Gene Expression Profiling of Renal Cell Carcinoma

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ABSTRACT
Renal cell carcinoma (RCC) is a histologically diverse disease, with variable and often unpredictable clinical behavior. The prognosis worsens dramatically with the onset of clinical metastasis, and current regimens of systemic therapy yield only modest benefits for metastatic RCC. Gene expression profiling is a promising technique for refining the diagnosis and staging of RCC, as well as for highlighting potential therapeutic targets. We review the recent advances in expression profiling of RCC and discuss the clinical and biological insights obtained from these studies.

INTRODUCTION
High-throughput gene expression profiling, in conjunction with the generation of comprehensive genomic sequences, has been instrumental in elucidating the genetic patterns of many biological networks. In clinical cancer research, expression profiling has unearthed new subclasses of cancer and identified novel markers of outcomes such as survival and drug response (1–5). In this review, we assess recent studies involving expression profiling of human renal cell carcinoma (RCC) tissue; we include results of expression profiling studies of RCC cell lines that have been corroborated by clinical studies. We organize the article into sections on diagnosis, prognosis, therapy, obstacles, and tumor biology. Each section concludes with our predictions for what studies are most likely to affect RCC research and management in the near future.

DIAGNOSIS AND SUBCLASSIFICATION
RCC is a heterogeneous disease, classified into various subtypes based on morphologic features identified with light microscopy (6). Clear cell RCC is the most common adult renal neoplasm, accounting for ~80% of kidney cancers. The other less common subtypes of RCC are papillary RCC (10–15%), chromophobe RCC (5%), collecting duct RCC (<1%), and unclassified (<2%). Sarcomatoid features characterize the advanced stage of all of the histologic subtypes and are thought to represent areas of active epithelial-mesenchymal transition (7).

Recent studies have demonstrated that the various subtypes of RCC are readily distinguishable with gene expression profiling (8–13). These results support the intuitive hypothesis that each subtype has its own individual biological features, clinical behavior, and, by extension, unique sensitivity to therapy. Although the discriminatory ability of gene expression profiling makes it potentially an excellent diagnostic tool, from a practical perspective the technology is currently not readily accessible to many pathologists in clinical practice. Microarrays have not been approved as diagnostic tests by the Food and Drug Administration because of concerns about standardization and interpretation (14, 15). Nevertheless, in renal pathology, microarrays have already been instrumental in discovering new immunohistochemical markers for distinguishing the different subtypes of RCC. For example, Yamazaki et al. (11) profiled several histologic subtypes of RCC and identified c-kit as being up-regulated in chromophobe RCC. Our group identified the following potential markers for the different histologic subtypes: glutathione S-transferase α for clear cell RCC, α-methylacyl racemase for papillary RCC, carbonic anhydrase II for chromophobe RCC, and K19 for transitional cell carcinoma (8).

These studies represent a direct effort to enhance the practice of pathology through the use of microarray technology (8, 16). In addition to building sets of individual gene classifiers, we have recently begun to use gene expression profiles to identify regional gene expression bias. Identification of regional expression biases often indicates underlying chromosomal abnormalities and allows the visualization of both cDNA and oligonucleotide expression data in terms of chromosomal structure. We identified regional expression biases with a technique called comparative genomic microarray analysis (CGMA; refs. 17, 18) from the cDNA microarray profiles of clear cell, papillary, and chromophobe RCCs matched with normal tissue (Fig. 1). CGMA identified consistent down-regulation of gene expression on chromosome 3p and frequent up-regulation of gene expression chromosome 5q in a set of clear cell RCC samples, and frequent gains in expression were demonstrated at chromosome 7, 16p and 17 in papillary RCC. Corresponding structural abnormalities have been previously identified with microsatellite and comparative genomic hybridization studies (19).

Gene expression profiling is not limited to tumor tissue. Interestingly, Twine et al. (20) profiled peripheral blood mononuclear cells (PBMCs), instead of tumor tissue, from RCC patients and healthy volunteers and demonstrated that these expression profiles could distinguish the PBMCs of RCC patients from those of healthy volunteers. Although the biological significance of the identified discriminatory genes is uncertain,
these preliminary findings are of considerable interest, because the authors indicate that their ongoing studies suggest that expression profiling of PBMCs can distinguish RCC patients from patients with other types of solid tumors. It is possible that additional work may establish the presence of disease-specific gene sets in PBMCs.

We predict that gene expression profiling is likely to result in rapid advances in our understanding of kidney development. A detailed knowledge of development is required for interpreting the complex genetic patterns of the various subtypes of RCC that are evident on gene expression profiling. Microarray studies that systematically examine the development of the rat and mouse kidney have been published (21, 22) and are freely available on the Internet. Using these resources, Li et al. (23) showed in an elegantly designed study that Wilms’ tumor (a pediatric developmental tumor that histologically recapitulates...
the nephrogenic zone of the fetal kidney) strongly resembles the earliest committed stage in the metanephric mesenchymal-epithelial transition. Because epithelial-mesenchymal transition is a major process within RCC and mesenchymal-epithelial transition is of such paramount importance in kidney ontogenesis (7, 24), the importance of these developmental studies in studying malignant transformation cannot be overstated.

PROGNOSIS

Refining prognostic systems to more accurately predict patient outcomes and thereby guide more effective treatment decisions is an ongoing process. To date, key prognostic factors identified include TNM staging, tumor grade, functional status, and various biochemical assessments (25–28). Integrated prognostic systems have been developed by several groups combining clinical and pathological data to better stratify patients and improve prognostic power (25, 26, 29). Further integration of molecular markers defined by expression and proteomic profiling into these prognostic systems is likely to further increase prediction accuracy.

We initially reported a study in which 29 specimens of clear cell RCC with patient-matched normal tissue were profiled with cDNA microarrays (30). In this study, unsupervised clustering demonstrated two subsets of tumors, with clear segregation by cause-specific survival at 5 years. Approximately 40 genes were identified that discriminated between these two groups. These results suggest that there are two distinct groups of clear cell RCC that vary in aggressiveness. One limitation of this study was the relatively small number of cases, resulting in the inability to stratify the analysis by stage or grade. In addition, the functional status of the patients was not available. However, the prediction accuracy of 5-year survival by using microarrays exceeded that of staging, and accurate cross-validated predictions were obtained for patients with clinically indolent metastatic RCCs and clinically aggressive localized RCCs, which suggested that the prognostic signature was not confounded by metastasis. In view of the relatively small numbers of this study, we believe that this study should be considered preliminary.

In a second, larger study involving 58 cases of stage IV kidney cancer, Vasselli et al. (31) identified a 45-gene signature defining poor outcome and highlighted vascular cell adhesion molecule 1 as a potential prognostic marker. Notably, this study controlled for functional status, restricting the case selection criteria to those patients with Eastern Cooperative Oncology Group status of 0 or 1. However, this study grouped clear cell, papillary, and unclassified stage IV cancers together in the final analysis. Because previous gene-expression-profiling studies have consistently highlighted the differences in gene expression among the various RCC subtypes (10, 11, 30), grouping the various subtypes may confound the analysis.

Although the design, methods, and case selection criteria of each study were fundamentally different, the absence of any overlap in the significant genes identified by these two studies is striking. We are currently performing a study on a larger cohort of patients with clear cell RCC from multiple centers to reconcile this discrepancy. A larger group of patients will permit a study that can adjust for subtype and prognostic factors, as well as allowing independent validation of various predictive models (32).

Until a classification model has been corroborated by additional follow-up studies, the application of expression-profiling studies of kidney cancer in determining prognosis, although highly promising, should be considered preliminary. In view of the currently limited options for RCC management, we expect that the most significant immediate clinical benefit in gene expression profiling of RCC will be the identification of patients with poor-prognosis, localized RCCs that are likely to relapse and metastasize. Systemic therapy for these patients in the framework of a clinical trial would be the ideal approach to improving current best practice. In addition, increasing the follow-up frequency for these patients might enable treatment to be initiated earlier, when the disease may potentially be more sensitive to treatment. Expression profiling will probably become a critical part of future trials of RCC, which will permit more effective subgroup analyses and follow-up studies in which genes related to response can be studied.

THERAPY

Surgical resection is the mainstay of therapy for patients with localized primary tumors. It is no exaggeration to say that new therapies are desperately needed for metastatic RCC, which is poorly responsive to chemotherapy and radiotherapy. Conventional treatment options currently available include immunotherapy regimens that use interferon-α, interleukin 2, or both; however, the therapeutic benefits of these agents are largely limited to a small percentage of patients with durable sustained complete remissions (33). A comprehensive meta-analysis of trials with at least one immunotherapeutic agent in one arm reported that immunotherapy yields an average response rate of 10.2%, a complete response rate of 3.2%, and a weighted average median survival improvement of 2.6 months (34).

Gene expression profiling could potentially be used to identify high-risk patients with localized RCC for early systemic therapy. Whether expression profiling will actually result in individually tailored therapeutic regimens in the near future is certainly much more arguable. On one hand, elevated levels of vascular endothelial growth factor (VEGF) have been documented in clear cell RCC tumors (35, 36). A phase II trial of the humanized anti-VEGF monoclonal antibody (bevacizumab) demonstrated a delay in progression in metastatic RCC (37), and a phase III trial investigating the addition of bevacizumab to conventional interferon therapy is currently under way (38). On the other hand, inhibition of a highly expressed gene may not translate to a favorable clinical outcome. High expression of c-kit was shown in chromophobe RCC, relative to clear cell RCC and normal kidney. In a phase II trial, a single case of metastatic chromophobe RCC with a high expression of kit did not show response to imatinib mesylate, a kit tyrosine kinase inhibitor (39). It is probably more prudent currently to assume that the inhibition of a highly expressed gene will not produce clinical benefit, without further evidence such as mutation or amplification. However, there is certainly room for flexibility in the setting of a rapidly fatal disease without any known treatment, such as renal medullary carcinoma (40). Although future developments may enable the use of microarray data to guide
treatment selections, currently such an approach should be discouraged outside the framework of a trial. At the very least, ethical boards should review all such customized therapy requests and the final outcome should be reported.

In the interim, we are working on defining gene predictors for patient groups that benefit in terms of survival from immunotherapy. Patient response to immunotherapy is unpredictable, and remission may not be sustained (33, 41). Currently, the only putative molecular marker identified to date is carbonic anhydrase IX, which was reported as being highly expressed in all patients (n = 8) who responded well to immunotherapy (42).

More small renal masses of indeterminate significance are being incidentally detected with the more liberal use of imaging studies (43, 44). A significant proportion of these masses are benign tumors (45–47). Expression profiling may allow a modified approach to the solitary renal mass in selected cases, in conjunction with image-guided biopsies that are accurate and can change clinical management (48–51). As further evidence accrues to the safety and durability of response of minimally invasive techniques (45, 52, or lack thereof, it is conceivable that gene expression profiling may be incorporated in a rational therapeutic algorithm. One such possibility may involve an initial biopsy, to obtain a histologic diagnosis, and a diagnostic and prognostic expression profile, allowing an individualized therapeutic strategy that may include open or laparoscopic nephron-sparing surgery, currently less-established minimally invasive techniques such as radiofrequency ablation or cryoablation, or even watchful waiting. However, such algorithms should be carefully evaluated for tangible clinical benefits. Until then, open surgical resection must remain standard therapy for patients with potentially curable RCC, because it has a low mortality and high success rate in the event of cancer (52).

**OBSTACLES**

Although gene expression profiling is of great potential importance, there are currently many impediments to incorporating this into research and practice. The requirement for high-quality RNA in fresh-frozen tissue demands optimization of work-flows in operating theaters and tissue banks to reduce time from resection to freezing. This requirement also severely limits tissue available for research. To address this, expression microarrays customized for formalin-fixed paraffin-embedded tissue have been constructed (53). Should the utility of these arrays be borne out in large-scale studies, large numbers of tissue specimens with defined clinical conditions and outcomes would be immediately available for clinical research.

Variability in expression profiling is a challenge for developing diagnostic and prognostic tools, both for research and clinical practice. Discrepant findings arise because of a plethora of possibilities, including differences in microarray platforms (54), laboratory techniques in handling RNA, and evolving statistical techniques used in analysis (55). Whereas these issues are likely to present ongoing challenges, MIAME standards (Minimum Information About a Microarray Experiment; ref. 56), a unified data exchange model (MAGE-OM) and format (MAGE-ML; ref. 57), and large public repositories of microarray data such as ArrayExpress at the European Bioinformatics Institute (58) and the Stanford Microarray Database (59) are important steps toward ensuring that microarray results can be readily exchanged, interpreted, and independently verified.

**TUMOR BIOLOGY**

In addition to its being relatively common, clear cell RCC has received much attention because 40 to 50% of sporadic tumors display mutations of the von Hippel-Lindau (VHL) tumor suppressor gene (60–62), which is a key player in a hypoxia-signaling pathway. Several groups have profiled RCC cell lines (63–68), seeking to dissect relevant tumorigenic pathways and the VHL protein (pVHL)-mediated hypoxia-signaling pathway. In view of the translational focus of this article, we review only those studies corroborated by clinical data.

Stallter et al. (69) established a link between the pathways of hypoxia signaling and metastasis by elucidating a relationship between pVHL and the chemokine receptor CXCR4 and subsequently demonstrated that high expression of CXCR4 receptor protein in RCC was associated with poor survival. In this study, gene expression profiling of pVHL-expressing and a pVHL-deficient RCC cell line was instrumental in identifying CXCR4 as a potential target of pVHL. CXCR4 was subsequently extensively validated in vitro as a hypoxia-inducible gene. Because VHL mutations occur early in RCC (70), the results imply that metastatic ability may be strongly influenced by particular mutations early in cancer growth. Struckmann et al. (71) used cDNA microarrays of cell lines and in situ hybridization in tissue microarrays to show that B-cell translocation gene 2 (BTG2) mRNA down-regulation occurs commonly in RCC. These studies demonstrate the utility of combining the high-throughput methods of cDNA microarrays and tissue microarrays to rapidly screen for and validate novel diagnostic and prognostic markers of RCC.

In a very interesting and unusual study, Copland et al. (72) first demonstrated stepwise sequential loss of type II and type III transforming growth factor-β receptor expression in patient-matched primary and metastatic RCC tissue before proceeding to show that the tumorigenicity of RCC cell lines could be modulated by manipulating the expression of these receptors. This approach showcases the considerable advantage of microarray technology in facilitating basic investigations of clinical observations rather than the more typical translational approach from the laboratory to the bedside.

**SUMMARY**

Gene expression profiling is likely to make inroads in clinical practice during the next few years as additional studies validate its utility in diagnosis and prognosis of various cancers. We expect that current clinical strategies in staging and managing RCC will be extensively modified with the refined diagnostic and prognostic data offered by this technology.

**OPEN DISCUSSION**

**Dr. Robert Figlin:** Do you think it’s possible to develop a protein expression profile for clear cell kidney cancers of either the mutated VHL gene or the wild type?

**Dr. Bin Tean Teh:** We have studied more than 50 clear cell RCCs by mutation analysis; we looked at point mutations, deletions, and loss of heterozygosity. About 70% have either...
one or a combination of these three, and about 25 to 30% don’t have any of these. At this moment, just by nonsupervised clustering analysis, we could not find any difference.

**Dr. Michael Atkins:** Do you have the histologic specimens to go along with your tumor tissue for these patients or is it just the frozen tissue?

**Dr. Teh:** For most of the cases, we have the histologic blocks, which were reevaluated by a urological pathologist.

**Dr. Figlin:** If you received 100 samples of kidney cancer for which the pathologist calls it clear cell, how many of those are really clear cell by protein expression?

**Dr. Teh:** Probably more than 90% are clear-cut “clear cell” type by gene expression, but there are definitely a number of cases that cluster outside the clear cell type. From our experience, most of these cases have mixed cell types.

**Dr. Figlin:** How about if you did that just for papillary tumors?

**Dr. Teh:** Thus far, we have not studied enough cases, and the project is still ongoing. What we would like to do also for papillary is to determine whether we can see the difference between type 1 and type 2 papillary RCC.

**Dr. James Yang:** Your unsupervised clustering data are among the cleanest I have ever seen for any histology. Is it that clear in your own experience?

**Dr. Atkins:** We have similar data from Tovia Lieberman at our institution, and, also, there are similar data from Switzerland from Dr. Moch’s group. I believe it is clear that different subtypes have distinct expression patterns.

**Dr. Teh:** The next challenge is to distinguish between the stages and grades in each subtype.

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