Vascular endothelial growth factor (VEGF) — part 2: in endocrinology and oncology

Naczyniowo-śródbłonkowy czynnik wzrostu (VEGF) — część 2: w endokrynologii i onkologii

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Abstract

Endocrine glands are well vascularised and the structure of their vessels facilitates the exchange of various substances, including hormones. These glands are a frequent experimental model in research on VEGF and angiogenesis. VEGF participates in the pathogenesis of diabetes. Diabetic nephropathy is in essence a microvascular disease that develops as a result of a confluence of haemodynamic and metabolic perturbations. Diabetic retinopathy is the commonest microvascular complication of diabetes mellitus and is the leading cause of blindness. In diabetic retinopathy, ischaemic states, and hence tissue hypoxia and angiogenesis, take place. The participation of angiogenesis and VEGF in the pathogenesis of neoplastic disease has been described in many papers. VEGF protein and mRNA have been found in cancers of the thyroid, bronchus, lungs, oesophagus, stomach, colon, liver, breast, ovary, uterus, kidney, and urinary bladder, and in malignant tumours of the brain and bone. There have been many reports of the connections between the degree of VEGF expression and tumour aggression and prognosis in patients. Richly vascularised are GEP NET. In neuroendocrine tumours, strong expression of VEGF, Flt-1 and KDR in relation to the unchanged surrounding tissues has been demonstrated. Depending on the disease entity or the degree of its severity, attempts to apply angiogenic and antiangiogenic therapy have being made. Antiangiogenic therapy (usually regarded as a form of cancer therapy) is based on: 1. inhibitory effects of proangiogenic ligands and their receptors; 2. stimulation or delivery of angiogenesis inhibitors; and 3. direct destruction of neoplastic tumour vasculature. **(Pol J Endocrinol 2011; 62 (5): 456–464)**

Key words: *VEGF, angiogenesis, KDR, Flt-1, endocrine glands, pituitary, thyroid, diabetes mellitus, liver, GEP NET, cancer, neoplasm, hepatocellular carcinoma, oncology, growth factor*

Streszczenie

Gruczoły wewnętrznego wydzielania są dobrze unaczynione, a struktura ich naczyń ułatwia wymianę różnych substancji, w tym hormonalnych. Gruczoły te są częstym modelem doświadczalnym w badaniach nad VEGF i angiogenezą. VEGF uczestniczy w patogenezie cukrzycy. Nefropatia cukrzycowa jest chorobą mikronaczyniową rozwijającą się w wyniku nakładania się zaburzeń hemodynamicznych i metabolicznych. Do niedokrwienia, a co za tym idzie niedotlenienia tkanek i angiogenezy dochodzi w retinopatii cukrzycowej, będącej jedną z przyczyn ślepoty. Udział angiogenezy i VEGF w patogenezie choroby nowotworowej opisano w wielu pracach. Obecność białka i mRNA VEGF stwierdzono w raku tarczycy, oskrzela, przełyku, płuc, żołądka, jelita grubego, wątroby, sutka, jajnika, macicy, nerki, pęcherza moczowego, guzach mózgu, kości. Powiązano stopień ekspresji VEGF z agresywnością guza i rokowaniem u chorych. Bogato unaczynione są GEP NET. W guzach neuroendokrynnych wykazano silną ekspresję VEGF, Flt-1 i KDR w stosunku do niezmienionych tkanek otaczających. W zależności od rodzaju jednostki chorobowej, bądź też stopnia jej zaawansowania podejmuje się próby zastosowania terapii angiogennej i antyangiogennej. Terapia antyangiogenna (najczęściej traktowana jako jedna z form terapii przeciwnowotworowej) opiera się na: 1. hamującym oddziaływaniu z proangiogennymi ligandami i ich receptorami; 2. pobudzeniu lub dostarczeniu inhibitorów angiogenezy; 3. bezpośrednim niszczeniu unaczynienia guza. **(Endokrynol Pol 2011; 62 (5): 456–464)**

Słowa kluczowe: *VEGF, angiogeneza, KDR, Flt-1, gruczoły endokrynne, przysadka, tarczyca, cukrzyca, wątroba, GEP NET, nowotwór, rak wątroby, onkologia, czynnik wzrostu*

VEGF and angiogenesis in endocrinology and diabetology

Endocrine glands are well vascularised and the structure of their vessels (fenestration of epithelium) facilitates the exchange of various substances, including hormones [1]. These glands are a frequent experimental model in research into angiogenesis.

The amount of vascular endothelial growth factor (VEGF) in the thyroid gland is greater in the frequently occurring parenchymal and nodular goitres compared to healthy subjects, although a difference between the tissues is not stated. TSH stimulation of the thyroid cells culture induces their proliferation [1]. Stimulation of human thyrocytes by the thyroid-stimulating hormone (TSH) and antibodies against TSH receptor (TRAb) leads

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to an increase in their mRNA VEGF expression and *in vivo* to an increased mRNA expression of VEGF, Flt-1 (fms-like tyrosine kinase-1, VEGFR1) and KDR (Flk-1, fetal liver kinase-1, VEGFR2) in the endothelial cells (EC) of thyroid rats. This points to the participation of VEGF in angiogenesis occurring in the thyroid gland, also in humans with Graves' disease [2, 3] in whom the constant stimulation of thyroid tissue by TRAb not only increases the production of thyroid hormones, but also enhances angiogenesis [1], leading to an increase in thyroid vascularity which has long been widely known. In people with Graves' disease, VEGF mRNA has been found to belocalised in the hyperplastic thyroid follicular cells, and mRNA and protein of Flt-1 in the EC of all thyroid tissues [4]. It has been found that serum VEGF levels are elevated in patients with untreated Graves' disease and Hashimoto's disease, and correlate positively with the degree of thyroid vascularity assessed by colour Doppler ultrasound. VEGF C and Flt-4 have also been observed in human tissues of goitre and autoimmune thyroiditis [1, 3]. Tissues of lymphocytic thyroiditis and differentiated thyroid carcinomas have a stronger expression of VEGF than healthy gland tissues [3, 5]. Increased VEGF expression in malignant thyroid tumours has been shown not only in comparison with healthy tissue, but also in relation to benign tumours, although there was no difference between the microcarcinoma papillary tissue and the tissue of healthy glands. In papillary and follicular cancers, growing VEGF expression coincides with increasing cancer cell proliferation assessed by Ki-67 index [1]. The thyroid carcinomas cells having more mRNA and protein of VEGF are characterised by increased mitotic activity [5]. It has been shown that the higher the VEGF expression, the larger the malignant thyroid tumour size [1] and that these tumours are more vascularised than normal thyroid tissue [1, 6]. A large number of small blood vessels have been detected in the tissues of recurrent papillary thyroid cancer. It has even been suggested that there is a correlation between the amount of vessels in the tumour tissue and the prognosis for patients with papillary cancer and medullary thyroid cancer [7]. In children and young adults, the expression of VEGF and Flt-1 in papillary thyroid cancer tissues is correlated with tumour size [8]. The thyroid cancer cells of primary tumours taken from patients with metastases had a higher VEGF expression compared to cells taken from primary tumours of patients without metastases [5], and thyroid cancer metastatic cells in lymph nodes had a higher VEGF expression from cells of primary focus [1]. It has been observed that the degree of VEGF expression within the tumour correlates with its aggression [1, 6]. These observations have been suggested to be clinically useful in identifying patients who are more likely to develop metastases. VEGF mRNA levels are similar in primary tumours and metastases, but in the cells of papillary and follicular carcinoma and in Hurthle cells, they are higher compared to the cells of medullary carcinoma, benign tumour, and tissue hyperplastic (in Graves' disease) [9]. Flt-1 and Flt-1 mRNA (identified using RT-PCR) have been located in the EC of both healthy and cancerous thyroid tissue (papillary and follicular carcinoma) [1]. The VEGF A, vascular endothelial growth factor receptors 1 and 2 (VEGFR1 and VEGFR2) immunoreactive proteins are overexpressed in medullary thyroid carcinoma lesions and might be implicated in tumour progression [10]. It has also been suggested that VEGF polymorphisms and mRNA expression may predict the level of aggression of thyroid cancer [11]. On the other hand, VEGF D serum levels are reduced in patients with metastases of differentiated thyroid cancer, regardless of the degree of metastatic spread. It is possible that some other molecule produced by the tumoural tissue could affect the VEGF D physiologically produced from different tissues, thus leading to a decrease of the VEGF D found in the blood of patients with evidence of metastatic differentiated thyroid cancer [12]. However, in the light of current knowledge, VEGF does not meet the criteria for a marker of thyroid cancer.

In discussing the pathology of parathyroid glands, it is worth noting that in the parathyroid tissue subjected to autotransplantation, angiogenesis occurs, and parathyroid hormone (PTH) regulates the expression of VEGF and metalloproteinases (MMPs) [6].

Primary pituitary tumours, in contrast to neoplastic changes of other endocrine organs, tend to be less vascularised than normal pituitary tissue and no relationship has been found between the intensity of tumour cell proliferation and microvessels density (MVD) [1, 13]. The mechanism for this is unknown [1]. These observations concern hormonally active pituitary tumours; in particular, VEGF mRNA expression in tyreotropinoma was lower than the expression in normal gland tissues. VEGF mRNA expression in hormonally inactive pituitary tumours was higher than the expression in normal tissues of the gland. KDR mRNA expression in all types of pituitary tumours — hormonally inactive, and secreting growth hormones (GH), prolactin (PR), adenocorticotropic hormone (ACTH), TSH — was higher than the expression in normal gland tissue, especially in tyreotropinoma. In pituitary tumours, there is a positive correlation between mRNA expression of VEGF and KDR and mRNA PTTG (pituitary tumour transforming gene) expression and between the expression of mRNA VEGF and mRNA FGF2. It appears that PTTG transcriptional activity contributes to VEGF stimulation [14]. In pituitary cell lines, transforming growth

factor beta 1 (TGFβ1) stimulates the production of VEGF in a dose-dependent manner, and this effect also depends on the type of target cell [15]. In patients with active acromegaly, the proangiogenic systemic effects of GH and insulin-like growth factor 1 (IGF-I), at least in part, depend on the activation of VEGF by IGF-I [1, 16]. Applied in the treatment of active acromegaly, somatostatin analogues inhibit angiogenesis directly through the receptors located on the endothelium cells (inhibition of EC proliferation) and indirectly through inhibition of secretion of GH, IGF-I, VEGF and other growth factors. It follows that somatostatin analogues, both directly and by inhibiting the proangiogenic effects of IGF-I and VEGF on tissue, protect the organism against the effects of widely understood angiogenesis (in this case the pathological) and can reasonably be expected to reduce GH secreting pituitary tumour (somatotropinoma) [17]. Ki-67, p53, and VEGF were evaluated concomitantly in GH-secreting adenomas to determine which of them could be useful in distinguishing an invasive adenoma from a non-invasive. Both Ki-67 and p53 expressions showed no correlation with the invasive character of adenomas, but VEGF expression in invasive adenomas was significantly higher with respect to the noninvasive group. The results from the study suggest that VEGF is an independent stimulator of angiogenic growth and progression for GH-secreting adenomas, with > 25% cytoplasmic immunoreactivity. This cut-off value may be useful in determining prognosis and appropriate treatment strategy. Additionally, preoperative octreotide treatment may be useful as an adjunctive therapy, especially for locally invasive GH-secreting adenomas [18]. Prolactinomas have higher VEGF protein expression compared to nonfunctioning or ACTH- and GH-secreting adenomas [13]. Large pituitary tumours secreting prolactin (macroprolactinoma) are better vascularised than microprolactinoma; invasive prolactinoma are better vascularised than noninvasive tumours; and pituitary cancers are better vascularised than benign tumours. Poor vascularity is characteristic of slow-growing pituitary adenomas. Invasive adenomas appear to be better vascularised than those that are not invasive [17]. The effectiveness of surgical treatment of poorly vascularised macroprolactinoma is greater than the well-vascularised. Dopamine agonists used to treat prolactinoma inhibit VEGF signalling. Dopamine agonists therefore have antiangiogenic activity. Dexamethasone also inhibits VEGF secretion by the majority of pituitary tumors, even when the use of certain glucocorticoids in their treatment is taken into account [7]. Oestrogen-induced pituitary prolactin-producing tumours (PRLoma) in rats express high VEGF levels and anti-VEGF antibodies exert an inhibitory effect on pituitary tumourigenesis in oestrogen-induced PRLomas. These inhibitory effects due to anti-VEGF antibody might be related to the autocrine/paracrine action of VEGF on the tumour cells, because VEGF and its receptor are coexpressed on the tumour cells [19]. The data obtained from a subsequent animal experiment demonstrated that the antiangiogenic approach was effective in inhibiting the growth of dopamine-resistant prolactinomas. No differences in VEGF protein expression were observed after either anti-VEGF treatment (direct intra-adenoma Flt-1/Fc chimera injection), and, although serum VEGF was increased in specific monoclonal antibody G6-31-treated mice (systemic VEGF neutralisation), pituitary activation of the KDR signalling pathway was reduced. These results indicate that even though the role of angiogenesis in pituitary adenomas is contentious, VEGF may contribute to adequate vascular supply and represent a supplementary therapeutic target in dopamine agonist-resistant prolactinomas [20]. Tumor necrosis factor alpha (TNFα) significantly correlates with intratumoural haemorrhage in pituitary adenomas and induces pituitary adenoma haemorrhage through up-regulation of VEGF and MMP-9. Based on these findings, it was concluded that TNF-α may play a role in the development of haemorrhage within pituitary adenomas [21].

Sex hormones also exert effects on angiogenesis — oestrogens may lead to both increased and reduced VEGF gene transcription, while decreasing prostate vascularity has been observed after castration [1].

VEGF participates in the pathogenesis of diabetes. Diabetic nephropathy is in essence a microvascular disease that develops as a result of a confluence of haemodynamic and metabolic perturbations. Angiogenic factors are prime candidates to explain the vascular and pathologic findings of diabetic nephropathy; however, analysis of their pathophysiology shows that they have a constellation of effects on the glomerulus that go beyond angiogenesis [22]. It has been shown that there is increased VEGF expression (its mRNA and protein) in the kidneys of patients with diabetic nephropathy, which probably helps to rebuild the structure of the kidney and preserve their integrity [22, 23]. Particularly abundant expression of VEGF has been found in the kidney glomerulus and podocytes. Changes in the VEGF in the kidney occur at an early stage of diabetes. In the early period of type 1 diabetes, there is an increase in plasma VEGF concentrations, which has not been observed in patients with proteinuria or in patients with reduced glomerular filtration [17, 22]. Diabetic retinopathy is the commonest microvascular complication of diabetes mellitus and is the leading cause of blindness. In diabetic retinopathy, ischaemic states and hence tissue

hypoxia and angiogenesis, take place [7, 24]. Serum inflammatory cytokines interleukin 1 beta (IL-1β), IL-6, TNFα and VEGF influence the development of diabetic retinopathy [25]. Streptozotocin-induced diabetes in rats coexists with increased mRNA and protein expression of VEGF and KDR in the kidneys, with KDR increased in the first three weeks of diabetes, later returning to baseline. In turn, *in vitro*, in mesangial rat cells lines, glucose in high concentration directly increases the expression of VEGF [17].

VEGF and angiogenesis in oncology

The observation that angiogenesis occurs around a neoplastic tumour was made more than a century ago [26], and a hypothesis that the tumour produces soluble angiogenic substances was presented in 1968 [27]. In later years, Folkman proposed that tumour growth and metastasis depend on the angiogenesis, and the blockage of angiogenesis may be one strategy for inhibiting tumour growth [28]. Mutations within protooncogenes lead to uncontrolled production of growth factors, including angiogenic factors. For example, mutations of ras and p53 gene increase the expression of VEGF [23]. It has been shown that cells of tissues at the preneoplastic stage require the acquisition of angiogenic capacity to become cancer cells, and that without blood vessels the tumours cannot grow and give metastases [7, 29, 30]. Tumour vessels are characterised by abnormal structure and function. In contrast to the normal, distribution of blood vessels is chaotic, the diameter is inadequate to the needs, they have irregular course, many connections, breaks in the wall (fenestration, lack of continuity, lack of basal membrane), are excessively branched and permeable. This is due to an imbalance between the regulators of angiogenesis. Accordingly, tumour blood flow is chaotic, leading to ischaemia and then hypoxia and acidosis of the tumour areas. Such conditions make it impossible to obtain the proper drug concentrations in certain tumour areas affecting the lack of therapy effectiveness, change the production of activators and inhibitors of angiogenesis, and facilitate metastasis [7, 29, 31]. In tumours with a low vascular permeability, overexpression of angiopoietin 1 (Ang-1) and/or low expression of VEGF have been found. On the other hand, in tumours with a high vascular permeability, no evidence of Ang-1 expression or overexpression of its antagonist angiopoietin 2 (Ang-2) have been observed. Intensification of vascular permeability and angiogenesis depend on the type of tumour and the organ in which the tumour grows, because each organ has a different environment surrounding the tumour. Hypoxia promotes the expansion of monoclonal cells, which have lost their ability to induce apoptosis in re-

sponse to hypoxia [7, 29]. Hypoxia does not always lead to the expression of VEGF in tumour tissues; VEGF also arises under conditions independent of hypoxia [32]. Pro- and antiangiogenic molecules are also synthesised in the cells of the tissue surrounding the tumour [33]. Monocytes, mast cells and lymphocytes, that infiltrate tumour and surroundings tissues, are an important source of angiogenic factors, including VEGF [34]. Adequate blood supply to the tumour and increased vascular permeability, mainly through VEGF, helping tumour cells reach the extracellular space and the bloodstream [30, 35] in consequence leads to the start of the process of metastasis formation [36].

The participation of angiogenesis and VEGF in the pathogenesis of neoplastic disease has been described in many papers [16, 30, 31, 37, 38]. The presence of VEGF has been found in cancers of the thyroid [10, 39], bronchus, stomach, colon, breast, ovary, kidney, and urinary bladder [39]. VEGF mRNA expression has been demonstrated in malignant tumours of the brain, oesophagus, stomach, colon, liver, breast, ovary, kidney, and urinary bladder [23, 40]. High VEGF concentrations in the blood have been found in patients with oesophageal cancer [41], colorectal cancer, cancer of the breast [42], ovary [43], uterus [44], bone [45], and hormone-resistant prostate cancer [46], although it has also been shown that the VEGF concentration in blood vessels draining the tumour is within the norm [47]. There has been a series of reports of connections between the degree of VEGF expression with tumour aggression and prognosis in patients with cancer of the uterus, ovary [32], breast [1, 32, 48], stomach [49], melanoma [50], head and neck neoplasms [32], and small cell lung cancer [1] . Similarly, high VEGF expression coexists with worse survival time and an increased probability of recurrence of malignant colon, rectum and kidney neoplasms [32]. Using methods of RT-PCR and in situ hybridisation, it has been demonstrated that invasive tumours of the colon are characterised by an increased VEGF expression in comparison with preneoplastic states [51]. Expression of VEGF mRNA in colon cancer correlates with its progression [52]. Moreover, the VEGF and KDR expression in colon cancer tissue correlates with the degree of tumour vascularisation, the presence of metastases and the intensity of cancer cells proliferation [53]. In cancer of the breast, stomach and urinary bladder, intensification of angiogenesis within the tumour (assessed as MVD) [17] is associated with the development of metastases, poor prognosis, and shorter survival time [54]. VEGF mRNA expression in breast cancer tissue is higher in comparison with the surrounding tissue, and its high levels correlate with worse prognosis, regardless of the presence of metastases in adjacent lymph nodes. The

amount of VEGF protein in breast ductal carcinoma tissue correlates with the density of blood vessels. It has also been found to be cyclical, depending on the phase of the menstrual cycle (modulated by the influence of oestrogen on the EC) and the variability of VEGF expression in breast cells and breast cancer cells [48]. In breast cancer, the number of metastases is dependent on the expression of VEGF and KDR, but not of Flt-1 [35]. The presence of Flt-1 and KDR in ovarian cancer cells has also been detected [55]. In patients with lung cancer (squamous cell), an inverse correlation between apoptosis and angiogenesis i.e. between the proapoptotic (Fas, caspase-3) and angiogenic (VEGF, MVD) parameters, has been demonstrated [56]. Studies *in vivo* and *in vitro* have shown that in keratinocytes, fibroblasts and capillaries, and neoplasms of skin (such as melanoma), 24-hour hypoxia increases VEGF secretion, stimulates Flt-1, and (unlike most tissues) inhibits KDR [57]. *In vitro* VEGF gene expression is higher in several cancer cell lines and tumours of the brain, gastrointestinal tract, liver, and kidney compared to healthy tissue [58–61]. *In vitro* synthesis of VEGF induced by basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) is inhibited by 50% after the administration of somatostatin analogues. These analogues inhibit VEGF expression in tumours of the colon and rectum, which coexists with decreased serum VEGF concentrations [17]. In a recent study, assessed by microarray, VEGF expression was higher in primary malignant tumours of the head, neck, breast, oesophagus, colon, rectum, ovary, cervix, kidney, skin and white blood cells compared to the corresponding normal tissue. Malignant neoplasms tissues of lung, ovary and thyroid which are found to hypoxia-inducible factor 1 alpha (HIF-1α) also showed higher expression of VEGF. However, VEGF expression was not reported in all neoplastic changes. Studies using DNA microarrays revealed that the expression is reduced in the tissues of benign prostate hyperplasia, and of primary malignant tumours in the tissues of the lymphatic system, prostate, stomach, and testes. The authors of these observations have drawn attention to the way the control tissues were matched. They noted that the control of tissue derived from the tumour margin may be involved in the mechanisms of paracrine interaction. Thus, the tissue surrounding the tissue of testicular cancer often shows histopathological changes. It has been found that there is no difference in the expression of VEGF between healthy tissue and the tissue surrounding the colon, ovary, breast, lung, and cervix [32].

Primary hepatocellular carcinoma resulting from chronic hepatitis and then liver cirrhosis is a common experimental model and also one of the cancers in which growth and metastasis are affected by angiogenesis and VEGF [30, 40, 62, 63]. Hepatocellular carcinoma (HCC) is a well-vascularised tumour [30, 31] and the vasculature is probably dependent on the size of the tumour and on the degree of histological differentiation. In HCC with a diameter exceeding 2 cm, arterial vessels and the capillary network are well developed, and the tumour is visible on angiography, unlike small tumours [40]. It has been suggested that the excessive vascularisation is characteristic of HCC, irrespective of its degree of differentiation [64], and MVD is similar in tumours < 5 cm and larger, but is greater in those that coexist with metastases [65]. VEGF expression in HCC tissues and metastases of other cancers of the liver is evident at both mRNA and protein level [30, 31, 40, 62, 65–67]. Yamaguchi et al. [40], using the RT-PCR technique, demonstrated that VEGF expression in HCC is closely related to the degree of histological differentiation of the tumour: in well-differentiated tumours it was highest (100% tumour cells) in comparison with moderately differentiated (88%) and poorly differentiated (67%) tumours. The opposite relationship was noted by Yao et al. [65]. An overexpression of mRNA [67] and protein of VEGF [67, 68] was found in HCC tissues compared to healthy subjects. In some studies, higher VEGF expression in HCC compared to cirrhotic liver tissue has been reported [65, 67], but in other works it has been reported to be lower [69]. VEGF expression is correlated positively with MVD, tumour size [65], with the HCC cells proliferation, increasing the tumour volume and its capsule formation [23], infiltration of the capsule [69], and the formation of intrahepatic metastases [65, 69]. This expression correlates with the mitotic index of HCC, but does not correlate with the biochemical profile, α-fetoprotein levels, severity of inflammation, gender or clinical stage of liver cirrhosis [23]. High expression of VEGF mRNA and its protein in HCC tissues (obtained by biopsy or following surgery) coexisted with a worse prognosis [69–71]. Serum VEGF concentration correlates with the VEGF protein level in the cytosol of the tumour and VEGF mRNA expression in the tumour, and these values were higher in a more advanced stage of HCC development [72]. Delivery of the VEGF gene into rat HCC *in vivo*, resulting in VEGF overexpression, tumour growth, intensification of angiogenesis but inhibition of VEGF expression, resulted in a reduction of tumour growth [73]. *In vitro* in a liver cancer cell line (HepG2), VEGF$_{121}$ and VEGF$_{165}$ overexpression were shown [74]. In another study, high expression of VEGF mRNA was reported in 52% of HCC. Flt-1 mRNA expression in 68% of HCC has also been demonstrated with significant differences in relation to the unaltered tissues and a positive

correlation between the amount of Flt-1 mRNA and VEGF mRNA. Due to the fact that MVD (assessed by immunohistochemistry using antibodies against CD34) was correlated negatively with the size of the tumour, it is believed that the assessment of angiogenesis by MVD measurement and VEGF expression seems to be more important in relation to the smaller size of HCC and its metastases [75]. However, it seems that this situation may be the result of slower — relative to the progression of tumour size — development of its vascularity (MVD). The mRNA expression of KDR and Flt-1 were detected respectively in 100% and 79% of HCC tissues, and in 29% and 36% of adjacent tissues, and there were found to be different amounts of KDR mRNA in HCC compared to non-cancerous tissue [76]. The amount of VEGF mRNA was 3.1 times greater than the amount of bFGF mRNA in rat HCC cells, and induced by bFGF, tumour growth was eliminated with KDR neutralising antibodies, which indicates that bFGF acts on HCC development and angiogenesis synergistically with VEGF and is the mediator of VEGF action [77]. It is possible that bFGF participates in the invasion of HCC into the surrounding tumour tissue [23]. In patients with HCC, very high VEGF blood concentrations were found compared to healthy controls, and chronic hepatitis and liver cirrhosis subjects [65, 78]. In patients with secondary liver cancer (with liver metastases), serum VEGF concentrations were higher (mean 503 pg/mL) compared to a group of cancer without liver metastases (mean 205 pg/mL) and to healthy people (mean 201 pg/mL) [79]. Preoperative serum VEGF levels were higher in those patients with HCC in whom the disease recurred after surgery [80]. Angiogenesis is closely associated with HCC metastasis and recurrence. In an experimental model, the comparison between a highly metastatic human HCC (LCI-D20) and a rare metastatic HCC (LCI-D35) showed that in the first there was development of blood vessels in the tumour. It was shown that serum concentrations of VEGF, intercellular adhesion molecule 1 (ICAM-1), plasminogen activator inhibitor 1 (PAI-1) levels correlated positively with invasiveness and ability to HCC metastasis [81]. A certain role in progression of HCC may be played by tissue factor (TF) [82] because in states of hypoxia the amounts of TF and VEGF are increased [83], and TF increases VEGF expression [82]. Assessment of MVD and VEGF expression in HCC could help in predicting the clinical course of HCC. The search for other prognostic markers is ongoing. In a microarray with material of HCC, 153 genes were assessed and a very clear osteopontin expression in HCC with metastatic potential was observed. In conjunction with this is the observation that antibodies to osteopontin *in vitro* inhibited the invasion of HCC cells and *in vivo* in mice inhibited metastasis to the lung [81].

Richly vascularised are GEP NET [84, 85]. In neuroendocrine tumours, strong expression of VEGF, Flt-1 and KDR in relation to the unchanged surrounding tissues has been demonstrated [86].

Angiogenic and antiangiogenic therapy

Depending on the disease entity or the degree of its severity, attempts to apply angiogenic and antiangiogenic therapy are being made.

Angiogenic therapy is based on the methods of VEGF delivery to ischaemic and hypoxic tissues — the injection of recombinant VEGF protein into the artery nourishing hypoxic tissue and administration of the VEGF cDNA into the area of ischaemia in order to stimulate the formation of new blood vessels. However, the new blood vessels may be more permeable, have a heterogeneous structure and chaotic course. It is uncertain whether the morphology of such modified vessels leads to an improvement of microcirculation. In gene therapy, viral vectors or plasmids have been used [29, 87]. With administration of exogenous VEGF [also in combination with hepatocyte growth factor (HGF)] is bound the hope for treatment of patients with liver damage in the course of severe inflammation [88] and cirrhosis. It has been shown that the delivery of VEGF plasmid to a cirrhotic rat liver induces fenestration formation and reduces pressure in the portal vein [87].

Antiangiogenic therapy (usually regarded as a form of cancer therapy) is based on: 1. inhibitory effects of proangiogenic ligands and their receptors; 2. stimulation or delivery of angiogenesis inhibitors; and 3. direct destruction of neoplastic tumour vasculature [20, 29–31, 36, 63, 89–94]. *In vivo* VEGF administration increased the formation of new capillaries by 236%, while applying antisense oligonucleotides Flt-1 or KDR before VEGF administration increased the formation of new capillaries by only 85% [95]. The recombinant soluble receptors sFlt-1 and sKDR inhibit *in vivo* angiogenesis in the retina, corpus luteum and neoplasms, and transfection (using retrovirus) a mutated KDR receptor inhibits VEGF signal transduction and growth of glioblastoma multiforme. Expression of sFlt-4 inhibits lymphangiogenesis in mouse embryos. Antiangiogenic therapy clinical trials in patients with Von Hippel-Lindau disease [1] and hepatic haemangiomas (bevacizumab) [89] are ongoing. Experimental evaluation of antiangiogenic substances such as suramin, TNP-470, endostatin, and interferon alpha (IFNα) has demonstrated their effectiveness in inhibiting the metastasis of human HCC [81], but also demonstrated that the anti-KDR antibodies marginally inhibit liver regeneration [96]. The recently completed

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clinical trials of sorafenib, an inhibitor of VEGF kinase, have confirmed its usefulness in the treatment of even advanced HCC [31, 63]. Practical applications of monoclonal antibodies anti-VEGF (bevacizumab, ranibizumab) have already been found [30, 36, 89, 90, 92–94], for example in colorectal cancer patients with metastases to the liver [89, 90]. Although there were previously doubts as to whether their use (in combination with chemotherapy) might not reduce the regenerative capabilities of the liver after surgery (embolisation) of metastases, these doubts have now been dispelled [90]. Bevacizumab is a recombinant humanised monoclonal antibody that targets VEGF. The addition of bevacizumab to chemotherapy has improved progression-free survival in the first- and second-line treatment of patients with advanced-stage breast cancer. The clinical trials testing the utility of bevacizumab for the treatment of metastatic disease are continuing [36]. An alternative strategy for the treatment of liver fibrosis and HCC may be ACE inhibitors and angiotensin receptor 1 blockers, which are widely used in clinical practice and do not have significant side effects. The renin-angiotensin system is frequently activated in patients with chronic liver disease, and angiotensin II has been shown to have proangiogenic effects and to induce VEGF. Angiotensin-converting enzyme (ACE) inhibitors that are also inhibitors of angiogenesis, and inhibitors of growth such as the experimental HCC, cause suppression of VEGF synthesis/secretion [97]. Moreover, in the process of liver fibrosis, angiotensin II stimulates the production of tissue inhibitor of metalloproteinases 1 (TIMP-1) in activated hepatic stellate cells (HSC) and HSC proliferation, while ACE inhibitors (enalapril, perindopril) have the opposite effect [97–99]. Antiangiogenic properties are exhibited by IFNα, which inhibits the migration of EC *in vitro* [100] and *in vivo* [101], inhibits the activity of molecules involved in the angiogenic response (e.g. bFGF, IL-8 and MMP-3) [86], reduces the VEGF expression in many types of human cancer cells [86, 102], and induces apoptosis in vessels EC of colon cancer metastases to the liver, following which the inhibition of division tumour cells is observed [103]. IFNα exhibits some efficacy in patients with haemangioma [104, 105], Kaposi's sarcoma [104], HCC [106] and GEP NET [84, 86]. The use of IFN in patients with GEP NET has led to a reduction in plasma VEGF levels and reduced VEGF mRNA levels and density of blood vessels in the material obtained during biopsy of the tumour metastases to the liver. In addition, *in vitro* on several cell lines of neuroendocrine tumours it has been shown that IFNα inhibits the transcriptional activity of the VEGF gene. IFNα remains one of the few agents discovered to date to effectively inhibit VEGF gene transcription. It has even been found that serum VEGF levels may be

a marker useful in the optimisation of IFNα therapy — providing the response to treatment, treatment duration and doses of IFNα [86]. Vandetanib is an orally active antagonist of VEGFR2, epidermal growth factor receptor (EGFR or HER1 or ErbB1) and RET kinase, and is now available in the US for the treatment of metastatic medullary thyroid cancer [91]. Changes in soluble VEGFR2 levels after initiation of therapy predicted response to motesanib in patients with advanced differentiated thyroid cancer or metastatic medullary thyroid cancer. Lower baseline VEGF levels have been associated with longer progression-free survival [107]. Results from recent clinical trials suggest a role for blockers of the renin-angiotensin system (ACE inhibitors and angiotensin II receptor blockers) and for fenofibrate in reducing progression and/or inducing regression of mild-to-moderate non-proliferative diabetic retinopathy. Intravitreal administration of anti-VEGF agents has been shown to reduce visual loss in more advanced stages of diabetic retinopathy, especially in macular oedema [94]. Ranibizumab (which binds to and inhibits a number of subtypes of VEGF A) should be considered for patients with diabetic macular oedema [92, 93].

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