# Next-generation sequencing to detect deletion of *RB1* and *ERBB4* genes in chromophobe renal cell carcinoma: A potential role in distinguishing chromophobe renal cell carcinoma from renal oncocytoma

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**ABSTRACT**

Overlapping morphological, immunohistochemical, and ultrastructural features make it difficult to diagnose chromophobe renal cell carcinoma (ChRCC) and renal oncocytoma (RO). Since ChRCC is a malignant tumor, whereas RO is a tumor with benign behavior, it is important to distinguish these two entities. We aimed to identify genetic markers that distinguish ChRCC from RO by using next-generation sequencing (NGS). NGS for hotspot mutations or gene copy number changes was performed on 12 renal neoplasms including seven ChRCC and five RO cases. Matched normal tissues from the same patients were used to exclude germline variants. Rare hotspot mutations were found in in cancer-critical genes (*TP53*, *PIK3CA*) in ChRCC but not RO. The NGS gene copy number analysis revealed multiple abnormalities. The two most common deletions were tumor suppressor genes *RB1* and *ERBB4* in ChRCC but not RO. Fluorescence *in situ* hybridization was performed on 65 cases (ChRCC, n=33; RO, n=32) to verify hemizygous deletion of *RB1* (17/33, 52%) or *ERBB4* (11/33, 33%) in ChRCC, but not in RO (0/32, 0%). In total, ChRCCs (23/33, 70%) carry either a hemizygous deletion of *RB1* or *ERBB4*. The combined use of *RB1* and *ERBB4* fluorescence *in situ* hybridization to detect deletion of these genes may offer a highly sensitive and specific assay to distinguish ChRCC from RO.

## INTRODUCTION

Both chromophobe renal cell carcinoma (ChRCC) and renal oncocytoma (RO) are tumors that originate from the intercalated cells of the collecting system. Microscopically, ChRCC contains polygonal cells with cytoplasm ranging from pale to eosinophilic and display nested, alveolar, or sheet-like growth patterns. RO shares many histologic features with ChRCC. Histologically, RO is composed of uniform round/polygonal tumor cells with abundant eosinophilic, granular cytoplasm and small round nuclei, arranged in nests, solid, and/or tubular patterns.[1, 2] Atypical features have been reported in RO despite the benign behavior associated with this tumor, and include chromophobe histology, significant nuclear atypia (bizarre cells with high nuclear:cytoplasm ratio and hyperchromatic nuclei), invasion into the renal sinus and perirenal fat and vessels, as well as rare mitoses and focal areas of necrosis.[1] These morphologic features may generate diagnostic dilemmas. In routine clinical practice, special and immunohistochemical stains are commonly used to distinguish ChRCC from RO, and include Hale's colloidal iron and CK7.[3] These markers are usually diffusely and strongly positive in ChRCC, whereas typically negative or show scattered positivity in RO. However, subsets of ChRCC are reported to be negative for these markers, whereas variable degree of positive staining has also been documented in RO. Therefore, the results of immunohistochemical studies are not always conclusive.[3-6]

It is well established that ChRCC and RO have different patterns of chromosomal anomalies, including copy number gains and losses at distinct chromosomal loci.[7-10] Specifically, ChRCC are frequently associated with gains in chromosomes 4, 7, 11, 12, 14q, and 18q, and losses in chromosome Y, 1, 2, 6, 10, 13, 17, and 21. Among the latter, losses of chromosomes 2, 6, 10, and 17 seem to be specific for ChRCC.[9, 11] RO occasionally exhibits losses of chromosomes 1 and Y and/or balanced translocation of the 11q13 breakpoint region.[12, 13]

Common losses of chromosomes in both ChRCC and RO suggest that there is a close relationship between ChRCC and RO.[14-16] The chromosomal anomalies are usually identified by conventional cytogenetic analysis, together with other approaches such as comparative genomic hybridization and restriction fragment length polymorphism analysis. [17-19] Here, next-generation sequencing (NGS) was used to distinguish these two entities from each other and fluorescence *in situ* hybridization (FISH) to confirm these findings.

## MATERIALS AND METHODS

Sixty-five cases (ChRCC, n=33; RO, n=32) were retrieved from the surgical pathology files of the Departments of Pathology at the University of Massachusetts Medical School, Worcester, MA and at the Indiana University School of Medicine, Indianapolis, IN. Relevant clinical records were reviewed for clinical presentation, tumor size, stage, treatment, and clinical outcome. Representative hematoxylin and eosin (H&E)-stained sections were reviewed to confirm the diagnoses by two genitourinary pathologists. At the University of Massachusetts Medical School, ChRCC cases (N=11) were selected between 1995 and 2010 and were comprised of three female and eight male patients, with ages ranging from 28 to 75 years (mean age, 50.8 years) with all localized tumors (T1, n=1; T2, n=5; T3, n=5); RO cases (N=10) were selected between 1999 and 2009 and included two female and eight male patients with ages ranging between 41 to 84 years (mean age, 64.7 years). At the Indiana University School of Medicine, ChRCC cases (N=22) were selected between 2015 and 2017 and were comprised of 12 female and 10 male patients, with ages ranging from 31 to 81 years (mean age, 58.3 years) with all localized tumors (T1, n=12; T3, n=10); RO cases (N=22) were selected between 2012 and 2017 and included six female and 16 male patients with ages ranging between 41 to 87 years (mean age, 66.1 years).

***Targeted mutational sequencing and copy number variation analysis by NGS***

Tumor and matched normal tissue from the same patient was identified on an H&E–stained slide, then macrodissected from sequential sections, typically three to five slides of formalin-fixed, paraffin-embedded (FFPE) tissue at 7 micron thickness. DNA was purified using the QIAamp DNA FFPE Tissue kit (Qiagen, Valencia, CA) then quantified by spectrometry (NanoDrop 1000 DNA/RNA Calculator, Thermo Scientific, Wilmington, DE) and fluorometry (Qubit DNA High-Sensitivity Assay Kit, Thermo Scientific). Pooling with short known nucleotides known as barcodes could be adapted to the amplicons with the IonXpress Barcode Kit (LIFE Technologies) if multiplexing of samples was desired.

Next-generation mutation sequencing and gene copy number analysis was performed as previously described.[20] Briefly, AmpliSeq Cancer Hotspot Panel v2 libraries were generated from genomic DNA (10 ng) using the Ion AmpliSeq Library Kit 2.0 (Thermo Scientific) and quantified using the Ion Library Quantification KIT (Thermo Scientific) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, CA). Genes in this panel are *AKT1, ALK, APC, ATM, ABL1, BRAF, FGFR1, GNAS, IDH1, FGFR2, KRAS, NRAS, PIK3CA, MET, RET, EGFR, JAK2, MPL, PDGFRA, PTEN, TP53, FGFR3, FLT3, KIT, ERBB2, EZH2, IDH2, GNA11, GNAQ, HNF1A, HRAS, RB1, CDH1, SMAD4, STK11, SRC, SMARCB1, VHL, MLH1, CTNNB1, KDR, FBXW7, CSF1R, NPM1, SMO, ERBB4, CDKN2A, NOTCH1, JAK3,* and *PTPN11*. Libraries were diluted, combined together and emulsion PCR performed on the One touch 2 system (Thermo Scientific) using the OT200 template kit to amplify library DNA onto IonSphere particles (ISP). Enrichment of ISPs with clonally amplified sequencing template relied on the OneTouch ES System (Thermo Scientific). Sequencing was performed on an Ion Torrent PGM (IC200 Sequencing Kit, Ion 318 chip, Thermo Fischer) which generates a FASTQ from the raw data file.

FASTQ files were analyzed using three pipelines to identify somatic mutations: Variant Caller v4 (Thermo Scientific), Ion Reporter v5 (Thermo Scientific), and NextGENe v2.4 (Softgenetics, State College, PA). Initial criteria used to call sequence variants relied on detection by multiple pipelines with amplicon coverage of >500x, a variant frequency greater the 3% with at least 20 reads in a wild-type background, and absence of the same variant in matched normal tissue. After review, variants were confirmed as somatic mutations in the COSMIC database,[21] or ruled out as a known germline single-nucleotide polymorphisms with the dbSNP database.[22]

Copy number variants (CNVs) were identified using Ion Reporter v5 and NextGENe v2.4. Specimens were excluded from copy number analysis for the following reasons: i) low sequencing coverage (n=4, <500x/amplicon), ii) poor quality DNA producing DNA sequencing artifacts (n=2, >50 low level variants with <2% allele frequency), or iii) an estimated tumor percentage of less than 50% (n=2). CNV detection was achieved by comparison of tumor amplicon coverage with matched normal tissue (paired-sample workflow) or comparison to a group average of 10 normal control tissues (multiple control workflow). The analysis incorporated the tumor percentage estimated by the pathologist to determine copy number. Any gene with a single amplicon probe was excluded from the analysis. Results from multiple probes were combined to generate a single gene gain/deletion score. Copy numbers greater than 3.3 were defined as a gain and a copy numbers less than 1.2 were defined as a loss. CNV calls made by both software packages were tabulated and reported both by gene and chromosome region. CNV changes present in >60% of ChRCC specimens were then confirmed by FISH.

For NextGENe v2.4 a coverage ratio (sample divided by sample plus control) is the basis for CNV detection. Coverage is converted to a normalized read count value to account for differences in coverage from sample to sample. A beta-binomial model is fit to the coverage ratio to model the amount of noise (dispersion). The likelihood a copy number variant is present is calculated from the

coverage ratio and dispersion value and can factor estimated tumor purity and expected CNV percentage. A hidden Markov model (HMM) makes the CNV classification of duplication, normal, or deletion with accompanying Phred-scaled probability scores.

The Ion Reporter predicts the copy number or ploidy state (0, 1, 2, 3, etc.) of the tumor by comparing normalized read coverage (after correction for GC bias) to a baseline constructed from control sample(s) with known ploidy. A statistical model adjusts for regions with low read coverage and significant variation, then calculates the likelihood a gene belongs to an alternate ploidy state. An HMM framework determines the optimal path through the ploidy state to maximize the total ploidy state likelihoods when state transition penalties are considered. A “medium setting” employing medium transition penalties was used to enable robust CNV detection with few false-positive calls. Confidence (log ratio of called ploidy versus expected ploidy state likelihoods) and precision (log ratio of assigned ploidy versus next-closest ploidy state likelihoods) scores accompany the copy number predictions.

***Fluorescence* in situ *hybridization (FISH)***

Fluorescence *in situ* hybridization (FISH) analysis was performed as described previously.[23-28] Briefly, multiple 4 µm sections were obtained from FFPE tissue blocks containing neoplastic tissue. An H&E-stained slide from each block was examined to identify areas of neoplastic tissue for FISH analysis. The slides were deparaffinized with two washes of xylene, 15 minutes each, and subsequently washed twice with absolute ethanol, 10 minutes each, and then air-dried in a fume hood. Next, the slides were treated with 0.1mM citric acid (pH 6.0) (Zymed, South San Francisco, CA) at 95 ℃ for 10 mi nutes, rinsed in distilled water for 3 minutes, followed by a wash of 2x standard saline citrate for 5 minutes. Digestion of nuclear proteins was performed by applying 0.4mL of pepsin (5 mg/mL in 0.01N HCl/0.9% NaCl)

(Sigma, St Louis, MO) at 37 ℃ for 40 minutes. The slides were rinsed with distilled water for 3 minutes, washed with 2x standard saline citrate for 5 minutes, and air-dried.

FISH employed a cocktail of dual-color DNA probes from Empire Genomics (Buffalo, New York) that targeted *RB1*-Orange (MAC clone RP11-893E5) and 13q11-Green (RP11-408E5). The second cocktail of dual-color probes consisted of *ERBB4*-Green (Empire Genomics) and CEP2-Orange (Abbott Molecular, Lake Bluff, IL). Probes were diluted at 1:25 with tDenHyb 2 (Insitus, Albuquerque, NM). Diluted probe (5 µL) was applied to each slide in reduced light conditions. The slides were then covered with a 22×22 mm cover slip and sealed with rubber cement. Denaturation was achieved by incubating the slides at 83 ℃ for 12 minutes in a humidified box followed by hybridization at 37 ℃ o vernight.

The cover slips were removed and the slides were washed twice with 0.1× SSC/1.5M urea at 45 ℃ (20 min for each), followed by a wash with 2× SSC for 20 minutes, and with 2× SSC/0.1% NP-40 for 10 minutes at 45 ℃. The slides were further washed with room temperature 2× SSC for 5 minutes. The slides were air-dried and counterstained with 10 mL 4,6-diamidino-2-phenylindole (Insitus, Albuquerque, NM), covered with cover slips, and sealed with nail polish.

***FISH Evaluation***

The slides were examined using a Zeiss Axioplan 2 microscope (ZEISS, Göttingen, Germany) with the following filters (Chroma, Brattleboro, VT): SP-100 for DAPI, FITC MF-101 for Spectrum Green and Gold 31003 for Spectrum Orange. The hybridized slides were analyzed and documented using a MetaSystem system (Belmont, MA) under a 100x oil objective. Four sequential focus stacks with 0.3 µm intervals were acquired and integrated into a single image to reduce thickness-related artifacts. Signals from each color channel (probe) were counted

under pseudocolor, with computerized translation of each color channel into blue, green, and red. A minimum of 100 non-overlapping cancer cells were evaluated for each case. Slides were counted only if >90% of cells demonstrated satisfactory signal quality.[23-28]

The deletion threshold for *RB1* and *ERBB4* copy number was based on previous literature including studies of chromosome 1p/19q deletions in oligodendrogliomas.[29-32] Homozygous deletions of *RB1*or *ERBB4* were defined as the absence of both gene-specific signals with intact control signals in ≥50% of cells. Hemizygous deletions were defined by the presence of only one of the gene specific signals in ≥50% nuclei.[31, 32]

## RESULTS

### *Rare hotspot mutations in 50 onco- and tumor suppressor genes identified in ChRCC but not RO*

It was first examined whether ChRCC (n=7) or RO (n=5) carried any hotspot somatic mutations in 50 onco- and tumor suppressor genes using NGS. The 50 onco- and tumor suppressor genes are those most commonly involved in human cancers (*Materials and Methods*). Approximately 2,800 COSMIC mutational loci in 50 genes are targeted and sequenced. These regions are described as hotspot as they are frequently mutated in human cancers. Single *TP53* or *PIK3CA* mutations were detected in two ChRCCs at allele frequencies of 9% and 21%, respectively. The tumor percentage of the *TP53* mutant ChRCC was estimated to be 50% indicating the mutation is present only in a subset of tumor cells.

### *Copy number analysis reveals hemizygous deletions of tumor suppressor genes* RB1*and* ERBB4 *in ChRCC, which were verified by FISH*

It was further examined if there were any copy number variations in these 50 cancer-critical genes in ChRCC and RO Genes that showed copy number gain or loss in at least 35% of ChRCC or RO (ie, not disomic) were considered. In the ChRCC subgroup, seven genes showed copy number loss and eight genes showed copy number gain, whereas the RO subgroup showed only two genes with copy number loss (Table 1). Both *RB1* and *ERBB4* showed loss of copy numbers in greater than 60% of ChRCC, but were not deleted in RO (Table 1).

The deletions of *RB1* and *ERBB4* genes were subsequently verified by FISH with chromosomal probes directed against *RB1* and *ERBB4*. It was confirmed that there is a good correlation between the NGS and the FISH results in each specific case (Table1 and Supplemental Table S1, Pearson correlation coefficient =1, $P < 0.01$). FISH studies demonstrated a hemizygous deletion of the *RB1*gene in 17 of 33 ChRCC (52%), but not in any of 32 RO cases (0%) (Table 2, Figure 1, Supplemental Table S1). Consistent with the NGS data, a hemizygous deletion of the *ERBB4* gene was detected in 11 of 33 ChRCC cases by FISH (33%), whereas none of the 32 RO cases showed *ERBB4* deletion (0%) (Table 2, Figure 1, Supplemental Table S1). Furthermore, there was no significant correlation between ChRCC tumor stages and *RB1* gene deletion (T1, 7/13, 54%; T2, 2/5, 40%; T3, 8/15, 53%, $P = 0.31$, chi square test). Similarly, ChRCC tumor stage did not correlate with ERBB4 gene deletion (T1, 2/13, 23%; T2, 2/5, 40%; and T3, 6/15, 40%, $P = 0.32$, chi square test). Taken together, 23 of 33 ChRCC carried either an *RB1* and/or *ERBB4* gene deletion (70%, Table 2). In contrast, none of RO cases showed deletion of these two genes (0%, Table 2).

**DISCUSSION**

In this study, NGS was used to detect mutations and copy number variation in ChRCC and RO. This approach showed deletion of tumor suppressor genes *RB1* and *ERBB4* in the majority of ChRCC, but not in RO. Based upon these NGS results, a simple FISH assay was adopted to confirm these findings. *RB1* and/or *ERBB4* deletions occurred in 70% of ChRCC but not in RO. Consequently, the combined use of *RB1* and *ERBB4* deletions by FISH yielded a highly specific and sensitive diagnostic assay to differentiate these tumors. Like other FISH assays, the *RB1* and *ERBB4* probes can be applied to surgical biopsy specimens or cell blocks derived from fine needle aspiration of renal tumors. If applied to clinical practice, the presence of *RB1* and *ERBB4* deletion may help to exclude a RO and rule in a ChRCC. Nevertheless, the results of this study are based on a small number of cases. The diagnostic significance of the combined use of *RB1* and *ERBB4* gene deletions as a diagnostic marker should be further examined in larger numbers of patients with ChRCC and RO.

The molecular mechanism of ChRCC tumorigenesis remains largely unclear. ChRCCs are frequently associated with large multiallelic copy number variations which include gains in chromosomes 4, 7, 11, 12, 14q, and 18q, and losses in chromosome Y, 1, 2, 6, 10, 13, 17, and 21.[33] It has been reported that the loss of chromosomes Y, 1, 2, 6, 10, 13, 17, and 21 is non-random and might lead to tumor suppressor gene inactivation, promoting tumorigenesis.[33, 34] In the FISH assay, accompanying losses of centromere signals were observed in ChRCC cases carrying *RB1* or *ERBB4* deletions. These data suggest either a whole chromosomal deletion or a large partial chromosomal deletion which comprises of *RB1* or *ERBB4* and the corresponding centromere region. The NGS data favor the latter as genes present in different areas of chromosomes 2 or 13 are identified in the same ChRCC cases with centromere loss (Table 1). Deletions that span a centromere will not affect chromosomal DNA replication since an origin of replication is found every 250 KB of human DNA. However, the resulting acentric chromosome will most likely be lost during cell division, unless there is a selective pressure that helps to

maintain the acentric chromosome in the genome. Centromere deletion has been described in naturally occurring pre-cancer and cancer genomes including myelodysplastic syndrome and acute myeloid leukemia.[35]

Previous reports indicate RO occasionally exhibits losses of chromosome 1.[12, 13] In this study, gene amplicons targeting chromosome 1did not reveal obvious alterations in the RO cohort. Copy number changes in *CDKN2A* and *NOTCH* on Chromosome 9 were observed in RO, but these abnormalities were also found in ChRCC.

In contrast to the numerous chromosomal anomalies found in ChRCC, this tumor type is reported to have a low rate of somatic mutation.[36] Similarly only two somatic mutations were observed in 10 ChRCC tested. Davis et al used mitochondrial DNA and whole-genome sequencing to show *TP53* mutations and *PTEN* mutations in 21 and six ChRCC cases (32% and 9%), respectively.[37] In this study, a *TP53* mutation was identified in one ChRCC (10%), but *PTEN* mutations were not observed. In agreement with previous studies, no *KIT* mutations were detected in either ChRCC or RO despite overexpression of C-kit in these two tumors.[38-40]

In summary, NGS demonstrated multiple gene copy abnormalities, the two most common being *RB1* and *ERBB4* in ChRCC, but not in RO. FISH was used to confirm these gene deletions in ChRCC. The FISH assay combining *RB1* and *ERBB4* probes for FFPE tissue sections offers a highly sensitive and specific genetic marker in distinguishing ChRCC from benign RO.

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**Figure legend**

**Figure 1.** Fluorescence *in situ* hybridization (FISH) analysis of *RB1* and *ERBB4* genes in chromophobe renal cell carcinoma (ChRCC) and renal oncocytoma (RO). **A:** Normal tissue with green signals indicating chromosomal centromere regions and red signals showing the presence of disomic *RB1* gene. Disomic *RB1* genes (red; **B**) and disomic *ERBB4* genes (green; **C**) in RO cells. A hemizygous deletion of *RB1* gene (loss of one red signal; **D**) and *ERBB4* gene (loss of one green signal; **E**) in ChRCC.

**Table 1. Next-generation sequencing data for renal oncytomoa (RO) and chromophobe renal cell carcinoma (ChRCC).**

| | | RO | | | | | | | | | | | | | ChRCC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr | Gene | % with LOSS | % with GAIN | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | % with LOSS | % with GAIN |
| **chr1** | ***NRAS*** | 0 | 0 | | | | | | **(–)** | | | | | | | 13 | 0 |
| **chr2** | ***ALK*** | 0 | 20 | | | | | | **(–)** | | **(–)** | | | **(–)** | | **38** | 0 |
| **chr2** | ***ERBB4*** | 0 | 0 | | | | | | **(–)** | | **(–)** | | **(–)** | **(–)** | | **63** | 0 |
| **chr3** | ***VHL*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr3** | ***PIK3CA*** | 0 | 0 | | | | | | | **(+)** | | | | | | 13 | 13 |
| **chr4** | ***FGFR3*** | 0 | 20 | | | | | **(+)** | | **(–)** | | | **(+)** | **(+)** | | 13 | **38** |
| **chr4** | ***PDGFRA*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr4** | ***KIT*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr4** | ***KDR*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr4** | ***FBXW7*** | 0 | 0 | | | | | | | | **(–)** | | | | | 13 | 0 |
| **chr5** | ***APC*** | 0 | 0 | | | | | | | | | | | | | 13 | 0 |
| **chr5** | ***CSF1R*** | 0 | 0 | | | | | | | | | | | | | 0 | 13 |
| **chr7** | ***EGFR*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr7** | ***MET*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr7** | ***SMO*** | 0 | 0 | | | | | | | | | | **(+)** | **(+)** | | 0 | **38** |
| **chr7** | ***BRAF*** | 0 | 0 | | | | | | | | | | **(–)** | **(–)** | | **38** | 0 |
| **chr8** | ***FGFR1*** | 0 | 0 | | | | | | | | | | | | | 0 | 0 |
| **chr9** | ***CDKN2A*** | **40** | 20 | **(–)** | **(–)** | | | **(+)** | | **(–)** | | **(–)** | | | | 25 | 0 |
| **chr9** | ***ABL1*** | 0 | 0 | | | | | | | | | | | **(+)** | | 0 | 25 |
| **chr9** | ***NOTCH1*** | **40** | 20 | **(–)** | **(–)** | | | **(+)** | | **(–)** | | | **(+)** | **(+)** | | 13 | **38** |
| **chr10** | ***RET*** | 20 | 0 | | **(–)** | | | | **(–)** | **(–)** | | | | | | 25 | 0 |
| **chr10** | ***PTEN*** | 0 | 0 | | | | | | **(–)** | | | | | **(–)** | | **38** | 0 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **chr10** | ***FGFR2*** | 0 | 0 | | | | **(–)** | | | | | | | 13 | 0 |
| **chr11** | ***HRAS*** | 0 | 20 | | | **(+)** | **(–)** | | | | **(+)** | **(+)** | | 13 | **38** |
| **chr11** | ***ATM*** | 0 | 0 | | | | **(–)** | | | | | | | 13 | 0 |
| **chr12** | ***KRAS*** | 0 | 0 | | | | | | | | | | | 13 | 0 |
| **chr12** | ***PTPN11*** | 0 | 0 | | | | | | | | | **(–)** | | 25 | 0 |
| **chr12** | ***HNF1A*** | 0 | 0 | | | | | **(–)** | | | **(+)** | **(+)** | | 13 | **38** |
| **chr13** | ***FLT3*** | 0 | 0 | | | | | | **(–)** | | | **(–)** | | 25 | 0 |
| **chr13** | ***RB1*** | 0 | 20 | | | **(+)** | | | **(–)** | **(–)** | **(–)** | **(–)** | **(–)** | **75** | 0 |
| **chr14** | ***AKT1*** | 20 | 0 | **(–)** | | | | **(–)** | | | **(+)** | **(+)** | | 13 | **38** |
| **chr16** | ***CDH1*** | 20 | 0 | | **(–)** | | | | | | | | | 0 | 13 |
| **chr17** | ***TP53*** | 0 | 0 | | | | **(–)** | **(–)** | **(–)** | **(–)** | **(+)** | | | **50** | 13 |
| **chr17** | ***ERBB2*** | 0 | 0 | | | | **(–)** | **(–)** | **(–)** | | **(+)** | | | **38** | 13 |
| **chr18** | ***SMAD4*** | 0 | 0 | | | | | | | | | | | 0 | 0 |
| **chr19** | ***STK11*** | 0 | 0 | | | | | **(–)** | | | **(+)** | **(+)** | | 13 | **38** |
| **chr19** | ***JAK3*** | 0 | 0 | | | | | | | | **(+)** | **(+)** | | 0 | **38** |
| **chr20** | ***GNAS*** | 0 | 0 | | | | | | | | | **(+)** | | 0 | 13 |
| **chr22** | ***SMARCB1*** | 20 | 0 | **(–)** | | | | | | | | | | 0 | 13 |

Chr, chromosome; LOSS (-): < 1.2 gene copies; GAIN (+), > 3.3 gene copies; %: loss or gain, percentage of gains or losses > 29% (two or more cases carrying loss or gain) of total cases tested are shown in bold.

**Table 2. Hemizygous deletions of *RB1* and *ERBB4* genes and their frequency of occurrence in renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC) by FISH study.**

| Diagnosis | Cases with hemizygous *RB1* deletion (%) | Cases with hemizygous *ERBB4* deletion (%) | Cases with either *RB1* or *ERBB4* deletions (%) | Total cases |
|---|---|---|---|---|
| **ChRCC** | 17/33 (52%) | 11/33 (33%) | 23/33 (70%) | 33 |
| **RO** | 0/32 (0%) | 0/32 (0%) | 0/32 (0%) | 32 |

ChRCC, chromophobe renal cell carcinoma; RO, renal oncocytoma.

**Figure 1**