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TP53, *EGFR*, and *KRAS* mutations in relation to *VHL* inactivation and lifestyle risk factors in renal-cell carcinoma from central and eastern Europe

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ABSTRACT

Renal-cell carcinomas (RCC) are frequent in central and eastern Europe and the reasons remain unclear. Molecular mechanisms, except for *VHL*, have not been much investigated. We analysed 361 RCCs (334 clear-cell carcinomas) from a multi-centre case-control study for mutations in *TP53* (exons 5–9 in the whole series and exons 4 and 10 in a pilot subset of 60 tumours) and a pilot 50 tumours for mutations in *EGFR* (exons 18–21) or *KRAS* (codon 12) in relation to *VHL* status. *TP53* mutations were detected in 4% of clear-cell cases, independently of *VHL* mutations. In non-clear-cell carcinomas, they were detected in 11% of *VHL*-wild-type tumours and in 0% of tumours with *VHL* functional mutations. No mutations were found in *EGFR* or *KRAS*. We conclude that mutations in *TP53*, *KRAS*, or *EGFR* are not major contributors to the RCC development even in the absence of *VHL* inactivation. The prevalence of *TP53* mutations in relation to *VHL* status may differ between clear-cell and other renal carcinomas.

1. Introduction

Renal-cell carcinoma (RCC) is the 14th most common cancer in the world in terms of incidence. It is more common in developed countries and its incidence has been increasing. Incidence rates of RCC in central and eastern Europe are among the highest in the world [1], and the reasons for this phenomenon remain unclear. The known risk

Abbreviations: RCC, renal-cell carcinoma; ccRCC, clear-cell renal carcinoma; OR, odds ratio; CI, confidence interval; *VHL*, Von Hippel-Lindau.

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factors for sporadic RCC are tobacco smoking, obesity, and the history of hypertension, all of which are only moderately associated with the disease [2–5].

The most common molecular event in renal carcinogenesis is inactivation of the *VHL* (Von Hippel-Lindau) gene, a tumour suppressor that is mutated in the germline of 99% of patients suffering from the Von Hippel-Lindau disease, a familial syndrome resulting in multiple tumours, including renal cancers [6], by mutations or promoter hypermethylation. *VHL* encodes a protein which regulates the expression of genes involved in angiogenesis and/or in the response to hypoxic and oxidative stress, such as *VEGF* and, especially. Hypoxia Inducible Factor 1-alpha. However, little is known about other genetic factors involved in the progression of RCC and their possible associations with the *VHL* status. In particular, data are scarce on the involvement of three genes that are often mutated in many other types of human cancer: *TP53*, *KRAS* and *EGFR*.

TP53 encodes the p53 tumour suppressor protein, which plays multiple roles in cell-cycle control, apoptosis and DNA repair in response to various types of stress. The IARC TP53 mutation database, which compiles *TP53* mutations reported in the scientific literature, records 68 *TP53* mutations found in 499 renal-cell carcinoma samples analysed (14%) [7], the mutation prevalence being relatively low as compared to many other human cancers. A variable prevalence of p53 protein over-expression has been shown in renal-cell carcinoma by a few studies [8,9]. Conflicting reports exist as to whether p53 overexpression is associated with the presence of inactivated *VHL* in renal cancer cell-lines [10,11]. The *KRAS* gene codes for a transducer of signals generated by tyrosine kinase growth factor receptors. Its status in renal cancers has not been extensively studied but several reports have shown that the promoter of RASSF1, a regulator of RAS biochemical activities, is often hypermethylated and downregulated in renal cancers [12], which may suggest that RAS inactivation is an important event in renal carcinogenesis. Finally, *EGFR*, encoding the Epidermal Growth Factor Receptor, is mutated in a wide range of epithelial cancers, in particular adenocarcinoma of the lung in never-smokers [13]. However, in the only study to have investigated the mutational status of *EGFR* in renal-cell carcinomas, no mutations were found in 19 patients from a Japanese population [14]. Of note, many studies on molecular mechanisms leading to renal cancer did not include only histologically confirmed clear-cell cases versus other RCC types, which could have hindered the detection of type-specific associations.

Here we have analysed *TP53* mutations (exons 4–10) in a series of 361 renal-cell carcinomas collected in the context of a case-control study in central and eastern Europe (exons 5–9 in the whole series and exons 4 and 10 in a pilot subset of 60 samples), the largest series ever screened for *TP53* mutations. In addition, we have performed a pilot analysis of *EGFR* mutations (exons 18–21) and *KRAS* mutations (codon 12) in 50 cases to determine whether these genes that are important in the carcinogenesis leading to many types of cancer are also involved in the development of renal-cell carcinoma.

2. Materials and methods

2.1. Patients and tumour specimens

A hospital-based case-control study of kidney cancer was conducted in seven centres of central and eastern Europe: Moscow (Russia), Bucharest (Romania), Łódź (Poland), Prague, Olomouc, Ceske Budejovice, and Brno (Czech Republic). Cases were recruited among kidney cancer patients (ICD-O code: C64) newly diagnosed at one of the participating hospitals. All cases were histologically confirmed by two independent expert pathologists (a local pathologist of each centre and a study pathologist) and a detailed histological diagnosis as well as the tumour stage according to the TNM cancer staging system were annotated. Controls were recruited among patients of the same hospitals as the cases, admitted for conditions unrelated to smoking or genitourinary disorders (except for benign prostatic hyperplasia), matched on age, sex, and the referral area. Both cases and controls had to be between 20 and 79 years old and residents of the study area for at least one year. No single disease made up more than 4% of the control group. The most common diagnoses were varicose veins of lower extremities (ICD-10 code: I83; 3.3%) and diseases of biliary tract NOS (ICD-10: K83; 3.0%).

A total of 1097 cancer cases and 1555 controls were recruited between August 1999 and January 2003. The response rates ranged from 90% to 99% for cases and from 90% to 96% for controls. All study subjects and their physicians provided written informed consent, and the study was approved by ethical committees in all participating centres. Each individual answered a standardised lifestyle and food frequency questionnaire that was administered face-to-face, in hospital, by a trained interviewer. Cases were interviewed within three months of diagnosis. Blood samples were collected from cases and controls, and fresh tumour pathological samples (frozen) from cases whenever possible.

2.2. DNA extraction, mutation and serology analysis

DNA was extracted from fresh (frozen) tumour tissue samples, following a pathological review and a manual macrodissection to remove non-tumour tissue. Only tissue areas that appeared to contain at least 70% tumour cells were used for DNA extraction. For each sample, 5 mm^3 of tissue was sectioned and digested with 0.4 μg Proteinase K per μl of digestion buffer (500 mM KCl, 100 mM Tris–HCl, 15 mM $MgCl_2$, 0.5% Tween 20) at 50 °C overnight. A standard protocol (http://cc.ucsf.edu/people/waldman/Protocols) was used to extract DNA from the digested samples. DNA was quantified using a ND-1000 spectrophotometer (Nanodrop, Wilmington, DE).

Three hundred and sixty one renal-cell carcinoma tumours from the initial tumour set which had the DNA extracted at the time the study was planned were screened for mutations in exons 5–9 of the *TP53* gene by Denaturing High Performance Liquid Chromatography (DHPLC) and sequencing as described elsewhere [15]. Briefly, PCR products were pre-screened for the presence of a mutation by