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Review Article

Role of hypoxia-inducible factor in clear cell renal cell carcinoma and its application in targeted therapy

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Abstract: Hypoxia is a common feature of many solid tumors such as renal cell carcinoma (RCC). Hypoxia-inducible factors (HIF), namely HIF-1α, -2α, and -3α, have been found to play important roles in tumors. As a key mediator of hypoxic responses, HIF-1α activates hypoxia-responsive genes, which are closely correlated with processes related to tumorigenesis and cancer progression, including cell proliferation, metabolism, angiogenesis, invasion, metastasis, and therapy. As 70-80% of RCCs are clear cell renal cell carcinomas (CCRCCs). With regard to the etiology of CCRCC, the article presents molecular pathways such as VHL-HIF-EPO pathway. The downstream effects of this pathway in RCC include upregulation of vascular endothelial growth factor production and increase in related inflammatory reactions. Importantly, HIF is a key component in this pathway. Previous findings indicate that upregulation of HIF-1α is associated with poor prognosis in CCRCC patients. Thus, the article discusses HIF-1α as a therapeutic target for CCRCC. The HIF-1α overexpression observed in CCRCC may be regarded as a proposing signaling or an indicator for the potential of HIF-α targeted therapy in CCRCC.

Keywords: Hypoxia-inducible factor, clear cell renal cell carcinoma, targeted therapy

Introduction

Currently, renal cell carcinoma (RCC) accounts for 3% of all adult tumors and 95% of all renal malignancies [1]. The number of new emerging cases is reaching 210,000 per year [2]. RCC has multiple subtypes with high heterogeneity [3], the classification of RCC has modified in 2004 [4]. The International Society of Urological Pathology (ISUP) 2012 consensus conference suggested modifications to the existing WHO 2004 categories [5]. The new classification includes 15 subtypes, of which three predominant types in malignant RCC tumors are clear cell renal cell carcinomas (CCRCCs, covering 70-80%), papillary renal cell carcinomas (10-15%) [6], and chromophobe renal cell carcinoma (5%). A small proportion of rare types, including papillary adenoma, oncocytoma, carcinoma of the collecting ducts of bellini, renal medullary carcinoma, MiT family translocation renal cell carcinoma, carcinoma associated with neuroblastoma, mucinous tubular and spindle cell carcinoma, tubulocystic renal cell carcinoma, acquired cystic disease associated renal cell carcinoma, clear cell (tubulo) papillary renal cell carcinoma, hereditary leiomyomatosis renal cell carcinoma syndrome-associated renal cell carcinoma, and unclassified renal cell carcinoma, also exist. Based on this classification, however, RCC has a variety of complicated histological variations with different histological subtypes. Each subtype also presents a wide range of clinical features such as morbidity, pathological character, prognosis, grade malignancy, drug susceptibility, and responses to therapy.

Gene mutations have accepted generally as the etiology of RCC. Among other causes, the von Hippel-Lindau (VHL) mutation and its protein product are an important component of the cell's response to hypoxia [7]. Mutations in this cancer suppressor gene, VHL, play a critical role in the development of the most prevalent CCRCCs by causing a loss of a segment of chromosome 3 [2, 8]. In the case of CCRCC, loss of function in both VHL alleles could be caused by chromosomal mutations or altered methylation in the promoter. Some studies have indicated

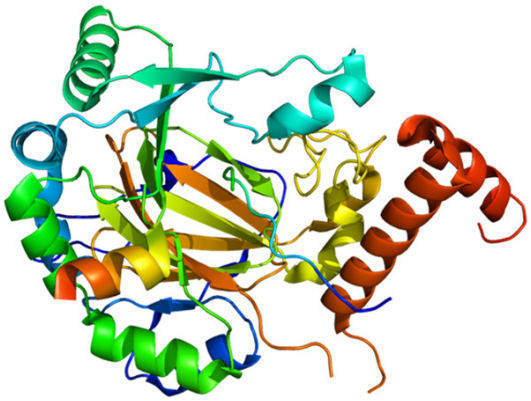

Figure 1. The schematic of HIF crystal structure.

that 19% of CCRCC cases present with promoter aberrant methylation, 27-56% present with gene mutation, and 12.5% present with both promoter methylation and gene mutation; however, in around 25% of the cases, none of these has been observed [9-11].

Hypoxia and hypoxia-inducible factors (HIFS)

Hypoxia is a common feature in locally advanced solid tumors that has been found to be associated with decreased response to treatment and malignant progression, causing an increasing probability of recurrence, spread, and metastasis. HIFs are a group of transcription factors for most hypoxia-inducible genes responding to changes in available oxygen in the cellular environment, decreases in oxygen levels, or hypoxia [12]. HIF is a major regulator of the adaptation of tumor cells to hypoxic stress.

Structure and function of HIFs

HIF is widely expressed in various tissues in mammals, and the nuclear transcription complex containing HIF mediates the hypoxia response of mammalian cells. Semenza, and Wang while extracting the nucleus from hypoxic cells [13] discovered HIF first in 1992. As a heterodimer, HIF is formed of two subunits, i.e., 120-kD HIF-α and 91-94-kD HIF-β (**Figure 1**). Being the active subunit, the coding region of the HIF-α gene is located in q21-24 on chromosome 14 and regulated by hypoxic signaling. The HIF-β gene is coded in q21 chromosome 1 and is expressed constantly in the nucleus and cytoplasm of both normal and hypoxic cells, where it functions as a cellular skeletal protein. Both the subunits belong to the family of basic-helix-loop-helix transcriptional factors (bHLH), which have the PER-ARNT-SIM (PAS) structure [14]. In the N-terminus of the subunits, there are bHLH and PAS structures, which are necessary for the formation of the heterodimer and for binding with DNA [15]. The consensus HIF-1 binding sequences (hypoxia response elements, HREs) have been published earlier, known as A/G TACGTGGG [16].

HIF is required for embryonic development in mice [17], and plays a key role in ischemic cardiovascular disease, stroke, and cancer [18-20]. The synthesized HIF protein, however, degraded rapidly by the intracellular, oxygen-dependent ubiquitin-proteasome under normoxic conditions, but is stably expressed under hypoxic conditions [12]. In mammals, active HIF-α subunits contains three subtypes, i.e., HIF-1α, -2α and -3α [21]. The activity of HIF is mainly regulated by oxygen through these subunits. HIF-1α is expressed throughout the whole body. Delineation of the mechanisms that regulate HIF-1 activity in these contexts has become a major challenge in contemporary molecular and cell biology [22]. In contrast, HIF-2α not only exists in some specific cell types including endothelia, hepatocytes, Type II alveolar cells, and macrophages [23], but can also be found in the nucleus of CCRCCs [24]. In the kidney, for example, HIF-1α expression can be detected predominantly in tubular cells. In contrast, renal interstitial fibroblasts, endothelial cells, and some glomerular cells show HIF-2α positivity [25]. Likewise, recent increasing evidence supports that different subunits play different roles. For example, HIF-1α is thought to be the main hypoxic inducible transcriptional factor in breast cancer and endothelial cancer, while HIF-2α, on the other hand, plays an important role in angiogenesis in hepatocellular carcinoma and RCC [26, 27]. It is accepted generally that HIF-1α and HIF-2α are involved in transcriptional regulation, and HIF-3α plays inhibits HIF-1α, HIF-2α, but has no ability of transcription activation. Interestingly, a recent study regarding the function of HIF-3α in zebra fish showed that HIF-3α has strong transactivation activity in this species. HIF-3α not only mediates hypoxia-induced growth and developmental retardation but also possesses hypoxia-independent activities. A recent report revealed that the transactivation activity is conserved in

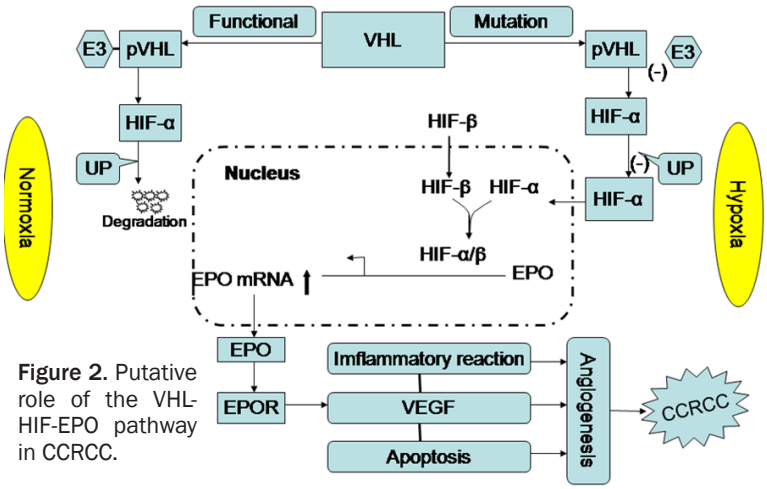

Figure 2. Putative role of the VHL-HIF-EPO pathway in CCRCC.

human HIF-3α, which is upregulated similar to HIF-1α and HIF-2α in human cells. These findings suggest that HIF-3α is also an oxygen-dependent transcription factor and activates the transcriptional response to hypoxia [28].

Usually, the stable expression of HIF-β in the cytoplasm plays a structural role. Under cellular hypoxic conditions, HIF-β translocates from the cytoplasm to the nucleus, thus causing more HIF-β to be present in the nucleus, which consequently forms dimers with HIF-α; when the oxygen tension returns to normal, HIF-β again translocates to the cytoplasm, and the intramuscular high HIF-α level rapidly vanishes [29].

Target genes of HIF

HIF is a transcriptional factor for many target genes. It is involved in the processes of cancer progression, including angiogenesis, erythropoiesis, glucose metabolism, cell proliferation, and apoptosis [30]. For example, the level of HIF-1α is significantly higher in primary and metastatic CCRCC than in benign tissues [31].

Most tumor cells become hypoxic under conditions of uncontrolled growth and in the hypermetabolic state. In response to this hypoxic environment, tumor cells adopt one or both of the following strategies: increasing glycolysis or angiogenesis. During these activities, HIFs play a very important role in maintaining the homeostasis of the tissue and cells under the hypoxic conditions.

Erythropoietin (EPO) is one class of HIF-target genes. Vascular endothelial growth factor (VEGF) is one of the EPOs, which is directly involved in angiogenesis [32]. Endothelin-1 (ET-1), platelet derived growth factor (PDGF), angiopoietin 2 (ANGPT2) and others also play important roles in angiogenesis, but they participate in the process through more complex mechanisms, rather than through the direct way as VEGF does [33].

Another essential process in cancer biology that HIF is associated with is glucose metabolism. HIF-α regulates the expression of many enzymes in the glycolytic pathway, which allows tumors to survive in hypoxic environments. In the absence of oxygen, tumors use anaerobic glycolysis for metabolizing glucose [34]. Glucose transporter 1, 3 (GLUT1, 3) and glycolytic enzymes, which include hexokinase (HK), lactate dehydrogenase A (LDHA), aldolase A (ALDA), enolase 1 (ENO1), monocarboxylate transporter 4 (MCT4), pyruvate dehydrogenase kinase 1 (PDK1), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), phosphofructokinase L (PFKL), phosphoglycerate kinase1 (PGK1), and MAX interactor 1 (MXI1), participate in glucose metabolism. HIF activates GLUT1 and 3, thus mediates cellular glucose uptake. Under hypoxia, pyruvate, a product of glycolysis, is converted to lactate by HK and LDHA [35].

Regulatory role of HIF in CCRCC

Several molecular pathways are involved in the oncogenesis of CCRCC. The VHL-HIF-EPO pathway is one of the well-studied pathways involved in the etiology of CCRCC. Under normoxic conditions, the VHL gene product, the ubiquitin ligase responsible for regulating HIF-α protein levels, efficiently targets HIF-α for rapid proteasome-dependent degradation. Usually, the subunit HIF-α has a shorter life, and HIF-α protein is rapidly degraded under normoxic conditions [36, 37], as it is degraded by an event largely mediated by a functional VHL [38, 39]. As shown in **Figure 2**, the functional protein product of VHL, pVHL, forms a pVHL-E3 ubiquitin ligase (pVHL-E3) complex with elongin B, elongin C, Cul2, and Rbx1 [40]. This pVHL-E3 complex can bind with HIF-1α under normal oxygen tension, which leads to its polyubiquitination

[41]. Its α subunit can be hydroxylated by three different prolylhydroxylases, which can be further ubiquitinated by the pVHL-E3 complex and degraded through the ubiquitin-proteasome proteolytic (UP) pathway. Therefore, HIF-α is hardly detected in normal cells.

Importantly, in the case of cancer hypoxia, the VHL gene is mutated; the formation of the VHL protein itself or that of the E3 ubiquitin ligase complex is either decreased or blocked. Degradation of HIF-α is decreased, leading to its accumulation in the cytoplasm [42]. HIF-α therefore translocates into the nucleus, and thus HIF-α can be observed in the cytoplasm as well as the nucleus of cancer cells [43]. In the nucleus, subunits HIF-α and HIF-β form dimers, which trans-activate the target genes of HIF, especially EPO. EPO mRNA forms EPOR with its receptors for participating in tumor progression, for example, by upregulating the production of VEGF, increasing inflammatory reactions, and decreasing intrinsic drug-induced apoptosis. In the end, these events eventually increase the likelihood of tumor angiogenesis and progression.

Under normoxic conditions, a functional VHL gene produces pVHL, which forms a pVHL-E3 ligase complex and mediates the polyubiquitination and proteasomal degradation of HIF. Under hypoxia, the degradation of HIF-α is decreased, leading to its accumulation in the cytoplasm. This HIF-α translocates into the nucleus, where it dimerizes with HIF-β. These dimers transactivate downstream target genes, especially EPO. EPO mRNA forms EPOR with its receptors for participating in tumor progression, for example by upregulating the production of VEGF, increasing inflammatory reactions, and decreasing intrinsic drug-induced apoptosis. In the end, these events eventually increase the likelihood of tumor angiogenesis and progression.

Therapy for CCRCC

Suppressing HIF expression

Tumors are formed by over-differentiation and proliferation of cancer stem cells (CSCs). The shrinkage of tumor was used as an efficient parameter in the clinic to determine after radiotherapy and/or chemotherapy. In fact, after a certain period of remission, tumors recur, as only the fast-differentiating tumor cells are killed but CSCs are not and they show strong resistance to radio- and/or chemotherapy. Therefore, traditional treatment methods such as those involving the use of interferon and interleukin have only seen 15-20% efficiency in CCRCC [44]. Thus, the cancer could not be really cured, as CSCs are not killed; at the same time, the CSCs may cause resistance to radiation and chemotherapeutic medication. There are several mechanisms underlying the resistance of CSCs to radio- and chemotherapy. One of these mechanisms is the upregulation of ATP-binding cassette (ABC)-transporting protein, which includes ATP-binding cassette B1 (ABCB1), C1 (ABCC1), and G2 (ABCG2). All three genes are multidrug resistance genes. These ABC-transporting proteins act by transferring the chemotherapeutic medicine out of the cells by utilizing the ATP energy generated in the cells, thus resulting in lower concentration of the drugs, which eventually compromises the effectiveness of the chemotherapy medications and confers drug resistance to the cells [45]. Furthermore, the ABC-transporting protein has also been reported as one of the direct downstream target genes of HIF in breast cancer. This is consistent with the finding that the ATP-binding cassette transporting protein expression in hypoxia-induced CSCs is one of the mechanisms underlying the chemotherapy resistance [46]. In addition, immunohistochemical analyses conducted to determine if HIF is involved in drug resistance through an indirect process revealed that HIF expression is also increased in other types of tumors. For example, in oropharyngeal cancer, the overexpression of HIF was found to be closely related to radiotherapy resistance and poor prognosis; in addition, the degree of its expression had a predictive and prognostic significance in individuals undergoing curative radiation therapy [47].

Given the important role of HIF, an HIF suppressor would provide a potential therapeutic strategy. In a different study aimed at identifying micromolecular HIF suppressors, it was found that 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) could inhibit the activity of HIF and the growth of the transplanted tumor [48]. After YC-1 was administered and reached to tumor tissue, the expression of HIF-α as well as angiogenesis was obviously decreased; furthermore, the expression of the downstream target genes

of HIF, such as VEGF, was also suppressed. HIF can also directly interact with heat shock protein 90 (HSP90), which binds to the HIF-α PAS domain. After separation of HIF-1α and HSP90, ubiquitin-proteasome-induced HIF-1α degradation is increased under normoxic conditions. HSP90 inhibitors such as geldanamycin, 17-allylaminogeldanamycin (17-AAG), and proteasomal can induce HIF-α degradation in a VHL-dependent way [49, 50]. In addition, 2-methoxylestradiol (2ME2) not only interrupts the aggregation of microtubules, but also decreases HIF levels and VEGF mRNA expression [51].

Targeted therapy for RCC

Targeted therapy involves the design of corresponding bio-micro medication at the cellular and molecular level according to certain specific signal transduction pathways or some oncogenic genes that have already assured from development of tumor. After administration into the body, these drugs for targeted therapy specifically interact with or interrupt relevant signal transduction pathways or oncogenic gene domains and kill tumor cells and/or attenuate vascular growth without disturbing the surrounding normal tissue and cells. Therefore, molecular targeted therapy is also called a bio-missile. Currently, targeted therapy has been applied in colorectal cancer, breast cancer, prostate cancer, and CCRCC. Medications used in such targeted therapy are classified as follows: 1) epidermal growth factor receptor (EGFR)-targeting blockers; 2) tumor angiogenesis inhibitors such as bevacizumab, a recombinant human anti-VEGF monoclonal antibody, and endostatin, an intrinsic anti-angiogenesis factor extracted from angioendothelioma; 3) monoclonal antibody against certain cellular oncological markers, such as cetuximab and trastuzumab for human epidermal receptor 2 (HER-2), and rituximab for CD20; 4) kinase inhibitors such as Bcr-Abl1 tyrosine kinase inhibitor imatinib and dasatinib, the insulin-like growth factor receptor-1 (IGFR-1) kinase inhibitor, hydrochloride (NVP-AEW541), the mTOR kinase inhibitor temsirolimus (CCI-779), and the aurora kinase inhibitor histone deacetylase (HDACs); and 5) the ubiquitin-proteasome inhibitor bortezomib.

For CCRCC, the main drugs used for targeted therapy are VEGF inhibitors, which are tumor angiogenesis inhibitors. In addition to VEGF that is extensively expressed and is a known oncogenic gene in CCRCC, platelet derived growth factor (PDGF) is also expressed in CCRCC. Moreover, VEGF and PDGF domains are critical components for angiogenesis and tumor cell proliferation. One of the downstream effects of the VHL-HIF-EPO pathway is the upregulation of VEGF production and an increase in inflammation reactions. There are two common groups of targeted therapy medications: small molecule tyrosine kinase inhibitors and VEGF monoclonal antibody.

Small molecule tyrosine kinase inhibitors, such as sunitinib, sorafenib and pazopanib, exert their functions by suppressing intracellular protein kinase activity of VEGF receptors [52]. In recent studies of CCRCC treatment using tyrosine kinase inhibitors of VEGFR and PDGRF (sunitinib, sorafenib), the mortality associated with CCRCC was reported to decrease [44, 53, 54]. The anti-tumor effect can be significantly improved by combined treatment with crizotinib and axitinib. Combination treatment also prolongs patient survival and significantly inhibits tumor growth by inhibiting angiogenesis [55].

In addition, as a tyrosine kinase inhibitor, glivec exerts its anti-tumor effects through two mechanisms: 1) directly attenuates tumor growth by suppressing the RAF/MEK/ERK signal transduction pathway; 2) inhibits tumor angiogenesis by blocking the receptors of VEGF and PDGF, resulting in the indirect attenuation of tumor growth. Although glivec has commonly used for gastrointestinal stromal tumor and chronic myelogenous leukemia, it can give us a few insights on the drug's possible application in CCRCC targeted treatment.

The second group of targeted therapy medications used is VEGF monoclonal antibodies such as bevacizumab, which suppresses tumor growth by blocking the interaction between VEGF and the intrinsic VEGF receptors [56]. A retrospective review from 2005 to 2008 to identify RCC patients who had undergone debulking nephrectomy followed by VEGF-targeted therapy with sunitinib, sorafenib, or bevacizumab showed a significant improvement in the progression-free survival of the patients [57].

Summary and perspectives

In summary, increasing number of studies have provided evidence that HIF plays an important

role in CCRCC tumorigenesis, progression, and metastasis, and that it is involved in cellular proliferation, angiogenesis, invasion, and resistance to radiotherapy and chemotherapy. Clinical data also indicate that HIF-1α overexpression is associated with poor prognosis in CCRCC [58]; therefore, HIF-α is strongly suggested as a target for CCRCC treatment. The HIF-1α overexpression observed in CCRCC may be regarded as a proposing signaling or an indicator for the potential of HIF-α targeted therapy in CCRCC. Accordingly, some small molecule inhibitors of the HIF pathway have been used in combination with other therapeutic drugs to improve anticancer efficacy [59]. However, these small molecule inhibitors are currently used only for late stage cancer patients; if this medication could be used for early stage cancer patients as well, it may improve anticancer efficacy and the overall survival rate. Further studies are needed to investigate this possibility.

Although different approaches to identify HIF pathway inhibitors that effectively inhibit renal cell carcinoma tumorigenesis have been applied, the application of the developed agents have some limitations such as the undesirable side effects, relatively low specificity, less obvious antitumor effects, and the lack of an evaluation system for clinical efficacy. Thus, further studies are needed to optimize the application of the existing therapeutic agents targeting HIF to improve clinical outcomes. Furthermore, it is an urgent task to search for novel targets for gene therapy for CCRCC.

The increasing number of studies on gene targeting treatments for RCC has provided us the directions for future studies in the following aspects: 1) as biomarkers being used to screen first-line medications for individualized therapy; 2) as targeting medication prescribed reasonably, which may achieve the better outcome when therapy combined with other targeting medications; 3) further studies may focus on reducing side effects and the tolerance of targeting medications ; 4) under the current health system, appropriate therapy needs to be developed for specific populations to get maximum-effectiveness with minimum cost; 5) the right time point for starting targeted therapy should be determined to achieve the ideal treatment effect.

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Disclosure of conflict of interest

None.

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References

[1] Morais C, Johnson DW, Vesey DA, Gobe GC. Functional significance of erythropoietin in renal cell carcinoma. BMC Cancer 2013; 13: 14-20.

[2] Young AC, Craven RA, Cohen D, Taylor C, Booth C, Harnden P, Cairns DA, Astuti D, Gregory W, Maher ER, Knowles MA, Joyce A, Selby PJ, Banks RE. Analysis of VHL gene alterations and their relationship to clinical parameters in sporadic conventional renal cell carcinoma. Clin Cancer Res 2009; 15: 7582-7592.

[3] Lopez-Beltran A, Scarpelli M, Montironi R, Kirkali Z. 2004 WHO classification of the renal tumors of the adults. Eur Urol 2006; 49: 798-805.

[4] Srigley JR, Delahunt B, Eble JN, Egevad L, Epstein JI, Grignon D, Hes O, Moch H, Montironi R, Tickoo SK, Zhou M, Argani P; ISUP Renal Tumor Panel. The international society of urological pathology (ISUP) vancouver classification of renal neoplasia. Am J Surg Pathol 2013; 37: 1469-1489.

[5] Mazzucchelli R, Galosi AB, Scarpelli M, Lopez-Beltran A, Cheng L, Montironi R. Contemporary update on pathology-related issues of adult renal neoplasms. Anal Quant Cytopathol Histpathol 2014; 36: 1-8.

[6] Curti BD. Renal cell carcinoma. JAMA 2004; 292: 97-100.

[7] Clark PE. The role of VHL in clear-cell renal cell carcinoma and its relation to targeted therapy. Kidney Int 2009; 76: 939-945.

[8] Ricketts CJ, Linehan WM. Intratumoral heterogeneity in kidney cancer. Nat Genet 2014; 46: 214-215.

[9] Arai E, Kanai Y. Genetic and epigenetic alterations during renal carcinogenesis. Int J Clin Exp Pathol 2010; 4: 58-73.

[10] Gossage L, Murtaza M, Slatter AF, Lichtenstein CP, Warren A, Haynes B, Marass F, Roberts I, Shanahan SJ, Claas A, Dunham A, May AP, Rosenfeld N, Forshew T, Eisen T. Clinical and pathological impact of VHL, PBRM1, BAP1, SETD2, KDM6A, and JARID1c in clear cell renal cell carcinoma. Genes Chromosomes Cancer 2014; 53: 38-51.

[11] Muscarella LA, D'Agruma L, la Torre A. VHL gene alterations in Italian patients with isolated renal cell carcinomas. Int J Biol Markers 2013; 28: 208-215.

[12] Smith TG, Robbins PA, Ratcliffe PJ. The human side of hypoxia-inducible factor. Br J Haematol 2008; 141: 325-334.

[13] Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992; 12: 5447-5454.

[14] Zhou W, Dosey TL, Biechele T, Moon RT, Horwitz MS, Ruohola-Baker H. Assessment of hypoxia inducible factor levels in cancer cell lines upon hypoxic induction using a novel reporter construct. PLoS One 2011; 6: e27460.

[15] Li QR, Shi YH. Research progress in degradation of HIF-1-ubiquitination. Chin J Cancer Prve Treat 2013; 20: 1371-1374.

[16] Imai M, Ishibashi H, Nariai Y, Kanno T, Sekine J, Onimaru M, Mori Y. Transfection of hypoxia-inducible factor-1 decoy oligodeoxynucleotides suppresses expression of vascular endothelial growth factor in oral squamous cell carcinoma cells. J Oral Pathol Med 2012; 41: 675-681.

[17] Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J 1998; 17: 3005-3015.

[18] Melillo G. HIF-1: a target for cancer, ischemia and inflammation--too good to be true? Cell Cycle 2004; 3: 154-155.

[19] Ran R, Xu H, Lu A, Bernaudin M, Sharp FR. Hypoxia preconditioning in the brain. Dev Neurosci 2005; 27: 87-92.

[20] Moeller BJ, Dewhirst MW. HIF-1 and tumour radiosensitivity. Br J Cancer 2006; 95: 1-5.

[21] Huang LE, Bunn HF. Hypoxia-inducible factor and its biomedical relevance. J Biol Chem 2003; 278: 19575-19578.

[22] Liu YV, Baek JH, Zhang H, Diez R, Cole RN, Semenza GL. RACK1 competes with HSP90 for binding to HIF-1α and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1α. Mol Cell 2007; 25: 207-217.

[23] Jain S, Maltepe E, Lu MM, Simon C, Bradfield CA. Expression of ARNT, ARNT2, HIF1 alpha, HIF2 alpha and Ah receptor mRNAs in the developing mouse. Mech Dev 1998; 73: 117-123.

[24] Qi Li. Significance and expression of hypoxia inducible factor-2α and thymidine phosphorylase in renal clear cell carcinoma. China Medicine and Pharmacy 2013; 3: 7-9.

[25] Schietke RE, Hackenbeck T, Tran M, Günther R, Klanke B, Warnecke CL, Knaup KX, Shukla D, Rosenberger C, Koesters R, Bachmann S, Betz P, Schley G, Schödel J, Willam C, Winkler T, Amann K, Eckardt KU, Maxwell P, Wiesener MS. Renal tubular HIF-2alpha expression requires VHL inactivation and causes fibrosis and cysts. PLoS One 2012; 7: e31034.

[26] Saponaro C, Malfettone A, Ranieri G, Danza K, Simone G, Paradiso A, Mangia A. VEGF, HIF-1α expression and MVD as an angiogenic network in familial breast cancer. PLoS One 2013; 8: e53070.

[27] Rankin EB, Rha J, Unger TL, Wu CH, Shutt HP, Johnson RS, Simon MC, Keith B, Haase VH. Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. Oncogene 2008; 27: 5354-5358.

[28] Zhang P, Yao Q, Lu L, Li Y, Chen PJ, Duan C. Hypoxia-inducible factor 3 is an oxygen-dependent transcription activator and regulates a distinct transcriptional response to hypoxia. Cell Rep 2014; 6: 1110-1121.

[29] Chilov D, Camenisch G, Kvietikova I, Ziegler U, Gassmann M, Wenger RH. Induction and nuclear translocation of hypoxia-inducible factor-1 (HIF-1): heterodimerization with ARNT is not necessary for nuclear accumulation of HIF-1alpha. J Cell Sci 1999; 112: 1203-1212.

[30] Burroughs SK, Kaluz S, Wang D, Wang K, Van Meir EG, Wang B. Hypoxia inducible factor pathway inhibitors as anticancer therapeutics. Future Med Chem 2013; 5: 553-572.

[31] Schultz L, Chaux A, Albadine R, Hicks J, Kim JJ, De Marzo AM, Allaf ME, Carducci MA, Rodriguez R, Hammers HJ, Argani P, Reuter VE, Netto GJ. Immunoexpression status and prognostic value of mTOR and hypoxia-induced pathway members in primary and metastatic clear cell renal cell carcinomas. Am J Surg Pathol 2011; 35: 1549-1556.

[32] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005; 23: 1011-1027.

[33] Simon MP, Tournaire R, Pouyssegur J. The angiopoietin-2 gene of endothelial cells is up-regulated in hypoxia by a HIF binding site located in its first intron and by the central factors GATA-2 and Ets-1. J Cell Physiol 2008; 217: 809-818.

[34] Semenza GL. HIF-1: upstream and downstream of cancer metabolism. Current Curr Opin Genet Dev 2010; 20: 51-56.

[35] Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem 2001; 276: 43407-43412.

[36] Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 1997; 272: 22642-22647.

[37] Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A 1998; 95: 7987-7992.

[38] Maynard MA, Qi H, Chung J, Lee EH, Kondo Y, Hara S, Conaway RC, Conaway JW, Ohh M. Multiple splice variants of the human HIF-3 alpha locus are targets of the von Hippel-Lindau E3 ubiquitin ligase complex. J Biol Chem 2003; 278: 11032-11040.

[39] Baldewijns MM, van Vlodrop IJ, Vermeulen PB, Soetekouw PM, van Engeland M, de Bruïne AP. VHL and HIF signalling in renal cell carcinogenesis. J Pathol 2010; 221: 125-138.

[40] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399: 271-275.

[41] Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001; 292: 468-472.

[42] Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG Jr. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Devm 2004; 18: 2893-2904.

[43] Zapatero A, Morente M, de Vidales CM, Adrados M, Lopez C, Nieto S, González MJ, Arellano R, Conde AC, Vicente FG. HIF1A expression in localized prostate cancer treated with dose escalation radiation therapy. Cancer Biomar 2015; 15: 41-46.

[44] Rini BI. Vascular endothelial growth factor-targeted therapy in metastatic renal cell carcinoma. Cancer 2009; 115: 2306-2312.

[45] Du J, Sun BC, Liu YX. Hypoxia inducing factor 1 in oncology research progress. J Clin Exp Pathol 2013; 29: 548-551.

[46] Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP, Schuetz JD. The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through inter-actions with heme. J Biol Chem 2004; 279: 24218-24225.

[47] Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, Semenza GL. Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001; 61: 2911-2916.

[48] Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, Kim MS, Park JW. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 2003; 95: 516-525.

[49] Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. J Biol Chem 2002; 277: 29936-29944.

[50] Isaacs JS, Jung YJ, Neckers L. Aryl hydrocarbon nuclear translocator (ARNT) promotes oxygen-independent stabilization of hypoxia-inducible factor-1alpha by modulating an Hsp90-dependent regulatory pathway. J Biol Chem 2004; 279: 16128-16135.

[51] Mabjeesh N J, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dys-regulating HIF. Cancer Cell 2003; 3: 363-375.

[52] Ciamporcero E, Miles KM, Adelaiye R, Ramakrishnan S, Shen L, Ku S, Pizzimenti S, Sennino B, Barrera G, Pili R. Combination strategy targeting VEGF and HGF/c-met in human renal cell carcinoma models. Mol Cancer Ther 2015; 14: 101-110.

[53] Sadeghi S, Albiges L, Wood LS, Black SL, Gilligan TD, Dreicer R, Garcia JA, Escudier BJ, Rini BI. Cessation of vascular endothelial growth factor-targeted therapy in patients with metastatic renal cell carcinoma: feasibility and clinical outcome. Cancer 2012; 118: 3277-3282.

[54] Macfarlane R, Heng DY, Xie W, Knox JJ, McDermott DF, Rini BI, Kollmannsberger C, Choueiri TK. The impact of kidney function on the outcome of metastatic renal cell carcinoma patients treated with vascular endothelial growth factor-targeted therapy. Cancer 2012; 118: 365-370.

[55] Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray LJ, Carver J, Chan E, Moss KG, Haznedar JO, Sukbuntherng J, Blake RA, Sun L, Tang C, Miller T, Shirazian S, McMahon G, Cherrington JM. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacody-

namic relationship. Clin Cancer Res 2003; 9: 327-337.

[56] Ferrar N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004; 3: 391-400.

[57] Barbastefano J, Garcia JA, Elson P, Wood LS, Lane BR, Dreicer R, Campbell SC, Rini BI. Association of percentage of tumor burden removed with debulking nephrectomy and progression-free survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted therapy. BJU Int 2010; 106: 1266-1269.

[58] Tóth K, Chintala S, Rustum YM. Constitutive expression of HIF-α plays a major role in generation of clear-cell phenotype in human primary and metastatic renal carcinoma. Appl Immunohistochem Mol Morphol 2014; 22: 642-647.

[59] Park JY, Lee JL, Baek S, Eo SH, Go H, Ro JY, Cho YM. Sarcomatoid features, necrosis, and grade are prognostic factors in metastatic clear cell renal cell carcinoma with vascular endothelial growth factor-targeted therapy. Hum Pathol 2014; 45: 1437-1444.