Molecular profiling of small renal masses: Current status and future directions

Balaji Kalyanaraman, Krishnanath Gaitonde, James F. Donovan
Division of Urology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

ABSTRACT

Small renal masses (SRMs) are renal tumors less than 4 cm in diameter. These account for the largest proportion of newly diagnosed renal cell cancers (RCC). Management of SRMs can be a dilemma if the patient is unfit to undergo partial nephrectomy. Molecular profiling enables better characterization of RCC and prediction of outcomes in terms of recurrence and progression. This article reviews the existing literature on molecular profiling of localized RCC, discusses limitations of molecular profiling, and presents the likely role that molecular profiling will play in guiding the treatment of SRMs.

Key words: Molecular profiling, percutaneous renal biopsy, renal cell cancer, small renal masses

INTRODUCTION

An estimated 57,760 new cases of cancer of the kidney and renal pelvis will be diagnosed in the United States in 2009, an increase of 3000 cases compared to the previous year. The overwhelming majority of these cancers will be renal cell cancers (RCCs). Widespread use of computed tomography (CT) for abdominal imaging has contributed to the increasing incidence of renal cell cancer by way of detecting incidental renal masses. For clinical staging purposes, localized renal cancers are classified by size as less than 4 cm (T1a), between 4 and 7 cm (T1b), and larger than 7 cm (T2). T1a tumors, also termed small renal masses (SRMs), account for the largest proportion of newly diagnosed renal cancers. Surgical excision in the form of partial nephrectomy (PN) is the standard of care for T1a tumors and confers oncologic outcomes similar to radical nephrectomy (RN), while offering distinct advantage in overall survival and noncancer-related mortality compared to RN. However, patient factors such as cardiopulmonary comorbidities, tumor in a solitary kidney, or presence of chronic kidney disease may force the surgeon to consider alternative treatment options, namely active surveillance (AS) and thermal ablation (TA) (i.e., cryoablation and radiofrequency ablation). Although there have been no prospective studies to date comparing the efficacy of these treatment modalities to PN, meta-analysis by Kunkle et al. indicates that TA is associated with increased risk of local recurrence but no greater risk of metastases when compared with PN.

In the process of determining the appropriate modality of therapy for a given SRM, it is also necessary to consider the natural history of that tumor. It is well known that RCCs are comprised of pathologically and genetically diverse population of cancers. This implies that regardless of tumor size and histology, the molecular attributes of a RCC can determine its risk of recurrence and metastasis. Therefore, characterizing the molecular biology of a tumor can provide vital information to the surgeon to help stratify risk and thereby guide therapy.

MOLECULAR PROFILING OF LOCALIZED RCC

Molecular profiling is defined as the classification of biological specimens, like tissues, blood, or urine, based on multiple molecule (like gene, protein, miRNA) expression patterns or genomic changes for diagnostic, prognostic, and predictive purposes. Several molecular markers have been investigated for their role in the pathogenesis and progression of RCC. Those which have been consistently correlated with the prognosis of localized RCC will be discussed in this section with the emphasis on implications for recurrence and/or progression.

p53: Tp53 is a tumor suppressor gene that regulates cell-cycle at the G1-S transition point. The normal gene product, p53,
induces apoptosis in proliferating cells that have undergone DNA damage. Mutations of p53 permit damaged cells to persist in the cell-cycle, thereby promoting carcinogenesis.\textsuperscript{10} Mutant p53 protein has a long half-life, and therefore can be detected by immunohistochemistry as a nuclear stain.\textsuperscript{11} In localized clear cell RCC (ccRCC), p53 mutation has been shown to negatively correlate with disease-free survival (DFS).\textsuperscript{12} In the study incorporating various histologic forms of RCC, mutant p53 expression was found to be higher in non-ccRCC compared to ccRCC, with papillary RCC showing the highest expression.\textsuperscript{12,13} In this study, p53-positive ccRCC had 56.3% progression after nephrectomy compared with 17.5% in p53-negative patients. In non-ccRCC, p53 expression did not have a statistically significant correlation with progression, although there was a positive trend. Of note, the median follow-up in this study was only 26 months and no cutoff was established for p53-positivity. Increasing the cutoff point for p53-positivity does not seem to alter the predictive ability of the biomarker, as shown by Shvarts et al.\textsuperscript{13} Conflicting evidence has been generated by Phuoc et al, whose data showed that mutant p53 expression in localized ccRCC was a significant prognostic factor on univariate analysis but not on multivariate analysis.\textsuperscript{14}

**Ki-67:** Ki-67 is a nuclear antigen that is present in all cycling human cells and is a marker of cell proliferation.\textsuperscript{15} Increased expression of Ki-67 has been associated with higher nuclear grade and worse prognosis in ccRCC.\textsuperscript{16} Several recent studies suggest that Ki-67 expression could serve as an independent predictor of DFS in localized ccRCC on multivariate analysis\textsuperscript{17,18} and that it may represent the true “molecular grade” of ccRCC.\textsuperscript{18}

**IMP-3:** IMP-3 is a member of insulin-like growth factor (IGF) m–RNA binding protein family. It is expressed at low or undetectable levels in normal tissues, and overexpressed in cancers of pancreas, lung, colon, stomach, and soft tissue sarcomas.\textsuperscript{19} In a study by Jiang et al.,\textsuperscript{20} IMP-3 was overexpressed in 10% of stage-I ccRCC. IMP-3 expression correlated negatively both with 5-year metastasis-free survival (IMP-3+ 44%; IMP-3− 98%) and overall survival (IMP-3+ 32%; IMP-3− 89%). External validation of this study confirmed the negative correlation of IMP3 expression with 10-year metastasis-free survival for TNM stage I ccRCC.\textsuperscript{21}

**Survivin:** Survivin is an antiapoptotic protein, which is overexpressed in almost all human cancers, including those of the kidney. Overexpression of Survivin has been shown to correlate negatively with DFS as well as overall survival in localized ccRCC.\textsuperscript{22,23} Five- and ten-year progression-free survival in the Survivin-positive group was 58.8% and 45.9%, respectively, compared with 86.8% and 81.2% in the Survivin-negative group.\textsuperscript{22} A smaller study showed that patients with Survivin-positive RCC had higher recurrence rate at 5 years compared with Survivin-negative RCC (72% vs. 93%), with no difference in overall survival.\textsuperscript{23}

### MOLECULAR MARKERS AND PROGNOSTIC MODELS FOR LOCALIZED RCC

Several nomograms and algorithms exist to help guide the followup and treatment of RCC.\textsuperscript{24–26} The current prognostic models use clinical variables such as tumor size, pathological stage, grade, nodal status, and histologic characteristics such as vascular invasion, to predict risk of recurrence and/or progression. The concordance index (CI), which represents accuracy of these models, ranges from 0.74 to 0.82. By integrating five molecular markers (Ki-67, p53, endothelial and epithelial VEGFR1, epithelial VEGF-D) with tumor T-stage and ECOG PS, Klatte et al. devised a nomogram to predict DFS in localized RCC, with CI of 0.90.\textsuperscript{18} The accuracy of this nomogram was higher than that of T classification alone (CI 0.74) or UISS nomogram alone (0.78). Parker et al. have recently described a biomarker panel for ccRCC consisting of Ki-67, survivin, and B7-H1 (named Bioscore), which increased the CI of UISS from 0.774 to 0.819 and the CI of SSIGN algorithm from 0.821 to 0.837.\textsuperscript{27} These demonstrate the contribution of molecular markers to enhance the power of clinical prognostic models in predicting long-term outcomes and risk of recurrent disease in localized ccRCC.

### LIMITATIONS OF MOLECULAR PROFILING OF RCC

Although biomarkers are a promising tool for the treatment and surveillance of RCC, it must be emphasized that the field of study is quite nascent and has numerous limitations. Most of the available data apply to ccRCC because it is the most commonly encountered variant. At best, limited information is available regarding the prognostic value of biomarkers in the context of papillary or chromophobe RCC – the data from ccRCC cannot be extrapolated to these other variants as they are biologically dissimilar.\textsuperscript{7,8} The evaluation of biomarkers is predominantly semiquantitative and can be subject to observer bias. The determination of cutoff point for clinical significance is sometimes arbitrary and can alter the prognostic significance in the final analysis. In addition, the studies that do show prognostic significance can be considered for clinical application only after they are externally validated because almost all initial studies are single-institution based. Except for IMP-3, no other molecular marker has been externally validated to date.\textsuperscript{21} Also, the molecular analyses carried out thus far have been on post-nephrectomy specimens and as such, biomarkers can be used only for surveillance purposes and not for primary treatment. There is an emerging need for research on preoperative molecular profiling of SRMs with the emphasis on percutaneous biopsy specimens. Finally, the cost of analyzing tumors for biomarkers in the clinical setting is prohibitive and these tests cannot be justified until their sensitivity and specificity significantly exceed those of current diagnostic methodologies.
Figure 1: Future role of molecular profiling in the management of SRMs.

FUTURE DIRECTIONS

As mentioned earlier, one of the challenges in the treatment of RCC is to decide which subset of SRMs needs surgical intervention.\(^{28}\) This is especially true as the population ages, and the surgeon is faced with comorbidities such as chronic kidney disease or solitary kidney in which case watchful waiting may be the best nephron-sparing strategy. Molecular profiling of percutaneous needle biopsy specimens could provide data on this population of RCC and potentially translate into clinically relevant nomograms. Recent studies on needle biopsy of SRMs for histopathologic diagnosis have reported sensitivities of 70–92% and specificity of 100% with no significant complications or needle-track seeding.\(^{29,30}\) Therefore, percutaneous tumor biopsies can be considered safe and effective for SRMs.\(^{31}\) High-throughput microarray analysis makes it possible to obtain molecular information from needle biopsy specimens\(^{32}\) and such information could be used in nomograms to stratify risk accurately and to guide treatment. This likely evolution of management of SRMs is illustrated in Figure 1. In summary, molecular profiling of renal tumors is poised to take the leap from “bench to bedside.”\(^{32}\)

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Et Al. Cancer Statistics, 2008. CA Cancer J Clin 2008;58:71.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin 2009;59:225.
3. Nguyen MM, Gill IS, Ellison LM. The Evolving Presentation Of Renal Carcinoma In The United States: Trends From The Surveillance, Epidemiology, And End Results Program. J Urol 2006;176:2397.
4. Antonelli A, Cozzioli A, Nicolai M, Zani D, Zanotti T, Perucchini L, Et Al. Nephron-Sparing Surgery Versus Radical Nephrectomy In The

Treatment Of Intracapsular Renal Cell Carcinoma Up To 7cm. Eur Urol 2008;53:803.
5. Zini L, Perrotte P, Capitiano U, Jeldres C, Shariat SF, Antebi E, Et Al. Radical Versus Partial Nephrectomy: Effect On Overall And Noncancer Mortality. Cancer 2009;115:1465.
6. Kunkle DA, Egleston BL, Uzzo RG. Excise, Ablate Or Observe: The Small Renal Mass Dilemma: A Meta-Analysis And Review. J Urol 2008;179:1227.
7. Aydin H, Zhou M. The Changing Face Of Renal Cell Carcinoma Pathology. Curr Oncol Rep 2008;10:235.
8. Soung SP, Rao J, Cheng L, Cote RJ. Classical Pathology Versus Molecular Pathology In Renal Cell Carcinoma. Curr Urol Rep 2007;8:5.
9. Ioannidis JP. Is Molecular Profiling Ready For Use In Clinical Decision Making? Oncologist 2007;12:301.
10. Elledge Sj. Cell Cycle Checkpoints: Preventing An Identity Crisis. Science 1996;274:1664.
11. Soussi T. The P53 Tumor Suppressor Gene: From Molecular Biology To Clinical Investigation. Ann N Y Acad Sci 2000;910:121-37; Discussion 137-9.
12. Zigeuner R, Ratschek M, Rehak P, Schips L, Langner C. Value Of P53 As A Prognostic Marker In Histologic Subtypes Of Renal Cell Carcinoma: A Systematic Analysis Of Primary And Metastatic Tumor Tissue. Urology 2004;63:651.
13. Shvarts O, Seligson D, Lam J, Shi T, Horvath S, Figlin R, Et Al. P53 Is An Independent Predictor Of Tumor Recurrence And Progression After Nephrectomy In Patients With Localized Renal Cell Carcinoma. J Urol 2005;173:725.
14. Phuoc NB, Ebara H, Gotot H, Nakano M, Yoko S, Deguchi T, Et Al. Immunohistochemical Analysis With Multiple Antibodies In Search Of Prognostic Markers For Clear Cell Renal Cell Carcinoma. Urology 2007;69:843.
15. Rioux-Leclercq N, Turlin B, Bansard J, Patard J, Manunta A, Moulinoux JP, Et Al. Value Of Immunohistochemical Ki-67 And P53 Determinations As Predictive Factors Of Outcome In Renal Cell Carcinoma. Urology 2000;55:501.
16. Visapaa H, Bui M, Huang Y, Seligson D, Tsai H, Pantuck A, Et Al. Correlation Of Ki-67 And Gelsolin Expression To Clinical Outcome In Renal Clear Cell Carcinoma. Urology 2003;61:845.
17. Sakai I, Miyake H, Takenaka A, Fujisawa M. Expression Of Potential Molecular Markers In Renal Cell Carcinoma: Impact On Clinicopathological Outcomes In Patients Undergoing Radical Nephrectomy. BJU Int 2009.
18. Klatte T, Seligson DB, Larochelle J, Shuch B, Said JW, Riggs SB, Et Al. Molecular Signatures Of Localized Clear Cell Renal Cell Carcinoma To Predict Disease-Free Survival After Nephrectomy. Cancer Epidemiol Biomarkers Prev 2009;18:894.
19. Zheng W, Yi X, Fadare O, Liang SX, Martel M, Schwartz PE, Et Al. The Oncofetal Protein IMP3: A Novel Biomarker For Endometrial Serous Carcinoma. Am J Surg Pathol 2008;32:304.
20. Jiang Z, Chu PG, Woda BA, Rock KL, Liu Q, Hsieh CC, Et Al. Analysis Of RNA-Binding Protein IMP3 To Predict Metastasis And Prognosis Of Renal Cell Carcinoma: A Retrospective Study. Lancet Oncol 2006;7:556.
21. Hoffmann NE, Sheinin Y, Lohse CM, Parker AS, Leibovich BC, Jiang Z, Et Al. External Validation Of IMP3 Expression As An Independent Prognostic Marker For Metastatic Progression And Death For Patients With Clear Cell Renal Cell Carcinoma. Cancer 2008;112:1471.
22. Parker AS, Kosari F, Lohe CM, Parker AS, Leibovich BC, Jiang Z, Et Al. High Expression Levels Of Survivin Protein Independently Predict A Poor Outcome For Patients Who Undergo Surgery For Clear Cell Renal Cell Carcinoma. Cancer 2006;107:37.
23. Byun SS, Yeo WG, Lee SE, Lee E. Expression Of Survivin In Renal Cell Carcinomas: Association With Pathologic Features And Clinical Outcome. Urology 2007;69:34.
24. Lam JS, Shvarts O, Leppert JT, Pantuck AJ, Figlin RA, Belldegrun AS.
25. Kalyanaraman et al.: Molecular profiling of small renal masses.
Postoperative Surveillance Protocol For Patients With Localized And Locally Advanced Renal Cell Carcinoma Based On A Validated Prognostic Nomogram And Risk Group Stratification System. J Urol 2005;174:466.

25. Kattan MW, Reuter V, Motzer RJ, Katz J, Russo P. A Postoperative Prognostic Nomogram For Renal Cell Carcinoma. J Urol 2001;166:63.

26. Zisman A, Pantuck AJ, Wieder J, Chao DH, Dorey F, Said JW, Et Al. Risk Group Assessment And Clinical Outcome Algorithm To Predict The Natural History Of Patients With Surgically Resected Renal Cell Carcinoma. J Clin Oncol 2002;20:4559.

27. Parker AS, Leibovich BC, Lohse CM, Sheinin Y, Kuntz SM, Eckel-Passow JE, Et Al. Development And Evaluation Of Bioscore: A Biomarker Panel To Enhance Prognostic Algorithms For Clear Cell Renal Cell Carcinoma. Cancer 2009;115:2092.

28. Boorjian SA, Uzzo RG. The Evolving Management Of Small Renal Masses. Curr Oncol Rep 2009;11:211.

29. Neuzillet Y, Lechevallier E, Andre M, Daniel L, Coulange C. Accuracy And Clinical Role Of Fine Needle Percutaneous Biopsy With Computerized Tomography Guidance Of Small (Less Than 4.0 Cm) Renal Masses. J Urol 2004;171:1802.

30. Volpe A, Mattar K, Finelli A, Kakcura JR, Evans AJ, Geddie WR, Et Al. Contemporary Results Of Percutaneous Biopsy Of 100 Small Renal Masses: A Single Center Experience. J Urol 2008;180:2333.

31. Lane BR, Samplaski MK, Herts BR, Zhou M, Novick AC, Campbell SC. Renal Mass Biopsy: A Renaissance? J Urol 2008;179:20.

32. Arsanious A, Bjarnason GA, Yousef GM. From Bench To Bedside: Current And Future Applications Of Molecular Profiling In Renal Cell Carcinoma. Mol Cancer 2009;8:20.

How to cite this article: Kalyanaraman B, Gaitonde K, Donovan JF. Molecular profiling of small renal masses: Current status and future directions. Indian J Urol 2009;25:485-6.

Source of Support: Nil, Conflict of Interest: None declared.