Eighty to eighty-five percent of renal cell cancers (RCC) are classified as clear cell renal cell carcinoma (ccRCC). This subtype is characterized by frequent mutation of the von Hippel–Lindau (VHL) tumor suppressor gene (for review, see ref. 1). When oxygen is present, the VHL protein (pVHL) binds hydroxylated hypoxia-inducible factor (HIF) α subunits and promotes their ubiquitination and proteosomal degradation (for review, see ref. 2). Under hypoxic conditions, however, or in the absence of pVHL, HIF1α and HIF2α are stabilized, and as a consequence modulate the transcription of various HIF target genes. Despite the fact that both subunits exhibit overlapping functions in the regulation of angiogenesis and extracellular matrix remodeling, HIF1α has been shown to act as a tumor suppressor in ccRCC through regulation of the glycolytic and apoptotic pathways, whereas HIF2α regulates proliferation and cell-cycle progression processes, indicative of its oncogenic potential (3; for review, see ref. 2).

MicroRNAs (miRNAs, miRs) are small (19–25 nucleotides), noncoding RNAs that play an important role in apoptosis, survival, proliferation, and differentiation processes through the posttranscriptional regulation of gene expression. It is estimated that 20% to 30% of all human mRNAs are miRNA targets. The complexity of this regulatory network may be additionally increased by the fact that one target mRNA may be regulated by multiple miRNAs and one miRNA may act as a specific regulatory molecule for numerous mRNAs involved in various pathways. The role of miRNAs has been intensively investigated, accounting for more than 30,000 publications by November 2013 in the PubMed database. Such a great interest in the research field has resulted in the identification of 1,872 precursors and 2,578 mature human miRNAs annotated in version 20 of the latest miRBase database. Numerous miRNAs have been reported to be significantly deregulated in ccRCC, including various hypoxia-related miRNAs (4, 5).

In this issue of Cancer Discovery, Mathew and colleagues (6) report miR-30c-2-3p and miR-30a-3p as a novel regulatory mechanism responsible for attenuation of the tumor-suppressive role of HIF1α in ccRCC (Fig. 1). miRNA profiling revealed that in tumors expressing both HIF1α and HIF2α, miR-30c-2-3p and miR-30a-3p were repressed as compared with tumors expressing HIF2α exclusively. Regulation of the aforementioned miRs proved to be pVHL-dependent but HIF-independent (6), contradicting the work of Huang and colleagues (7) that showed miR-30c to be regulated by HIF1α. Using cell lines as well as mouse models, Mathew and colleagues (6) showed that ectopic expression of both tested miRs induced downregulation of HIF2α and inhibition of xenograft tumor growth in mice. Moreover, application of a miR-30a-3p antagonim induced a higher tumor-cell proliferation rate and enhanced tumor growth (6).

HIFα proteins belong to the helix-loop-helix PAS family of DNA-binding transcription factors. They bind to HIF target genes at a 5′-RCGTG-3′ (where R is a purine) core sequence and activate their transcription (for review, see ref. 2). HIFα proteins regulate genes in a large variety of tissues and cells; however, expression of their targets may be restricted to specific locations. An example is erythropoietin, which is expressed only in the kidney in adults. Moreover, although HIF1α and HIF2α regulate some of the same genes, their targets are not identical and may differ in different cell types. For example, VEGF is regulated by HIF1α in VHL-deficient RCC cells, but HIF1α is responsible for the transcription of the VEGF gene in breast cancer cells (8). Another level of complexity is added by the fact that HIFα pathway members are targets of various miRNAs. Significant upregulation of hypoxia-related miRNAs, such as miR-21 and miR-210, was reported in ccRCC. On the contrary, miR-200c, which binds multiple targets in the VHL/HIFα pathway, including VEGF, as well as PI3K/AKT and mTOR pathway members, was shown to be consistently downregulated in numerous ccRCC studies (5, 9).

Despite the frequent mutations occurring within chromosome 14q, to which HIF1A maps, ccRCC tumors often maintain one wild-type HIF1A allele (10). A unique function of the
Hence, genes preferentially regulated by HIF2α confirming its oncogenic potential (for review, see ref. 2). Molecules (e.g., sunitinib and sorafenib) or with monoclonal therapy option is so-called targeted therapy with small tumor response, accompanied by significant toxicity. A novel levels of HIF2α suppression of anabolic biosynthesis. Contrary to HIF2α of the c-Myc oncoprotein. In contrast to HIF1α inhibits cell-cycle progression by posttranslational inhibition with accumulation of DNA damage in early-stage disease. It was shown that HIF2α is responsible for the growth of human ccRCC xenografts in mice (3). In addition, a single-nucleotide polymorphism within pVHL, blocking HIF2α, was associated with accumulation of DNA damage in early-stage disease that may also contribute to genomic instability in tumors. It was shown that HIF2α is responsible for the growth of human ccRCC xenografts in mice (3). In addition, a single-nucleotide polymorphism within HIF2A was associated with an increased risk of kidney cancer. Similar to restoration of pVHL, blocking HIF2α expression suppresses tumorigenesis, confirming its oncogetic potential (for review, see ref. 2). Hence, genes preferentially regulated by HIF2α have been shown to be especially oncogenic as compared with HIF1α-regulated genes. Mathew and colleagues (6) elegantly demonstrated a more pronounced upregulation of HIF2α in tumors expressing both HIF1α and HIF2α compared with tumors expressing HIF2α exclusively. Thus, it was concluded that the elevated levels of HIF2α in HIF1α/HIF2α-positive tumors are a direct consequence of miR-30c-2-3p and miR-30a-3p downregulation (6).

The standard procedure for ccRCC tumors is partial or complete nephrectomy. The surgical procedure is usually followed by systemic therapy in metastatic RCC. Immunotherapy (e.g., IFN-α and interleukin-2) showed relatively low tumor response, accompanied by significant toxicity. A novel treatment option is so-called targeted therapy with small molecules (e.g., sunitinib and sorafenib) or with monoclonal antibodies (e.g., bevacizumab) that inhibit tumor angiogenesis and cell viability by targeting VEGF and platelet-derived growth factor receptor pathways in endothelial and RCC cells. As has been suggested, tumors expressing HIF1α and HIF2α could be more susceptible to these drugs (3). Systemic therapies may also target the mTOR pathway (e.g., temsirolimus and everolimus). Despite the fact that these treatment options show an improvement in progression-free survival, the response rate is low. Therefore, new therapies for metastatic ccRCC are needed. An increasing number of publications have shown successful reduction of tumor size after application of various miRNAs or antagonirs in in vivo systems. Mathew and colleagues (6) elegantly demonstrated that tumors developed in immunocompromised mice were bigger if xenografts originated from miR-30a-3p antagonir-expressing cells compared with control tumors. Therefore, the authors concluded that application of the synthetic miRNAs could be beneficial for patients with ccRCC (6). Although prospective studies are needed, this study sheds light on potential new therapeutic tools for ccRCC treatment.

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**REFERENCES**

miR-30c-2-3p and miR-30a-3p: New Pieces of the Jigsaw Puzzle in HIF2 α Regulation

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