Chapter 6
von Hippel–Lindau Tumor Suppressor, Hypoxia-Inducible Factor-1, and Tumor Vascularization

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Cast of Characters

von Hippel–Lindau (VHL) disease is an autosomal dominant, familial cancer syndrome that is characterized by the development of various benign and malignant tumors. The most frequent tumors are hemangioblastoma (HB) in the central nervous system (CNS), pheochromocytoma (Pheo), and renal-cell carcinoma of the clear-cell type (RCC). VHL families have been subdivided into those with a low risk of pheochromocytoma (type 1 VHL disease) and those with a high risk of pheochromocytoma (type 2 VHL disease). VHL type 2 disease is further classified into three categories: type 2A, type 2B, and type 2C. Type 2A VHL disease has pheochromocytoma and hemangioblastoma in the CNS, but not RCC. Type 2B exhibits pheochromocytoma, RCC, and hemangioblastoma. Type 2C disease has only pheochromocytoma, without hemangioblastoma or RCC.

Individuals with VHL disease harbor a germline mutation in one allele of the VHL gene and somatic inactivation or silencing of the remaining wild-type allele results in tumor development (Kim and Kaelin 2004). Type 2 families almost invariably have missense VHL mutations, while type 1 VHL disease is linked to many different types of mutations, including nonsense mutations and deletions. In type 1, type 2A, and type 2B VHL diseases, VHL alleles encode proteins that are at least partially defective with respect to the regulation of hypoxia-inducible factor (HIF) 1α and 2α, whereas the products of type 2C VHL alleles are not defective in this regard (Clifford et al. 2001; Hoffman et al. 2001). However, the products of type 2C VHL alleles are defective with respect to another VHL function, i.e., down-regulation of atypical protein kinase C activity (Pal et al. 1997; Okuda et al. 1999, 2001). Increased atypical protein kinase C activity and consequent upregulation of JunB seem to promote the survival of pheochromocytoma cells (Lee et al. 2005).
The most common sites for hemangioblastoma (HB) development are the cerebellum and spinal cord. The symptoms of this disease are largely characterized by the expansion of the tumor in the cranial space or the spinal cord. Pheochromocytoma develops in the adrenal gland or paraganglia. RCC develops in the kidney and is the tumor that most commonly metastasizes to other organs in VHL disease.

Introduction

In adult life, little angiogenesis occurs in the absence of disease (Hanahan and Folkman 1996). However, the growth of cancers is dependent on angiogenesis (Folkman 1995; Carmeliet and Jain 2000). During the earliest stages of tumor growth, tumors do not demonstrate significant angiogenesis. At sizes up to approximately 1–2 mm tumors can obtain oxygenation via passive diffusion. When tumors grow beyond a volume of several cubic millimeters, passive diffusion cannot provide enough oxygen, and the availability of $O_2$ and nutrients is limited by competition among actively proliferating cells, and diffusion of metabolites is also inhibited by high interstitial pressure (Stohrer et al. 2000). Thus, tumors are required to establish their own vascular supply, which is also referred as tumor neovascularization. New vessels are required not only to provide oxygen, but also to provide nutrients and dispose of cellular metabolic waste. Thus, a major event in tumor development is the angiogenic switch, an alteration in the balance between pro- and anti-angiogenic factors that leads to tumor vascularization, following which the tumor assumes a more aggressive form characterized by rapid growth (Hanahan and Folkman 1996).

Angiogenesis, which refers to the budding of new capillary branches from preexisting capillaries, may be stimulated by changes within the endothelial cell microenvironment including genetic change, trauma, hypoxia, oxidative stress, and mechanical strain. Hypoxia is perhaps the best-characterized initiator of angiogenesis, and HIF-1-regulated factors are involved in different steps in angiogenesis (Semenza 2000). The rapid growth of solid tumors creates an hypoxic microenvironment. Hypoxia-induced and HIF-1-mediated angiogenic growth factor production plays a major role in tumor vascularization. HIF-1 gain-of-function in human cancer cells resulted in increased vascularization of tumor xenografts (Ravi et al. 2000). The following chapter will describe molecular events underlying hypoxic responses and angiogenesis in RCC.

Renal-Cell Carcinoma

RCC is a highly vascular tumor which originates from the proximal tubule cells of nephrons, accounts for approximately 2.6% of all cancers in the United States, and is the sixth leading cause of cancer deaths in developed nations. A quarter of the patients present with advanced disease, including locally invasive or metastatic
RCC. A third of the patients who undergo resection of localized disease will have a recurrence. Although with the emergence of nephron-sparing surgery and other non-surgical techniques, such as radiofrequency ablation, early stage RCC is becoming a curable condition, the median survival for patients with metastatic disease is only 13 months. Each year in the United States, there are approximately 36,000 new cases of RCC and 13,000 related deaths (Cohen and McGovern 2005). Though there are different pathologic subtypes, the majority (≈75%) of RCC cases are referred to as “conventional” or “clear-cell” type (ccRCC) (Cohen and McGovern 2005). More than 95% of clear-cell kidney cancers occur sporadically within the population, while the remainder occur as part of relatively rare, inherited genetic syndromes (Choyke et al. 2003; Cohen and McGovern 2005), which arise from one inherited mutated VHL allele and the inactivation or silencing of the remaining normal (wild-type) VHL allele. Thus, the primary genetic defect of clear-cell kidney cancer (in both sporadic and hereditary forms) involves inactivation of the VHL gene pathway. Remarkably, in sporadic clear-cell renal carcinomas, somatic VHL gene defects are detected in 60–90% of patients with this cancer and up to 20% exhibit decreased VHL expression due to hypermethylation (Gallou et al. 1999; Brauch et al. 2000; Ma et al. 2001; Kondo et al. 2002). However, VHL mutations are not observed in non-clear-cell (papillary or chromophobe) histologies. In this chapter, the term RCC will be used to refer to the clear-cell type renal-cell carcinoma.

The defining feature of RCC is the histologic appearance of large cells with abundant cytoplasm packed with glycogen and neutral lipids that do not stain with hematoxylin/eosin. The accumulation of immense quantities of glycogen probably results from the high level of glucose metabolism observed in RCC; moreover, the neutral lipid may be contributed by the expression of adipose differentiation-related peptide (ADRP). ADRP is a HIF-1 target gene that encodes a cell surface lipid transport molecule, which may promote the cytoplasmic neutral lipid accumulation (Yao et al. 2005). Additional tumor-specific metabolic characteristics, such as the elevated lactate levels within RCC, may relate to the high rates of glucose metabolism and the impaired oxidative phosphorylation process for generating ATP.

VHL Gene

The VHL tumor-suppressor gene, which is located on chromosome 3p25–26, was identified in 1993 (Latif et al. 1993). The gene consists of 3 exons and encodes a short protein (pVHL) with 213 amino acids. pVHL is a potent tumor suppressor, as demonstrated by the introduction of a wild-type VHL cDNA into VHL-null RCC cells, which represses the growth of tumor xenografts in immunocompromised mice (Iliopoulos et al. 1995). There are two start codons in the first exon of the VHL gene. Thus, an alternate N-terminal truncated version is produced by utilization of an in-frame internal ATG translation start site located 54 codons downstream of the 5′-most ATG, producing a 19-kDa product in addition to the 30-kDa 213-amino acid pVHL product (Blankenship et al. 1999). This second gene product retains the
tumor-suppressor function of the full-length protein, but its specific role is not clear. The relative levels of the two pVHL proteins appears to be of little consequence with respect to the promotion of cancer, as few disease-causing mutations have been localized in this N-terminal 54-amino acid region. Instead, the tumor-suppressor activity of pVHL is relegated to the central and C-terminal portions of the protein (Gao et al. 1995). This gene is evolutionarily conserved in organisms ranging from Caenorhabditis elegans to humans. Vhl<sup>−/−</sup> mouse embryos are not viable due to defective placental vasculogenesis, and conditional, systemic inactivation of Vhl in adult mice is also lethal (Gnarra et al. 1997; Ma et al. 2003).

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 cancer, whereas somatic inactivation of both tumor-suppressor alleles led to the sporadic cases (Knudson 1971). Individuals carrying one wild-type VHL allele and one mutated VHL allele in their germ line develop VHL syndrome, which is associated with an increased risk of a variety of tumors, including central nervous system (especially cerebellum and spinal cord) hemangioblastomas, pheochromocytomas, as well as RCC. Whereas individuals with sporadic RCC usually have unilateral kidney involvement, patients with VHL syndrome often have bilateral multifocal disease of early onset.

**VHL Protein**

pVHL functions in the proteolysis of HIF-1α and HIF-2α by the ubiquitin proteasome system (Fig. 6.1). The ubiquitin proteasome system is highly regulated and involves several steps: initiation by a ubiquitin-activating enzyme (E1), transfer of activated ubiquitin to a ubiquitin-conjugating enzyme (E2), and conjugation of ubiquitin to target proteins by an E3 ubiquitin–ligase complex. The E3 ligase complex contains an adaptor molecule, such as pVHL, that determines the substrate specificity. Successive transfers of activated ubiquitin to lysine-48 of the previously conjugated ubiquitin molecule lead to the formation of polyubiquitin chains, which serve as recognition markers for degradation by the 26S proteasome. pVHL functions as the substrate recognition component of an E3 ubiquitin–ligase complex that contains elongin B, elongin C, Ring box protein 1 (Rbx1), and Cul2 (Pause et al. 1997). X-ray crystallographic analysis of pVHL has revealed two major protein domains: an α domain and a β domain. The surface of the α domain (residues 155–192) is primarily responsible for the interaction between pVHL and Elongin C. The surface of the β domain consists of a seven-stranded β sandwich (residues 63–154) and α helix (residues 193–204) and is primarily responsible for binding target proteins for ubiquitination (Stebbins, Kaelin and Pavletich 1999).

Known and putative substrates of the pVHL E3 ubiquitin–ligase complex include atypical protein kinase C; hyperphosphorylated large subunit of RNA polymerase II; VHL deubiquitinating enzymes (VDU)-1 and -2; and HIF-1α and HIF-2α. The substrates that have been most extensively studied are HIF-1α and HIF-2α, both
Fig. 6.1 HIF-1α protein degradation pathways. Two pathways regulate HIF-1α protein stability. Under normoxic condition, HIF-1α is hydroxylated at proline residue(s) 402 and/or 564. Hydroxylated HIF-1α is recognized by pVHL, recruited to the E3 ubiquitin–ligase complex containing Elongin C, Elongin B, Cullin 2, and RBX1, polyubiquitinated, and targeted for proteasomal degradation. RACK1 competes with heat shock protein 90 (HSP90) for binding to HIF-1α. RACK1 also binds to Elongin C and thereby (as in the case of pVHL) recruits an ubiquitin–ligase complex. Inhibitors of HSP90, such as 17-AAG, dissociate HSP90 from HIF-1α and thus promote RACK1 binding. Under hypoxic conditions, HIF-1α is not hydroxylated, not ubiquitinated, and accumulates at high levels to form transcriptionally active HIF-1, leading to the transcription of downstream target genes.

Of which are induced in response to hypoxia. In the presence of O2, one (or both) of two prolyl residues in the HIF-α oxygen degradation domain (Pro-402 and Pro-564 in human HIF-1α) is hydroxylated by members of the EGLN family of prolyl hydroxylases. Hydroxylated HIF-α is recognized by pVHL, recruited to the E3 ubiquitin–ligase complex, polyubiquitinated, and targeted for proteasomal degradation (Maxwell et al. 1999; Bruick and McKnight 2001; Ivan et al. 2001; Jaakkola et al. 2001; Yu et al. 2001). Under hypoxic conditions, HIF-α is not hydroxylated, cannot bind to pVHL, and accumulates in the cell. When VHL is lost or mutated, as in RCC, the pVHL target proteins, HIF-1α and HIF-2α are not degraded and accumulate at high levels to form transcriptionally active HIF-1, leading to the transcription of downstream target genes (Iliopoulos et al. 1996; Kaelin 2004).

Hypoxia-inducible genes regulated by HIF-1 encode proteins involved in angiogenesis (e.g., vascular endothelial growth factor, VEGF), cell proliferation...
(e.g., transforming growth factor α, TGF-α) and migration (e.g., C-MET), glucose uptake (e.g., the GLUT1 glucose transporter), and acid–base balance (e.g., carbonic anhydrase IX, CA9). Overproduction of such hypoxia-inducible mRNAs is a hallmark of pVHL-defective tumors. When VHL protein is lost, these proteins are overexpressed, creating a microenvironment favorable for cell proliferation, migration, and invasion. Thus, cells deficient in VHL behave as if they are hypoxic, even in conditions of normoxia. The hypervascularity of these tumors can be explained by a pVHL-dependent defect in ubiquitin-mediated degradation of HIF-α proteins, leading to increased HIF-1 transcriptional activity with consequent upregulation of VEGF and other angiogenic growth factors that promote tumor progression (Semenza 2003).

HIF-1

HIF-1, which functions as a global regulator of oxygen homeostasis in all metazoan species, is a heterodimeric transcription factor that consists of an oxygen-regulated subunit, which is designated HIF-1α, and a constitutively expressed subunit, which is designated as HIF-1β (Semenza and Wang 1992; Wang et al. 1995). Both HIF-1 subunits are members of the basic helix-loop-helix (bHLH) PER-ARNT-SIM (PAS)-domain family of transcription factors (Wang et al. 1995). The HLH and PAS domains mediate heterodimer formation between the HIF-1α and HIF-1β subunits, which is necessary for DNA binding by the basic domains (Jiang et al. 1996). Exposure of cells to graded hypoxia revealed that HIF-1α protein expression induced by hypoxia was half-maximal at 1.5 – 2% O₂ and maximal at 0.5% O₂. A second oxygen-regulated protein, which is designated HIF-2α (also known as endothelial PAS domain protein 1, EPAS1) can also dimerize with HIF-1β. The specific cellular biological processes or tissue-specific contexts that dictate the utilization of HIF-1α vs HIF-2α remain incompletely understood. HIF-1α and HIF-2α regulate distinct, but overlapping, batteries of target genes (Tian et al. 1997; Elvidge et al. 2006). Another related protein, HIF-3α (also known as inhibitory PAS domain protein, IPAS), is expressed in certain cell types in the eye and brain and functions as an inhibitor that is involved in the negative regulation of transcriptional responses to hypoxia (Makino et al. 2001), but its role in cancer pathophysiology has not been established.

VHL protein binds the transcriptional factors HIF-1α and HIF-2α directly and destabilizes them (Maxwell et al. 1999). Under normoxic conditions, HIF-1α (as well as HIF-2α and HIF-3α) is subjected to O₂-dependent ubiquitination that is initiated by the binding of the pVHL and its recruitment of an E3 ubiquitin–ligase complex that contains Elongin C, Elongin B, Cullin 2, and RBX1 (Pause et al. 1997; Ohh et al. 2000). The binding of pVHL is dependent on the hydroxylation of proline residue(s) 402 and/or 564 of HIF-1α within the so-called oxygen-dependent degradation domain (Bruick and McKnight 2001; Ivan et al. 2001; Jaakkola et al. 2001;
Yu et al. 2001). The HIF-1α prolyl hydroxylases are dioxygenases that utilize O2 and α-ketoglutarate as substrates. A family of three human HIF-1α prolyl hydroxylases (HPHs), alternatively designated prolyl hydroxylase domain-containing proteins (PHDs), was identified and shown to be encoded by the EGLN2, EGLN1, and EGLN3 genes, respectively (Epstein et al. 2001). Under hypoxic conditions, the hydroxylase activity is inhibited and HIF-1α and HIF-2α accumulate in the cell as a result of decreased hydroxylation, ubiquitination, and degradation. In the absence of VHL, which occurs in the majority of clear-cell type renal-cell carcinoma, HIF-1α and HIF-2α are hydroxylated but pVHL-dependent ubiquitylation does not occur, which thus results in the accumulation of high levels of HIF-1α and HIF-2α protein even under non-hypoxic conditions (Kaelin 2004).

Besides the O2-dependent degradation of HIF-1α mediated by PHD/VHL/Elongin-C/B/Cul2 E3 ubiquitin ligase and proteasome, the receptor for activated C kinase 1 (RACK1), which was originally identified as an anchoring protein for activated protein kinase C, promotes the O2/PHD/VHL independent but proteasome-dependent degradation of HIF-1α (Fig. 6.1). Inhibitors of heat shock protein 90 (HSP90) dissociate HSP90 from HIF-1α and induce O2/PHD/VHL-independent degradation of HIF-1α. RACK1 competes with HSP90 for binding to the PAS-A domain of HIF-1α. RACK1 also binds to Elongin C via an amino acid sequence with striking similarity to the region of pVHL that interacts with Elongin C. Thus, RACK1 recruits an ubiquitin–ligase complex similar to that which is recruited by pVHL, establishing a parallel but O2-independent pathway for the proteasomal degradation of HIF-1α (Liu et al. 2007).

In addition to prolyl hydroxylation, HIF-1α is also subjected to O2-dependent hydroxylation of asparagine residue 803 in the carboxyl-terminal transactivation domain by factor inhibiting HIF-1 (FIH-1), which is another dioxygenase that utilizes O2 and α-ketoglutarate (also known as 2-oxoglutarate) (Lando et al. 2002). The prolyl and asparaginyl hydroxylation reactions require O2, Fe (II), and α-ketoglutarate and generate succinate and CO2 as side-products. Hydroxylation of asparagine-803 prevents the interaction of HIF-1α with the co-activators p300 and CBP. Thus, both the half-life and transcriptional activity of HIF-1α are regulated by O2-dependent hydroxylation events that provide a direct mechanism by which changes in O2 concentration can be linked to changes in the gene expression mediated by HIF-1.

In addition to VHL loss of function, many other genetic alterations that inactivate tumor suppressors or activate oncoproteins have been shown to increase HIF-1 activity in cancer cells through a variety of molecular mechanisms (Semenza 2003). Immunohistochemical analysis of human tumor biopsies has revealed overexpression of HIF-1α in the majority of common cancers (Zhong et al. 1999; Talks et al. 2000). High HIF-1α levels in tumors reflect the frequent presence of intratumoral hypoxia and the fact that many common genetic alterations in cancer cells upregulate HIF-1α expression. In general, these changes serve to increase the basal levels of HIF-1α in cancer cells, which serves to amplify the physiological response of cancer cells to hypoxia.
Target Genes Transcriptionally Regulated by HIF-1

A recent study of global gene expression using DNA microarrays indicates that more than 2% of all human genes are directly or indirectly regulated by HIF-1 in arterial endothelial cells (Manalo et al. 2005). HIF-1 binding sites, designated as hypoxia response elements, all contain the core consensus nucleotide sequence 5′-RCGTG-3′, and can be located in the 5′-flanking region of the gene, introns, or 3′-flanking region. One major function of HIF-1 is to increase O₂ delivery to cells subjected to reduced O₂ availability (hypoxia). When the entire organism is hypoxic, HIF-1 activates transcription of the gene encoding erythropoietin EPO, the glycoprotein hormone that controls the production of red blood cells and thereby determines blood O₂-carrying capacity. When hypoxia results from inadequate perfusion of a specific tissue (ischemia), vascular endothelial growth factor (VEGF) and other angiogenic cytokines are produced to stimulate new blood vessel formation and/or the remodeling of existing blood vessels to increase blood flow. HIF-1 also promotes cell survival under conditions of O₂ deprivation. HIF-1 activates the transcription of genes encoding glucose transporters and glycolytic enzymes, such as the glucose transporter (GLUT1), enzymes of glucose metabolism, such as hexokinase and lactate dehydrogenase A, and the lactate transporter MCT-4, and thereby increases the capacity for anaerobic ATP synthesis. In addition, HIF-1 controls the expression of survival factors that can block hypoxia-induced apoptosis, including insulin-like growth factor 2 (IGF-2) and adrenomedullin (Semenza 2003).

HIF-1α expression levels are correlated with an increased risk of mortality in several types of carcinoma. The basis for this association is that many genes that are regulated by HIF-1 play critical roles in many key aspects of cancer biology, especially angiogenesis, metabolic reprogramming, invasion/metastasis, and drug resistance (Semenza 2003).

Angiogenesis/Hypoxia

HIF-1α is necessary and sufficient for the hypoxia-induced expression of multiple angiogenic growth factors including angiopoietin 1, angiopoietin 2, placental growth factor, platelet-derived growth factor B, stromal-derived factor 1, and VEGF (Kelly et al. 2003). These factors promote the proliferation, migration, and maturation of endothelial cells and pericytes during angiogenesis, which is necessary to establish and maintain blood supply to the growing tumor mass (Fig. 6.2). Collectively, these results implicate dysregulation of HIF target genes playing a causal role in the pathogenesis of different tumors, especially the VHL-defective RCC.

Consistent with a major role for hypoxia in the overall process, many genes involved in different steps of angiogenesis are independently regulated by hypoxia/HIF-1. Those genes include vascular endothelial growth factor (VEGF), angiopoietin 1 and angiopoietin 2, platelet-derived growth factor B (PDGFB), placental growth factor (PLGF) and stromal-derived factor 1, and genes involved
Fig. 6.2 Tumor angiogenesis regulated by VHL and HIF-1. Hypoxia or VHL loss-of-function stabilizes HIF-1α protein, leading to increased HIF-1 transcriptional activity. HIF-1 activates the transcription of genes encoding pro-angiogenic factors including vascular endothelial growth factor (VEGF), placental growth factor (PLGF), platelet-derived growth factor B (PDGFB). PLGF and VEGF bind to the receptor tyrosine kinases, VEGFR1 and/or VEGFR2, which mediate mobilization and recruitment of circulating angiogenic cells as well as endothelial cell proliferation, survival and activation. PDGFB interacts with its receptor PDGFR, modulating the interaction of endothelial cells with smooth muscle cells/pericytes.

in matrix metabolism, including matrix metalloproteinases, plasminogen activator receptors and inhibitors, and procollagen prolyl hydroxylases, and lysyl oxidase (Kelly et al. 2003, Semenza 2003; Alvarez et al. 2006).

Under hypoxic conditions HIF-1 activates the transcription of genes encoding pro-angiogenic factors, most notably VEGF (Wang et al. 1995). VEGF is dramatically overexpressed throughout RCC tissue and may be the most important tumor angiogenic factor. VEGF is a pleiotropic growth factor that mediates multiple functions including regulation of vessel permeability, endothelial cell activation, survival, proliferation, invasion and migration. In order to exert biological effects, VEGF binds to the receptor tyrosine kinases, VEGFR1 and VEGFR2, which are expressed on the surface of endothelial cells. VEGFR2 mediates the majority of VEGF downstream angiogenic effects, leading to the most robust upregulation of angiogenesis, while VEGFR1 is critical in developmental angiogenesis. VEGF can also bind to neuropilin 1 and 2, which function as co-receptors to activate endothelial cells and promote angiogenesis.

Another angiogenic factor that is regulated by hypoxia/HIF-1 is PDGF-B (Alvarez et al. 2006). Mature PDGF proteins are the dimers PDGF-AA, PDGF-AB, and PDGF-BB, which interact with two cognate receptors, PDGFRα and PDGFRβ, the activation of which is critical for pericyte proliferation and survival (Alvarez...
et al. 2006). Pericytes are an important supporting cell in blood vessels that maintain endothelial cell viability. 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.

Angiopoietins, which are also HIF-1 target genes, appear to modulate angiogenesis in concert with VEGF. Angiopoietin 1 binds to a tyrosine kinase receptor, Tie-2, to promote endothelial cell pericyte interaction. Angiopoietin 2 binds to the same Tie-2 receptor, but blocks endothelial cell interaction with pericytes, which is required for endothelial cell sprouting. Several matrix metalloproteases (MMPs) are also regulated by HIF-1. The best-characterized proteases regulating tumor angiogenesis are MMP-2 and MMP-9, which are encoded by HIF-1 target genes, as is the membrane-type MMP, MT1-MMP.

**Anti-angiogenic Therapy**

Prior to the development of anti-angiogenic therapy, treatment for renal cancer was limited to the immunotherapeutic agents IFN-α and interleukin-2, which have had only modest success. Given the paucity of angiogenesis in adults, the growing vessels in tumors present a therapeutic target with fewer potential side effects than traditional chemotherapies. Besides, endothelial cells have a stable genome and may not develop drug resistance as rapidly as tumor cells.

Avastin (Bevacizumab), a humanized VEGF-neutralizing antibody, is the first Food and Drug Administration (FDA) approved drug for anti-angiogenic therapy in metastatic RCC (Yang et al. 2003). Other strategies have targeted the VEGFR family. Recently, the FDA approved two new agents for the treatment of advanced kidney cancer, Sutent (Sunitinib) and Nexavar (Sorafenib). Both agents are small molecule tyrosine kinase inhibitors that can selectively inhibit both VEGFRs and PDGFRs (Motzer et al. 2006a,b; Escudier et al. 2007).

As aforementioned, hypoxia/HIF-1 is a potent initiator of tumor angiogenesis, and inhibitors designed specific for HIF-1 may be useful in combination with anti-angiogenic therapy. A large number of novel compounds have been shown to inhibit HIF-1. 2-Methoxyestradiol, which inhibits HIF-1α protein accumulation, is already in clinical trials (Mabjeesh et al. 2003). Other HIF-1 inhibitors, such as PX-478, YC-1, and chetomin, also have shown anti-cancer activity in tumor xenograft models (Kung et al. 2004; Macpherson and Figg 2004; Welsh et al. 2004). Importantly, as mentioned above, small molecule inhibitors of HSP90 promote degradation of HIF-1α proteins (Fig. 6.1) in a pVHL-independent manner (Liu et al. 2007). The HSP90 inhibitors 17-AAG (17-allylamino-17-geldanamycin) and 17-DMAG (17-N-allylamino-17-demethoxygeldanamycin), which inhibit HIF-1 activity, are currently in clinical trials in patients with RCC (Isaacs et al. 2003). Inhibitors of the mTOR (mammalian target of rapamycin) pathway reduce HIF-1α protein levels and thereby inhibit HIF-1 transcriptional activity (Hudson et al. 2002). Clinical trials have shown that the novel mTOR inhibitor CCI779 (temsirolimus, Torisel) has promising activity in patients with advanced RCC (Atkins et al. 2004).
Conclusions

Advanced renal cancers are notoriously resistant to chemotherapy and radiotherapy, and novel therapeutic approaches are desperately needed. Multi-drug regimens that include angiogenesis inhibitors, e.g., targeting of both mTOR and VEGF/PDGF pathways simultaneously, will need to be studied. The identification of drug combinations that are safe and effective remains a major challenge.

References


