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Urologic Oncology: Seminars and Original Investigations ▮ (2017) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Original article

Hypoxia and renal cell carcinoma: The influence of *HIF1A + 1772C/T* functional genetic polymorphism on prognosis

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Received 25 February 2017; received in revised form 30 March 2017; accepted 3 April 2017

Abstract

Objectives: Hypoxia-inducible factor (HIF-1) is a key regulator of the genes involved in the cellular response to hypoxia. Overexpression of HIF-1 has been implicated in the pathogenesis of renal cell carcinoma (RCC), and functional polymorphisms of the *HIF1A* gene may confer susceptibility to RCC. Our purpose was to assess the influence of *HIF1A + 1772C/T* (rs11549465) polymorphism on RCC prognosis.

Material and methods: This study evaluated the associations of the *HIF1A + 1772C/T* (rs11549465) polymorphism with clinicopathologic prognostic factors, recurrence/progression, and survival in a cohort of 179 patients with RCC treated at Portuguese Oncology Institute of Porto. Genotyping analysis, using DNA extracted from peripheral blood, was performed by real-time polymerase chain reaction allelic discrimination. The genotype associations with clinicopathologic parameters and recurrence/progression were analyzed by the chi-square or Fisher tests. Genotypes influencing cancer-specific survival were compared using Cox proportional hazard regression, Kaplan-Meier curves, and Breslow test.

Results: None of the genotypes (CC, CT, or TT) were significantly associated with clinicopathologic prognostic factors. The TT genotype and T allele were associated with recurrence/progression ($P = 0.042$ and $P = 0.02$, respectively). Patients with CT and CT + TT genotypes tend to have an increased risk to RCC-related death (hazard ratio = 2.79; 95% CI: 0.88–8.82; $P = 0.08$ and hazard ratio = 2.76; 95% CI: 0.93–8.22; $P = 0.07$, respectively) and showed worse cancer-specific survival curves than those with the CC genotype ($P = 0.012$ and $P = 0.018$, respectively).

Conclusions: These results suggest that *HIF1A + 1772C/T* (rs11549465) polymorphism may have effects on RCC recurrence/progression and survival.

Keywords: Hypoxia-inducible factor; Renal cell carcinoma; Single nucleotide polymorphism; Prognosis

1. Introduction

Renal cell carcinoma (RCC) accounts for 3.8% of all adult cancers. It is the most common cancer of the kidney and the most lethal urologic cancer [1,2].

The natural history of RCC varies widely. However, up to 30% of patients with RCC present with metastatic disease at diagnosis, and it is estimated that 20% to 40% of patients, treated with nephrectomy for a localized tumor, will develop metastases [3]. Actually, the main prognostic factors are *performance status* (PS), tumor histological subtype, tumor grade, TNM stage, sarcomatoid or rhabdoid differentiation or both, tumor necrosis, and microvascular invasion. Other pathological (tumor size, number of metastatic sites, and nonpulmonary metastases) and laboratory (low serum hemoglobin level, elevated corrected serum calcium level, and elevated serum lactase dehydrogenase level) factors have been associated with poor prognosis in RCC [4–7]. The variation in RCC's clinical course is

This study was funded by an educational grant for basic research from AstraZeneca Foundation 2010, Portugal.

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http://dx.doi.org/10.1016/j.urolonc.2017.04.002
1078-1439/

unpredictable, which has led to the development of different prognostic models for the assessment of the patient's individual risk in localized or metastatic disease [4–7]. Recently, molecular markers are being investigated to be included in the established models that already include histological and clinical parameters, which may lead to additional benefits in distinguishing between poor and favorable risk patients with RCC.

Similarly, with other solid cancer, RCC is frequently characterized by hypoxic conditions due to local imbalance between oxygen supply and consumption. Indeed, hypoxia and compensatory hyperactivation of angiogenesis are thought to be particularly important in RCC compared to other tumor types, given the highly vascularized nature of kidney tumors and the specific association of inactivation of von Hippel-Lindau (*VHL*) gene with RCC carcinogenesis. The *VHL* tumor suppressor gene is inactivated in up to 80% of sporadic cases of clear cell carcinoma (the predominant histological subtype of RCC) by deletion, mutation, or methylation [8–10]. The VHL protein serves as recognition site of ubiquitin ligase complex, targeting the hypoxia-inducible factor 1 (HIF-1) for degradation in normoxic conditions [11].

HIF is a heterodimeric transcription factor consisting of α and β subunits that sense the oxygen tension within the cell, which regulates the expression of genes involved in the cellular response to hypoxia, including the vascular endothelial growth factor, the transforming growth factor α, or the glucose transporter type 1 genes [12]. The α subunit is constitutively hydroxylated in 2 proline residues under normoxic conditions, which allows binding and subsequent ubiquitin-mediated degradation by the VHL protein, limiting the transcription of HIF-dependent gene targets necessary for angiogenesis. Under hypoxia or VHL dysfunction, the α subunit is stabilized, resulting in an overaccumulation of HIF heterodimer and increased transcription of a number of downstream genes involved in crucial pathways related to tumorigenesis including angiogenesis, invasion, cell metabolism, and cell growth [13,14]. This HIF activation, promoter of a hypoxia state, leads to cancer cell changes that contribute to an aggressive tumor behavior, tumor progression, and treatment resistance [15].

Overexpression of HIF-1 has been implicated in several human cancers, such as head-neck, colon, breast, stomach, pancreas, prostate, esophagus, endometrial, non–small cell lung cancer and RCC [16,17]. Studies have shown that *HIF1A* functional polymorphisms in the oxygen-dependent degradation domain of HIF-1α may affect the oxygen regulation of the protein via hydroxylation by HIF-α prolyl hydroxylases and the stabilization and transactivation functions of this transcription factor [16]. A functional polymorphism in the *HIF1A* gene characterized by a C > T (rs11549465) transition in exon 12, locus +1772, results in an amino acid change from a proline to serine at codon 582. Furthermore, functional studies showed that *HIF1A + 1772C/T* (rs11549465) polymorphism causes significant increase in transcriptional activity of HIF-1 under normoxic and hypoxic conditions and is associated with increased tumor microvessel density [18,19].

Several studies investigated the possible association between the *HIF1A + 1772C/T* (rs11549465) polymorphism and the cancer risk, including susceptibility, with controversial results. Moreover, recent meta-analysis showed that this polymorphism is significantly associated with overall cancer risk [20]. However, the influence of *HIF1A + 1772C/T* (rs11549465) polymorphism in RCC development and progression must be clarified. Our purpose was to assess the effect of *HIF1A + 1772C/T* (rs11549465) functional polymorphism on RCC prognosis and its association with the clinicopathologic prognostic factors, tumor recurrence/progression, and survival in patients with RCC.

2. Material and methods

2.1. Patients

This retrospective study evaluated *HIF1A + 1772C/T* (rs11549465) single nucleotide polymorphism (SNP) in a cohort of 179 native Portuguese patients with histopathologically confirmed sporadic RCC treated at Portuguese Oncology Institute of Porto (IPO-Porto), from January 1999 to March 2009. The patients with other cancers before the diagnosis of RCC were excluded.

Clinicopathologic- and follow-up data were obtained from medical records. The clinical- and pathologic characteristics of the 179 patients with RCC are presented in Table 1. The patients included 120 (67%) men and 59 (33%) women, with a median age of 62 years at diagnosis. All patients were staged according to the TNM staging system of American Joint Committee on Cancer 2006, and tumors were graded according to the Fuhrman nuclear grading system.

All patients were treated with radical or partial nephrectomy (97.8% curative, 1.7% cytoreduction, and 0.6% palliative surgery) and 2 patients also underwent metastasectomy (1 synchronous and other metachronous). Only 5 patients after nephrectomy received adjuvant interferon α. Palliative treatment with interferon α was offered to 3 patients after cytoreduction nephrectomy, and 1 patient after nephrectomy and metachronous metastasectomy continued the palliative treatment with interferon α. The patient submitted to palliative nephrectomy continued palliative treatment with sunitinib that was previously started.

After nephrectomy, patients were followed up at the discretion of physician by chest x-rays and abdominopelvic CT scans or ultrasound every 6 months or annually for a minimum period of 5 years.

The study was conducted according to the principles of the Helsinki Declaration and was approved by the ethics committee of IPO-Porto. Written informed consent was obtained from all individuals to participate in molecular