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Identification of copy number alterations and its association with pathological features in clear cell and papillary RCC

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Abstract

We report and characterize the copy number alterations (CNAs) in 35 clear cell and 12 papillary renal cell carcinomas (RCC) using Affymetrix 100K SNP arrays. Novel gain and loss regions are identified in both subtypes. In addition, statistically significant CNA are detected and associated with the pathological features: *VHL* mutation status, tumor grades, and sarcomatoid component in clear cell RCC and in types 1 and 2 of papillary RCC. Florescence *in situ* hybridization confirmed the copy number gain in the transforming growth factor, beta-induced gene (*TGFBI*), which is a possible oncogene for clear cell RCC.

Keywords: Clear cell RCC; Papillary RCC; Copy number alterations; Pathological features; Transforming growth factor; Beta-induced gene

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1. Introduction

Renal cell carcinoma (RCC) is the most common malignancy of the adult kidney. In the United States, approximately 54,000 new cases of RCC are diagnosed and approximately 13,000 deaths are attributed to this disease each year [1]. RCC is a heterogeneous disease. It has several morphological subtypes, namely clear cell (80%), papillary (12%), chromophobe (5%), collecting duct (<1%), and unclassified, all of which are malignant tumors. Papillary RCC is further divided into type 1 and type 2. A sarcomatoid component can be identified in all RCC subtypes and serves as an indicator of poor outcome. The von Hippel-Lindau gene (*VHL*) mutation in clear cell RCC can be associated with good prognosis. Each RCC subtype has a unique clinical feature set and is believed to be regulated by a different mechanism. Therefore, ideally, different therapeutic strategies and targets should be selected for each specific RCC subtype for effective treatment, according to their clinical features.

Genomic DNA copy number alterations (CNAs) have been known to contribute to the development and progression of human cancer. Chromosomal losses and gains in RCC have been reported in a number of studies using cytogenetic, microsatellite, loss-of-heterozygosity, and comparative genomic hybridization (CGH) approaches. Recently, high-density and high-throughput single nucleotide polymorphism (SNP) microarrays showed newly identified gain and loss regions in clear cell RCC [2]. However, the association of CNA with the clinicopathological features of RCC remains unknown. In this study, we used the Affymetrix 100K SNP mapping array to detect the genomic changes in 35 clear cell and 12 papillary RCC. Our main objective is to identify the CNA regions and relate them to different pathological features in RCC.

2. Materials and methods

2.1. RCC samples

Tissue samples of 35 clear cell RCC, 12 papillary RCC, and 7 normal specimens as controls were obtained from the Spectrum Health Hospital of Grand Rapids, Michigan, the French Kidney Cancer Study Group, and the Cooperative Human Tissue Network (CHTN). The tumor specimens were snap-frozen in liquid nitrogen immediately after nephrectomy and stored at −80 °C. Genomic DNA was isolated using a Jetquick tissue DNA maxi spin kit (Genomed, Lohne, Germany) according to the manufacturer's protocol. DNA concentration was measured with the Nanodrop model ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Informed consent of all patients was obtained, and this study was approved by the Institutional Review Board of the Van Andel Institute. Clinicopathological data for the patients is listed in Table 1.

2.2. Affymetrix 100K SNP array

The Affymetrix GeneChip Mapping 100K array (Affymetrix, Santa Clara, CA, USA) includes 116,204 SNPs in two chips, 50K Xba240 and 50K Hind240. The SNP mapping assay was performed according to the manufacturer's instructions. Briefly, 250 ng of genomic DNA was digested with either XbaI or HindIII and then ligated to XbaI or HindIII adaptors. Adaptor-ligated restriction fragments were amplified in the 250- to 2000-bp size range by PCR with a thermocycler (MJ Research, Watertown, MA, USA) and purified with the MinElute 96 UF PCR purification kit (Qiagen, Valencia, CA, USA). The amplicons were then quantified, fragmented, labeled, and subsequently hybridized to either an Xba240 or Hind240 array for 16 h. After hybridization, the arrays were washed and stained in the Affymetrix GeneChip fluidics station 450 and scanned with a GeneChip Scanner 3000

Table 1
Clinicopathological features of 35 clear cell and 12 papillary RCC

	Clear cell RCC	Papillary RCC
Gender (male/female)	14/21	8/4
Age (year, mean ± SD)	63.0 ± 13.1	62.0 ± 13.9
Pathological grade		
1	3	0
2	10	7
3	13	3
4	9	2
VHL mutation		
+	18	NA
−	17	NA
Sarcomatoid component		
+	7	1
−	28	11
Papillary type		
Type1	NA	6
Type2	NA	6

Abbreviations: NA, not applicable; SD, standard deviation.