Review paper

Role of angiogenesis and angiogenic factors in colorectal cancer

BARBARA J. BAŁAN[1], ROBERT SŁOTWIŃSKI[1,2], EWA SKOPIŃSKA-RÓŻEWSKA[3]

[1]Department of Immunology and Nutrition, Medical University, Warsaw, Poland; [2]Department of Surgical Research Center Polish Academy of Science, Warsaw, Poland; [3]Department of Pathology, Biostructure Center, Medical University, Warsaw, Poland

Abstract

Colorectal cancer, the second most frequent cause of death worldwide, is due to genetic changes in gastrointestinal cells Carcinogenesis depends on/is promoted/induced/initiated by/activation of oncogenes or inactivation of tumour suppressor genes. Microscopic human cancers may remain dormant for lifetime Tumor progression depends on a cascade of events during which the non-angiogenic phenotype switches to the angiogenic one. The switch promotes immediate recruitment of new blood vessel and, ultimately, tumor development.

Key words: *colorectal cancer, angiogenesis, angiogenic factors, VEGF.*

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Introduction

Colorectal cancer, or cancer of the colon and rectum, is second only to breast cancer in women, and to lung cancer in men in Poland. It is also the leading cause of death from malignant disease in men and women almost worldwide, particularly in highly developed and industrialized countries [1].

Irrespective of the gender, morbidity and mortality rates in Poland show a steady rise reaching 3% of the general population annually. In view of a late detectability of the disease, only 25% of patients with colorectal cancer have a five-year survival [2].

Numerous previous studies of patients with colorectal cancer demonstrated that mortality from the cancer may be reduced by prevention, screening tests, early diagnosis and treatment of the disease [3, 4]. New anti-angiogenic (anti-VEGF) therapy is also promising as a support to the classical adjuvant therapy, due to many correlations found between the VEGF secretion, new vessel growth, cancer cell apoptosis, lymph node metastases and overall survival prognosis [5, 6]. This knowledge has directed many gastroenterological, oncological and research centers to recommend screening in asymptomatic, high-risk adults over the age of 50 years and to initiate clinical trials with the so-called anti-angiogenic agents [7].

It is widely known that prevention and early screening are crucial/ of utmost significance in detection and diagnosis of colorectal cancer. However, such investigations as fecal occult blood test (FOBT), fecal DNA assays, or lower endoscopy (flexible sigmoidoscopy, colonoscopy) are not routinely used even in high risk groups of patients [8]. Diagnosis is also difficult due to a low diagnostic yield of FOBT and DNA assays which detect less than 20% of advanced adenomas. However, in approximately 50% of cases, colorectal cancer is located distal to the splenic flexure, that is within reach of flexible sigmoidoscopy [9]. Also, unfortunately, rectal examination is still not performed by medical professionals other than surgeons. Detectable tumors (almost all adenocarcinomas) are only those which form exophytic masses in the lower intestinal segments or annular constricting lesions [10-12].

All the above reasons, i.e. poor and late detectability, high cancer invasiveness, poor response to the conventional adjuvant therapy in advanced stages of the disease and uncontrollable angiogenesis, required finding new, more effective agents for the treatment of colorectal cancer. Therefore, inhibition of angiogenesis appears to be the adequate type of the therapy.

Etiopathology and risk factors

A number of factors increase the risk of developing colorectal cancer. Recognition of those has an impact on

Correspondence: Barbara J. Bałan, Department of Immunology and Nutrition, Warsaw Medical University, Pawińskiego 3, 02-023 Warsaw, Poland. Email: barbara.balan@wum.edu.pl

screening strategies and also treatment. However, almost 75% of all cancer cases occur in persons with no known predisposing factors [13].

The etiopathology of colorectal cancer is complex and appears to involve interactions between age, inherited susceptibility, nutritional habits and different environmental factors as well as angiogenic potential. The incidence of colorectal cancer rises significantly after the age of 40 years. Almost 90% of cases occur in persons over the age of 50 years. Many specialists have regarded diet, particularly fat intake, as the most contributory nutritional factor exerting a major impact on colon cancer onset. It is suggested that diets rich in total fat, chiefly saturated fats, increase the risk of colorectal cancer, whereas diets rich in fish and fish oils reduce the risk. Certain unsaturated fats (omega 6, linoleic acid) may have a stimulating effect on the VEGF secretion, angiogenesis and tumor growth. Furthermore, a high-fat diet stimulates dysplastic lesions in the colon. It is currently considered that the majority of colorectal cancers arise from malignant transformation of adenomatous polyps. Following the transformation, the angiogenic potential of mutated cells increases and tumor growth is initiated [14-16].

A high intake of saturated fats increases colon tumor promotion by changing membrane phospholipids. Arachidonic acid released from the membrane phospholipids affects prostaglandin synthesis by cyclooxygenase enzymes (COX). In human colon tumors one of the COX isoforms (COX-2) is considered to be the most important. It has been shown that the expression of the COX-2 gene in the colon epithelial cells leads to resistance to apoptosis, and its inhibition is crucial for the growth of any tumor. Recent studies have demonstrated that COX-2 may also induce tumor angiogenesis, although the association still remains unclear. COX-2 has also been shown to mediate the VEGF expression in numerous cell lines; the effect, however, is not evident in all tumors [17, 18]. Recently, the FDA has approved one of the selective inhibitors of COX-2, Celecoxib (Celebrex), as an addition to the treatment of familial adenomatous polyposis (FAP), a syndrome which accounts for 0.5% of colorectal cancers. The recommended dose is 400 mg orally twice a day [19-23]. However, clinical trials have shown that the risk of serious cardiovascular and TIA (transient ischemic attacks) events is nearly four times higher among patients treated with Celecoxib. Hence, it is considered that the agent should not be used for colorectal cancer chemoprevention except in patients with familial polyposis. Extensive cohort and case-control studies also show that chronic and regular use of acetylsalicylic acid (aspirin) and other non-steroid anti-inflammatory drugs (NSAIDs) is associated with a 30-50% reduction in the development of adenomas and colon cancer [24-28].

A positive family history of colorectal cancer or adenomatous polyps is one of the most important risk factors of developing colon cancer. Hereditary factors are considered to contribute to 20-30% of colorectal cancers, however, the genes responsible for the majority of the cancer cases have not yet been identified.

Colorectal cancer usually develops from areas of dysplasia and adenomatous polyps which become malignant. Approximately 85% of sporadic colorectal cancers demonstrate a loss of function of one or more tumor suppressor genes (e.g. p53, DCC, APC) due to a combination of a spontaneous mutation of one allele combined with chromosomal instability and abnormalities in the DNA content [29-31]. Up to 5% of colorectal cancers are caused by inherited germline genetic mutations resulting in polyposis or HNPCC (hereditary non-polyposis colorectal cancer).

Two most common forms of hereditary cancer are among genetic factors which may increase the risk of colorectal cancer, i.e.:

- familial adenomatous polyposis (FAP) caused by autosomally dominant inherited mutations in the adenomatous polyposis coli (APC) gene on chromosome 5q21 which results in the lack of synthesis of protein critical during cell adhesion and apoptosis [32-34];
- hereditary non-polyposis colorectal cancer (HNPCC) [35, 36].

Approximately 90% of FAP patients have a mutation in the APC gene. Colorectal polyps develop by a mean age of 15 years, and cancer, at 40 years. Patients with untreated FAP are at a 100% risk of developing colon cancer. The cancer is also diagnosed in nearly 80% of patients with HNPCC by the age of 80 years [37, 38].

Colorectal cancer may also develop in patients with Peutz-Jeghers syndrome (approximately 50% of cases), and juvenile polyposis (2-13%) [39].

Similarly to those, with a positive family history of colorectal cancer, patients with other cancer diseases are also at an increased risk of developing intestinal malignancy. The risk of cancer is proportionate to the number and age of first-degree family members with colonic cancer. Persons with one first-degree relative with colorectal cancer have an increased risk of approximately twice that of the general population. Those with two first-degree family members, have a fourfold increased risk of developing the disease. Women with a history of breast, ovarian or endometrial cancer also have a higher risk of developing colorectal malignancy.

The risk also depends on a person's age at the time of the diagnosis. The risk is nearly fourfold if the family member was diagnosed with cancer before the age of 45 years, twice higher if diagnosed with malignancy at 49-59 years of age, and only 1.8 times higher, if diagnosed over the age of 60 years.

The second most common risk factor of colorectal cancer is represented by the group of inflammatory bowel diseases (IBDs), i.e. ulcerative colitis or Crohn's disease. The risk of adenocarcinoma of the colon begins to rise after 7-10 years following the IBD recognition. The cumulative risk appro-

aches 2-8% after 10 years, above 10% after 20 years, and about 20% in persons with a 30 year history of IBD [40].

Dietary habits, described above, particularly the diets rich in fats and red meat, are responsible for a high risk of colorectal adenomas and cancer, whereas diet rich in vegetables, fruits and fibers is associated with a decreased risk. Unexpectedly, many clinical prospective research studies have not demonstrated any beneficial effects of the antioxidants, vitamins A,C,E, and beta-carotene on colonic cancer development [41-45].

Other factors such as ethnicity, obesity, metabolic syndrome, cigarettes, alcohol, low physical activity and some of the endocrine disorders (acromegaly) and bacterial diseases (sepsis due to streptococcus) are controversial and are not regarded as risk factors of colorectal cancer. However, adenocarcinoma incidence rates in black persons are higher than those in the white population; it is still not clear whether this is due to genetic or socioeconomic factors [46].

A different aspect, as it seems today, of high significance as risk and predictive factors are development of new blood vessels and secretion of VEGFs during carcinogenesis. Highly angiogenic colorectal tumors (producing high amounts of factors stimulating angiogenesis, i.e. VEGFs) are associated with aggressive histopathological features and poor survival [10, 16].

Nearly 40 years ago, Dr. Judah Folkman formulated his hypothesis of treating solid tumors by inhibiting tumor angiogenesis. The process of new blood vessel formation, which may usually occur in normal physiology, is also found in pathological conditions. Ultimately, angiogenesis, and probably also lymphangiogenesis, are underlying processes in the pathogenesis and invasion of neoplasms. Metastases to local lymph nodes via the lymphatic vessels are a common step in the spread of solid tumors [47, 48].

In the early seventies of the 20th century, J. Folkman proposed a then revolutionary theory that tumors are unable to grow beyond 1-3 millimeters in diameter unless they have a blood supply. To accomplish this, tumors can secrete an unknown substance, i.e. tumor angiogenic factor (TAF), which induces growth of new blood vessel or angiogenesis. The process helps transform a tumor from a small group of mutated cells into a large, malignant growth. Folkman believed that the tumor growth might be stopped when its blood vessels were not allowed to grow, which would be a giant step in oncology [49, 50].

At present, a large number of pro-angiogenic factors have been discovered, and they include. chemokines, growth factors, and other cytokines, i.e. FGF, TGF, TNF, PDGF, HGF, EGF, EGFR, angiogenin, angiopoetin-1, IL-8 and others, however, the VEGF family plays the most important role [51-54]. The VEGF is the most specific stimulator of vascular endothelial cell proliferation. Although, it has now been recognized that tumor cells can produce and secrete more than one type of protein stimulating angiogenesis [55]. The substances usually affect different stages of the cellular signaling process, which leads to the formation of blood and lymphatic vessels The knowledge of the substances may bring the day when it might be possible to defeat cancer. It is also expected that, apart from chemo- and radiotherapy, agents blocking the stimulatory effect of VEGF on endothelial cells, would prove their beneficial impact to the patients. So far a correlation has been found between an increased VEGF expression and a poor prognosis for patients with colorectal cancer [56-60].

Diagnosis and staging

Assessment of the stage of colorectal cancer is crucial. not only because of its correlation with patient survival, but also to determine the treatment strategy (especially the adjuvant therapy) [61].

Patients with colorectal cancer usually receive the conventional therapy, i.e. surgery, adjuvant chemotherapy, radiotherapy, focused on tumor cells. However, in advanced stages of the disease the management is mainly palliative and usually involves a combination of specialist treatments, symptom control and psychological support, therefore it should be supported by an anti-angiogenic therapy targeted at tumor supplying blood vessels [62-64,].

There are a few classification systems used in staging cancer disease: Duke's score, Astler-Coller score and TNM. Duke's classification was widely used in the past, however, the TNM staging system developed and recommended by the American Joint Committee on Cancer (AJCC) has now been more commonly applied [79, 85].

The TNM staging scheme describes three key clinical criteria:

T (tumor) describes how far the main (primary) tumor has grown into the wall of the intestine and whether it has grown into adjoining areas.

N (nodes) describes the extent of spread to regional lymph nodes.

M (metastasis) indicates whether the cancer has spread to other organs of the body. Colorectal cancer may spread almost anywhere in the body, but the most common sites of spread are the liver and lungs.

The numbers or letters after T, N, and M provide more details about each category stage [19].

T categories for colorectal cancer	
Tx	no description of the tumor extent is possible because of incomplete information
Tis	the cancer is in the earliest stage. It involves only the mucosa and has not grown beyond the inner muscle layer (tumor *in situ*)
T1	the cancer has grown through the inner muscle layer and extends into the submucosa
T2	the cancer has grown through the submucosa and extends into the outer muscle layer

T3	the cancer has grown through the muscle layer and into the subserosa but not to any neighboring organs or tissues
T4	the cancer has grown through the wall of the colon or rectum and into nearby tissues or organs
	N categories for colorectal cancer
Nx	no description of lymph node involvement is possible because of incomplete information
N0	no lymph node involvement is found
NI	cancer cells found in 1 to 3 nearby lymph nodes
N2	cancer cells found in 4 or more nearby lymph nodes
	M categories for colorectal cancer
Mx	no description of distant spread is possible because of incomplete information
M0	no distant spread is seen
M1	distant spread is present

STAGE	TNM (AJCC)	Duke's score	Astler-Coller score
0	Tis, N0, M0	–	–
I	T1-2, N0, M0	A	A, B1
IIA	T3, N0, M0	B	B2
IIB	T4, N0, M0	B	B3
IIIA	T1-2, N1, M0	C	C1
IIIB	T3-4, N1, M0	C	C2, C3
IIIC	Any T, N2, M0	C	C1, C2, C3
IV	Any T, any N, M1	–	D

Stage	5-year survival rate[1]	5-year survival rate (Poland)[2]
I	93%	85-100%
IIA	85%	50-80%
IIB	72%	
IIIA	83%	30-60%
IIIB	64%	
IIIC	44%	
IV	8%	<5%

Percentage survival rate – from a study of the National Cancer Institute's SEER database, analysis includes nearly 120,000 patients diagnosed with colon cancer between 1991 and 2000[1] [2]. Polish data published in the TERAPIA 2009; 1[2].

The role of angiogenesis and angiogenic factors in colorectal cancer

Angiogenesis is a crucial process in tumor growth, development and dissemination with significant implications for clinical management. Inhibition of angiogenesis has the potential to enhance the effectiveness of treatment for this disease. As in other cancers, new blood vessels supply nutrients and oxygen to proliferating colorectal cancer cells. The regulatory process of tumor angiogenesis is highly complex. It depends on the balance between pro- and anti-angiogenic factors, secreted by endothelial, tumor and host-infiltrating cells. Numerous studies have indicated that assessment of the angiogenic activity by microvessel density or expression of angiogenic factors in colorectal cancer may provide prognostic information, predict cancer response to chemotherapy or radiotherapy and bring therapeutic benefits to patients [67-69]. However, the most important clinical implication of tumor angiogenesis is the development of a novel strategy of anticancer therapy targeting tumor vessels instead of cancer cells [70]. Such antiangiogenic therapy in colorectal cancer aims at inhibiting the tumor growth, and the current evidence indicates that it is effective when combined with the conventional cytotoxic chemotherapy [71].

Since inhibition of tumor angiogenesis might control cancer growth, antiangiogenic drugs are used in the clinical treatment of patients with advanced cancer. The statement is based on drug capacity to improve survival rates of cancer patients in clinical trials. Unfortunately, there are still no validated biomarkers of angiogenesis available for routine clinical protocol. Such biomarkers might improve the clinical use of currently available antiangiogenic drugs, and also the development of new therapeutic agents [72].

One of the most important factors in activation of angiogenesis is VEGF which is produced in response to different cellular and environmental stimuli. VEGF is the endothelial cell (EC) mitogen and permeability factor with proangiogenic and antiapoptotic abilities since it may induce expression of Bcl-2 protein, and decrease caspase-3 expression in EC, which results in cell survival [16, 58, 73-75].

VEGF is known to be involved in the growth and development of colorectal cancer and is expressed early in the progression of the tumor. The factor has been shown to facilitate survival of existing blood vessels, to contribute to vascular abnormalities inhibiting effective delivery of antitumor compounds, and to stimulate new blood and lymphatic vessel growth. The VEGF expression also correlates with invasiveness of colorectal cancer cells, tumor vascular density, appearance of metastasis, tumor recurrence, and poor prognosis for patients, including early death. Furthermore, a very close correlation between colorectal cancer cell apoptosis and angiogenesis was noted. As cancer progresses, microvessel density increases, which results in a decreased apoptotic index [76-78]. High VEGF levels may also predict a poor response to the conventional systemic therapy in patients with colon cancer and the preoperative neoadjuvant radiotherapy in persons with rectal cancer. The VEGF overexpression correlates with a lack of response to radiotherapy and it is also significantly correlated with lymph

node and liver metastases, as reported in many research papers. Twenty seven research studies showed that overexpression of the growth factor in colorectal cancer was significantly correlated with an increased risk of relapse. The increased VEGF expression may also have a negative impact on survival in colorectal cancer patients. Those with overexpressed VEGF tumors had significantly poorer survival than the patients whose tumors did not overexpress the growth factor. The studies also showed, that the VEGF overexpression was closely correlated with the overall survival of patients with colorectal cancer [79-81].

Although, the role of VEGF in the colon cancer biology is quite well understood, further investigations need to be performed to clearly explain the function of VEGFs and antagonists of their receptors in the tumor development.

Other growth factors, i.e. PDGF, TGF, HGF, EGF and bFGF which are potent endothelial cell mitogens, are relatively nonselective for the endothelium and may also stimulate division in any other type of cells [82-86].

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