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Differential Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptors in the Sequence of Hyperplastic Polyp, Serrated Adenoma and Adenocarcinoma of Colorectum

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AIM: The aim of this study was to investigate the role for vascular endothelial growth factor (VEGF) and its receptors, VEGFR-1 and -2, in the hyperplastic polyp (HP)- serrated adenoma (SA)-adenocarcinoma (AC) sequence of the colorectum.

Methods: Thirty-six HPs, 33 SAs and 7 ACs (which contained HP and/or SA) were immunohistochemically examined for the expression of VEGF, VEGFR-1, and VEGFR-2.

Results: VEGF protein was expressed in the cytoplasm of SA and AC tumor cells, and VEGFR-1 and VEGFR-2 were expressed both in the cytoplasm and on the membrane of these tumors, while there was faint or no expression of VEGF, VEGFR-1 and VEGFR-2 in HPs. Immunohistochemical staining revealed that 8.3% (3 of 36) HPs, 87.9% (29 of 33) SAs and 100% (7 of 7) ACs were positive for VEGF; 2.8% (1 of 36) HPs, 97.0% (32 of 33) SAs and 100% (7 of 7) ACs were positive for VEGFR-1; 16.7% (6 of 36) HPs, 100% (33 of 33) SAs and 100% (7 of 7) ACs were positive for VEGFR-2. The expression of VEGF, VEGFR-1 or VEGFR-2 was statistically correlated with the sequence of HP, SA and AC ($P < 0.0001$, respectively)

Conclusion: Our results suggest that the VEGF pathway may play an important role in the HP-SA-AC sequence.

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Keywords: VEGF; VEGF receptor; Hyperplastic polyp; Serrated adenoma; Adenocarcinoma

Introduction

The hyperplastic polyps (HPs)-serrated adenomas (SAs)-adenocarcinoma (AC) sequence in the colorectal tumorigenic pathway has been proposed as the different way from the traditional adenoma-carcinoma sequence suggested by Vogelstein.[1-4] HPs are rounded and usually sessile polypoid lesions of a few millimeters in diameter showing elongated crypts with a tendency to cystic dilatation. The epithelium of serrated gland in HPs is constituted by a single layer of cells without the dysplasia such as swollen nuclei and crowding of nuclei.[5] Therefore, it has been believed that HPs are absolutely benign lesions which lack any malignant potential. However, recently the case has been reported the occurrence of AC drived from HP.[6] SAs are also flat and/or sessile polypoid lesions in the endoscopic appearance and demonstrate the histopathological characteristics of saw-tooth appearance in those glands,[7] and it may be difficult to distinguish between HPs and SAs in the morphologic spectrum.[1] Longacre *et al*. described that the general architecture of SAs was similar to that of HPs,[8] however the cytologic features were different since surface mitotic activity, nuclear pseudostratification, and nuclear/cytoplasmic ratio were greater than in classic hyperplastic lesions. Although the prevalence of high grade dysplasia, which could be construed as a measure of such risk, was reported with 37% SAs containing foci of "significant dysplasia" and 11% containing intramucosal carcinoma,[8] the natural history of SAs and their risk for progression to malignancy is not well understood.

Vascular endothelial growth factor (VEGF) has been identified as a key regulator of tumor angiogenesis, and VEGF receptors (VEGFRs) are the major mediators of the mitogenic and permeability-enhancing effects of VEGF in endothelial cells.[9,10] In addition, VEGF is a survival factor for endothelial cells and a marked

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dependence on VEGF has been shown in newly formed but not established tumor vessels.[11] Although tumor angiogenesis is an area of extensive research, the consequence of enhanced angiogenesis and its reversion on tumor growth and progression are only partially elucidated.[12-14] Recently, coexpression of VEGF and its receptor, either VEGFR-1 (Flt-1) or VEGFR-2 (Flk-1/KDR), has been reported in tumor cells, suggesting the presence of an autocrine and/or a paracrine VEGF/VEGFR growth pathway in solid tumors.[15-17] Further, the expression levels of VEGF and VEGFR have been shown to correlate with progressive tumor growth and development of metastasis in many carcinomas.[18]

The present study aims to evaluate the expression and distribution of VEGF and VEGFR in HPs, SAs and ACs and to ascertain their possible role in the HP-SA-AC sequence considered from the VEGF/VEGFR axis.

Materials and methods

Tissue specimens

The material consisted of 64 paraffin-embedded tissue sections of colorectal lesions from 64 patients in Nagasaki University Hospital. The definition of SA was based on the description by Longacre and Fenoglio-Preiser as follows: a neoplastic lesion composed of a monotonous cell population with atypical nuclei proliferating in a serrated glandular architecture.[8] According to the Vienna classification, we diagnosed tumors in category 5 as carcinomas.[19] The material consisted of 57 benign solitary polyps and 7 ACs including 6 well differentiated and 1 moderately differentiated adenocarcinoma. The histopathological differentiation of colorectal adenocarcinomas determined according to the WHO classification for tumors. All of the colorectal carcinomas were consisted in SA and/or HP. Of the solitary polyps, 31 were HPs and 26 were SAs. Two independent pathologists (M. Taba and T. Nakayama) determined the diagnosis of HP, SA and AC.

Immunohistochemistry for VEGF and VEGFRs

The subcellular location of VEGF, VEGFR-1 and VEGFR-2 was determined in HPs, SAs and ACs using polyclonal antibodies directed against unique sequences of these proteins. These antibodies were devoid of any cross-reaction with other proteins in the VEGF family.[20] Formalin-fixed, paraffin-embedded tissues were cut into 4-μm thick sections, deparaffinized in xylene and rehydrated in PBS. Deparaffinized sections were preincubated with normal bovine serum to prevent non-specific binding and then incubated overnight at 4℃ with primary polyclonal antibody to human VEGF (1μg/ml), VEGFR-1 (Flt-1(C-17), 1mg/L) or VEGFR-2 (Flk-1(C-20), 1μg/ml) (Santa Cruz Biotechnology, Inc, USA), followed by alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (0.4μg/ml; Santa Cruz Biotechnology, Inc.). The reaction products were visualized using a mixture of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium chloride (BCIP/NBT; Roche Diagnostic Corp., Indianapolis, IN, USA). Negative controls consisted of replacing the primary antibody with non-immunized rabbit serum. Antigen absorption for anti-VEGF, VEGFR-1 and VEGFR-2 antibody using excess recombinant VEGF, VEGFR-1 and VEGFR-2 in each samples (R&D Systems Europe, MN, USA), and human breast cancer tissue served as the positive control.[21] VEGF, VEGFR-1 and VEGFR-2 expressions were classified into 2 categories depending upon the percentage of the cells stained and/or the intensity of staining (-: 0% to 15% positive tumor cells; +: >15% positive tumor cells).

Statistical analysis

The Stat View program (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analysis. Analyses comparing the degree of VEGF, VEGFR-1 or VEGFR-2 expressions in HPs, SAs and ACs were performed using Kruskal-Wallis test.

Results

Figure 1 shows a case with AC (1C) complicated with HP (1A) and SA (1B). Figure 2, 3 and 4 demonstrated the results of immunohistochemical staining for VEGF, VEGF-1 and VEGFR-2 in HP, SA and AC, respectively. VEGF, VEGFR-1 and VEGFR-2 expressions were heterogenous in SAs (Figure 3) and ACs (Figure 4), and localized to the cytoplasm and/or cell membrane of tumor cells, while the expression of these proteins was not detected in HP cells (Figure 2) in the specimens of AC with HP and/or SA. Immunohistochemical staining further revealed VEGF expression in the cytoplasm of SA (Figure 3B) and AC (Figure 4B) cells. VEGFR-1 expression was shown in the membrane and cytoplasm of SA (Figure 3C) and AC (Figure 4C) cells. VEGFR-2 was expressed in the membrane and cytoplasm of SA (Figure 3D) and AC (Figure 4D) cells. However, in HP cells, there was faint or almost no positive staining of VEGF (Figure 2B), VEGFR-1 (Figure 2C), and VEGFR-2 (Figure 2D). Immunohistochemical staining was positive for VEGF in 8.3% (3 of 36) HP, 87.9% (29 of 33) SA and 100.0% (7 of 7) AC. Positive staining for VEGFR-1 was detected in 2.8% (1 of 36) HP, 97.0% (32 of 33) SA and 100% (7 of 7) AC. Immunohistochemical staining was positive for VEGFR-2 in 16.7% (6 of 36) HP, 100% (33 of 33) SA and 100% (7 of 7) AC. There were statistical correlations in the expressions of VEGF, VEGFR-1 or VEGFR-2 between the sequence of HP, SA and AC ($P < 0.0001$).

Table. VEGF, VEGFR-1 and VEGFR-2 expression in HP, SA and AC. n (%)

		VEGF*		VEGFR-1*		VEGFR-2*	
	n	+	-	+	-	+	-
HP	36	3(8.3)	33(91.7)	1(2.8)	35(97.2)	6(16.7)	30(83.3)
SA	33	29(87.9)	4(12.1)	32(97.0)	1(3.0)	33(100)	0(0)
AC	7	7(100)	0(0)	7(100)	0(0)	7(100)	0(0)

*; p<0.0001, Kruskal-Wallis test.

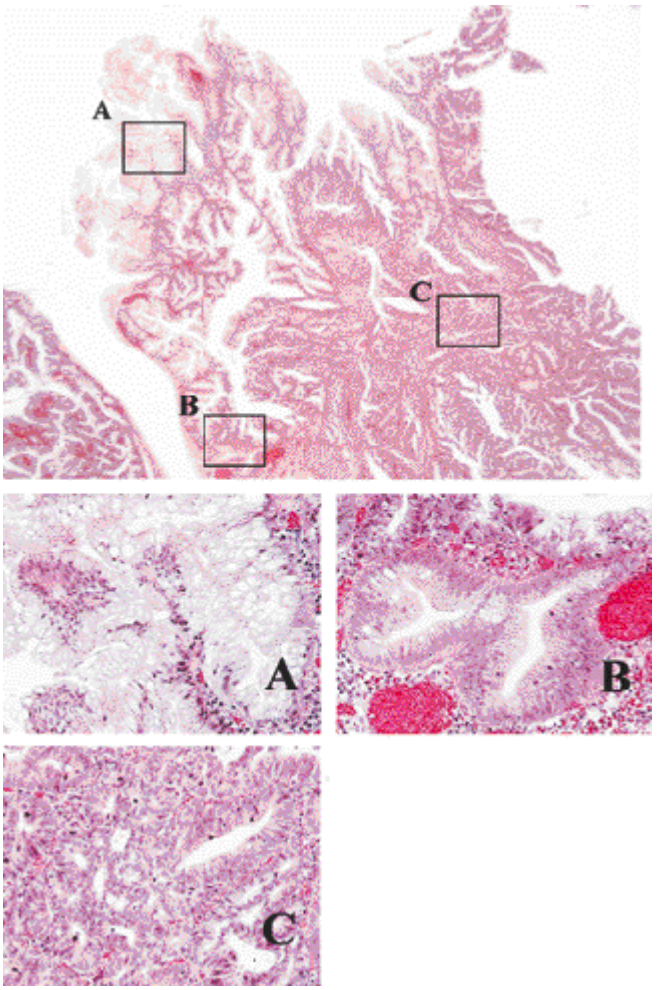

Figure 1. Hematoxylin and eosin staining of a case with AC (1C) complicated with HP (1A) and SA (1B).

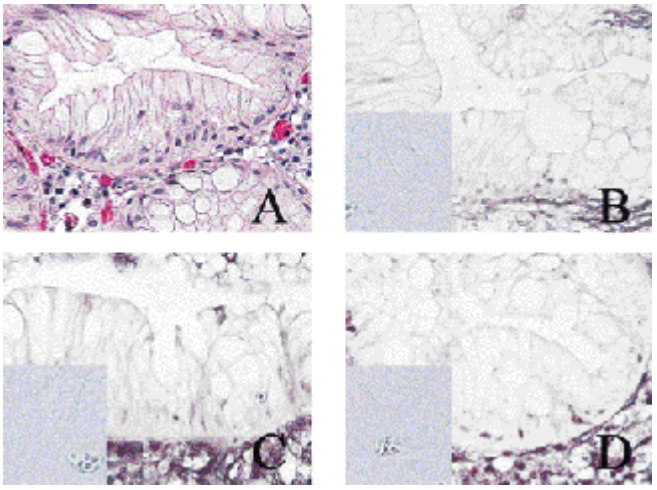

Figure 2. Hematoxylin and eosin staining of HP (A). Immunohistochemical staining of HP with antibodies to VEGF (B), VEGFR-1 (C) and VEGFR-2 (D). VEGF, VEGFR-1 and VEGFR-2 expressions were not detected in the epithelium of HP (B, C, and D). (magnification: x 400)

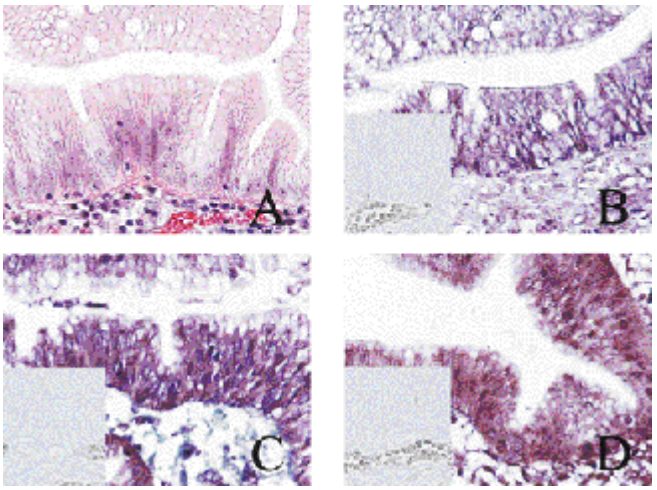

Figure 3. Hematoxylin and eosin staining of SA (A). Immunohistochemical staining reveals VEGF expression in cytoplasms of the epithelium of SA (B); VEGFR-1 and VEGFR-2 expression in the membrane and cytoplasms of epithelium of SA (C and D).

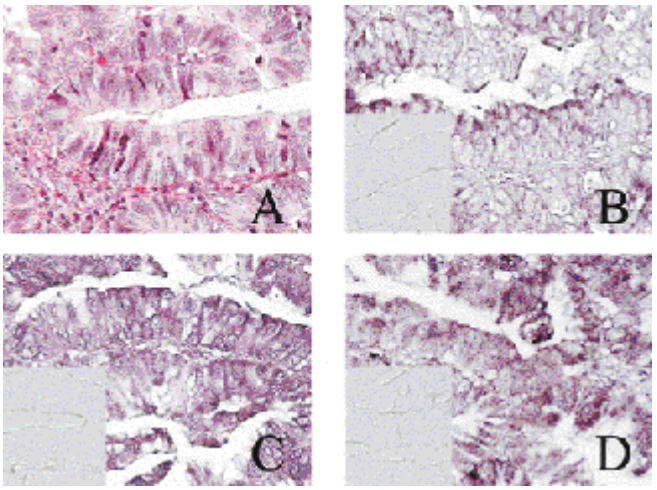

Figure 4. Hematoxylin and eosin staining of AC (A). Immunohistochemical staining reveals VEGF expression in cytoplasms of the epithelium of AC (B); VEGFR-1 and VEGFR-2 expression in the membrane and cytoplasms of the epithelium of AC (C and D).

Discussion

The more prevalent cancers of the traditional adenoma-carcinoma sequence accounted for upwards of two-thirds of all colorectal cancers.[22,23] These originate in adenomas (or aberrant crypt foci) instigated by mutations of the APC or β-catenin gene that lead to disruption of the Wnt signaling pathway, and the end point carcinomas are frequently characterized by chromosomal instability.[24,25] As the traditional adenoma-carcinoma sequence, the HP-SA-AC sequence was accounted for genetic abnormalities. In the HP-SA-AC sequence, deoxyribonucleic acid (DNA) mismatch repair resulting in microsatellite instability (MSI) plays an important role,[5] however, they also include some proportion of carcinomas

that are microsatellite stable (MSS).[22] The penultimate stage in the progression to carcinoma in this pathway is likely to be SA.[26]

Recently, angiogenesis, or the development of a vascularised stroma, is essential for tumors to grow beyond a minimal size[27,28] and metastasis.[29,30] In support of this hypothesis, expression of VEGF has been shown to correlate positively with microvessel count and metastasis.[31] In our study, there were no significant differences between vessel counts and the VEGF, VEGFR-1, and VEGFR-2 expression (data not shown). The reason of this remains unclear, however, there is strong evidence that the VEGF/VEGFR pathway has tumor growth and progression effects, which are angiogenesis-independent manners.

It was previously reported that the angiogenic switch occurs at the adenoma stage of the adenoma-carcinoma sequence in colorectal cancer.[32] VEGF and tissue factor were correlated in the early stages of the development of colorectal cancer. In this study, VEGF was higher expressed in SAs and ACs than in HPs. From these results, we concluded that the expression of VEGF might be activated in the adenoma stage of both the adenoma-carcinoma and the HP-SA-AC sequences. The production of VEGF, which began during the step of adenoma or SAs, may contribute to the progression and/or growth of colorectal cancer.

The coexpression of VEGF with VEGFR-1 and -2 has been reported in tumor cells, suggesting the presence of an autocrine and/-or paracrine VEGF/VEGFR growth pathway in solid tumors.[15-17,20] VEGF has already been shown to play a role in the colorectal ACs.[33] However, there have been no studies on VEGF and VEGFR expressions in the HP-SA-AC sequence. Our data show that the expressions of VEGF and VEGFR were significantly elevated in ACs and SAs compared with HPs. Moreover, expressions of VEGF and VEGFRs were detected in the lesions of SAs and ACs, although these proteins were minimally detected in the lesions of HPs. These data suggest that the VEGF/VEGFR pathway may play an important role in the HP-SA-AC sequence.

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