SELECTED ASPECTS OF ANGIOGENESIS IN HAEMATOLOGICAL MALIGNANCIES

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Summary: It is well established that angiogenesis is necessary in solid tumours development. Interestingly, the role of angiogenesis in haematological malignancies has been also recognized. Recent publications indicate that microvessel density in bone marrow and angiogenic factors like endoglin, placental growth factor and cyclooxygenase 1 are increased in lymphoproliferative disorders and suggest that angiogenesis is a part of pathomechanism of these malignancies. However, it has not been identified how angiogenesis can be involved in progression of haematological disorders. There is a strong need for further investigations in this area, since antiangiogenic therapy is a potential adjunct to conventional therapy of lymphoproliferative disorders.

Key words: angiogenesis, acute lymphoblastic leukemia, acute myeloid leukemia, angiogenic factors, microvessel density

ANGIOGENESIS – DEFINITION, MECHANISM AND SIGNIFICANCE

Development of blood vessels represents an indispensable condition for body development and differentiation during embryogenesis. Therefore, in vertebrata organ of circulation develops first [12]. Also during extrauterine life development of blood vessels carries an immense significance for a normal body functioning. Abnormal blood vessels as well as disturbances in vascular structure and function may cause several diseases or even be lethal [14].

Development of new blood vessels includes three processes: vasculogenesis, angiogenesis and arteriogenesis.

The first of them is typical for embryonal stage and involves formation of blood vessels from endothelial stem cells, haemangioblasts, developing in haematogenic islands of embryonal yolk sac. This results in development of a primordial vascular plexus in the third week of embryogenesis in regions not supplied with blood vessels [7, 73]. In the extrauterine life blood vessels do not develop by vasculogenesis [73]. Exceptions include certain pathological conditions, such as neoplastic diseases, injured cornea or ischaemia of lower extremities, in which development of new blood vessels is noted with involvement of endothelial precursor cells [59].

In subsequent stages of foetal development blood vessels develop due to angiogenesis (called also neovascularisation) and arteriogenesis. arteriogenesis involves transformation of existing collateral arterioles into functional arteries due to thickening of their muscle layer which provides the blood vessels with vasomotoric properties [41]. Transformation of small blood vessels yields conducting arteries of a 20-fold higher diameter. Arteriogenesis occurs also in the extrauterine life e.g., in a mature ischaemic and anoxic heart [95].

FIGURE 1. The steps of blood vessels formation

In turn, angiogenesis represents a process in which new blood vessels develop from already existing ones, which takes place due to budding of parietal

endothelium cells and endothelial cells of capillary ends. Angiogenesis represents the main way of blood vessel formation in extrauterine life. In such cases it is typical of specific physiological situations. It develops in certain stages of menstruation cycle: during remodelling of uterine mucosa and formation of corpus luteum. It is also indispensable during implantation of embryo in endometrium and formation of placenta [85]. Physiological angiogenesis is observed also in intestinal mesentery and during endochondral ossification [29]. Process of neovascularization accompanies also development of granulation tissue during wound healing [97]. Angiogenesis represents also a key component in pathogenesis of several diseases. It plays a significant role in development of chronic inflammation: psoriasis, rheumatoid arthritis or asthma [15, 24]. It constitutes one of elements engaged in development and course of gastrointestinal diseases (Crohn's disease, ulcerative colitis), cardiological diseases (arteriosclerosis, myocardial ischaemia, cyanotic cardiac defects, neuroinfections and diabetes mellitus) [14]. The process is also involved in formation of adipose tissue, which suggests its involvement in development of obesity [11]. Without doubt, angiogenesis plays an immense role in development of neoplastic diseases, determining both size of the tumour and development of metastases [24].

Angiogenesis represents a complex, multi-stage process [14]. At the first stage blood vessels become more flaccid and vascular endothelial cells become activated due to various physical and humoral factors (hypoxaemia, hypoglycaemia). This induces release and binding of pro-angiogenic factors to their receptors on the surface of endothelial cells. A principal role in initiation of angiogenesis is played by proteins of VEGF family: vascular endothelial growth factor and fibroblast growth factor (FGF). Due to action of locally activated proteolytic enzymes, such as metalloproteinases (MMP), basement membrane providing support for endothelial cells and extracellular matrix (ECM) undergo degradation. This provides an indispensable condition for penetration of endothelial cells to new locations. In addition, proteolysis of ECM yields products inducing release of ECM-trapped growth factors and compounds of chemotactic activity, promoting the subsequent migration of endothelial cells. At this stage adhesion molecules are active, located at the surface of endothelial cells (integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, selectin E), which interact with specific components on extracellular matrix (vitronectin, fibronectin, laminin) [22]. In parallel, endothelial cells proliferate. At rest, cell cycle of endothelial cells exceeds 1000 days but during angiogenesis it becomes abbreviated to 5 days. Divisions of endothelial cells assure continuity of a blood vessel and development of its intima. At the subsequent stage vascular lumen develops: elongated pipe-like structures are formed which anastomose at ends forming capillary loops. Basement membrane becomes produced also. Development of a blood vessel of a full value requires recruitment and binding of adventitia cells (pericytes) and, in cases of large vessels, also of smooth muscle cells. This stage is controlled by platelet-derived growth factor

(PDGF) [77]. At the late phase of angiogenesis, endothelial cells become linked to the surrounding mesenchymal cells and stable cellular and biochemical interactions are formed, in which angiopoetins and receptors Tie 1 and 2 play principal role [54].

CONTROL OF ANGIOGENESIS

Angiogenesis represents a complex process, requiring engagement of several factors. Initiation of the process requires cooperation of: 1) various cell types, 2) components of extracellular matrix and 3) compounds which stimulate or inhibit angiogenesis [84]. It should be mentioned that the latter group of factors plays a key role. Angiogenesis is thought to be controlled by a balanced expression of factors manifesting pro- and anti-angiogenic character and in healthy adult individuals the balance is shifted toward the latter factors [33]. In turn, mobilization of angiogenetic programme is induced by augmented expression of stimulators and/or a decreased expression of inhibitors. Acquisition of angiogenic phenotype used to be termed the angiogenic switch [33].

The factors of pro-angiogenic character include also a number of cytokines and mediators, which stimulate growth and proliferation of endothelial cells, degrade extracellular matrix or induce maturation of blood vessels.

FAMILY OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor (VEGF) plays a key role in initiation of physiological and pathological angiogenesis, lymphangiogenesis and also vasculogenesis. It was discovered in 1983 by Dvorak and described as a factor affecting vascular permeability [79]. VEGF is the most powerful and the most specific factor of endothelial cell growth. The family of VEGF consists of VEGF-A (habitually termed VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placenta growth factor (PlGF) [90]. The factors bind to their specific receptors VEGFR-1, VEGFR-2 and VEGFR-3 on endothelial cells, as well as also to neuropilin 1 and 2 (Nrp-1 and Nrp-2), representing receptors for semaphorins, factors of a directed axon growth. Interactions between molecules of VEGF family and their receptors are complex. Depending on the type of linkage formed, the interactions initiate distinct pathways, as illustrated in Figure 2.

FIGURE 2. Interactions between members of VEGF family and their receptors. Specific binding ligand/receptor causes different effect on endothelial cells

VEGF gene contains 8 exons. Its alternate splicing during transcriptions yields at least 6 isoforms of the protein: VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉ and VEGF₂₀₆, of which isoforms VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉ are most frequently produced by several cell types [23]. In some isoforms, presence of heparin-binding domains favours formation of VEGF – proteoglycan compounds in extracellular matrix, which promotes cumulation of the molecules in ECM.

Synthesis of VEGF is induced in conditions of anoxia by HIF (hypoxia inducible factor). In such a situation, inhibition takes place of oxygen-dependent constitutive HIF-1 α degradation. HIF binds to the promoter of VEGF gene and stimulates its transcription [49].

Effect of VEGF on angiogenesis is comprehensive and manifests in several areas. It induces proliferation, growth, migration of endothelial cells and affects their spatial organization during formation of a blood vessel [23]. It increases permeability of blood vessels, i.e., due to formation of gaps between the cells [6].

Activating nitrogen oxide synthase (eNOS) and linked to its production of nitrogen oxide it induces vasodilation during preliminary stages of angiogenesis [34]. It also stimulates expression of proteases engaged in degradation of extracellular matrix [64]. Moreover, it activates expression of anti-apoptotic factors, acting as a factor which secures survival of endothelial cells [9].

Several factors which stimulate angiogenesis are thought to act indirectly, through induction of VEGF expression. Such factors include, i.a., transforming growth factor (TGF β), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), interleukins 1 β and 6 [82]. It was also claimed that nitrogen oxide may stimulate expression of VEGF, forming in this way an autocrine loop with VEGF [74].

PLACENTAL GROWTH FACTOR (PlGF)

Placental growth factor (PlGF), belonging to VEGF family was discovered in 1991 by Graziella Perscio [53]. In parallel, PlGF was found to bind to receptor 1 of VEGF. Moreover, in mice with no PlGF gene the process of angio- and arteriogenesis was found to be significantly inhibited despite high levels of VEGF [52].

PlGF gene is located on the long arm of chromosome 14, in locus 24 and it encompasses 7 exons. Amino acid sequence of PlGF is 42% identical with sequence of VEGF and PlGF demonstrates a high structural similarity to VEGF [53]. PlGF functions as a dimer. Until now, its 4 isoforms were identified, differing in their number of amino acids: PlGF-1 (PlGF₁₃₁), PlGF-2 (PlGF₁₅₂), PlGF-3 (PlGF₂₀₃) and PlGF-4 (PlGF₂₂₄), formed due to an alternate splicing. The isoforms manifest also affinity to other receptors. PlGF-1, PlGF-3 and PlGF-4 bind to receptor 1 of VEGF, while PlGF-2 in addition binds to neuropilin 1 and 2. Moreover, isoforms 2 and 4, which carry heparin-binding domains, may bind to proteoglycans of ECM and be stored there.

At first, presence of PlGF was demonstrated in placenta, in which it was supposed to control growth and differentiation of trophoblast [53]. Immunocytochemical studies demonstrated presence of PlGF in media of large placental blood vessels. Studies demonstrating expression of PlGF in early stages of embryogenesis in mice suggest its involvement in initiation and coordination of vascularization [2]. PlGF is present also in the heart, lungs, thyroid gland, skeletal muscles and adipose tissue [66].

Placental growth factor represents a positive regulator of angiogenesis. It is thought to stimulate the process by a few mechanisms [Figure 4]:

- by a direct effect on endothelial cells through VEGFR-1 [52],
- by separation of VEGF from receptor 1, in parallel allowing interaction of VEGF with VEGFR-2 [49],

- by sensitization of cells to activity of VEGF-VEGFR-2 through interaction of PlGF-VEGFR-1 [96],
- by recruitment of monocytes/macrophages engaged in the process of vascular growth [16]
- through mobilization of haemopoietic progenitor cells from bone marrow [16].

Placental growth factor may also act as an inhibitor of angiogenesis. This happens when PlGF forms a heterodimer with VEGF. Such a complex manifests 20- to 50-fold less pronounced angiogenesis-stimulating effect than that induced by VEGF homodimer. Experiments on animal models demonstrated that PlGF may block angiogenesis stimulated by VEGF by decreasing the pool of VEGF homodimers [98].

FIGURE 3. VEGF and PlGF dependent angiogenesis can be initiated by binding to VEGFR-1 and VEGFR-2 receptors, both present on endothelial cells (EC). VEGFR-1 is also present on macrophages (MA) and haematopoietic progenitor cells (HPC).

ENDOGLIN (ENG) / CD105 ANTIGEN

Endoglin/CD105 represents a homodimeric transmembrane protein of 180 kDa molecular weight. It was identified in 1985 by the team of Latarte [30]. The gene of CD105 is located on chromosome 9q34 and contains 14 exons. The protein contains short cytoplasmic and transmembrane domains and an extensive extracellular domain, containing RGD (arginine-glycine-aspartic acid) motif. It undergoes N- and O-glycosylation. Two isoforms of endoglin are known, differing in cytosolic terminus: L-CD105 (633 aa, 47 forms the intracellular domain) and S-CD105 (600 aa, 14 at the cytoplasmic terminus) [8]. The form L is suggested to prevail.

Endoglin is thought to represent a protein engaged in angiogenesis, maturation of blood vessels and maintenance of body homeostasis. CD105 manifests a pronounced expression in endothelial cells at the early stages of embryogenesis (4th-8th week) and during formation of septa and valves in myocardium [69]. Absence of endoglin in mouse embryos is lethal [45]. In human tissues CD105 manifests expression mainly in cells of vascular endothelium and of stroma [26] and in placental syncytiotrophoblast [89]. Its poor expression is noted also in activated monocytes, macrophages, erythrocyte precursors, fibroblasts, mesangial cells, dendritic cells and melanocytes [26, 18]. Endoglin level manifests positive correlation with the extent of proliferation of human umbilical vein endothelial cells (HUVEC) and with expression of proliferation markers, Ki-67 or cyclin A [27]. CD 105 manifests an overexpression in endothelial cells of newly formed blood vessels of neoplastic tumours [27]. It is present also in immature blood vessels and blood vessels undergoing reconstruction.

Endoglin demonstrates an extensive homology with receptor III (betaglycan) of transforming growth factor β and it functions as its adjuvant receptor, modulating cell response to TGF β action [17]. It is able to bind TGF β 1 and 3 (but not TGF β 2) and other ligands, e.g. activin A or bone morphogenetic protein (BMP) 7 and 2 [17]. It was demonstrated that CD105 in order to bind the ligand it requires presence of TGF β receptor II. Moreover, ENG interacting with TGF β receptor I and II controls degree of their phosphorylation and in this way affects their ability to transmit signals [30]. However, it may function independently on cell surface.

Mechanism of endoglin functioning as a molecule of pro-angiogenic character has not been fully recognised. Endoglin seems to stimulate TGF β /ALK1 signalling pathway, which induces phosphorylation of Smad 1 and 5 molecules which, in the result, promotes proliferation and migration of endothelial cells to the site of blood vessel formation [31]. In parallel, it indirectly inhibits the Smad 2 and 3 phosphorylating TGF β /ALK5 pathway, responsible for inhibition of cell divisions.

Endoglin is also suggested to represent a component of the pathway activating synthesis of nitrogen oxide and a modulator of cyclooxygenase 2 (COX-2) and in this way, indirectly, it controls tension of blood vessel wall [38]. Probably, it influences also activation of expression involving molecules of anti-apoptotic activity in vascular endothelium cells during neoplastic angiogenesis [44].

Involvement of endoglin in neoplasia of blood vessels in a neoplastic tumour has not been confirmed but *in vitro* an elevated expression level of the protein was demonstrated in metastases of prostate tumours and ovarian cancer [48, 61]. Moreover, a deficit in the molecule in CD105 $\pm$ mice, carrying Lewis' lung tumour, was linked to a significantly decreased blood vessel supply to the tumour and size of the tumour [19]. Evaluation of microvessel density (MVD) in a tumour

using anti-CD105 antibodies is thought to provide a good tool to evaluate prognosis [43].

FIGURE 4. A hypothetical role of endoglin/CD105 in TGF-β/ALK5 and TGF-β/ALK1 signalling pathways in endothelial cells.

CYCLOOXYGENASE 1 COX-1/PTGS1

Cyclooxygenase (termed also synthase of prostaglandins G/H) is a key enzyme catalyzing transformation of arachidonic acid (AA) to prostaglandins G₂ and H₂, engaged, i.a., in control of inflammation, fever and pain [91]. Two isoforms of the enzyme include COX-1 and COX-2. Expression of COX-1 is constitutive, while COX-2 is activated by stimulators of inflammation and proliferation [86].

Studies on cyclooxygenase 1 have continued since 1970-ties but sequence of human COX-1 was determined in 1989 roku [100]. Gene of the enzyme is located on the long arm of chromosome 9 in locus 32 and it includes 11 exons. The

produced protein contains 576 amino acids and manifests molecular weight of 70 kDa.

COX-1 undergoes expression in almost all types of human tissues. A particularly high level of the protein is present in endothelium of blood vessels, smooth muscle cells, seminal vesicles, blood platelets, monocytes, mesothelial cells and nephron collecting tubules [91]. COX-1 stimulates synthesis of PGs required to maintain homeostasis (i.a., in alimentary tract or in haemostasis). Despite the claims that PTGS1 is permanently present in tissues, induction of the enzyme was observed in several cell lines, in which cells differentiate (i.a., in endothelial cells) [57]. Also, augmented expression of COX-1 was demonstrated in acute cholecystitis in guinea pigs [10]. Moreover, elevated level of the enzyme was documented in some tumours (breast cancer, oesophageal adenocarcinoma, cancer of uterine cervix) as well as during development of polyps in large intestine [75, 46, 58]. The fact was explained by involvement of COX-1 in the process of angiogenesis.

Role of cyclooxygenase in angiogenesis is far from clear. The protein seems to be engaged in formation of blood vessels. The data stem mainly from experimental models. This function of COX-1 is confirmed mainly by the fact that selective inhibitors of COX-1 block also neovascularization [58, 75, 76, 46]. Moreover, expression of PTGS1 expression was found to correlate with factors of pro-angiogenic character: VEGF, bFGF, angiopoetins 1 and 2 [46, 58, 76]. Moreover, release of the factors was found to be blocked by inhibitors of COX-1. The mechanism through which COX-1 controls angiogenesis has not been recognized. Probably it is linked to engagement of the enzyme in synthesis of prostaglandins, which significantly affect formation of blood vessels [92]. COX-1 probably participates in one of the terminal stages of angiogenesis, i.e. formation and elongation of vascular lumen [92].

ANGIOGENESIS AND NEOPLASTIC PROCESS

A crucial step in tumour biology involved the thesis, posed by the American scientist, Judah Folkman in 1971, related to significance of angiogenesis for development of tumours [25]. At the first stage of tumour progression, it represents an accumulation of around one million cells of not more than $1 - 2 \text{ mm}^3$ in size. At the phase of growth and development, the tumour is independent of vascular supply since oxygen and nutrients rich tumour cells by diffusion. On the other hand, at later stages of carcinogenesis tumour stimulates formation of new blood vessels since the till now available manner of tumour supply becomes insufficient and in its central portion necrosis may develop. In parallel, the tumour takes advantage of the vascular network to spread throughout the body by development of metastases [25]. Reports of recent years indicate that the newly

formed blood vessels play additional functions: they provide source of growth factors, cytokines and hormones, which stimulate tumour growth [71], as well as manifest proteolytic activity which promotes tumour invasiveness [13]. The process of angiogenesis, in physiological conditions strictly controlled, in a neoplastic disease by-passes control mechanisms. A stable production develops of factors, manifesting pro-angiogenic activity while expression of neovascularization inhibitors becomes reduced: the angiogenic switch becomes eliminated [33].

The strict relationship between angiogenesis and tumour development provided a starting point for studies on a new manner of treating neoplastic diseases, the anti-angiogenic therapy. The idea surfaced already within a year after the critical discovery of Folkman. After over 30 years later, the first drug blocking tumour angiogenesis (Avastin/bevacizumab) became accepted for clinical use [36].

ANGIOGENESIS IN SOLID TUMOURS

A pronounced angiogenesis is observed in a number of solid tumours, both in adults and in children. In adults intensity of blood vessel formation manifests a strict correlation with clinical advancement of a disease, prognosis and frequency of metastasis development [56]. The process is monitored mainly by evaluation of expression related to pro-angiogenic type factors. Studies *in vitro* and *in vivo* confirm elevated levels of vascular endothelial growth factor and its receptors in breast tumours, colorectal cancer, oesophageal cancer, cancers of gallbladder, pancreas or prostate [51, 80, 5, 67, 88]. Elevated levels are also noted of other molecules participating in the process of angiogenesis, such as : fibroblast growth factor (FGF), angiogenin (Ang), transforming growth factor (TGF β), tissue factor (TF) and other [55]. In cases of many tumours, microvessel density (MVD) seems to provide a prognostic factor [55].

Tumours developing in children manifest a distinct biology than that characterizing tumours of adults. They manifest lower differentiation of cells, more intense growth and a pronounced blood supply [56] and, therefore, they are discussed separately. In many tumours of childhood, similarly as in adults, i.a. in malignant gliomas, neuromas, sarcomas, liver tumours elevated levels of VEGF are noted [62]. Also, an increased expression is noted of bFGF, angiopoietin, PDGF-A in embryonal neuromas [21] and of hepatocyte growth factor (HGF) in nephroblastoma [83]. Elevated levels of pro-angiogenic factors and of VEGF in particular represent an unfavourable prognostic factor [47].

ANGIOGENESIS IN LYMPHOPROLIFERATIVE TUMOURS

In 1994 the study of Vacca's team was published, demonstrating a relationship between progression of multiple myeloma (MM) and intensified development of new blood vessels in bone marrow [94]. The observations as well as later reports of the team [93] provided a starting point for studies on angiogenesis in proliferative diseases in haemopoetic system. Subsequent studies brought confirmation of angiogenesis development in tumours of the type [1, 28]. The observations of many research teams pertained to adults and children, in whom haematological tumours represent in the period of life the most frequent group of tumours [70].

The intensified angiogenesis in haemopoetic tumours is manifested, first of all, by augmented number of blood vessels in bone marrow, as observed for the first time by Perez-Atayde in 1997 [65] in children with acute lymphoblastic leukemia, and which was later confirmed by other investigators [68, 60]. Moreover, an increased production is also noted of pro-angiogenic factors (an intensified expression of genes and proteins) both in tumour cells and endothelial cells and cells of bone marrow stroma. This is expressed by elevated levels of angiogenesis stimulators in body fluids [4, 99]. A decisive majority of such studies pertain to leukemias, representing the most frequent type of haematologic tumours.

Thus, angiogenesis seems to form a fraction of the pathomechanism leading to lymphoproliferative tumours. However, while the cause for development of new blood vessels seems clear in solid tumours, in haematological hyperplasia determination of angiogenesis role poses an enigma. Few hypotheses attempt to clarify the matter. One of them assumes that a number of auto- and paracrine loops links cells of bone marrow stroma (also endothelial cells) with tumour cells, stimulating growth and proliferation of the two types of cells (Figure 5) [39]. It is suggested also that the newly formed blood vessels of bone marrow undergoing no destruction during chemotherapy provide a type of niche in which tumour cells find a shelter. In parallel, they provide factors of a paracrine type, which allow for growth of the „saved” cells [72].

FIGURE 5. The model of auto- and paracrine loops between different cell types in bone marrow.

ANGIOGENESIS AND ACUTE LYMPHOBLASTIC LEUKEMIA

Studies on potential involvement of angiogenesis in pathophysiology of haemopoetic tumours pertain to a significant extent to acute lymphoblastic leukemia in children. Investigations using immunohistochemical techniques demonstrated stimulated formation of blood vessels in bone marrow of ALL

children already at the moment of diagnosis [65, 68, 60]. Interestingly, the reports related to the number of blood vessel densities in bone marrow during remission are contradictory: some investigators detected their decrease [68], while other authors detected no differences [65]. However, such a measurement of MVD manifested no prognostic value: only Norén-Nyström observed that a high proportion of blood vessels in bone marrow may be treated as a factor predisposing to relapse and only in patients qualified to the group of a high risk [60]. A decisive majority of reports related to angiogenesis in ALL analyzed expression of neovascularization-stimulating factors. Evaluation of the factor concentrations, both at the mRNA and protein levels, provides an indirect proof for significance of the process. The experiments are focused mainly on principal controllers of angiogenesis: VEGF and bFGF. Studies are available which demonstrated an elevated level of bFGF in body fluids of children with ALL, as compared to a control group [65, 50], but other reports denied such an effect [99, 78, 87]. A similar situation can be noted in the case of vascular endothelial growth factor. Numerous reports documented an elevated level of VEGF protein and an augmented expression of the gene, demonstrating also a prognostic significance of the variable [42, 78, 4, 50, 87]. Nevertheless, some authors failed to observe an increased production of VEGF [99]. An increased expression of other pro-angiogenic factors was also observed, such as TGF β [87] and receptor 3 of VEGF [40]. Discrepancies between the obtained results are explained by distinct techniques of measurement and inhomogeneity of studied groups. Thus, beyond doubt, discrepancies in the results point to the need of a continuing monitoring of angiogenesis in acute lymphoblastic leukemia.

ANGIOGENESIS AND ACUTE MYELOID LEUKEMIA

Literature reports suggest also a relationship between vasculogenesis and pathogenesis of acute myeloid leukemia (AML). Aguayo et al. [3] observed an increased number of blood vessels in bone marrow, i.e., in patients with AML. The team detected also elevated levels of pro-angiogenic type factors (VEGF, bFGF, HGF, TNF α) in sera of AML patients, as compared to the control. The team of Hussong et al. [37] confirmed the reports. The investigators, using endothelial markers, including von Willebrand's factor, evaluated blood vessel density in bone marrow of adult patients with AML and control patients and found their significantly higher number in AML. They detected also expression of VEGF in cells originating from patients with AML and in cell lines of acute myeloid leukemia. Elevated numbers of blood vessels in bone marrow were detected also by Padro et al., [63]. Interestingly, they observed a reduction in number of capillaries in the course of chemotherapy: in the 16th day after induction chemotherapy MVD was reduced by 60%, and in patients at remission vascular

density reached the level noted in the control group. In acute myeloid leukemia not only an augmented angiogenesis is noted in bone marrow and an elevated level of angiogenesis stimulators but also their prognostic value is seen in AML patients. de Bont et al. [19] observed VEGF production by leukaemic cells and proved that its level in the group of high risk AML manifested a relationship with survival time and that the variable may serve as an independent prognostic factor. In turn, Hu et al. [35] found that levels of the soluble forms of VEGF receptor 1 carries a prognostic significance. Moreover, it was found that evaluation of microvessels using magnetic resonance may prove extremely helpful in determination of prognosis for a patient, suggesting that an augmented number of blood vessels in bone marrow represents an unfavourable prognostic factor [81]. The observations clearly point to formation of new blood vessels in bone marrow of AML patients and suggest an involvement of angiogenesis in development of the disease.

ANGIOGENESIS IN HAEMATOLOGIC MALIGNANCIES: CLINICAL SIGNIFICANCE

In recent years, physicians in charge of patients carrying haematologic tumours encounter the basic dilemma of how to qualify patients to individual risk groups and how to conduct treatment in patients manifesting an unfavourable course of the disease [manifesting, e.g., resistance to steroid drugs, delayed remission or relapse of the disease]. In several situations, therapeutic management is based on variables accepted by specific medical community therapeutic protocols. Nevertheless, in a certain group of patients employment of the corroborated therapeutic protocols proves to be insufficient. Such patients require a much more individualized treatment and monitoring of its effects. Current status of the therapy leads to the situation that a patient with relapse of the disease is subjected to consecutive new chemotherapy schedules (including transplantation of haemopoietic cells), which inevitably leads to progressive cachexia and an increased propensity to potentially lethal infectious agents.

Could such a status be altered? The most desirable element of therapeutic individualization in such cases seems to involve not exacerbation of the applied therapeutic protocols but attempts to target chemotherapy, basing first of all on knowledge of tumour biology. Such a type of treatment finds nowadays a restricted application. It takes into account, first of all, evaluation of tumour cell sensitivity to various chemotherapeutic agents and attempts to apply therapy using antibodies, which selectively destroy specific population of tumour cells. The commendable but solitary exception in such actions is application of drugs which block translation in patients with t(9:22) translocation (Philadelphia chromosome).

Not much can be cited on practical aspects of applying drugs which block neoplastic angiogenesis in lymphoproliferative diseases. Although the problem is

not new, it is difficult to encounter examples of generally accepted therapeutic protocols which apply anti-angiogenic drugs in patients with therapeutic failure. The principal cause for such a status is not the fear of undesirable side effects of angiogenesis-blocking drugs, but absence of a sufficient proof for involvement of pro-angiogenic factors in pathogenesis of lymphoproliferative diseases.

Biology of lymphoproliferative tumours continues to pose a puzzle. In contrast to solid tumours, in which neoangiogenesis remains fully logical and understandable, development of new blood vessels in haematologic tumours remains a matter of conjectures and assumptions. The question arises of how to explain the need for development of the blood vessels in tumours of haemopoietic system, when the tumour itself develops in the biotope of bone marrow or in the site of blood formation. Certainly, the process of developing new blood vessels is not needed to supply the proliferating neoplastic cells with nutrients, oxygen or factors stimulating their growth. This factor is also indicated by the clinical course in this group of tumours: no primary tumour can be detected and the disease is primarily of a diffuse character. In addition, in bone marrow neoplastic cells enjoy a permanent and an easy access to already existing blood vessels in sinusoidal compartment of bone marrow.

Nevertheless, which surprised investigators already in 1990-ties, also haematologic tumours, including acute lymphoblastic leukemia and acute myeloid leukemia, manifest an increased number of blood vessels in bone marrow and elevated serum levels of several pro-angiogenic factors. The question which can be posed at this moment is whether this is a purposeful action in the course of the diseases or, perhaps, tumour cells, copying behaviour of other tumour cells in solid tumours, begin to produce blood vessels without the intention to use them. Solution of such a problem seems complex: first, in no tumour purposeful actions can be encountered, which would reflect optimum use of energy and available components, as it can be observed in a healthy tissue. A tumour represents a chaos, the principal effect of which involves destabilization of existing structures and relationships. Against such a background, development of new blood vessels in a haematologic tumour may be treated as a clear accident. An accident which, however, does not remain without lethal consequences: the newly formed blood vessels, possibly made of neoplastic cells themselves, are not destroyed in the course of pharmacotherapy. It suggests that they might create a specific niche, a reservoir for blasts, located beyond the reach of the applied chemotherapeutic drugs [72]. In parallel, apart from the undoubted benefit of the shelter, tumour cells obtain an additional source of growth factors: endothelial cells begin to produce substances which stimulate growth, differentiation and proliferation. It should be mentioned that blood vessels synthesize also angiogenesis regulators, factors of stimulator and inhibitor types, which represents their *de facto* natural function, intensified in neoplastic diseases. In parallel, in line with the theory related to cancerogenesis-associated angiogenesis, also tumour cells are responsible for production of pro-angiogenic factors. In cases of lymphoproliferation, the

producers include also stromal cells of bone marrow. The facts permitted to construct a model demonstrating angiogenesis-linked relationships between various cell populations in haematologic tumours. Function of several auto- and paracrine loops is postulated, produced and acting on the above cell types. Such events result in development of a microenvironment in bone marrow which is rich in newly formed blood vessels, which promotes tumour development [39]. As it was already mentioned, neoangiogenesis in lymphoproliferative tumours manifests not only elevated number of blood vessels in bone marrow but also increased concentrations of angiogenesis-stimulating factors in body fluids [65, 4]. Aguayo et al. [3] demonstrated, among other, an increased concentration of VEGF in bone marrow and peripheral blood which represented an independent prognostic factor in patients with acute myeloid leukemia. Similar results were obtained by Avramis et al., [4] who showed that not only high level of VEGF in serum could be noted in children with ALL who suffered from a relapse of the disease, but in addition it manifested negative correlation with timing of the relapse. The data were confirmed by the team of Lyu [50], who observed a significantly higher levels of VEGF and fibroblast growth factor in children with acute leukemia. Investigations determining concentrations of angiogenesis regulators also belong to the molecular aspects. Koomagi et al. [42] noted elevated levels of mRNA coding for vascular endothelial growth factor during relapse of ALL, which was corroborated later by Stachel et al. [87]. Moreover, duration of relapse-free survival and total survival time manifested a negative correlation with VEGF concentration. Obviously, the most reliable method determining intensity of angiogenesis involves a direct estimation of microvessel density in bone marrow. Already the last 1990-ties brought information on augmented microvessel density in bone marrow of patients with acute leukemia [65, 37]. The elevated MVD was found to be linked to prognosis of unfavourable events in the course of ALL, such as relapse or death [60]. Against this background, it seems justified to monitor indirect (expression of pro- and anti-angiogenic factors) and direct (MVD in bone marrow) parameters of neovascularization, aimed at attempting to modify therapeutic management of patients with haematologic tumours.

The problem of angiogenesis in hyperplasias of haemopoetic system is relatively new: the first reports originated from mid 1990-ties. The studies were focused mainly on evaluation of expression shown by principal regulators of angiogenesis, the VEGF family, seldom encompassing evaluation of other factors, the role of which in this type of tumours can be far greater. The very term of „haematologic tumours” is extremely broad, including tumours originating from various cell types of an acute or a chronic course. It should also be born in mind that biology of the diseases differs markedly between adults and children. Difficulties originate also from contradictory reports and absence of knowledge related to function of various factors. Therefore, studies on angiogenesis in haematologic tumours seem to be immensely interesting from both the clinical and the cognitive point of view.

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