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Czynniki proangiogenne i antyangiogenne w guzach nadnerczy

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Streszczenie
Formowanie nowych naczyń krwionośnych jest istotnym czynnikiem w procesie rozwoju nowotworu oraz dawaniu przerzutów odległych. Dlatego też badania nad czynnikami proangiogennymi oraz antyangiogennymi przyciągają uwagę wielu badaczy. W niniejszej pracy przedstwiamy czynniki pro- i antyangiogenne oraz ich rolę w tworzeniu nowych naczyń krwionośnych w guzach nadnerczy. Ocena procesów angiogenezy, jak również ocena wzoru naczyniowego w guzach nadnerczy, może być istotna dla rozróżnienia pomiędzy zmianami złośliwymi a łagodnymi. Wiedza na temat angiogenezy może być zatem pomocna w poszukiwaniu nowych strategii leczenia u chorych ze złośliwymi guzami nadnerczy.

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Słowa kluczowe: *angiogeneza, guzy nadnerczy, czynniki proangiogenne, czynniki antyangiogenne*

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Angiogenic and anti-angiogenic factors in adrenal tumours

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Abstract
It appears that neoangiogenesis is an important factor in tumour invasion and the formation of metastases in several human cancers, and studies on pro-angiogenic and anti-angiogenic factors are therefore of considerable interest to researchers. In this review we present pro-angiogenic and anti-angiogenic factors and other growth factors and their role in the formation of new blood vessels in adrenal tumours. Assessment of the angiogenic status of adrenal tumours and their vascular pattern may be useful for discriminating benign from malignant lesions and knowledge of their angiogenesis may be essential to the drawing up of promising treatment strategies for patients with malignant tumours.

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Key words: *angiogenesis, adrenal tumours, pro-angiogenic factors, anti-angiogenic factors*

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Introduction

Neoangiogenesis appears to be an important factor in tumour invasion and the formation of metastases in several human cancers, and studies on pro-angiogenic and anti-angiogenic factors are therefore of considerable interest to researchers. In this review pro-angiogenic and anti-angiogenic factors and other growth factors [1] are presented along with their role in the formation of new blood vessels in adrenal tumours.

Pro-angiogenic factors can be classified into several groups. As shown in Table I, these are heparin-binding growth factors, mediators of inflammation, hormones and adhesive molecules.

Vascular endolethial growth factor (VEGF) is one of the most important pro-angiogenic factors. VEGF is expressed on the endothelial cells [2–5] and in the stroma [6]. It acts through a VEGF receptor. The signalling pathway after the binding of VEGF to its receptor includes dimerisation and activation of thyrosine kinase [7]. So far three VEGF receptors have been investigated. VEGFR1 is involved in the signalling pathway, VEGFR2 appears to play a role in regulatory processes and VEGFR3 is connected with the process of lymphangiogenesis. The regulatory agents of VEGF gene expression include hypoxia, growth factor cytokines, hormones [8] and pharmacological agents. In conditions of hypoxia VEGF gene expression is increased. This overexpression is mediated via hypoxia-inducible factor-1 (HIF-1). HIF-1 consists of an HIF-1 alpha subunit and an HIF-1 beta subunit. The former is stable in conditions of hypoxia but unstable in the presence of oxygen. The latter is constitutively expressed. Dimer HIF-1 binds to the hypoxia response element in the VEGF gene promoter and induces VEGF gene transcription. Expression of VEGF is also regulated by other factors such as EGF (epidermal growth factor), TGF beta (transforming growth factor beta), FGF (fibroblast growth factor), IGF-1 (insulin growth factor-1) and IL-1 (interleukin-1) [9–13]. Within the VEGF gene there are two untranslated regions (near the 3′ end and the 5′ end). Oestrogens are able to bind to these regions and develop an inhibitory or stimulatory effect on VEGF gene expression [14]. Other hormones involved in the regulation of VEGF gene expression are growth hormone and IGF-1. It is known that growth hormone decreases VEGF gene expression. Some authors [15] suggest that GH and IGF-1 have an influence on VEGF gene expression via p44/42 MAPK (mitogen activated protein kinase) activation.

There have also been studies which provide evidence that testosterone may increase VEGF gene expression [16]. Misztal-Dethloff et al. observed a stimulatory effect of leptin on the secretion of VEGF from cultured endothelial cells [17]. Komorowski et al. revealed that thalidomide decreased secretion of VEGF in endothelial cell cultures [18]. There are also data to suggest that psychotropic agents (diazepam and chlorpromazine) can inhibit secretion of VEGF from cultured endothelial cells [19].

Among the anti-angiogenic factors there are natural fragments of proteins, polypeptide modulators and hormones (Tab. II) Angiostatin is one of the most po-

Table I
Pro-angiogenic factors

Tabela I
Czynniki proangiogenne

Heparin-binding growth factors	Vascular endothelial growth factor (VEGF) Basic fibroblast growth factor (bFGF) Platelet-derived endothelial cell growth factor (PDGF) Hepatocyte growth/scatter factor (HGFSF) Pleiothropin
Mediators of inflammation	Tumour necrosis factor alpha (TNF-alpha) Interleukins (IL-8, IL-3)
Hormones	Oestrogens Proliferin Substance P Erythropoetin
Adhesive molecules	Vascular cell adhesion molecule 1 (VCAM-1) Intracellular adhesion molecule 1 (ICAM-1) E-selectin
Other growth factors	Transforming growth factor beta (TGF-beta) Epidermal growth factor (EGF) Insulin-like growth factor Agiopoetins 1,2,3

Table II
Anti-angiogenic factors

Tabela II
Czynniki antyangiogenne

Natural fragments of proteins and polypeptide modulators	Angiostatin Endostatin AaAT (fragment of antithrombin 3) Prolactin (16 kDa fragment) Trombospondin 1 Troponin I Interferons (IFN-α, IFN-β) Platelet factors-4 Interleukins (IL-12, IL-4) Tissue inhibitors of matrix metalloproteinase (TIMP-1, TIMP-2)
Hormones	Testosterone Methoxyestradiol Somatostatin Melatonin

tent anti-angiogenic factors. It is a product of the enzymatic cleavage of plasminogen, which binds to the alpha subunit of ATP synthase and disturbs ATP synthesis. Angiostatin may therefore render endothelial cells more sensitive to hypoxic stress [20].

Angiogenesis in normal adrenal glands and in adrenal tumours

Normal adrenal glands are highly vascularised endocrine glands located in the retroperitoneal space just above or alongside the superior poles of the kidneys. They are supplied with blood by the phrenic artery, renal artery, and aorta. Thanks to the fenestrated epithelium lining their vessels they are permeable to the hormone produced in these glands [21, 22]. This permeability of the epithelium enables hormones and cytokines to be transported from the cells surrounding the vessels and from the blood to the perivascular environment. During foetal life vascularisation of the adrenal glands is first observed at the eighth week of pregnancy [23]. Shifren et al. examined the foetal adrenal glands at mid-gestation, between the 16th and 20th weeks of pregnancy and assessed the expression of VEGF mRNA. They revealed that immunocytochemical staining for VEGF mRNA was most abundant in the foetal zone of the foetal adrenal gland. Moreover, VEGF expression and secretion by the foetal adrenal cortical cells was up-regulated by ACTH. High expression of VEGF mRNA was associated with rich vascularisation of the foetal adrenal glands, as confirmed by positive immunocytochemical staining for the von Willebrand factor A marker of endothelial cells [24]. Bernini et al. [25] examined the expression of cytoplasmatic VEGF using antibodies anti-VEGF in human normal adrenal glands, aldosterone-producing adenomas (APA), cortisol-producing adenomas (CPA), non-functioning cortical adenomas (NFA) and adrenal cortical carcinomas (ACC). The highest expression of cytoplasmatic VEGF was exhibited by cells of ACC and the lowest by those of NFA. Expression of VEGF in APA was higher than in normal cortex cells, while in CPA it was comparable to expression in normal cells. It is interesting that in patients with adrenal tumours (benign or malignant) serum VEGF is also increased. After surgical resection serum VEGF was decreased (measured one month after operation) [26].

The measurement of circulating plasma levels of VEGF and its soluble receptors, sVEGFR-1 and sVEGFR-2 (acting as an inhibitor for VEGF), in patients with adrenal tumours appears to be of use in the prediction of tumour malignancy. Korzeniewska et al. [27] revealed that patients with adrenocortical carcinoma had the highest VEGF and the lowest sVEGFR-2 serum concentrations when compared to the control group. Patients with adrenocortical adenomas had VEGF serum concentrations comparable to the control group, but sVEGFR-1 and sVEGFR-2 were lower than in the control group. Of patients with metastases to the adrenal glands VEGF concentration was higher than in controls but not as high as in patients with adrenocortical carcinoma. Increased serum concentrations of VEGF were also observed in other endocrine tumours such as thyroid [28] and pituitary tumours [29].

Fraipont et al. [30] measured the cytosolic concentrations of three proteins (VEGF, trombospondin and platelet-derived endothelial growth factor) involved in angiogenesis in adrenal tumours and assessed the correlation between the concentration of these proteins and genetic alterations occurring in adrenocortical tumours. From an examination of 18 adenomas, 12 transitional tumours and 13 carcinomas they revealed that platelet-derived endothelial cell growth factor (thymidine phosphorylase) cytosolic concentration did not differ between these groups. VEGF concentration was increased in the carcinomas, but, in contrast, in adenomas and transitional tumours concentrations of VEGF were not elevated. Expression of trombospondin 1(TSP1), an anti-angiogenic factor measured in this study, was decreased in carcinomas and transitional tumours as compared to adenomas. The authors of this research suggest that a low concentration of TSP1 is the first event in the multi-step process of evolution from benign to malignant lesions. Allelic loss at the 11p15 accompanied by overexpression of IGF-II is the most frequent genetic disturbance observed in adrenocortical tumours. In this study a correlation was observed between overexpression of IGF-II and a higher concentration of cytosolic

VEGF and a lower concentration of TSP-1. This genetic disturbance occurs most frequently in localised malignant adrenocortical tumours and transitional tumours but is not common in benign lesions [31]. The authors also suggest that a high cytosolic concentration of VEGF may be a marker of tumour recurrence, but in this study the group examined was too small to establish the predictive value of this finding.

Zacharieva et al. [32] assessed circulating levels of VEGF, active renin and urinary prostaglandin E2 (PGE2) excretion in patients with adrenal tumours. The highest plasma levels of VEGF were found in adrenocortical carcinomas (statistically significant when compared to VEGF level in patients with pheochromocytoma and primary hyperaldosteronism). VEGF levels were also elevated in patients witch Cushing's syndrome, primary hyperaldosteronism and pheochromocytoma. The former group of patients had higher plasma VEGF levels than cases with pheochromocytoma and primary hyperaldosteronism. Plasma active rennin levels were highest in patients with pheochromocytoma and lowest in patients with primary hyperaldosteronism as compared to control, patients with Cushing's syndrome and patients with adrenocortical carcinoma. Urinary excretion of PGE2 did not differ between these groups. The authors of this work suggest that the angiogenic status of adrenal tumours is associated with tumour malignancy and is also related to the hormonal activity of the tumour. VEGF has a mitogenic effect on endothelial cells and also induces expression of matrix metalloproteinases (MMPs), which are involved in angiogenesis [33]. MMPs are zinc-dependent endopeptidases that are able to dissolve extracellular matrix. It is known that MMP-2 and MMP-9 play the most important roles in the process of degradation of the major component of the extracellular matrix, namely type IV collagen [34]. The activity of MMPs is regulated by their tissue inhibitors (TIMPs, tissue inhibitors of MMPs). TIMPs bind active MMPs with a 1:1 stoichiometry and it is due to this binding that MMPs do not exhibit endopeptidase activity [35].

There have been studies suggesting that growth factors and cytokines are involved in regulation of the expression of MMPs. Randeva et al. revealed that growth hormone decreased serum levels of MMP-2 and MMP-9 in adults deficient in growth hormone [36]. Activation of some MMPs requires the presence of plasmin, a peptide that is formed during enzymatic cleavage of plasminogen [37]. Increased levels of some MMPs and an imbalance between MMPs and their inhibitors (TIMPs) are described in several human cancers. Maruayma et al. revealed increased serum concentrations of MMP-2 and MMP-9 and undetectable concentrations of TIMP-2 in patients with papillary thyroid carcinoma [38]. Extensive studies by Komorowski et al. showed [39] elevated serum levels of MMP-2 and slightly elevated serum levels of TIMP-2 in patients with all histological types of thyroid cancer. Additionally, increased levels of MMP-3 and MMP-9 were observed in patients with medullary thyroid cancer. It is reported that MMPs are also involved in angiogenesis in adrenal tumours by degradation of the extracellular matrix. MMP-2 is present in the cells of malignant cortical tumours but not in stroma [40]. Kjellman et al. [40] examined 30 adrenal tumours for gelatinase A, membrane type 1 matrix metalloproteinase (MT 1-MMP) and collagenase-3. The tests revealed that gelatinase A mRNA was expressed in stromal cells in most malignant tumours (13 out of 16 carcinomas) but not in benign lesions (only 1 out of 14). Kołomecki et al. [41] compared serum levels of MMP-3 between patients with adrenocortical carcinoma and benign adrenal tumours (hormonally inactive), revealing that the former group of patients had significantly higher levels of MMP-3 than patients with benign lesions. They also assessed serum levels of MMP-3 after surgical treatment of adrenal tumours and observed a normalisation of these. Serum MMP-3 levels increased again only in patients with recurrence.

There are also data to confirm the anti-angiogenic properties of MMP-9 (one of the enzymes that convert plasminogen to angiostatin) [42]. Grosset et al. [43] examined pheochromocytoma in von Hippel-Lindau disease (VHL). VHL is a cancer syndrome inherited in an autosomal dominant manner. The most common alterations occurring in VHL syndrome are renal cell carcinoma, retinal angiomas and cerebellar and spinal cord haemangioblastomas. It is interesting that patients with VHL syndrome exhibited high expression of VEGF mRNA. This is due to the unresponsiveness of VEGF to normoxia/hypoxia regulation, the effect of mutation in VHL, which is one of the suppressor genes. In this study expression of MMPs and collagen 1 (the major components of the extracellular matrix [44, 45]) was assessed in pheochromocytoma derived from patients with VHL syndrome. After surgical treatment fragments of tumour were transplanted s.c. to nude mice and treatment with halofunginone (an anti-tumoral agent which exhibits anti-angiogenic properties) was performed. Levels of collagen 1 and vascular density were assessed in the tumour before and after halofunginone treatment with the use of immunohistochemical assays. This study revealed decreased levels of collagen type 1 and decreased expression of MMP-2 and MMP-9 after halofunginone treatment. Additionally, tumour size was diminished after halofunginone treatment. Another pro-angiogenic factor is bFGF, which is presumably involved in angiogenesis in pheochromocytoma and chemodetectoma [46]. Statuto et al. examined normal adrenal medulla, pheochromocytoma and chemodetectoma.

This study showed that levels of immunoreactive and biologically active bFGF were elevated in pheochromocytoma and chemodetectoma as compared to normal adrenal medulla. Pheochromocytoma exhibited immunostaining for bFGF in the nuclei of the chief cells and the endothelial cells (likewise normal adrenal medulla). Cytoplasmatic bFGF in endothelial cells was detected in the pheochromocytoma but not in the normal gland.

Investigation of SW-13 human adrenocortical carcinoma cells [47, 48] revealed that endothelins might be involved in angiogenesis and tumour cell growth. SW-13 exhibits a low ability to synthesise steroid hormones owing to a lack of the organelles which are involved in this process. Because of the expression of two vasoactive peptides, proendothelin-1 (pro ET-1) and adrenomedullin (AM) and their receptors, SW-13 is a good model for investigating autocrine/paracrine control of the growth of adrenocortical carcinoma. Takahashi et al. [49] examined the influence of cytokines such as TNF alpha (tumour necrosis factor alpha), IFN-gamma (interferon gamma) and IL-1 on the secretion of AM and endothelin-1 by SW-13 human adrenocortical carcinoma cells. They revealed that TNF-alpha stimulated synthesis of both vasoactive peptides. IFN-gamma induces synthesis of endothelin-1 but not AM, while IL-1 increases the synthesis of adrenomedullin but has no effect on the synthesis of endothelin-1.

Donckier et al. [50] described patients with Cushing's syndrome in the course of adrenocortical carcinoma. Immunohistochemical analysis of the excised tumour revealed a high expression of endothelin-1. Endothelins may also be involved in the process of angiogenesis in pheochromocytomas.

Favier et al. [51] revealed overexpression of endothelin receptor ETB in endothelial cells and endothelin receptor ETA in pericytes or tumour cells in malignant tumours. They suggest that this overexpression and the vascular pattern of tumours are the most important factors in distinguishing benign from malignant lesions. The precursors of endothelins, PPET-1 and PPET-3, were not overexpressed in malignant tumours. The authors of this research suggest that migration and proliferation of endothelial cells is stimulated by ET-3 acting via ETB receptors. They also investigated the strong positive correlation between expression of VEGF and EPAS-1 (transcriptive factor), but no correlation was observed between expression of VEGF and HIF 1 alpha. The authors of this study also suggest that the angiopoetin system does not take part in angiogenesis in pheochromocytomas.

Some authors suggest that prokineticins (endocrine gland vascular endothelial factors) may take part in angiogenesis [52]. Chi-Hong Lin et al. [53] described two receptors for prokineticins, PKR1 and PKR2, and their tissue distribution. These receptors are expressed in many endocrine (adrenal gland, thyroid gland, pituitary gland, testis and ovary) and non-endocrine (small intestine, colon and rectum) tissues and are G-protein-coupled receptors. Activation of these receptors via prokineticins leads to phosphorylation of p44/42MAPK, which is crucial for angiogenesis [54].

Some studies [55] provide evidence that the loss of cell adhesion may result in cell invasiveness. There is a family of proteins (the CCN family) that include ctg f [56], cyr 61[57] and nov H. Proteins that belong to this family possess adhesive, angiogenic, mitogenic and chemotactic properties. Of the endocrine tissues the adrenal glands are the major site of nov H expression, both foetal and adult adrenal glands being involved (nov H is predominantly expressed in the adrenal cortex.) It is likely that nov H is associated with cell adhesion and participates in a signalling pathway involving the extracellular matrix. Martinerie et al. [55] examined expression of nov H mRNA in 12 benign and 18 malignant adrenocortical tumours. They revealed that expression of nov H is significantly lower in a malignant tumour than in a benign lesion, suggesting that cell invasiveness is related to loss of cell adhesion as a result of a low level of expression of nov H. They also revealed that benign lesions possessed another N-glycosylation profile of nov H when compared to malignant lesions, but the site of expression of nov H did not differ between benign and malignant tumours.

It has been reported that an increased level of soluble forms of immunoglobulin-like adhesive molecules (sICAM-1, sVCAM-1) is related to a malignant course of many endocrine tumours. ICAM-1 and VCAM-1 are expressed on the surface of the lymphocytes and are able to bind to the corresponding molecules (such as integrins) on the neoplastic cells. They are, therefore, involved in the process of intercellular adhesion. Their soluble forms can bind to the corresponding molecules and decrease adhesion between cells and also allow neoplastic cells to avoid immunological response. These alterations have been observed in several human cancers such as thyroid cancer [58], lung cancer [59], ovarian cancer [60] and breast cancer [61]. There are also unpublished data (Stępień et al.) to indicate that in patients with adrenal cortex carcinoma levels of sICAM-1 and sVCAM-1 are significantly increased.

Fraipont et al. [62], using cDNA microarrays, described the gene profile related to the malignant course of adrenal tumours. They identified two clusters of genes, termed the IGF II-cluster and the steroidogenesis cluster. It is interesting that in the IGF-II cluster there are the FGFR1 and FGFR4 genes (chromosome location 8p11.2-p11.1 and 5q35.1-qter respectively), which encode thyrosine kinase receptors type 1 and 4. FGF1 and

FGF 2 expressed in the adrenal cortex act via these receptors and may develop a mitogenic effect on endothelial, mesenchymal and steroidogenic adrenocortical cells [63]. In this report the authors suggest that overexpression of FGFR1 and 4 in adrenocortical carcinomas may be linked to increased proliferation and vascularisation of these tumours. The TGF beta gene and the gene encoding its type 1 receptor (TGF beta R1) also cosegregate with the IGF-II cluster. TGF beta 2 (resembling TGF beta 1) is known to have an inhibitory effect on adrenocortical steroidogenesis. The authors of this work suggest that overexpression of TGF beta-2 and TGF beta R-1 in adrenocortical carcinomas may interfere with the differentiation of these tumours. The IGF-II cluster also includes the MST1R gene, encoding a thyrosine kinase receptor for macrophage-stimulating protein 1, a potent mitogen for the adrenomedullary cells. The IGF-II cluster also includes the KCNQ10T1 gene and the IGF-II gene, located in the same region of the 11p15.5. The product of KCNQ10T1 is a non-coding anti-sense transcript, which is presumably involved in regulatory processes linked with the paternal imprinting of the centromere domain of the 11p15 region. The high expression of IGF-II in malignant tumours is due to loss of the maternal allele and duplication of the paternal allele (paternal isodisomy) [64, 65]

Ciquel et al. [64] examined mutations at the 11p15 locus in sporadic adrenocortical tumours (6 carcinomas and 17 adenomas) and revealed that mutations at this locus are frequent in malignant tumours but rare in benign lesions. The most frequent disturbance occurring at 11p15 was uniparental disomy. Tumours with uniparental disomy also exhibited a high serum IGF-II concentration.

Research into adrenal tumours in genetic syndromes [65] (Beckwith-Wiedemann, Li-Fraumeni, McCune-Albright, Carney) and multiple endocrine neoplasia type I has demonstrated that mutations at the 11p15 locus have been associated with overexpression of growth promotion factors. It is reported that some mutations in mitochondrial genes are related to the angiogenesis and malignant course of pheochromocytoma [66]. Mitochondrial complex II is involved in the Krebs cycle and aerobic electron transport chain. It contains four subunits: subunit A (flavoprotein) and subunit B (iron-sulphur) protein, which possess catalytic properties, while subunits C and D are an anchorage domain. Mutations in the SDHD and SDHB gene in mitochondrial complex II result in a loss of activity of the complex and activation of the hypoxic/angiogenic pathway. The markers of this activation are endothelial PAS domain protein 1 (EPAS -1), hypoxia inducible factor 1 alpha (HIF-1alpha) and VEGF) [67]. The authors described a germline mutation at the R46Q position of the SDHD gene related to a malignant course of pheochromocytoma. Astuti et al. [68] described a frameshift mutation within exon six of the SDHB gene linked with a benign course of pheochromocytoma. Assessment of vascular pattern in pheochromocytomas may be a useful tool in distinguishing benign from malignant lesions. Favier et al. [51] examined 19 pcheochromocytomas (ten benign, nine malignant). This study showed evidence that all malignant pheochromocytomas presented an abnormal vessel pattern of irregular blood vessels, flattened between tumour nodules, with larger diameters than normal blood vessels. Sasano et al. [69] reported that the vascular density of adrenocortical carcinoma measured as the number of vessels per mm^2 did not differ from the vascular density of adrenocortical adenomas and the normal cortex, suggesting that adrenocortical carcinomas are not highly vascularised tumours but that they have an increased angiogenic potential expressed by the greater endothelial area of each vessel (EA, $microm^2$/vessel) and a greater vascular area (the percentage of EA per field).

In summary, assessment of the angiogenic status of adrenal tumours and their vascular pattern may be useful for discriminating benign from malignant lesions and knowledge of angiogenesis may be essential in drawing up promising treatment strategies in patients with malignant tumours.

PRACE POGLĄDOWE

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