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FLUORESCENT IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY FOR SUBTYPING "NON-CLASSIFIABLE" RENAL CELL CARCINOMAS

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Abstract: Renal tumors account for about 3% of the malignancies in adults. Clear cell subtype renal cell carcinoma (RCC) and papillary RCC are the most common renal tubular epithelial carcinomas and their differentiation is important because they have a different prognosis and are associated with different treatment protocols. In most cases, histological features allow accurate diagnosis of renal cell carcinomas. There are also overlapping morphological findings between certain kidney neoplasms that make their subtyping extremely difficult. Some of them display papillary architecture but also have a clear cell component and it is not clear whether they should be classified as clear cell RCC or papillary RCC.
In our study we performed an immunohistochemical and genetic analysis of 24 cases of RCC classified as non-classifiable with mixed papillary and clear cell components treated at Clinic of Urology in University Hospital "St. George "-Plovdiv. The mean age of patients was 54.5 years, and gender distribution: 60% male and 40% female.
Based on the results of immunohistochemistry and fluorescence in situ hybridization (FISH), patients were stratified in 2 groups. The first group included 16 of the cases where strong immunoreactivity was found for alfa-methyl coenzyme A racemase (AMACR), with cytokeratin 7 (CK7) being present in 15 of these. In all cases in this group, FISH proved trisomy 7 and 17, in 4-9p deletion, and in 2- 3p deletion. The remaining 8 cases were stratified in the second group - all negative for CK7 and only one positive for AMACR. Genetic analysis showed a lack of trisomy 7 and 17 in all cases, as well as a deletion of 3p and 9p in 7 of them.
The combination of immunohistochemical and genetic analyzes allows with a high accuracy to differentiate cases of papillary RCC from those with clear cell RCC.
Keywords: Renal cell cancer; subtypes; immunohistochemistry; fluorescence in situ hybridization

1. INTRODUCTION

Renal tumors account for about 3% of the malignancies in adults. Clear cell subtype renal cell carcinoma (RCC) and papillary RCC are the most common renal tubular epithelial carcinomas and their differentiation is important because they have a different prognosis and are associated with different treatment protocols. According to the 2004 World Health Organization (WHO) classification, ccRCC show a solid alveolar growth pattern composed of clear cells, while pRCC are characterized by papillae recovered by small basophilic cells (type 1 pRCC) or by large eosinophilic cells (type 2 pRCC). In most cases, histological features allow accurate diagnosis of renal cell carcinomas. There are also overlapping morphological findings between certain kidney neoplasms that make their subtyping extremely difficult. Some of them display papillary architecture but also have a clear cell component and it is not clear whether they should be classified as clear cell RCC or papillary RCC (Fig.1).

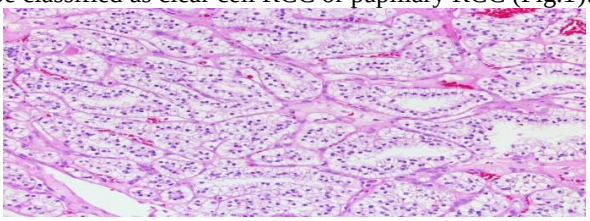

Fig. 1 Non-classifieble RCC

Most common types of RCC are characterized by recurrent chromosomal abnormalities, the detection of which is very useful for confirming or completing the diagnosis. In order to improve the diagnostics of such "unclassified" cases we evaluated the immunophenotypes of 24 renal neoplasms with papillary architecture and extensive components of neoplastic cells with clear cytoplasma combining it with an additional fluorescence in situ hybridization (FISH) in order to clarify their cytogenetic characteristics.

2. MATERIALS AND METHODS

In our study we performed an immunohistochemical and genetic analysis of 24 cases of RCC classified as non-classifiable with mixed papillary and clear cell components treated at Clinic of Urology in University Hospital "St. George"-Plovdiv. The mean age of patients was 54.5 years, and gender distribution: 60% male and 40% female.

Tumor material was fixed in 10% phosphate-buffered formalin and embedded in paraffin. Histological sections were stained with hematoxylin–eosin–saffron. Immunohistochemical analysis was done using the following monoclonal antibodies: CK7, CD10 and AMACR.

Series of 4-mm slides were prepared from buffered formalin-fixed, paraffin-embedded tissue blocks. 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 the hood. Next, the slides were treated with 0.1 mM citric acid (pH 6.0) at 95°C for 10 minutes, rinsed in distilled water for 3 minutes followed by a wash of standard saline citrate (SSC) for 5 minutes. Digestion of the tissue was performed by applying 0.4 mL of pepsin (5 mg/mL in 0.1 N HCl/0.9 NaCl) at 37°C for 40 minutes. The slides were rinsed with distilled water for 3 minutes, washed with SSC for 5 minutes, and air-dried Fig.2).

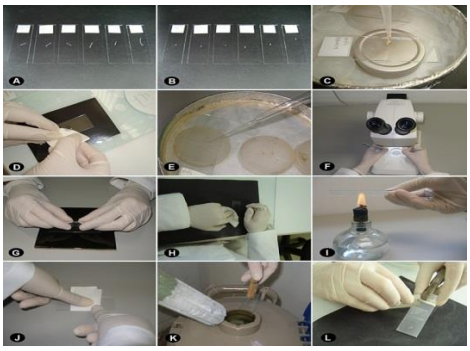

Fig. 2 Preparation of slides for FISH

FISH was performed with centromeric a-satellite DNA probes for 3p25.3 (VHL - Von Hippel-Lindau)(Spectrum Orange) ; 9p21.3(Spectrum Orange) ; Chromosome 7 Alpha Satellite Probe (Spectrum Green) ; Chromosome 17 Alpha Satellite Probe (Spectrum Red), respectively. The slides were examined using a Zeiss Axioplan 2 microscope (ZEISS, Gottingen, Germany) with the following filters from Chroma (Chroma, Brattleboro, VT): SP-100 4,6-diamidino-2- phenylindole, FITC MF-101 for Spectrum Green (CEP 7), and Gold 31003 for Spectrum Orange (CEP 3 , 9and 17). The images were acquired with a CCD camera and analyzed with MetaSystem Isis Software (MetaSystem, Belmont, MA). Five sequential focus stacks with 4mm intervals were acquired and then integrated into a single image to reduce thickness-related artifacts (Fig.3).

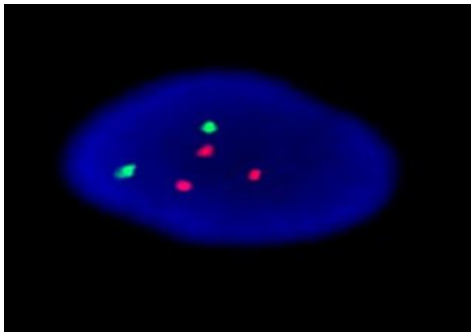

Fig. 3 FISH analysis

We performed FISH analysis with the same probes (CEP 7, 17, 3, and 9) in classic clear cell renal cell carcinoma and classic papillary renal cell carcinoma as controls.

3. RESULTS

Based on the results of immunohistochemistry and fluorescence in situ hybridization (FISH), patients were stratified in 2 groups: Group 1- patients with pRCC and Group 2- patients with ccRCC. The results received into both groups are summarized in Fig. 1 and 2.

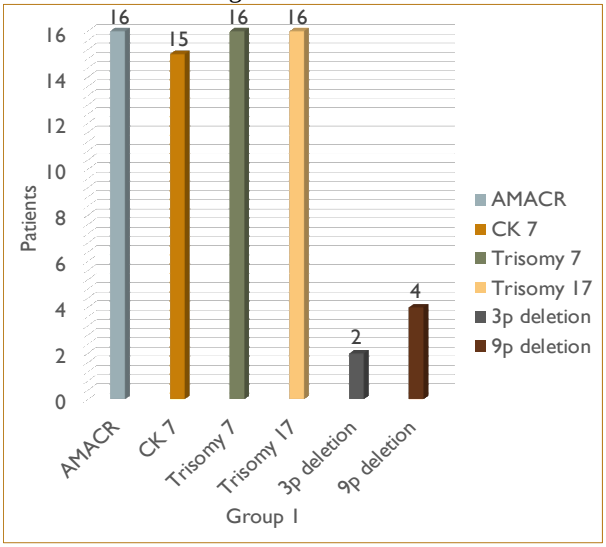

Fig. 3 Group 1- patients with pRCC

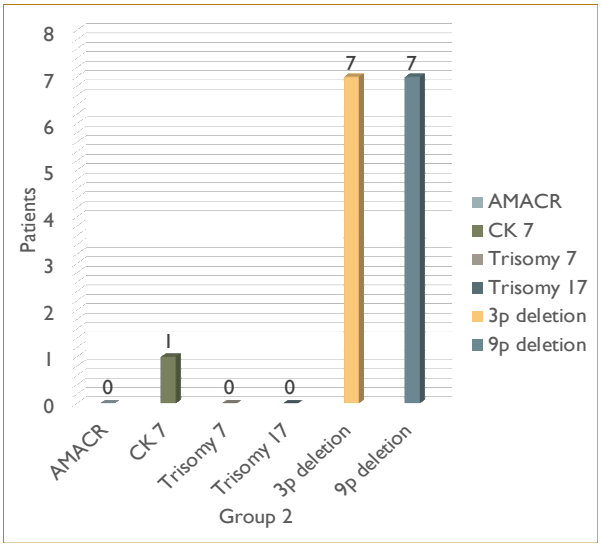

Fig. 4 Group 4- patients with ccRCC

The first group included 16 of the cases where strong immunoreactivity was found for alfa-methyl coenzyme A racemase (AMACR), with cytokeratin 7 (CK7) being present in 15 of these. In all cases in this group, FISH proved trisomy 7 and 17, in 4-9p deletion, and in 2- 3p deletion. The remaining 8 cases were stratified in the second group - all negative for CK7 and only one positive for AMACR. Genetic analysis showed a lack of trisomy 7 and 17 in all cases, as well as a deletion of 3p and 9p in 7 of them.

The proposed panel of immunohistochemical and cytogenetic studies allowed us to provide a definitive diagnosis of 22 of 24 cases of RCC with mixed papillary and clear cell components.

4. DISCUSSION

The immunohistochemical panel we used in this study enables one to distinguish accurately between papillary renal cell carcinoma, clear cell renal cell carcinoma, Xp11.2 translocation renal cell carcinoma, and the recently described clear cell papillary renal cell carcinoma. Papillary renal cell carcinomas often show positive immunostaining for AMACR and CK7 whereas clear cell renal cell carcinomas usually lack immunoreactivity for AMACR and CK7.

The cytogenetic findings in the renal cell carcinomas in the current study were those typically seen in papillary renal cell carcinoma. Gains of chromosomes 7 and 17, which were observed in the first group of our cases, are commonly identified in papillary renal cell carcinoma and these numerical aberrations occur early in the evolution of papillary renal cell neoplasia. The second group presented deletion of chromosome 3p, which is considered one of the primary events in the development of clear cell renal cell carcinoma.

A few reports describing RCC with both clear cells and papillary component are available in the literature. A new subtype of RCC, characterized by both a papillary pattern and the presence of clear cells, was described in five patients . This entity showed a particular immunohistochemical profile characterized by positivity for CK7 and negativity for CD10 and AMACR. The authors called these tumors “clear cell papillary RCC”. However, the genomic characteristics of these five cases were different from our cases since FISH analysis did not detect trisomies 7 and 17 or 3p deletion. The RCC associated with Xp11 translocations are also composed of cells with clear cytoplasm to faintly eosinophilic cytoplasm, arranged in nest and papillary structures. These tumors are generally observed in teenagers and young adults and show positive immunostaining for TFE3 or TFEB.

Fuzesi et al studied 3 such cases with classic cytogenetic analysis, and demonstrated clonal aberrations of chromosome 3, leading to loss of terminal 3p segments, in all of them. Salama et al included in their study only renal cell carcinomas, with papillary architecture, showing more than 75% of cells with clear cytoplasm. They analyzed 7 neoplasms with FISH using centromeric probes for chromosomes 7 and 17 and with loss of heterozygosity analysis, with markers mapping in the short arm of chromosome 3. They found no trisomy of

chromosome 7 or 17 and in 6 cases there was loss of heterozygosity of 3p. Consequently, the neoplasms analyzed in these 2 studies were regarded as clear cell renal cell carcinomas, on the basis of cytogenetic studies alone. Neither of these investigations was done in concert with immunostainings of these renal cell carcinomas.

5. CONCLUSION

Our results suggest that some RCC that are diagnosed only on the basis of morphological analysis are probably misdiagnosed. The genomic analysis is of crucial importance for establishing the final diagnosis and subsequently giving the most appropriate treatment to the patient. In summary, we report a series of renal cell carcinomas with papillary architecture and areas of clear cell morphology. Their immunohistochemical profiles and cytogenetic patterns allowed us to distinguish between papillary renal cell carcinomas and clear cell renal cell carcinomas in the great majority of cases. We present a suggested panel of immunohistochemical and cytogenetic findings that are useful in establishing an accurate diagnosis in these cases and in distinguishing them from other renal neoplasms with similar histologic findings.

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