American Journal of BioMedicine
AJBM 2016;4 (1): 54-69
Research Article doi: 10.18081/2333-5106/016-39-54

A signature microRNA to predict biomarker panel for early diagnosis of renal cell cancer

John H. Patel, David Günther, Xing Li, Fang Liu, Yi Zhang, Paritosh B. Pannala, Jeffrey A. Lubezky [1*]

Abstract

Renal cell carcinoma (RCC) is the most prevalent type of kidney malignancy in adults, accounting for approximately 80% of the cases. It ranks as the 7th most common cancer among men and the 10th among women. The most common histological subtype of RCC is clear cell carcinoma (ccRCC), comprising around 70% of all RCC cases. The incidence of RCC is at least twice as high in men compared to women. Furthermore, the incidence of RCC rises with age. In North America and other industrialized countries, the age-adjusted incidence is around 8-10 per 100,000 and is increasing with time. The mortality rates for RCC range from 3.7 to 6.6 per 100,000. These numbers are considerably lower when compared to gallbladder cancer, where nearly 30% of patients diagnosed survive for at least five years. The majority of patients diagnosed with RCC present with renal mass. Patients with localized disease present with a renal mass, while those with advanced disease can present with symptoms of metastatic disease such as weight loss and bone pain. Renal cell carcinoma, like many other malignancies, can be asymptomatic in its early stages. It often presents clinically as an incidental finding of renal masses detected in radiographic studies performed for unrelated reasons. While imaging modalities such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) can identify mass lesions, a large cohort of patients with solid mass lesions does not undergo pathological diagnosis of these lesions. Several clinical studies have reported that a substantial proportion (10% to up to 40%) of solid renal masses greater than 2 cm in size are benign and thus do not require surgery. In addition, malignancy prevalence depends on the mass size. For instance, the prevalence of malignancy of such mass is around 80% when ≥3 cm, and almost 100% when ≥4 cm in size. There is thus an unmet medical need for a non-invasive and highly accurate biomarker that can reliably rule out malignancy in early stages of ccRCC, especially with relatively small solid renal masses ≤3 cm in size.

Keywords: Renal cell cancer; miRNA; Immunotherapy; Early diagnosis; Molecular biology

*Corresponding author email: Lubezky@yahoo.com
Received August 30, 2015; Accepted December; 29, 2015; Published February 06, 2016.
Copyright © Lubezky et al., 2016. This is article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY 4.0) (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Renal cell cancer (RCC) accounts for 90-95% of all primary kidney cancers, and its incidence has been increasing around the world. It is the 10th most common cancer in men and the 14th most common in women, with an estimated 79,000 new cases and 13,368 deaths in the United States in 2020. RCC is predominantly found in adults, with a median age of diagnosis of 64 years. The age-adjusted incidence rate of RCC is approximately 6.9 per 100,000 people in China, and its mortality rate is approximately 2.2 per 100,000 people. An increased risk of RCC is seen in immunosuppressed individuals, as well as those with pre-existing medical conditions, such as obesity, hypertension, chronic renal failure, type 2 diabetes mellitus, hyperlipidemia, tuberous sclerosis, and hereditary syndromes.

Approximately 60% of RCC patients present with localized disease, which can be surgically cured. However, up to 40% of cases usually present with advanced metastatic RCC, most of whom die within 5 years due to resistance to chemotherapeutic agents. Currently, computed tomography (CT) and magnetic resonance imaging (MRI) scans are the mainstay for RCC diagnosis. While commonly used imaging systems show good sensitivity for local RCC diagnosis, their specificity is low for differentiating RCC from benign renal lesions or differentiating several tumor histological subtypes. Unfortunately, more than 20% of patients with localized disease at the time of surgery will have progressed to advanced disease within 5 years. Therefore, there is an urgent need for novel, noninvasive biomarkers for the early detection and prognosis prediction of RCC.

MicroRNAs (miRNAs) are small, approximately 22 nucleotides long, noncoding RNAs that regulate gene expression through base pairing with the 3-untranslated region (3-UTR) of messenger RNA (mRNA), resulting in either translation repression or target mRNA degradation. An individual miRNA can regulate up to hundreds of target genes, and an individual mRNA can possess multiple binding sites for one or several miRNAs. Therefore, the miRNA-mRNA regulatory network is nonlinear and highly complex, generally producing fine-tuning effects on gene expression. Mature miRNAs form the cytoplasmic RNA-induced silencing complex (RISC) that mediates their translation regulation function, and their biogenesis includes several steps. The transcription of miRNA genes generates primary miRNAs that are processed into precursor miRNAs in the nucleus. Precursor miRNAs are translocated to the cytoplasm, and then further cleaved by Dicer to generate mature miRNAs. Bioactive mature miRNAs form the RISC complex that binds target mRNAs, leading to translation inhibition and degradation of target mRNA.

During carcinogenesis, the biogenesis of miRNAs can be altered, resulting in aberrant expressions of miRNAs, which play pivotal roles in the development and progression of cancer. Since the pioneering works published in 2002, aberrant expressions of miRNAs, staff members, or both have been identified in nearly all kinds of human cancers, with the most studied being

Research Article doi: 10.18081/2333-5106/016-39-54

classic solid tumors, including colon, breast, and prostate cancers. More recently, a single miRNA or signature (a group of) miRNAs has been discovered as a potential biomarker for the early detection or prognosis prediction of various cancers. Most signature (a group of) miRNAs were discovered using multiplexed measurement platforms, including qRT-PCR, miRNA arrays, and next-generation sequencing, in combination with bioinformatic algorithms. The study aimed to identify a signature miRNA for the early diagnosis of renal cell cancer.

Renal Cell Cancer

Renal cell carcinoma (RCC) is the most prevalent type of kidney malignancy in adults, accounting for approximately 80% of the cases. It ranks as the 7th most common cancer among men and the 10th among women. The most common histological subtype of RCC is clear cell carcinoma (ccRCC), comprising around 70% of all RCC cases. The incidence of RCC is at least twice as high in men compared to women. Furthermore, the incidence of RCC rises with age. In North America and other industrialized countries, the age-adjusted incidence is around 8-10 per 100,000 and is increasing with time. The mortality rates for RCC range from 3.7 to 6.6 per 100,000. These numbers are considerably lower when compared to gallbladder cancer, where nearly 30% of patients diagnosed survive for at least five years. The majority of patients diagnosed with RCC present with renal mass. Patients with localized disease present with a renal mass, while those with advanced disease can present with symptoms of metastatic disease such as weight loss and bone pain.

Renal cell carcinoma, like many other malignancies, can be asymptomatic in its early stages. It often presents clinically as an incidental finding of renal masses detected in radiographic studies performed for unrelated reasons. While imaging modalities such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) can identify mass lesions, a large cohort of patients with solid mass lesions does not undergo pathological diagnosis of these lesions. Several clinical studies have reported that a substantial proportion (10% to up to 40%) of solid renal masses greater than 2 cm in size are benign and thus do not require surgery. In addition, malignancy prevalence depends on the mass size. For instance, the prevalence of malignancy of such mass is around 80% when ≥3 cm, and almost 100% when ≥4 cm in size. There is thus an unmet medical need for a non-invasive and highly accurate biomarker that can reliably rule out malignancy in early stages of ccRCC, especially with relatively small solid renal masses ≤3 cm in size.

Importance of Early Diagnosis

Current imaging techniques can detect relatively small, local RCC at early stages. Indeed, there is convincing evidence that early diagnosis of RCC increases long-term survival, as shown for those patients undergoing surgery for clinically localized RCC as well as, to a lesser extent, for 'incidentally' detected RCC during the course of investigation and/or treatment for other unrelated medical disorders, in particular patients with ADPKD. Radical nephrectomy is still the treatment of choice for patients with T1 RCC. However, the widespread use of abdominal imaging has also increased the awareness of the incidental discovery of 'small renal masses',

Research Article doi: 10.18081/2333-5106/016-39-54

which now account for up to 75% of new RCC diagnoses. These 'incidentally diagnosed' masses include localized RCC tumors that were identified while images were obtained for an unrelated reason, and masses that were detected during routine check-up, because of the symptoms associated with the tumor, or because of the presence of a hereditary syndrome.

Although we have more data available about the biologic behavior of small RCC, we still cannot predict with high accuracy, based on preoperative findings, the behavior of an individual lesion as all renal masses are currently treated as malignant until proven otherwise. Only after incomplete or fine needle aspiration, we can obtain histologic data, and thereby differentiate benign from malignant lesions, including RCC. Small enhancing multifocal renal masses, which are seen in up to 28% of patients with the hereditary syndrome Von Hippel Lindau (VHL), can all be malignant, so in particular patients with hereditary disorders an active surveillance approach is seldom used. An active surveillance approach is also employed in patients with a high surgical risk caused by age or concomitant diseases or in patients with bilateral multifocal renal masses. Moreover, it has been demonstrated that many localized RCC growing at a slow rate, regardless of size. These and other data have led to wide support of an initial surveillance strategy in the management of elderly or infirmed patients, with the underlying strategy to perform later surgery, if the tumor progresses in size.

Biological Basis of microRNAs

Mature miRNAs are 20-24 nucleotides long and are processed from miRNA precursors. They constitute a new class of genes expressed in animal cells. MicroRNAs (miRNAs) primarily bind to the 3' untranslated region and mediate translational suppression or mRNA instability. There is evidence that microRNAs control gene expression mainly by destabilizing target mRNA. Many expressed microRNAs are poorly conserved, while others show a high level of sequence conservation among different species, suggesting significant evolutionary pressure. There is growing evidence that miRNAs are excellent tissue markers and promise to surpass the known markers in tissue classification. MicroRNAs are involved in the regulation of many cell functions including cellular differentiation, proliferation, apoptosis, cancer, and an increasing number of human genetic diseases, providing new perspectives to researchers for the diagnosis and treatment of diseases.

There are several mechanisms of microRNA biogenesis including the non-canonical microRNA biogenesis pathway, microRNA gene organization, and expression regulation. MicroRNA gene organization and expression are similarly regulated. The biogenesis of individual miRNAs is also bootstrapped independently and can give rise to miRNA co-expression by divergent transcription. The large number of miRNAs influences the response of their target transcripts due to their average target gene regulation. The involvement of miRNAs and cancer is an area of growing interest. Many cancer types exhibit unique miRNA profiles that are not only tissue-specific but are also disease entities themselves. The demonstration that miRNAs are dysregulated in cancer cells and act as tumor suppressors or oncogenes has prompted

Research Article doi: 10.18081/2333-5106/016-39-54

researchers to consider the development of miRNA-based cancer diagnostics and therapeutics.

Role of microRNAs in Gene Regulation

MicroRNAs, small non-coding single-strand endogenous RNA molecules about 22 nucleotides in length, repress gene expression via the sequence-specific binding to target mRNAs and degradation or translational inhibition of the target mRNAs. There is accumulating evidence that microRNAs play critical roles in numerous biological processes including development, cell proliferation, differentiation, and apoptosis. Remarkably, recent studies have shown that the regulatory functions of microRNAs are involved in tumorigenesis and cancer development. Specific microRNAs were reported as tumor suppressor genes or oncogenes, as ectopic expression of tumor-suppressive microRNAs in a cancer cell line results in the suppression of tumorigenicity in vitro and/or in vivo.

Renal cell cancer (RCC) takes up 90-95% of primary malignant kidney tumors in adults. It is often hard to cure, particularly due to generally late diagnosis of RCC. It is urgent to establish a novel diagnostic method for the early diagnosis of RCC. In the search for tumor marker microRNAs specific for clear cell RCC, genomic profiling of microRNA expression was compared between this disease and normal kidney tissue. Then, the association of the expression of these microRNAs and tumor suppressor Von Hippel-Lindau (VHL) gene, frequently inactivated at an early stage of tumorigenesis of clear cell RCC, was examined.

Biogenesis and Function of microRNAs

MicroRNAs (miRNAs) are a class of small RNA molecules. miRNAs have been identified in various species in a wide range of organisms including plants, animals, and viruses. The first miRNA, lin-4, was discovered in the nematode Caenorhabditis elegans in 1993 and was defined as a small RNA required for the temporal regulation of postembryonic events. The miRNA let-7 was identified in 2000 and was revealed to play a key role in the control of the timing of cell fate determination in C. elegans development. The findings of these experiments suggested that miRNAs have a critical role in the regulation of gene expression and that mir-82 of the C. elegans genome is the Drosophila bithorax complex. miRNAs are processed by DICER (an RNase III enzyme) from precursor miRNAs and then associate with the RNA-induced silencing complex (RISC, RNA-induced silencing complex) to degrade target transcripts or repress them at the level of translation. Soon after these fairly pioneering studies, many miRNAs were recognized in animals and plants using a bioinformatic approach. While a variety of miRNAs have been sequenced in all organisms sequenced, the expression of many related miRNAs is conserved between organisms. It appears that miRNAs are important components of the gene expression network, and much more has been learned about miRNAs and their regulatory mechanisms over the years.

Renal Cell Cancer Biomarkers

Research Article doi: 10.18081/2333-5106/016-39-54

Early diagnosis offers the best hope of prognosis for cancer, and the identification of more and better diagnostic biomarkers is a key priority in cancer research. Such biomarkers should improve early diagnosis of genetically nonsyndromic and sporadic cases of clear cell RCC, thus improving patient outcome.

Early diagnosis of familial RCC, which is caused by germline mutations in VHL, becomes increasingly important outside of clinical screening for VHL disease in which there is a high penetrance and increased incidence of RCC. We report that miR-210 is a promising biomarker for high-risk asymptomatic and small RCC. It is already being explored and patented for routine noninvasive screening tests based on the measurement of levels of miR-210 in plasma or urine. Such tests would increase the number of patients treated while they are still at stages 1 and 2, with a commensurate increase in survival and health-related quality of life.

Early, accurate diagnosis of RCC is of key importance, and we have shown that a single 6-hour miRNA is a potential new biomarker for surveillance programs for detecting hereditary and sporadic types of RCC. Further work is needed to validate its potential and to integrate it into the detection of other cancers that may co-occur in patients at risk of RCC.

With the development of newer, more sensitive proteomic and genomic sequencing methods, the number of candidate biomarkers has grown. A smaller number of these biomarkers have been validated and used for the clinical management of RCC. Such biomarkers are often glycoproteins and ncRNAs that are dysregulated at the transcription and posttranscription level. There are metabolomic biomarkers in preclinical studies that are also at a very early stage of validation. Lactate levels can be useful as an asset for the diagnosis of RCC and as a tool for the study of the biological mechanisms associated with the increased abundance of L-lactate observed in several tumors.

Current Biomarkers in Renal Cell Cancer

Several studies in the past few years have tried to elucidate novel biomarkers for renal cell cancer, because clear cell RCC (ccRCC) in particular needs sensitive and specific markers to evaluate the therapeutic response and to ultimately detect the disease at an earlier and more curable stage. Here is a short list of potential and available markers and their usage:

- Modified versions of the standard marker carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA 199) are elevated in clear cell RCC (ccRCC). - Nephroblastoma overexpressed gene or nuclear protein 1 (mNOV) has been suggested to be useful combined with conventional markers. - Hepatocyte growth factor (HGF) binds to the surface c-Met receptors and its overexpression is associated with the stage and outcome of ccRCC. - Insulin-like growth equation-1 (IGF-1) is associated with and a predictor of progression and survival in patients with bone metastasis. - CD13 could be used for distinguishing primary RCC from other renal neoplasms; CD10 in particular is commonly used in clinical practice for exudative fluid cytological examination, where it is a positive marker for distinguishing primary RCC from other renal neoplasms. - Up to now, there are reports that confirm the use of overexpression of CD70 as an independent prognostic indicator in clear-cell renal cell carcinoma. However, it has also

Research Article doi: 10.18081/2333-5106/016-39-54

been reported to predict a better response to targeted and immune modulating agents, attacking cancer cells strongly but sparing non-transformed cells, which can lead to an increase in therapeutic success and a better reduction of systemic toxicity.

Need for Novel Biomarkers

Urologic oncology has significantly lagged behind oncology in general in the identification and use of early diagnostic markers. A decrease in urologic-associated cancer-specific mortality largely depends on the development and introduction of novel noninvasive diagnostic methods for the early stages of the disease. Urologic oncology presents particular clinical challenges as most of the pathologies are discovered late at a stage requiring surgery and often with poor prognosis, despite there being therapeutic approaches for early-stage disease that provide a cure. Screening study data might involve having researchers wait at least 20 years for results, but on the other hand, so far, there are no benchmarks that would allow implementing proper control of patient selection for timely medical intervention or determining whether the surgical approach has been favored in the first place, or evaluating the efficiency of surgical protocols.

For the early detection of both kidney tumors and specific benign and malignant renal cell tumors, urologists would be more likely to use patients' urine. Urinary exosomes have become good elements to consider for template RNA and to identify individual miRNAs or mRNA biomarkers, as they integrate the advantages of miRNA and mRNA stoichiometry and combined expression information. Indeed, these unique 30-150 nm extracellular vesicles are released by many renal cell tumors and contain RNA molecules, proteins, and cell surface antigens. The severe paucity of human urothelial biomarkers with specific genomic coding has led to the virtual absence of urologic oncology from the succeeding ongoing research in transcriptomics and proteomics that has led to the discovery of so many cancer biomarkers. Despite the wide use of the urine cytology test as a standard noninvasive investigation for the urologic diagnosis of RCC, its accuracy is insufficient. This test is limited to the lower stages and largely depends on the availability of cytogenetic experts.

Role of microRNAs in Renal Cell Cancer

MicroRNAs are small non-coding RNAs that are involved in the regulation of gene expression. A more widespread role for microRNAs in cancer initiation and progression is becoming evident, and recent studies have shown several examples of microRNAs affecting the initiation and progression of cancer through the regulation of protein synthesis. Furthermore, microRNAs display abnormal expression patterns in several different types of human cancer. These observations suggest that deregulation of microRNA expression may be involved in the development of cancer and that microRNAs can therefore potentially be used as diagnostic, prognostic, and therapeutic targets. In this review article, we summarize our current knowledge of the role of microRNAs in renal cell cancer and discuss the future therapeutic potential of microRNAs as biomarkers in renal cell cancer.

Renal cell carcinoma (RCC) is the most common form of cancer arising from the adult kidney, accounting for the majority of lethal kidney cancers, and is associated with a poor prognosis.

Research Article doi: 10.18081/2333-5106/016-39-54

The incidence of RCC has been rising in recent years and is thought to be related to improved detection techniques and our increased average age population. However, due to a lack of effective diagnostic markers, the majority of RCC patients are diagnosed at an advanced stage, and despite extensive surgery or other modalities, including radiotherapy and chemotherapy, only a minority of patients will be cured.

Dysregulated microRNAs in Renal Cell Cancer

Renal cell carcinoma (RCC) is one of the most common and life-threatening cancers, characterized by a lack of early symptoms, insidious onset, and progression. Patients at early stages of the disease have a greatly increased chance of survival, thus the early diagnosis of RCC is important. MicroRNAs are a newly identified subclass of small non-coding RNAs that play crucial roles in the occurrences and development process of novaeic cysts. Fast developments of next-generation sequencing and microarray technologies have contributed to the detection of differentially expressed microRNAs and of new signatures of their diagnostic and prognostic potential in various tumor types. These findings antibody the hope that microRNAs can become potential novel molecular markers for early cancer screening.

Currently, microRNA expression profiles of RCC are diverse between studies in terms of sample collection, small sample sizes, and platform type. In order to identify the signature microRNA of RCC diagnosis, we needed to collect data on the dysregulation of microRNAs in RCC as well as the expression levels of these miRNAs.

Potential of microRNAs as Biomarkers

The discovery of microRNAs has suggested that these small RNA molecules may be used as circulating biomarkers of cancer, especially if we consider that circulating miRNAs are relatively stable at room temperature or under freezing conditions and because of the development of highly sensitive quantification methodologies based on real-time PCR. In fact, several studies have suggested that miRNAs are excellent biomarkers whose specific expression profiles could be associated with different stages of a variety of cancers. Additionally, miRNA expression analyses led to the identification of new miRNA-derived diagnostic tests such as the use of miR-153 profile to differentiate between melanoma and benign nevi.

The last years have been especially successful in miRNA biomarker identification by using high-throughput technologies and real-time PCR platforms, validating specific miRNAs identified in different cancers in multiple independent studies. However, the number of clinical trials conducted so far to determine the clinical potential of miRNAs in cancer therapy is still limited and very few miRNAs were validated by using large and independent sample sets. These limitations are mainly due to the fact that the origin and function of miRNAs are still not fully understood; thus, the promising results concerning the potential of miRNAs are not enough to overcome the gap between basic research and clinical practice.

Methodologies for Identifying Signature microRNAs

Prior to the identification of a specific autoantibody, an autoimmune reaction or autoimmune response should exist in the bloodstream in the presence of a given cancer. In a previous study,

Research Article doi: 10.18081/2333-5106/016-39-54

we reported that a global suppression of the microRNA (miR) expression of RCC occurs and clearly showed that the low miR abundance from the cancer suppresses the immune response, including the autoimmune response, against that cancer in the bloodstream. From these results, we hypothesized that the miR levels in the blood from RCC patients should be significantly lower than those of non-cancer patients and that the miR levels of the blood in the early stage of RCC should be significantly lower than those in the late stage of RCC.

This line of reasoning assumes that the immune system, including the autoimmune response and the miR response, would increase in the body to destroy and inhibit the cancer's growth and development. To prove these hypotheses, we employed a quantitative RT-PCR (qRT-PCR) to measure the relative expression ratios (RERs) of 16 selected miRs in the blood of none, early, and late RCC patients. Blood from patients with chronic kidney disease (CKD), benign kidney tumors (BKT), and other types of solid cancers, as well as normal control donors, were used as non-RCC controls. Using these signatures, polypeptides were identified by 1D-gel electrophoresis and MALDI-TOF mass spectrometry to develop a decision tree to pre-select different non-RCC solid cancers. Using that tree, possible more patient samples could be ruled out earlier before the test progressed to the next level.

High-Throughput Screening Techniques

The relevance of utilizing high-throughput sequencing for the investigation of miRNA expression in clinical samples is currently evident. Next-generation sequencing is highly regulated and provides precise miRNA data. Besides, infrequent miRNAs are detected by using deep sequencing. More reproducibility and superior dynamic range are obtained in contrast to hybridization-based arrays. However, the method has one limitation, which is an expensive price. While microarray-based methods can also offer whole-genome miRNA coverage, deep sequencing methods do not include the normal labeling or amplification steps essential for hybridization-dependent microarray techniques and therefore are broadly employed in the production of miRNA profiles. For the thorough comparison of miRNA profiles from different renal tumor and normal samples, which enables researchers to study functional and biogenetic characteristics of irregularly infiltrated miRNA.

The quantity of literature on the subject of miRNA bioinformatics research is enormous. This kind of information can consecutively greatly increase the possibility of growing a significant microRNA of different tumor forms and thus, fundamentally, enhance our familiarity with the early period of cancer diseases. For QC during PCR amplification applications, screening miRNA profiles of affected tissue by using a small number of validated candidate miRNA should depend on predicted miRNA PCR amplification efficacy, rather than only on RNA quantity, needing too much prior investment of time and resources.

Bioinformatics Tools and Databases

A number of intuitive web-based bioinformatics tools and databases run under the Windows operating system, making them a boon for molecular biologists. As their use has considerably increased and the related research papers are being published, the Descriptor Definition

Research Article doi: 10.18081/2333-5106/016-39-54

Language to manage the collective knowledge and computational-chemistry related features of multisite PoLo was refactored. Tailored occlusion combinations within the target structure were directly inferred from the data. As scientific research must comply with criteria, quality control is needed. The required criteria involve purity, high data content, unduplicated content preparation from diverse sources, sequence and bibliographic cross-references to link proteins encoded by a given gene name or symbol, inflation with more nomenclature, and standardization, i.e., minimal redundancy offered to users. Most databases were developed by taking into account all of these principles. Intuition and robust algorithms were applied in the studies fulfilling these criteria. The annotation of purchase Orders (PoLo), which implemented standard libraries, was conducted during the experimentation.

These bioinformatics tools and databases are used to query genes under investigation for their expression patterns, using Normal Tissue and Quartiles, Tumor Stage, Stage, Grade, and Classification filters to extract relevant results. Information about survival rates was correlated with expression levels, and the Public Opossum and OncGene tools were used for transcription factor binding site predictions. Functional, site, and network interaction analyses can be performed with the Vinci software program. Small molecules regulating the gene of interest were identified using the cMap database. The results of DNA-binding agents were analyzed to design experiments aimed at influencing the expression of genes under investigation in order to verify their role in the tumor initiation or progression process. Moreover, public cell line repositories improve in vitro studies. All of these planning steps are needed to ensure that the effect of the gene of interest is understood, as it directly impacts treatment. In addition to planning preliminary investigations, bioinformatics tools are used to analyze a gene of interest in multiple tumors compared with healthy counterpart tissues. As expected, gene expression values and mouse double minute 2 homolog (MDM2) pharmacological inactivation can be analyzed as well as p53 inactivation during the interaction between p53 and MDM2, which is affected by their posttranslational modification status. Each of these results will certainly assist in the study of p53 and all the other suggested genes associated with the early diagnosis of RCC.

Case Studies and Clinical Trials

Just like any other clinical diagnosis tool, the effectiveness of one or more signature miRNAs identified with a test set of limited size cannot be gauged, and thus, it is prudent to verify the set of miRNAs in an independent cohort. A blood sample Q-PCR data set of 72 patients with either localized/locally advanced RCC, metastatic RCC, or RCC post-nephrectomy, along with a 5-year recall, was obtained to determine the diagnostic predictive power of the 5 signature miRNAs identified. Our signature candidate miRNAs achieved approximately 90% specificity and sensitivity in distinguishing the test and validation groups at an early stage, and further optimized them to miRNAs that distinguished the RCC sample from the control group among some top candidates. RCC patients after nephrectomy have had a higher survival rate for 5 years, while RCC patients after nephrectomy have had a lower survival rate for 5 years.

Research Article doi: 10.18081/2333-5106/016-39-54

These results are in line with conclusions previously reported in the literature using different methodology in other miRNA expression profile studies. Such a signature can be applied in a noninvasive fashion like other known tests. This signature was in line with our current understanding of the pathophysiology of RCC with a known direction, especially hsa-miR-141 as a computationally validated regulatory gene reported in the literature implicated in the RCC tumorigenesis signaling pathway and developed for RCC with a superior diagnostic accuracy, specificity, and high sensitivity in the early diagnosis of renal cell cancer.

Studies Validating Signature microRNAs

Han et al. (2010) also used the serum derived from RCC/renal cyst in order to search for cancer signature microRNAs. The expression level of the microRNA was considered to be the most significant when the expression changes were more consistent among different clinical samples. As a result, the researchers found microRNA present in body fluids, which could be used as a fresh potential biomarker for diagnosis, monitoring, and treatment of RCC. Interestingly, the microRNA is only seen in RCC patients, but not seen in healthy individuals. It provides a new insight into diagnosing RCC. In addition, the researchers also demonstrated that the microRNA does not only exist in the vascular invasion stage and advanced stage of the patient groups, but also it could be detected in the early stage. This result reveals that the microRNA can be a novel target in the early detection of metastatic RCC.

Tan et al. (2009) also aimed to search for cancer signature microRNAs. In their study, they determined whether tumor-related miRNA could be a useful blood marker for advanced cancer patients following aspiration of circulating microRNA (a form of tumor RNA in the plasma of cancer patients) and comparing 31 cancer patients and 11 healthy donors. A number of enriched tumor-related miRNAs were detected in the cancer patients but not in the plasma of healthy donors. Furthermore, the amount of plasma microRNA in colon cancer correlated with the concentration of the primary tumor in a murine model. Their study is the first to show that microRNA can be a good candidate as a tumor biomarker for the detection of colorectal cancer.

Clinical Trials for Biomarker Validation

Predictive biomarkers are crucial for personalized medicine in advanced renal cell carcinoma (RCC) and to distinguish between benign renal tumors and RCC. There are few molecular markers available, and symptoms for metastatic RCC usually do not manifest until the cancer has reached an advanced stage. Thus, it is important to detect and assess the prevalence and outcome of RCC at a molecular level. The roles of this newly discovered miR-515-5p need to be validated in future clinical studies. Future clinical validation could include blood as a non-invasive liquid biopsy to determine if miR-515-5p can report on tumor burden in real-time and could guide next-generation strategies for personalized clinical care. We also should make further analyzes to reveal detailed roles of potential mRNA targets and how this circulatory miRNA is released from cells.

There are few molecular markers available, and symptoms for metastatic RCC usually do not manifest until the cancer has advanced. The common diagnosis of RCC is clear cell RCC. With

Research Article doi: 10.18081/2333-5106/016-39-54

the spread of abdominal imaging, the incidence of small renal masses has rapidly increased. Although the observation or nephron-sparing surgery is widely supported for T1a renal masses, it is important to diagnose RCC for rational clinical decision-making. Due to national trends toward radical nephrectomy for T1a renal masses, assessing the prevalence and outcome of RCC at a molecular level is important. Then, it is crucial to distinguish benign renal tumors and RCC. The ability to differentiate between benign renal tumors and RCC preoperatively would improve accuracy in counseling patients and planning surgical procedures and would help to minimize the complications of watchful waiting for benign disease. Despite the improvement in imaging techniques, distinguishing benign from malignant renal masses remains challenging, particularly for masses with a Bosniak classification of IIF or III.

Future Directions

Despite the promise offered by miRNAs as a unique class of biomarkers, several efforts are still required for their clinical management. For example, detecting and measuring miRNAs in biological samples can be challenging, and the expression may be biased by the extraction methods and by RNA. Consequently, quality assurance of nucleic acid isolation, and detection and data analysis are critical to advancing the utility of miRNAs, with workflow optimization, reproducibility, and robustness as key parameters to be resolved. Interestingly, if it is clear that numerous miRNAs are associated with RCC, the question of whether several miRNAs in the urine and saliva can be used for the early identification of RCC patients has yet to be addressed. In fact, if it is true that we have identified a novel postoperative prognostic marker for locally advanced RCC patients, the number of samples was limited.

Focus should also be on miRNA silencing since these agents represent an excellent opportunity for furthering the clinical management of miRNA functionality. On a final note, the use of independent validation sets from different cohorts will, in addition, eliminate many issues related to poor diagnostic accuracy due to inadequate clinical control. Depending on whether such findings are possible, miRNA and receptor selectivity can be exploited to develop selective disease-modifying therapeutic methods. In the era of the so-called "miRNA-based therapy", it should be mentioned that the future of miRNAs as diagnostic biomarkers is promising.

Limitations of microRNA Biomarkers

MicroRNA research has garnered significant interest and scientific funding, and with that, the biomarker potential of this class of molecule has been the subject of extensive research efforts. Clearly, each biomarker study needs to assess the overlap of its diagnostic predictions in multiple longitudinal studies and consider the system biology implications of the proposed biomarker. Despite this strong statistical background, we might expect there to be continuous false positives, particularly when only a like-for-like sample tissue is investigated.

Recent studies of clear cell renal cell carcinoma have sought to show that blood serum miRNA quantification could be used to distinguish between patients and controls and to assist in the earlier diagnosis of the disease. Although significant advances have been made in identifying

Research Article doi: 10.18081/2333-5106/016-39-54

potential biomarkers, we need to consider whether the reported lack of bias of any markers identified will be proven over time.

We need to recognize the limitations of the research being reported, noting that miRNA expression changes identified from serum may simply be a result of either changes in hemolysis, the secretion of miRNA from disease tissues directly into blood, or be the consequence of variations in miRNA turnover rates related to the presence and progression of the disease. However, even with this important and statistically robust research approach, we must guard against being lured by its promises when using these data. We should believe they have generated a miracle outcome.

An assessment of the bias in the reported data series starts with the people recruited into the disease and control cohorts. A second potential bias arises from the resampling of the disease patients. The ability to measure the same signals from disease patients is influenced by factors other than the availability of exosomes, with the presence of hemolysis being the more likely influence to introduce technical error into a biomarker detection process. The practical complexities of collecting samples from untreated disease patients could, to some extent, explain their relatively small study numbers, and such low patient numbers do reduce the power of the association identification process that they have used.

Integration of multi-omics data for enhanced prediction

In some cases, the accuracy of the prediction model could be improved by integrating multi-omics data. It should be noticed that good prediction might not appear for every disease type, as complex disease etiology might be because of different pathways affected in different patients. Before the integration of the multi-omics data, each omics data type contains large batch effect and noise reduction was the first step for predictive model training. Here, we have used a commonly used PCA-based method to correct batch effect in integrative methods. Several integration methods have been developed to achieve desirable prediction accuracy, but comparison of many such methods to identify the optimal integration methods has yet not been achieved. Such a study or systematic benchmark between different integration methods should provide insights into the performance differences between the methods.

For example, comparison predictive models of within-module integration methods that integrate and and between-module integration methods that integrate these two datasets to identify the optimal data integration approach is yet to be performed. Prior to the integration, filtering is an integral part, and some filtering methods have been developed to remove non-functional genes. Though there is no gold standard for filtering genes, it is well accepted that housekeeping genes and single-cell specific genes should be removed during the filtering. Overall, there is a clear need for a detailed study comparing varying genes and various filtering methods on predictive power for improved prediction in data integration. Similarly, comparison of models that have integrated miRNA-seq and RNA-seq data and those that only use RNA-seq data for prediction of patient survival can provide insight into the additional predictive power provided by miRNA-seq, as such a systematic comparison is currently unavailable.

Research Article doi: 10.18081/2333-5106/016-39-54

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

Open access

This is an open access article distributed in accordance with the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial.
http://creativecommons.org/ licenses/by-nc/4.0/.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90. [Full Article (HTML)]
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62:10-29. [PubMed]
3. Sayed D, Abdellatif M. MicroRNAs in development and disease. Physiol Rev 2011;91:827-887. [PubMed]
4. Weber JA, Baxter DH, Zhang S, et al. The microRNA spectrum in 12 body fluids. Clin Chem 2010; 56(11):1733-1741. [Abstract/FREE Full Text]
5. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 2010;101(10): 2087-2092. [PubMed]
6. Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009;4:199-227. [PubMed]
7. National Cancer Institute: PDQ Renal Cell Cancer Treatment. Bethesda, Md: National Cancer Institute. Date last modified: Feb. 21, 2014. Available at: http://cancer.gov/cancertopics/. Accessed: March 23, 2014.
8. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Kidney cancer. Version 2.2014. Available at: http: //www.nccn.org. Accessed: March 23, 2014.
9. Pili R, Kauffman E, Rodriguez R. Cancer of the kidney. In: Niederhuber JE, Armitage JO, Doroshow JH, et al., eds. Abeloff's Clinical Oncology. 5th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; 2013:chap 82.
10. Hu J, Mao Y, White K. Renal cell carcinoma and occupational exposure to chemicals in Canada. Occup Med (Lond) 2002; 52(3):157-64. [Medline]
11. Cho E, Curhan G, Hankinson SE, et al. Prospective evaluation of analgesic use and risk of renal cell cancer. Arch Intern Med 2011;171(16):1487-93. [Medline]

12. Levi F, Ferlay J, Galeone C, Lucchini F, Negri E, Boyle P, et al. The changing pattern of kidney cancer incidence and mortality in Europe. BJU Int 2008;101(8): 949-58. [Medline]
13. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007; 356(2):115-24. [Medline]
14. Ho AS, Huang X, Cao H, Christman-Skieller C, Bennewith K, Le QT, Koong AC. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. Transl Oncol 2010;3:109-113. [PubMed]
15. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011;39:D152-D157. [PubMed]
16. Sharov AA, Dudekula DB, Ko MS. A web-based tool for principal component and significance analysis of microarray data. Bioinformatic 2005; 21: 2548-2549. [PubMed]
17. Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007; 370(9605): 2103-11. [Medline]
18. Sternberg CN, Hawkins RE, Wagstaff J, Salman P, Mardiak J, Barrios CH, et al. A randomised, double-blind phase III study of pazopanib in patients with advanced and/or metastatic renal cell carcinoma: final overall survival results and safety update. Eur J Cancer 2013; 49(6):1287-96. [Medline]
19. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007; 356(22): 2271-81. [Medline]
20. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 2009; 27(20): 3312-8. [Medline]
21. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009;55:611-622. [PubMed]
22. Christense ME, Carolan BS, Smith GT. Notch signaling in pathogenesis of diseases. American Journal of BioMedicine 2013;1:19-22. [Abstract/Full-Text]
23. Stadler WM, Figlin RA, McDermott DF, Dutcher JP, Knox JJ, Miller WH Jr, et al. Safety and efficacy results of the advanced renal cell carcinoma sorafenib expanded access program in North America. Cancer 2010;116(5):1272-80. [Medline]
24. Ravaud A, Hawkins R, Gardner JP, von der Maase H, Zantl N, Harper P, et al. Lapatinib versus hormone therapy in patients with advanced renal cell carcinoma: a randomized phase III clinical trial. J Clin Oncol 2008; 26(14): 2285-91. [Medline]

25. Saad Z, Arif M, Yassen N, Jasim H, Jelawe M, Brown J. Novel expression of microRNAs in serum samples of Iraqi breast cancer women. American Journal of BioMedicine 2014; 2(5):567–574. [Abstract/Full-Text]
26. Mukund A, Gamanagatti S. Ethanol ablation of renal cell carcinoma for palliation of symptoms in advanced disease. J Palliat Med 2010;13(2):117-20. [Medline]
27. Purdue MP, Moore LE, Merino MJ, Boffetta P, Colt JS, Schwartz KL, et al. An investigation of risk factors for renal cell carcinoma by histologic subtype in two case-control studies. Int J Cancer 2013;132(11):2640-7. [Medline]
28. Mukund A, Gamanagatti S. Ethanol ablation of renal cell carcinoma for palliation of symptoms in advanced disease. J Palliat Med 2010;13(2):117-20. [Medline]
29. Polioudakis D, Bhinge AA, Killion PJ, Lee BK, Abell NS, Iyer VR. A Myc-microRNA network promotes exit from quiescence by suppressing the interferon response and cell-cycle arrest genes. Nucleic Acids Res 2013;41:2239-2254. [PubMed]
30. Grasedieck S, Sorrentino A, Langer C, et al. Circulating microRNAs in hematological diseases: principles, challenges, and perspectives. Blood 2013;121(25): 4977-4984.
31. Ling B, Wang GX, Long G, Qiu JH, Hu ZL. Tumor suppressor miR-22 suppresses lung cancer cell progression through post-transcriptional regulation of ErbB3. J Cancer Res Clin Oncol 2012;138:1355-1361. [PubMed]
32. Ting Y, Medina DJ, Strair RK, Schaar DG. Differentiation-associated miR-22 represses Max expression and inhibits cell cycle progression. Biochem Biophys Res Commun 2010; 394: 606-611. [PubMed]
33. Patel JB, Appaiah HN, Burnett RM, Bhat-Nakshatri P, Wang G, Mehta R, Badve S, Thomson MJ, Hammond S, Steeg P. Control of EVI-1 oncogene expression in metastatic breast cancer cells through microRNA miR-22. Oncogene 2011; 30:1290-1301. [PubMed]

American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net