growth factor)</td>
<td>2-metoksiöstradiol (2-ME2)</td>
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<td>Interleukin 8 (IL 8)</td>
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<td>Prostaglandin E1 and E2 (PG-E1, -E2)</td>
<td>TNFα (in high concentrations)</td>
</tr>
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### VEGF Family and VEGF-A

VEGF Family is a family composed of VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E (viral factor) and placental growth factor (PGF) proteins.22
The functions of VEGF-B, VEGF-C and VEGF-D are not completely known. VEGF-A is mainly responsible for angiogenesis and increase in vascular permeability, also known as vascular permeability factor. Apart from altering permeability, VEGF leads to vasodilation by increasing nitric oxide synthesis in endothelial cells. Additionally, it stimulates cell migration and suppresses apoptosis.

VEGF is a 35-45 kDa dimeric glycoprotein bound with disulphide bonds. It is secreted by many tissues including brain, liver, kidney and spleen apart from endothelium cells. Hypoxia is one of the most important regulators of VEGF expression, because increased VEGF expression due to hypoxia is observed in many diseases characterized with angiogenesis including cancer.

**VEGF Receptors**

The obtained information until now indicate that VEGF and its receptors are important in both regulation of neovascularization and pathological angiogenesis. The most well known disease associated with angiogenesis is cancer. The relation between tumour formation and angiogenesis was noticed almost a hundred years ago. However cancer investigators start to pay more attention to this topic after finding some hints showing that tumour cells can change their own growth by releasing angiogenic factors. Prevention of tumour blood circulation by blocking these angiogenic factors has brought new approaches to fight with cancer. Indeed some studies demonstrated that not only prevention of blood circulation but also lymphatic circulation inhibited both tumour growth and metastatic invasion.

VEGF receptor family consists of three members: VEGF-R1 (Flt-1), VEGF-R2 (KDR/Flk-1) and VEGF-R3 (Flt-4) which belong to receptor tyrosine kinase super family. VEGF-R1 and VEGF-R2 beside single tyrosine kinase transmembrane domains have extracellular immunoglobulin-like domains and are expressed in a variety of cells.

Each VEGF family member show different affinity to different receptors. For example, while VEGF-A binds strongly to VEGF-R1, it does not bind to VEGF-R3. But, VEGF-R-3 binds only VEGF-C and VEGF-D. Different expression patterns are present for the receptors and each receptor has also different in-vivo biological functions.

**VEGF-R1 (Flt-1)**

VEGF-R1 is a 180 kDa receptor that is present both in vascular endothelial cells and non-endothelial haematopoietic stem cells, macrophages and monocytes. It binds VEGF-A, VEGF-B and PIGF with high affinity. VEGF-R1 is also expressed in spermatogenic cells, leydig cells and osteoclasts. Therefore, it can be involved in male sterility due to the increase production of VEGF-A.

VEGF-R1 gene codes 2 types of polypeptides: a membrane protein with exact length and soluble form that misses intracellular and transmembranous pieces. Soluble VEGF-R1 is highly expressed in placenta. sVEGF-R1 plays an important role in maintenance of placental blood vessel net and permeability in normal borders during pregnancy.

Many studies reported that VEGF-R1, receptor of both VEGF and PIGF, can be expressed in many cancer types including NSCLC.

**VEGF-R2 (KDR, Flk-1)**

It is a 200-230 kDa receptor with high affinity to VEGF-A, -C, -D, and -E. It is expressed both in vascular and lymphatic endothelium. Its presence has been also shown in megakaryocytic and haematopoietic stem cells.

**VEGF-R3 (Flt-4)**

It is an 195 kDa receptor which takes part as a receptor for VEGF-C and VEGF-D. It is expressed only in lymphatic epithelium. Activation of VEGF-R3 induces proliferation, migration and survival of lymphatic cells. Soluble form of VEGF-R3 has been demonstrated to suppress lymph angiogenesis and, therefore, reduce metastasis to regional lymph nodes.
VEGF-Receptors and Lung Cancer

VEGF and its receptors are expressed in cancer cells, in both NSCLC and SCLC. VEGF expression is significantly correlated with neovascularization in resected NSCLC tissues and may be used as a prognostic factor. According to the report of Nishi et al, VEGF overexpression in surgically resected adenocarcinomatous lung tissue was indicative of earlier postoperative relapse. Overall, patients with lower serum VEGF levels had longer survival compared to the patients with higher VEGF levels. The measurement of serum VEGF has also been shown to be a marker for the response of patients to chemotherapy, as decreases in VEGF levels at week 12 after the initiation of chemotherapy was demonstrated to be correlated with response to therapy. In a recent study, pre-treatment VEGF serum levels proved to be an independent prognostic factor in patients with metastatic NSCLC. Lung cancer represents an area where the role of VEGF in prognosis tends to be more established, and further therapeutic implications targeting VEGF are already in progress.

VEGF-R1 expressions were shown in several tumor tissues. Decaussin et al showed that VEGF and its two receptors are expressed not only in tumor cells but also in fibroblasts and endothelial cells. In tumor cells, the levels of expression of VEGF and the VEGF-R1 in lung cancer were correlated, suggesting an autocrine function of VEGF on tumor cells via the VEGF-R1 receptor. According to the results of the study performed by Ilhan et al, high levels of serum VEGF and sVEGF-R1 before the treatment might give partial prognostic information in patients with various types of 42 lung cancer patients. They found that high levels of VEGF and sVEGF-R1 were observed when the stage of disease was high and the prognosis was worst. In agreement with the latter, our study demonstrated that the pretreatment sVEGF-R1 levels were significantly lower in progressive disease.

Seto et al. also examined the prognostic value of the expression of VEGF and of the VEGF-Rs, fms-like tyrosine kinase receptor-1 (flt-1) and kinase insert domain-containing receptor (KDR) in NSCLS. And their data suggested that expression of VEGF and VEGF-Rs are associated with a poor prognosis via autocrine and paracrine growth stimulation of cancer cells. Moreover, tumors expressing both flt-1 and KDR may have greater malignant potential and are associated with a poor prognosis. Furthermore, the synergy between the VEGF-R1 and VEGF-R2 specific ligands, indicative of “cross-talk” between the receptors, allowing modulation of a variety of VEGF-R dependent signals are reported by Carmeliet and coworkers.

As a conclusion carcinogenesis, inflammation, and atherosclerosis tumor growth and metastasis is stimulated by VEGF-R1. For suppression of cancer metastasis the VEGF-R1 signaling can be an important target.

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