

# An Overview of Omics Technologies for Renal Cell Carcinoma Diagnosis and Profiling

**Laura-Ancuța Pop<sup>1</sup>, José Antonio López-Guerrero<sup>2</sup> and Ioana Berindan-Neagoe<sup>1,3,4\*</sup>**

<sup>1</sup>Research Center for Functional Genomics, Biomedicine and Translational Medicine, University of Medicine and Pharmacy Iuliu Hatieganu, Romania

<sup>2</sup>Laboratory of Molecular Biology, Fundación Instituto Valenciano de Oncología Valencia, Spain

<sup>3</sup>Research Institute for Advanced Medicine Med Future, University of Medicine and Pharmacy Iuliu Hatieganu, Romania

<sup>4</sup>Department of Functional Genomics and Experimental Pathology, The Oncology Institute, Romania

**\*Corresponding author:** Ioana Berindan-Neagoe, Research Center for Functional Genomics, Biomedicine and Translational Medicine, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania. Email: ioananeagoe29@gmail.com

**Published Date:** September 20, 2016

## ABSTRACT

Renal cell carcinoma is the twelfth type of cancer worldwide with 140000 deaths annually. Clear cell renal cell carcinoma is the most frequent subtype of renal cell carcinoma (80%) and is one of the most aggressive subtypes. Taking into consideration that there are no validated biomarkers for early diagnosis of renal cell carcinoma or targeted therapies, the “omics” technologies development could help improve the diagnosis and future therapies. “Omics” technologies analyze genomics, transcriptomics, proteomics and metabolomics by next generation sequencing, microarray analysis, or proteomic tools. All these technologies have been applied with success in early diagnosis, prognosis and treatment evaluation of several other cancer types like, breast, ovarian, prostate, lung or colon cancer, so their application in renal cell carcinoma should be successful. This chapter proposes introspection on the application of “omics” technology in the molecular diagnosis, prognostic, treatment evaluation or subclassification of patients with renal cell carcinoma. Even though microarray technology is well established for renal cell carcinoma

evaluation, proteomics and next generation sequencing are still immersing as high-throughput technologies employed for renal cell carcinoma analysis.

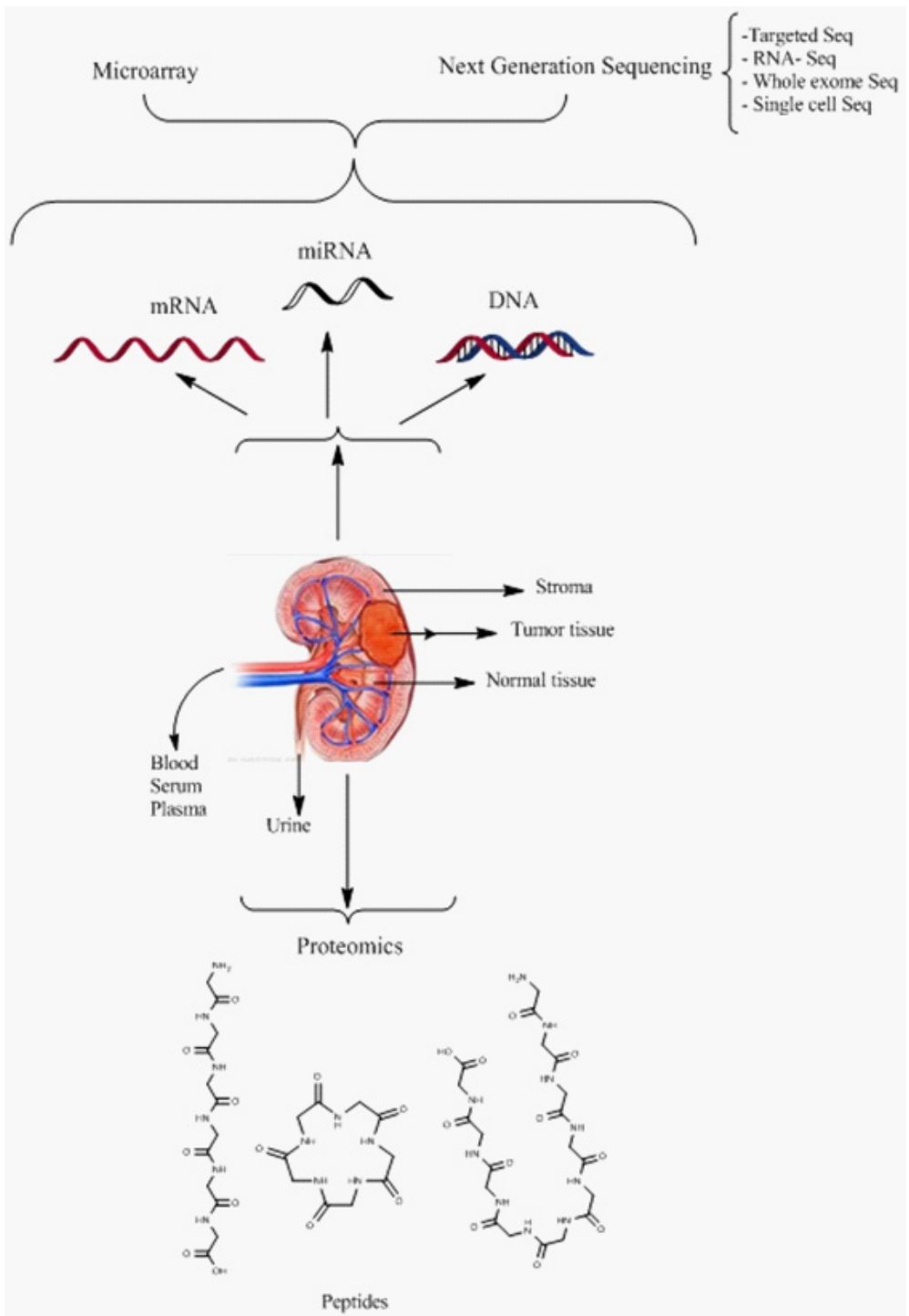
**Keywords:** Renal carcinoma; Next generation sequencing; Microarray; Proteomics; Early diagnosis; Personalized treatment

**Abbreviations:** **CT:** Computer tomography; **NGS:** Next generation sequencing; **DNA:** Deoxyribonucleic acid; **dNTPs:** Dinucleotides; **PGM:** Personal genome machine; **PCR:** Polymerase chain reaction; **RCC-** Renal cell carcinoma; **SETD2:** Histone-lysine N-methyltransferase; **BAP1:** Ubiquitin carboxyl-terminal hydrolase; **PBRM1-** Protein polybromo-1; **ccRCC-** Clear cell renal cell carcinoma; **ERBB4-** Erb-B2 receptor tyrosine kinase 4; **HRAS:** Ras family small GTP binding protein H-Ras; **IDH1:** Isocitrate dehydrogenase (NADP(+)) 1, Cytosolic; **MET:** MET proto-oncogene, receptor tyrosine kinase; **PTEEN:** Phosphatase and tensin homolog; **RET:** Ret proto-oncogene; **SMAD4:** SMAD family member 4; **STK11:** Serine/Threonine kinase 11; **ccpRCC:** Clear cell papillary renal cell carcinoma; **EGF-** Epidermal growth factor; **PDGF:** Platelet derived growth factor; **FGF:** Fibroblast growth factor; **PI3K:** Phosphatidylinositol-4,5-bisphosphate 3-kinase; **VHL:** Von hippel-lindau tumor suppressor; **KDM5C:** Lysine demethylase 5C; **MTOR:** Mechanistic target of rapamycin; **PIK3CA:** Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; **ARID1A:** AT-rich interaction domain 1A; **TP53:** Tumor protein P53; **MST1:** Macrophage stimulating 1; **ZNF800:** Zinc finger protein 800; **NPNT:** Nephronectin; **BTNL3:** Butyrophilin like 3; **TXNIP:** Thioredoxin interacting protein; **CCNB2:** Cyclin B2; **RHEB:** Ras homolog enriched in brain; **TCEB1:** Transcription elongation factor B subunit 1; **DNHD1:** Dynein heavy chain domain 1; **MUC4:** Mucin 4, Cell surface associated; **MLLT10:** Myeloid/Lymphoid or mixed-lineage leukemia, translocated To 10; **TCEB1:** Transcription elongation factor B subunit 1; **ATM:** ATM Serine/Threonine kinase; **HIF1A:** Hypoxia inducible factor 1 alpha subunit; **BHLHE41:** Basic helix-loop-helix family member E41; **IL-11:** Interleukin 11; **STAT3:** Signal transducer and activator of transcription 3; **HIF-1 $\alpha$ :** Hypoxia inducible factor 1 alpha subunit; **FFPE:** Formalin fixed paraffin embedded; **RNA:** Ribonucleic acid; **miRNA:** Micro ribonucleic acid; **WGS:** Whole genome sequencing; **WES:** Whole exome sequencing; **NF2:** Neurofibromin 2 (Merlin); **prCC:** Papillary renal cell carcinoma; **sc:** Single cell; **mRCC:** Metastatic renal cell carcinoma; **AHNAK:** AHNAK Nucleoprotein; **LRRK2:** Leucine-rich repeat kinase 2; **SRGAP3:** SLIT-ROBO Rho GTPase activating protein 3; **USP6:** Ubiquitin specific peptidase 6; **TCGA:** The cancer genome atlas; **OS:** Overall survival; **ISPD:** Isoprenoid synthase domain containing; **MAN2A2:** Mannosidase alpha class 2A member 2; **OTOF:** Otoferlin; **SLC40A1:** Solute carrier family 40 member 1; **MICA:** Major histocompatibility complex class I-related chains A; **CD1d:** Non-polymorphic major; **NKT:** Natural killer T-cell; **CNV:** Copy number variation; **FOXE3:** Forkhead box E3; **PITX1:** Paired like homeodomain 1; **RIN1:** Ras and rab interactor 1; **TWF2:** Twinfilin actin binding protein 2; **EHBP1L1:** EH domain binding protein 1 Like 1; **UQCRH:** Ubiquinol- cytochrome C reductase hinge protein; **SLC16A3:** Solute carrier family 16 member 3; **MS:** Mass spectrometry; **LC:** Liquid chromatography; **PIM:** Proto-Oncogene, Serine/Threonine kinase; **MAPKAPK:** Mitogen-Activated protein kinase-activated protein kinase; **JNK2:**

c-JUN N-terminal kinase-2; **CDK1**: Cyclin-Dependent kinase 1; **iTRAQ**- isobaric tags for relative an absolute quantitation labeling; **Hsp27**- Heat shock protein beta-1; **HSC71**- Heat shock cognate 70 kDa protein; **MALDI**: Matrix-assisted laser desorption ionization; **TOF**: Time of flight; **ZYX**: Zyxin; **SRGN**: Serglycin; **TMSL3**: Thymosin beta-4-like protein 3; **FIBA**: Fibrinogen alpha chain; F13A-Coagulation Factor XIII A Chain; **THRB**: Prothrombin; **IC1**: Plasma protease C1 inhibitor; **SDPR**: Serum deprivation response protein; **CD146**: Melanoma cell adhesion molecule; **ECM1**: Extracellular matrix protein 1 precursor; **SELL**: Selectin L; **SYNE1**: Spectrin repeat containing nuclear envelope protein 1; **HSPG2**: Heparan sulfate proteoglycan 2; **VCAM1**: Vascular cell adhesion molecule 1; **IMS**: Imaging mass spectrometry; **S100A11**: S100 Calcium binding protein A11

## INTRODUCTION

Renal carcinoma represents approximately 3% of all human cancers and constitutes the twelfth most common cancer in the world (joint position with pancreatic cancer) with more than 140000 deaths every year globally [1]. The high number of deaths is due to the fact that this type of cancer is asymptomatic and is mainly diagnosed accidentally, when patient perform CT scans or are symptoms like positive for blood in urine or have abdominal pain [2,3]. The main investigation for renal cancer diagnosis is imagistic, even in most developed countries, which means that in some cases this type of cancer is difficult to be discovered at early stage. Also, the main treatment option in renal carcinoma is surgery, which in early stage increases the patient's survival rate, but for advanced stages this method is not bulletproof and the prognosis of such patients is poor, due to chemo and radiotherapy resistance [4]. High throughput technology development gives the opportunity to overcome this kind of limitations. In this way by employing different technologies like, next generation sequencing (**NGS**), microarray tools and/or proteomic approaches, researchers and clinicians can develop new early diagnosis biomarkers and profiling methods for different types of cancers including renal carcinomas. Furthermore, by using these "omics" technologies one can reach a precision medicine correlated to each patient's molecular characteristics and giving them the possibility to receive the optimal treatment option. The application of "omics" techniques in renal cell carcinoma is presented in (Figure 1).



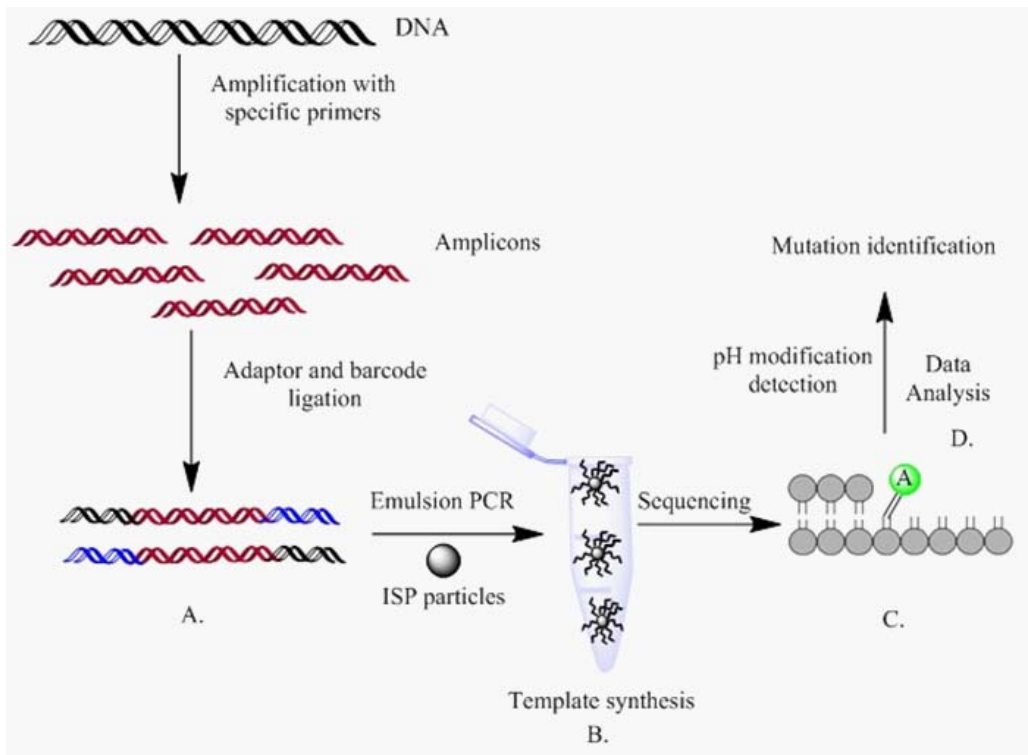
**Figure 1:** Renal cell carcinoma and “omics” technology. Types of biological samples that could be used for microarray, NGS and proteomic analysis in the case of renal cell carcinoma.

# NEXT GENERATION SEQUENCING ANALYSIS OF RENAL CELL CARCINOMA

## Fundamentals

Next generation sequencing (**NGS**) term was introduced for describing new sequencing technologies developed for overcoming the limitations of Sanger sequencing [5]. If Sanger sequencing is based in separating DNA fragments linked to differently marked dNTPs by capillary electrophoresis, NGS is based on the massive parallel amplification of the interest DNA fragments. The DNA fragments identified by specific instruments either reading fluorescent signals or difference in pH. The advantages brought by the NGS technology are reduced time of analysis, higher sensitivity, selectivity and performance, sample multiplexing and lower cost. The limitation of this technique is the high amount of data that can be obtained after sequencing, data that need to be analyzed with specific bioinformatics software and needs special storage equipment [6].

On the market are several types of NGS platforms, but the most used in clinic are Illumina Miseq and Hiseq or Life Technologies Ion Torrent Personal Genome Machine and Ion Proton. Each platform has its own method of sequencing, some use sequencing by synthesis, which mean constructing a complementary DNA strand to the template strand using polymerase enzyme. Each platform has its pros and cons, and type of application that is suitable for. If you are interested in targeted sequencing and short run time, Ion Torrent PGM is the most suited both for price and run time. Illumina MiSeq runs take more time, but give longer reads length of up to 60bp, whereas Ion PGM can go only to 400bp. In case of whole exome, whole genome or mRNA sequencing the preferred platform is Illumina HiSeq. Regarding data analysis and results the Illumina platforms show substitution errors, while Life Technologies platforms present errors in the homopolymer regions [7,8, 9]. One type of NGS workflow is presented in figure 2.



**Figure 2:** An example of a Next Generation Sequencing workflow representative of Ion PGM platform from Life Technology. In this figure are presented the basic steps used for Targeted NGS sequencing, like A. Library preparation, B. Template synthesis, C. Sequencing and D. Data analysis.

## Approaches

### Gene panels

When seeking to identify the heterogeneity of different types of RCC, Singh et al. presented both microarray and NGS technologies as valuable tools that provided high quality data, which could be easily correlated to a specific type of RCC. They studied the genomic and gene expression profile of rhabdoid RCC and observed that by contrast to other study the mutation frequency was higher in rRCC in the case of *SETD2* and *BAP1*, which are correlated to a highly aggressive behavior of such tumors, while the mutation frequency of *PBRM1* was lower than that in the case of Fuhrman grade 4 ccRCC. Regarding the gene expression profile they did not observe high differences between rRCC and ccRCC [10]. Lawrie et al. used NGS technology for clear cell tubule papillary renal cell carcinoma analysis. They used the Ion Torrent PGM platform and the Ion Ampliseq Cancer Hotspot Panel v2 and DNA for formalin-fixed paraffin embedded tissue (FFPE). They observed somatic mutations in both intronic and exonic areas in genes like: *ERBB4*, *HRAS*, *IDH1*, *MET*, *PTEN*, *RET*, *SMAD4*, *STK11* in ccRCC patients [11].

Huang et al. used Illumina HiSeq 2000 instrument for the evaluation of the subclonal genomic profile of metastatic renal carcinoma. They observed several deregulation of critical pathways like *EGF*, *PDGF* and *FGF* in primary RCC, but not in metastatic RCC. While deregulation in *PI3K* pathway were observed in both primary and metastatic RCC. Also they observed that the subclone of the metastasis occurred in late stage of renal cancer progression [12].

A review on the tumor and patient factors in renal cell carcinoma made a classification of the most frequently mutated genes in renal cell carcinoma, the data were obtained from studies of next generation sequencing technology application in renal cell carcinoma. The mutated genes are presented in table 1.

**Table 1:** Most frequently mutated genes in renal cell carcinoma identified in NGS studies.

Gene	Percentage of mutations	Number of patients	Study
VHL	52.3	417	TCGA [13]
	39.6	107	Sato et al. [14]
	51.5	342	Daiglish et al. [15]
	27.6	98	Guo et al. [16]
	53.7	67	Arai et al. [17]
	73.4	94	Scelo et al.[18]
PBRM1	32.9	417	TCGA [13]
	26.4	107	Sato et al. [14]
	20.4	98	Guo et al. [16]
	32.8	67	Arai et al. [17]
	58.5	94	Scelo et al. [18]
SETD2	11.5	417	TCGA [13]
	11.3	107	Sato et al. [14]
	4.4	342	Daiglish et al. [15]
	4.1	98	Guo et al. [16]
	9	67	Araiet al. [17]
	39.4	94	Scelo et al. [18]
BAP1	10.1	417	TCGA [13]
	7.5	107	Sato et al. [14]
	8.2	98	Guo et al. [16]
	6	67	Arai et al. [17]
	19.2	94	Scelo et al. [18]

In the same review as above, also other mutated genes were presented, but the level of mutations were lower, these genes are: *KDM5C*, *PTEN*, *MTOR*, *PIK3CA*, *ARID1A*, *TP53*, *SLTRK6*, *MST1*, *NEF2L2*, *ZNF800*, *NPNT*, *BTNL3*, *TXNIP*, *CCNB2*, *RHEB*, *TCEB1*, *DNHD1*, *MUC4*, *MLLT10*, *TCEB1*, *ATM*, *HIF1A* and others [19].

## RNA sequencing

In a functional assay to study the effect of rs7132434 on RCC risk, Bigot et al. used RNA seq to identify the effect of this variant on the *BHLHE41* and observed that over expression of *BHLHE41* is correlated to higher growth rate of RCC tumors in mice and that this molecule acts through the induction of IL-11, which further activates *STAT2* for cell cycle progression and apoptosis inhibition [20]. *BHLHE41* is described to be involved in the control of circadian rhythm, cell differentiation and apoptosis [21]. Also one study has shown that dysregulations in genes involved in circadian clock circuitry are related to disease progression and patient outcome [22].

Schokrpur et al. used RNAseq for the evaluation of the transcriptome in a *VHL* deficient cell line and observed that this cell line shows over expression of several regulators of *HIF-1 $\alpha$* , which is correlated to an aggressive form of ccRCC [23]. Some studies used RNA seq for identification of translocations from FFPE [24,25] or frozen tissue [26], evaluation of miRNA profile of RCC tumors from FFPE tissue [27], evaluation of the expression of different genes in disease progression and overall survival of RCC patients [28], or invasion and angiogenesis [29]. Also the identification of novel biomarkers for treatment of RCC are identified by RNA seq [30,31].

Pflueger et al. used RNA seq for the correlation of Chimeric read-through RNAs to patients' outcome and showed that they regulate the cellular mechanisms related to patient outcome [32].

Using whole exome and RNA seq data Matsushita et al. observed that non-silent or missense mutation or higher expression of immune related genes and effector genes are not correlated to the prognostic of ccRCC patients, but A-neo (hi)/HLA-A(hi) or ABC-neo(hi)/ $\beta$ 2M(hi) phenotype predicts a better clinical outcome for these patients. [33]

## Whole exome sequencing

Whole genome sequencing (**WGS**) is an analysis that determines the entire DNA sequence of the genome of one organism, including chromosomal DNA and mitochondrial DNA or chloroplast DNA for plants, whereas whole exome sequencing (**WES**) is the analysis of the DNA sequence of all the expressed genes in an organism. This technique analyzes the DNA sequencing contained in the all exons of the studied organisms, but not in the intronic area or the chromosomes.

A study on patients treated with VEGF- targeted therapy using whole exome sequencing observed that patients that have *PBRM1* mutations have a better response to this therapy. *PBRM1* is part of the *SWI/SNF* chromatin remodeling complex, which is involved in the development transition and cell-type specific transcription [34]. Using whole exome sequencing Ji et al. showed that synchronous tumors in RCC patients do not start from germ line mutation, but rather from *de novo* mutations in each kidney. This study was done only in two patients so their findings need to be validated on a larger patient cohort [35]. Another study used whole exome sequencing in order to evaluate the genetic alterations that appear in the sarcomatoid phenotype of a ccRCC tumor. They observed that this type of phenotype harbors higher number of *TP53* mutations



or *NF2* mutations, but these mutations are exclusive. Also these tumors present *VHL* and *NF2* simultaneous mutations [36]. Also, Ren et al. observed high differences in common RCC component and the sarcomatoid component, with main altered pathways being embryonic digestive tract morphogenesis and signal transduction regulation [37].

When comparing a benign renal disease and papillary RCC (**pRCC**) Zhang et al identify a somatic missense mutation in the proto-oncogene *MET* by using whole exome sequencing, this mutation being specific to pRCC tumors, which mean that the pathogenesis of this cancer involves the *MET* signaling pathways for progression [38].

One study compared two types of papillary RCC and observed that type 1 RCC is characterized by *MET* alteration, while type 2 RCC is characterized by *SETD2* mutations [39]. Liu et al. observed, in a whole exome sequencing study on type 2 pRCC, genetic alterations in genes involved in cell adhesion, microtubule-based movement, the cell cycle, polysaccharide biosynthesis, muscle cell development and differentiation, cell death, and negative regulation when compared to normal renal tissue [40].

As we can see whole exome sequencing can be easily applied to different applications and give highly specific data, other applications of whole exome sequencing are the evaluation of the heterogeneity of synchronous tumors in *VHL* mutated RCC patients [41], identification of the origin of the chromophobe renal cell carcinomas [42], screening for new therapy targets in metastatic RCC [43] or identification of new subtypes of RCC based on the genetic profile of this type of cancer [44].

## Single cell sequencing

The used of single cell technology is starting to be more and more used in the field of genetics, this technology is based on the isolation of either DNA or RNA from a single cell of the sample. There are several methods used for single cell DNA isolation, but the most used are micro-fluidic based. This technology provides high-resolution views of the genome of an organism by using the data only from a single cell [45].

This is an important development in “omics” due to the fact that it can help scientists and clinicians to discriminate between the heterogeneity of the tumor sample. Kim et al., studied the single cell RNA sequencing application in metastatic RCC. For the single cell RNA extraction they used the Fluidigm technology and then the extracted RNA was used in a NGS method using the Illumina HiSeq 2000 instrument. They observed that by using sc-RNA sequencing the heterogeneity of the drug target pathway was better screen in refractory mRCC patient. Also they observed that by using co-targeting strategies, which showed better results that mono-therapy [46]. Another study used single-cell exome sequencing of a RCC tumor of a Chinese patient, and observed mutations in genes like *AHNAK*, *LRRK2*, *SRGAP3* and *USP6*, and also they showed that this type of technology could give detailed and specific information about the development and lineage origin of a specific tumor [47].

## Main Findings in RCC with Special Focus on the TCGA Results

When using TCGA RNA-seq data Zhao et al. observed that androgen receptor (**AR**) and its associated gene expression is correlated to the prognostic of ccRCC, and the higher the expression of these gene the longer the survival of patients. Also they proposed that therapies that promote the retention of AR signaling could be beneficial for ccRCC patients [48]. Another study described a model for prognostic evaluation of RCC patients composed of the expression level of a set of five genes. The five genes chosen for this model were *CKAP4*, *ISPD*, *MAN2A2*, *OTOF*, and *SLC40A1*, which presented the higher specificity and significance related to the prognostic of RCC patients [49].

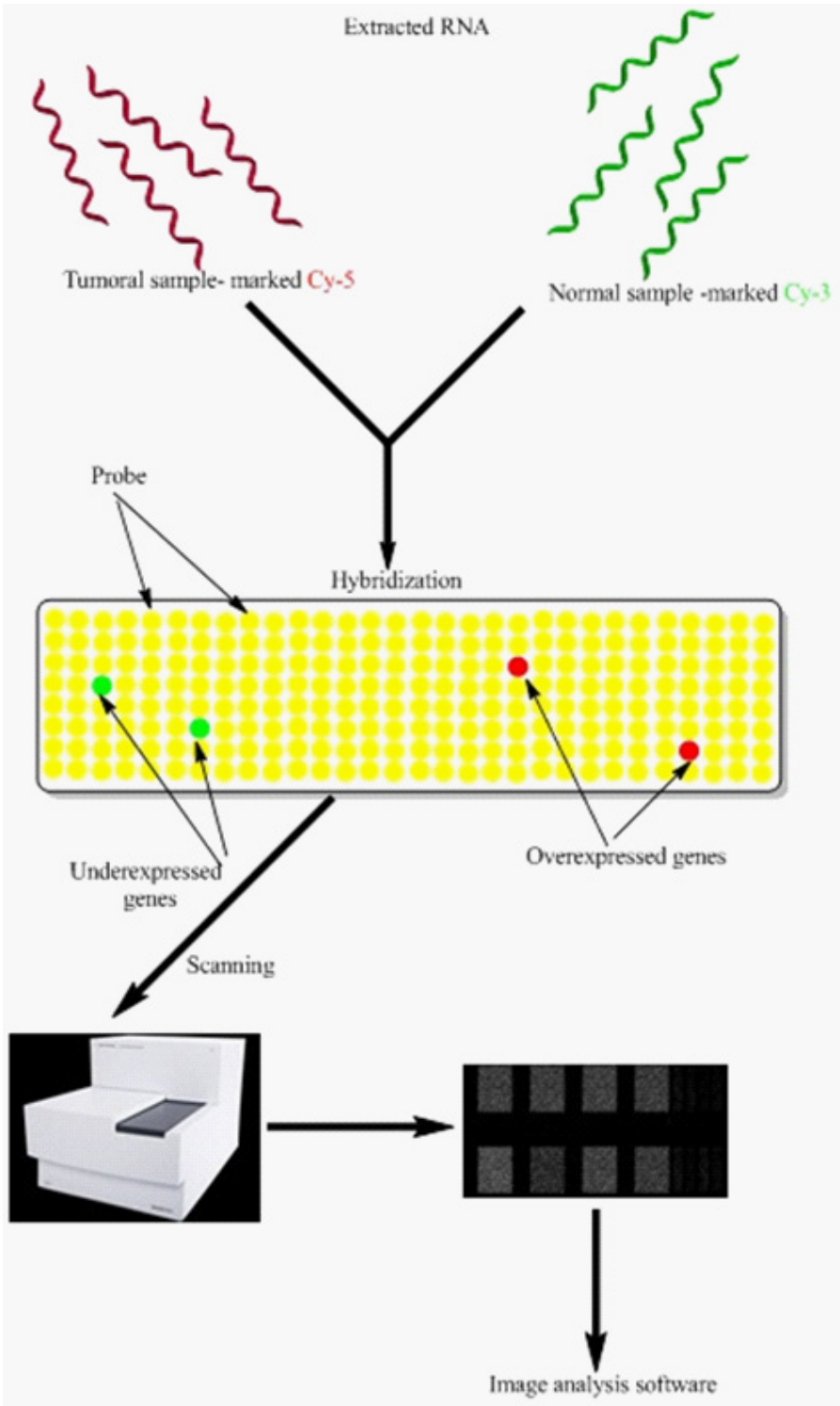
In another study that used TCGA RNA-seq data in order to evaluate the role of the Major histocompatibility complex class I-related chains A (**MICA**) in RCC, Zhang et al. observed that MICA is associated to epithelial -mesenchymal transition genes, observation that could be used for the characterization of the cancer progression in RCC patients [50]. Regarding the correlation of specific miRNA with prognostic of RCC patients, Fritz et al. showed by an analysis TCGA deep sequencing data that miR21/10b are independent prognostic factors in metastatic free patients [51].

TCGA sequencing data was used also for the identification of a novel therapeutic compound for ccRCC. In this study identify TGX221 as suitable treatment option for *VHL* and *SETD2* mutated ccRCC tumors. For this study they used sequencing data, CNV data and protein expression data from the TCGA [52].

## MICROARRAY ANALYSIS FOR RENAL CELL CARCINOMA DIAGNOSIS AND PROFILING

### Fundamentals

The microarray technology is a very versatile method of analysis that can be employed for gene expression analysis, miRNAs analysis or mutation and CNV analysis. Using such technology one can evaluate at different levels oncogenes and tumor suppressor genes, involved in cancer progression and response to therapy [53,54]. Using microarray we can evaluate a high number of transcripts, genes or miRNAs, which in turn produces high amount of data. The data obtained by microarray needs bioinformatics software analysis. The results could be integrated with the clinical data of the patients or with other results obtained by other analysis methods. The result obtain by microarray analysis could be correlated with the clinical outcome of the patients, when it gives information about the response to therapy or the progression of the disease, or it can be correlated to the molecular pattern and be used for staging or for identification of diagnosis biomarkers. A generic workflow for a microarray analysis is presented in figure 3.



**Figure 3:** Microarray workflow.

When searching for microarray analysis in renal carcinoma on PubMed we obtain 202 results. In renal carcinoma studies have showed that microarray can be used for evaluating the prognosis of patients, progression of cancer, and identification of specific biomarkers for different types of renal carcinoma or identification of biomarkers for early diagnosis.

## Approaches

### Transcriptome microarray analysis

The first study using microarray for identification of potential prognosis parameters in RCC was done in 1999 by Moch et al. [55] after this pioneer study in 2001 several other researcher used microarray for RCC classification [56], evaluation of new diagnosis and prognosis biomarkers or treatment evaluation biomarkers [57]. Among these studies, Takahashi et al. identify 109 differently expressed gene in ccRCC and selected 40 of them in correlation to the clinical outcome of 29 patients, and observed that the accuracy exceeded that of staging [58]. When trying to identify biomarkers for RCC subtypes classification Young et al. observed 189 differently expressed genes among the 7075 tested between the four subtypes of RCC tumors [59]. In 2004 another study compared the expression level of 7129 genes both in tumor samples and RCC cell lines and concluded that the expression of 74 genes was altered. Those genes were involved in cell adhesion and cellular transportation. The most up-regulated genes in RCC are part of the cell adhesion pathways and are highly related to carcinogenesis, tumor invasion and metastasis [60].

Chong et al. showed in their study on 138 renal cell carcinoma (**RCC**) patients that an elevate expression of *CD1d* was associated to a poor prognosis of renal cell carcinoma patients. In their study the microarray platform used was the Affymetrix instrument and the Affymetrix Gene Chip Human Genome U133 Plus 2.0 Array, which evaluates the expression of over 47000 human transcripts [61]. *CD1d* is mainly expressed in monocytes, B cells, macrophages and dendritic cells. Also they have a major role in presenting glycolipids to NKT cells and it has been showed that the *CD1d* positive tumor cell is sensitive to NKT cell killing [62].

Another study conducted on microarray data available in the TCGA database was able to obtain a signature consisting of 22 differently expressed miRNA and showed to be an independent prognostic factor for ccRCC. The study was conducted on tumors samples from 411 Caucasian patients. Ge et al. showed ccRCC patients could be divided in 2 groups, low risk and high risk according to the expression of this miRNA signature. Of the 22 miRNA, 13 were negatively correlated to patient's OS and they were described as "risky" miRNA, and the other 9 miRNA were positively correlated to patient's OS and were described as positive miRNA. The "risky" miRNA were mir-223, mir-21, mir-130b, mir-183, mir-365-2, mir-18a, mir-335, mir-149, mir-9-2, mir365-1, mir-9-1, mir-625, mir-146b, while the positive correlated miRNA are mir-584, mir-10b, mir27b, mir-769, mir-181a-2, mir-23b, mir204, mir-24-1 and mir-139. The authors concluded that this miRNA signature could be used for ccRCC prognostic and helps also to predict treatment outcome [63].

## CNV and epigenetic microarray analysis

One study on CNV analysis of sporadic papilloma renal cell carcinomas showed that 46 % of type II RCC and 81% of type I RCC have CNV alterations, mainly gains. They used the Agilent technology for the experiment and also observed a highly statistic correlation between the CNV alterations and mRNA expression level [64].

Another study, using CpG-methylation microarray based assay, observed that by using a five genes corresponding to five - CpGs: *FOXE3*, *PITX1*, *RIN1*, *TWF2* and *EHBP1L1*; could be used as a practical and reliable prognostic tool for ccRCC [65].

## Main findings in RCC with special focus on the TCGA results

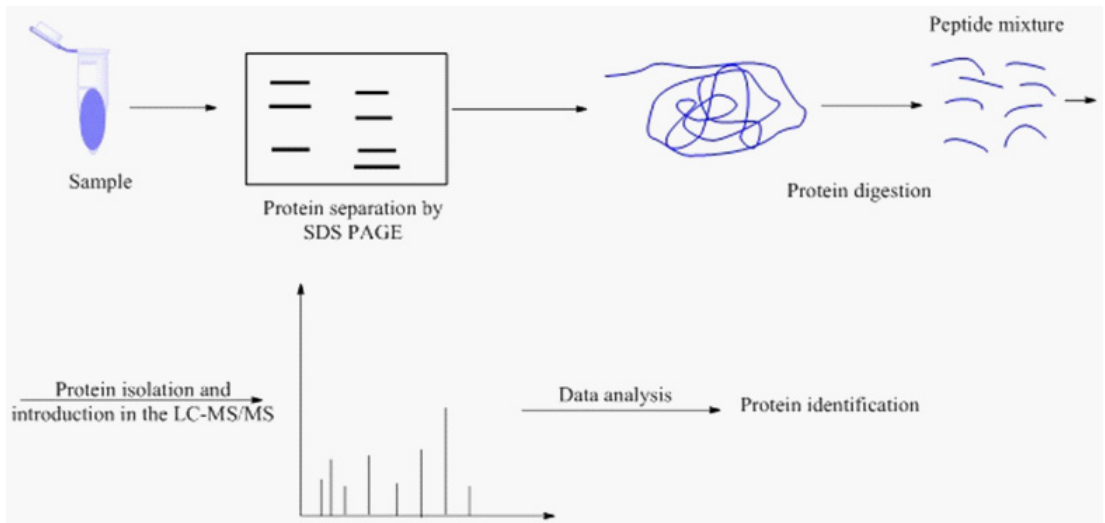
Looking at the studies that used TCGA microarray data we identify two major studies that used epigenetic data for the evaluation of prognostic of RCC patients. In the first study authors observed that aberrant hypermethylation of genes like *VHL* and *UQCRH* are predictors of adverse prognosis in ccRCC [66]. The other study identify that a low *SLC16A3* DNA methylation at specific CpG sites is related to worst patient survival of ccRCC patients [67].

## PROTEOMIC ANALYSIS OF RENAL CELL CARCINOMA

### Fundamentals

The term proteomics was first introduced in 1995 and meant the large-scale characterization of whole proteins found in a cell line, tissue or organism [68]. First studies that could be called proteomic studies were conducted in 1975 when the bidimensional electrophoresis technique was developed [69]. The advantages of this technology were: separation and visualization of a large number of proteins from a mixture, but still its main disadvantage was the fact that you could not identify the proteins you separated. In order to overcome this limitation the protein sequencing technology was developed. This technology was based on Edman protein degradation method [70]. Even with this technology the process was long and a high amount of sample was needed. These disadvantages were overcome when the mass spectrometry (**MS**) technology was launched [71]. The MS technology is more sensitive and can identify proteins even at a concentration of femto-molars. Due to its performance MS applied proteomics was used in studies of proteomic profile identification, post-translational modification identification, evaluation of protein-protein interactions, protein structure, functional proteomics or discovery of new targets for new drugs development [72]. The workflow for a proteomic experiment using LC-MS/MS technology is presented in figure 4.

In a search on PubMed for proteomic studies using MS technology in RCC 45 articles were identify on the evaluation of biomarkers for early diagnosis, overall survival, discovery of new drug targets, prognosis, subtype classification or treatment evaluation.



**Figure 4:** LC-MS/MS workflow for protein identification.

## Approaches

A study on ccRCC showed that kinase activity could be used for ccRCC classification. They studied the kinase activity in ccRCC by protein microarray of 41 ccRCC patients. By employing several bioinformatics analyses, they were able to identify three clusters separated by different kinase activity in inflammation pathways (cluster A), translocation initiation (cluster B), and immune response and cell adhesion pathways (cluster C). From these three clusters, patients from cluster C showed the higher recurrence rate. In this study, authors also compared ccRCC tumors to normal renal tissue and observed that kinase activity increased in the case of PIM's and MAPKAPK's and decreased in the case of JNK2 and CDK1. Also, when correlated the kinase activity pattern to tumor progression, a significant difference was observed only in the case of serine/threonine kinase, which was reduced in non-progressed tumors [73].

When looking for noninvasive diagnosis biomarkers, White et al. showed that Hsp27 is elevated in RCC patient serum in contrast to normal serum. They also observed in their study that 55 proteins are deregulated in RCC tissue, and that these proteins are playing an important role in carcinogenesis. Also, 39 of these proteins are secretory proteins that, if found in serum or urine, could be used as specific RCC diagnosis biomarkers. In their study, White et al. used isobaric tags for relative and absolute quantization labeling (iTRAQ) and LC-MS/MS analysis for protein identification in 199 patients [74]. Zhang et al. also used iTRAQ proteomics in their study on 30 serum samples (10 controls and 20 RCC patients) and identified HSC71 as a potential noninvasive early diagnosis biomarker for RCC [75]. Using MALDI-TOF and LC-MS/MS analysis, Gianazza et al. were able to identify specific deregulated proteins specific for RCC, and benign renal tumors compared to normal controls. Their study was conducted on serum samples from 92 controls (68 men, 24 women), 85 ccRCC patients (54 men, 31 women), and 29 non-ccRCC patients (15 men,

14 women). They identified 32 proteins, from which SDPR and ZYX were down regulated, while SRGN and TMSL3 were up-regulated. In ccRCC patient FIBA was presented in high concentration and F13A, THRB and IC1, SRGN are observed in low concentrations [76].

In a study regarding the prognosis of patients with RCC, Muller et al. observed that the concentration of vitamin B6 can be used as a prognostic factor. More exactly low concentration of vitamin B6 in blood at diagnosis is associated to bad prognosis, while high concentration of vitamin B6 in blood at diagnosis is correlated to a better survival, independently of RCC stage. The study was conducted on 630 patients and the vitamin B6 levels were determining using LC-MS/MS technology [77]. Sandim et al. used three different proteomic approaches to identify different secreted proteins in the urine of ccRCC patients and healthy individuals. They tested 30 urine samples from healthy individuals (**CG**), 35 ccRCC Fuhrman grade I/II patients (**GP**) and 34 ccRCC Fuhrman grade IV patients (**PP**). From the differently secreted proteins, kininogen-1, uromodulin, apolipoprotein D, polyubiquitin and CD59 glycoprotein were down-expressed in the study groups as follows CG>GP>PP, while apolipoprotein A, fibrinogen and haptoglobin were up-secreted in the patient groups, then in CG [78]. Kim et al. used LC-MS/MS for a metabolomic study on patients with RCC and healthy individuals observed that there were 10 differently expressed metabolites in the urine of the two groups, only three showed statistical significant difference. Quinolate levels were increased in patient group, while 4-hydroxybenzoate and genisate were decreased when compared to the healthy individual group. These metabolites are involved in benzoate degradation via hydroxylation pathway and upstream of glycolysis pathway [79].

Gbormittah et al. compared the proteom, glycoproteom and N-glycome of 20 plasma samples of patients with ccRCC before and after curative nephrectomy and observed that HSPG2, CD146, ECM1, SELL, SYNE1 and VCAM1 have significant different expression levels in the studied samples. Also they observed a strong correlation of the differently expressed proteins and the clinical status of the patients [80].

Na et al tried to identify specific proteins for differentiation of the papillary renal cell carcinoma by using matrix-assisted laser desorption ionization (**MALDI**) mass spectrometry based imaging mass spectrometry (**IMS**). In their study S100A11 and ferritin light chain were identify to be over-expressed in tumor tissue then in normal one [81].

In conclusion there three “omics” technology can be successfully used for the early diagnostic and progression biomarkers evaluation, as well as for biomarkers specific for each subtype of renal cell carcinoma identification.

## ACKNOWLEDGMENT

This work was supported by the National Plan II Program PN-II-PT-PCCA-2011-3.1-1221 IntelUro, contract no. 125/2012: “Intelligent systems of prediction of recurrence and progression in superficial bladder cancer based on artificial intelligence and microarray data: tumor mRNA and plasmatic microRNA-IntelUro”.



## References

1. Facts Sheet by Population.
2. Banks RE, Craven RA, Hamden P, Madaan S, Joyce A, et al. Key clinical issues in renal cancer: a challenge for proteomics. *World J Urol.* 2007; 25: 537-556.
3. Rathmell WK, Godley PA. Recent update in renal cell carcinoma. *Curr Opin Oncol.* 2010; 22: 250-256.
4. Mulders P, Figlin R, deKernion JB, Wiltout R, Linehan M, et al. Renal cell carcinoma: recent progress and future directions. *Cancer Res.* 1997; 57: 5189-5195.
5. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America.* 1977; 74: 5463-5467.
6. Holt RA, Jones SJ. The new paradigm of flow cell sequencing. *Genome research.* 2008; 18: 839-846.
7. Michael AQ, Miriam S, Paul C, Thomas DO, Simon RH, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers *BMC Genomics.* 2012; 13: 341.
8. Lin L, Li Y, Li S, Hu N, He Y, et al. Comparison of Next Generation Sequencing Systems *Journal of Biomedicine and Biotechnology.* 2012.
9. Lin E, Chien J, Ong FS, Fan JB. Challenges and opportunities for next-generation sequencing in companion diagnostics. *Expert Rev. Mol. Diagn.* 2015; 15: 193-206.
10. Singh RR, Murugan P, Patel LR, Voicu H, Yoo S-Y, et al. Intratumoral Morphologic and Molecular Heterogeneity of Rhabdoid Renal Cell Carcinoma: Challenges for Personalized Therapy. 2015; 28: 1225-1232.
11. Lawrie CH, Larrea E, Larrinaga G, Goicoechea I, Arestin M, et al. Targeted next-generation sequencing and non-coding RNA expression analysis of clear cell papillary renal cell carcinoma suggests distinct pathologic mechanisms from other renal tumor subtypes. *J Pathol.* 2014; 232:32-42.
12. Huang Y, Gao S, Wu S, Song P, Sun X, et al. Multilayered molecular profiling supported the monoclonal origin of metastatic renal cell carcinoma. *Int. J. Cancer.* 2013; 135: 78-87
13. Cancer Genome Atlas Research, Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature.* 2013; 499: 43-49.
14. Sato Y, Yoshizato T, Shiraishi Y, Maekawa S, Okuno Y, et al. Integrated molecular analysis of clear cell renal cell carcinoma. *Nat. Genet.* 2013; 45: 860-867
15. Dalgliesh G, Furge K, Greenman C, Chen L, Bignell G, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature.* 2010; 463: 360-363
16. Guo G, Gui Y, Gao S, Tang A, Hu X, et al. Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. *Nat. Genet.* 2012; 44: 17-19
17. Arai E, Sakamoto H, Ichikawa H, Totsuka H, Chiku S, et al. Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. *Int. J. Cancer.* 2014; 135: 1330-1342
18. Scelo GL, Riazalhosseini Y, Greger L, Letourneau L, González-Porta M, et al. Variation in genomic landscape of clear cell renal cell carcinoma across Europe. *Nat. Commun.* 2014; 5: 5135
19. Haddad AQ, Margulis V. Tumour and patient factors in renal cell carcinoma-towards personalized therapy. *Nat Rev Urol.* 2015; 12: 253-262.
20. Bigot P, Colli LM, Machiela MJ, Jessop L, Myers TA, et al. Functional characterization of the 12p12.1 renal cancer-susceptibility locus implicates BHLHE41. *Nat Commun.* 2016; 7: 12098.
21. Kato Y, Kawamoto T, Fujimoto K, Noshiro M. DEC1/STRA13/SHARP2 and DEC2/SHARP1 coordinate physiological processes, including circadian rhythms in response to environmental stimuli. *Curr. Top. Dev. Biol.* 2014; 110: 339-372.
22. Montagner M, Enzo E, Forcato M, Zanconato F, Parenti A, et al. SHARP1 suppresses breast cancer metastasis by promoting degradation of hypoxia-inducible factors. *Nature.* 2012; 487: 380-384.
23. Shokrpur S, Hu J, Moughon DL, Liu P, Lin LC, et al. CRISPR-Mediated VHL Knockout Generates an Improved Model for Metastatic Renal Cell Carcinoma. *Sci Rep.* 2016; 6: 29032.
24. Just PA, Letourneur F, Pouliquen C, Dome F, Audebourg A, et al. Identification by FFPE RNA-Seq of a new recurrent inversion leading to RBM10-TFE3 fusion in renal cell carcinoma with subtle TFE3 break-apart FISH pattern. *Genes Chromosomes Cancer.* 2016; 55: 541-548.



25. Huang W, Goldfischer M, Babyeva S, Mao Y, Volyanskyy K, et al. Identification of a novel PARP14-TFE3 gene fusion from 10-year-old FFPE tissue by RNA-seq. *Genes Chromosomes Cancer*. 2015.
26. Pflueger D, Sboner A, Storz M, Roth J, Compérat E, et al. Identification of molecular tumor markers in renal cell carcinomas with TFE3 protein expression by RNA sequencing. *Neoplasia*. 2010; 15:1231-1240.
27. Weng L, Wu X, Gao H, Mu B, Li X, et al. MicroRNA profiling of clear cell renal cell carcinoma by whole-genome small RNA deep sequencing of paired frozen and formalin-fixed, paraffin-embedded tissue specimens. *J Pathol*. 2010; 222: 41-51.
28. Pei X, Li M, Zhan J, Yu Y, Wei X, et al. Enhanced IMP3 Expression Activates NF- $\kappa$ B Pathway and Promotes Renal Cell Carcinoma Progression. *PLoS One*. 2015; 10: e0124338.
29. Pflueger D, Mittmann C, Dehler S, Rubin MA, Moch H, et al. Functional characterization of BC039389-GATM and KLK4-KRSP1 chimeric read-through transcripts which are up-regulated in renal cell cancer. *BMC Genomics*. 2015; 16: 247.
30. Xiong Z, Yu H, Ding Y, Feng C, Wei H, et al. RNA sequencing reveals upregulation of RUNX1-RUNX1T1 gene signatures in clear cell renal cell carcinoma. *Biomed Res Int*. 2014; 450621.
31. Pal SK, He M, Tong T, Wu H, Liu X, et al. RNA-seq reveals aurora kinase-driven mTOR pathway activation in patients with sarcomatoid metastatic renal cell carcinoma. *Mol Cancer Res*. 2015; 13: 130-137.
32. Salama MF, Carroll B, Adada M, Pulkoski-Gross M, Hannun YA, et al. A novel role of sphingosine kinase-1 in the invasion and angiogenesis of VHL mutant clear cell renal cell carcinoma. *FASEB J*. 2015; 29: 2803-2813.
33. Matsushita H, Sato Y, Karasaki T, Nakagawa T, Kume H, et al. Neo-antigen Load, Antigen Presentation Machinery, and Immune Signatures Determine Prognosis in Clear Cell Renal Cell Carcinoma. *Cancer Immunol Res*. 2016; 4: 463-471.
34. Malouf GG, Ali SM, Wang K, Balasubramanian S, Ross JS, et al. Genomic Characterization of Renal Cell Carcinoma with Sarcomatoid Dedifferentiation Pinpoints Recurrent Genomic Alterations. *Eur Urol*. 2016; 70: 348-357.
35. Ren Y, Liu K, Kang X, Pang L, Qi Y, et al. Chromophobe renal cell carcinoma with and without sarcomatoid change: A clinicopathological, comparative genomic hybridization, and whole-exome sequencing study. *Am J Transl Res*. 2015; 7: 2482-2499.
36. Zhang W, Tan AY, Blumenfeld J, Liu G, Michael A, et al. Papillary renal cell carcinoma with a somatic mutation in MET in a patient with autosomal dominant polycystic kidney disease. *Cancer Genet*. 2016; 209:11-20.
37. Linehan WM, Spellman PT, Ricketts CJ, Creighton CJ, Fei SS, et al. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. *Cancer Genome Atlas Research Network, N Engl J Med*. 2016; 374: 135-145.
38. Liu K, Ren Y, Pang L, Qi Y, Jia W, et al. Papillary renal cell carcinoma: A clinicopathological and whole-genome exon sequencing study. *Int J Clin Exp Pathol*. 2015; 8: 8311-8335.
39. Fisher R, Horswell S, Rowan A, Salm MP, de Bruin EC, et al. Development of synchronous VHL syndrome tumors reveals contingencies and constraints to tumor evolution. *Genome Biol*. 2014; 15: 433.
40. Davis CF, Ricketts CJ, Wang M, Yang L, Cherniack AD, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. *Cancer Cell*. 2014; 26: 319-330.
41. Voss MH, Hakimi AA, Pham CG, Brannon AR, Chen YB, et al. Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy. *Clin Cancer Res*. 2014; 20: 1955-1964.
42. Peña-Llopis S, Vega-Rubín-de-Celis S, Liao A, Leng N, Pavía-Jiménez A, et al. BAP1 loss defines a new class of renal cell carcinoma. *Nat Genet*. 2012; 44: 751-759.
43. Fay AP, de Velasco G, Ho TH, Van Allen EM, Murray B, et al. Whole-Exome Sequencing in Two Extreme Phenotypes of Response to VEGF-Targeted Therapies in Patients with Metastatic Clear Cell Renal Cell Carcinoma. *J Natl Compr Canc Netw*. 2016; 14: 820-824.
44. Ji Z, Zhao J, Zhao T, Han Y, Zhang Y, et al. Independent Tumor Origin in Two Cases of Synchronous Bilateral Clear Cell Renal Cell Carcinoma. *Sci Rep*. 2016; 6: 29267.
45. Gowad C, Koh W, Quake SR. Single-cell genome sequencing: Current state of the science. *Nature Reviews. Genetics*. 2016; 17: 175-188.
46. Kim KT, Lee HW, Lee HO, Song HJ, Jeong da E, et al. Application of single-cell RNA sequencing in optimizing a combinatorial therapeutic strategy in metastatic renal cell carcinoma. *Genome Biol*. 2016; 17: 80.
47. Zhao H, Leppert JT, Peehl DM. A Protective Role for Androgen Receptor in Clear Cell Renal Cell Carcinoma Based on Mining TCGA Data. *PLoS One*. 2016; 11: e0146505.
48. Zhan Y, Guo W, Zhang Y, Wang Q, Xu XJ, et al. A Five-Gene Signature Predicts Prognosis in Patients with Kidney Renal Clear Cell Carcinoma *Comput Math Methods Med*. 2015; 2015: 842784.

49. Zhang X, Yan L, Jiao W, Ren J, Xing N, et al. The clinical and biological significance of MICA in clear cell renal cell carcinoma patients *Tumour Biol.* 2016; 37: 2153-2159.
50. Fritz HK, Lindgren D, Ljungberg B, Axelson H, Dahlbäck B. The miR(21/10b) ratio as a prognostic marker in clear cell renal cell carcinoma. *Eur J Cancer.* 2014; 50:1758-1765.
51. Feng C, Sun Y, Ding G, Wu Z, Jiang H, et al. PI3K $\beta$  inhibitor TGX221 selectively inhibits renal cell carcinoma cells with both VHL and SETD2 mutations and links multiple pathways. *Sci Rep.* 2015; 8: 9465.
52. Xu X, Hou Y, Yin X, Bao L, Tang A, et al. Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. *Cell.* 2012; 148: 886-895.
53. Slonim DK, Yanai I. Getting started in gene expression microarray analysis. *PLoS computational biology.* 2009; 5: e1000543.
54. Berretta R, Moscato P. Cancer biomarker discovery: The entropic hallmark. *PLoS one.* 2010; 5: e12262.
55. Moch H, Schrami P, Bubendorf L, Mirlacher M, Kononen J, et al. Identification of Potential Prognostic Parameters for Renal Cell Carcinoma by Tissue Microarray Analysis and cDNA Microarray Screening *Verh Dtsch Ges Path.* 1999; 83:225-223.
56. Boer JM, Huber WK, Sultmann H, Wielmer F, von Heydebreck A, et al. Identification and Classification of Differentially Expressed Genes in Renal Cell Carcinoma by Expression Profiling on a Global Human 31500-Element cDNA Array. *Genome Research.* 2001; 11: 1861-1870.
57. Zhou M, Rubin MA. Molecular Markers for renal cell carcinoma: Impact on diagnosis and treatment; *Seminars in Urologic Oncology.* 2001; 18: 80-87.
58. Takahashi M, Rhodes DR, Furge KA, Kanayama HO, Kagawa S, et al. Gene expression profiling of clear cell renal cell carcinoma: Gene identification and prognostic classification. *Proc Natl Acad Sci.* 2001; 98: 9754-9759.
59. Young AN, Amin MB, Moreno CS, Lim SD, Sohen C, et al. Expression Profiling of Renal Epithelial Neoplasms: A Method for Tumor Classification and Discovery of Diagnostic Molecular Markers. *Am J Pathol.* 2001; 158:1639-1651.
60. Liou LS, Shi T, Duan ZH, Sadhukhan P, Der SD, et al. Microarray gene expression profiling and analysis in renal cell carcinoma. *BMC Urology.* 2004; 4: 1-11.
61. Chong TW, Goh FY, Sim MY, Huang HH, Thike DAA, et al. CD1d expression in renal cell carcinoma is associated with higher relapse rates, poorer cancer-specific and overall survival. *J Clin Pathol.* 2015; 68: 200-205.
62. Fais F, Morabito F, Stelitano C, Callea V, Zanardi S, et al. CD1d is expressed on B-chronic lymphocytic leukemia cells and mediates alpha-galactosylceramide presentation to natural killer T lymphocytes. *Int J Cancer.* 2004; 109: 402-411.
63. Albiges L, Guegan J, Le Formal A, Verkarre V, Rioux-Leclercq N, et al. MET is a potential target across all papillary renal cell carcinomas: result from a large molecular study of pRCC with CGH array and matching gene expression array. *Clin Cancer Res.* 2014; 20: 3411-3421
64. Ge YZ, Wu R, Xin H, Zhu M, Lu T-Z, et al. A tumor-specific microARN signature predicts survival in clear cell renal cell carcinoma. *J Cancer Res and Clin Oncol.* 2015; 141: 1291-1299.
65. Shenoy N, Vallumsetla N, Zou Y, Galeas JN, Shrivastava M, et al. Role of DNA methylation in renal cell carcinoma. *J Hematol Oncol.* 2015; 8: 88.
66. Fisel P, Kruck S, Winter S, Bedke J, Hennenlotter J, et al. DNA methylation of the SLC16A3 promoter regulates expression of the human lactate transporter MCT4 in renal cancer with consequences for clinical outcome. *Clin Cancer Res.* 2013; 19: 5170-5181.
67. Wei JH, Haddad A, Wu KJ, Zhao HW, Kapur P, et al. A CpG methylation based assay to predict survival in clear cell renal cell carcinoma. *Nature Communication.* 2015; 6: 8699.
68. Wasinger VC, Cordwell SJ, Cerpa PA, Yan JX, Gooley AA, et al. Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium.* *Electrophoresis.* 1995; 16:1090-1094.
69. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. *The Journal of biological chemistry.* 1975; 250: 4007-4021.
70. Edman P. A method for the determination of amino acid sequence in peptides. *Archives of biochemistry.* 1949; 22: 475.
71. Andersen JS, Mann M. Functional genomics by mass spectrometry. *FEBS letters.* 2000; 480: 25-31.
72. Graves PR, Haystead TA. Molecular biologist's guide to proteomics. *Microbiology and molecular biology reviews: MMBR.* 2002; 66: 39-63;
73. Anderson JC, Willey CD, Metha A, Walaya K, Chen D, et al. High Throughput Kinomic Profiling of Human Clear Cell Renal Carcinoma Identifies Kinase Activity Dependent Molecular Subtypes; *PLoS One.* 2015; 10: e0139267

74. White NMA, Masui O, DeSoua LV, Krakovska O, Metias S, et al. Quantitative proteomic analysis reveals potential diagnostic markers and pathway involved in pathogenesis of renal cell carcinoma. *Onco target*. 2014; 5: 506-518.
75. Zhang Y, Cai Y, Yu H, Li H. iTRAQ-based Quantitation Proteomic Analysis Identified HSC71 as a Novel Serum Biomarker for Renal Cell Carcinoma. *BioMed Research International*. 2015; 10: 210-219.
76. Gianazza E, Chinello C, Mainini V, Cazzaniga M, Squeo V, et al. Alterations of the serum peptidome in renal cell carcinoma discriminating benign and malign kidney tumors. *Journal of Proteomics*. 2012; 76: 125-140.
77. Muller DC, Johansson M, Zardze D, Moujeria A, Janout V, et al. Circulating Concentration of Vitamin B6 and Kidney Cancer Prognosis: A Prospective Case-Cohort Study. *PLoS One*. 2015; 10: e0140677.
78. Sandim V, de Abreu PD, Kalume DE, Oliveira CAL, Ornellas AA, et al. Proteomic analysis reveals differentially secreted proteins in the urine from patients with clear cell renal cell carcinoma. *Urologic Oncology*. 2016; 24: 5e11-5e25.
79. Kim K, Taylor SL, Ganti S, Guo L, Osier MV, Weiss RH. Urine Metabolomic Analysis Identifies Potential Biomarkers and Pathogenic Pathways in Kidney Cancer. *OMICS*. 2011; 15: 293-303.
80. Gbormittah FO, Lee LY, Taylor KO, Hancock WS. Comparative Studies of the Proteome, Glycoproteome, and N-Glycome of Clear Cell Renal Cell Carcinoma Plasma before and after Curative Nephrectomy. *J Proteom Res*. 2014; 13: 4889-4900.
81. Na CH, Hong JH, Kim WS, Shanta SR, Bang JY, et al. Identification of Protein Markers Specific for Papillary Renal Cell Carcinoma Using Imaging Mass Spectrometry. *Mol. Cells*. 2015; 38: 624-629.