Evaluation of Angiogenesis in Colorectal Carcinoma by CD34 Immunohistochemistry Method and its Correlation with Clinicopathologic Parameters

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Abstract- The basic pathogenic step in the process of tumor growth, invasion and metastasis is tumor-induced angiogenesis. The aim of this study was to evaluate the angiogenesis in colorectal carcinoma by microvascular density (MVD) determination with IHC (immunohistochemistry) method and to determine if and how angiogenesis correlates with clinicopathologic parameters. Sixty two archival, paraffin embedded tissue samples of colorectal carcinoma from Omid Hospital (Mashhad, Islamic republic of Iran) were selected. Microvessels were identified immunohistochemically, using monoclonal CD34 antibody. Two investigators examined the microvessel density then the median value of MVD was determined and correlated with clinicopathologic parameters. Tumor-induced angiogenesis of colorectal carcinoma statistically correlated with histological tumor grade (P=0.000). There was no significant correlation between intratumoral microvessel density and sex and age of patients, localization, and stage and histologicalal tumor type ($P > 0.1$). Intratumoral microvessel density quantification in histologic specimens of colorectal carcinoma reflects the grade of tumors and may be a useful additional prognostic factor.

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Key words: Angiogenesis, antigens, CD34, colorectal neoplasm, immunohistochemistry, clinical pathology

Introduction

Colon cancer is the third prevalent cancer in adults and is a worldwide health problem. Tumors stimulate the growth of host blood vessels, a process called angiogenesis, which is essential for supplying nutrients of the tumor. Tumors cannot enlarge beyond 1 to 2mm in diameter or thickness unless they are vascularized. Beyond this size the tumor fails to enlarge without vascularization because of hypoxia-induced cell death. Angiogenesis is a requisite not only for continued tumor growth but also for Metastasis (1).

Weidner and colleagues (2) in 1991 reported the first study showing the prognostic influence of neovascularization in breast carcinoma. Their results led several groups to analyze the possible prognostic role of this factor in many kinds of solid tumors, such as gastric or large bowel tumor (3, 4).

Several studies have noted that microvascular density (MVD) correlates with stage of disease and histological grade of tumor (5-9). In this study, we investigated correlation of MVD and grade in the tumor tissue of patients with colorectal carcinoma.

Patients and Methods

Study population

We retrospectively studied 62 patients who were treated by colectomy for colorectal carcinoma at Omid Hospital (Mashhad, Iran) between 1997 and 2003.

The patients included 31 (50%) men and 31 (50%) women, and their mean age at surgery was 56 years (range, 26 to 83).

The clinical records were obtained at admission. All of them were treated with colectomy and none received preoperative radiation therapy.

Primary study

A section from paraffin embedded tissue of colorectal carcinoma was stained with H&E for histological

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evaluation. Histological types of tumors were classical, signet ring and mucinous variants. The grade of classical type tumors (well, moderately and poorly differentiated) was also determined.

Immunohistochemical staining

Immunostaining was performed on paraffin-embedded tissues, by avidin-biotin peroxidase technique.

Sections measuring 4-5 µm thick were cut, air–dried for 15 min, heat-fixed at 42^{oc} and then air-dried overnight at room temperature. The slides were stored at room temperature until use. After deparaffinization with xylene, endogenous peroxidase activity was eliminated by treating the slides with EtOH for 10 min at room temperature. Then the slides were incubated with citrate for 12 min and placed in wash solution bath. H_2O_2 was then added for 10min and washed. Primary antibody (CD34-clone QBEnd 10.Dako) was added and incubated for 30 min and rinsed with wash solution.

Labeled polymer HRP was used for 30 min and washed. Finally, prepared DAB and substrate chromogen solution were used for 5-10 min with xylene and EtOH.

Vascular counts

We chose special areas of tumor in 4× magnification that did not have necrosis, ulceration or inflammation, as vascular hot-spot.

Following the first experimental design reported by Weidner and Colleagues (1991) (2), we counted the number of vessels in three hot spots with 400× magnification and obtained median value of them.

Statistical analysis

Students *t*-test was used for quantitative parameters (age of the patient) and the chi-squared test was used for qualitative parameters, such as histological grades, stages, location and sex.

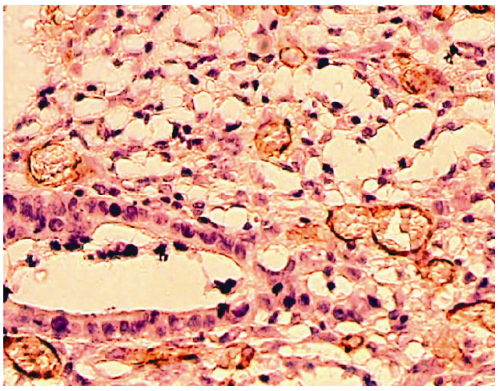
Results

Sixty two colon adenocarcinomas were studied. All the specimens were from surgical colon resection. The tumors were categorized as left-sided (77.41%) and right-sided (22.58%). There were 51 cases with the histological diagnosis of conventional adenocarcinomas (which included 30 well-differentiated and 17 moderately-differentiated grade and 4 poorly differentiated adenocarcinomas), and 10 mucinous carcinoma and one case of signet ring carcinoma. The stages (Astler-Coller staging) of the tumors were as follows: stage A = 0, stage B_1 = 3, stage B_2 = 27, stage C1 = 1, stage C2 = 25 and stage D = 6. Because of the low number of cases in stage A and C1 , for statistical analysis we considered the cases as stage B (no metastases to lymph node), stage C (with metastases to lymph node) and stage D (distant metastases). The main clinicopathological characteristics of our patients are summarized in table 1.

Table 1. Clinicopathologic characteristics of colon cancer cases.

Patients	**Total number** **Mean age (range)**	**62** **56(26-83)years**
Sex	Male	31
	Female	31
Site	Right	14
	Left	48
Adenocarcinoma	Conventional	51
	Non-conventional	11
Grade	W.D*	30
	M.D*	17
	P.D*	4
Dukes stage	A	0
	B	30
	C	26
	D	6

W.D indicates; Well differentiated; M.D: Moderately differentiated; P.D, Poorly differentiated.



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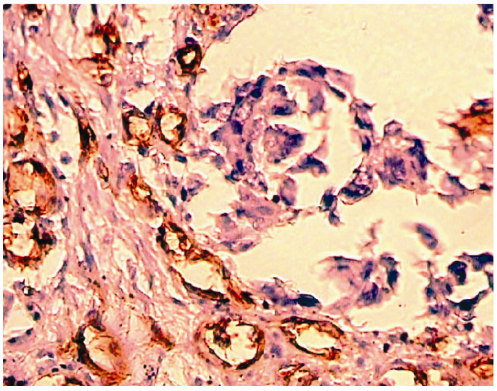

B

Figure 1. Microvascular density in colorectal cancer specimen

Table 2. Relationship between clinicopathologic factors and MVD .

Parameter	n	More than median (median=28.50) n (%)	Less than median (median=28.50) n (%)	*P Value*
Sex				*P = 0.127*
Male	31	13(41.9)	18(58.1)	
Female	31	19(61.3)	12(38.7)	
Location				*P = 0.176*
Right	14	5(35.7)	9(64.3)	
Left	48	27(56.3)	21(43.8)	
Stage				*P = 0.544*
A	0	0	0	
B	30	15(50)	15(50)	
C	26	15(57.7)	11(42.3)	
D	6	2(33.3)	4(66.7)	
Type				*P = 0.379*
Conventional	51	25(49)	26(51)	
Non-conventional	11	7(63.6)	4(36.4)	
Histological grade		**More than Median (Median=27.5)**	**Less than Median (Median=27.5)**	***P Value***
	n	n(%)	n(%)	*P = 0.000*
Well differentiated	30	6(20)	24(80)	
Moderate differentiated	17	17(100)	0(0)	
Poor differentiated	4	4(100)	0(0)	

Any single brown-stained cell that indicates an endothelial cell stained with CD34 counted as a single vessel (Figure 1).

The median MVD for total adenocarcinoma was 28.50 but for conventional adenocarcinoma were 27.50. A significant correlation was found between the MVD and different grade (*P = 0.000*), and there was no significant relationship between the MVD and sex, tumor location, tumor type and stage (Table 2).

Discussion

Carcinoma of the large bowel is common in Northwest Europe, North America, and other Anglo-Saxon areas and low in Africa, Asia, and some parts of South America.

In the United States, it is by far the most common and most curable carcinoma of the gastrointestinal tract. During the last thirty years the occurrence of major GI cancers has changed in Iran with sharp increase in colon cancer, slight to moderate increase in stomach cancer and sharp decrease in esophageal cancer (10). A similar anatomic distribution pattern and left shift of colorectal adenomas and cancers in Iranian population is compatible with most other Asian countries (11).

The staging systems, which represent a combination of the criteria of local extent (and lymph node involvement) have proved to be a powerful way of predicting the prognosis of patients and there is a definite relationship between the microscopic grade of the tumor and its prognosis (12), on the other hand tumor angiogenesis is essential for carcinogenesis and facilitates the process of tumor development and Metastasis (7).

The aim of our study was to analyze correlation between microvascular density and clinicopathologic parameter. There are many methodological differences between studies that make their comparison difficult. First, some authors have employed factor VIII as the endothelial marker (4, 13), others chose either CD31 (5, 14, 15) or CD34 (6, 16). In addition some authors have chosen patients with tumors in all Dukes stages (4, 14-16), others have only studied certain stages.

For this reason it extremely difficult to design a study about vascularization that could be easily compared with other reports.

We performed manual counts as all the other reports have employed this kind of measure. We chose CD34 as a vascular marker due to its increased sensitivity.

In our study we considered a vessel to be any structure stained with the chromogen that could be separated from adjacent vessels and stromal cells, although it did not show a lumen.

The microvalcular density only showed significant association with the histological grade (P = 0.000). The poorly and moderately differentiated tumors had a significantly higher number of vessels than the well-differentiated ones. Microvascular density doesn't have any correlation with sex, age, localization, stage and histological tumor type ($P > 0.1$). Microvascular density may be valuable in stratifying patients in planning appropriate adjuvant, antiangiogenic and antitumor antibody-directed therapy (9, 17-20) after surgery.

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References

1. Kumar V, Assas AK, Fausto N, editors. Robbins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia, PA: Elsevier Saunders; 2005.
2. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. N Engl J Med 1991; 324(1): 1-8.
3. Maeda K, Chung YS, Takatsuka S, Ogawa Y, Sawada T, Yamashita Y, et al. Tumor angiogenesis as a predictor of recurrence in gastric carcinoma. J Clin Oncol 1995; 13(2): 477-81.
4. Lindmark G, Gerdin B, Sundberg C, Påhlman L, Bergström R, Glimelius B. Prognostic significance of the microvascular count in colorectal cancer. J Clin Oncol 1996; 14(2): 461-6.
5. Fenjvesi A. Prognostic significance of tumor-induced angiogenesis in colorectal carcinoma. Med Pregl 2003; 56(5-6): 263-8.
6. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol 2003; 9(6): 1227-30.
7. Cristi E, Perrone G, Toscano G, Verzì A, Nori S, Santini D, et al. Tumour proliferation, angiogenesis, and ploidy status in human colon cancer. J Clin Pathol 2005; 58(11): 1170-4.
8. Hollingsworth SJ, Drye ER, Tou SI, Boulos PB. Expression of angiogenic VEGF-A (soluble isoforms 121, 165) and lymphangiogenic VEGF-C in colorectal cancers with micro-satellite instability. J Surg Oncol 2005; 92(4): 317-25.
9. Grantab R, Sivananthan S, Tannock IF. The penetration of anticancer drugs through tumor tissue as a function of cellular adhesion and packing density of tumor cells. Cancer Res 2006; 66(2): 1033-9.
10. Bafandeh Y, Daghestani D, Esmaili H, Aharizad S. Distribution of cancer and adenomatous polyps in the colorectum: study in an Iranian population. Asian Pac J Cancer Prev 2006; 7(1): 65-8.
11. Yazdizadeh B, Jarrahi AM, Mortazavi H, Mohagheghi MA, Tahmasebi S, Nahvijo A. Time trends in the occurrence of major GI cancers in Iran. Asian Pac J Cancer Prev 2005; 6(2): 130-4.
12. Rosai J. Rosai and Ackerman's Surgical Pathology. 9th ed. Edinburgh: Mosby; 2004.
13. Fox SH, Whalen GF, Sanders MM, Burleson JA, Jennings K, Kurtzman S, et al. Angiogenesis in normal tissue adjacent to colon cancer. J Surg Oncol 1998; 69(4): 230-4.
14. Bossi P, Viale G, Lee AK, Alfano R, Coggi G, Bosari S. Angiogenesis in colorectal tumors: microvessel quantitation in adenomas and carcinomas with clinicopathological correlations. Cancer Res 1995; 55(21): 5049-53.
15. Engel CJ, Bennett ST, Chambers AF, Doig GS, Kerkvliet N, O'Malley FP. Tumor angiogenesis predicts recurrence in invasive colorectal cancer when controlled for Dukes staging. Am J Surg Pathol 1996; 20(10): 1260-5.
16. Tanigawa N, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K, et al. Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res 1997; 57(6): 1043-6.
17. Boxer GM, Tsiompanou E, Levine T, Watson R, Begent RH. Immunohistochemical expression of vascular endothelial growth factor and microvessel counting as prognostic indicators in node-negative colorectal cancer. Tumour Biol 2005; 26(1): 1-8.
18. Wang HM, Zhang GY. Indomethacin suppresses growth of colon cancer via inhibition of angiogenesis in vivo. World J Gastroenterol 2005; 11(3): 340-3.
19. Liang P, Hong JW, Ubukata H, Liu HR, Watanabe Y, Katano M, et al. Increased density and diameter of lymphatic microvessels correlate with lymph node metastasis in early stage invasive colorectal carcinoma. Virchows Arch 2006; 448(5): 570-5.
20. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer 2006; 94(12): 1823-32.