Changes in Serum Soluble VEGFR-1 and Tie-2 Receptors in Colorectal Cancer Patients Following Surgical Resections

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Abstract. Aim: Determination of changes in serum levels of soluble (s) VEGFR-1 and Tie-2 receptors in colorectal cancer patients following resection in the search for novel tumour markers. Patients and Methods: Forty-five patients with primary colorectal cancer and 29 normal subjects were recruited. Serum sVEGFR-1 and sTie-2 receptors were assayed using ELISA. Results: sVEGFR-1 was detectable in 27% (10/37) and 12.5% (1/8) of cancer patients prior to curative and palliative resections, respectively, whilst 65.5% (19/29) of normal controls had detectable sVEGFR-1 levels. sTie-2 receptor levels were significantly raised in patients when compared with normal controls (p=0.0018). Furthermore, sTie-2 receptor levels were significantly higher in patients with metastases than those without (p=0.02). sTie-2 receptors demonstrated a significant drop in patients undergoing both curative (p<0.0001) and palliative resections (p=0.012). Conclusion: sVEGFR-1 levels were suppressed and sTie-2 receptor levels were raised in colorectal cancer patients. This data supports the potential use of sTie-2 receptor as a tumour marker.

Angiogenesis is crucial for the growth of solid tumour and dissemination of metastases (1). There is increasing evidence that the regulation of angiogenesis is dependent on a complex, tightly regulated interaction between the various pro-angiogenic and anti-angiogenic factors in the tumour microenvironment (2, 3). A main mechanism by which angiogenic factors are regulated is through the level of the relevant soluble receptor, which acts by competitively blocking access to cell surface receptors.

The vascular endothelial growth factor (VEGF) family and angiopoietin (4) are key regulators of angiogenesis with their soluble receptors, fms-like tyrosine kinase (Flt-1/VEGFR-1) and Tie-2 respectively, acting either as negative inhibitors or reservoirs of ligand. Inhibition of tumour angiogenesis could be achieved by inhibiting the VEGF receptor pathway (5, 6). The VEGF receptor family consists principally of three receptor-type tyrosine kinases, denoted VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4) (7, 8). VEGFR-1 has been described as one of the physiological receptors for VEGF (VEGF-A, VEGF-B) with high affinity (9). An alternatively spliced variant of the flt-1 gene results in short VEGFR-1 mRNA (3.0-kb, 2.2-kb), which is designated as soluble VEGFR-1 (10). It has been shown that the soluble VEGFR-1 protein exists in vivo and is capable of binding to VEGF acting as an inhibitor (11). It can also act as a 'decoy' receptor, forming heterodimers with full length VEGFR-2, thereby preventing VEGF binding to VEGFR-2 (11). This decoy function can regulate the activity of VEGF on vascular endothelium in a negative fashion (12).

The Tie receptor tyrosine kinase is expressed on endothelial cells (13, 14) and hematopoietic progenitor cells (15, 16). Gene knock-out studies have demonstrated that Tie receptors (Tie-1, Tie-2) support the integrity and survival of mature vascular endothelium, especially in the regions undergoing angiogenic growth of capillaries (17, 18). In particular, the Tie-2 receptor has been shown to be expressed both during development and in the quiescent vasculature of adult mice and rats, reflecting its role in maintaining the integrity of vascular trees in addition to its role in angiogenesis (19, 20).

Although VEGF-specific antagonists can inhibit the growth of most tumours, there appears to be a specific group of tumours which are not affected by such an approach (21).
Siemeister et al. have demonstrated that the Tie-2 receptor could act as an alternative pathway for angiogenesis, independent of VEGF (22). Takahama et al. have shown that the expression of Tie-2, its ligand angiopoietin-1, VEGF and CD31 were enhanced in human non-small cell lung carcinoma when compared with adjacent non-cancerous tissues. This is in contrast to the VEGFR-1, Flt-4, VEGF-C, Tie-1 and thrombin receptor, which showed no difference (23). Additionally, the expression of the Tie-2 receptors has been shown to increase in the endothelium of metastatic melanoma (24) and breast carcinoma (25). Hayes et al. have shown that soluble Tie-2 receptors can, in five-fold molar excess, block Ang-1-induced tubule formation (26). Similarly, interference of the Tie-2 pathway also results in the inhibition of tumour growth and angiogenesis (27).

It is unclear whether the soluble receptors of both pathways are acting as natural inhibitors (anti-angiogenically) or as a reservoir of ligand (pro-angiogenically). Whichever situation exists, it is possible that either or both receptors may have a role as a tumour marker. The purpose of this study was to compare the levels of the sVEGFR-1 and sTie-2 receptors in the serum of colorectal cancer patients with normal, non-cancer controls and to study changes following resection.

### Patients and Methods

**Study population.** This was a prospective, sequential cohort study in which forty-five patients with primary colorectal cancer undergoing curative resections at Castle Hill Hospital, Hull, U.K., were recruited. Twenty-nine age-matched healthy subjects were also recruited prior to their hernia operations as normal controls. The Hull and East Yorkshire research ethics committee granted approval and written informed consent was obtained from all patients prior to their inclusion in this study.

None of the patients with colorectal cancer had received blood transfusion, radiotherapy or chemotherapy prior to the surgery. All the cancer patients underwent routine pre-operative staging CT scan of the liver for colonic cancer and MRI scan of the abdomen and pelvis for rectal cancer. The patients were considered to have undergone curative resections when there were no metastases detected by the pre-operative staging scan and intra-operative assessments. A single consultant pathologist staged all tumour samples according to TNM, UICC, JASS and Dukes’ classifications.

**Preparation of samples.** Venous blood (7ml) was collected from the patients prior to surgery and 4-6 hours after surgery. The serum was separated by centrifugation (400g for 10 min) after 30 minutes of coagulation, immediately aliquotted and stored at -80°C until immunoassay.

**Detection of soluble VEGFR-1 and soluble Tie-2 receptors.** Soluble VEGFR-1 and sTie-2 receptors were assayed using a sandwich ELISA. For sVEGFR-1 a polyclonal, biotinylated secondary antibody was used, whereas the captured sTie-2 was detected using two biotinylated murine anti-human Tie-2 monoclonal antibodies. Baculovirus-produced recombinant protein was used to generate the standard curves. The minimum detectable level for sVEGFR-1 and sTie-2 is 0.2 ng/ml and 1 ng/ml, respectively.

**Statistical analysis.** The proportions of patients with detectable levels of soluble VEGFR1-receptors in different groups were compared using the Chi-Square test for trend. Continuous variables are presented as median [range]. The Mann-Whitney U-test was used for data with a non-normal distribution. Continuous variations before and after surgery were compared using Wilcoxon Signed Ranks test. Soluble VEGFR-1 receptor levels that were undetectable were assigned a value equal to the lower limit of detection, which is 0.2 ng/ml. Correlation was evaluated by the Spearman rank correlation test. A two-tailed p value of <0.05 was considered statistically significant.

### Results

Forty-five consecutive patients undergoing complete resection of the primary colorectal cancer were recruited in this prospective cohort study. Of these, 37 underwent curative resections whilst 8 patients were considered to have palliative surgery due to the presence of liver or peritoneal metastases. The Duke’s staging was: 7 Dukes’ A, 10 Dukes’ B and 20 Dukes’ C.

**Detectability of serum sVEGFR-1.** Serum sVEGFR-1 was detectable in 27% (10/37) and 12.5% (1/8) of colorectal cancer patients prior to curative and palliative resections, respectively, whilst 65.5% (19/29) of normal controls had detectable sVEGFR-1 levels. There was a significant association between the detectability of serum sVEGFR-1 and the disease status (p=0.0016). A significant linear trend was observed for the association, which suggests that the proportion of patients with a detectable level of serum sVEGFR-1 decreases with the disease status (p=0.0005; Table I).

**Distribution of serum sTie-2 receptors.** Serum sTie-2 receptors were detectable in all patients. The levels of serum sTie-2 receptors were significantly raised in colorectal

### Table I. Association of detectability of soluble VEGFR-1 receptors with the disease status.

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Colorectal cancer without metastases</th>
<th>Colorectal cancer with metastases</th>
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<tbody>
<tr>
<td>sVEGFR-1 detectable</td>
<td>19</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>sVEGFR-1 non-detectable</td>
<td>10</td>
<td>27</td>
<td>7</td>
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Chi-squared test for trend, p=0.0005.
cancer patients when compared with the normal controls (median [range], 57.7 [27-108] ng/ml vs 48.6 [33-65] ng/ml, p=0.0018). Pre-operative levels of serum sTie-2 receptors were significantly higher in colorectal cancer patients who presented with metastases than those without (median [range], 70.7 [52-99] ng/ml vs 56.9 [27-108] ng/ml, p=0.02).

Correlation of changes in serum sVEGFR-1 and sTie-2 receptors in colorectal cancer following surgical resections. Patients who had undergone curative resections demonstrated a significant increase in post-operative serum sVEGFR-1 levels compared with the pre-operative sVEGFR-1 levels (Figure 1; Median [range], 0.20 [0.20-1.01] ng/ml vs 0.20 [0.20-0.45] ng/ml, p=0.03). In contrast, there was no significant increase in patients having palliative resections (Figure 2; Median [range], 0.21 [0.20-0.56] ng/ml vs 0.20 [0.20-0.71] ng/ml, p=0.5). On the other hand, the level of serum sTie-2 receptors demonstrated a significant
The present study demonstrated that the activities of sVEGFR-1 receptors were suppressed, whereas the levels of sTie-2 receptors were elevated, in colorectal cancer patients when compared with normal controls. sTie-2 receptors demonstrated a significant drop in patients undergoing both curative and palliative resections. SVEGFR-1 activity appeared to "bounce back" to levels comparable to normal controls following curative resection.

One hypothesis that would explain these results is that the suppressed sVEGFR-1 levels would promote angiogenesis by reducing the inhibitory effects on VEGF activities. Evidence already exists that supports the use of sVEGFR-1 as an anti-angiogenic agent for blocking tumour angiogenesis in vivo (6, 28-31).

Given the significant rise in the levels of sTie-2 receptors in colorectal cancer, it is possible that this soluble receptor may play a role in stabilizing the activities of angiopoietin-1 by forming a soluble receptor-ligand complex, which will be protected from proteases, resulting in a longer half-life of the pro-angiogenic cytokines. The discovery that two ligands, Ang1 and Ang2, behave in a reciprocal fashion indicates that a tightly regulated activation of Tie-2 receptor is required in vascular developments. Ang-1 is a predominately pro-angiogenic factor, whilst Ang-2 acts as an antagonist for the Tie-2 receptor (4, 32-34). It is already known that the expression of both Ang-1 and Tie-2 receptors are increased in various tumour tissues (23, 35). Such an increase in the expression of Tie-2 mRNA could also result in a proportional increase in the release of its shorter extra-cellular domains, i.e. the soluble receptors.

This study has also shown that the increase in levels of sTie-2 receptors was much higher in metastatic colorectal cancer than non-metastatic disease. This finding, together with the observed drop in sTie-2 receptor level after a curative or palliative resection, strongly suggests that the main source of sTie-2 receptors is the tumour and that the serum level reflects tumour load. The magnitude of drop may be useful as a marker of resection of primary tumours with adequate oncological clearance. Patients who had residual metastatic disease also demonstrated a significant drop in the levels of sTie-2 receptors after surgery. Similarly, in patients who have undergone curative resections, their sVEGFR-1 activities return to normal levels. This is not so in patients with metastatic disease who have persistently suppressed sVEGFR-1 levels.

The observed correlation between the two soluble receptors suggests that both biomarkers, despite not interacting directly, have inter-related functions through two distinct pathways which are both essential for normal development of "vascular trees". This preliminary study indicated the potential use of soluble receptors as tumour markers, which will be particularly useful in monitoring the response of anti-angiogenic gene therapy in clinical practice. However, for the soluble receptor to be a useful tumour marker, a further prospective study is needed to investigate their prognostic value in conjunction with VEGF.

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References


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