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REVIEW ARTICLE

Angiogenesis and hemostasis in colorectal cancer

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Abstract Worldwide, colorectal cancer is the third cause of death in men and the second cause of death in women, with nearly 1.2 million newly-diagnosed cases and 600,000 estimated deaths. A significant proportion of patients have metastatic disease at diagnosis. Neoangiogenesis is the formation of new blood vessels from those already existing, which play an important role in tumor growth and progression. Factors related to endothelial growth have been identified including vascular endothelial growth factor. Different studies have demonstrated coagulation and fibrinolysis systems activation participation in tumor angiogenic development. Some of these factors are Von Willebrand factor, fibrinogen, type I plasminogen activator inhibitor and receptor, in addition to D-dimer and platelets. Serum concentrations of these proteins are considered to be predictors of treatment response, disease progression and survival. Interaction between tumor cells, angiogenesis and coagulation activation is a positive feedback, and strategies interfering in this relationship, such as the use of chemotherapy in combination with new specifically targeted agents, can prevent or treat cancer. In addition, the role of anticoagulant or antiplatelet agents in the treatment of cancer has not yet been determined.

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INTRODUCTION

Colorectal cancer (CRC) is a public health problem. At the moment of diagnosis, 15% of patients already have metastasis, and 50% with initially localized disease will develop metastasis regardless of the treatment used. It is important to have minimally invasive techniques that allow for CRC patients survival prognosis to be known, as well as their response to treatment[1]. CRC is the fourth most common malignancy and the second cause of death in the United States. In 2013, there were 96,830 new cases of colon cancer and 40,000 cases of rectum cancer recorded, as well as 50,310 related deaths. Incidence and mortality have decreased as a result of prevention and early diagnosis[2]. Some factors have been associated with a decreased incidence of CRC, including low body mass index and exercise, with increased incidence being associated with (neoplastic) polyps, the diet (high fat content, low consumption of fiber, and high caloric intake), inflammatory bowel disease (chronic ulcerative colitis and Crohn's disease), genetic factors (Lynch syndrome and familial adenomatous polyposis), smoking, personal or family history of cancer in other anatomical locations (breast, endometrium, and ovary).

CRC left colon localization accounts for 60% of cases and right colon for 30%, with the rest being located in the rectum. Clinical manifestations are associated with tumor size and location. Lesions in the right colon are usually asymptomatic, with subsequent pain, hemorrhage and anemia. Left colon lesions usually produce changes in bowel habits, hemorrhage, pain, decreased caliber stools, and obstruction. In 40-70% of cases, there is regional lymph node involvement, and most common metastases are to the liver, peritoneum, and lung[3].

CRC is staged with the tumor-node-metastasis system[4]. Stage is the most important prognostic factor, and some characteristics influence on survival, such as histological grade, anatomical localization, obstruction, and perforation at diagnosis[5]. The American Society of Clinical Oncology recommends systematic carcinoembryonic antigen detection as a means to identify early relapse.

Surgical treatment is potentially curative in CRC. By means of colectomy and locoregional lymph node *en bloc* resection, a complete surgery can be curative in patients without metastasis[6].

Patients with clinical Stage I disease do not require adjuvant therapy[7].

Adjuvant chemotherapy is recommended in CRC in the following circumstances:

Patients with clinical Stage II low-risk disease can be maintained on observation or receive treatment with capecitabine or 5-fluorouracyl/leucovorin.

Patients with clinical Stage II high-risk disease, those with poor prognosis: T4 tumors (clinical stage IIB/IIC); poor histological differentiation, lymphovascular invasion, perineural invasion, and bowel obstruction; lesions with perforation, positive margins or inadequate lymph node specimen (<12 lymph nodes) can receive 5-fiuorouracyl/leucovorin, oxaliplatin (FOLFOX), capecitabine/oxaliplatin (Cape/Ox), or 5-fluorouracyl/leucovorin/oxaliplatin (FLOX)[7].

For patients with clinical Stage III disease, 6-month chemotherapy is recommended, and they can receive FOLFOX or CapeOx; FLOX, or capecitabine monotherapy or 5-fluorouracyl/leucovorin in those in whom oxaliplatin is contraindicated[8].

Out of 60% of CRC patients that will develop metastasis, 80% will develop unresectable liver disease, with liver disease being the most common cause of death in these patients. Their treatment includes systemic chemotherapy. Pre-operative chemotherapy provides early treatment of micrometastatic disease and allows for sensitivity to chemotherapy to be determined. Treatment includes 5-fluorouracyl/leucovorin, capecitabine, irinotecan, oxaliplatin, bevacizumab, cetuximab, panitumumab, and regorafenib[9].

THE IMPORTANCE OF ANGIOGENESIS IN CANCER

Maintaining tumor growth requires sufficient nutrient supply, which is accomplished by angiogenesis. Angiogenesis is the formation of new vessels from those already existing[10].

Blood vessel cells are maintained at rest, but they are able to divide in response to stimuli and generate neoangiogenesis. Angiogenesis positive-regulation molecules are vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor, epidermal growth factor, transforming growth factor, matrix metalloproteinases (MMPs), tumor necrosis factor, and angiopoietins. Some endogenous angiogenesis inhibitors are interferon, interleukins, tissue inhibitors of matrix metalloproteinases, and angiostatin and endostatin[11]. Angiogenesis biological process is initiated when there is hypoxic stress in the tumor cell, and it activates hypoxia-inducible factor-1 bis transcription, which promotes VEGF expression. The secreted VEGF binds to its receptor on the surface of endothelial cells and, in addition, increases MMP expression in tumor cells. This generates the angiogenesis process and triggers endothelial cells growth, proliferation, and migration[10].

Four stages subsequent to endothelial cells activation can be distinguished: (1) Basement membrane and extracellular matrix degradation is produced by proteolytic enzymes, (2) endothelial cell proliferation, (3) pericytes retraction and endothelial cell migration, which form solid cellular cords, and (4) formation of new vessels, anastomosing with preexisting vessels[12,13]. With the formation of cords, the endothelial cell that is found at the advancement site navigates across the stromal surroundings, while the group of proliferating endothelial cells pushes from the rear, with this advancement across the stroma connecting with other newly formed vessels. The dynamical aspect of this tip-cell has been documented with confocal microscopy. There is intercommunication between angiogenesis and the extracellular matrix, with endothelial cells producing soluble and insoluble paracrine signals that modulate and direct growth, and with the matrix providing a biomechanical environment of stability that favors new vessels growth and morphology[14]. In tumor angiogenesis, endothelial cell activation is induced by tumor originating angiogenic factors (autocrine), and by cells of the tumor stroma, mast-cells, fibroblasts, and macrophages, which are recruited (tumor chemotaxis), in addition to angiogenic factors that are sequestered in the extracellular matrix (paracrine)[12]. The angiogenic process is regulated by activating and inhibiting factors, between which there is a state of balance that can be altered in physiological or pathological conditions[15]. Tumor cells alter this

angiogenic balance, the pro- and anti-angiogenic balance, which allows for the "angiogenic switch" to be turned on, triggered by tumor hypoxia and oncogene activation, which would facilitate angiogenesis by an increase in activators or loss of suppressor genes, which would in turn decrease inhibitors[16].

In experimental models, some oncogenes (v-ha ras, v-raf, k-ras) stimulate angiogenesis by inducing VEGF formation and reducing thrombospondin 1, which is a potent angiogenesis inhibitor; the loss of p53 causes a decrease in thrombospondin 1 and an increase in VEGF. The presence of oncogenes and the loss of p53 would result in tumor cells shifting toward an angiogenic phenotype. Acquisition of this angiogenic phenotype takes place at early stages of tumor development[16]. Cancer biological process starts with the loss of cell proliferation control, which gives origin to carcinoma in situ. Folkman et al. demonstrated that solid tumors cannot continue to grow beyond 2-3 mm in diameter without inducing their own vasculature[12]. Carcinoma *in situ* can acquire an angiogenic phenotype that induces new capillary formation and starts to invade surrounding tissues. This angiogenic phenotype can be acquired by high production of growth factors or low expression of negative modulators[12]. The last stage of tumor growth is the formation of metastasis. Angiogenesis, which is an initial process in growth, facilitates tumor cells entering the circulation and spread[17]. Extracellular matrix components are regulated by the angiogenic cascade. Factors related to endothelial growth have been identified, including VEGF[18]. VEGF intervenes in three basic functional processes of tumor angiogenesis: Coagulation system activation, adhesion interactions between endothelial surface integrins and extracellular matrix, and extracellular proteolysis control. The VEGF family is comprised by six members: VEGF-A (also known as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PlGF or placental growth factor. Its structure is composed of homodimers, which contain polypeptide chains that, in their general structure and in the cysteine residues spacing, are relayed to PDGF[19].

VEGF biological effects are mediated by its binding to three structurally-related cell-surface specific receptors: VEGF-R1 or Fit-1, VEGF-R2, KDR or Fik-1, and VEGF-R3 or Fit-4. R1 and R2 are essential for vascular development and are mainly expressed in the vascular endothelium, while R3 is found in the lymphatic endothelium[20]. In the past few years, an intense search for new biologic markers, such as VEGF, has been carried out, to allow for CRC patients' evolution follow-up[21]. However, only a few studies have so far been conducted trying to elucidate the progression predictive and survival prognostic role of VEGF levels prior and after chemotherapy in patients diagnosed with CRC[22]. Patients with VEGF elevated levels have worse prognosis in terms of response to chemotherapy[23]. VEGF determination is made both in serum and plasma[24]. Patients whose concentrations of these factors, such as VEGF, before antineoplastic treatment is elevated, might benefit from chemotherapy in combination with new specific targeted agents.

HEMOSTASIS IN CRC AND PROGNOSIS

Hemostasis is defined as hemorrhage arrest. The term originates from hema = blood and stasis = to stop; its concept is: Thrombohemorrhagic balance maintained by interactions between coagulation, the fibrinolytic system, platelets and vascular wall[25]. Coagulation activation requires the participation of platelets, endothelium, monocytes, and coagulation factors and it can occur by the extrinsic and intrinsic pathways, both comprised in the cell-based model of coagulation[26].

The elements that constitute hemostasis can be divided into thrombogenic elements, which include the exposed endothelium, tissue factor, prothrombin, fibrinogen, collagen, platelets, platelet-activating factor and von Willebrand factor (vWF), and anti-thrombogenic factors, which include heparin, thrombomodulin, plasminogen, tissue plasminogen activator, antithrombin III, C-protein, and S-protein[25]. Cancer induces a prothrombotic state, which occurs as venous thrombosis or often as a subclinical state that manifests itself by alterations in thrombotic biomarkers that show coagulation and fibrinolysis activation, with these changes paralleling tumor growth and dissemination[22]. Tumor cells express tissue factor, other procoagulant proteins and MMP, which activate coagulation and also activate host cells (endothelium, platelets, and leukocytes); these, in turn, release soluble and contact factors and express their procoagulant phenotype by forming a structure that favors platelet adhesion and thrombin generation and activation. Some thrombotic factors are related to cancer patients, such as demographic factors, treatment-related factors and central venous catheter[27]. The use of predictive risk assessment models (RAM) is recommended to identify patients at high risk for thrombosis, such as the deep venous thrombosis RAM in patients who will receive chemotherapy. Risk is assessed with the following data: Type of tumor, platelets >350 cells/µL, hemoglobin <10 g/dL or use of erythropoietin, white blood cell >11 thousand cells/µL, and body mass index of 35 kg/m[28]. Urokinase-type plasminogen activator (uPA), its inhibitor plasminogen activator inhibitor-1 (PAI-1) and its cell receptor (uPAR), play a key role in pericellular proteolysis and interact with extracellular matrix proteins, as well as with transmembrane receptors and, by means of these, they induce intracellular signaling and modulate cell migration and extracellular cell-matrix interactions[29]. uPAR functional activity inhibition significantly reduces endothelial cells invasive potential, and the absence of PAI-1 prevents cancer invasion and metastasis. The fibrinolytic system acts on extracellular matrix degradation by forming an endocytic complex between uPA/PAI-1/uPAR with integrins and low-density lipoprotein-type receptor, which binds matrix proteins. The complex is endocyted by epithelial cells and degraded by endosomes. In this process, uPAR can recirculate, in addition, that VEGF can induce uPAR overexpression[29,30]. Hypercoagulability state is known to be associated with clinical progression and prognosis in patients with cancer. Some biomarkers are correlated with progression and poor prognosis: Thrombocytosis, hyperfibrinogenemia, D-dimer, fibrin degradation products, or vWF[31]. Gil-Bazo et al. report vWF elevated plasma concentrations in patients with breast, prostate, bladder, head and neck, ovary, cervix, larynx, and colon cancer[32]. Elevated vWF is associated with worse prognosis in patients with metastatic CRC, as well as D-dimer and fibrinogen[33]. Beer et al. point out the predictive and prognostic value of D-dimer plasma concentrations in cancer patients[34]. Kawai et al.[31] report that pre-operative thrombosis is associated with tumor size and invasiveness in

patients with CRC, and that this suggests tumor invasive activity. In addition, they found that platelets, D-dimer and fibrinogen are related to CRC progression and poor prognosis[31].

CONCLUSIONS

In spite of the continuous increase in the number of potential biomarkers in CRC, standardization of their determination is necessary. The interaction between cancer and coagulation activation is a positive feedback, and strategies interfering with this relationship may modify the outcomes. Anticoagulant or antiplatelet agents' efficacy in the treatment of cancer has not yet been clearly determined, and study on this relationship is required to be continued.

DECLARATION INTEREST

The authors declare not having any conflicts of interest.

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