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DIAGNOSIS AND TREATMENT OF SMALL RENAL MASSES: THE ROLE FOR MOLECULAR BIOLOGY

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Summary.- Renal cell carcinoma (RCC), the most common type of kidney cancer, is increasing in incidence and is the most lethal genitourinary cancer. Due to the increasing use of abdominal imaging, incidentally detected, asymptomatic small renal masses (SRMs), most of which are RCC, have become the most common presentation of kidney cancer. Most RCC SRMs initially grow slowly or not at all, but others progress to advanced and metastatic cancer. Several diagnostic and prognostic genomic, transcriptomic and proteomic studies have been completed in RCC, however signatures for SRM progression have not been identified. In the absence of useful factors to distinguish those tumors requiring treatment for progression from those that can be managed by active surveillance alone, most SRMs are treated as RCC with surgery. Currently, the only prognostic factor at diagnosis is tumor size. Tumor growth rate also appears to identify potential progressive tumours. Identifying signatures for progression and the utilization of needle biopsies will be important for SRM patients and will guide therapy.

Keywords: Renal cell carcinoma. Small renal masses. Proteomic tumor suppression. Micro. RNA. Molecular biology.

Resumen.- El carcinoma de células renales (CCR), el tipo más común de cáncer renal, está aumentando en incidencia y es el cáncer genitourinario más letal. Debido al incremento de la utilización de las pruebas de imagen abdominales, las masas renales pequeñas detectadas incidentalmente, de las que la mayoría son CCR, se han convertido en la forma más común de presentación del cáncer renal. La mayoría de las pequeñas masas renales con CCR crecen despacio inicialmente o no crecen nada, pero otras progresan a cáncer avanzado o metastásico. Se han completado varios estudios diagnósticos y pronósticos de genómica, transcriptómica y proteómica en CCR, sin embargo no se han identificado marcadores para la progresión de las masas renales pequeñas. En ausencia de factores útiles para distinguir aquellos tumores que requieren tratamiento por progresión de aquellos que pueden manejarse sólo con vigilancia activa, la mayoría de las masas renales pequeñas se tratan como CCR con cirugía. Actualmente, el único factor pronóstico al diagnóstico es el tamaño del tumor. El crecimiento del tumor también parece identificar los tumores potencialmente progresivos. Identificar
Until recently, an annual 2% worldwide increase in renal cell carcinoma (RCC) incidence has been occurring, primarily due to increased use of imaging (1). This rise in incidence is mostly due to stage migration, with the increased detection of small renal masses (SRMs) (2). More recently this trend seems to have peaked, and in some European countries, even a decrease in incidence has been observed (3). Recent kidney cancer epidemiological studies have demonstrated an improvement in survival rates in all ethnic groups and genders, and have shown that stage migration predicted better survival (4,5). Therefore, increased SRM detection has played a fundamental role in the rising RCC incidence, in RCC stage migration, and therefore, should contribute to improved survival.

The proportions of histological subtypes of RCC are different in malignant SRMs compared to the classical distribution in RCCs treated by nephrectomy. Remzi et al analyzed the histopathological features of 227 RCCs less than 4 cm that were diagnosed by imaging and removed surgically. Their results showed that although clear cell RCC (ccRCC) remained predominant, its proportion was reduced as compared to the classical distribution with an increase in the rate of papillary RCC (pRCC) in cases of SRMs less than 3 cm (65% ccRCC and 25% pRCC respectively) (6). Needle biopsies of SRMs show a similar trend when a diagnosis of RCC is confirmed (7).

**DIAGNOSTIC APPROACH TO SRMS**

- **Presentation and imaging**

Local and paraneoplastic symptoms which are independent prognostic factors in more advanced RCC are rare with SRMs (8,9). Most SRMs are incidentally discovered but the lack of symptoms is not a guarantee of uniformly good outcome (10).

Physical examination has a negligible role in SRM assessment. Imaging with ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) can all contribute to detection and characterization of SRMs. Abdominal and chest CT scans provide evaluation of possible metastasis and contralateral kidney morphology but a minimum of a chest X-ray is recommended to rule out metastatic disease as presentation, particularly after a biopsy that reveals RCC (11). The presence and pattern of enhancement on CT and other imaging characteristics can help to differentiate RCC from a benign SRM (11). For the evaluation of cystic renal masses and the differentiation of cystic RCC from simple renal cysts, the Bosniack classification is frequently used although it is not a well validated system (12). Bone and brain imaging examination is only indicated if the patient exhibits symptoms of metastasis to these sites (13). In patients where intervention is considered, renal function should also be estimated particularly in those with a solitary kidney, bilateral tumors, chronic kidney disease (CKD) or in those at risk of future renal dysfunction from comorbid disorders (13).

- **Role of biopsy**

With the increase in availability and safety of image-guided biopsies, expanded use of nephron-sparing surgery, probe ablation treatments, and most recently, of active surveillance programs, the resistance to percutaneous needle core biopsies of tumor masses is decreasing. In addition, the accuracy and confidence that SRM biopsy will not produce wound implants have improved substantially. False negative rates are low (about 1%), symptomatic complications requiring interventions occur in less than 2% of the cases, needle-tract seeding is exceedingly rare (14,15). However, even though biopsies were shown to be safe and with high specificity and sensitivity for detection of renal malignancy and tumor grade, more than 20% of initial biopsies remain non-diagnostic (14,16). Of some concern, genomic studies on multiple regions within primary RCC tumors and metastatic RCC deposits showed intra-tumor clonal heterogeneity, with only discrete regions of primary tumors contributing to metastatic lesions genomically. This highlights the importance of multiple biopsies when examining RCC tumors, as lower grade information from a single core may be falsely reassuring (17,18).

The decision to perform a renal biopsy should be driven by tumor size, patient age, comorbidities and planned treatment. Histological diagnosis is only useful if the result will change patient management. Renal biopsy of SRMs is recommended (a) to help in discriminating benign from malignant lesions; (b) at the time of ablative therapy such as radiofrequency ablation or cryotherapy to guide follow-up; or (c) during follow-up after ablative therapies, to define treatment success or failure. Biopsy is also increasingly being used in surveillance strategies to stratify patients.
into different protocols of follow-up (19). Limitations of renal tumor biopsies are multifocality, mixed histology type tumors, and cystic tumors.

**PROGNOSIS AND TREATMENT**

- **Clinicopathological parameters**
  Tumor grade and TNM stage remain the best predictors of survival after treatment of localized RCC (20). SRMs are early stage tumors, present with a lower grade and are associated with an excellent survival (20). For SRMs, the only prognostic factor is size at presentation (21,22). Although rare in SRMs, the presence of symptoms is an independent prognostic factor with a significantly worse pathological and long-term behaviors (9). Postoperative prognostic models or nomograms that combine independent prognostic factors have been developed and externally validated for RCC (23). Similarly, ECOG performance status at presentation, cachexia, anemia and platelet count have been shown to predict survival, especially in patients with metastatic disease (20).

- **Active surveillance of SRMs**
  In contrast to other solid malignancies, relatively large volume primary renal carcinomas, such as SRMs presenting at up to 4 cm in diameter, usually exhibit slow growth, suggesting that initial surgical treatment may cause more harm than good, especially in elderly or morbid patients. A 3 cm breast carcinoma evokes concern, a 3 cm melanoma provokes alarm, but a 3 cm RCC can be managed conservatively with caution. Investigation of the natural history of SRMs has suggested the possibility of active surveillance (AS) as appropriate initial management, particularly in the elderly or patients with significant comorbidities. A number of case series have shown that overall, at least 20% of SRMs are benign, though the risk of a SRM being malignant rises 17% for every 1 cm increase in the mass size. Nevertheless, 20-30% of malignant SRMs show aggressive behavior, but accurate, clinically applicable prognostic biomarkers have not been yet identified (6,10).

For SRMs, the median growth rate and clinical outcome have been investigated. Many retrospective studies have shown that most SRMs grow slowly and do not behave aggressively (24-27). Recent prospective multicenter studies evaluating AS as primary management for SRMs have corroborated those studies. Mason et al reported a mean growth rate of 0.25 cm/year in a prospective cohort of 82 SRM patients. The mean follow-up was 36 months and one patient developed metastatic RCC (21). We reported the results of our multicenter prospective phase 2 clinical trial of AS of SRMs in 178 patients, correlating progression patterns with histology (from biopsy) with a follow-up period of 28 months on average (22). This showed a lower growth rate (0.13 cm/year) than previously reported, with 12% of patients showing local progression (growth >4 cm or doubling time <12 months) and only 1% progressing to metastasis. Initial histology was poorly discriminating of subsequent radiological behavior; biopsy-proven RCC SRMs were observed to grow at the same rate as benign tumors (angiomyolipoma and oncocytoma), implying that growth alone is insufficient to define malignant SRMs.

There is therefore a lack of strong radiological, molecular and pathological prognostic factors for progression of SRMs so that in the absence of curative therapies for metastatic RCC, initial AS may increase the risk of progression in healthier and younger patients. However, for elderly patients and for those with significant comorbidities, there is evidence that the rate of metastatic progression, as well as overall survival (OS) are similar when comparing primary AS to other management strategies (28,29). Therefore, AS with delayed or, alternatively, no treatment, is appropriate in patients that are elderly, those with medical comorbidities or in patients with decreased life expectancy (13,30).

**MOLECULAR MARKERS AND SIGNATURES IN SRMS**

Molecular studies have included investigation of RCC genetics, genomics, gene expression, epigenetics and proteomics.

- **VHL gene alterations**
  Clear cell RCC (ccRCC) is the most common form of RCC with the worst prognosis when subtypes are matched for stage and grade. The major gene inactivated in ccRCC is the von Hippel Lindau gene (VHL) on 3p25-26, which was initially identified from familial studies of VHL disease (31,32). VHL inactivation is considered the initial step of ccRCC development, playing the role of tumor suppressor gate-keeper and is inactivated in 80% of sporadic ccRCCs (1,32-34). Allelic inactivation of VHL occurs as a result of missense and nonsense mutations, frameshift deletions and insertions, promoter methylation and/or loss of heterozygosity (LOH). Mutation analysis in sporadic ccRCC reported between 20-82% VHL allelic mutations, 5-20% VHL promoter methylation and over 90% loss of heterozygosity (LOH) (2,35-43). VHL mutations are distributed stochastically throughout the gene.

Several studies have investigated associations between VHL genetic and epigenetic alterations and disease progression, prognosis and response to
therapy. Some showed a correlation between VHL alterations and advanced pathologic stage (3,44) and between frequency of VHL mutations and higher Fuhrman nuclear grade (4,5,45). ccRCC tumors with distant metastasis were seen to have more double mutations, nonsense mutations and mutations on exon 3 than those without metastasis. Nonsense mutations also correlated with Fuhrman nuclear grade, node positivity and self-reported family history of RCC (6,42).

Conversely, VHL alterations were also shown to correlate with positive patient outcome. Patard et al. reported that VHL mutation status stratified ccRCC patients into poor, intermediate and good prognosis groups. Absence of a detectable VHL mutation and low carbonic anhydrase IX (CAIX, a downstream marker of hypoxia and VHL inactivation) expression correlated with advanced tumor stages and presence of metastasis. The intermediate prognosis group had either VHL mutation or high CAIX, while the good prognosis group was defined by detectable VHL mutation and high CAIX levels and correlated with progression free survival and disease-specific survival (8,9,46). VHL alterations and low VEGF expression have also been shown to correlate with decreased tumor aggressiveness and good patient outcome (10,47). Finally, other studies have shown no correlation between VHL genetic or epigenetic alterations and clinico-pathological factors including Fuhrman nuclear grade, pathological stage, tumor size as well as patient outcome (11,38,40,41,48).

Attempts to relate VHL alterations to therapeutic response also show conflicting results. Kim et al. observed that VHL missense mutations, and not overall VHL alterations, correlated with better response to immunotherapy (11,40), while Rini et al. showed that there may be prolonged time to tumor progression with VEGF-targeted therapy in patients with loss-of-function VHL mutations (12,49). Gad et al. reported that there was no association between VHL mutation status and response to anti-angiogenic therapy (13,50), but a larger study by Choueiri et al. showed that VHL loss-of-function mutations correlated with increased radiological response to VEGF-targeted therapy in metastatic ccRCC, though progression free survival and overall survival were not significantly affected by VHL status (13,51).

The disparity in the results obtained on the association of VHL alterations with clinico-pathological factors, patient outcome and response to therapy could be explained by the differences in patient populations, methods of VHL alteration detection and our incomplete understanding of potential driver and passenger VHL mutations (14,15,52). For example it was recently reported that a particular haplotype of germline VHL SNPs in patients who developed ccRCC was associated with a higher probability of VHL promoter hypermethylation, suggesting a role for these SNPs in promoting epigenetic changes in the kidney leading to tumourigenesis (14,16,53). This interplay of genetic and epigenetic factors adds an additional level of complexity to interpreting associations between VHL status and clinical outcome in RCC.

VHL mutation testing using biopsies has been recently reported to have similar sensitivity to testing using whole tumor tissue and laser capture microdissection did not show an increased advantage (17,54). As VHL aberration is an initial change in ccRCC tumorigenesis, integrating VHL mutation testing of SRMs using biopsies could potentially help in the diagnosis and prognosis of these masses and guide therapy.

- Other chromosomal and genetic aberrations

VHL inactivation appears to be the first step in ccRCC initiation and has been detected in the smallest ccRCC tumors, but VHL mutation itself may confer genomic instability leading to alterations in other oncogenes and tumor suppressor genes which could be important for ccRCC progression (18, 34, 55, 56). Conventional cytogenetic, allelotyping, comparative genomic hybridization (CGH) studies and high-density and high-throughput single nucleotide polymorphism (SNP) array studies have been used to study chromosomal aberrations in ccRCC. Most common changes include loss of 3p, 4q, 6q, 8p, 9p, 10q, 13q, 14q, Y and a gain of 1q, 5q and 7 (19,57-62). Regions of chromosomal loss or gain harbor potential tumor suppressor genes and oncogenes. Tumor suppressor genes including PTEN on 10q as well as CDKN2A (p16Ink4A) and MDM2 on chromosome 9 may be very important in ccRCC progression (20,58,60,63). A potential oncogene TGFB1 was found in the 5q23.2-34 gain region and was also overexpressed at the RNA level in ccRCC tumors (20,59).

Chromosomal alterations have been associated with clinico-pathological parameters and patient outcome. Loss of 3p and 13q correlated with recurrence free survival and 3p loss was also present in early stage and low grade tumors (21,22,58,59,64). Loss of 9p was an independent prognostic variable of metastasis and poor survival (9,58,65), and was found to be predictive of recurrence in non-metastatic ccRCC and tumors of larger size, advanced stage and high grade (23,58,60,64,66). 4p and 14q loss were associated with advanced stage, higher grade, larger tumor size, poor survival and propensity for
metastasis (20,58,60,66,67). 2q loss and 7 gain were observed in high grade tumors and 8p in worse stage and grade tumors (6,10,59,66). Loss of chromosome Y was correlated with better progression free survival in patients with metastatic RCC (24-27,58). Gain of chromosome 5 has been associated with low tumor grade and improved disease specific survival (21,67,68), but other studies have shown no association with clinicopathological parameters or patient outcome (22,60,64).

Chromatin and histone modifications, such as acetylation and methylation, can regulate transcriptional activity and may also influence RCC tumorigenesis. Exome sequencing has identified mutations in several genes mainly involved in histone modification in ccRCC including SETD2, a histone H3 lysine 36 methyltransferase, and JARID1C (also known as KDM5C), a histone H3 lysine 4 demethylase, as well as mutations in the histone H3 lysine 27 demethylase, UTX (KMD6A) (28,69,70).

A recent study identified mutations in the SWI/SNF chromatin remodelling complex gene PBRM1. Mutations were observed in 41% of ccRCC tumors, making it the second most common gene inactivated in ccRCC after VHL (29,71). Knockdown of PBRM1 in ccRCC cell lines resulted in increased aggressiveness in the transformed phenotype, suggesting a tumor suppressor role for PBRM1 in ccRCC. The clinical implication of these chromatin and histone modifying genes is being actively studied.

All these genes presented and many others that are present on the aberrant chromosomal regions could act as diagnostic or prognostic biomarkers and play essential roles in ccRCC development and progression. Therefore they need to be validated as biomarkers and their mechanisms of action explored for identifying novel therapeutic targets.

With the advances in next generation sequencing technologies, exome and whole genome sequencing will allow the use of small samples of tissues such as biopsies to generate information that can be translated to the clinic, providing an important impact on SRM diagnostics and prognostication.

- **RNA and proteomic alterations**

- **mRNA expression profiles**

Transcriptional profiling has shown specific genetic signatures that could predict progression and metastasis in RCC tumors as well as clinical outcome confirming the potential use of gene expression signatures as predictors of clinical outcome and response to therapy (13,30,72-77).

For example, Vasselli et al. showed that stage IV ccRCC primary tumors in patients with metastatic disease can be subdivided into two groups with distinct gene expression patterns of which high expression of VCAM1 predicted longer survival time (31,32,78). A study by Jones et al. compared the expression profile of T1 ccRCC tumors to normal tissue and identified a set of genes including angiogenic factors, chemokine and tyrosine kinase receptors that defined early ccRCC and segregated it from other RCC histological types. They also determined signatures of progression consisting of 31 genes by comparing normal kidney to T1 primary tumours to metastatic tumors and importantly identified a metastatic signature of 155 genes by comparing T1 tumors to metastatic ones (79).

Brannon et al. aimed to identify subsets of ccRCC using gene expression data without applying any classifying clinical information, an unsupervised hierarchical clustering approach. Using a number of bioinformatic analysis techniques including principal component analysis (PCA), consensus clustering and logical analysis of data (LAD), they identified two robust subtypes of ccRCC, ccA and ccB with ccA having significantly better prognosis than ccB. The ccA group was enriched for genes involved in hypoxia, angiogenesis and metabolism while genes overexpressed in the ccB cluster were involved in cell differentiation, epithelial-mesenchymal transition and response to wounds. As both ccA and ccB had a similar frequency of VHL inactivation, they speculate that different activation pathways may have led to different gene signatures (80). The same group recently performed a meta-analysis of ccRCC expression data that contained 480 tumors to determine whether other ccRCC subtypes beyond ccA and ccB existed that could be related to VHL inactivation, HIF hypoxia inducible factor (HIF) status, tumor histology or gender. They identified a third cluster in addition to their previously identified ccA and ccB subtypes. This third cluster overexpressed genes involved in mitochondrial bioenergetics and estrogen-related receptor alpha targets. Cluster 3 tumors had atypical histologies as compared to ccA and ccB, downregulated hypoxia and angiogenic genes and most intriguingly were found to be ccRCC with wild-type VHL (81).

Several other studies have also attempted to integrate genomic with transcriptomic data to better understand and stratify tumors. Beroukhim et al. studied the genomic profiles of germline VHL-associated ccRCC and sporadic ccRCC with or without VHL biallelic inactivation. They observed that sporadic ccRCC with VHL biallelic inactivation resembled VHL-disease associated tumors with VHL inactivation. The sporadic ccRCC tumors without VHL...
biallelic inactivation were subdivided into a group that had a similar genomic and gene expression profile to sporadic ccRCC with VHL inactivation and another that was dissimilar documenting heterogeneity of RCC tumors even within same subtype (62). A sequencing study looking at 3544 protein-coding genes in ccRCC tumors showed a set of tumors with VHL mutations and another with NF2 mutations along with mutations in other genes. Hypoxia-related gene expression analysis segregated tumours with VHL mutations, from NF2 mutants with low expression of hypoxia related genes (69).

Identification of gene expression signatures in SRMs that would predict growth or aggressive behavior is currently feasible with AS protocols where patients have been biopsied and observed with time. Biopsies were shown to yield RNA that can be used in RT-PCR studies and potentially in microarrays or nanostring assays (54). Such results will be invaluable in SRM diagnostics and guide treatment modalities.

- miRNA in RCC

MicroRNAs (miRNAs) are transcriptionally regulated single-stranded RNA molecules that regulate their target genes either by mRNA degradation or suppression of protein expression (82,82). A host of studies have documented the dysregulation of miRNAs in renal cell carcinoma (RCC) (83-88). Recently, a comprehensive list of miRNAs dysregulated in RCC was compiled (89).

Accumulating evidence is showing the involvement of miRNAs in RCC pathogenesis and that they represent a new dimension in controlling tumor progression. Proposed mechanisms of involvement of miRNAs in kidney cancer were recently reviewed (84,90-93). Bioinformatics analysis has shown that many key molecules in RCC pathogenesis e.g., VHL, HIF, VEGF, and members of the PI3K and the mTOR pathways are potential targets of miRNAs that are dysregulated in RCC (87,94). Also, miRNA alterations are predicted to be associated with key mechanisms of RCC, like the hypoxia pathway and epithelial-mesenchymal transition (95). Recently, Neal et al. showed that certain miRNAs are regulated by VHL in either a hypoxia-inducible factor (HIF)-dependent or HIF-independent manner in RCC (96). Two other miRNAs (miR-5b and miR-20a) were shown to have an oncogenic effect by enhancing tumor proliferation in RCC cell lines (97). Also, miR-205 can inhibit Src-mediated oncogenic pathways (98), and miRNAs were shown to target kallikrein-related peptidases with a downstream effect on tumor proliferation (99). miR-23b was also shown to target proline oxidase, a known tumor suppressor in RCC (100). Finally, miRNAs are downstream effector molecules of the hypoxia pathway (101). An interesting study also showed that a single miRNA can target multiple molecules in RCC pathogenesis (102). A recent study identified a miRNA signature that is associated with RCC tumor progression and metastases (89), and another study showed that tumor suppressor miR-584 can directly target the actin-stabilizing molecule Rock-1 and decrease the invasive ability of RCC cell lines (103). The relationship between miRNAs and carcinogenesis is more complex than previously appreciated. miRNAs can function as regulators of oncogenes or tumor suppressor gene, but can also act as downstream effectors of these molecules. For instance, the TP53 gene acts as a transcriptional regulator of many miRNAs (104). To add to the complexity, recent reports have also proposed that miRNAs can be a part of defense mechanism of the body against carcinogenesis (105).

Although recent evidence suggests that miRNAs are not initiating events in carcinogenesis but rather “fine tuning” molecules with only moderate suppression of their targets, the effect of miRNA on carcinogenesis can still be significant since a single miRNA can simultaneously target multiple molecules.

Finally, the integration of miRNAs with other molecular levels of analyses (including proteomics, transcriptomic, and cytogenetic changes) can significantly enhance our understanding of the pathogenesis of RCC (106-108).

The spectrum of potential clinical applications of miRNAs in cancer is broad and continues to evolve, as outlined recently (109). Of particular interest is the potential clinical utility of miRNAs in the management of SRMs. First, miRNAs can help as diagnostic markers for RCC (89), both in body fluids or at the tumor tissue level, especially for small biopsies obtained from SRMs, where the material might not be adequate for histological evaluation. Recently, miRNA signatures that can accurately distinguish between the different RCC subtypes were identified (110). This can be further utilized to provide more insight about the biological differences between the different types of SRMs with different biological behavior. Finally, it can be also the basis for developing new-targeted therapies for each of these subtypes. Another interesting frontier is the utilization of miRNAs as prognostic markers in RCC. Recent studies identified miRNA signatures of tumor progression and metastases (89). Moreover, individual miRNAs, including miR-106b (111) and miR-21 (112), were shown to be prognostic markers in kidney cancer. Also, miR-9 methylation status was shown to be associated with cancer development and metastatic recurrence in ccRCC (113).

More recently, miRNAs have drawn attention for their potential use in cancer therapy. miRNAs
represent attractive targets in cancer therapies since altering the level of a single miRNA can have an impact on multiple gene targets along the same pathway, producing a coherent physiological response via multiple parallel perturbations (114). Technically, reconstruction or suppressing of miRNA expression (using a complementary sequence) is more feasible compared to other targeted therapies due to their small size, easier transfection, and their stability in blood (115). Interestingly, local miRNA therapy might be an attractive option for SRMs with benign behavior. Other emerging fields of miRNA applications in RCC include the use of miRNAs as predictive markers for treatment efficiency and the utilization of miRNA sequence SNPs to predict cancer predisposition (109).

- **Proteomic signatures**

Proteomic studies in RCC have been performed on tumor tissues, blood and urine using various screening platforms such as mass spectrometry, protein microarrays, and tissue microarrays to identify potential biomarkers for diagnosis, prognosis and targets for therapy. Data from high throughput studies can then be validated by assays such as western blot analysis, ELISA, immunohistochemistry, and also through functional in vitro and in vivo experiments.

For example, LC-MS/MS and ELISA studies showed an abundance of thrombospondin-1 (TSP1) and enolase 2 (ENO2) proteins in the serum of ccRCC patients as compared to normal controls (116). A study using 2D gels and MALDI-TOF/TOF analysis followed by network analysis, identified changes in metabolic pathways such as glycolysis, propanoate, pyruvate, urea and arginine/proline metabolism and in p53 and FAS pathways. Downstream of these metabolic changes, an abnormal glycolysis metabolite, sorbitol, was increased five-fold in the urine of RCC patients urine compared to normal controls (117). A first study of quantitative protein analysis using isotope affinity tags (iTAQ) detected proteomic changes in ccRCC by direct tissue analysis. The study showed upregulation of major vault protein (MVP), adipose differentiation-related protein (ADFP), nicotinamide N-methyltransferase (NNMT), prothymosin alpha and poly (rC)-binding protein with translational regulatory function (118).

Pathway analyses in RCC are very important and are getting more integrated with the high through-put studies. They determine how protein sets are connected to each other through the diverse biological pathways, thus giving insight on RCC initiation and progression and providing targets for therapeutic intervention (118,119).

Proteomic studies have aimed to stratify tumors similarly to DNA and RNA studies. In an elegant correlative study it was shown that ccRCCs can be segregated as follows: I) absence of a VHL abnormality and absent HIF-α expression ("VHL WT"), II) VHL deficient tumors expressing high HIF-1α and HIF-2α protein levels ("H1H2") III) and tumors with VHL loss and high HIF-2α levels exclusively (H2). "VHL WT" and "H1H2" tumors showed increased AKT/mTOR and ERK/MAPK signaling whereas "H2" RCC exhibited elevated c-Myc activity, yielding an in vitro phenotype with increased proliferation and resistance to replication stress. These results provide a rationale basis for stratification of ccRCC in trials of targeted therapies (120).

Biomarkers that are downstream targets of VHL and HIF include CAIX and VEGF. High CAIX levels in ccRCC were found to predict good outcome and response to treatment while low levels were found to be independently associated with poor survival (121,122). VEGF levels in serum were associated with tumor grade and stage and high levels correlated with poor survival (122,123). Phuoc et al. reported that low tumor CAIX levels and high VEGF levels in ccRCC tumors were each associated with poor prognosis. They found that both, CAIX expression and the co-expression of CAIX-VEGF, were independent prognostic factors of disease specific survival (124). Identifying proteomic signatures of progression would be important in guiding treatment of patients presenting with SRMs. Biopsies were found to provide adequate amounts of protein for studies including western blot analysis and high through-put protein arrays, thus facilitating the translation of these results to the clinic (54).

**CONCLUSIONS**

Overall, there is both a need to identify and better understand tumors destined to be fatal in order to reduce mortality by early intervention in patients whose SRMs will progress yet also reducing treatment morbidity for the majority of patients now presenting with indolent SRMs. Identifying molecular prognostic biomarkers or signatures of lethality will be achieved by integrating genomic, transcriptomic and proteomic signatures to individualize early stage kidney cancer management.

**REFERENCES AND RECOMMENDED READINGS**

(*of special interest, **of outstanding interest)


122.疵

123.疵

124.疵