Chromosomes, Hypoxia, Angiogenesis, and Trial Design: A Brief History of Renal Cancer Drug Development

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The recent development of effective therapy for renal cancer is a fascinating story of successful translational research that encompasses fundamental studies of oncogenesis and hypoxia, preclinical investigations of antiangiogenic therapy, and the use of novel clinical trial designs. In fact, no single investigator or group of investigators is independently responsible for changing the treatment paradigm in a disease that had been resistant to multiple previous therapeutic approaches. Prior to the development of antiangiogenic therapy, treatment for renal cancer was limited to the immunotherapeutic agents IFN-α and interleukin-2, which have been only modestly successful. The lack of success with other approaches and the well known vascular phenotype of renal cancer thus led to the interest in antiangiogenic therapies. The development of effective antiangiogenic drugs, however, awaited further basic discoveries.

Renal Cancer Oncogenesis and 3p Chromosomal Losses

In 1971, Knudson hypothesized that germ line inactivation of one tumor suppressor allele in a hereditary cancer syndrome, followed by somatic inactivation in the remaining allele, led to disease in the hereditary version of the cancer, whereas somatic inactivation of both tumor suppressor alleles led to the spontaneous version of the disease (1). von Hippel-Lindau disease is a familial cancer syndrome characterized by multiple primary renal cell carcinomas. Exhaustive chromosomal mapping and linkage analysis of affected families led to the identification of the *VHL* gene on chromosome 3 (2). The initial tumor suppressor hypothesis was confirmed upon identification of *VHL* gene inactivation in spontaneous renal cancers, which, importantly, are confined to clear cell carcinomas, thus validating pathologic investigations showing that renal cancer can be subdivided into distinct histologic categories (3–5).

Response to Hypoxia and the *VHL* Gene

Simultaneously, a separate and unrelated series of investigations focused on assessing the cellular mechanism of the hypoxic response, which requires a coordinated set of gene and protein expression that allows cells to survive and adapt to this environmental perturbation. These studies led to the identification of hypoxia-inducible factor (HIF) as the chief transcriptional regulator of this response (6). HIF has since been shown to be a complex of the stably expressed HIF-β subunit and the labile HIF-α subunits that stimulate the transcription of a large number of genes and their corresponding proteins including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (7). Although two HIF-α proteins have been identified and HIF-2α seems to be most relevant in renal cancer, both have similar biology and function (8).

The relationship between *VHL* and HIF became clear in 1999 when the VHL protein (pVHL) was identified as the most important regulator of HIF-α stability (9). Under normoxic conditions, HIF-α is hydroxylated on a specific proline leading to binding by the pVHL multiprotein complex, which in turn, activates the ubiquitin ligase activity of the complex targeting HIF-α for proteasomal degradation (10, 11). Under hypoxic conditions, HIF-α is not hydroxylated, is not bound by pVHL, and thus accumulates in the cell (12).

The *VHL* mutations identified in von Hippel-Lindau disease families and in spontaneous renal cancer abolish the protein's ability to bind HIF-α, which then accumulates in renal cancers even under normoxic conditions (12).

Tumor Angiogenesis

Simultaneously, investigators studying normal and tumor angiogenesis identified key regulatory molecules and pathways, including VEGF, which is the critical survival and growth factor for endothelial cells. It has since been determined that VEGF consists of five specific proteins (A-F, each with multiple splice variants), which, along with placenta growth factor, interact with three VEGF receptors VEGFR1, VEGFR2, and VEGFR3 (see ref. 13 for review). PDGF is composed of two individual proteins that can dimerize into the three active growth factors PDGF-AA, PDGF-AB, and PDGF-BB, which interact with two separate PDGF receptors, PDGFRα and PDGFRβ, the activation of which is thought to be critical for pericyte proliferation and survival (14). Pericytes are an important supporting cell in blood vessels that had been somewhat underappreciated until a series of elegant studies showed that inhibition of both pericytes and endothelial cells synergistically inhibited tumor angiogenesis (15).

VEGFR and PDGFR are both membrane-associated tyrosine kinase receptors that, like other growth factor receptors, signal through a complex series of partners including downstream serine/threonine kinases such as Raf (13, 14). Although the cell biology of angiogenesis has been recognized to be far more complicated than originally anticipated, the VEGF and PDGF systems are still thought to occupy a central role in tumor angiogenesis (Fig. 1).

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Cytostatic Drugs and Clinical Trial Design

Effective antiangiogenic agents could be expected to be growth-inhibitory rather than causing frank tumor shrinkage. Although unlikely to be curative, tumor growth inhibition, especially if accompanied by minimal toxicity, would be expected to lead to improvements in survival and morbidity. The development of a growth-inhibitory agent, however, introduces a new complexity into clinical testing. Specifically, the traditional phase II trial end point has been a somewhat arbitrary degree of tumor shrinkage (16). Lack of growth or tumor shrinkage less than this standard is thus typically not categorized as a response.

"Response" for a growth-inhibitory agent, however, means less growth than would be expected in the absence of therapy, which is not possible to evaluate without a concurrent control group. This follows from the fact that the growth rate of tumors, and especially renal cell cancers, is highly variable and therefore it cannot be determined whether lack of growth over an arbitrary time frame in an individual patient is a drug effect.

The standard controlled evaluation is a trial in which patients are randomized upfront to a novel agent versus placebo. This design is, however, complicated by the reluctance of patients and physicians to randomize patients to placebo, the lack of a clearly identified population that benefits from the drug, and the relatively large number of patients required to identify an effect. To address the first two problems, we proposed a randomized discontinuation trial design (Fig. 2). In this design, all patients receive the growth-inhibitory agent upfront (17). Patients who experience tumor shrinkage after a certain time frame continue on the trial whereas those who experience tumor growth are removed from the protocol. Those patients who experience stable disease are randomized to continuing or discontinuing therapy in a double-blind placebo-controlled manner, with the primary end point being the fraction of randomized patients who maintain stable disease at a second time point following randomization. This trial design thus exposes fewer patients to placebo, and most importantly, allows the drug to select the potentially benefiting population rather than forcing the investigator to use incomplete biological knowledge to select the appropriate population.

Design and Serendipity in the Identification of Renal Cell Cancer Therapeutics

Based on the science of tumor angiogenesis discussed previously, specific targeted therapies for renal cancer were pursued, including VEGFR kinase inhibitors. Interestingly, the initial highly specific VEGFR inhibitors were not clinically useful (18, 19). It remains unclear whether this was because of their too specific molecular spectrum or because they had unfavorable pharmaceutical properties. Compounds with broader spectrum tyrosine kinase inhibitory activity against both VEGFR and PDGFR, such as sunitinib, however, had very dramatic activity in patients with clear cell renal cancer as shown by dramatic tumor shrinkages, including 35% to 40% of whom that met the arbitrary criteria of partial response (20, 21). Although this degree of tumor shrinkage was unexpected, it is noteworthy that the even larger effect of sunitinib on time to progression in the subsequent phase III trial can only be explained if additional patients beyond those experiencing a partial response experienced an antitumor effect (22).

The identification of sorafenib as an active agent is perhaps most instructive. This drug was originally identified as a Raf kinase inhibitor and its initial clinical development plan was focused on tumors, such as colorectal cancer, in which

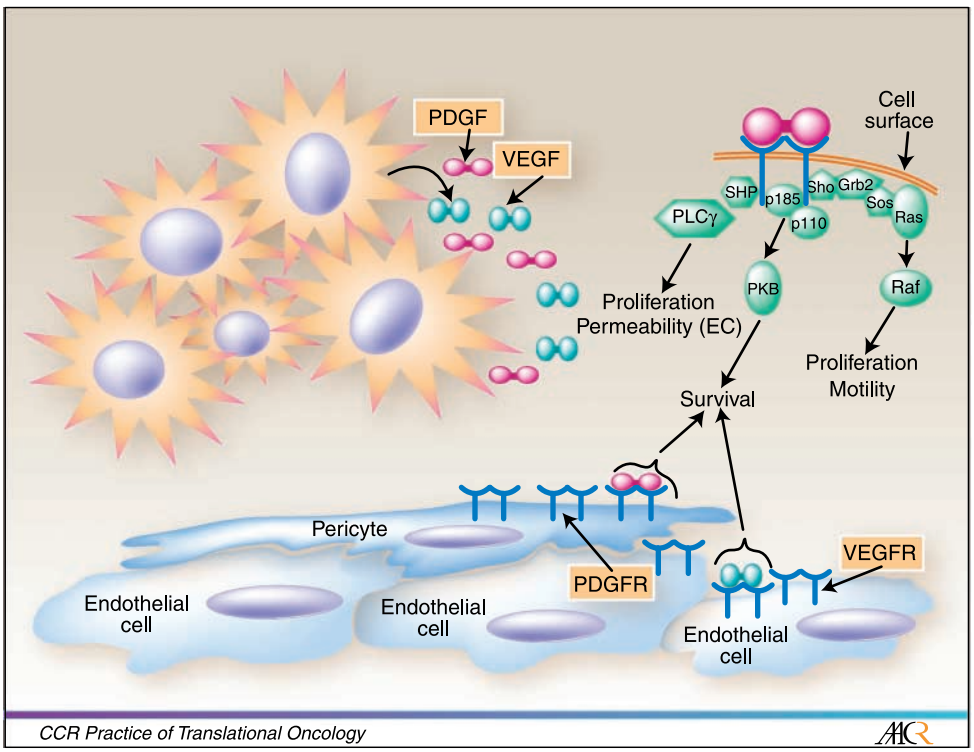

Fig. 1. Tumors secrete a number of proangiogenic factors, including various VEGF family members of which the VEGF-A isoforms are the most important, as well as PDGF family members. The receptors for both are classical tyrosine kinases that mediate growth factor activity through kinase cascades that then lead to cellular growth, inhibition of apoptosis, motility, and increases in vascular permeability. The principal receptors and targets for sorafenib and sunitinib thought to be responsible for their antitumor activity are VEGFR2 on the surface of endothelial cells and PDGFRα and PDGFRβ on the surface of pericytes.

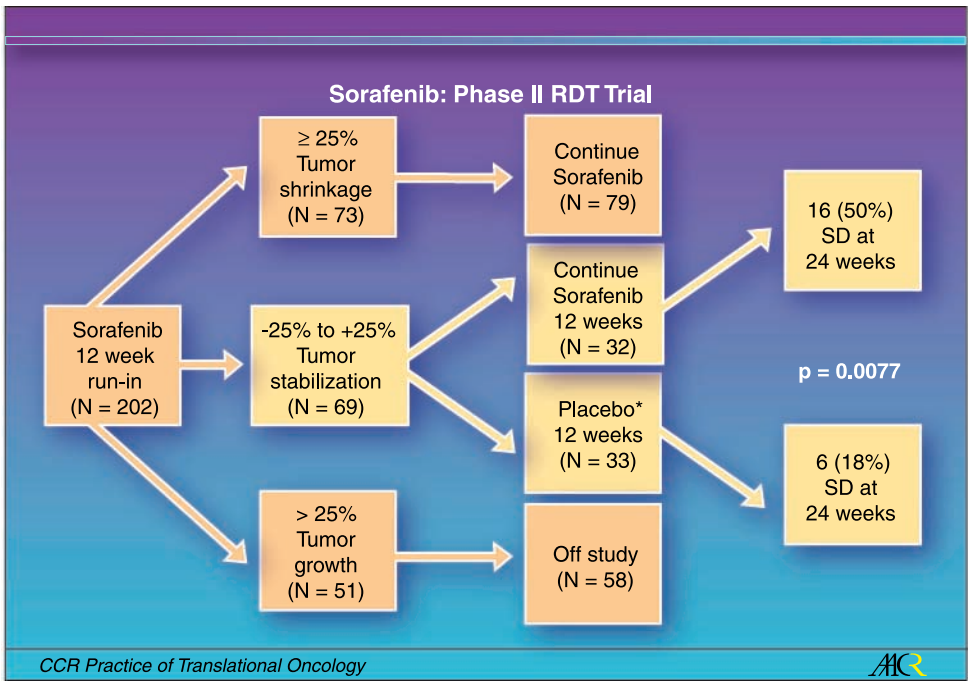

Fig. 2. The schema for the sorafenib phase II randomized discontinuation design. A total of 202 patients with renal cell cancer were enrolled, of which 69 with protocol-defined stable disease were randomized to continuing or discontinuing therapy in a double-blind manner. The resultant difference in freedom from progression 12 wks after randomization was significant and supported the conduct of the confirmatory phase III trial.

activated Raf was expected (23). Because sorafenib was anticipated to be a growth-inhibitory agent, however, a randomized discontinuation phase II trial was initiated that allowed the enrollment of multiple tumor types (24). This trial quickly identified the renal cell cancer population as a therapeutic target and a subsequent phase III trial showed true patient benefit (25). Importantly, tumor shrinkages were also observed in the phase II randomized discontinuation trial, but the objective response rate by standard criteria was only 11%, a rate that is generally insufficient to justify further phase III evaluation. In addition, the VEGFR and PDGFR inhibitory properties of sorafenib, which are now thought to be critical for its mechanism of action in renal cancer were, in fact, not identified until the phase II clinical trial was under way.

Based on the noted biology of tumor angiogenesis, inhibition of VEGF binding to VEGFR could also be anticipated as an effective strategy in renal cancer. A randomized placebo-controlled phase II design using a more traditional upfront randomization in fact did show that the VEGF-binding agent bevacizumab slows disease progression (26). Once again, tumor shrinkages were observed, but the objective response rate by the usual arbitrary criteria was 10%, a rate that would not support the conduct of phase III studies. The definitive phase III studies of bevacizumab versus the combination with IFN-α have been completed and preliminary results are promising (December 11, 2006; Genentech Press Release, South San Francisco, CA).

Conclusions

It is evident that a broad spectrum of basic, translational, and clinical investigations have led to the identification of sunitinib and sorafenib (and probably bevacizumab) as effective agents in the treatment of renal cancer. This, along with the identification of mTOR inhibitors as additional active agents (27), are without a doubt, the most significant developments in the treatment of renal cancer in three decades. Furthermore, it is clear that these developments could not have been accomplished without the contribution of several disciplines, as well as serendipity. Despite this success, a number of critical questions remain. For example, it is likely that the tyrosine kinase inhibitors target additional kinases beyond those already identified. The ones most important for their toxicity and the ones most important for antitumor activity is not clear. Whether targeting of both mTOR and VEGF/PDGF pathways or whether targeting both the ligand and the receptor simultaneously provides a therapeutic advantage will also need to be determined. This is especially critical because complete responses with sorafenib or sunitinib are extremely rare and the disease eventually progresses in all patients. Whether the answer to these questions will come from further directed clinical studies, from translational-based laboratory studies, or from fundamental basic science investigation cannot be determined at this time. It is clear, however, that none of these pursuits can be ignored.

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