# Review

# Angiogenesis Factors Involved in the Pathogenesis of Colorectal Cancer

## A. Mihalache[1], I. Rogoveanu[2]

[1]*Emergency Department, County Emergency Hospital Slatina*

[2]*Department of Internal Medicine, University of Medicine and Pharmacy of Craiova*

**ABSTRACT:** Colorectal cancer stands at the top of oncologic pathology in the world, and in the same measure in Romania because is the third most frequent cancer diagnosed in men and women. Colorectal cancer develops as a result of mutations in genes that control proliferation and cell death. It was established that in the development of a tumor there is originally a prevascular phase followed by a phase of tumor angiogenesis. In the future it is necessary to develop new clinical protocols that angiogenesis inhibitors are associated with chemo or radiotherapy, conventional or other methods such as immunotherapy and gene therapy.

*KEYWORDS: colorectal cancer, angiogenesis, oncogenes*

## Introduction

Colorectal cancer develops as a result of genetic and epigenetic changes occurring within 10-15 years, leading to the transformation of normal colonic epithelium. Approximately 75% of patients with colorectal cancer are sporadic cases without presenting evidence that would have inherited the disease. The remaining 25% have a family history of colorectal cancer or suggesting the contribution of genetic factors or common exposure to environmental factors in favor of colorectal cancer or a combination of both factors. Whether occurs spontaneously in a single individual or multiple people manifest from the same family, the same location or different locations, cancer is a genetic disease because the development of tumors involving different genes controlling the major cellular physiological processes: cell proliferation, DNA repair, mitotic cycle, cell death.

Colorectal cancer develops as a result of mutations in genes that control proliferation and cell death. Appear abnormal changes in oncogenes and tumor suppressor genes of growth (GST) and apoptosis [1] (Table 1, Table 2, Table 3)

.

*Table 1. Oncogenes and their role in the colorectal cancer*

| The marker | Function | Prognostic significance |
|---|---|---|
| Ras | as a G-protein signal given cell proliferation | - Gene mutations may/may not be a predictor of a poor prognosis (conflicting studies).<br>- Can predict response to chemotherapy with 5-fluorouracil. |
| EGFR | tyrosine kinase activity | - Found no prognostic role.<br>- AntiEGFR inhibitors and antibodies are being studied as potential therapeutic agents |
| Erb-B2 | cell proliferation stimulated tyrosine kinase | - Increased expression can be a predictor of survival decrease.<br>- Has not been studied role in the response to chemotherapy |
| TGFα | cooperates with EGFR as growth promoters | - Tumors with<25% positive cells for. TGFα have a worse prognosis than those with>25% positive cells (global data are still unclear). |
| TGF β-1 | inhibits tumor growth but stimulates mesenchyme cell proliferation and migration | - Increased expression of TGF-β 1 is associated with tumors of advanced stages (limited studies). |

***Table 2. Tumor suppressor genes and their role in colorectal cancer prognosis***

| The marker | Function | Prognostic significance |
|---|---|---|
| p53 | Produce cell cycle gene promoters that induce, or inhibit apoptosis. | - P53 mutations are associated with relapse and survival downward trend. |
| p27 | Regulates G1-S phase progression | - Increase of 2.5 times the risk of death from cancer;<br>- The absence of p27 to tumors in stage I and II is that they have the same prognosis as those in stage III; |
| MSI Microsatellite instability | DNA repair system defects. | - For HNPCC, MSI + tumors have demonstrated an increase in the asymptomatic period and overall survival;<br>- Role in the response to chemotherapy<br>- However, general prognostic role uncertain |
| 18q LOH loss of heterozigozity | heterozigozity 18q gene inhibits tumor growth by an unknown mechanism | - Stage II with 18q deletion have a similar prognosis std. III with 3-7 fold increased risk of death from cancer;<br>- There was no prognostic role in patients with curative liver resection for colorectal metastases |
| Allele deletion 5q | tumor suppressor | - Initial data suggests an improvement in survival in the case of normal expressions. |
| DNA hyper methylation | - Promoters CpG methylation inhibits gene expression and gene repeating units<br>- Can inhibit tumor suppressor genes. | - Insignificant as independent prognostic factor<br>- along with other molecular markers may play a complementary role, eg. MSI. |

***Table 3. Apoptosis and Oxygen Radical enzymes in colorectal cancer prognosis***

| The marker | Function | Prognostic significance |
|---|---|---|
| Bcl-2 | Stabilizes mitochondrial membrane function to the detriment of apoptosis | - Possible favorable prognosis for Bcl-2+/p53 - |
| SOD superoxide dismutase | Converts superoxide anion to H2O2 (antioxidant). | - Increased MnSOD corresponds to increasing the risk of death from cancer by 1.9 times. |
| GST-π glutathione S-transferase | antioxidant | - Increase the antioxidant activity involves an increased risk of death from cancer 3 times. |

Once formed cells malignant vascular components of the primary tumor must invade vascular and lymphatic structures to form emboli into the bloodstream, survive interaction with elements of the blood and immune system and be transported organic distant sites [2, 3]. At this level will be extravasated, will join the target and will achieve secondary tumors [2-4].

Initiation and progression phenomena occurring in new locations involves a series of dynamic interactions host-tumor [5]. Study intimate mechanisms of carcinogenesis and metastasis paved the understanding of biological properties of tumor cells [2-24]. Following the work of different research groups focused on this area pathophysiological model has been developed which involves the following sequence of events:

- angiogenesis;
- impairment of intercellular adhesion in the primary tumor;

- destruction of the basement membrane and initiation of tumor invasion;
- Seizure and adhesion of tumor cells in target organs.

An extremely important responsibility tumor microenvironment [2-4, 7], as described in recent years many molecular and genetic factors (potential therapeutic targets) present at this level and being involved in modulation of immunological protumoral purposes.

## Angiogenesis

The term angiogenesis, defined as the formation of new blood vessels was introduced in 1787 by John Hunter [1].

Correlation of angiogenesis with carcinogenesis was done hundreds of years later, in 1971, when Folkman has advanced the hypothesis that tumor growth is angiogenesis-dependent, based on initial experiments in the early '60s following comments related to the

limitation in the absence of tumor extension an own vasculature [2,3].

It was suggested that tumor cells and endothelial cells in cancer achieved an ecosystem, and endothelial cells can be activated from a latent status in a rapid growth phase, a chemical released by tumor cells [5].

It was established that the development of a tumor is prevascular initial phase followed, with increasing tumor volume 2-3 mm3-the equivalent of 100-300 tumor cells [1], a phase of tumor angiogenesis.

The formation of new blood vessels and thus translating to tumor angiogen phenotype is controlled by stimulators and inhibitors of angiogenesis factors, release both cancer cells and the host cells or extracellular matrix [5-9].

This phenomenon is dependent on the direct action of angiogen factors, such as endothelial growth factor, placental growth factor, angiopoietin and by the indirect action of angiogen factors (fibroblast growth factor, vascular endothelial growth factor derived from platelets, interleukins 1, 2 and 8 [10-13]. neovascularization is extremely important in the evolution of a tumor as by increasing perfusion in the territory of malignant cells provide nutrients and oxygen intake, the abolition catabolics, resulting in stimulation of tumor growth [14]. In a primary tumor, increased vascular micro density areas are where metastatic cells capable of expressing the angiogen phenotype [15] factors involved in tumor angiogenesis are summarized in Table 4.

**Table 4. Molecular markers involved in angiogenesis, tumor metastasis colorectal cancer and their prognostic significance**

| The marker | Function | Prognostic significance |
|---|---|---|
| VEGF | Promoting angiogenesis. | - increase by 2 the risk of death due to cancer. (Strong evidence in the literature.) |
| PD-ECGF platelet derived-endothelial cell growth factor | Is an enzyme (phosphorylase) which reversibly phosphorylates thymidine and deoxyuridine; dezoxiflouridine converted to fluorouracil. | - 8-fold increased risk of cancer death, although it would decrease the incidence of invasion and lymph nodes metastasis. Therefore, the prognostic role uncertain. |
| CAMs cell adhesion molecules | Glycoprotein that facilitates adhesion and intercellular interactions. | - Reduction cams on the surface of tumor cells are associated with increased lymph node metastasis and liver and the decrease of survival without symptoms. Role remains uncertain. |

## Vascular endothelial growth factor expression (VEGF) and intratumoral vascular micro density (MVD)

The concept of tumor angiogenesis has been supported by numerous experimental and clinical evidence, resulting from investigations focusing on studying the factors pro-and anti-angiogen and that endothelial receptor [19].

Research, mainly geared to identify cells responsible for the production of angiogen factors and further molecular and genetic mechanisms of stimulation/inhibition of angiogenesis process [14, 16-18] had direct impact in increasing the prognostic value of tumor angiogenesis [19]. There are currently over 12 described angiogen factors, most being proteins or cytokines [20]. They act either directly by stimulating endothelial or indirectly

through paracrine mechanisms involving other cells [22].

Angiogenesis can be assessed through a wide range of markers of endothelial really use the factor VIII (von Willebrand factor), CD31 (PECAM-1), CD34, CD105, Tie [14]. Because these markers are expressed by endothelial cells, both normal tissues and in the tumor, only the tumor microvasculature identification difficult. Concerns specific markers specialists focused primarily malignant status has recently been identified by comparing DNA from endothelial cells lining the colon of normal endothelial cells derived from colorectal tumors, 9 endothelial tumor markers (TEMS-endothelial Tumour Markers) directly involved in tumor angiogenesis [21, 22].

Of angiogen factors involved in regulating tumor angiogenesis, the best known are VEGF (Vascular endothelial Growth Factor-vascular endothelial growth factor), FGF (Fibroblast

Growth Factor-fibroblast growth factor), TGFα/β (Transforming Growth Factor-conversion factor increasing α/β) and angiogenin. VEGF has been extensively studied in relation to colorectal cancer and corresponding liver metastases [9, 17].

For other factors, roles in the promotion of colorectal cancer metastasis is not clearly established, it has multiple activities and a variety of effects on different cells [12]. VEGF is a protein of 40-45 kDa hemodynamic secreted by a wide variety of cells and by the majority of tumor cells. It was originally described as a product that induce vascular leakage (which is why it was named vascular permeability factor) that trigger an important angiogen response [23-25].

In addition, VEGF induces collagenase of plasminogen activators as well as from their inhibitors and stimulates hexose transport in these cells [15]. 5 types of VEGF gene produces mRNA which encodes the VEGF variants differing in molecular weight and biological properties [26]. VEGF family (growth factors that bind heparin) [25-28] include 5 different molecules of glycoprotein homodimer structure: VEGF-A,-B,-C,-D, and PLGD (placental growth factor-like), with specific receptor tyrosine kinases: VEGFR1, R2 and R3.

The biological effect of VEGF is mediated by activation of these receptors located in the endothelium [29-32]. VEGF-A and VEGF-B are ligands for Flt-1 or VEGF-R1 [32, 35], VEGF-A and VEGF-C or VEGF-R2 Flk-1/KDR [7, 11], and VEGF-C VEGF-D and Flt-4 or VEGFR3 [38-40]. VEGF-R3 is the same as the structure of the other two receptors, but that does not bind VEGF-A, VEGF-B and PlGF. VEGF-R1 and VEGF-R2 is expressed predominantly in blood vessel endothelium, whereas VEGF-R3 is restricted to the lymphatic endothelium, describes the process of lymphangiogenesis [12].

The tumor angiogen activity is closely related to biological properties and the presence of VEGF family members. VEGF as a prognostic factor value is out specialists, numerous studies retrospectively evaluated the published literature - sometimes with different results and conclusions due to the relatively small number of patients with various research teams are reporting [33].

VEGF expression has been extensively studied in normal mucosa, primary colon cancer and metastatic colon cancer and experimental [34, 35].

VEGF and receptor expression correlates with neovascularization and default extension with vascular micro density [36, 37], considered not only intratumoral, but also on the outskirts of invasion and vascular micro density is higher in metastatic tumors, compared with non-metastatic [38, 39]. More research on colorectal carcinomas showed strong correlation between microvascular density and metastases in regional lymph nodes and distant. Microvascular density determination requires the use of immunohistochemical techniques for highlighting endothelial cells. Most markers are used colorectal factor VIII antigen, CD31 and CD34 [40]. Because expression of other angiogen factors did not differ between primary and secondary tumors, VEGF is considered an important angiogen factor in metastatic colon cancer, VEGF receptors and prognostic indicators that the increased metastatic risk and survival [41-43].

VEGF levels are significantly higher in distant metastases compared with those near and VEGF expression is associated with a significantly higher survival rate compared with high VEGF expression [42]. KDR and FLT1 are strongly expressed by tumor endothelial cells located in the liver metastases.

The role of VEGF in tumor angiogenesis has a direct impact in the development of anti-angiogen therapy [44], administration of anti-VEGF monoclonal antibody inhibits tumor growth in vivo and experimental, reducing the number and size of liver metastases [27, 45-48].

## PD-ECGF (platelet derived-endothelial cell growth factor)

Endothelial cell growth factor derived from platelets (platelet-derived endothelial cell growth factor) is the enzyme thymidine phosphorylase, which ensures the reversible phosphorylation of thymidine and deoxyuridine and converted to basic compounds, also, deoxifluorouridine to fluorouracil [49]. In a study on a group of 163 patients with colorectal carcinoma, intratumoral expression of PD-ECGF was found to increase nearly 8 times the deaths from cancer, independent or newly formed microvessels density of tumor stage [50]. As other studies [51] have found that, in contrast to colorectal tumors staged as T2 or T3, elevated levels of PD-ECGF is correlated with a low incidence of lymphatic dissemination and distant metastasis, the role of PD-ECGF as a factor prognosis remains uncertain.

## Cams-cell adhesion molecules

There are some glycoproteins on the cell surface which is essential to cell-cell interactions. Fractures of cellular interconnectivity were reported in neoplastic cell proliferation and metastasis in tumor biology, effects on the immune response defense cams can trigger tumor genesis [49]. CAM markers have been reported in colorectal cancers, but their prognostic role remains to be studied more.

Family component of CAM (VCAM-1, ICAM-1, ELAM-1), the most important role to have prognostic marker ICAM-1. Thus, in a study of 96 patients with colorectal cancer [52] ICAM-1 negative tumors were associated with the presence of lymph node metastasis and liver another study conducted on 41 patients in stages II-III, low ICAM cells positive -1 was associated with a shorter period of disease-free [53].

## COX-2 expression in colon cancer

COX is a key enzyme involved in the conversion of arahidonic acid to prostaglandins and identified two isoforms-COX-1 and COX-2. COX-1 is expressed in many tissues and are believed to be involved in various physiological functions, whereas COX-2 is induced by pathological stimuli such as inflammation, various growth factors and cytokines produced by tumor cells. There have been numerous studies aimed at determining the relationship between COX-2 and tumor angiogenesis, and the development and progression of colon cancer. COX-2 expression correlated closely with micro vascular density, indicating the possible involvement of COX-2 in tumor angiogenesis in colorectal cancer. It is possible that angiogenesis induced by COX-2 is one of the mechanisms by which it promotes cancer invasion and metastasis. Several studies have revealed the prophylactic effect of NSAIDs on colorectal carcinoma and their therapeutic effects on colon polyps. The mechanism of action of NSAIDs is the inhibition of COX-2.

Many researchers have tested the relationship between COX-2 and angiogenesis and inhibitory effects of NSAIDs on vascular endothelial cells. COX-2 inhibitors realize their anticancer effects by suppressing the antiapoptotic Bcl-2 gene expression and reduced angiogenesis, thus affecting nutrient intake of the tumor, inhibiting proliferation and inducing apoptosis of cancer cells.

## Peculiarities of antiangiogen therapy

So far there have been many clinical trials on the effect of angiogen inhibitors as anti VEGF, angiostatin and endostatin, metalloproteinase inhibitors, TNP-740 and many others in patients with metastatic malignancies.

Angiogen therapy is directed mainly on small foci of capillary endothelial cell proliferation in the tumor bed or metastatic sites. Therefore, angiogenesis inhibitors generally do not suppress bone marrow does not cause gastrointestinal symptoms. Since angiogenesis inhibitors reduce neovascularization by inhibiting proliferation and migration of endothelial cells and not by a direct cytotoxic effect on them, their administration is longer compared to conventional cytotoxic agents.

Unlike conventional chemotherapy, requiring rest periods that allow normal cell regeneration in the spinal antiangiogen therapy is administered continuously, experimental studies revealing a cumulative effect. So as the inhibitor is administered for a longer period, the possibility of recurrence of the tumor after treatment discontinuation is lower. The phenomenon of resistance to angiogen inhibitors has not been a major problem in long-term experimental studies and clinical trials. This therapy has been proposed as a strategy for prevention of acquired resistance to classic anticancer agents. It was observed, on experimental methods, a higher efficiency for an angiogen and cytotoxic combination therapy (curative effect) than for each used separately (inhibitory effect). In addition anti-angiogen therapy may be used in combination with other treatment modalities, such as gene therapy or immunotherapy. Angiogenesis research have greatly expanded in recent years. Thus, genes that have been elucidated stimulates or inhibits angiogenesis. Also angiogenesis inhibitors are currently studied for their possible use in non-neoplastic diseases. Currently there are some fundamental questions: can be detected in blood or other fluids for diagnostic onset of angiogen activity? These are just some of the questions that incite further research on angiogenesis.

## Conclusions

Tumour angiogenesis is the formation of new vessels by sprouting of preexisting essential for tumor growth beyond microscopic.

The process of angiogenesis is the result of the balance between a number of angiogen factors and angiogenesis inhibitors.

VEGF and MVD, as an expression of tumor angiogenesis plays an important role in the biological behavior of tumor progression and lymph node metastasis are relevant for prognosis, tumor differentiation, etc.

In the future it is necessary to develop new clinical protocols that angiogenesis inhibitors are associated with chemo-or radiotherapy, conventional or other methods such as immunotherapy and gene therapy. Also, it may be possible angiogen inhibitor therapy in the early stages of neoplasia or as maintenance therapy to prevent recurrence.

## References

1. Rmali KA, Puntis MCA, Jiang WG. Tumour-associated angiogenesis in human colorectal cancer. Colorectal Dis 2006; 9: 3-14
2. Folkman J. Tumour angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182-1186
3. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumours. Ann Surg 1972; 175: 409-416
4. Folkman J, Merler E. Isolation of tumor factor responsible of angiogenesis.J Exp. Med 1971:133:275-288
5. Folkman J. The role of angiogenesis in tumour growth. Semin Cancer Biol 1992; 3: 65-71
6. Liekens S, De Clercq E, Neyts J. Angiogenesis: regulators and clinical applications. Biochem Pharmacol 2001; 61(3): 253-270
7. Ellis LM. Angiogenesis and its role in colorectal tumor and metastasis formation. Semin Oncol 2004; 31(6 Suppl 17): 3-9
8. Sharma S, Sharma MC, Sarkar C. Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis. Histopathology 2005; 46(5): 481-489
9. Khosravi Shahi P, Fernández Pineda I. Tumoral angiogenesis: review of the literature. Cancer Invest 2008; 26(1): 104-108
10. Kerbel RS. Tumor angiogenesis; past, prezent and the near future. Carcinogenesis,2000:21:505-515
11. Ribatti D. Vacca A, Presta I, The discovery of angiogenic factors. A historical review, General Pharmacol, 2002; 35: 227-231
12. Shih SC. Robinson GS,Perruzzi CA et al. Molecular profiling of angiogenesis markers. Amer J Pathol 2002; 161(1);35-41.
13. Takeda A, Stoeltzing O, Ahmad SA, Reinmuth N, Liu W, Parikh A, Fan F, Akagi M, Ellis LM. Role of angiogenesis in the development and growth of liver metastasis. Ann Surg Oncol 2002; 9(7): 610-616
14. Bresalier RS. The biology of colorectal cancer metastasis. Gastroenterol Clin North Am 1996; 25(4): 805-820
15. Bouck N. Tumour angiogenesis: the role of oncogenes and tumour suppressor genes. Cancer Cells 1990; 2: 179-185
16. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumourigenesis. Cell 1996; 86: 353-366
17. Ellis LM. A targeted approach for antiangiogenic therapy of metastatic human colon cancer. Am Surg 2003; 69(1): 3-10
18. Tomanek RJ, Schatteman GC. Angiogenesis: new insights and therapeutic potential. Anat Rec 2000; 261(3): 126-135
19. Folkman J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med 1995; 333(26): 1757-1763
20. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW. Genes expression in human tumour endothelium. Science 2000; 289: 1197-1201
21. Carson-Walter EB, Watkins DN, Nanda A, Vogelstein B, Kinzler KW, St Croix B. Cell surface tumour endothelial markers are conserved in mice and humans. Cancer Res 2001; 61: 6649-6655
22. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989; 15: 851-858
23. Plouet J, Schilling J, Gospodarowicz D. Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. EMBO J 1989; 8: 3801-3806
24. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr Rev 1992; 13: 18-32
25. Neufeld G, Tessler S, Gitay-Goren H, Cohen T, Levi BZ, Cohen T, Gengrinovitch S. Vascular endothelial growth factor (VEGF) and its receptors. Faseb J 1999; 13: 9-22
26. Shinkaruk S, Bayle M, Laïn G, Déléris G. Vascular endothelial cell growth factor (VEGF), anemerging target for cancer chemotherapy. Curr Med Chem Anticancer Agents 2003; 3(2): 95-117.
27. Breen EC. VEGF in biological control. J Cell Biochem 2007; 102(6): 1358-1367
28. Terman BI, Carrion ME, Kovacs E, Rasmussen BA, Eddy RL, Shows TB. Identification of a new endothelial cell growth factor receptor tyrosine kinase. Oncogene 1991; 6: 1677-1683
29. Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 1992; 255: 989-991
30. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Bohlen P. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun 1992; 187: 1579-1586
31. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Bohlen P. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993; 72: 835-846
32. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer: A critical review. J Clin Pathol 2008; doi:10.1136/jcp.2007.05.48-58
33. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Rep 2008; 41(4): 278-286

34. Abdou AG, Aiad H, Asaad N, Abd El-Wahed M, Serag El-Dien M. Immunohistochemical evaluation of vascular endothelial growth factor (VEGF) in colorectal carcinoma. J Egypt Natl Canc Inst 2006; 18(4): 311-322

35. Weidner N. Intratumour microvessel density as a prognostic factor in cancer. Am J Pathol 1995; 147: 9-19

36. Nakasaki T, Wada H, Shigemori C, Miki C, Gabazza EC, Nobori T, Nakamura S, Shiku H. Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. Am J Hematol 2002; 69(4): 247-254

37. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995; 55: 3964-3968

38. Boxer GM, Tsiompanou E, Levine T, Watson R, Begent RH. Immunohistochemical expression of vascular endothelial growth factor and microvessel counting as prognostic indicators in nodenegative colorectal cancer. Tumour Biol 2005; 26(1): 1-8

39. Du JR, JiangY, Vascular endothelial growth factor of microtumors by antivascular density in gastric carcinomas. Word JGastroenterol 2003,9(7):1604-1606

40. Tokunaga T, Oshika Y, Abe Y, Ozeki Y, Sadahiro S, Kijima H, Tsuchida T, Yamazaki H, Ueyama Y, Tamaoki N, Nakamura M. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. Br J Cancer 1998; 77: 998-1002

41. Ellis LM, Takahashi Y, Liu W, Shaheen RM. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. Oncologist 2000; 5(Suppl. 1): 11-15

42. Takeda A, Shimada H, Imaseki H, Okazumi S, Natsume T, Suzuki T, Ochiai T. Clinical significance of serum vascular endothelial growth factor in colorectal cancer patients: correlation with clinicopathological factors and tumour markers. Oncol Rep 2000; 7: 333-338

43. Folkman J. Endogenous angiogenesis inhibitors. APMIS 2004; 112(7-8): 496-507

44. Rennel ES, Hamdollah-Zadeh MA, Wheatley ER, Magnussen A, Schüler Y, Kelly SP, Finucane C, Ellison D, Cebe-Suarez S, Ballmer-Hofer K, Mather S, Stewart L, Bates DO, Harper SJ. Recombinant human VEGF(165)b protein is an effective anti-cancer agent in mice. Eur J Cancer 2008; 44(13): 1883-1894

45. Goodin S. Development of monoclonal antibodies for the treatment of colorectal cancer. Am J Health Syst Pharm 2008; 65(11 Suppl 4): S3-7

46. Agrez MV. Cell adhesion molecules and colon cancer. Aust N Z J Surg 1996; 66(12): 791-798

47. Ngan CY, Yamamoto H, Seshimo I, Ezumi K, Terayama M, Hemmi H, Takemasa I, Ikeda M, Sekimoto M, Monden M. A multivariate analysis of adhesion molecules expression in assessment of colorectal cancer. J Surg Oncol 2007; 95(8): 652-662

48. Kahlenberg MS, Sullivan JM, Witmer DD, Petrelli NJ. Molecular prognostics in colorectal cancer. Surg Oncol. 2003; 12(3): 173-186

49. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F, Aikou T. Clinicopathologic and prognostic significance of an aniogenic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst. 1996; 88(16): 1110-1117.

50. Saito S, Tsuno N, Nagawa H, Sunami E, Zhengxi J, Osada T, Kitayama J, Shibata Y, Tsuruo T, Muto T. Expression of platelet-derived endothelial cell growth factor correlates with good prognosis in tients with colorectal carcinoma. Cancer. 2000; 88(1): 42-49.

51. Maeda K, Kang SM, Sawada T, Nishiguchi Y, Yashiro M, Ogawa Y, Ohira M, Ishikawa T, Hirakawa YS, Chung K. Expression of intercellular adhesion molecule-1 and prognosis in colorectal cancer. Oncol Rep. 2002; 9(3): 511-514.

52. Mulder W, Stern PL, Sukart MJ, de Windt E, Butzelaar RM, Meijer S, Adér HJ, Claessen AM, Vermorken JB, Meijer CJ, Wagstaff J, Scheper RJ, Bloemena E. Low intercellular adhesion molecule1and high 5T4 expression on tumor cells correlate with reduced disease-free survival in colorectal carcinoma patients. Clin Cancer Res. 1997; 3(11): 1923-1930.

53. Joo YE, Oh WT, Rew JS, Parc CS, et al. Cyclooxygenase-2 expression is associated with well-differentiated and intestinal type pathways in gastric carcinogenesis. Digestion: 2002; 66: 222-229.

*Corresponding Author: Alexandru Mihalache, Emergency Department, County Emergency Hospital Slatina, 9-11 Crisan St., e-mail: alexandru_mihalache2004@yahoo.com*