Abstract
Renal cell cancer (RCC) is the most lethal of the urological cancers and accounts for 3% of all adult malignancies. Despite numerous recent advances in diagnostic imaging, surgical therapy, and basic molecular understanding, many patients still experience metastatic disease. For metastatic disease patients, response rates to conventional therapies rarely exceed 15% to 25% and are associated with serious adverse effects. The recent development of novel targeted therapies based on the precise biological pathways deregulated in a particular patient has paved the way for individualized, targeted patient management. Nevertheless, to achieve this goal, it is important to delineate the molecular mechanisms underlying cancer development and progression. Genomic approaches have revolutionized the field of cancer research and have led to the rapid discovery of multiple, parallel disease hypotheses, which ultimately have to be validated in large cohorts of patients and in downstream biological experiments for translation into clinical applications. The variable course of RCC and, until recently, a paucity of therapeutic options in the event of metastasis have led to the search for diagnostic and prognostic markers. We and others have used transcriptional profiling to classify different subtypes of RCC and to identify subtype- and metastasis-specific gene signatures predictive for outcome. We discuss herein recent genomic approaches to RCC and the emerging biological pathways underlying RCC development and progression. We also speculate how genomics may affect drug development and the management of patients with RCC.

Renal cell cancer (RCC), the most common malignancy of the adult kidney, was responsible for 35,700 new diagnoses and 12,500 deaths in the United States in 2004 (1–3). Histologic classification divides RCC into different subtypes (clear cell, papillary, chromophobe, and collecting duct), which have recently been associated with distinct molecular alterations and different clinical outcomes, with clear cell RCC (cRCC) being the most common (70–80%) and most aggressive subtype (4).

RCC presents with up to 30% metastatic cases at initial diagnosis, and ~30% of initially organ-confined cases develop metastases during follow-up at variable intervals (5, 6). Although surgery is highly effective for treating localized cRCC, interleukin-2 immunotherapy in metastatic disease showed durable response rates in only a small subset of patients (7).

Metastatic RCC poses a therapeutic problem because this disease is chemotherapy resistant and radiotherapy is only palliative (8–10). Aggressive treatment with radical nephrectomy and interleukin-2 immunotherapy seems to provide a survival benefit for a small subset of patients with metastatic cRCC, although this has not yet been shown prospectively (11).

One of the major latest successes in the war against cancer is the development of novel targeted therapies based on the precise biological pathways deregulated in a particular patient due to genetic and epigenetic abnormalities. Although in its infancy, molecularly targeted therapy holds promise for a more individualized therapy that contrasts previous approaches with less specific drugs (12–16).

Recent clinical trials in metastatic RCC with targeted therapies for angiogenesis inhibition or inhibition of receptor tyrosine kinases and the mammalian target of rapamycin look promising (16, 17). The development of most of these new agents has been driven by the progress in understanding the biological mechanisms that are involved in oncogenesis.

Several opportunities for targeted intervention are available, including those focusing on inhibition of von Hippel-Lindau, hypoxia-inducible factor-1α, vascular endothelial growth factor (VEGF), epidermal growth factor receptor, mammalian target of rapamycin, Raf, and c-Met (14–16, 18). The mutation of the von Hippel-Lindau gene in high-risk patients with von Hippel-Lindau disease leads to a hereditary form of cRCC but is also seen in 60% to 80% of patients with sporadic cRCC (14, 15, 19, 20). Although the most common RCC subtype (cRCC), mainly with its deranged von Hippel-Lindau pathway, is the one best understood, much less is known for the other subtypes due to their decreased prevalence.
Two of the main problems in the management of RCC are the lack of enough established diagnostic targets and the unsatisfactory therapeutic options for advanced disease. The study of gene interactions and whole-genome transcriptional profiling (genomics) has revolutionized the field of cancer research and has led to rapid discovery of multiple, parallel disease hypotheses that ultimately have to be validated in large cohorts of patients and in downstream biological experiments. Genome-wide investigation is necessary to enhance our understanding of critical pathways in RCC and to identify other potential therapeutic targets (14, 15).

Lack of Consistency in Microarray Data?

Although thousands of microarray data sets have been published over the last 7 years, study-to-study variation and lack of consistency across different platforms and different laboratories have dampened the enthusiasm for transcriptional profiling experiments. Some of these concerns mirror inter-laboratory differences, which are often due to the use of different microarray platforms (cDNA versus oligonucleotide microarrays) in combination with differences of analytic algorithms applied to the data sets. In the context of RCC, Lenburg et al. (21) reviews this topic in detail and ultimately postulates the need for rigorous statistical approaches in microarray studies. Given the differences between the studies, the failure of a gene to be reported as differentially expressed in different studies does not indicate that the gene is in fact not differentially expressed in RCC.

Despite these considerable limitations, several genes have been repeatedly identified in numerous studies of RCC, providing a short list of validated, differentially expressed genes in RCC. Differentially expressed genes repeatedly observed in RCC reveal deregulation of entire biochemical and cellular processes (e.g., glycolysis, cell adhesion, signal transduction, or nucleotide metabolism). Examples of commonly reported genes that are highly expressed in the different subtypes are the following: VEGF, endothelin 1, solute carrier family 2 (facilitated glucose transporter), member 3, insulin-like growth factor–binding protein 3 (in cRCC), α-methyl-CoA racemase [in papillary RCC (pRCC)], or KIT (CD117) in chromophobe RCC (chrRCC).

Coregulated groups of genes associated with clinical prognosis, prognostic “gene signatures,” have the potential of providing relevant information for clinical decision making and have been proposed previously (22). Validation and further refinement of these signatures is a step toward clinical applicability but can be limited by the aforementioned lack of uniform study design, experimental setup, platform differences, or differences in bioinformatics analysis. Nevertheless, successful external validations of microarray data generated on different platforms and by different research groups have been recently shown (23, 24) and have the potential to further facilitate translational research by implementing existing microarray results into new clinical studies.

Is It RCC or Not?

Several recent reports on genomics in RCC have enhanced our knowledge of the biology behind this heterogeneous tumor. In particular, Takahashi et al., Vasselli et al., and Sultmann et al. made significant contributions (22, 23, 25, 26). Early studies showed changes in gene expression in RCC when compared with normal kidney tissue (27). Subsequent studies confirmed the known histologic and genetic differentiation in several subtypes by the analysis of transcriptional profiling alone (25, 28). Although Lenburg et al. (21) analyzed only a relatively small set of RCC samples, this study compared RCC gene expression changes across several previously published transcriptional profiling data sets. In contrast to most previous publications, Lenburg et al. (25) confirmed a subset of 37 genes that were consistently differentially expressed in RCC in at least three different microarray studies. Furthermore, Sultmann et al. (23) showed a clear distinction of cRCC and chrRCC in a study of 87 RCC and 25 normal kidney samples on RCC-specific cDNA microarrays containing 4,207 genes and expressed sequence tags.

Our analysis of 23 normal kidney samples and 32 cRCC samples showed that, under stringent conditions, 1,957 of >18,400 genes were up-regulated in cRCC and 623 genes were down-regulated (24). A significant overlap of our gene signature with published data sets from Lenburg et al. (21) and other studies was observed despite different microarray platforms, different algorithms for bioinformatics analysis, and different compositions of RCC samples. We also analyzed other RCC subtypes (pRCC, chrRCC, and oncocytomas) and transitional cell cancer of the renal pelvis and showed distinct clustering according to the known histomorphologic phenotypes (24).

Interestingly, comparison of all renal tumors in our study to normal kidney identified a common set of 31 genes that were overexpressed in all RCC subtypes and other renal tumors (transitional cell cancer and oncocytomas; Fig. 1). This 31-gene list includes several genes that play critical roles in cancer, such as transforming growth factor–β2 (TGF–β2), a disintegrin and metalloproteinase 12, and protein kinase Cη, indicating that some common biological mechanisms may be involved in most renal tumors, although RCC, transitional cell cancer, and oncocytomas are different clinical entities. A disintegrin and metalloproteinases have been recently suggested to play a role in tumorigenesis and clinical outcome in RCC (29). Disregulation of the TGF–β signaling pathway has also been proposed to play a role in RCC carcinogenesis and progression [refs. 30–32; e.g., through activation of nuclear factor-κB (33)], and a disintegrin and metalloproteinase 12 expression in human activated hepatic stellate cells seems to be regulated by TGF–β (34), possibly linking TGF–β and a disintegrin and metalloproteinase expression in RCC.

Which Subclass of RCC Is It?

Molecular classification of cancer by genomics for diagnostic purposes has become a reality and is starting to move into the clinic. Although histopathologic diagnosis for different RCC subtypes is not a major problem for most RCC cases, a considerable number of patients present with RCC that cannot easily be classified by histopathologic analysis (35). Subtype-differentiating gene signatures on a RCC microarray for molecular diagnosis may provide an additional tool for difficult-to-diagnose cases, whereas subtype-specific gene signatures may also provide improved molecular understanding for the individual subtype and identification of potential drug
Fig. 1. The colorgram of the 31 genes enriched in all renal tumors. Rows, 31 genes; columns, tumor samples. Colorgram depicts high (red) and low (blue) relative levels of gene expression.
targets that may eventually lead to a more specific and targeted therapy (28). Yang et al. (36) recently identified two subtypes of pRCC that differed in patient survival. Some of the differentiating genes were validated with immunohistochemistry. Interestingly, Affymetrix in conjunction with Roche has recently released the first Food and Drug Administration–approved clinical microarray platform that could bring genomic diagnostics to the clinic.

Most early published reports focused on the most common RCC subtypes (cRCC, pRCC, and chrRCC) are limited by small sample sizes for the less common subtypes (pRCC and chrRCC). Various highly accurate subtype-specific gene signatures were recently presented in a larger sample size (23–25, 28, 37). We showed that a 150-gene signature can distinguish each RCC subtype from normal kidney and all other subtypes with 100% accuracy not only in our data set but also in an independent validation set from a different microarray platform in a different laboratory (24, 28). Several other studies (23, 37) also identified RCC subtype-specific gene signatures in large cohorts of RCC samples, supporting the application of genomics for molecular subtype classification of RCC, but came short of providing signatures for other non-RCC tumors.

Further analysis of subtype-specific overexpressed genes revealed a significant overlap of 65 genes among the three RCC subtypes (cRCC, pRCC, and chrRCC; Fig. 2). In addition, each of the subtypes showed additional overlaps of subsets of genes with other subtypes. Detailed molecular modeling and pathway analysis will help to identify common and subtype-specific biological pathway deregulations.

**Early-Stage Gene Expression Changes: A Window into Tumorigenesis and Targeted Therapy?**

Currently, prognostic information in oncology is provided by the use of staging systems. The tumor-node-metastasis staging system for RCC relies solely on anatomic information (38). Tumor staging, therefore, does not take into account molecular variability within a tumor, which could explain the diversity in clinical outcome. Recent reports have suggested reevaluation of the cutoff point between T1 and T2 RCC because larger T1 tumors have an outcome closer to higher T stage tumors (39–41). Consequently, the 2002 tumor-node-metastasis T1 category was subdivided into T1a and T1b. However, this subclassification may not reliably distinguish the outcomes of T1a from T1b tumors (42).

Among the four main histologic subtypes, cRCC has been associated with the worst prognosis, whereas chrRCC seems to be the least aggressive subtype (4). However, only cRCC seems to respond to interleukin-2 therapy, whereas chrRCC and pRCC do not respond to immunotherapy (43). As a result of the uncertainty for individual prognostication, new staging systems with implemented clinical variables have been proposed. The University of California at Los Angeles Integrated Staging System is useful for predicting postoperative outcome to establish appropriate follow-up regimens or clinical trials (44).

None of the microarray data sets was able to clearly identify gene signatures that distinguished different cRCC stages, suggesting that either stage definitions are not related to stringent biological differences or the analyzed sample sizes were too small. We have identified gene signatures that differentiated early-stage T1 cRCC tumors from normal kidney, providing early gene expression changes during tumor development (24). Gene patterns that distinguish distant metastases from T1 stage tumors were determined as a tool to identify genes that change expression during progression and metastasis, and we used the T1 gene signature and the metastasis gene signature to differentiate the intermediate stages of chrRCC independent of the clinical staging.

By focusing purely on the T1 stage cRCC tumors, we identified 1,359 genes that were significantly up-regulated in T1 and 493 genes that were significantly down-regulated in T1 stage cRCC tumors compared with normal kidney. These genes help to define the biological changes that occur during early stages of cRCC development and may provide new clues into the biology of cRCC development and a list of biomarkers and targets for therapeutic intervention.

**Which Genomic Changes Are Associated with Aggressiveness and Metastasis?**

Prediction of metastasis in patients with RCC would have a high effect in their clinical management. The use of transcriptional profiling to build RCC-specific outcome signature has been proposed previously (25). Kosari et al. (45) used transcriptional profiling to identify gene signatures associated with tumor aggressiveness of cRCC. They derived a gene signature of 35 genes, most of them being down-regulated in aggressive cRCC and metastases, and validated their results by real-time PCR in an independent set of cRCC tumors.

We presented a metastatic 155 gene signature (by comparing distant metastases with T1 stage RCC) that accurately distinguished the primary tumor cRCC patients with distant metastasis at the time of surgery from patients without distant metastasis (Fig. 3; ref. 24). This result suggests that cRCC patients presenting with distant metastasis at the time of surgery may have a distinct cRCC subtype with a different underlying biology that can be identified by transcriptional profiling of the primary tumor. Our metastatic gene signature

**Fig. 2.** Venn diagram displaying the relationship of significantly overexpressed genes in cRCC, pRCC, and chrRCC. Numbers outside and inside the circles, number of significantly overexpressed genes.
was successfully validated in an independent published data set (from Sultmann, \( P < 0.05; \) ref. 23) on a different platform, showing cross-laboratory and cross-platform comparison (24). Other studies have postulated that the metastatic potential of human tumors is encoded in the primary tumor (23, 24, 45, 46).

Several of the 155 genes within this metastatic signature have been linked to metastasis and cancer. Protein kinase C\( \alpha \), up-regulated in distant metastases, plays a role in breast cancer and melanoma metastasis and in the mammalian target of rapamycin pathway (47, 48). Mammalian target of rapamycin, a downstream effector of the phosphatidylinositol 3-kinase/protein kinase B signaling pathway, is involved in protein kinase C signaling and is a potential drug target for cRCC (49, 50). The transcription factor \( POU5F1/OCT4 \) is one of the highest and most consistently up-regulated genes in the cRCC distant metastases in our analysis (24). Under normal physiologic conditions, \( POU5F1 \) is exclusively expressed in stem cells (51–53). None of the primary cRCC tumors from patients without metastasis at the time of surgery expresses \( POU5F1 \). However, all distant metastases as well as primary tumors of patients with distant metastases at the time of surgery express increased levels of \( POU5F1 \). This finding suggests that a cancer stem cell may play a role in cRCC metastasis and more aggressive behavior.

### Can Genomic Signatures Predict Outcome of RCC?

In the first prognostic RCC genomic study, Takahashi et al. (25) attempted a prognostic classification in 29 cRCC patients using 21,632 cDNA microarrays. They showed that 40 genes distinguished two subsets with opposite prognosis within cRCC. Among those genes, \( TGF-\beta II \), \( TGF-\beta III \), and \( TIMP3 \) were already known to be associated with tumor progression. Interestingly, down-regulation of \( TGF-\beta II \) and \( TGF-\beta III \) and of several \( TGF-\beta \)–regulated genes was observed during progression of cRCC, although \( TGF-\beta 2 \) itself is up-regulated (24, 30). A study by Vasselli et al. (26) identified a 45-gene signature in 58 metastatic RCC patients associated with poor outcome. Vascular cell adhesion molecule 1 was highlighted as a potential prognostic marker (26).

Yao et al. (54) analyzed the transcriptional profile of 33 RCC samples and 9 normal kidney samples, resulting in the identification of adipose differentiation-related protein as a potential prognostic biomarker for cRCC. Validation in 103 cRCC cases confirmed that elevated levels of adipose differentiation-related protein correlate with better survival. Although all these transcriptional profiling studies provide a glimpse of what may be happening in RCC, there is clearly a need for a concerted effort to validate any of the genes or gene signatures in a multicenter large cohort study.

### Which Biological Pathways Are Deregulated in RCC?

Inactivation of the \( von Hippel-Lindau \) gene in sporadic cRCC tumors leads to activation of the hypoxia pathway via hypoxia-inducible factor-1\( \alpha \) and hypoxia-inducible factor-2\( \alpha \), which in turn activates expression of genes involved in hypoxia response, angiogenesis, and other signaling pathways involving VEGF, \( TGF-\alpha \), GLUT1, CXC-R4, and HIG2 (55–58). Indeed, transcriptional profiling data show a strong increase in VEGF, \( TGF-\alpha \), CXC-R4, and HIG2 expression and the induction of angiogenic pathways, including a whole set of angiogenic growth factors, such as VEGF, \( TGF-\alpha \), placental growth factor, angiopoietin-2, and angiopoietin-like 4 (24). Various growth factor signaling pathways are also turned on...
in cRCC tumors, such as the receptors for VEGF and TGF-α, FLT-1 and epidermal growth factor receptor, and their ligands, suggesting autocrine or paracrine activities as observed in other types of cancer (24). Activation of these two pathways also suggests potential therapeutic interference with VEGF/VEGF receptor inhibitors, such as bevacizumab (Avastin), and epidermal growth factor receptor, such as erlotinib (Tarceva) and gefitinib (Iressa). Epidermal growth factor receptor inhibitors, however, failed to show sufficient clinical activity in RCC (58).

Further evidence for involvement of the WNT pathway in RCC indicating an overall enhanced activation of Wnt signaling (58). Nizes Wnt activity, is down-regulated in cRCC tumors (Fig. 4B), at the same time, secreted frizzled-related protein 1, which antagonizes oncogenic signaling of the Wnt pathway (58). Elevated CXCR4 expression seems to correlate with poor outcome in cRCC patients (60). CXCR4 inhibitors are presently being evaluated for several cancers and might enter into clinical trials for cRCC (61, 62).

The Wnt/β-catenin pathway seems to be highly deregulated in cRCC and indirectly links to the hypoxia pathway. Genomic analysis of cRCC tumors revealed that the hypoxia-inducible genes HIG2 and frizzled homologue 1 are up-regulated (Fig. 4A). HIG2, a secreted protein, directly interacts with frizzled homologue 10 and possibly other frizzled proteins and enhances oncogenic signaling of the Wnt pathway (58). At the same time, secreted frizzled-related protein 1, which antagonizes Wnt activity, is down-regulated in cRCC tumors (Fig. 4B), indicating an overall enhanced activation of Wnt signaling (58). Further evidence for involvement of the Wnt pathway in RCC was provided in kidney-specific APC knockout mice that developed RCC (63).

**Open Discussion**

**Dr. Atkins:** What drives the von Hippel-Lindau (VHL) signature in VHL wild-type tumors? Is the effort to VHL type kidney cancers and correlate VHL mutational status with response to various therapies misguided?

**Dr. Libermann:** I don’t know if it’s misguided, but it is likely that there are several different subtypes for clear cell renal cell cancer. Only a certain fraction of these cancers appear to contain mutations in the VHL gene; nevertheless, our data suggest that the VHL gene signature distinguishes all clear cell renal cell cancers from normal kidney tissue. Therefore, it is possible that other components of the VHL signaling pathway may be modified in tumors with wild-type VHL due to either mutation or changes in expression, and this could have the same effect as a mutation of VHL.

**Dr. Kaelin:** The VHL signature is effectively the hypoxia-inducible factor (HIF) signature. Since all solid tumors have hypoxic cells, if you compare the VHL signature in pancreatic cancer and a normal pancreas, you will see all the carcinomas clustering on the right and normal clustering on the left, but that would be misleading. You are just detecting the hypoxic signature.

**Dr. Figlin:** We have observed in lung cancer that the metastatic potential of the tumor is driven by the biology of the stem cell. You noted that the signature is often driven by CXCR4 because the percentage of CXCR4-positive cells in the original tumor may drive distant metastases, since those have receptors on different organs. What you may be observing as a transition of tumors from stem cell to metastases may be nothing more than compartmentalization of a small subset of tumor cells that are destined to develop metastatic disease that expresses certain chemokine receptors.

**Dr. Libermann:** What is striking in our metastasis gene signature is the difference seen in the T3 stage, where we have a subset of patients who already have the metastatic signature in the bulk of the primary tumor at the time of surgery. These T3 stage patients are the ones presenting with distant metastases at the time of surgery. Since none of the T1 stage tumors express the metastasis gene signature, it is possible that upon progression from the T1 stage some tumors convert to a more aggressive and metastatic phenotype that takes over the primary tumor and rapidly seeds metastatic cells.

**Dr. Figlin:** That is because many of the organs that kidney cancers metastasize to have biological homing devices for selecting cells to target those organs. The question I have not yet resolved is whether there are better preventive molecules for metastatic spread or whether they can be used once the metastatic disease has been identified. If the cell is already established, I’m not sure you can make the cell disappear by chemokine inhibition.

**Dr. Libermann:** There is no drug that can by itself prevent disease. What is needed is combination therapy that targets different pathways at the same time.

**Dr. Vieleg:** CRX4 is a relevant molecule because the natural ligand of CRX4 is SDF-1, which is critical for pulling T cells into the tumor site. Inhibition studies have shown that if you inhibit this pathway, you achieve better T-cell infiltrations in tumors. Also interesting is that this interaction is HIF regulated. We have to look at this as a whole, although it may still boil down to hypoxia as the overriding driver in this entire...
Dr. Ochoa: Are you planning to look at the same genetic signatures, not so much in the tumor itself, but in the cells of the immune system in the responder versus nonresponders to interleukin 2?

Dr. Libermann: If we can get them, we would love to.

Dr. Ernstoff: I was intrigued by the profiles between the primary and the metastases where there was down-regulation of genes. Would that lend itself to the hypothesis that there are some genes that are required for establishment of primary tumors but have to be lost for metastases to occur?

Dr. Libermann: In any biological process, there are genes that have to be inhibited and genes that have to be activated to induce an effect.

Dr. Ernstoff: But there would be genes that would be required for establishment of tumor but then have to be lost for metastases to occur.

Dr. Libermann: Definitely. This is analogous to tumor suppressor genes that become inactivated during tumor formation.

References
Genomics of Renal Cell Cancer: The Biology Behind and the Therapy Ahead

Jon Jones and Towia A. Libermann