Inhibitors of angiogenesis in therapy of ovarian cancers

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Summary

The capacity to induce growth of blood vessels represents one of the phenotypic traits of neoplastic cells. Several preclinical studies prove that the inhibition of growth of peri-neoplastic blood vesssels leads to restricted growth of primary tumours and of metastases. Nevertheless, clinical studies indicate that angiogenesis inhibitors are not such effective drugs as might be expected on the basis of studies conducted on animals. In this article we would like to draw the readers' attention to divergencies between preclinical and clinical results, in particular to those related to ovarian cancers. In the treatment of ovarian cancers, angiogenesis inhibitors combined with other drugs may prove to represent a relatively effective therapeutic approach.

Key words: Angiogenesis; Angiogenesis inhibitors; Ovarian cancer.

Introduction

The belief that the ability to induce growth of new blood vessels represents one of the phenotypic traits of tumour cells seems to be well grounded [1]. Pre-clinical studies indicate that:

(1) avascular tumours fail to develop until they are reached by newly formed blood vessels [2];

(2) dormancy of tumours can be interrupted by transfection of neoplastic cells with a gene coding for one of the pro-angiogenic factors [3];

(3) tumour growth may be blocked by inhibitors of endothelial cell proliferation, by so-called anti-angiogenic factors [4, 5].

However, clinical studies more and more often demonstrate that angiogenesis inhibitors are not such effective drugs as could be anticipated from the results obtained in animal models [6]. The question is why the results of anti-angiogenic therapy in animal models are so encouraging but anti-angiogenic therapy remains so ineffective in humans and how to explain the divergence.

Angiogenesis and anti-angiogenic drugs

Angiogenesis, the process of the formation of new blood vessels from already existing ones, represents a relatively complex sequelae [7-10]. Greatly simplified, it is composed of three stages:

– formation of space for newly forming vessels (involving activation of enzymes which participate in digestion of the extra-cellular matrix);

– initiation of proliferation of the resting "till now" vascular endothelial cells and migration of the latter towards the source of pro-angiogenic signals;

– differentiation of proliferating endothelial cells and formation of vascular lumen.

The process of angiogenesis results from the coordinated action of multiple normal cells, including macrophages, pericytes and cells circulating in blood: monocytes, endothelial cells and thrombocytes. The process is initiated by specific growth factors which stimulate endothelial cells. Under regular conditions, e.g., in the course of wound healing, angiogenesis remains strictly controlled. Controlled secretion of various pro-angiogenic factors and anti-angiogenic factors from various cells leads to the restriction of growth of the vessels. In pathological conditions, however, e.g., in neoplasms, a highly uncontrolled release of pro-angiogenic factors develops: "tumours represent a never healing wound" [12]. Neoplastic cells, which cause growth factors and thus enforce the uncontrolled proliferation of endothelial cells, are supposed to be responsible for the effect [13].

Apart from genetic variables which participate in the induction of angiogenesis, also epigenetic factors may induce the formation of blood vessels. Hypoxia, a deficit of oxygen in tissue, represents one of such epigenetic factors which may affect expression of genes coding for pro-angiogenic factors [14].

The process of the development of new peri-neoplastic blood vessels is continuous, uncoordinated and uncontrolled. The peri-neoplastic vessels may develop from already existing ones or may appear due to so-called co-opting of the already existing vessels [15]. In the formation of peri-neoplastic blood vessels, precursors of endothelial cells may also participate, originating from bone marrow and circulating in the blood [16]. In the latter case, angiogenesis resembles rather vasculogenesis, i.e. formation of vessels from precursors of endothelial cells, as it happens, e.g., in embryogenesis. Walls of peri-neoplastic blood vessels may also be formed and lined by tumour cells (known as mosaic vessels) [17].

The newly formed peri-neoplastic blood vessels markedly differ from regular blood vessels [18]. The most significant traits of neoplastic blood vessels include persistent proliferation of endothelial cells, a sinusoidal course of the vessels, numerous anastomoses and dead ends, in some regions absence of the supporting cells vessels, pericytes and muscularis, absence of lymphatic drainage and pronounced permeability of their walls. The last trait and the absence of lymphatic drainage represent some of the causes of intra-parenchymatic pressure in the tumours [19].

The changes taking place in the genetic material of neoplastic cells are the cause of vascular induction [1]. In a significant way, tumour cells start to activate several genes, including the VEGF gene, coding one of principal growth factors for endothelial cells. The change in genotype of neoplastic cells was preliminary known as the angiogenic switch [13].

The angiogenic switch promotes the development of a previously avascular tumour [13]. Tumour progression takes place when neoplastic cells begin to stimulate development of their own vascular supply.

If the development of blood vessels represents a pre-requirement or an indispensable condition for the development of tumours or metastases, the question arises whether the inhibition of vascular network development could suppress the growth of primary tumours and of metastases.

Multiple experimental data indicate that in fact this is so [7, 8, 10, 20]. Disturbed equilibrium between pro- and anti-angiogenic factors with a prevalence of the latter one not only inhibits the growth of new vessels, which may bring about a slowed down growth of tumours and metastases, but in some cases it results in regression of the blood vessels.

Inhibitors of angiogenesis have recently become a novel class of anti-neoplastic drugs. The drugs, by their nature, are cytostatic agents: inhibiting proliferation of endothelial cells they restict formation of new blood vessels. The drugs may, e.g., affect secretion of pro-angiogenic factors by tumour cells. The classical example of this category, called, trastuzumab, is the antibody capable of blocking ERBB2 (HER2/neu). Inhibited activity of the receptor results in restricted secretion by tumour cells of various pro-angiogenetic factors, including angiopoietin 1 and VEGF. Anti-angiogenic drugs may bind or sequester free extracellular pro-angiogenic factors. Such drugs may include, e.g., soluble VEGF receptors and antibodies which inactivate VEGF. Finally, some drugs may affect various stages of mitogenic signal transduction, initiated by the stimulated receptor of the growth factor. This group encompasses, e.g., inhibitors of tyrosine kinases, inhibitors of endothelial cell proliferation and migration.

Obviously, other classifications of anti-angiogenic drugs are available. In a few recent reviews not only new classifications of anti-angiogenic drugs can be found but also the accurate descriptions of their mode of action [20-22].

Among the preclinical studies which demonstrated efficacy of angiogenesis inhibitors in the therapy of tumours, in our opinion a few reports deserve special emphasis. One of them provided evidence for an effective action of endostatin (proteinaceous endogenous inhibitor of angiogenesis) in the therapy of selected tumours in experimental animals [23]. The study supported a conviction that anti-angiogenic therapy represents an approach which is not accompanied by the development of resistance to anti-angiogenic drugs. The studies of Browder *et al.* [24] and those of Klement *et al.* [25] demonstrated that certain cytostatic drugs (e.g., cyclophosphamide or vinblastin) may inhibit angiogenesis and indirectly the growth of tumours. The drugs, when combined with anti-angiogenic factors (TNP-470 or antibodies inactivating VEGF receptor), may even result in a complete cure. In turn, the studies of Kuo *et al.* [26] demonstrated that VEGF-sequestering agent (soluble receptor of VEGF), at least in experimental conditions employed by the authors, proved to be a much more effective drug than endostatin or angiostatin. It was thought to represent the strongest anti-angiogenic drug. Independently of the critical comments related to the application of endostatin in

several clinical trials [27], the conviction that anti-angiogenic drugs represent an effective approach in preclinical studies has won broad support.

Nevertheless, the clinical studies conducted with several drugs, either in the form of monotherapy or in combined therapies, indicate that the therapeutic benefit is not as promising as expected [6, 22, 28, 29].

Anti-angiogenic drugs in therapy of ovarian cancers

Thalidomide, an inhibitor of angiogenesis, was one of the first drugs introduced to anti-angiogenic therapy. In foetuses delivered by mothers who used the drug for sedation, it induced inborn defects of the extremities. In pilot studies, the drug has failed to demonstrate the expected therapeutic efficacy even if it counteracted the influences of pro-angiogenic cytokines, such as TNF-α, VEGF and bFGF. Among 19 women with ovarian cancer who participated in phase II trials only one has experienced stabilization of the disease [30].

In another study, among ten patients with ovarian cancer who were resistant to taxanes and platinium derivatives as well as to standard cytostatic drugs a positive reaction was obtained in three patients (37.5%) and it persisted for up to 60 weeks [31]. Side-effects of the drugs fit grades 1 and 2 in the WHO scale [30, 31]. In the therapy of other malignant tumours (e.g., of multiple myeloma) thalidomide was administered in parallel to cytostatic drugs or interferon and the response was noted in approximately 70% of patients. However, side effects were also evident, including thromboembolic disease, neurological disturbances and disturbances of cardiac rhythm [32].

It has not been disclosed up till now, which of the thalidomide activities played a principal role in the therapy of neoplasma: inhibition of angiogenesis, modulation of immunological functions, blocked expression of molecules, participating in intercellular adhesion or inhibition of cyclooxygenases (Cox-2) thus decreasing production of prostaglandins [32].

A new therapeutic approach in phase II trials of the Gynaecological Oncology Group (GOG) involved IV administration of an angiogenesis inhibitor, recombined human interleukin 12 (rh IL-12). Partial response was obtained in one patient and stabilization of the disease in 26 out of 28 patients subjected to the test. However, it should be noted that all the patients subjected to the therapy suffered from a relapse of the disease or they were resistant to a previously applied chemotherapy. In 21% of the patients the fourth degree of myelotoxicity was noted, according to WHO [33].

An attempt was also made in the therapy to employ CAI (carboxy-amidotriazole), a synthetic small molecular weight inhibitor of non-inducible calcium channels. The drug inhibits angiogenesis, proliferation of neoplastic cells and decreases the metastasizing potential of the tumour. However, in 39 patients with tumours, including five patients with ovarian and oviductal cancer, poor response to the treatment was noted. Clinical studies on the application of CAI are being continued [34].

In around 70% of ovarian cancers, the cell membrane receptor for EGF (EGFR) undergoes expression and its presence correlates with poor prognosis. The receptor undergoes expression not only on tumour cells but also on endothelial cells in the tumour [35]. In the multicentre phase II GOG studies response was detected by EGFR inhibitor, ZD1839 (Iressa, Gefinitib). Iressa represents a small molecular weight compound (anilinquinazoline derivative) capable of blocking signal transmission by inhibition of EGFR tyrosine kinase. Among 27 women with ovarian cancer, positive response was noted in only one patient (3.7%), who was resistant to platinium, while in three other patients (11.1%) stabilization of the disease was obtained. Until now, the other EGFR-blocking drug, C225 humanised mouse antibody, has not been tested on patients with ovarian cancer. The antibody combined with chemotherapy, has been already applied in cases of patients with malignant tumours of other localization (e.g., in cancers of the neck and head regions). In preclinical *in vivo* and *in vitro* tests blocked EGFR inhibited production of the three pro-angiogenic factors, VEGF, IL-8 and bFGF. The factors seem to play a significant role in the growth of ovarian cancer and in the development of ascites connected with ovarian cancer [36, 37].

Clinical trials concerning the application of other angiogenesis inhibitors in ovarian cancer are being continued. These include, e.g., Squalamine originating from the shark, *Squalus acanthias,* Atrasentan, an antagonist of endothelin capable of binding its receptor, or Bevacizumab (Avastin), the humanised monoclonal antibody reacting with VEGF [35, 38].

In the studies of Li *et al.* [39] on ovarian cancer cell lines, Squalamine amplified effects of cisplatin or carboplatin independently of HER-2 status of the tumour. Cells with ovarian cancer with HER-2 overexpression demonstrated resistance to cisplatin. Attempts are continuing to combine chemotherapy with the application of Squalamine in patients with ovarian carcinoma, but the respective results have not been published up to now [39].

At present, ovarian carcinoma patients are being recruited in the treatment combining administration of cisplatin with bryostatin. Bryostatin is a lactone isolated from the sea plant of *Bugula nerutina.* Over 300 patients have been subjected to treatment with this PKC agonist which is a family of protein kinases C but the results have not been published yet [40].

Recently, a former tendency to use interferons as anti-neoplastic drugs has been noted. The cytokines may control cellular cell cycles. Interferon α inhibits tumour cell proliferation in hairy-cell leukaemia, chronic myeloid leukaemia in lymphomas and Kaposi sarcoma. It is capable of decreasing levels of MMP-2, MMP-9, bFGF, VEGF and of IL-8 as well as inducing apoptosis in endothelial cells. Due to its side-effects, the so-called pegylated interferon began to be used in phase I trials at Anderson Cancer Center in women with ovarian cancer. When applied together, pegylated interferon and paclitaxel proved to have additional effects on xenografts of ovarian cancer [41].

Problems linked with application of anti-angiogenic drugs in clinical studies

It remains difficult to explain the divergence between the efficacy of anti-angiogenic drugs in preclinical tests and the poor response to the drugs in clinical studies in a clear way. Possibly, the blood vessels of human tumours, which used to be defined as immature and leaky, also exhibit a distinct morphology. The latter may possibly cause functional insufficiency and insufficient supply of oxygen and nutrients to the tumours. Moreover, they may not react to angiogenic and anti-angiogenic factors in the way that normal vessels do [17]. Metabolism of anoxic tumour cells causes acidification of the environment: due to the low partial pressure of oxygen, some drugs are relatively ineffective and others may even show no therapeutic effect at all [6, 35].

Perhaps, the growth of tumours is not necessarily linked with development of the peri-neoplastic vessel network [42]. Several data indicate that the so-called resistance to anti-angiogenic drugs may be linked to multiplicity of pro-angiogenic factors and, therefore, inhibition of one of the factors does not necessarily represent an effective therapeutic approach [8]. Possibly, even in anoxia, neoplastic cells may live and their growth is not preconditioned by the newly formed blood vessels [18].

The so-called heterogeneous character of ovarian cancers should also be taken into account. According to See *et al.* [35], this denotes that distinct conditions of equilibrium may exist among cyclins, cyclin-dependent kinases (CDK) and inhibitors of CDK. Excessively high levels of cyclins and of their kinases may lead to persistent proliferation, e.g., elevated level of cyclin D abbreviates G_1 phase and reduces cellular dependence on growth factors. Various cell cycle-controlling proteins may undergo different expressions in ovarian cancers and may modify the effects of both cytostatic drugs and anti-angiogenic agents. In mammary cancer, Trastuzumab (Herceptin) is used in cases when HER-2/neu expression is detected in the tumour. However, a proportion of cancers without HER-2/neu expression will be resistant to the drug. Most probably, the proportion of mammary cancer cases with HER-2/neu expression which fail to respond to Herceptin treatment exhibit expression of another system of, e.g., apoptotic proteins, p53. This further complicates the variable mechanism of action of the drug. It should be noted that expression of p53 in neoplasms is linked with a more pronounced response to anti-angiogenic therapy [43]. Therefore, before starting anti-angiogenic therapy markers of sensitivity to the therapy should be investigated.

Heterogeneous expression of various receptors to growth factors within the cancer may restrict the efficacy of the therapy targeted at one of the receptors. The number of variables may lead to a conclusion that, e.g., TGFβ exhibits a double and reciprocal effect: it may inhibit growth of cancer cells and, on other occasions, may promote neoplastic growth [35]. According to Sömmezer *et al.* [44], in angiogenesis of ovarian cancer prognostic factors include VEGF (in agreement with other authors) and TGFβ, which may provide the cause for resistance to anti-proliferative treatment [45].

A number of pro-angiogenic factors, including VEGF, bFGF, IL-8, PDGF may provide targets for new anti-angiogenic drugs.

It seems that markers which may be useful in the selection of ovarian cancer patients responding to anti-angiogenic therapy will have to be determined. Such therapy may not be effective enough to cure the cancer patient. However, in light of the presented studies, this therapy, if improved and selective, may provide us with an approach capable of controlling the growth of primary tumours and metastases.

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