Angiogenesis as a target for therapy in colorectal cancer

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Abstract

Colorectal cancer is the second most common cause of death in cancer patients worldwide and difficult to treat, especially in advanced stages. Angiogenesis is a crucial process in colorectal cancer growth, development and dissemination, with significant implications for clinical management. Surgery followed by chemo- and radiotherapy is still the treatment of choice for most patients with colorectal cancer, although in advanced stages its results are frequently not satisfactory. However, inhibition of angiogenesis has been implicated as the potential, clinically effective, additional treatment strategy in advanced colorectal cancer, as antiangiogenic drugs allow controlling the tumor growth. Accordingly, the number of clinical trials confirmed, that anti-angiogenic therapy prolonged survival of colorectal cancer patients. However, the process of tumor angiogenesis and its regulation are highly complex. It depends on the balance between pro- and anti-angiogenic factors, secreted by endothelial, tumor and host-infiltrating cells. Therefore, development of efficient anti-cancer treatment targeting primarily tumor angiogenesis might be a major step in oncology.

Key words: angiogenesis, anti-angiogenic drugs, colorectal cancer.

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Introduction

As stated in the paper on "The role of angiogenesis and angiogenic factors in colorectal cancer", colorectal cancer is a severe disease, characterized by a high mortality rate. The major reason for that is not sufficient diagnostic management. When the disease is detected the cancer is usually at a very advanced stage, the treatment is very difficult and often ineffective. There are many reasons which may contribute to the development of colonic and rectal cancers, which were also listed in the previous part; however the angiogenic switch and angiogenesis seem to be the most important issues. Each tumor growth, as established by Folkman and his coworkers, depends on angiogenesis. Therefore, an anti-angiogenic therapy may be a key treatment strategy in advanced stages of this severe cancer disease.

Therapy of colorectal cancer

Resection of the involved segment of the colon (colectomy), along with its blood supply and regional lymph nodes, is still the treatment of choice for almost all the patients with resectable colorectal cancer. Regional lymph node dissection should be performed to determine staging, which guides decisions about adjuvant therapy. Radiation, before or after surgery, appears to have a significant impact only on a local recurrence.

Chemotherapy and radiotherapy have been demonstrated to improve the overall and tumor-free survival in colon cancer patients. Colorectal cancer chemotherapy involves two major parts, adjuvant chemotherapy and its palliative analogue for more advanced tumors. Although, the standard chemotherapy regimen is given over a short period of time, it usually depends on large doses of highly toxic drugs. They are administered at nearly maximum doses, which the patient can tolerate, and, due to their toxicity, a resting period is required between the treatment sessions to allow the patient's own cells to recover. Therefore, there is a great need to find new anticancer agents, that may be used effectively, in fighting the malignancy. New anti-angiogenic drugs may probably solve the problem, since

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large clinical trials demonstrated, that an angiogenesis inhibitor added to the standard chemotherapy augments effectiveness and allows decreasing drugs doses, which then may be more frequently repeated. The treatment is also less toxic, and acquired drug resistance may be avoided. Added, anti-VEGF antibodies display their anti-angiogenic effects, which prevents vessel and tumor growth more effectively than the standard chemotherapy alone [1, 2].

Over many years, the treatment of colorectal cancer has been based upon pyrimidine antagonist synthesized by Robert Duschinsky, *i.e*. 5-fluorouracil (Adrucil, Fluorouracil, Efudex, Fluoroplex) and leucovorin, and later also on irinotecan (Camptosar), and oxaliplatin (Eloxatin – an organoplatinum complex). Although 5-FU was synthesized in 1957, it still remains the most widely used agent in treating both, early and advanced colorectal cancer. It was shown, that 5-FU, apart from its chief action to decrease the biosynthesis of pyrimidine nucleotides due to an inhibiting enzyme, thymidylate synthase, that catalyzes reactions in DNA synthesis, resulting in death of rapidly growing cells, dose-dependently increases the level of one of the anti-angiogenic proteins, thrombospondin-1 (TSP-1), in human colon cancer cell lines. Thrombospondin-1 is a potent natural inhibitor of angiogenesis. Although, TSP-1 has been reported to induce endothelial cell apoptosis and downregulate neovascularisation, the molecular mechanisms linking those two processes have not yet been established. Mediation by TSP-1 of the EC apoptosis is probably associated with alteration by this molecule of the profile of survival genes expression, by activation of caspase-3. Thrombospondin-1 increases expression of Bax, decreases expression of Bcl-2. Both, proapoptotic and antiangiogenic abilities of TSP-1 are blocked by caspase-3 inhibitors [3]. Leucovorin increases binding of 5-FU to thymidylate synthase, and thereby increases 5-FU T = 0,5 [4, 5]. Irinotecan is an inhibitor of topoisomerase I [6–10].

Combination of the drugs depends on clinical stages of the disease

There is no adjuvant therapy recommended in stage I of the disease because of the excellent 5-year survival rate (90-100%).

Stage II (node-negative disease) with an estimated 80% of 5-year survival rate. Patients with this disease stage, after surgical treatment, who are at a higher recurrence risk, may benefit from adjuvant chemotherapy.

Also, with surgical resection alone, patients with **stage III** (node-positive disease) colorectal cancer have the expected 5-year survival rate of 30-50%. Postoperative adjuvant chemotherapy significantly increases the disease-free survival. It also increases the overall survival, and is recommended for all patients with this cancer stage. Clinical trials showed that in colorectal cancer patients with one to three involved nodes treated with fluorouracil (5FU) and leucovorin (folinic acid), 5-year survival has increased to

65%. In patients with more than three nodes involved the survival has reached 40% [11].

Recently, a new 5-FU (Capecitabine) has been developed. Capecitabine (Xeloda) is an orally administered systemic prodrug, which is converted in the body to 5-FU, and at present, it is indicated as a single-agent adjuvant therapy for stage III (Dukes stage C) colon cancer patients and also for those with metastatic colorectal cancer (stage IV). The therapy is beneficial for patients since the drug may not only be taken orally, but it also produces fewer side-effects.

The FDA has also approved oxaliplatin for the adjuvant chemotherapy in combination with fluorouracil and leucovorin (FOLFOX). Disease-free survival at 3-4 years of follow up was 76%. In spite of neutropenia and sensory neuropathy, which are reversible, FOLFOX, in general, is currently a preferred adjuvant chemotherapy for most stage III cancer patients. The role of the combined adjuvant chemotherapy (FOLFOX) and anti-angiogenic agents (bevacizumab, cetuximab, panitumumab) in this stage of disease is still under clinical investigation [12–14].

Stage IV of colorectal cancer is a metastatic disease. Approximately 20% of patients have distant metastases when diagnosed with the cancer; another 30% will develop them over months. The long-term and median (less than 6 months) survival is very poor (only 5%). Patients with unresectable hepatic metastases may only be offered a long-term tumor control therapy; it usually includes local ablative techniques, such as radiofrequency or microwave coagulation, embolization, cryosurgery or intra-arterial hepatic chemotherapy.

FOLFOX and FOLFIRI (5-FU + irinotecan) extend the patients' survival up to 15-20 months, and are the preferred first-line treatment for most patients with metastatic colorectal cancer disease. Treatment with oral Capecitabine (instead of intravenous 5-FU) in combination with oxaliplatin or irinotecan is still under investigation [15].

The most recent advance in the treatment of metastatic colorectal cancer, involves the development of the biological anti-angiogenic agents, whose role has been rapidly evolving. Two novel and most promising targets in colorectal cancer treatment are the receptor for epidermal growth factor (EGFR) and vascular endothelial growth factor (VEGF). Two new drugs approved to be combined with FOLFOX and FOLFIRI therapy are bevacizumab (Avastin) and cetuximab (Erbitux). As single anti-angiogenic agents, they are also the first-line treatment for patients with metastatic colorectal cancer. Bevacizumab (Avastin) was the first monoclonal antibody targeted against VEGF, approved by the FDA in 2004. At present, adjuvant chemotherapy for colorectal cancer includes drugs of different mechanisms of action, supported by different anti-angiogenic monoclonal antibodies [13, 16-20].

Anti-angiogenic drugs – new possibility for cancer patients

Despite the optimal treatment with surgery, radiotherapy and adjuvant chemotherapy, prognosis for most patients with advanced colorectal cancer is still poor.

According to Folkman's theory, inhibition of angiogenesis might be an effective therapeutic strategy, and angiogenesis inhibitors will probably be the most important drugs in cancer therapy in the near future. Almost 50 molecules, which might switch off angiogenesis in tumors, are being tested at different phases of clinical trials involving patients with all types of cancer including those with gastrointestinal malignancy (colorectal cancer patients).

Since VEGF is one of the most important pro-angiogenic factors in patients with colorectal cancer, drugs targeting either VEGF or its cell surface receptor are still attracting a major interest [21].

However, except for this cytokine and its receptors, there is another most promising new target in colorectal cancer disease, i.e. epidermal growth factor receptor (EGF-R) [21-24]. Combination of drugs against both of them, associated with chemotherapy in stage IV colorectal cancer, which is usually incurable, resulted in a complete remission of the disease. It is believed that such therapy may increase survival rates in patients with inoperable cancer [16, 25, 26].

Following the clinical trials, drugs inhibiting EGFR and VEGF (antibodies bevacizumab, cetuximab, panitumumab) were approved for treatment of advanced colorectal cancer.

Bevacizumab (Avastin), recombinant humanized monoclonal IgG antibody, binds to free human vascular endothelial growth factor (VEGF) and blocks its binding to receptors (VEGF-R1, VEGF-R2) on the endothelial cell surface. Thus, it prevents the interaction of VEGF with cell receptors. In this way bevacizumab inhibits the biological activity of VEGF. When VEGF is targeted and bound to this monoclonal antibody it cannot stimulate the endothelial cells, which, consequently, leads to inhibition of endothelial cell proliferation, migration and new blood vessels formation (angiogenesis). As shown earlier, oxaliplatin and irinotecan chemotherapy combined with 5-FU or leucovorin is indicated as the first- or second-line treatment in patients with colonic or rectal metastatic cancer [12, 14, 28-31].

Cetuximab (Erbitux) is a recombinant, chimeric monoclonal antibody which binds specifically to the extracellular domain of the human epidermal growth factor receptor (EGFR). Cetuximab blocks the binding of EGF and transforming growth factor α (TGF-α) to EGFR, and is approved by FDA, as a second-line agent for metastatic colonic or rectal cancer. It is also is indicated to be administered in combination with other drugs, e.g. irinotecan, or to be used singly if patients cannot tolerate chemotherapy [32, 33].

Panitumumab (Vectibix) is a human IgG monoclonal antibody which also targets the EGF-R, a protein playing an important role in cancer cell signaling. EGF-R present on the surface of bowel tumor cells, is activated by binding of proteins naturally occurring in the body, i.e., EGF or TGF-α. This binding changes the shape of EGF-R, which, in turn,
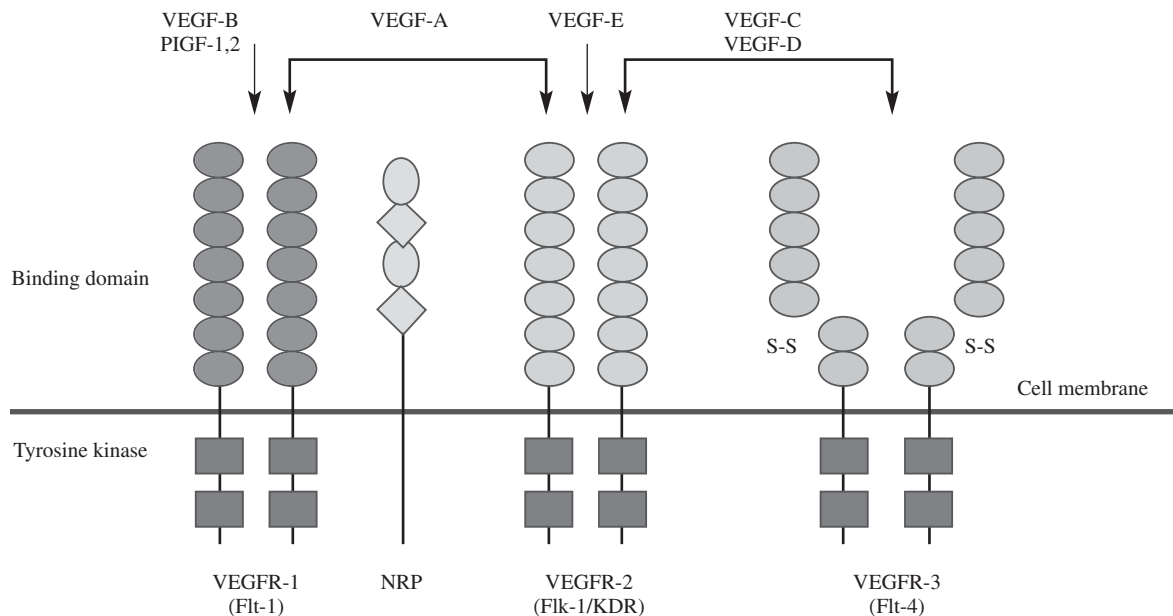

Fig. 1. Receptors for different VEGFs (Hicklin DJ, Ellis LM. J Clin Oncol 2005; 23: 1011-1027)
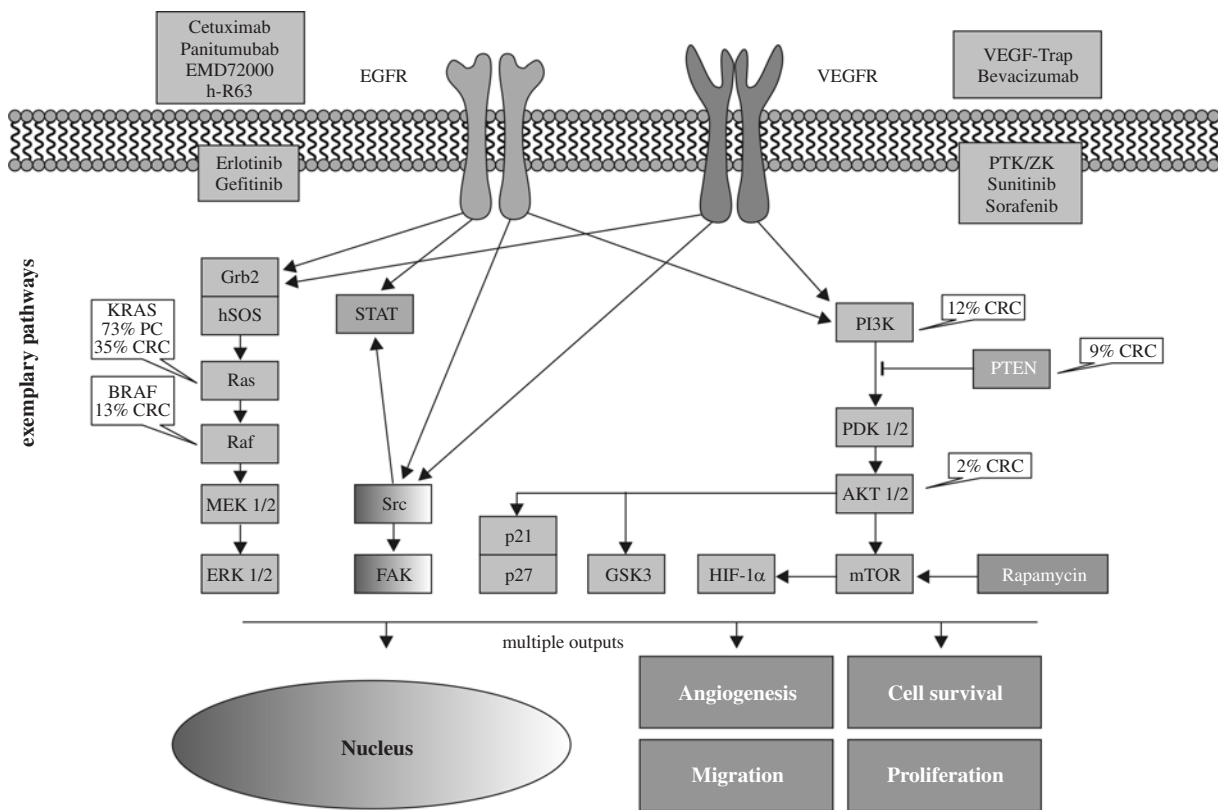
Fig. 2. Exemplary components of the EGFR and VEGR dependent signal transduction pathways (Cell Commun Signal 2009; 7: 19)

triggers internal cellular signals stimulating tumor cell growth. Panitumumab binds to EGF-R, preventing the natural ligands such as EGF and TGF-α from binding to the receptor and interfering with the signals that would otherwise stimulate growth of the cancer cell and allow it to survive. Panitumumab binds to the EGF-R with high affinity and inhibits the growth of cancer cells which require EGF-R-mediated signals for their survival. Panitumumab is indicated for EGF-R metastatic colorectal cancer with disease progression during or following the treatment with 5-FU, oxaliplatin, and irinotecan [20, 34-36].

The drugs are used alone as well as an addition to standard chemotherapy due to their pro-apoptotic and anti-angiogenic activities. According to the knowledge of signal transduction pathways, cell proliferation and the fact that for growth beyond a few millimeters tumors depend on new blood vessel formation, further clinical trials will probably focus on investigation of new antagonists of receptors, antibodies against growth factors, false mediators, and inhibitors of cell signaling drugs, appendant to different groups. However, all those agents play an important role in mechanisms decreasing or inhibiting tumor growth. Addi-

tionally, angiogenic inhibitors reduce the ability of tumors to develop new blood vessels, delaying or inhibiting its growth, not necessarily by targeting tumor cells but by interacting with tumor-related endothelial cells.

Antiangiogenic treatment is generally safe and well tolerated although there are some common adverse effects including hypertension and proteinuria, but also potentially more serious adverse effects, such as thrombotic disease and hemorrhage

Apart from old chemical drugs, e.g. Thalidomide and others, monoclonal antibodies, which are the novel angiogenesis inhibitors, and different molecules and peptides occurring naturally in the body, various exogenic factors (e.g., some herbal drugs, or compounds – like plant polyphenols, methyloxantins, catechins, oils and fatty acids) also exert their effect on angiogenesis. Therefore, further understanding of anti-angiogenic activities of those substances may play a pivotal role in the future treatment of different diseases including neoplasms. Moreover, all of them are consumed as a part of the daily diet, and the nutrients may act directly in the gastrointestinal tract.
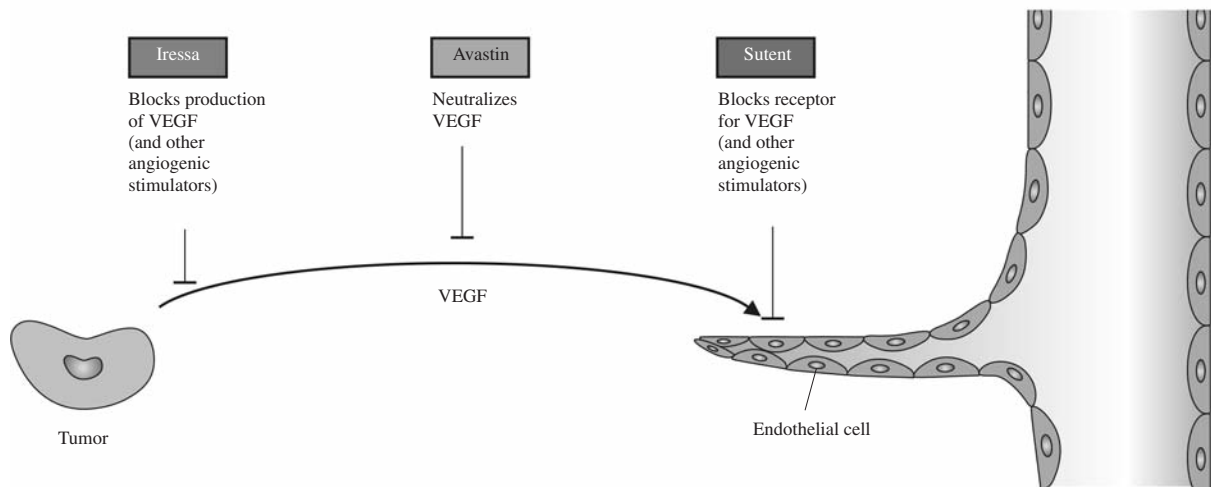
Fig. 3. Inhibition of angiogenesis by anti-VEGF drugs.(Judah Folkman Angiogenesis: an organizing principle for drug discovery? Nature Reviews Drug Discovery 2007; 6: 273-286)

New fields of anticancer drug investigation

Colorectal cancer has become an excellent tumor model for evaluating new therapeutic strategies. It has been found that in colorectal cancer, the VEGF expression correlates with invasiveness, vascular density, metastasis, recurrence, and prognosis. Due to adequate understanding of the development and growth of the colorectal cancer, it is possible to approach the treatment of all stages of the cancer disease, i.e. prevention, adjuvant therapy, and therapy for advanced disease. Thus, specific molecular processes are continuously targeted for therapeutic intervention, including growth factor receptors, proliferation signaling, cell cycling, apoptosis, angiogenesis, and the immune system. Several new biological agents are being tested in phase 2 and 3 of clinical trials. Probably, in not too far future, we will see their contribution to the therapy in patients with colorectal cancer. The agents tested have been recruited from different groups:

1. Small-molecule tyrosine kinase inhibitors of vascular endothelial growth factor receptor (VEGF-R) like Valatinib, Semaxanib (Sugen), Sunitinib (Sutent), and new inhibitors in early development jet without own names (AAL 993 Novartis, CEP 7055 Sanofi-Aventis, CP 547632 OSI, GW 654652 GlaxoSmithKline, AMG 706 Amgen, AZD 2171 AstraZeneca). These potent inhibitors of VEGF-R-1, -2, -3 and platelet-derived growth factor receptor (PDGF-R) tyrosine kinase activity have shown significant antitumor activity [37, 38].
2. Combined inhibitors of VEGF, tyrosine kinase, and others: Zactima, Sorafenib (Nexavar), Semaxanib, AEE 788.

These agents inhibit endothelial cell proliferation by blocking VEGF-induced signaling and inhibit cancer cell growth by blocking EGFR autocrine signaling [39].

3. Insulin growth factor receptor inhibitors: NIMC-A12, CP-751871, AVE 1642, 19D2. Increased expression of type I IGF-1-R is associated with cancer, and inhibitors of IGF-1-R are now a target of intensive research. The IGF ligand-receptor system is important in multiple mechanisms that mediate human colon cancer growth, including regulation of VEGF and angiogenesis.
4. Tyrosine kinase inhibitors (potential of inhibiting insulin receptors): NVP-ADW 742, NVP-AEW 541, BMS 536924, BMS 554417.
5. Src-oncogene kinase inhibitors: BMS 354825, AP 23846, TG 100598, AZD 0530, SKI-606. Compared with the normal tissues, Src expression is often increased in epithelial tumors, i.e. the colon, breast, pancreas, lung, and ovarian cancers. Increased levels of Src-kinase expression were found in human colon cancer, where elevated Src expression correlates with malignant potential and is associated with metastatic disease. It was shown, that in colon cancer, an increased Src expression was linked to its malignant potential [40-44].

Over the past few years, new drugs targeted at different elements of carcinogenesis pathways and associated with angiogenesis were tested in clinical trials and approved for the treatment of colorectal cancer. Their efficacy depends on the fact that they are targeted at growth inhibition of tumor or its vasculature [45-47].

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