

REVIEW PAPER

REVIEW ON SIGNIFICANCE OF GDNF, PTCH1 AND RNF213 IN CHROMOPHOBE RENAL CELL CARCINOMA ILLUSTRATED BY THE CASE OF 71-YEARS-OLD MAN

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Here we review the role of GDNF, PTCH1, RNF213 illustrated by a case of renal cell carcinoma, chromophobe type (pT2a 8th pTNM edition) of the left kidney of 71-year-old man. Status of potential hotspots in 409 tumor genes were studied by means of next generation sequencing (NGS) technology (IonTorrent – Thermo Fisher Scientific, USA) using Ion AmpliSeq™ Comprehensive Cancer Panel. Next-generation sequencing (NGS) revealed mutations of *GDNF* (NM_001190468: c. 328C>T, p.R110W, allelic frequency 46%), *PTCH1* (NM_001083607:c. 2969C<T, p.A990V, allelic frequency 7%) *RNF213* (NM_001256071: c.6967C<T, p.Q2323X, allelic frequency 7%). Our findings in relation to concise description of significance of *GDNF*, *PTCH1*, *RNF213* supplement molecular characterization in area of gene profiling of renal cell carcinoma, chromophobe type, which is going to certainly deepen our knowledge on hazards in development of this peculiar kind of renal cancer.

Key words: GDNF, PTCH1, RNF213, chromophobe renal cell carcinoma.

Introduction

The chromophobe renal cell carcinoma (ChRCC) is often a subject of accurate differential diagnosis. In this aspect it should be kept in mind that this renal cancer can be a malignant component of hybrid oncocytic/chromophobe tumors (HOCT) which are found sporadically or in patients with renal oncocytomatosis or with Birt-Hogg-Dubé syndrome (BHD) with

distinct morphology in the third clinic-pathological setting [1]. Both oncocytoma and chromophobe renal cell carcinoma express CK7 and CD117 [1]. However the pattern of staining is different and thus of great significance in the distinction between ChRCC and renal oncocytoma (RO) as well as clear cell renal cell carcinoma (CRCC) [2]. The immunohistochemical panel of DOG1 (positive in ChRCC and RO, negative in CRCC), cyclin D1 (mainly positive in

RO but negative in ChRCC and CRCC), CK7 (pattern of mainly negative staining with only scattered single positive cells and small groups of cells in RO, positive in ChRCC and generally negative in CRCC with only focal positivity noted in a couple of cases), CD117 (mainly positive in RO and ChRCC but negative in and CRCC) and vimentin (mainly positive in CRCC and RO but negative in ChRCC) was investigated in the differential diagnosis of renal epithelial tumors [2]. In addition ChRCC are characterized by membranous CD117 (KIT) expression in opposition to cytoplasmic one in papillary renal cell carcinomas (pRCC), which could be related to a specific point mutation of the c-kit intron 17 T->A that was present in pRCC and absent in ChRCC [3]. However, in our simplest point of view ChRCC is more prone to present membranous staining as its cells contain less cytoplasm than pRCC and RO after tissue processing. Of course if positive, the cells with little cytoplasm are more prone to present with cytoplasmic reaction for non-nuclear immunohistochemical marker. Considering cytoplasmic vs. membranous pattern of immunoexpression, as another example to support our simple point of view, prominent membranous staining was found for vimentin in CRCC, which also shows paucity of cytoplasm after tissue processing [2]. In everyday economically justified practice we believe that appliance of panel CK7, CD117 and vimentin is sufficient for differential diagnosis of ChRCC, particularly if Hales colloidal iron histochemical staining is challenging and might appear ambiguous in some laboratories. Anyway, studies of molecular biologists and pathologists are united in attempt to deepen a characteristics of tumors. In this manner, considering some overlap of morphologic, immunohistochemical, and ultrastructural features of ChRCC and RO, Liu *et al.* decided to search for some distinct genetic markers by appliance of next-generation sequencing (NGS) [4]. They found hotspot mutations were found in cancer-critical genes (*TP53* and *PIK3CA*) and deletions in tumor-suppressor genes *RB1* and *ERBB4* in ChRCC further confirmed in fluorescence *in situ* hybridization (FISH) in ChRCC in opposition to RO [4]. Our aim was to screen the tissue of ChRCC for presence of hotspots of 409 tumor genes by appliance of next generation sequencing (NGS) technology (IonTorrent – Thermo Fisher Scientific, USA) using Ion AmpliSeq™ Comprehensive Cancer Panel.

Material and methods

The postoperative specimen contained left, 11 × 6 × 6 cm-large kidney with a renal tumor of 71-year-old man with 4cm-long part of ureter. Representatives samples underwent staining with haematoxylin and eosin, vimentin, CK7, CD117 Hale's colloidal iron. Simultaneously gene profiling incorporated

examination of status of hotspots found in 409 tumor genes by means of next generation sequencing (NGS) technology (IonTorrent – Thermo Fisher Scientific, USA) using Ion AmpliSeq™ Comprehensive Cancer Panel in tumor material from paraffin-embedded slides.

DNA isolation

In order to isolate DNA from formalin-fixed paraffin embedded tissue (FFPE), the commercially available Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega, USA), dedicated to the Maxwell 16 MDx (Promega, USA), was used. The DNA concentration was measured using a Qubit 2.0 fluorometer and the HS Assay Kit dsDNA (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The average concentration for the obtained DNA isolates was 25 ng/μl, while the purity was 260/280 1.8-2.0.

Next generation sequencing

The isolated DNA was then used to detect mutations by the NGS method, using the Ion AmpliSeq™ Comprehensive Cancer Panel kit (Thermo Fisher Scientific, USA) that allows the study of 409 tumor genes. This panel includes tumor suppressor genes and oncogenes that are most often found mutated in tumor tissues. The DNA was diluted to 10 ng/μl. The libraries were prepared using the Ion AmpliSeq™ Library Kit 2.0, the Ion AmpliSeq™ Comprehensive Cancer Panel and the Ion Xpress Barcode Adapters Kit (Thermo Fisher Scientific), according to the manufacturer's instructions (Thermo Fisher Scientific). Then, with Ion Chef (Thermo Fisher Scientific) and Ion 520 & Ion 530 Kit-Chef and Ion 530™ Chip Kit (Thermo Fisher Scientific), emPCR was performed, enrichment and two 530 chips were loaded (4 samples per chip). Sequencing was performed on an Ion S5 sequencer (Thermo Fisher Scientific). The raw data generated during sequencing was processed using the Torrent Server Suite 5.6-TSS (Thermo Scientific, USA). The obtained sequences were matched (mapped) to the reference sequence of the human genome (hg19). Searching for different variants (SNP, deletions, insertions) was carried out using the Variant Caller 5.6 program, which is part of Torrent Server Suite 5.6. Additionally, Torrent Server Suite 5.6 generated FASTQ files that were used for analysis using the CLC Biomedical Workbench 5.0 (QIAGEN). Detected mutations were annotated using wANNOVAR software (www.wannovar.usc.edu). COSMIC database, dbSNP database and Varsome database (<https://varsome.com/>) were used as well.

Results

The yellowish-whitish, solid tumor was found in renal parenchyma did not exceed the fibrous capsule

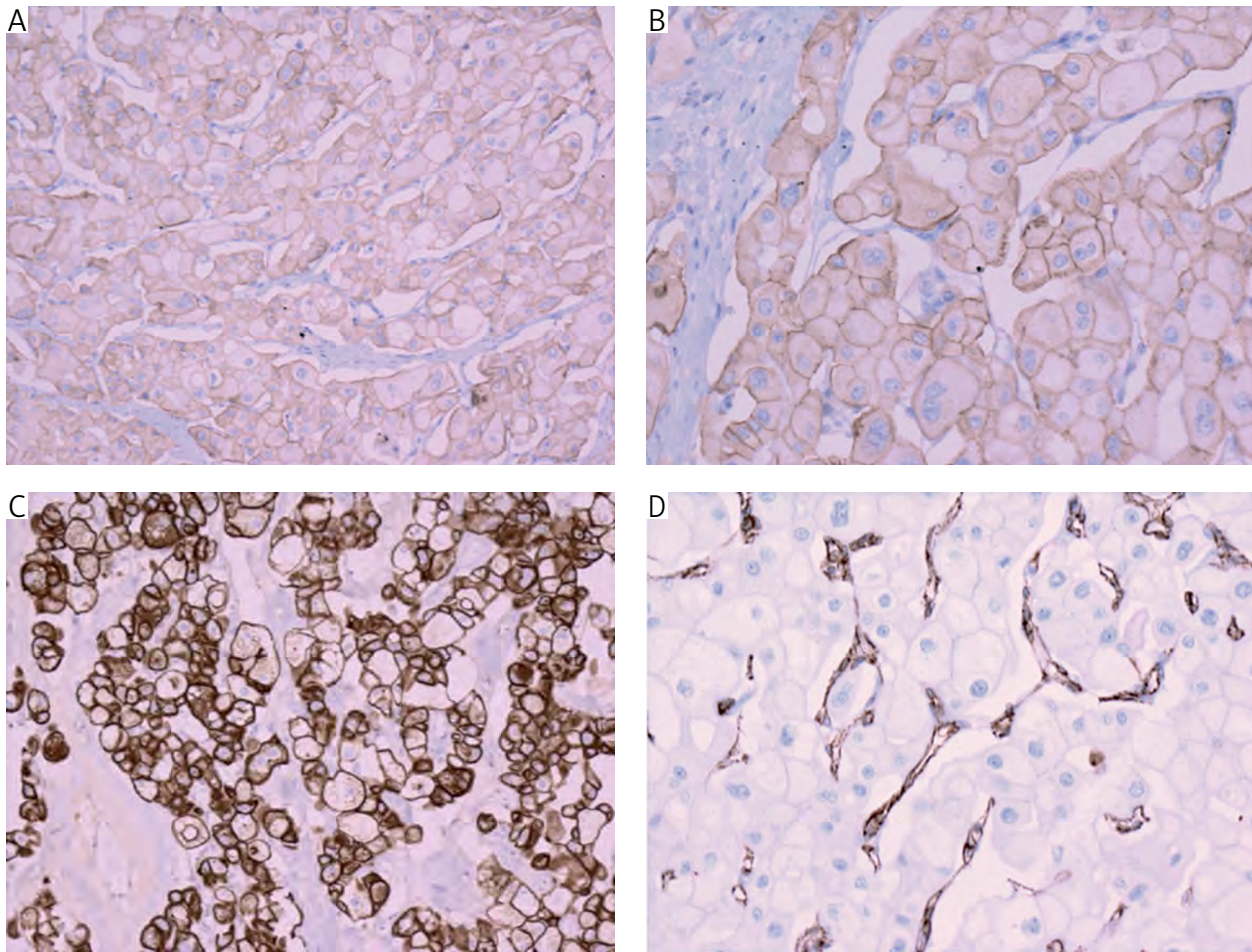


Fig. 1. Immunoprofile of chromophobe renal cell carcinoma. A) Membranous pattern of immunostaining of renal cancer cells for CD117 (CD117, magnification 100 \times). B) Membranous pattern of immunostaining of renal cancer cells for CD117 (CD117 staining, magnification 400 \times). C) Mixed membranous and cytoplasmic immunoreactivity in renal cancer cells to CK7 (CK7 staining, magnification 200 \times). D) Positive staining to vimentin only in vascular framework adjacent to vimentin negative cancer cells (Vimentin staining, magnification 400 \times)

of the kidney. It measured 7.2 cm, pT2a (8th pTNM edition) and contained a 2 cm-wide calcification. The neoplasm was composed of pale cells with distinct cellular borders, finely reticular pale or clear cytoplasm while some of nuclei were wrinkled to present appearance of raisinoid nuclei and were surrounded by perinuclear halos. There was a strong coexpression of membranous and cytoplasmic CK7 and membranous CD117 in the tumor as confirmation of cancer histological type diagnosis, while cancer cells lacked vimentin reactivity. The diagnosis of chromophobe renal cell carcinoma (chromophobe type) pT2a (8th pTNM edition). Ureter and hilar vessels as well as renal fibrous capsule showed no cancer invasion. However, atherosclerosis of the renal artery and minor microcalcifications within the tumor were noted. Next-generation sequencing (NGS) revealed mutations of *GDNF* (NM_001190468: c. 328C<T, p.R110W, allelic frequency 46%), *PTCH1* (NM_001083607:c. 2969C<T, p.A990V, allelic frequency 7%), *RNF213* (NM_001256071:c.

6967C<T, p.Q2323X, allelic frequency 7%). Various classified mutations detected in *GDNF* and *PTCH1* as variants of unknown significance and *RNF213* as likely pathogenic [5].

Discussion

GDNF

To our knowledge the molecular abnormalities in *GDNF*, *PTCH1*, *RNF213* are hereby reported in chromophobe carcinoma for the first time in the literature. Our findings supplement molecular characterization in area of gene profiling of renal cell carcinoma, chromophobe type, which is going to certainly deepen our knowledge on hazards in development of this peculiar kind of renal cancer. The glial cell line-derived neurotrophic factor (GDNF) family includes a variety of ligands (GFLs) like GDNF which is pivotal for morphogenesis of kidney [6].

GDNF is present in adjacent to the RET-enriched tips of the ureteric bud in nephrogenic mesenchyme during renal development [7]. *Gdnf* knockout mice die soon after birth due to lethal defects associated with lack of GDNF that is essential particularly for renal development [8]. Furthermore, GDNF activates the RET receptor tyrosine kinase that mediates cell migration and chemotaxis with potential recruitment of phosphatidylinositol 3-kinase (PI3K) in branching morphogenesis in the developing kidney [9, 10].

PTCH1

PTCH1 is a receptor for secreted evolutionary conserved morphogen Hedgehog (HH), which regulates activity of Smoothed SMO and the Ci/Gli transcription factor family involved in body organization [11]. HH signaling has been implicated in the proliferation and regulation of stem-cell maintenance. Misregulation in HH signaling pathway has been shown to cause numerous cancers including basal cell carcinoma, medulloblastoma, small cell lung cancer and pancreatic adenocarcinoma [12]. As stated above HH regulates key target genes involved in development, it has also impact on modulation of the tumor microenvironment to prepare a tumor-suitable niche. Collectively this promotes tumor progression and metastasis [13]. The first HH pathway inhibitor Vismodegib a specific and potent SMO antagonist was approved by FDA in USA for the treatment of locally advanced or metastatic basal cell carcinoma [14]. Immunohistochemical analysis of patched 1 (PTCH1) showed higher expression in high in comparison to low grade tumors in clear cell renal cell carcinomas and associated with shorter cancer specific survival [15]. An oncogenic nature of PTCH1 has been suspected in renal cell carcinoma on the basis of limited *in vitro* analyses as well [15]. Detection of *PTCH1* mutation in our case of ChrCC additionally signalize the role of PTCH1 in renal oncogenesis, which still remains obscure with only few reports being published on abnormalities of this factor in particular renal tumors. To the best to our knowledge mutation detected in our case has not been described so far. Bioinformatic analysis by Varsome showed that its significance is unknown. However, several *in silico* prediction scores (e.g. FATHMM, Mutation taster) points that it has damaging impact.

RNF213

RNF213 encodes an E3 ubiquitin-protein ligase that is known to degrade NFAT1. NFAT1 is a transcription factor that activate expression of MDM2 which is involved in p53 degradation [16]. RNF213

protein functions in mediating protein-protein interactions and also possesses ATPase activity. Single nucleotide variation in *RNF213* has been linked with onset Moyamoya disease (MMD – specific intracranial vascular disorder) in both familial and sporadic form [17]. In *RNF213* gene there were described point mutations (missense and nonsense), frameshift deletion and fusions in different cancers [17, 18]. RNF213 negatively regulates matrix metalloproteinase genes expression (MMP) in endothelial cells. Repressing RNF213 activity revealed increased expression of MMP, which are known to cause epithelial to mesenchymal transition (EMT). EMT has deleterious impact on endothelial cells in maintenance of vascular structures [19]. In our case of chromophobe renal cell carcinoma we detected nonsense mutation in *RNF213* gene, which cause premature stop protein synthesis. It is interesting to note that *TP53* mutations are characteristic events for ChrCC [4]. In our case we detected mutation in *RNF213*, which disrupts this protein. As was described above lack of functional RNF213 indirectly causes TP53 degradation. This could be another mechanism of ChrCC development by inactivation of TP53 pathway. In addition to *RNF213*, LOH was detected in one case in the same cited study [4]. Lately it was revealed that RNF213 deficiency promotes tumor survival in hypoxic environment [20]. Based on above information RNF213 deficiency in tumor cells could have triple impact by leading to most important tumor suppressor degradation (TP53), disrupts proper angiogenesis through upregulation of MMP and survival promotion under hypoxic environment. Deregulated angiogenesis is one of the hallmarks of development of renal cell carcinoma.

In our opinion, presentation novel molecular factors in genetic landscape of chromophobe renal cell carcinoma in this review is going to contribute to further determination of next biological agents, which could be variably expressed in renal cell carcinomas like it was shown for *TRPM3*, *AQP1*, *FBP1*, *ITPKA*, *LOX*, *TUBB3*, *IGFBP1*, *ALDOB*, *FLRT3*, *ACE2*, *OGDHL*, *EYA1*, *STEAP3*, *GPRC5A*, *COMP*, *MIOX*, *TINAG* and *ANGPTL3* in clear cell renal cell carcinoma of different pT1 and pT3 stage [21].

In conclusion, mutations detected in our ChrCC case could have negative impact on renal morphogenesis (GDNF), deregulation of HH signaling (PTCH1) and deregulation of proliferation and angiogenesis and survival promotion under hypoxic state (RNF213) leading to tumor development and progression.

The authors declare no conflict of interest.

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