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Vascular endothelial growth factors and receptors in colorectal cancer: Implications for anti-angiogenic therapy

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ARTICLE INFO

Article history:
Received 9 August 2005
Received in revised form
12 September 2005
Accepted 14 September 2005
Available online 29 November 2005

Keywords:
VEGF
VEGFR
Colorectal cancer
Liver metastasis
Lymph nodes metastasis
Anti-VEGF antibodies

ABSTRACT

There are conflicting associations between growth factor expression and clinicopathological variables in colorectal cancer. This study aimed to define the expression of members of the VEGF family and the receptor, VEGFR2, in primary and metastatic sites of colorectal cancer and their relationship to metastatic potential. Thirty colorectal cancers, 12 lymph node metastases and 9 liver metastases were immunostained for VEGF-A, VEGF-C, VEGF-D and VEGFR2. VEGFR2 was expressed by endothelial cells and by the malignant epithelium. VEGF-C and VEGFR2 were co-expressed in the same territory and correlated throughout the primary tumour and in metastatic lymph nodes, but not in liver metastases. Their expression at the invasive tumour edge correlated with expression in metastatic nodes. The benefit of anti-VEGF antibodies might be increased by directing additional therapies against VEGF-C or against the kinase receptors to target redundancy in the system. A component of the therapeutic benefit might be due to a direct anti-tumour effect as well as an anti-angiogenic effect.

1. Introduction

The Vascular Endothelial Growth Factor (VEGF) family is important for the process of angiogenesis which is central to the growth and metastasis of cancer [1]. Indeed, recent data have shown that the addition of an anti-VEGF antibody to conventional cytotoxic chemotherapy confers a survival advantage to patients with metastatic colorectal cancer [2]. The most studied member of the VEGF family is VEGF (VEGF-A) [3], which is vital at all stages of human development. Newer members of the VEGF family include VEGF-C and VEGF-D [4,5], which possess lymphangiogenic effects in addition to their angiogenic action. The VEGF family exert their effects through activation of one or more of three related VEGF tyrosine kinase receptors (VEGFRs) [6].

The principal lineage that expresses VEGFRs is the endothelial cell, but increasingly, it is becoming apparent that the receptors can be expressed on malignant cell types, both on human cancer cell lines in vitro [7,8] and in human tissues including ovarian cancer [9], renal cell carcinoma [10], squamous cell carcinomas of the head and neck [11] and pancreatic cancer [12]. Co-expression of functional VEGFRs with their corresponding ligands in tumours raises the possibility of autocrine loops, whereby a tumour is capable

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doi:10.1016/j.ejca.2005.09.018

of stimulating its own growth, progression and survival. The existence of autocrine loops has important implications for the development of novel anti-VEGF and anti-VEGFR compounds that could have potential anti-angiogenic and direct anti-tumour effects in human malignancy [13].

The association between tumour growth factor expression and clinicopathological variables, including outcome, is not straightforward. Various authors have examined expression of VEGF-A in colorectal cancer and concluded either that increased expression is associated with negative clinicopathological variables [14] or alternatively, that no such association could be demonstrated [15]. Similarly, expression of VEGF-C and VEGF-D is noted in a variety of human malignancies and tends to correlate with negative clinicopathological variables, in particular, lymph node involvement and lymphatic invasion, although the relationships observed are not always consistent [16]. The reported inconsistencies may be due to the balance between the different members of the VEGF family and their relative levels of expression together with receptor availability.

The aim of this study was to define the expression pattern of the VEGF family members and VEGFR2 in primary and secondary sites of colorectal cancer and examine their influence on metastatic spread.

2. Materials and methods

Thirty primary colorectal cancer specimens, 12 associated lymph node metastases and 9 subsequent liver metastases were examined for growth factor and receptor expression using immunohistochemical techniques. The clinicopathological details of patients studied are shown in Table 1.

Tissue sections were mounted on 3-aminopropyl-triethoxysilane coated slides and immunostained for the antigens VEGF-A, VEGF-C, VEGF-D and VEGFR2 using a standard immunoperoxidase technique. Briefly, the sections were dewaxed in xylene and rehydrated through a graded ethanol series. Subsequently, endogenous peroxidase was blocked by immersion in 1% hydrogen peroxide for 30 min at room temperature. Following serum blocking, the primary antibody was applied and incubated overnight at 4 °C. A biotinylated secondary antibody was applied and incubated at room temperature for 45 min, followed by horse-radish peroxidase conjugated streptavidin for 45 min. Finally, the chromogenic substrate 3,3-diaminobenzidine tetrahydrochoride (DAB) was added. The primary antibodies and dilutions used were: VEGF-A: rabbit anti-human VEGF-A IgG (Santa Cruz, CA, USA), 1:400; VEGF-C: rabbit anti-human VEGF-C IgG (Zymed, San Francisco, USA), 1:50; VEGF-D: mouse monoclonal anti-human VEGF-D IgG (R&D Systems, Abingdon, UK), 1:500; VEGFR2: mouse monoclonal anti-VEGFR2 IgG (Santa Cruz, CA, USA), 1:50; and rabbit anti-VEGFR2 IgG (Abcam, Cambridge, UK), 1:50 to verify the findings.

The slides were reviewed independently by two observers (SED, MJ) blinded to clinical details and to which lymph nodes and/or liver metastases belonged to which primary tumour. The intensity of tissue staining was scored on a semi-quantitative scale from 0 to 5 (0: no stain; 5: strongest stain). Assessment was made at different areas of the tissue, including the normal colonic mucosa (N), mucosa at the junction between normal and malignant tissue (J), superficial part of the tumour (TS), central tumour (TC) and tumour at the invading edge (TI). In the event of discrepancies between the scorers, the slides were reviewed and scoring agreed by consensus.

Table 1 – Clinicopathological details of patients studied

Parameter	Group 1 (patients with non-metastatic tumours or with lymph node metastasis)	Group 2 (patients with liver metastasis)
Number of patients	21	9
Age (years)[a]	71 (49–86)	53 (39–76)
Gender (male:female)	10:11	7:2
T stage		
T1	1	0
T2	4	0
T3	13	8
T4	3	1
N stage		
N0	9	5
N1	7	3
N2	5	1
Differentiation		
Well differentiated	0	0
Moderately differentiated	13	9
Poorly differentiated	8	0
Dukes' stage		
A	1	0
B	7	4
C	11	4
D	2	1

a Median (range).

The study was approved by the local research ethics committee and informed consent sought in accordance with the recommendations of the committee.

Non-parametric tests were used for statistical analysis. Comparisons between medians of related variables were made with the Wilcoxon signed rank test for 2 variables and the Friedman test for greater than 2 related variables. Correlations between variables were examined with Spearman's rank correlation coefficients. All statistical tests were two-sided and $P < 0.05$ was taken as statistically significant.

3. Results

Primary tumours were stained for VEGFR2 using a monoclonal anti-VEGFR2 antibody. Weak staining was identified on vascular endothelial cells (Fig. 1a) and strong immunostaining was seen on malignant colorectal epithelium (Fig. 1b). Such intense expression in colorectal epithelial cells was unexpected, so in order to confirm this finding immunostain-