Abstract. Background: Hypoxia-inducible factor-1 (HIF-1) is the key regulator of cellular responses to hypoxia and plays a central role in tumour growth. Recently, two single nucleotide polymorphisms (SNPs) in the HIF-1α gene, C1772T and G1790A, were shown to cause significantly higher transcriptional activity than did the wild-type. This study aimed to investigate the effect of these SNPs on the prognosis of colorectal cancer (CRC). Patients and Methods: DNA from 336 CRC patients was genotyped. Genotypes of each polymorphism were tested for association with disease-free survival (DFS) using univariate and multivariate Cox-regression analysis. Results: Genotype frequencies were: CC 75.6%, CT 18.8% and TT 1.8% for HIF1A1 C1772T and GG 93.2%, GA 2.7% and AA 0% for G1790A. A statistically significant association between DFS and clinicopathological features was observed. However, no association was found between HIF1A1 C1772T (p=0.44; risk ratio of recurrence, RR=1.19, 95% confidence interval, CI=0.77 to 1.83) and G1790A (p=0.89; RR=0.92, 95% CI=0.29 to 2.90) polymorphisms and DFS in univariate and multivariate Cox-regression analysis. Conclusion: These results suggest that HIF1A1 C1772T and G1790A polymorphisms are not involved in the progression or metastasis of CRC.

Significant progress has been made in recent years towards reducing the worldwide incidence and mortality rates of patients with colorectal cancer (CRC). However, CRC still remains one of the most common malignancies with one million cases worldwide in 2002 (1-3). Understanding the development and progression of CRC is crucial for successful therapeutic interventions. By better knowledge of its underlying mechanisms, powerful prognostic and predictive markers might be identified, allowing optimisation of treatment modalities. Genetic polymorphisms are responsible for inter-individual variation and diversity, and have been recently considered as the main genetic elements involved in the development and progression of cancer (4). A number of polymorphisms have been identified within the hypoxia-inducible factor (HIF)-1α gene (5). HIF-1 is a key regulator of cellular response to hypoxia and plays an important role in tumour growth by trans-activating various genes that are related to regulation of angiogenesis, energy metabolism, cell survival and apoptotic and proliferative responses (6-9). HIF-1 is a heterodimeric transcription factor composed of α and β subunits, both of which are members of the basic-helix-loop-helix-Per-Arnt-Sim (PAS) family of proteins (10). HIF-1α is the oxygen-regulated component and determines HIF-1 activity, while HIF-1β is expressed constitutively (11). Under normoxic conditions, HIF-1α protein is hydroxylated within the oxygen-dependent degeneration domain (ODDD) and degraded via the ubiquitin-proteasome degradation system (12-15). This process is mediated by the von Hippel-Lindau tumour suppressor protein (pVHL), which predominantly targets the minimal N-terminal trans-activation domain (N-TAD) within the ODDD (16, 17). In contrast, in a hypoxic microenvironment, degradation of HIF-1α is suppressed and it is rapidly accumulated in the cell (18). The gene for the HIF-1α subunit carries two common missense mutations, P582S (C1772T, rs11549465) and A588T (G1790A, rs11549467), which both have been related to an increased trans-activation capacity of HIF-1α (19). Recent studies suggest that HIF1A1 polymorphisms may be related to the risk and/or prognosis of several cancer types (20-24).
CRC, these polymorphisms have been associated with the development of ulcerative tumours (25). To the best of the Authors’ knowledge, no data about the prognostic influence of HIF1A1 polymorphisms on CRC have been reported. This study was designed to elucidate the possible effect of HIF1A1 C1772T and G1790A polymorphisms on disease-free survival (DFS) of patients with CRC.

Patients and Methods

Patients. Between January 2008 and June 2009, a total of 381 blood samples from patients with CRC were collected at the Division of Oncology, Department of Internal Medicine, Medical University of Graz, Austria. All participants’ tumours were verified histologically with specimens obtained from endoscopic biopsy or surgical resection. In the study collective, CRC was diagnosed between May 1993 and September 2008. Forty-five patients were excluded from the study because of secondary tumours. Complete clinical and tumour biological details, therapy data, CRC relapse information and genotyping results were available for 317 patients (Table I). The study was performed according to the Austrian Gene Technology Act and has been approved by the Ethics Committee of the Medical University of Graz. Written informed consent was obtained from all participants; all were Caucasian.

Isolation of genomic DNA and determination of single nucleotide polymorphisms (SNPs). Anticoagulated EDTA blood from 336 CRC patients was stored at –20˚C. Genomic DNA was isolated by a fully automated procedure on a GeneMole instrument (Mole AS, Lysaker, Norway). HIF1A1 genotypes were determined by a 5’-exonuclease assay (TaqMan, Applied Biosystems, Applera, Austria). Primer and probe sets were designed and manufactured using Applied Biosystems ‘Assay-by-Design’ custom service. For analysis of the HIF1A1 P582S polymorphism, sequences of primers and probes were: forward primer TTCCAGTTACGTTCCCTCTAGCAG, reverse primer CTTTGAGGACTTGCGCTTTCAG, 582P probe VIC-ACTGCTTTCTAATGTTTCA-NFQ, and 582S probe FAM-ACTGCTTTCTAATGATTTCA-NFQ. The A588T polymorphism was analysed using: forward primer 588F GTTTAGGACCTGCTGCTTTCAG, reverse primer 588R CTTTGAGGACTTGCGCTTTTCAG, 588A probe VIC-CAGTTCCGCAAGCC-NFQ, and 588T probe FAM-CAGTTCCGCAAGCC-NFQ. Fluorescence was measured in a Lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Penzberg, Germany) using excitation/emission filters of 485/530 nm for FAM-labelled probes and 530/572 nm for VIC-labelled probes. The data were exported into Excel format and depicted and analysed as a scatter plot. Samples were analysed in batches, each containing 95 samples and a negative control (water instead of DNA). Fifty samples were reanalysed and the results were identical for all samples. Genotypes were determined successfully in 322 patients.

Statistical analysis. The primary outcome was DFS. The endpoints included local cancer recurrence and/or metastasis. Follow-up information was available for all patients at the 5-year time point. The genotypes were coded assuming an allele dose-effect (wild-type=0, heterozygous carrier of the mutated allele=1, homozygous carrier of the mutated allele=2). DFS curves were generated by the Kaplan-Meier method and verified by the log-rank test. Cox’s proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. SNPs were re-evaluated in a model adjusted for known CRC prognostic factors, which included age at diagnosis, Karnofsky index, tumour size, histological grade, number of lymph nodes evaluated after resection, number of metastatic lymph nodes, clinical stage (according to the AJCC TNM classification) and application of 5-fluorouracil-based chemotherapy. Differences were considered significant when a p-value <0.05 was obtained. All analyses were performed using the SPSS 14.0 statistical software package (SPSS Inc., Sunnyvale, USA).

Results

Frequency data for clinical and tumour biological factors and therapy modalities are described in Table I. Mean follow-up time was 41.7 months (median 31, range 3-208 months) and
mean DFS was 30.9 months (median 20, range 0-200 month). The genotype frequencies among patients were: C/C 75.6% (wild-type), C/T 18.8% and T/T 1.8% for \textit{HIF1A1} \textit{C1772T} and G/G 93.2% (wild-type), G/A 2.7% and A/A 0% for \textit{HIF1A1} \textit{G1790A}. These frequencies did not deviate from the Hardy-Weinberg disequilibrium. The C/T heterozygous and T/T homozygous, and the G/A heterozygous and A/A homozygous genotypes were combined due to the small number of the T/T and A/A genotypes. In this study population, a statistically significant association was observed between DFS and Karnofsky index, tumour size, clinical stage, number of metastatic lymph nodes and application of 5-FU-based chemotherapy (Table II). However, no association was found between \textit{HIF1A1} \textit{C1772T} (p=0.44, risk ratio of recurrence [RR]=1.19, 95% confidence interval [CI]=0.77 to 1.83) and \textit{HIF-1α} \textit{G1790A} (p=0.89, RR=0.92, 95% CI=0.29 to 2.90) polymorphisms and DFS in univariate and multivariate Cox regression analysis including known CRC prognostic factors (Figures 1 and 2).

**Table II. Univariate Cox’s model (disease-free survival).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1772T</td>
<td>1.185</td>
<td>0.768-1.827</td>
<td>0.443</td>
</tr>
<tr>
<td>G1790A</td>
<td>0.921</td>
<td>0.292-2.901</td>
<td>0.888</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>0.997</td>
<td>0.979-1.014</td>
<td>0.707</td>
</tr>
<tr>
<td>pT</td>
<td>2.089</td>
<td>1.495-2.918</td>
<td>0.001</td>
</tr>
<tr>
<td>Evaluated N</td>
<td>0.991</td>
<td>0.971-1.012</td>
<td>0.414</td>
</tr>
<tr>
<td>Positive N</td>
<td>1.069</td>
<td>1.031-1.108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histological grading</td>
<td>1.265</td>
<td>0.876-1.825</td>
<td>0.210</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>5.030</td>
<td>3.543-7.140</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5-FU-based CTX</td>
<td>0.279</td>
<td>0.159-0.489</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Karnofsky-Index</td>
<td>0.970</td>
<td>0.0951-0.989</td>
<td>0.002</td>
</tr>
</tbody>
</table>

RR: Relative risk; CI: confidence interval; pT: primary tumour size according to AJCC-TNM system; evaluated N: number of lymph nodes evaluated after resection; positive N: number of metastatic lymph nodes; 5-FU-based CTX: 5-fluorouracil-based chemotherapy.

**Discussion**

This study assessed whether \textit{HIF1A1} polymorphisms are associated with DFS in colorectal cancer. A significant association was observed between DFS and known prognostic factors, however no association was found for the \textit{HIF1A1} SNPs. Both \textit{HIF1A1} polymorphisms showed significantly higher transcription activity under normoxic and hypoxic conditions. The mechanisms by which these SNPs promote activation of \textit{HIF1A1} transcription, even under normoxia, are still unclear, but since HIF-1α is activated by a multiple-step pathway, it is possible to hypothesize as to several mechanisms of the increased transactivation. Because of the location of the substituted
amino acids within or near the N-TAD and the interaction with the pVHL, the alteration of protein stability of the variant proteins may be one of the possible mechanisms for the enhancement of transactivation capacity (20). Furthermore, recruitment of transcriptional co-factors such as CBP/p300 and SRC-1 that interact with HIF-1α may be enhanced by the variant forms via conformational changes caused by amino acid substitution (26). Both possibilities imply an important role for HIF1A1 polymorphisms in generating individually different tumour progression. In 2003, Tanimoto et al. found that HIF1A1 polymorphisms show an increased number of microvessels and a higher disease stage among head and neck squamous cell carcinoma patients (20). Recently, Li et al. reported an association of the HIF1A1 G1790A polymorphism with gastric cancer in Tibetans (24). Moreover, both HIF1A1 polymorphic variants may confer susceptibility to renal cell carcinoma (21). In CRC, Fransen et al. found that patients carrying one or more polymorphic alleles in either the HIF1A1 C1772T or the G1790A polymorphisms display a significantly increased risk for the development of ulcerative CRCs (25). Furthermore, Kuwai et al. reported a significant difference in the HIF1A1 C1772T genotype distribution between CRC patients and healthy controls (27). In contrast, no risk association was found for oesophageal squamous cell carcinoma, prostate and breast cancer (28-30). Studies evaluating the prognostic significance of HIF1A1 polymorphisms are few, however both these polymorphisms may have a significant influence on poor prognosis of patients undergoing radical cystectomy for bladder cancer and G1790A may be a marker of unfavorable prognosis in early stages of oral cancer (31, 32). The variable results of these studies do not necessarily contradict the findings presented here, as the aetiology and progression of malignant diseases and CRC in particular is multifarious. A limitation of this study is its retrospective design, whereby a survival bias cannot be excluded. The strengths of the study are its relatively high number of participants, as well as the clinically validated phenotypes. Prospective studies are needed to learn more about the role of HIF1A1 polymorphisms in cancer. This would lead to a better understanding of tumour biology and behaviour and would possibly clarify the inconsistency of data found in recent studies.

References


