The role of angiogenic factors in endometrial cancer

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Abstract

Endometrial cancer is the most common malignancy within the female reproductive system (37.7%). The incidence increases with age. Frequently this type of cancer is diagnosed in peri- and post-menopausal women. 60-70% of cancers occur in women over 60 years of age, and less than 5% in women below 40 years of age.

Angiogenesis is a process of formation of new microvessels from existing capillaries. There are four different mechanisms of new vessel growth: sprouting, intussusception, vessel elongation and incorporation of endothelial progenitor cells into new microvessels. Angiogenesis plays important roles in growth of endometrial cancers. This process is controlled by many angiogenic factors, for example vascular endothelial growth factor (VEGF). VEGF is the most powerful and most specific endothelial cell growth factor. It plays a crucial role in the initiation of physiological and pathological angiogenesis, lymphangiogenesis, and vasculogenesis. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and PLGF (placental growth factor). The effects of VEGF are mediated through binding to the two specific and homologous receptors VEGFR-1 (FLT-1) and VEGFR-2 (KDR). Placental growth factor (PLGF) belongs to the VEGF family and it is also a very important growth factor. So far four isoforms of PLGF have been identified: PLGF-1 (PLGF131), PLGF-2 (PLGF152), PLGF-3 (PLGF203) and PLGF-4 (PLGF224).

Key words: endometrial cancer, angiogenesis, growth factors, vascular endothelial growth factor, placental growth factor.

Endometrial cancer

Endometrial cancer is one of the most common cancers in women in Poland and around the world. Every year about 190 thousand new cases of endometrial cancer and about 45 thousand deaths are registered. It is commonly believed that it is a tumor that occurs in highly developed and developing countries, and that it is closely related to the so-called "Western way of life" [1].

Endometrial cancer is the most common malignancy within the female reproductive system (37.7%) [2]. The incidence increases with age. Frequently this type of cancer is diagnosed in peri- and post-menopausal women. 60-70% of cancers occur in women over 60 years of age, and less than 5% in women below 40 years of age [1, 3].

Factors that increase the risk of developing endometrial cancer include early menarche, late menopause, age, obesity, diabetes, menstrual disorders, anovulatory cycles, polycystic ovary syndrome, childlessness, hormone replacement therapy (HRT), and estrogen-secreting tumors [4].

Malignant tumors of the uterus may vary according to histological structure. Most of the cells derive from the endometrium. 80% of endometrial cancers are adenocarcinomas. 60-65% of them are endometrioid cancers [1, 5, 6]. The remaining 20% of endometrial cancers are serous and clear cell adenocarcinomas. Mixed type of endometrial cancer represents about 10%. A rare form of endometrial cancer is mucinous adenocarcinoma – about 9% [5]. Other rare kinds of endometrial cancer include endometrioid cancer with squamous metaplasia, small cell neuroendocrine carcinoma, squamous cell carcinoma, transitional cell carcinoma, and sarcomas [1, 5].

Bokham, based on years of clinical observations of endometrial cancer, divided it into 2 types. Type 1 – the most common one – accounts for about 80% of diagnoses. These are mainly endometrial endometrioid carcinomas. These types of tumors are estrogen-dependent. They develop on the basis of hypertrophic endometrium. They are characterized by slow growth and a good prognosis. These types of tumors occur in both menopausal and pre-menopausal women. In endometrial endometrioid cancers, characteristic mutations in the PTEN gene, K-ras, and microsatellite instability inception occur. Type II – nonendometrioid – tumors are not estrogen-dependent, growing on the substrate atrophic

endometrium. From the biological point of view, these tumors are very aggressive and have a poor prognosis. These tumors occur predominantly in women after menopause. In the cases of type II tumors, mutations in p53 and HER2/neu [7] occur.

Angiogenesis versus vasculogenesis

Neovascularization involves two processes – vasculogenesis and angiogenesis.

Vasculogenesis means formation of a vascular plexus by differentiation of hemangioblasts into endothelial cells without a pre-existing vascular system. The process of vasculogenesis occurs for example in the fetal life.

Angiogenesis is a process of formation of new microvessels from existing capillaries. Angiogenesis occurs in fetal life. In adults angiogenesis is a rare process, except for wound healing, menstrual cycle, and some diseases for example endometriosis, diabetic retinopathy, and rheumatoid arthritis [8]. Angiogenesis is necessary for the formation and development of all cancers. In 1971, Folkman pointed out the importance of angiogenesis for the development of cancer, which was a milestone in the field of cancer biology [9].

In the initial phase of cancer progression, the tumor is a cluster consisting of about 1 million cells and does not exceed 2 mm^3. At this stage of its growth and development it is independent from the vascular network. Nutrients and oxygen are transported to the tumor cells by diffusion. In the later stages of carcinogenesis, the tumor stimulates the production of new blood vessels, because the current supply becomes insufficient – in the central part of the tumor necrosis may occur. The newly formed vessels in the tumor are necessary, since they provide essential nutrients and oxygen to the tumor cells and allow continuous growth and unlimited proliferation. Simultaneously, the tumor vascular network is used to spread the cancer in the body – metastasis [9-12].

There are four different mechanisms of new vessels' growth: sprouting, intussusception, vessel elongation and incorporation of endothelial progenitor cells into new microvessels. Sprouting is also called classic angiogenesis. It is associated with active endothelial cells, that produce proteases. Proteases break down the basement membrane, and allow endothelial cells to migrate toward the circulating angiogenic factors. New microvessels are stabilized by pericytes and smooth muscle cells. This process is controlled by angiopoietin-1, which binds to endothelial Tie-2 receptors [13, 14].

The second mechanism is intussusception, which consists in partitioning the lumen of the vessels into two separate vessels. Endothelial cells migrate inwards and create a network of new vessels [13]. Elongation is the mechanism most frequently occurring during organism growth.

Growth factors and angiogenesis

The newly formed vessels, with many additional features, are a source of growth factors, cytokines, and hormones that stimulate the development of the tumor [15]. They show proteolytic activity, which increases tumor invasiveness [16, 17]. Angiogenesis under physiological conditions is strictly regulated. In healthy subjects, the relationship between pro-angiogenic and anti-angiogenic factors points in the direction of the latter. In cancer, these processes are getting out of control. This leads to constant production of pro-angiogenic factors; at the same time activity of antiangiogenic factors and their expression are reduced – the "angiogenic switch" is turned on [8]. The fact that angiogenesis is closely associated with the development of cancer became the starting point for research on a new method of treatment in the fight against cancer – anti-angiogenic therapy. This idea already appeared in the year after the groundbreaking discovery by Folkman [18]. More than 30 years later, the first tumor angiogenesis inhibitory drug (Avastin/ bevacizumab) was approved for clinical use [19, 20].

Vascular endothelial growth factor family

Vascular endothelial growth factor (VEGF) plays a crucial role in the initiation of physiological and pathological angiogenesis, lymphangiogenesis, and vasculogenesis. VEGF is the most powerful and most specific endothelial cell growth factor. VEGF synthesis is stimulated under conditions of hypoxia by hypoxia inducible factor (HIF). HIF binds with the promoter of the gene and stimulates its transcription [21].

VEGF was discovered in 1983 by Dvorak [22]. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and PLGF (placental growth factor) [23]. The effects of VEGF are mediated through binding to the two specific and homologous receptors VEGFR-1 (FLT-1) and VEGFR-2 (KDR). Other receptors by means of which VEGF works are VEGFR-3 and neuropilin 1 and 2, which are semaphorin receptors. Specific ligand-receptor interaction induces a different effect on endothelial cells. VEGF-A, VEGF-B and PLGF act through VEGFR-1 and are responsible for the formation of new vessels. VEGF-A, VEGF-B, VEGF-C and VEGF-D work through VEGFR-2 and are responsible for angiogenesis, proliferation and migration. VEGF-C and VEGF-D act through VEGFR-3 and are responsible for the processes of lymphangiogenesis, proliferation and migration [24-26].

The VEGF gene consists of 8 exons. During transcription 6 protein isoforms are formed: VEGF$_{121}$, VEGF$_{145}$, VEGF$_{165}$, VEGF$_{183}$, VEGF$_{189}$, VEGF$_{206}$.

VEGF$_{121}$, VEGF$_{165}$, VEGF$_{189}$ isoforms are most commonly produced by various cells types [27, 28].

Many factors that stimulate angiogenesis act indirectly through the induction of VEGF expression, for

example transforming growth factor (TGF), endothelial growth factor (EGF), and platelet-derived growth factor (PDGF) [29, 30].

Placental growth factor

Placental growth factor belongs to the VEGF family. It was discovered in 1991 by Maria Graziella Perscio [31]. The human PLGF gene is located on 14 chromosome and consists of seven exons. The PLGF amino acid sequence is 42% identical with the sequence of VEGF and has significant structural similarity [32]. So far four isoforms of PLGF have been identified: PLGF-1 (PLGF$_{131}$), PLGF-2 (PLGF$_{152}$), PLGF-3 (PLGF$_{203}$) and PLGF-4 (PLGF$_{224}$). These four isoforms are the result of alternative splicing overlap [32, 33]. PLGF isoforms differ in terms of both their section properties and their binding affinities. PLGF-1 is a dimeric protein with a molecular mass of about 46 kDa. It is composed of 131 amino acids. PLGF-1 binds to VEGFR-1 and is predominantly expressed by vascular endothelial cells. PLGF-2 consists of 170 amino acid residues prior to signal peptide (18 amino acid residues in length) cleavage. A highly basic 21 amino acid insertion in the carboxy-terminal region of PLGF-2 results in high heparin binding affinity. PLGF-2 binds neuropilin 1 and 2, which are receptors for the semaphorin family of proteins. PLGF-3 contains an insertion of 216 nucleotides coding for a 72-amino acid sequence between exons 4 and 5 of the PLGF gene. PLGF-3 like PLGF-1 binds to VEGFR-1. PLGF-4 consists of the same sequence as PLGF-3. PLGF-4 like PLGF-2 has a heparin-binding domain [33-35].

PLGF is a positive regulator of angiogenesis. It can stimulate this process by several mechanisms, for example a direct impact on endothelial cells by VEGF-1, sensitization of cells to VEGF–VEGFR by the interaction of PLGF–VEGFR-1, separation of the VEGF from receptor-1, simultaneously enabling the binding of VEGF to VEGFR-2, mobilization of hematopoietic progenitor cells from bone marrow, and recruitment of monocytes/macrophages which are involved in the process of vessel growth [31, 36-38].

PLGF activity is not detectable in healthy organs. PLGF activity is highly unregulated in pathological conditions. PLGF was originally demonstrated in the placenta, but it is also present in heart, lungs, thyroid, muscles and adipose tissue [39, 40]. Placental growth factor levels correlate with poor prognosis of cancers, for example colorectal cancer, hepatocellular cancer or renal cancer [41, 42]. Many reports have suggested that PLGF might by a useful prognostic marker of cancer progression. Plasma PLGF levels correlate with tumor grade and survival in patients with renal cell carcinoma [43]. High preoperative PLGF serum level is a prognostic factor of recurrence and survival in patients with colorectal cancer [44]. Zhang *et al.* suggest that PLGF mRNA and

protein in tumor cells are correlated with tumor stage in lung cancer [41]. Parr *et al.* came to similar conclusions in patients with breast cancer [45].

Circulating levels of PLGF are elevated not only in patients with different kinds of cancers but also in patients with atherosclerosis and ischemic heart disease. A high plasma level of PLGF in patients with acute coronary syndrome within 12 hours of the onset of symptoms is a poor prognostic factor [46, 47]. In the skin, PLGF expression is upregulated during wound healing [48].

PLGF may also act as an inhibitor of angiogenesis. That happens when PLGF creates a heterodimer with VEGF. This complex has from 20 to 50 times less stimulatory effect on angiogenesis as compared to VEGF homodimer [49, 50]. PLGF can block VEGF-stimulated angiogenesis by reducing the pool of VEGF homodimers [51].

Vascular endothelial growth factor and placental growth factor in endometrial cancer

Angiogenesis plays important roles not only in growth of endometrial cancers but also in the menstrual cycle. The endometrium is a very dynamic organ that constantly proliferates and breaks down. However, angiogenesis in uterine endometrial cancers is complicated because steroid hormone modifies the angiogenic potential in their growth.

VEGF-A/VEGFR-1 expression has been investigated as an angiogenic factor in benign and malignant diseases of uterine endometrium. It is involved in physiological angiogenesis in the menstrual cycle and in pathological angiogenesis. VEGF-A/VEGFR-1 expression is affected by exogenous hormone therapy [52-54]. Zhang *et al.* reported greater VEGF expression in the endometrial epithelium than in stromal cells. VEGF expression in greater in the secretory phase than in the proliferative phase [55].

Saito *et al.* studied the role of angiogenic factors in normal endometrium and endometrial adenocarcinoma. One of the examined angiogenic factors was VEGF. They proved that VEGF expression was recognized in all phases. It was higher in the functional layer of early and mid-secretory phases. In the basal layer VEGF was expressed in the mid to late secretory phases. In the G1 and G2 adenocarcinoma samples, VEGF expression was higher than in the proliferative phases of normal endometrium. VEGF expression in the endothelial cells was not correlated with the histological grade [56]. Fujimoto *et al.* reported that in normal endometrium and endometrial cancers mainly VEGF$_{165}$ and VEGF$_{121}$ and their mRNA were detected. VEGF protein and mRNA were expressed in normal uterine endometrium. Their levels were significantly higher in normal uterine endometrium than in endometrial cancers. VEGF protein and

mRNA levels were inversely proportional to histological grade, myometrial invasion and clinical stages of endometrial cancer [57]. Giatromanolaki *et al.* found no association between VEGF expression and histological type and grade, depth of myometrial invasion or lymph vascular space invasion [58].

The role of PLGF in the development of endometrial cancer has not been thoroughly investigated. There are not many publications concerning this subject. The role of PLGF has been investigated in several cancer types, for example breast and colorectal cancer. For endometrial cancer the prognostic function of PLGF has to be investigated. Coenegrachts *et al.* conducted research on the role of PLGF in endometrial cancer, and the correlation between PLGF expression and histological and clinical data. They found that serum PLGF levels are increased only in late stages of cancer. Only in high-grade endometrial carcinomas is local PLGF expression increased. They did not observe any statistically significant correlation between serum PLGF levels and clinical stage of endometrial cancer. They reported that serum PLGF levels cannot be used as a prognostic or diagnostic marker of endometrial cancer [59].

Acknowledgements

Disclosure

Authors report no conflicts of interest.

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