

MicroRNAs associated with Von Hippel–Lindau pathway in renal cell carcinoma: a comprehensive review

Lisa-Maria Schanza, Maximilian Seles, Michael Stotz, Johannes Fosselteder, Georg Hutterer, Martin Pichler, Verena Stiegelbauer

Angaben zur Veröffentlichung / Publication details:

Schanza, Lisa-Maria, Maximilian Seles, Michael Stotz, Johannes Fosselteder, Georg Hutterer, Martin Pichler, and Verena Stiegelbauer. 2017. "MicroRNAs associated with Von Hippel–Lindau pathway in renal cell carcinoma: a comprehensive review." *International Journal of Molecular Sciences* 18 (11): 2495. <https://doi.org/10.3390/ijms18112495>.



Review

MicroRNAs Associated with Von Hippel–Lindau Pathway in Renal Cell Carcinoma: A Comprehensive Review

Lisa-Maria Schanza ^{1,2}, Maximilian Seles ³, Michael Stotz ¹, Johannes Fosselteder ^{1,2},
Georg C. Hutterer ³, Martin Pichler ^{1,2,4} and Verena Stiegelbauer ^{2,3,*}

¹ Division of Oncology, Department of Internal Medicine, Medical University of Graz, 8036 Graz, Austria; Lisa-Maria.Schanza@klinikum-graz.at (L.-M.S.); michael.stotz@klinikum-graz.at (M.S.); johannes.fosselteder@edu.uni-graz.at (J.F.); martin.pichler@medunigraz.at (M.P.)

² Research Unit of Non-Coding RNA and Genome Editing in Cancer, Division of Oncology, Department of Internal Medicine, Medical University of Graz, 8036 Graz, Austria

³ Department of Urology, Medical University of Graz, 8036 Graz, Austria; maximilian.seles@medunigraz.at (M.S.); georg.hutterer@medunigraz.at (G.C.H.)

⁴ Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX 77054, USA

* Correspondence: verena.stiegelbauer@medunigraz.at; Tel.: +43-316-385-13115; Fax: +43-316-385-13355

Received: 11 October 2017; Accepted: 17 November 2017; Published: 22 November 2017

Abstract: Renal cell carcinoma (RCC) are the most common renal neoplasia and can be divided into three main histologic subtypes, among which clear cell RCC is by far the most common form of kidney cancer. Despite substantial advances over the last decade in the understanding of RCC biology, surgical treatments, and targeted and immuno-therapies in the metastatic setting, the prognosis for advanced RCC patients remains poor. One of the major problems with RCC treatment strategies is inherent or acquired resistance towards therapeutic agents over time. The discovery of microRNAs (miRNAs), a class of small, non-coding, single-stranded RNAs that play a crucial role in post-transcriptional regulation, has added new dimensions to the development of novel diagnostic and treatment tools. Because of an association between Von Hippel–Lindau (VHL) genes with chromosomal loss in 3p25-26 and clear cell RCC, miRNAs have attracted considerable scientific interest over the last years. The loss of VHL function leads to constitutional activation of the hypoxia inducible factor (HIF) pathway and to consequent expression of numerous angiogenic and carcinogenic factors. Since miRNAs represent key players of carcinogenesis, tumor cell invasion, angiogenesis, as well as in development of metastases in RCC, they might serve as potential therapeutic targets. Several miRNAs are already known to be dysregulated in RCC and have been linked to biological processes involved in tumor angiogenesis and response to anti-cancer therapies. This review summarizes the role of different miRNAs in RCC angiogenesis and their association with the VHL gene, highlighting their potential role as novel drug targets.

Keywords: renal cell carcinoma; Von Hippel–Lindau; angiogenesis; microRNAs

1. Introduction

Renal cell carcinoma (RCC) represents the most common renal neoplasia, whereby particularly advanced RCC remains a very aggressive and fatal disease. Several histologic subtypes of this heterogeneous tumor entity and its associated distinct molecular alterations and different clinical outcomes are described [1–4]. Three main different morphotypes of RCC exist: clear cell RCC accounts for approximately 70–80% of all RCCs, while 6–15% and 2–5% are papillary and chromophobe RCCs, respectively [5]. Clear cell RCC is the most common form of kidney cancer presenting with metastases

in 30% of patients at the time of initial diagnosis [6,7]. Surgical resection (partial or radical nephrectomy) remains the principal treatment option for localized disease; nevertheless, advanced ccRCC has a poor prognosis not least because its resistance to chemotherapy and radiotherapy [8]. Despite life prolonging therapeutic improvements, such as the introduction of tyrosin, multi-kinase inhibitors (e.g., sunitinib etc.), and immunotherapeutic agents (e.g., nivolumab) [9], metastatic RCC still remains an incurable disease in almost all patients [10]. Hence, it is of paramount interest to identify novel therapeutic strategies in the context of increasingly personalized cancer care. The discovery of microRNA (miRNA) signatures in various cancerous tissues has provided novel diagnostic and prognostic insights [11,12]. MiRNAs are a class of small, endogenous, non-coding RNAs that play a crucial role in post-transcriptional regulation of their target mRNAs [13]. Non-coding RNAs in general and especially miRNAs might serve as promising biomarkers for the diagnosis, prognosis, therapy efficacy prediction, as well as act as novel RCC drug targets themselves [14–17].

2. Von Hippel–Lindau/Hypoxia Inducible Factor (HIF)

The genetic association of RCC with the Von Hippel–Lindau (VHL) gene due to chromosomal loss in 3p25-26 was discovered in 1993 in hereditary kidney cancer families [18]. Alterations of this gene occur in the majority of clear cell RCC through mutations, deletions, and/or hypermethylation of its promoter region [19]. Mutations of VHL were also found in 60–80% of sporadic RCC cases [20]. The loss of VHL function leads to a constitutional activation of the hypoxia inducible factor (HIF) pathway. Subsequently, missing proteasomal degradation of HIF α results in increased expression of angiogenic and growth factors, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor B chain (PDGF-B), that contribute to the growth and proliferation of RCC tumor cells [21,22]. The hypoxia inducible factor-1 (HIF1) is a heterodimer with an HIF1 α and an HIF1 β subunit. HIF1 β represents the major activated transcriptional factor in response to hypoxia. Although HIF1 β is constitutively expressed, the protein level of HIF1 α is tightly regulated by the oxygen level. When adequate oxygen levels are reached, HIF1 α is hydroxylated by prolyl hydroxylase proteins (PHDs). It is then recognized by the VHL tumor suppressor, which is an E3 ubiquitin protein ligase for ubiquitin-mediated degradation [23]. Because of the low hydroxylase activity of PHDs under hypoxia, HIF1 α is stabilized and dimerized with HIF1 β to form HIF1, which in turn is translocated to the nucleus. The heterodimer HIF1 activates in combination with the coactivator protein CREB binding protein/p300 the transcriptional activity of target genes within the hypoxia response element [24,25] (Figure 1).

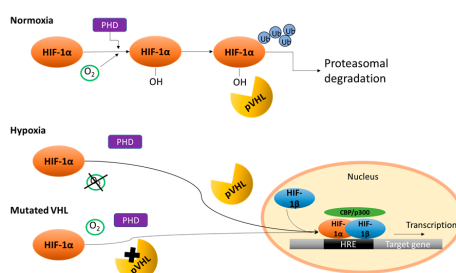


Figure 1. Regulation of HIF1 α under normoxia and hypoxia conditions. Under normoxia conditions, the prolyl hydroxylase domain (PHD) hydroxylates HIF1 α via molecular oxygen. Hydroxylated HIF1 α becomes polyubiquitylated (Ub) for proteasomal degradation by binding the Von Hippel–Lindau protein (pVHL). Under hypoxic conditions, the activity of PHD is reduced. HIF1 α is directly translocated to the nucleus where it binds HIF1 β and CREB binding protein/p300 (CBP/p300) at the hypoxia response element (HRE) to act as transcription factor. If the VHL gene is mutated, it is not capable to bind HIF1 α . HIF1 α leads to the transcription of the target genes as described under hypoxia conditions.

Most cancer cells rely on aerobic glycolysis—a phenomenon called the “Warburg effect” [1,2]. Several glycolytic enzymes, such as glucose transporter isoform 1 (GLUT1), hexokinase 2, and lactate dehydrogenase A, show an elevated expression in many types of human cancer [3,4]. HIF1 α is the key mediator of cellular adaption of oxygen stress and acts as a transcription factor for these genes [26]. HIF is also responsible for upregulation of pro-angiogenic genes like epidermal growth factor, VEGF, PDGF, and other proangiogenic factors, resulting in enhanced blood vessel growth [27]. In summary, the HIF pathway plays an important role as a possible driver in VHL mutant clear cell RCC.

3. Angiogenesis in Renal Cell Carcinoma (RCC)

Histopathological evaluations of RCC have shown that highly vascularized neoplasms evolve out of abundant angiogenesis and abnormal blood vessel development [28]. Angiogenesis is a process that has been shown to be important in embryonic development, tissue growth, as well as in wound healing [29]. It is controlled by a balance of factors that promote or inhibit angiogenesis [30]. In RCC cells, frequent inactivation of the VHL gene contributes to HIF expression, which accelerates angiogenesis through the transcription of its target genes. During the process of angiogenesis, several growth factors interact with endothelial cells. The growth factors most frequently mentioned in the literature are the fibroblast growth factor (FGF), VEGF, transforming growth factor β (TGF- β), and cytokines as well as nitric oxide to form new vessels [31–33]. Angiogenic stimuli are released by tumor cells, stromal cells, as well as inflammatory cells and are recruited to the tumor site [34]. Several recent studies were able to demonstrate a strong link between miRNAs and angiogenic growth factors. For example, Zhu et al. described a miRNA called miR-146a, whose overexpression leads to an upregulation of angiogenesis and cytokine activity-associated genes, including FGF2 by directly targeting cyclic AMP-responsive element-binding protein 3-like 1 (CREB3L1). CREB3L1 functions as an inhibitor of fibroblast growth factor binding protein 1 (FGFBP1) by binding to two cyclic adenosine monophosphate (AMP)-responsive element-like sites. It prevents the expression of FGFBP1, which acts as a chaperone molecule to positively modulate the biological activities of autocrine FGF, thus supporting tumor growth and angiogenesis [35]. Hence, angiogenesis might represent a therapeutic target for the treatment of various human diseases. Moreover, the promotion of angiogenesis might be used to treat cardiovascular heart diseases, whereas anti-angiogenesis treatments might provide benefits in various tumor therapies, in particular in RCC [36,37].

4. Current Anti-Angiogenic Therapy

In recent years, a better understanding of the mechanisms of angiogenesis in the pathogenesis of RCC led to the development of a number of targeted therapies in the metastatic setting. The VEGF pathway plays a crucial role in tumor vascular development [36]. Its inhibition is used as one of the primary therapeutic approaches for metastatic RCC. One of these anti-angiogenic agents is bevacizumab, a VEGF-targeted monoclonal antibody that blocks the binding of VEGF-A to its receptor. Other agents, such as sunitinib, pazopanib, sorafenib, lenvatinib, and axitinib, act as inhibitors of the tyrosine kinase activity of the intracellular domain of the VEGF receptor [38]. Axitinib as a second-line treatment for metastatic RCC is a selective and second-generation multitarget TKI that inhibits the receptors VEGFR1-3. Its effectiveness may be due to the co-inhibition of further enzymes like Kit and PDGFR, which promote cell growth and proliferation [39,40].

Another class of therapeutic agents to treat metastasized RCC exhibits anti-tumor effects by inhibition of the mammalian target of rapamycin (mTOR). This pathway affects cellular functions including cell growth, proliferation, metabolism, and angiogenesis [41]. Temsirolimus and everolimus are analogues of rapamycin, which have been applied in the treatment of various solid, as well as hematologic malignancies [42,43]. In spite of directly affecting the mTOR kinase, temsirolimus and everolimus bind to FK-binding protein 12, an intracellular immunophilin, resulting in an inhibition of the kinase activity of mTOR complex 1 [44,45]. These agents have proven limited efficacy in

metastasized RCC [46]. Due to an almost certain occurrence of resistances towards these agents over time, combined or novel therapeutic approaches in advanced RCC are strongly warranted [47].

5. MiRNA Biogenesis and Function

MiRNAs are endogenous small regulatory non-coding RNA molecules of around ~22 nucleotides in length that regulate the activity of specific mRNA targets and play an important role in a wide range of physiological and pathological processes [48–50].

The processing of miRNAs starts with the transcription into a long primary nuclear pri-miRNA, regulated by polymerase II or RNA polymerase III. By the Drosha/DGCR8 complex pri-miRNA will be cleaved to a 70–80 bp long pre-miRNA and is then exported into the cytoplasm via exportin-5/RanGTP [51,52]. After cleaving the pre-miRNA to double-stranded mature miRNA by the RNase III Dicer, the mature miRNA is separated into two strands: the miRNA-guide strand and the miRNA-passenger strand, which will be degraded. The guide strand is loaded into a silencing complex called RNA-induced silencing complex and is transported to the target mRNA [51,53] (Figure 2).

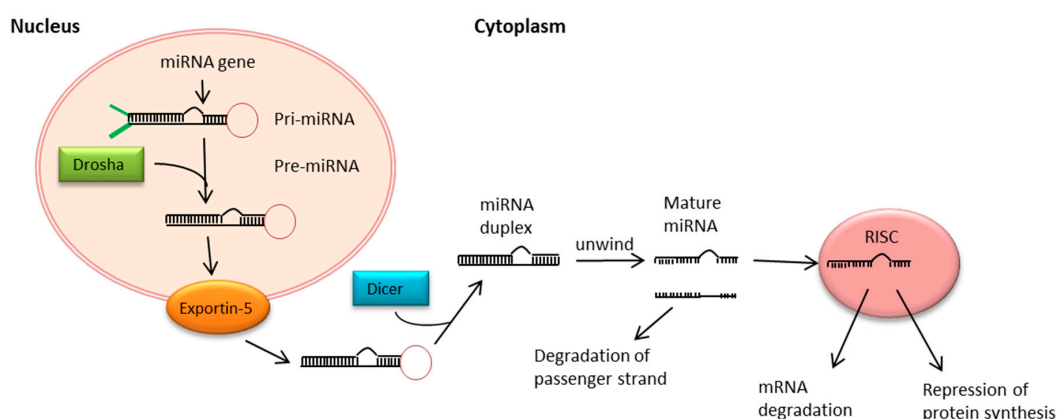


Figure 2. MicroRNA biogenesis and processing pathway. Arrows are indicating the way how microRNAs are processed and how they influence protein expression. Primary-miRNA (pri-miRNA) is transcribed from DNA and cleaved into precursor-miRNA (pre-miRNA), which is exported into the cytoplasm by Exportin-5 for further processing. The mature microRNA (miRNA) strand is assembled into the RNA-induced silencing complex (RISC) to bind mRNA and negatively regulates gene expression by mRNA degradation or by repression of the protein translation (as shown by the arrows pointing away from RISC respectively).

MiRNAs function as post-transcriptional regulators of their target genes by inducing mRNA degradation or translation inhibition, through binding to complementary nucleotides in the 3'UTR region of target mRNAs. The miRNA can be released into the cytoplasm from normal or tumor tissue, where their expression in serum and plasma is relatively stable [54–56]. A dysregulation of miRNAs has been extensively implicated in cancer pathogenesis in various tumor types [56]. It has been shown that miRNAs are involved in the pathogenesis of solid tumors and can act either as tumor promotor (“oncoMiR”) or tumor suppressive miRNA [57,58]. The following paragraphs will discuss several miRNAs that have been experimentally proven to be associated with the VHL gene and might serve as novel drug targets in RCC (Table 1).

Table 1. MicroRNAs associated with Von Hippel–Lindau (VHL) in clear cell renal cell carcinoma (RCC). mesenchymal transition (EMT)

| MicroRNA | Chromosomal Location | Tumor Suppressor/OncomiR | Proven Target Genes | Pathway Involved |
|------------|----------------------|--------------------------|---|--|
| MiR-30c | Unknown | Tumor suppressor | Unknown | Hypoxia, Epithelial-mesenchymal transition |
| MiR-182-5p | 7q32.2 | Tumor suppressor | HIF2 α | Hypoxia |
| MiR-92a | Unknown | OncomiR | Unknown | Unknown |
| MiR-210 | 11p15.5 | OncomiR | HIF1 α | Hypoxia |
| MiR-17-5p | 13q31.3 | Unknown | HIF1, VHL, HIF1AN, VEGFA, EGLN3, PI3K, MAP3K1 | Hypoxia |
| MiR-224 | Xq28 | OncomiR | VHL, SMAD4/5 | Hypoxia |
| MiR-28-5p | 3q28 | OncomiR | Mad2 | Mitotic checkpoint |
| MiR-204 | 9q21.12 | Tumor suppressor | MAP1LC3B | Macroautophagy |
| MiR-155 | 21q21.3 | OncomiR | CAIX | Hypoxia |

6. MiRNAs Associated with VHL in Clear Cell RCC

6.1. MiR-30c

MiR-30c is involved in many biologic events, including cell apoptosis, growth and differentiation [59]. In a recent study of Huang et al. a downregulation of miR-30c in RCC by using microarray analysis was shown. Through quantitative RT-PCR analysis, a downregulation in 32 pairs of RCC tissues was accomplished, whereby 23 were tested positive. Furthermore, the authors observed a reduction of miR-30c in three RCC-cell lines after culturing under hypoxia conditions for 24 h: ACHN, Caki-1, A498 and 786-O cells. By transfecting siRNAs against HIF1/-2 α , they rescued the downregulation of miR-30c. These results suggested that hypoxia might be responsible for the downregulation of miR-30c in RCC in an HIF-dependent manner. In addition, miR-30c revealed a correlation with VHL. Lower levels of miR-30c in VHL-deficient RCC cell lines A498 and 786-O were experimentally determined, indicating that VHL might regulate miR-30c expression. Huang et al. transfected Flag-VHL, a VHL expressing plasmid, into VHL-deficient cells to examine miR-30c expression. They were able to show an increase of miR-30c expression in FlagVHL-transfected A498 and 786-O cells compared to their control group. A mutation or loss of VHL in 12 of 32 RCC tissues and a significant correlation between miR-30c expression and VHL status in RCC tissues was also demonstrated. Since the loss of VHL results in an epithelial–mesenchymal transition (EMT), the downregulation of miR-30c might affect it. Through Western blot analysis Huang et al. showed a significant inhibition of E-cadherin by miR-30c downregulation in ACHN and Caki-1 cells, whereas α -SMA and vimentin were highly induced. The transwell assay showed promoted migration of RCC cells after inhibition of miR-30c. In conclusion, these results indicate that downregulation of miR-30c promotes EMT in RCC cells. Moreover, the authors investigated the impact of miR-30c overexpression on EMT and were able to detect an increased expression of E-cadherin. The expression of α -SMA and vimentin were inhibited in A498 and 786-O cells. Taken together, these results demonstrated that a high expression of miR-30c could inhibit EMT of RCC cells and therefore might prevent the spread of RCC cells from the primary tumor into the blood stream [60]. MiR-30c might be used as a first therapeutic approach at an early time stage of RCC to sustain the expression of E-cadherin, inhibiting EMT.

6.2. MiR-182-5p

Fan et al. demonstrated that Dicer inhibits the expression of HIF2 α , which is a direct target of Dicer-dependent miR-182-5p in VHL-deficient clear cell RCC [61]. The enzyme Dicer is responsible for processing pre-miRNAs into their mature forms [62]. The authors demonstrated that the levels of pre-miRNA were increased in VHL-deficient clear cell RCCs in contrast to mature miRNA. There is a strong body of evidence that the enzyme Dicer is downregulated by VHL deficiency in clear cell RCC. A correlation between the enzyme and HIF2 α has already been described. An overexpression of Dicer leads to reduced levels of HIF2 α mRNA and protein, as well as the downstream target genes

VEGFA and *GLUT-1*. In the VHL-deficient clear cell RCC cell line, OS-RC-2, that stably expresses both *HIF1 α* and *HIF2 α* , *HIF1 α* levels were not affected by Dicer overexpression. Dicer knockdown following transfection of short hairpin Dicer (shDicer) RNA into OS-RC-2 cells upregulated *HIF2 α* , *VEGFA*, and *GLUT-1* expression. Fan et al. [63] performed the same overexpression and knockdown experiment in 786-O cells, with restored pVHL levels and confirmed that Dicer suppresses *HIF2 α* expression only in VHL-deficient clear cell RCCs compared to wild-type VHL clear cell RCC cells. The levels of mature miR-182-5p, a direct target of *HIF2 α* , were decreased and the levels of the precursor molecule were increased in pVHL knockdown Caki-1 cells compared to wild type VHL cells. Furthermore, the authors were able to show an association between *HIF2 α* and miR-182-5p, indicating an inhibition of the *HIF2 α* expression in clear cell RCC cells through this Dicer-dependent miRNA. The overexpression of Dicer also has a suppressing influence on tumor growth and angiogenesis in VHL-deficient clear cell RCCs by reducing *HIF2 α* expression, demonstrated in vivo and in vitro. In conclusion, Fan et al. demonstrated that reduced Dicer levels predict poor survival in VHL-deficient clear cell RCC patients [61]. According to this methodological approach, the enzyme Dicer could be used as a supporting agent in combined therapies to reduce tumor-promoting factors like *HIF2 α* . Thus, the expression of *HIF2 α* associated targets (*VEGFA* and others) responsible for angiogenesis might be limited in VHL-deficient clear cell RCC.

6.3. MiR-92a/ MiR-210

Valera et al. showed that 786-O and UOK117 tumor cell lines with VHL mutations had low transcript levels of *VHL* mRNA compared to two normal kidney cell lines they identified on the VHL gene. Higher levels of miR-92a were seen in higher-grade clear cell RCCs. Since the increased amount of miR-92a in clear cell RCC and decreased levels of *VHL* mRNA, the authors assume that miRNAs may influence the transcript abundance of protein-coding target genes in RCC [63].

Furthermore, they examined the activation of hypoxia in clear cell RCC. MiR-210 is upregulated by *HIF1 α* in response to hypoxic conditions and its overexpression is associated with poor cancer-specific survival (CSS) following RCC resection [64]. Valera et al. tested whether the hypoxia-induced miRNA-210 is part of the clear cell RCC phenotype. Clear cell tumors, compared with tumors of non-clear cell histology, had significantly higher miR-210 expression levels. Mutations of VHL in exon 1, deletions, and stop mutations, as well as multiple (more than one) mutations, increased miR-210 expression. Similarly, a higher miR-210 expression was seen in high-grade tumors and in lesions with positive lymph nodes [63]. Both miR-92a and miR-210 might play an important role in high-grade tumors. The downregulation of these miRNAs could be a promising approach due to the resistance of advanced clear cell RCC against currently used agents.

6.4. MiR-17-5p/MiR-224

HIF1 and *VHL* are predicted to be direct targets of miR-17-5p. Lichner et al. confirmed this hypothesis by Western blot analysis, the luciferase interaction assay, and qRT-PCR. Several factors that play a crucial role in clear cell RCC, such as hypoxia-inducible factor 1- α inhibitor (*HIF1AN*), Egl nine homolog 3 (*EGLN3*), *VEGF-A*, phosphatidylinositol-3-kinase (*PI3K*), phosphatase and tensin homolog, and mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*), might be potential targets of miR-17-5p. *VEGF-A* is a target of the *HIF1 α* / β transcription factor complex that induces signaling through the *MAP3K1* and *PI3K* pathways. *HIF1AN* and *EGLN3* are regulators of the ubiquitin ligase activity of the VHL-Cullin-RING complex [65].

Another miRNA that is dysregulated in clear cell RCC and other types of cancer is miR-224 [66]. It showed a 21.06-fold overexpression in RCC tumor tissue samples compared to adjacent, healthy kidney cortex [65]. This miRNA is already known to provide oncogenic potential in many types of cancer, such as ovarian cancer or for regulating TGF-mediated proliferation of granulosa cells [67]. However, little is known about the involvement of miR-224 in hypoxia and RCC. After treatment of SKOV-3 cells with miR-244, Lichner et al. observed a significant decrease of *VHL* and *HIF1 α* protein levels. According to

bioinformatic analysis, VHL is likely to be a direct target, whereas HIF1 α raises the possibility to be regulated indirectly. Lichner et al. also demonstrated that miR-224 regulates the TGF- β pathway by inhibiting *SMAD4/5* [65]. Dysregulation of miR-17-5p and miR-224 might influence many pathways by targeting several factors, which lead to cancer development and tumor progression. Affecting different pathways at the same time might represent a supportive treatment modality for clear cell RCC patients.

6.5. MiR-28-5p

Chromosomal instability often results from defects in the mitotic checkpoint [68]. The central component herein is the mitotic spindle checkpoint protein (Mad2), a mitotic checkpoint protein, which prevents cells with incompletely assembled spindles from leaving mitosis. Both increased and decreased levels of *Mad2* can lead to tumor progression in consequence of chromosomal instability [69,70]. Hell et al. characterized the miRNA miR-28-5p as regulator of Mad2 by binding to specific target sequences in the 3'UTR region. This mechanism is triggered by the inactivation of VHL. It has been shown that in VHL-positive cancer cells, an overexpression of miR-28-5p reduced *Mad2* levels, causing chromosomal instability [71]. In contrast, the inhibition of miR-28-5p in checkpoint-deficient VHL-negative cancer cells restored *Mad2* levels. By bioinformatic analyses, the authors strengthened the hypothesis that a loss of VHL is associated with enhanced miR-28-5p expression and chromosomal instability, resulting in tumor progression in clear cell RCC [71]. Taken together, diminished levels of this miRNA would sustain chromosomal stability on the basis of functional mitotic checkpoints such as Mad2. This might provide a new therapeutic strategy to prevent clear cell RCC development and progression at the chromosomal level.

6.6. MiR-204

Autophagy is a survival-promoting complex to eliminate defective organelles and molecules [72]. The recycling of these intracellular constituents in lysosomes can serve as an alternative energy source during periods of metabolic stress [73]. Cancer cells use autophagy in response to metabolic stress to sustain cell viability and an increased aggressiveness [72]. Using human clear cell RCC specimens, VHL-deficient cells, and xenograft models, Mikhaylova and colleagues identified miR-204 as an inhibitor of macroautophagy, induced by VHL [74]. Thereby, MAP1LC3B (LC3B) functions as a direct target. In higher tumor grades of clear cell RCC, a decrease of miR-204 and an increase of LC3B levels were shown. LC3B-mediated macroautophagy seems to be necessary for RCC progression. In conclusion, VHL-induced miR-204 overexpression leads to suppression of tumor growth by targeting LC3B and therefore inhibiting macroautophagy [74]. Influencing the autophagy mechanism could significantly support currently used treatment options of clear cell RCC. Nutrient supply during periods of metabolic stress is crucial for cell survival, tumor growth, and aggressiveness [75]. For an effective tumor therapy, it is necessary to halt the intake of nutrients, which might be achieved through autophagy inhibition.

6.7. MiR-155

Higher expression levels of miR-155 were seen in RCC tissues compared to adjacent normal tissues [64]. Neal et al. exhibited a significant increase of miR-155 in RCC4 cells without a functional VHL gene (RCC4 – VHL compared to RCC4 + VHL). Reduced levels of miR-155 were shown through the treatment with HIF1 α and HIF2 α siRNAs. The induction of the miRNA in cells lacking VHL appears to be mediated mostly via HIF induction. Furthermore, miR-155 showed a weak correlation with carbonic anhydrase IX (CAIX), a transcriptional target of HIF1 α , indicating that miR-155 is regulated by VHL through HIF1 α [76]. The dysregulation of miR-155 might play an important role in the VHL/HIF pathway, and its downregulation could be a promising therapeutic approach for RCC, but the involvement of miR-155 in the VHL/HIF pathway remains largely unknown.

7. Conclusions

Several therapeutic agents, such as tyrosine kinase and mTOR inhibitors as well as immunotherapeutics (sunitinib or sorafenib), have demonstrated significant improvements regarding response rates, cancer-specific progression-free survival rates, and overall survival rates of advanced RCC patients. However, many of these patients are either primarily resistant or they develop secondary resistances towards these agents. Another issue is the possibility of increased adverse effects in combination therapies and the challenge of choosing an appropriate treatment for each RCC patient, highlighting the importance for the identification of novel prognostic markers concerning drug resistances and the development of new therapeutic strategies in RCC. Since miRNAs act as important regulators of gene expression, they show a high potential as predictive markers for the therapeutic response towards chemotherapeutic drugs. Dysregulated patterns of miRNAs appear to be tissue-specific in human cancer, and miRNAs are present in human plasma in a relatively stable form that is protected from endogenous RNase activity. Therefore, miRNA might be useful biomarkers in treatment of cancer patients. Targeting miRNAs that are involved in drug resistance mechanisms might improve the therapeutic efficacy in therapy-resistant tumor patients. It is important to find new strategies to address challenges around drug specificity, drug resistance, drug stability, and adverse effects.

Acknowledgments: Maximilian Seles received a funding for non-coding RNA research in RCC from the Austrian Society of Urology and Andrology (Richard Übelhör-Grant). Michael Stotz was supported by the funding of Hans and Blanca Moser Foundation. Georg C. Hutterer's research was supported by a research grant of the Austrian National Bank (No. 15888).

Author Contributions: All authors contributed to the drafting, writing, and reviewing of this article.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| | |
|--------------|--|
| RCC | renal cell carcinoma |
| VHL | Von Hippel Lindau |
| HIF | hypoxia inducible factor |
| miRNAs | microRNAs |
| VEGF | vascular endothelial growth factor |
| FGF | fibroblast growth factor |
| PDGF-B | platelet-derived growth factor B chain |
| TGF- β | transforming growth factor β |
| GLUT1 | glucose transporter isoform 1 |
| EMT | epithelial-mesenchymal transition |
| PI3K | phosphatidylinositol-3-kinase |
| CREB3L1 | cyclic AMP-responsive element-binding protein 3-like 1 |
| PHDs | prolyl hydroxylase proteins |
| FGFBP1 | fibroblast growth factor binding protein 1 |
| mTOR | mammalian target of rapamycin |
| HIF1AN | Hypoxia-inducible factor 1-alpha inhibitor |
| EGLN3 | Egl nine homolog 3 |
| MAP3K1 | activated protein kinase kinase kinase 1 |

References

1. Cairns, R.A.; Harris, I.S.; Mak, T.W. Regulation of cancer cell metabolism. *Nat. Rev. Cancer* **2011**, *11*, 85–95. [[CrossRef](#)] [[PubMed](#)]
2. Lunt, S.Y.; Vander Heiden, M.G. Aerobic glycolysis: Metabolic. *Annu. Rev. Cell Dev. Biol.* **2011**, *27*, 441–464. [[CrossRef](#)] [[PubMed](#)]

3. Koppenol, W.H.; Bounds, P.L.; Dang, C.V. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer* **2011**, *11*, 325–337. [[CrossRef](#)] [[PubMed](#)]
4. Hirschhaeuser, F.; Sattler, U.G.A.; Mueller-Klieser, W.; Warburg, O.; Altenberg, B.; Greulich, K.; DeBerardinis, R.; Levine, A.; Puzio-Kuter, A.; Lu, H.; et al. Lactate: A metabolic key player in cancer. *Cancer Res.* **2011**, *71*, 6921–6925. [[CrossRef](#)] [[PubMed](#)]
5. Ljungberg, B.; Bensalah, K.; Canfield, S.; Dabestani, S.; Hofmann, F.; Hora, M.; Kuczyk, M.A.; Lam, T.; Marconi, L.; Merseburger, A.S.; et al. EAU guidelines on renal cell carcinoma: 2014 update. *Eur. Urol.* **2015**, *67*, 913–924. [[CrossRef](#)] [[PubMed](#)]
6. Zbar, B.; Klausner, R.; Linehan, W.M. Studying Cancer Families to Identify Kidney Cancer Genes. *Annu. Rev. Med.* **2003**, *54*, 217–233. [[CrossRef](#)] [[PubMed](#)]
7. Bukowski, R.M. Natural history and therapy of metastatic renal cell carcinoma: The role of interleukin-2. *Cancer* **1997**, *80*, 1198–1220. [[CrossRef](#)]
8. Singer, E.A.; Gupta, N.G.; Srinivasan, R. Update on targeted therapies for clear cell renal cell carcinoma. *Curr. Opin. Oncol.* **2011**, *23*, 283–289. [[CrossRef](#)] [[PubMed](#)]
9. Motzer, R.J.; Escudier, B.; McDermott, D.F.; George, S.; Hammers, H.J.; Srinivas, S.; Tykodi, S.S.; Sosman, J.A.; Procopio, G.; Plimack, E.R.; et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N. Engl. J. Med.* **2015**, *373*, 1803–1813. [[CrossRef](#)] [[PubMed](#)]
10. Buonerba, C.; Di Lorenzo, G.; Sonpavde, G. Combination therapy for metastatic renal cell carcinoma. *Ann. Transl. Med.* **2016**, *4*, 100. [[CrossRef](#)] [[PubMed](#)]
11. Pichler, M.; Calin, G.A. MicroRNAs in cancer: From developmental genes in worms to their clinical application in patients. *Br. J. Cancer* **2015**, *113*, 569–573. [[CrossRef](#)] [[PubMed](#)]
12. Calin, G.A.; Croce, C.M. MicroRNA signatures in human cancers. *Nat. Rev. Cancer* **2006**, *6*, 857–866. [[CrossRef](#)] [[PubMed](#)]
13. Bartel, D.P. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* **2004**, *116*, 281–297. [[CrossRef](#)]
14. Rigoutsos, I.; Lee, S.K.; Nam, S.Y.; Anfossi, S.; Pasculli, B.; Pichler, M.; Jing, Y.; Rodriguez-Aguayo, C.; Telonis, A.G.; Rossi, S.; et al. N-BLR, a primate-specific non-coding transcript leads to colorectal cancer invasion and migration. *Genome Biol.* **2017**, *18*, 98. [[CrossRef](#)] [[PubMed](#)]
15. Smolle, M.A.; Calin, H.N.; Pichler, M.; Calin, G.A. Noncoding RNAs and immune checkpoints—Clinical implications as cancer therapeutics. *FEBS J.* **2017**, *284*, 1952–1966. [[CrossRef](#)] [[PubMed](#)]
16. Goblirsch, M.; Richtig, G.; Slaby, O.; Berindan-Neagoe, I.; Gerger, A., P.M. MicroRNAs as a tool to aid stratification of colorectal cancer patients and to guide therapy. *Pharmacogenomics* **2017**, *18*, 1027–1038. [[CrossRef](#)] [[PubMed](#)]
17. Seles, M.; Hutterer, G.C.; Kiesslich, T.; Pummer, K.; Berindan-Neagoe, I.; Perakis, S.; Schwarzenbacher, D.; Stotz, M.; Gerger, A.; Pichler, M. Current insights into long non-coding RNAs in renal cell carcinoma. *Int. J. Mol. Sci.* **2016**, *17*, 573. [[CrossRef](#)] [[PubMed](#)]
18. Latif, F.; Tory, K.; Gnarr, J.; Yao, M.; Duh, F.; Orcutt, M.; Stackhouse, T.; Kuzmin, I.; Modi, W.; Geil, L.; et al. Identification of the von Hippel—Lindau disease tumor suppressor gene. *Science* **1993**, *260*, 1317–1320. [[CrossRef](#)] [[PubMed](#)]
19. Kaelin, W.G. The von Hippel—Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin. Cancer Res.* **2007**, *13*, 680–684. [[CrossRef](#)] [[PubMed](#)]
20. Mena, A.C.; Pulido, E.G.; Guillén-Ponce, C. Understanding the molecular-based mechanism of action of the tyrosine kinase inhibitor: Sunitinib. *Anticancer Drugs* **2010**, *21*, 3–11. [[CrossRef](#)] [[PubMed](#)]
21. Shenoy, N.; Pagliaro, L. Sequential pathogenesis of metastatic VHL mutant clear cell renal cell carcinoma: Putting it together with a translational perspective. *Ann. Oncol.* **2016**, *27*, 1685–1695. [[CrossRef](#)] [[PubMed](#)]
22. Gudas, L.J.; Fu, L.; Minton, D.R.; Mongan, N.P.; Nanus, D.M. The role of HIF1 α in renal cell carcinoma tumorigenesis. *J. Mol. Med.* **2014**, *92*, 825–836. [[CrossRef](#)] [[PubMed](#)]
23. Forristal, C.E.; Wright, K.L.; Hanley, N.A.; Oreffo, R.O.; Houghton, F.D. Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions. *Reproduction* **2010**, *139*, 85–97. [[CrossRef](#)] [[PubMed](#)]
24. Kaelin, W.G.; Ratcliffe, P.J. Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway. *Mol. Cell* **2008**, *30*, 393–402. [[CrossRef](#)] [[PubMed](#)]
25. Shimoda, L.A. 55th Bowditch Lecture: Effects of chronic hypoxia on the pulmonary circulation: Role of HIF-1. *J. Appl. Physiol.* **2012**, *113*, 1343–1352. [[CrossRef](#)] [[PubMed](#)]

26. Brahimi-Horn, M.C.; Chiche, J.; Pouyssegur, J. Hypoxia signalling controls metabolic demand. *Curr. Opin. Cell Biol.* **2007**, *19*, 223–229. [[CrossRef](#)] [[PubMed](#)]
27. Arjumand, W.; Sultana, S. Role of VHL gene mutation in human renal cell carcinoma. *Tumour Biol.* **2012**, *33*, 9–16. [[CrossRef](#)] [[PubMed](#)]
28. Yoshino, S.; Kato, M.; Okada, K. Clinical significance of angiogenesis, proliferation and apoptosis in renal cell carcinoma. *Anticancer Res.* **2000**, *20*, 591–594. [[PubMed](#)]
29. Wang, Z.D.; Wei, S.Q.; Wang, Q.Y. Targeting oncogenic KRAS in non-small cell lung cancer cells by phenformin inhibits growth and angiogenesis. *Am. J. Cancer Res.* **2015**, *5*, 3339–3349. [[PubMed](#)]
30. Howe, G.A.; Kazda, K.; Addison, C.L. MicroRNA-30b controls endothelial cell capillary morphogenesis through regulation of transforming growth factor beta 2. *PLoS ONE* **2017**, 1–20. [[CrossRef](#)] [[PubMed](#)]
31. Cross, M.J.; Claesson-Welsh, L. FGF and VEGF function in angiogenesis: Signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol. Sci.* **2001**, *22*, 201–207. [[CrossRef](#)]
32. Goto, F.; Goto, K.; Weindel, K.; Folkman, J. Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. *Lab. Investig.* **1993**, *69*, 508–517. [[PubMed](#)]
33. Li, Y.; Zhu, H.; Wei, X.; Li, H.; Yu, Z.; Zhang, H.M.; Liu, W.C. LPS induces HUVEC angiogenesis in vitro through miR-146a-mediated TGF- β 1 inhibition. *Am. J. Transl. Res.* **2017**, *9*, 591–600. [[PubMed](#)]
34. Takano, S.; Yoshii, Y.; Kondo, S.; Suzuki, H.; Maruno, T.; Shirai, S.; Nose, T. Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. *Cancer Res.* **1996**, *56*, 2185–2190. [[PubMed](#)]
35. Zhu, H.; Bai, W.; Liu, J.; Zheng, Z.; Guan, H.; Zhou, Q.; Su, L.; Xie, S.; Wang, Y.; Li, J.; Li, N.; Zhang, Y.; Wang, H.; Hu, D. Up-regulation of FGF1 signaling contributes to miR-146a-induced angiogenesis in human umbilical vein endothelial cells. *Sci. Rep.* **2016**, *6*, 25272. [[CrossRef](#)] [[PubMed](#)]
36. Ferrara, N.; Kerbel, R.S. Angiogenesis as a therapeutic target. *Nature* **2005**, *438*, 967–974. [[CrossRef](#)] [[PubMed](#)]
37. Simons, M.; Bonow, R.O.; Chronos, N.A.; Cohen, D.J.; Giordano, F.J.; Hammond, H.K.; Laham, R.J.; Li, W.; Pike, M.; Sellke, F.W.; Stegmann, T.J.; Udelson, J.E.; Rosengart, T.K. Clinical trials in coronary angiogenesis: Issues, problems, consensus: An expert panel summary. *Circulation* **2000**, *102*, E73–E86. [[CrossRef](#)] [[PubMed](#)]
38. Battelli, C.; Cho, D.C. mTOR inhibitors in renal cell carcinoma. *Therapy* **2011**, *8*, 359–367. [[CrossRef](#)] [[PubMed](#)]
39. Roskoski, R. Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. *Pharmacol. Res.* **2016**, *103*, 26–48. [[CrossRef](#)] [[PubMed](#)]
40. McTigue, M.; Murray, B.W.; Chen, J.H.; Deng, Y.L.; Solowiej, J.; Kania, R.S. Molecular conformations, interactions, and properties associated with drug efficiency and clinical performance among VEGFR TK inhibitors. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18281–18289. [[CrossRef](#)] [[PubMed](#)]
41. Wullschlegel, S.; Loewith, R.; Hall, M.N. TOR signaling in growth and metabolism. *Cell* **2006**, *124*, 471–484. [[CrossRef](#)] [[PubMed](#)]
42. Meric-Bernstam, F.; Gonzalez-Angulo, A.M. Targeting the mTOR signaling network for cancer therapy. *J. Clin. Oncol.* **2009**, *27*, 2278–2287. [[CrossRef](#)] [[PubMed](#)]
43. Panwalkar, A.; Verstovsek, S.; Giles, F.J. Mammalian target of rapamycin inhibition as therapy for hematologic malignancies. *Cancer* **2004**, *100*, 657–666. [[CrossRef](#)] [[PubMed](#)]
44. Guertin, D.A.; Sabatini, D.M. Defining the Role of mTOR in Cancer. *Cancer Cell* **2007**, *12*, 9–22. [[CrossRef](#)] [[PubMed](#)]
45. Abraham, R.T.; Gibbons, J.J. The mammalian target of rapamycin signaling pathway: Twists and turns in the road to cancer therapy. *Clin. Cancer Res.* **2007**, *13*, 3109–3114. [[CrossRef](#)] [[PubMed](#)]
46. Voss, M.H.; Molina, A.M.; Motzer, R.J. mTOR inhibitors in advanced renal cell carcinoma. *Hematol. Oncol. Clin. N. Am.* **2011**, *25*, 835–852. [[CrossRef](#)] [[PubMed](#)]
47. Motzer, R.J.; Hutson, T.E.; Glen, H.; Michaelson, M.D.; Molina, A.; Eisen, T.; Jassem, J.; Zolnieriek, J.; Maroto, J.P.; Mellado, B.; et al. Lenvatinib, everolimus, and the combination in patients with metastatic renal cell carcinoma: A randomised, phase 2, open-label, multicentre trial. *Lancet Oncol.* **2015**, *16*, 1473–1482. [[CrossRef](#)]
48. Kloosterman, W.P.; Plasterk, R.H.A. The Diverse Functions of MicroRNAs in Animal Development and Disease. *Dev. Cell* **2006**, *11*, 441–450. [[CrossRef](#)] [[PubMed](#)]
49. Stefani, G.; Slack, F.J. Small non-coding RNAs in animal development. *Nat. Rev. Mol. Cell. Biol.* **2008**, *9*, 219–230. [[CrossRef](#)] [[PubMed](#)]

50. Ling, H.; Vincent, K.; Pichler, M.; Fodde, R.; Berindan-Neagoe, I.; Slack, F.J.; Calin, G.A. Junk DNA and the long non-coding RNA twist in cancer genetics. *Oncogene* **2015**, *34*, 5003–5011. [[CrossRef](#)] [[PubMed](#)]
51. Romero-Cordoba, S.L.; Salido-Guadarrama, I.; Rodriguez-Dorantes, M.; Hidalgo-Miranda, A. miRNA biogenesis: Biological impact in the development of cancer. *Cancer Biol. Ther.* **2014**, *15*, 1444–1455. [[CrossRef](#)] [[PubMed](#)]
52. Yi, R.; Qin, Y.; Macara, I.G.; Cullen, B.R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* **2003**, *17*, 3011–3016. [[CrossRef](#)] [[PubMed](#)]
53. O’Kelly, F.; Marignol, L.; Meunier, A.; Lynch, T.H.; Perry, A.S.; Hollywood, D. MicroRNAs as putative mediators of treatment response in prostate cancer. *Nat. Rev. Urol.* **2012**, *9*, 397–407. [[CrossRef](#)] [[PubMed](#)]
54. Benson, E.A.; Skaar, T.C. Incubation of whole blood at room temperature does not alter the plasma concentrations of microRNA-16 and -223. *Drug Metab. Dispos.* **2013**, *41*, 1778–1781. [[CrossRef](#)] [[PubMed](#)]
55. Mitchell, P.S.; Parkin, R.K.; Kroh, E.M.; Fritz, B.R.; Wyman, S.K.; Pogosova-Agadjanyan, E.L.; Peterson, A.; Noteboom, J.; O’Brian, K.C.; Allen, A.; et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10513–10518. [[CrossRef](#)] [[PubMed](#)]
56. Croce, C.M. Causes and consequences of microRNA dysregulation in cancer. *Nat. Rev. Genet.* **2009**, *10*, 704–714. [[CrossRef](#)] [[PubMed](#)]
57. Pichler, M.; Stiegelbauer, V.; Vychytilova-Faltejskova, P.; Ivan, C.; Ling, H.; Winter, E.; Zhang, X.; Goblirsch, M.; Wulf-Goldenberg, A.; Ohtsuka, M.; et al. Genome-Wide miRNA Analysis Identifies miR-188-3p as a Novel Prognostic Marker and Molecular Factor Involved in Colorectal Carcinogenesis. *Clin. Cancer Res.* **2016**, *23*, 1323–1333. [[CrossRef](#)] [[PubMed](#)]
58. Stiegelbauer, V.; Vychytilova-Faltejskova, P.; Karbiener, M.; Pehserl, A.M.; Reicher, A.; Resel, M.; Heitzer, E.; Ivan, C.; Bullock, M.; Ling, H.; et al. MicroRNA-196b-5p regulates colorectal cancer cell migration and metastases through interaction of HOXB7 and GALNT5. *Clin. Cancer Res.* **2017**, *23*, 5255–5266. [[CrossRef](#)] [[PubMed](#)]
59. Karbiener, M.; Neuhold, C.; Opriessnig, P.; Prokesch, A.; Bogner-Strauss, J.G.; Scheideler, M. MicroRNA-30c promotes human adipocyte differentiation and co-represses PAI-1 and ALK2. *RNA Biol.* **2011**, *8*, 850–860. [[CrossRef](#)] [[PubMed](#)]
60. Huang, J.; Yao, X.; Zhang, J.; Dong, B.; Chen, Q.; Xue, W.; Liu, D.; Huang, Y. Hypoxia-induced downregulation of miR-30c promotes epithelial-mesenchymal transition in human renal cell carcinoma. *Cancer Sci.* **2013**, *104*, 1609–1617. [[CrossRef](#)] [[PubMed](#)]
61. Fan, Y.; Li, H.; Ma, X.; Gao, Y.; Bao, X.; Du, Q.; Ma, M.; Liu, K.; Yao, Y.; Huang, Q.; et al. Dicer suppresses the malignant phenotype in VHL-deficient clear cell renal cell carcinoma by inhibiting HIF-2 α . *Oncotarget* **2016**, *7*, 18280. [[CrossRef](#)] [[PubMed](#)]
62. Kim, V.N. MicroRNA biogenesis: Coordinated cropping and dicing. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 376–385. [[CrossRef](#)] [[PubMed](#)]
63. Valera, V.A.; Walter, B.A.; Linehan, W.M.; Merino, M.J. Regulatory effects of microRNA-92 (miR-92) on VHL Gene expression and the hypoxic activation of miR-210 in clear cell renal cell carcinoma. *J. Cancer* **2011**, *2*, 515–526. [[CrossRef](#)] [[PubMed](#)]
64. Tang, K.; Xu, H. Prognostic value of meta-signature miRNAs in renal cell carcinoma: An integrated miRNA expression profiling analysis. *Sci. Rep.* **2015**, *5*, 10272. [[CrossRef](#)] [[PubMed](#)]
65. Lichner, Z.; Mejia-Guerrero, S.; Ignacak, M.; Krizova, A.; Bao, T.T.; Girgis, A.H.F.; Youssef, Y.M.; Yousef, G.M. Pleiotropic action of renal cell carcinoma-dysregulated miRNAs on hypoxia-related signaling pathways. *Am. J. Pathol.* **2012**, *180*, 1675–1687. [[CrossRef](#)] [[PubMed](#)]
66. Ling, H.; Pickard, K.; Ivan, C.; Isella, C.; Ikuo, M.; Mitter, R.; Spizzo, R.; Bullock, M.D.; Braicu, C.; Pileczki, V.; et al. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. *Gut* **2016**, *65*, 977–989. [[CrossRef](#)] [[PubMed](#)]
67. Yao, G.; Yin, M.; Lian, J.; Tian, H.; Liu, L.; Li, X.; Sun, F. MicroRNA-224 Is Involved in Transforming Growth Factor- β -Mediated Mouse Granulosa Cell Proliferation and Granulosa Cell Function by Targeting Smad4. *Mol. Endocrinol.* **2010**, *24*, 540–551. [[CrossRef](#)] [[PubMed](#)]
68. Gordon, D.J.; Resio, B.; Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* **2012**, *13*, 189–203. [[CrossRef](#)] [[PubMed](#)]
69. Michel, L.S.; Liberal, V.; Chatterjee, A.; Kirchwegger, R.; Pasche, B.; Gerald, W.; Dobles, M.; Sorger, P.K.; Murty, V.V.; Benezra, R. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* **2001**, *409*, 355–359. [[CrossRef](#)] [[PubMed](#)]

70. Sotillo, R.; Hernando, E.; Díaz-Rodríguez, E.; Teruya-Feldstein, J.; Cordon-Cardo, C.; Lowe, S.W.; Benezra, R. Mad2 Overexpression Promotes Aneuploidy and Tumorigenesis in Mice. *Cancer Cell* **2007**, *11*, 9–23. [[CrossRef](#)] [[PubMed](#)]
71. Hell, M.P.; Thoma, C.R.; Fankhauser, N.; Christinat, Y.; Weber, T.C.; Krek, W. Mir-28-5p promotes chromosomal instability in VHL-Associated cancers by inhibiting mad2 translation. *Cancer Res.* **2014**, *74*, 2432–2443. [[CrossRef](#)] [[PubMed](#)]
72. White, E. The role for autophagy in cancer. *J. Clin. Investig.* **2015**, *125*, 42–46. [[CrossRef](#)] [[PubMed](#)]
73. Mathew, R.; Karantza-Wadsworth, V.; White, E. Role of autophagy in cancer. *Nat. Rev. Cancer* **2007**, *7*, 961–967. [[CrossRef](#)] [[PubMed](#)]
74. Mikhaylova, O.; Stratton, Y.; Hall, D.; Kellner, E.; Ehmer, B.; Drew, A.F.; Gallo, C.A.; Plas, D.R.; Biesiada, J.; Meller, J.; Czyzyk-Krzeska, M.F. VHL-Regulated MiR-204 Suppresses Tumor Growth through Inhibition of LC3B-Mediated Autophagy in Renal Clear Cell Carcinoma. *Cancer Cell* **2012**, *21*, 532–546. [[CrossRef](#)] [[PubMed](#)]
75. Sato, K.; Tsuchihara, K.; Fujii, S.; Sugiyama, M.; Goya, T.; Atomi, Y.; Ueno, T.; Ochiai, A.; Esumi, H. Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. *Cancer Res.* **2007**, *67*, 9677–9684. [[CrossRef](#)] [[PubMed](#)]
76. Neal, C.S.; Michael, M.Z.; Rawlings, L.H.; Van der Hoek, M.B.; Gleadle, J.M. The VHL-dependent regulation of microRNAs in renal cancer. *BMC Med.* **2010**, *8*, 64. [[CrossRef](#)] [[PubMed](#)]



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).