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Vascular endothelial growth factor (VEGF) in physiological and pathological conditions

Vascular endothelial growth factor (VEGF), primarily described in 1989, is the major endothelial cell-specific stimulatory molecule involved in both physiological and pathological conditions (2, 3, 6, 9, 10, 11, 13). This growth factor is capable to regulate multiple endothelial cell functions, including mitogenesis, vascular permeability, endothelial cell survival and production of other vasoactive molecules (1, 3, 5, 6, 9, 10, 11). Although a lot of biologically active agents are involved in angiogenesis, VEGF appears to be a key paracrine regulator of this complex and multi-step process (2, 5, 6, 9, 10, 11). The unique role of VEGF is due to its high specificity for vascular endothelium and strong mitotic potency (2, 6, 9, 11). The powerful mitogenic activity of VEGF is directed to both blood and lymphatic vessel endothelium (9, 10, 11). However, various structural forms of this growth factor are capable to exert their mitogenic influence differently. Vascular endothelial growth factor is a heparin-binding homodimeric glycoprotein with a molecular weight of 34–45 kDa, which exists in at least five different isoforms consisted of 121, 145, 165, 189 and 206 amino acids (1, 2, 3, 9). The predominant isoforms are 165 and 121 VEGF (2, 5). The VEGF gene is located on the short arm of chromosome 6 (5). VEGF gene expression is regulated by several mechanisms, important of which is hypoxia (4, 5, 7, 9, 11). Other known factors capable to upregulate the VEGF expression include cytokines, among them EGF, TGF-α, TGF-β, KGF, IL-6 some oncogenes, like H-ras and also several transmembrane tyrosine kinases, such as EGF receptor (2, 6, 7, 8, 11). What is interesting, VEGF itself can influence the production of other active molecules, since it up-regulates the expression of plasminogen activator, plasminogen activator type I inhibitor and interstitial collagenase in the *in vitro* conditions (9). It is well established that the production of different forms of VEGF is controlled by the alternative mRNA splicing and proteolytic digestion (2, 5, 13). The VEGF family, of which VEGF-A is the main member, includes also VEGF-B, -C, -D, -E, and placental growth factor (2, 6, 9, 10, 11). Moreover, they share structural homology with the platetet-derived growth factor (6). The biological activities of VEGF are mediated by three tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3 (5, 6, 9, 11). The expression of these receptors is mostly restricted to the vascular endothelium (2, 5, 11, 13, 19), but VEGFR-1 is also expressed on monocytes and can induce their migration (5, 9). The proliferative and mitogenic activities of VEGF, as well as vascular permeability, are mediated primarily through VEGFR-2 (3, 5, 9, 10, 11). On the other hand, VEGFR-3, which is homologous with the neutrophilin-1 receptor, is believed to mediate lymphoangiogenesis (5, 13). All of these tyrosine kinase receptors bind VEGF with high affinity and have seven immunoglobulin-like chains in the extracellular domain, a single transmembrane region and a common tyrosine kinase sequence that is interrupted by a kinase insert domain (9). Recently, a soluble form of a VEGF type-1 receptor has

been identified (7). Soluble receptor binding VEGF with high affinity inhibits its mitogenic activity for endothelial cells and thus may have a regulatory role in angiogenesis (7). VEGF, like some other angiogenic agents, including b-FGF and angiogenin, needs copper as a essential co-factor (12). It has been observed that in the absence of copper neovascularization mediated by these cytokines is markedly reduced (12).

The cellular and tissue sources of VEGF have been investigated by various authors. It has been found that VEGF can be produced by normal healthy cells in various organs to maintain a good blood supply (1, 6, 9, 11). VEGF is expressed in macrophages, T-cells, smooth muscle cells, kidney cells, mesangial cells, keratinocytes, astrocytes, osteoblasts and tumor cells of various origin (1, 3, 6, 7, 11, 13, 14, 22). In the peripheral blood VEGF mRNA has ben shown in platelets, B cells, T cells, granulocytes, monocytes (7). It is believed that the majority of circulating VEGF is associated with platelets, and what is more, some amount of VEGF may be internalized from the plasma when platelets are travelling in the tumor vessels (7). So, it is suggested that platelets may not only transport VEGF to the target tissue but that they also have ability to protect this circulating protein from proteolysis as well (7). In circulation VEGF can be inactivated by binding to alpha-2 macroglobulin, and this binding can be inhibited by heparin and also by b-FGF (7). What is interesting, VEGF like another angiogenic factor b-FGF, binds to extracellular heparin-containing proteoglycans and can be released into a soluble and bioactive form by heparin and plasmin (7). So, the extracellular matrix may also serve as a biological reservoir for VEGF and the content of membrane heparan sulfate proteoglycans is likely to modulate the cell affinity for this growth factor (7, 9). Various forms of VEGF can preferentially influence different activities of this cytokine directed to target cells. Thus, VEGF-A is capable to induce angiogenesis, but lymphangiogenesis is regulated by VEGF-C and -D (5, 13). What is more, VEGF-C can induce either angiogenesis or lymphangiogenesis through binding to VEGFR-2 or VEGFR--3 on lymphatic endothelium, respectively (2, 13). Angiogenesis begins when injured cells release VEGF and other proangiogenic growth factors, such as FGF or PDGF into the surronding tissue (11, 10). VEGF released from wound epithelium and from the extracellular matrix by endothelial-derived proteases, stimulates endothelial cell proliferation and increases vascular permeability (2, 8). It has been evidenced that VEGF may be up-regulated in response to nitric oxide (NO), which also influences vasodilatation, a very early step of angiogenesis (8, 9). An increase in vascular permeability mediated by VEGF, is believed to be essential for angiogenesis in the wound healing and tumors (9, 11). It enables the extravasation of plasma proteins by inducing fenestrations on the endothelium which results in formation of an extravascular fibrin gel which serves as a substrate for endothelial and tumor cell growth (9, 11, 13). Extravasated proteins create a temporary support for migrating endothelial cells and monocytes (2, 9, 11, 13). Several agents are capable to block the permeability effect of VEGF. One of them is angiopoietin-1 – a natural antagonist for VEGF, inhibiting excessive plasma leakage (13). Moreover, it has been found that the permeability induced by VEGF may be also prevented by the inhibition of MAP kinase pathway (9).

VEGF is capable to influence all vital biological functions of the endothelial cells. Literature data indicate that this cytokine is not only essential for survival of human endothelial cells in newly formed vessels but can also delay their senescence and prevent the endothelial cell apoptosis induced by TNF-α as well (2, 3, 5, 9, 11, 13). The results of further studies have demonstrated that vascular endothelial cell is not an only target for VEGF. It has appeared that VEGF can stimulate the proliferation of T lymphocytes, the colony formation of granulocyte-macrophage progenitor cells and can inhibit the differentiation of dendritic cells (5, 7, 9). VEGF is also an autocrine growth factor for retinal epithelial cells, and stromal cells cultured from neonatal hemangiomas (9). In addition, VEGF is chemotactic for several cell types, including monocytes, lens epithelial or corneal endothelial cells as well as a differentiation factor for osteoblasts (9). Some other biological properties of VEGF have

been recognized recently. It may be of interest that acute and severe hypotension can result from VEGF-induced vasodilatation through an NO-dependent pathway (9).

Special attention of various authors has been directed to these tissues where angiogenesis is a continual or continuous phenomenon. Since the *in situ* hybridisation has demonstrated that VEGF and its receptor are temporally expressed during the menstrual cycle in the ovaries and uterus, which may suggest that VEGF is involved in hormonally regulated angiogenesis (9). Hair follicle growth also requires cyclical angiogenesis to initiate the proliferation and survival of the dermal papilla (9). Data of the immunohistochemical studies indicate that VEGF may in addition act as an autocrine factor for hair dermal papilla cells (9). The decreased expression of VEGF observed in alopecia patients seems to support this belief (9). As a key angiogenic inducer, VEGF is the main participant of the wound repair acting together with other potent mediators, among them FGFs, TGFs, TNF-a and IL-8 (6, 10, 11). VEGF has not only been found in tissue fluids of wounds but also is elevated in the epidermis overlying healing wounds (6, 10). Simultaneously, its type-1 and type-2 receptors have be found up--regulated in newly formed vessels (10). Apart from this, it appeared that the wound-edge keratinocytes and macrophages can actively release VEGF, possibly in response to KGF and TGF-α (6, 8). Interestingly, at least one of the VEGF receptors, has been also observed to be up-regulated on endothelial cells at the site of cutaneous injury (8).

VEGF, due to its central role in tumor angiogenesis, is associated with the growth and development of malignancies of various origin (6, 9, 11–15). Numerous reports have evidenced the elevated expression of VEGF and its receptors in a variety of human solid tumors, including breast cancer, central nervous system neoplasms, melanoma, gastrointestinal cancer, urinary tract cancer and ovarian cancer (3, 5, 7, 11–14). Considerably elevated levels of VEGF, both cellular and circulated, have been also observed in many hematologic malignancies, including non-Hodgkin disease and myeloid leukemia (5, 11) Although, the origin of circulating VEGF in the cancer patients is not clearly elucidated yet, it is strongly suggested that the major source of this cytokine is probably the tumor (4, 7, 9, 11, 14). Another evidenced sources of VEGF expression are tumor-infiltrating inflammatory cells in several histological types of cancer (7, 9, 11, 14). Results obtained by various authors have confirmed that the VEGF production in tumor cells is stimulated by several factors,including hypoxia, oncogenes, inactivation of tumor supressor genes, and other cytokines (4, 11, 13). Tumor cells and infiltrating cells such as macrophages, T lymphocytes and fibroblasts activate the endothelial cells, thus initiating angiogenesis by expressing factors such as VEGF and b-FGF (7, 9, 11). VEGF is usually overexpressed in the hypoxic periphery of necrotic areas, whereas its type-1 and type-2 receptors are up-regulated in the endothelial cells localized in the close vicinity (9).

Results of the recent studies indicate that VEGF is implicated not only in tumor development but also in metastasis formation (11, 13). It is believed that the up-regulation of angiogenic factors and the down-regulation of their inhibitors are the important steps in a progression from the premalignant quaiescent cells to a malignant invasive lesion (11, 13). It is already well known that tumor needs angiogenesis to grow beyond 1 to 2 mm^3 in size and to metastases formation and furthermore tumor can produce some active mediators, including VEGF, promoting its own invasiveness (11). VEGF secreted by tumor cells can induce the release of matrix metalloproteinases (MMPs) by endothelial cells (9, 13). Elevated levels of membrane MMPs have been demonstrated to degradate the cellular matrix, thus facilitating the migration of endothelial cells into the surrounding tissue (2, 9, 13). Furthermore, MMPs can release angiogenic factors like VEGF and b-FGF from their extracellular stores, increasing in this way the bioavailability of these two potent angiogenic and mitogenic cytokines (2, 13).

Data of many reports indicate that VEGF levels appear to have prognostic significance in a variety of human malignancies (5, 7, 11, 13–15). Overexpression of VEGF is associated with poor prognosis in acute myeloid leukemia, non-Hodgin disease, breast, lung, colonorectal, esophageal, gastric,

pancreatic, laryngeal, hepatic cancers (5, 7, 11, 12, 14). In these neoplasms, high levels of circulating VEGF are correlated with significantly shorter survival (5, 7, 11, 12). It is worth to stress that the highest prognostic power, especially in some hematologic proliferative diseases, has been observed when serum VEGF and b-FGF levels were examined in combination (5). It has been reported, that the elevated serum VEGF levels have been more frequently observed in patients with disseminated cancer than in subjects with localized disease, independently of the histological form of tumor (7). Additionally, the decrease in serum VEGF level parallel with partial remission due to the GM-CSF gene therapy in patient with metastatic renal cancer was observed (7). This finding suggests that serum VEGF could be a useful maker in the treatment monitoring of highly angiogenic tumors (7). Taking into consideration the well known high specificity of VEGF towards endothelial cells, the role of this growth factor in the development of neoplasms associated with the blood or lymphatic vessels is of special interest. It has been reported that proliferating haemangiomas show overexpression of VEGF-among other angiogenic activators, such as b-FGF, monocyte chemoattractant protein-1 (13). Many cytokines that can promote angiogenesis, lymphangiogenesis and vascular remodelling are known to be up-regulated in the Kaposi Sarcoma (KS) lesions (13). Among them are VEGF-A, FGF-2, IL-6, IL-8 (13). Some data indicate that the migration and proliferation of KS cells can be stimulated by VEGF *in vitro* (13). Recently, it has been suggested that VEGF-C acts as a paracrine growth factor for KS because its receptors VEGFR-2 and VEGF-3 have been demonstrated to be overexpressed by KS tumor cells (13).

Activity of VEGF and other powerful angiogenic inducers including b-FGF, IL-8 and placenta--derived growth factor has been studied in human melanoma cells and tumors. An elevated level of VEGF in the peripheral blood as well as its high expression in the tumor cells have been observed in the melanoma patients (7, 13, 14). Additionally, VEGF receptors have also shown the aberrant expression in human melanoma cells, but not in normal melanocytes (13, 14). The origin of elevated VEGF in melanoma patients is not clearly elucidated yet. Some authors believe that high VEGF amounts are partly derived from activated T-cells, macrophages and keratinocytes, but the others in contrast, suggest not only a strong correlation of VEGF levels with the quantity of tumor tissue but also believe that melanoma tissue is the main source of elevated circulating VEGF in these patients (14). Weber et al. (14) have found the significant correlation of serum VEGF level with the clinical stage of melanoma. They observed that serum VEGF levels in patients with distant metastases were significantly higher than those of patients without distant metastases (14). It is believed that, although the measurement of serum VEGF is not sufficiently specific or sensitive to serve as a reliable tumor marker for early detection of melanoma, but it can be rather useful prognostic tool in the later clinical stages (7, 13, 14). VEGF overexpression has been found to be associated with metastasis formation in melanoma patients (14). It seems specially interesting that the increased VEGF serum levels have been observed after surgery for pulmonary metastasis, which suggests that VEGF may induce the growth of latent micrometastasis (7).

Recent years provided data that VEGF is strongly overexpressed in many skin disorders (1, 2, 3, 6, 9). Previous studies showed that cultured normal keratinocytes synthesize this cytokine in its all biological forms (1). VEGF as the endothelial cell-specific mitogen represents a central regulator of cutaneous angiogenesis and its level is an important factor in maintaining balanced vascular homeostasis (6). VEGF in the cutaneous environment is a stimulator of hair growth, its production has been demonstrated in various hair follicle structures (9). It has been shown previously that the induction of VEGF in skin keratinocytes is mediated also by ultraviolet B radiation (3). Blaudschun et al. (3) found the significantly induced VEGF expression in the normal epidermis following UVB irradiation. What is more, they also evidenced that repetitive UVB irradiation during three days can result in enhanced angiogenesis in the skin of normal healthy volunteers (3). These results strongly indicate that VEGF plays a causal role in the UVB-dependent angiogenic response in skin (3).

VEGF activity has been studied especially in these skin diseases which are believed to be angiogenesis-dependent cutaneous disorders and where a dense network of microcapillaries is produced accompanied by inflammatory cells. Special interest is directed to psoriatic skin in which angiogenesis is the prominent phenomenon. Not only elevated levels of VEGF have been found in psoriatic sera but also the increased expression of this cytokine was detected in psoriatic skin (1, 3, 6, 13). It has been found that VEGF is produced predominantly by keratinocytes and to a minor degree by fibroblasts, in the clinically involved skin of patients with psoriasis (1, 2, 6). Moreover, the VEGF type-1 receptor levels were enhanced significantly in the psoriatic plaques as compared with uninvolved skin and normal healthy skin (1). In psoriatic skin elevated VEGF expression has been detected mostly in the suprabasal keratinocytes of psoriasis plaques, where TGF-α potently up-regulates the levels in the paracrine fashion (2, 6). Furthermore, elevated levels of VEGF observed in sera of patients with erythrodermic psoriasis may account for the vascular leakage and exudative symptoms observed in this severe form of psoriasis (1, 2).

The angiogenic ability of VEGF also plays a role in a variety of other pathological cutaneous conditions, including wound healing, rosacea, viral and seborrhoic warts, keratoacanthoma, bullous diseases, allergic contact dermatitis, non-melanoma neoplasms and melanoma (1, 2, 3, 6, 13). Last years, the possible involvement of VEGF has been carefully studied in the skin tumor growth. VEGF family has been shown to play a fundamental role in the growth and invasion of squamous cell carcinoma (SCC) (13). It has been evidenced that blocking function of the VEGF type-2 receptor by a neutralizing antibody can result in the inhibition of angiogenesis and invasion of malignant SCC (13). It is worth to stress that VEGFR-2 expression which was transiently up-regulated in precancerous lesions of actinic keratosis, has been found to be continuously overexpressed in malignant tumor (13). This finding strongly suggests an essential role of this receptor in SCC development and growth (13).

The exact role of VEGF in many pathological conditions is not fully recognized yet. Understanding of the VEGF biological properties, even if it is not complete, can provide some useful therapeutic tools in better controlling of the diseases connected with angiogenesis and cell proliferation. In recent years VEGF has become a target for experimental anticancer therapy (14). In some clinical cases, inhibition of tumor-secreted VEGF may be sufficient to significantly impair tumor growth and the forthcoming metastasis of the developed cancer (4, 6, 9, 11, 14, 15).

Up till now, several attempts to inhibit VEGF availability have proved to be succesful, including: monoclonal neutralizing antibodies, antisense oligonucleotides, soluble VEGFR1 fusion proteins, anti-VEGFR2 antibodies or immunotoxins against VEGFR2 (4, 6, 9, 14, 15). These methods have often resulted in inhibition of neovascularization, tumoral progression and metastasis formation (4, 6, 9, 11, 14, 15). Recombinant human monoclonal antibody against VEGF is being tried as a antitumor agent in a wide range of malignancies, including lung, breast, prostate, colorectal and renal cancers (4, 15). VEGF inhibition can be an attractive noninvasive therapeutic strategy because it is highly specific and may be less toxic than cytotoxic therapy (11). Generation of new potential therapeutic agents that can inhibit angiogenesis is now intensely investigated.

On the other hand, apart from the malignant tumor treatment, VEGF is believed to have also an increasing role as a positive regulator of angiogenesis in the diseases where its angiogenic propertes are needed. The possibility of using VEGF in the angiogenic therapy of human coronary heart disease and severe peripheral ischemic vascular disease has been reported (4). Good results of enhancing the local angiogenesis to produce neovascularization and reestablish perfusion to ischemic areas have already introduced these new therapeutic strategies into clinical practice.

REFERENCES

1. Bhushan M. et al.: Levels of endothelial cell stimulating angiogenesis factor and vascular endothelial growth factor are elevated in psoriasis. Br. J. Dermatol., 141, 1054, 1999.
2. Bhushan M. et al.: Recent advances in cutaneous angiogenesis. Br. J. Dermatol., 147, 418, 2002.
3. Blaudschun R. et al.: Vascular endothelial growth factor causally contributes to the angiogenic response upon ultraviolet B irradiation in vivo. Br. J. Dermatol., 146, 581, 2002.
4. Gibaldi M.: Regulating angiogenesis: a new therapeutic strategy. J. Clin. Pharmacol., 38, 898, 1998.
5. Giles F. J.: The vascular endothelial growth factor (VEGF) signaling pathway: a therapeutic target in patients with hematologic malignancies. The Oncologist, (suppl. 5), 32, 2002.
6. Gille J. et al.: Transforming growth factor-α-induced transcriptional activation of the vascular permeability factor (VPF/VEGF) gene requires AP-2-dependent DNA binding and transactivation. EMBOJ, 16,750, 1997.
7. Kuroi K., Toi M.: Circulating angiogenesis regulators in cancer patients. Int. J. Biol. Markers, 16, 5, 2001.
8. Martin P.: Wound healing – aiming for perfect skin regeneration. Science, 276, 75, 1997.
9. Ortega N. et al.: Signal relays in the VEGF system. Frontiers in Biosciences, 4, 141, 1999.
10. Paavonen K. et al.: Vascular endothelial growth factor receptor-3 in lymphogenesis in wound healing. Am. J. Pathol., 156, 1499, 2000.
11. Rosen L. S.: Clinical experience with angiogenesis signaling inhibitors: focus on VEGF blockers. Cancer Control, 9, 2 (suppl.), 36, 2002.
12. Teknos T. N.: A novel anti-angiogenesis strategy for the treatment of head and neck cancer. Michigan Oncology Journal (MOJ) Online, ed.: L.H. Baker, 2001.
13. Velasco P., Lange-Asschenfeld B.: Dermatological aspects of angiogenesis. Br. J. Dermatol., 147, 841, 2002.
14. Weber F. et al.: Vescular endothelial growth factor and basic fibroblast growth factor in melanoma. Br. J. Dermatol., 142, 370, 2000.
15. Zgodziński W. et al.: Angiogenesis inhibitors, new anticancer strategy. Pol. J. Pharmacol., 51, 455, 1999.

SUMMARY

Vascular endothelial growth factor (VEGF) is the major endothelial-cell specific stimulatory factor regarded as the most important positive regulator of angiogenesis and vascular permeability. Due to its powerful angiogenic and mitogenic properties, VEGF takes part in a variety of crucial biological processes, including wound repair and tumorigenesis. The elevated expression of VEGF and its receptors has been found in malignancies of various origin as being associated with metastasis formation and poor prognosis. Recently, some possibilities appeared to block the VEGF action when it is undesirable or promote its activity when it can be useful. These new therapeutic strategies based upon the knowledge of VEGF biological role, are being tried and up till now they proved to be successful, especially in anticancer therapy and the coronary heart disease treatment.

Śródbłonkowy czynnik wzrostu (VEGF) w procesach fizjologicznych i stanach chorobowych

Śródbłonkowy czynnik wzrostu (Vascular endothelial growth factor - VEGF) jest wysoce swoistym czynnikiem pobudzającym wzrost komórek śródbłonka naczyń, uważanym za najważniejszy dodatni regulator angiogenezy i przepuszczalności naczyń. Dzięki swoim właściwościom angiogennym i mitogennym VEGF bierze udział w wielu zasadniczych procesach biologicznych, między innymi w gojeniu ran i powstawaniu nowotworów. Uważa się, że zwiększona ekspresja VEGF i jego receptorów, obserwowana w przebiegu nowotworów złośliwych różnego pochodzenia, związana jest z powstawaniem przerzutów i złym rokowaniem. W ostatnich latach pojawiły się możliwości zmniejszania aktywności VEGF, gdy jest ona niepożądana, lub pobudzania działanie tej cytokiny, gdy jest to korzystne w leczeniu chorego. Te nowe nożliwości terapeutyczne, oparte na poznaniu biologicznej roli VEGF, są stale badane i okazały się przydatne zwłaszcza w leczeniu nowotworów i choroby wieńcowej serca.