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Tumour's angiogenesis – the function of VEGF and bFGF in colorectal cancer

The regular cell in order to undergo neoplastic transformation must escape from being controlled by signals regulating proliferation and apoptosis (8). The feature of malignant tumours is their ability to form metastases. To enable neoplastic cells to develop in the remote places from the primary lesion, they must come through a series of complex stages – so-called metastases cascade. These stages are the result of the complex interraction between the tumour and the surroundings (7). The basis for the growth of the tumour and metastasis formation is the development of the network of blood-vessels, which is called angiogenesis (13). This is the result of capillar increasing from already existing vessels and their shaping within the tumour. It involves migration and proliferation of microcirculation endothelial cells and also proliferation of tumour cells. Experimentally, not only the kinetics of angiogenesis but also its precise relationship with the transformation from limited growth phase into invasive growth phase are well known. An example can be transformation from a regular epithelium into a non-vascularized transformed epithelium, and next into a vascularized neoplastic tumour (7). It is accompanied by disordering of the balance between proliferation and apoptosis in the neoplastic cell's population. Mutations activating oncogenes and also elimination of suppressor genes' functions are the cause of activating cell proliferation, with decreasing of apoptosis index. It has been proved that, for example K-RAS oncogene activation is the parallel process to stimulation of vascular endothelial growth factor (VEGF) secretion. It directly stimulates angiogenesis (14).

According to Radzikowski (14) at the initial stage of the neoplastic growth the cells are nourished by means of diffusion. The growth factors are also provided in this way. When the population of neoplastic cells reaches the volume of 2–3 mm (about one million of cells), as a result of their anoxia it comes to the activation of the genes coding the production of different proteins, including VEGF, and also proteolytic enzymes. The process of blood-vessels neoplasia changes the conditions of oxygenation and nourish-

ment within the neoplastic cells. It also changes penetration of the growth factors into these cells. By making the structure of extracellular matrix more loose it makes it easier for the neoplastic cells to migrate outside the primary lesion. Apart from this it enables the neoplastic cells to penetrate through the blood-vessels wall. It is the moment when the cascade initiating progressive tumour growth and forming its metastasis starts.

Angiogenesis in neoplastic tumour causes that neoplastic cells are the source of the factors stimulating this process. They directly have an influence on endothelium cells, stimulating their proliferation and migration (6). In malignant tumour, neoplastic cells are the source of so-called angiogenic factors stimulating the process of vessels development. Proliferative tumour and microcirculation endothelial cells are detected in the whole infiltration. It has been found out that proliferation is the highest on the tumour margin and is 10 times as big as in the adjoining to the tumour tissue. It can be explained by the fact that endothelial cells proliferation in the tumour is 40 times bigger than in the normal tissue.

Vascular endothelial growth factor (VEGF) is one of the best known angiogenic factors, which is produced by endothelium and neoplastic cells. It is trimeric glycoprotein binding hepar which is one of the factors stimulating endothelium cell divisions (10). Additionally, it increases permeability of blood-vessels which makes the macromolecules penetration easier than that of fibrinogen, out of circulation. It results in penetrating of fibrin substratum or its reticulum and helps in organising endothelium cells and also tumour cells. VEGF is produced by numerous tumours. It helps then to grow and fosters the development of metastases which consequently makes them more vascularized. There are five already known VEGF isoforms: 121, 145, 165, 198, 206 – that are produced by different places of mRNA. Most neoplastic cells produce several isoforms at the same time. However, VEGF 121 and VEGF 165 are usually the dominant forms (13). This factor acts through the paracrinic effect by means of two tyrosine kinases' receptors flt-1 (VEGF R1) and KDR (VEGF R2) which can be found mainly in the endothelium cells. VEGF stimulates divisions of endothelial cells and also inhibits their apoptosis (10).

Basic fibroblast growth factor (bFGF) is a mitogenic polypeptide which plays an important part in autocrinic and paracrinic growth in different tumours coming from mesoderm and neuroderm (5, 11). This polypeptide stimulates vascular, endothelial cells' proliferation. In fact, all these cells produce or possess receptors for bFGF. It has been found out that bFGF is engaged in neoplastic angiogenesis of several tumour types (melanoma, glioblastoma, Kaposi sarcoma, tumours of pancreas, kidney, breast, lungs) (11).

The prognostic importance of VEGF has been observed in many malignant tumours. The immunohistochemical method was employed in order to state the presence of VEGF in the tissue. Its growth correlates with the spreading within blood-vessels and also with metastases to lymph nodes and liver. It certainly casts a light on the worse prognosis (2, 3, 4, 6). In the studies of Kumar et al. (10) preoperative VEGF level in the serum was examined. It was achieved by evaluating the level of progression of colorectal cancer.

That level was measured in 108 patients being ill with colorectal cancer and in 136 healthy people (who were the control group). The results showed meaningful differences within the T marker (T1-T4), UICC and the Duke's classification. Comparing VEGF levels in the serum of the control group, they were significantly higher at patients with the trait T2 ($P = 0.003$), T3 i T4 ($P < 0.0005$); UICC I ($P= 0.001$), UICC II, III and IV ($P < 0.0005$); Duke's A ($p = 0.001$), Duke's B and Duke's C ($P< 0.0005$). The patients with metastases to lymph nodes had a higher VEGF level in comparison to the ill with colorectal cancer without metastases ($P = 0.008$). It proves that the preoperative VEGF level can detect colorectal cancer, but not at the early stage (e.g. T1 – $P < 0.06$). Similar results were achieved by other scientists (3, 9).

Takahashi et al. (15) showed that VEGF and its KDR receptors' expression was higher at those sick with metastases. The aforementioned authors observed the correlation between the level of neoplastic cells' proliferation and the vascularization of tumour. Another important discovery made by the Japanese scientists was also finding higher VEGF levels in the mesenteric blood in comparison with the areas distant from the colorectal tumour (15). Statistical analysis of these studies proved that there is a correlation between the VEGF levels and the progression of rectum tumour, acording to TNM and Duke's classification. In the Dirix's et al. works (2, 3) it was proved that bFGF level in the serum at a higher level may indicate an advanced form of colorectal tumour. The bFGF level was examined at 44 patients with colorectal tumour and it was stated that its higher level is always present with those patients where the tumour is growing very quickly and there are metastases. Galzy's team (5) demonstrated a relationship between the production and the size of bFGF and the growth and the spreading of neoplastic cells causing colorectal tumour.

Studies conducted by other scientists proved the usefulness of VEGF and bFGF determination as far as predicting the survival time of people suffering from colorectal tumour is concerned. These examinations proved the existence of the correlation between the progression of the tumour and the chance for survival. The expression growth of the examined factors indicated the worse prognosis in relation to the length of survival (1, 9, 12).

Landriscina et al. (11) presented quite a contrary opinion about the prognostic importance of VEGF and bFGF. The results achieved by them did not show any correlation between expression value of markers and the prognosis in colorectal cancer.

Galzy's et al. observations (5) showed that high preoperative levels of both factors (VEGF and bFGF) in patients with colorectal cancer were reduced to the correct level after the removal of primary tumour. It could suggest that VEGF and bFGF are considerably produced by the tumour itself. Moreover, they pointed out that 60% of the patients had one or even both of these factors at a higher level. With the fast progression of this disease this number increased to more than 90%. These authors also indicated that there was a connection between the kinetics of metastases and the growth of VEGF and bFGF levels in the blood serum, which is independent of the tumour weight.

Table 1

Endogenous angiogenic factors that regulate angiogenesis	
Vascular endothelial growth factor	Acidic and basic fibroblast growth factors
Angiogenin	Platelet-derived endothelial cell growth factor
Epidermal growth factor	Hepatocyte growth factor
Transforming growth factors	Platelet-activating factor
Interleukin – 8	Tumor necrosis factor – α
Granulocyte colony-stimulating factor	Vascular intergrin
Matrix metalloproteinases	Prostaglandins E1, E2

The application of angiogenic factors determination and also molecules engaged in the neoplastic angiogenesis for both prognostic and diagnostic purposes is an extremely important use of all the examination results concerning the role of angiogenesis in the dynamics of neoplastic process. The angiogenic factors are presented in Table 1. Apart from the determination of the blood supply level in primitive and metastatic changes (based on the bloodvessels detection, for example determination of von Willebrand factor), diagnostic and prognostic methods are enriched by denoting of proteolytic enzymes, adhesive molecules, growth factors. All these factors take part in angiogenesis (14). Among others, interleukins, adhesive molecules, CD 44, CD 8, TGF, TNF are denoted. On the basis of hitherto existing medical examinations conducted on the patients with colorectal cancer it can be assumed that the most specific cytokines for its detection and progression evaluation are VEGF, bFGF and interleukins (IL-6, IL-7, IL-8, IL-10) (6).

Table 2

Endogenous antiangiogenic factors that regulate angiogenesis	
Thrombospondin	Interferon
Interleukin – 12	Angiostatin
Endostatin	Tissue inhibitors of metalloproteinase
Angiopoietin 2	Platelet factor 4

The knowledge about the factors that can influence the inhibition of the neoplastic process is becoming better known. This fact is associated with hope for applying factors inhibiting angiogenesis in the antineoplastic treatment. The factors which inhibit angiogenesis are presented in Table 2. It seems that in the near future preparations inhibiting at different levels the process of angiogenesis will become the ones which supplement already existing methods of curing cancers (13). The main assumption of this strategy is inhibiting the neoplastic disease by restoring the balance between proliferation and apoptosis. It should involve no direct destroying or damaging activity on neoplastic cells. However, it requires futher examinations.

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SUMMARY

The main objective of this paper was to present the view on the vascular endothelial growth factor (VEGF) and basis fibroblast growth factor (bFGF) in the angiogenesis in the colorectal carcer. The analysis includes the works of these scientists, who proved the relationship between VEGF and bFGF expression and the advancement level of colorectal cancer and the survival of the patients ill with this disease. It was stated that the highest levels of these factors were connected with the advancement of neoplastic process, especially when metastases coexisted at the same time. It was also proved that the higher levels of VEGF and bFGF gave worse prognosis as far as survival was concerned. Apart from this, other factors effecting angiogenesis and inhibing factors were also presented.

Angiogeneza w guzie – rola VEGF i bFGF w raku jelita grubego

Celem pracy było przedstawienie poglądu na temat roli naczyniowo-śródbłonkowego czynnika wzrostu (VEGF) i zasadowego czynnika wzrostu fibroblastów (bFGF) w procesie angiogenezy w raku jelita grubego. Analizą objęto prace badaczy procesu angiogenezy nowotworowej, którzy wykazali związek między ekspresją VEGF i bFGF a stopniem zaawansowania raka jelita grubego oraz długością przeżycia chorych z tym schorzeniem. Stwierdzono, że podwyższone poziomy tych czynników w większości doniesień były powiązane z zaawansowaniem procesu nowotworowego, szczególnie przy współistnieniu przerzutów, oraz że wzrost ten wskazywał na gorsze rokowanie w odniesieniu do długości przeżycia. Przedstawiono także inne czynniki wpływające na angiogenezę nowotworową oraz czynniki hamujące (inhibitory angiogenezy), odnosząc się do ich roli w tym procesie.