



REVIEW ARTICLE

# The paradoxical roles of miR-4295 in human cancer: Implications in pathogenesis and personalized medicine

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## KEYWORDS

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**Abstract** MiR-4295, located on chromosome 10q25.2, is a unique miRNA with a wide range of biological functions. miR-4295 is widely expressed *in vivo*, participating in the biological processes of multiple cancers. Although miR-4295 is dysregulated in various cancers, it has also been found to have the function of inhibiting cancer. At the same time, the expression of miR-4295 is related to prognosis and can be affected by numerous factors connecting to the therapeutic effects of various drugs. This article is to better summarize the role of miR-4295 in cancer and review the potential diagnostic, prognostic, and therapeutic value of miR-4295, which may provide insight into subsequent research.

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## Introduction

MicroRNAs (miRNAs) are small non-coding RNAs, usually 20–22 nucleotides in length.<sup>1</sup> miRNA generates pri-miRNA through transcription, which is then recognized by the Drosha/DGCR8 complex and cleaved into pre-miRNA, which is transported to the cytoplasm through Exportin-5.<sup>2</sup> Pre-miRNA is further cleaved by Dicer enzyme and cofactors

to form mature miRNA. The RNA-induced silencing complex (RISC) and mature miRNA bind to the Argonaute (AGO) protein to guide the complex to its target mRNA.<sup>3</sup> Most miRNAs inhibit gene translation by recognizing and binding the 3'-UTR of the gene.<sup>4</sup> miRNAs can regulate various biological processes such as cell differentiation, apoptosis, cell migration, and cell cycle.<sup>5,6</sup> miRNAs can be artificially divided into two types of miRNAs, miRNA with tumor suppressor function and miRNA with cancer-promoting function.<sup>7</sup> However, sometimes two mature miRNAs of the same pre-miRNA may have opposite effects in cancer.<sup>8</sup> A mature miRNA may also have a dual impact on cancer development.<sup>9</sup> In addition, more and more pieces of evidence show

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that miRNAs are not only reliable diagnostic markers of diseases<sup>7,10</sup> but also potential therapeutic targets.<sup>11</sup>

miR-4295 is located in the intron region of the *VT11A* gene. The precursor miR-4295 (85 bp) can be cleaved by the Dicer enzyme to form an active miRNA (miR-4295-5P, 18 bp).<sup>12</sup> The apoptosis protein X-linked inhibitor of apoptosis protein (XIAP) targets the *VT11A* promoter region through the transcription factor SP1 to promote the expression of miR-4295.<sup>13</sup> miR-4295 can target to inhibit the expression of downstream genes<sup>14</sup> and has abnormal expression in a variety of cancers including prostate cancer, gastric cancer, bladder cancer.<sup>15–17</sup> Here, this review summarizes the biological functions of miR-4295 and its role in cancer pathogenesis and treatment.

## The biological function of miR-4295

Currently, target genes of miR-4295 include *TP63*, *USP28*, *RUNX3*, *GPC5*, *CDKN1A*, *BTG1*, *NPTX1*, *LRIG1*, and *PTPN14*. In addition, downstream genes of miR-4295 also include *SOS2*, *EREG*, *VEGF*, *VEGF-A*, *FLT1*, *FLT2*, and *FOXF1*. The relationship among these genes and miR-4295 remains to be studied, and it will be an interesting question that whether there are potential intermediate pathways between these genes and miR-4295. We summarize the molecular functions of these downstream genes, indicating that miR-4295 has a wide range of biological functions (Fig. 1).

### Binding function, molecular modulator and signal receptor activity of miR-4295 related genes

In the urothelial cell UROTsa, XIAP increases miR-4295 expression through the transcription factor SP1. It is well known that miR-4295 targets the expression of *TP63* (p63 $\alpha$ ), which ultimately down-regulates the expression of epidermal growth factor (EGF). Low expression of EGF eventually induces malignant transformation of cells.<sup>13</sup> *TP63* is a gene on chromosome 3q27-29, which has strong homology with the tumor suppressor gene *p53* and the related gene *p73*.<sup>18</sup> Studies have shown that *TP63* can bind to the anti-apoptotic factor BclxL through its DNA binding domain (p63-DBD).<sup>19</sup>

Analysis of KEGG and GEO databases revealed that low expression of miR-4295 reduced the expression of *SOS2* and *EREG*, which can activate the *ERBB2* pathway to promote cancer.<sup>20</sup> Both *SOS2* and *EREG* are related proteins of the *ERBB2* pathway, and *EREG* is a ligand for *EGFR*.<sup>20</sup> *SOS2* forms a molecular complex with *GRB2*, thereby coupling *EGFR* to *RAS* activation.<sup>21</sup>

The expression of *FLT1* is regulated by miR-4295 as the overexpression of miR-4295 in human umbilical vein endothelial cells (HUVEC) significantly upregulates the protein level of *FLT1*. Propranolol can down-regulate the expression of fms-associated tyrosine kinase 1 (*FLT1*) by down-regulating the expression of miR-4295, which exerts a tumor suppressor effect.<sup>22</sup> *FLT1* is a receptor for *VEGF/PIGF* and has a protein binding function. It is expressed on vascular endothelial cells and plays a key role in angiogenesis and subsequent cancer progression.<sup>23</sup>

The miR-4295/*PTPN14*/*YAP* axis plays an important role in the development of osteosarcoma. In osteosarcoma tissue, dual luciferase experiments showed that overexpressed miR-4295 can target the expression of Non-receptor tyrosine phosphatase 14 (*PTPN14*).<sup>24</sup> The protein encoded by *PTPN14* is a member of the protein tyrosine phosphatase (PTP) family, which is widely present in the nuclear and cytoplasmic localization.<sup>25</sup> It mainly functions through a linker that includes a FERM domain, a linker domain, and a C-terminal catalytic PTP domain. The two PPxY motifs contained in the linker can bind to the WW domain of *YAP* and regulate its phosphorylation. Inactivation of *YAP* phosphorylation is an important mechanism by which *PTPN14* inhibits tumor proliferation and metastasis and exerts a tumor suppressive effect.<sup>26</sup>

### Catalytic activity of miR-4295 target gene

miR-4295 directly targets *USP28* expression, and reduces the proliferation of non-small cell lung cancer (NSCLC) cells and induce its cell apoptosis.<sup>14</sup> In addition, the stability of the oncoprotein *MYC* is regulated by *SCF<sup>Fbw7</sup>* ubiquitin ligase-mediated protein regulation of degradation. Since *USP28* and *SCF<sup>Fbw7</sup>* bind to the same phosphorylation motif,<sup>27</sup> *USP28* can antagonize the protein degradation activity of *SCF<sup>Fbw7</sup>* and promote the growth of cancer cells.<sup>28,29</sup> *USP28* antagonizes *SCF<sup>Fbw7</sup>* and can also

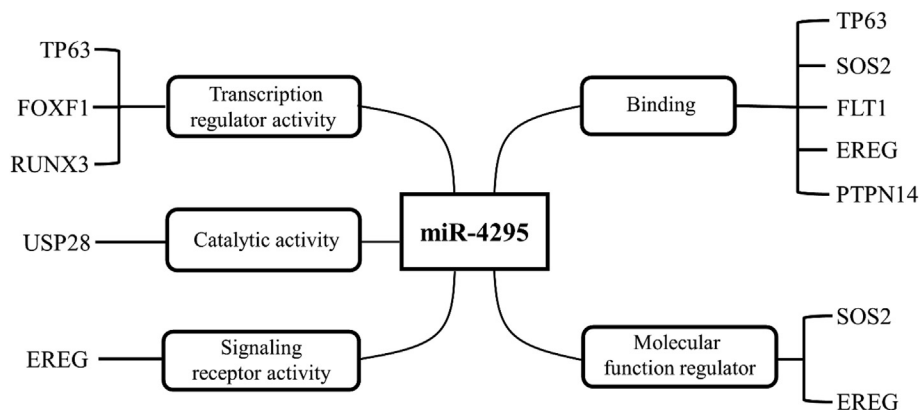


Figure 1 Molecular functions of the downstream genes of miR-4295.

stabilize HIF-1 $\alpha$ , which plays an important role in cell growth, migration, and angiogenesis.<sup>30</sup> USP28 can stabilize the squamous cell carcinoma cell protein by removing the ubiquitin chain interaction between K48 and  $\Delta$ Np63 protein, and the catalytic domain of USP28 is required for this activity.<sup>31</sup> USP28 is also related to cell apoptosis caused by DNA damage.<sup>32</sup> USP28 antagonizes cell apoptosis induced by ubiquitination by binding to the complex formed by Chk2 and ubiquitin E3 ligase PIRH2.<sup>33</sup> As mentioned earlier, the miR-4295 expression is down-regulated in NSCLC tissues.<sup>14</sup> Due to the low expression of miR-4295, the inhibitory effect of this miRNA on the expression of USP28 is relieved. As a target gene of miR-4295, the high expression of USP28 will promote the occurrence of non-small cell lung cancer,<sup>14</sup> promote the proliferation, metastasis, and angiogenesis of cancer cells, and inhibit the apoptosis of cancer cells.<sup>34</sup> To sum up, the low expression of miR-4295 will weaken the targeted inhibitory effect on USP28, thus showing a cancer-promoting effect.

### Transcription regulator activity of miR-4295 related genes

miR-4295 affects the transcriptional regulatory activity of RUNX3, FOXF1, and TP63. RUNX3 expression is regulated by a variety of microRNAs including miR-4295.<sup>35–37</sup> The RUNX3-encoded protein can form a protein complex that binds to the core DNA sequence 5'-PYGPYGGT-3' of enhancers or promoters and then activates or inhibits gene transcription. In addition, RUNX3 interacts with other transcription factors and acts as a tumor suppressor.<sup>38</sup> Li X et al discovered a potential signaling pathway of N-myc/miR-4295/RUNX 3. Among them, the up-regulation of miR-4295 can directly inhibit RUNX3 expression and its tumor suppressor function.<sup>37</sup> Studies have shown that FOXF1 acts as a transcription factor and can activate the transcription of genes such as *VEGF-A* and *SNAL1D*.<sup>39,40</sup> Similarly, propranolol down-regulates the expression of FOXF1 by down-regulating miR-4295 and inhibiting its transcriptional activation, exerting the anticancer function of miR-4295.<sup>22</sup> In addition, TP63, which has transcription factor activity that binds to DNA, can be targeted and inhibited by miR-4295.<sup>13</sup>

### The dual function of miR-4295 in cancer

As shown in Table 1, the expression of miR-4295 in various cancers is not completely consistent. miR-4295 is down-regulated in NSCLC tissues of 10 smoking patients<sup>14,20</sup> while it is up-regulated in bladder cancer tissue, bladder cancer cell lines (Hcv29, UM-UC-3, Blu87, T24, and Hbc) and UROtsa cells. At the same time, it is up-regulated in the drug-treated bladder cancer cells (T24T cells).<sup>13,17,41</sup> miR-4295 is also upregulated in the prostate cancer cell lines (PC3 and DU145)<sup>16</sup> and in various pancreatic ductal adenocarcinoma (PDAC) cell lines (AsPC-1, Panc-1, BxPC-3, and SW1990).<sup>42</sup> Besides, miR-4295 is up-regulated in various gastric cancer cell lines (MKN-28, NCI-N87, SGC-7901, MKN-45, and BGC-823)<sup>15</sup> and 24 infantile hemangioma tissues and HUVEC cell lines.<sup>22</sup> miR-4295 is also up-

regulated in 20 gliomas tissues and the gliomas cell lines (U87 and U251).<sup>37</sup> Beyond that, miR-4295 is up-regulated in 92 head and neck squamous cell carcinoma tissues and various head and neck squamous cell carcinoma (HNSCC) cell lines (FaDu, Hep2, SCC22B, and SCC154).<sup>43</sup> miR-4295 is also up-regulated in 25 osteosarcoma tissues.<sup>24</sup> Moreover, low expression of miR-4295 is currently only found in NSCLC tissues and cell lines, which requires more validation studies, but at least we have discovered the possibility of miR-4295 as a double-edged miRNA. In addition, the different effects of miR-4295 on prognosis also provide evidence for this.

### Tumor-promoting function of miR-4295 related genes

miR-4295 has been found to be involved in multiple cancer pathways (Fig. 2), and at the same time, it promotes cancer by affecting seven cellular activities including cell proliferation, cell migration, cell cycle, cell invasion, epithelial–mesenchymal transition (EMT) and apoptosis. In detail, miR-4295 suppresses or promotes the expression of specific genes primarily, and then through certain pathways taking effect at the cellular level. Finally, miR-4295 can promote the development of cancer. The genes and pathways related to miR-4295 are shown in Fig. 3. The association of these genes with miR-4295 has been experimentally proven, and we will describe them below.

### Cell proliferation regulation of miR-4295 related genes

In the PDAC cell lines (AsPC-1 and BxPC-3), overexpression of miR-4295 down-regulated the expression of GPC5 and promoted cell proliferation by promoting Wnt/ $\beta$ -catenin signaling.<sup>42</sup> In the human glioma cell lines (U87 and U251), N-myc binds to the promoter of miR-4295 to up-regulate its expression, and over-expression of miR-4295 will inhibit the expression of RUNX3 and promotes colony formation and cell growth viability subsequently.<sup>37</sup> In prostate cancer cell lines (PC3 and DU145), ginsenoside Rh2 down-regulates the expression of miR-4295, and it is proved that miR-4295 can directly inhibit CDKN1A and reduces cell proliferation of oncocytes.<sup>16</sup> In bladder cancer cell lines (T24 and Hbc), overexpression of miR-4295 down-regulates BTG1 expression and significantly promotes cell proliferation in bladder cancer.<sup>44</sup> In the HNSCC cell line, overexpression of miR-4295 down-regulates the expression of NPTX1 and promotes cell proliferation.<sup>43</sup> In osteosarcoma Saos-2 and MG-63 cell lines, inhibition of miR-4195 expression increased PTPN14 expression and significantly inhibited the proliferation of these two cells. This shows that miR-4295 promotes cancer cell proliferation through targeted inhibition of PTPN14.<sup>24</sup> The specific mechanism of PTPN14 inhibiting cancer cell proliferation is related to YAP. The Hippo signaling pathway is involved in regulating cell proliferation, and YAP is one of its family members. The combination of PTPN14 and YAP promotes YAP phosphorylation, prevents YAP nuclear translocation, thereby inhibiting YAP-mediated transcriptional activity and inhibiting cell proliferation.<sup>45</sup>

**Table 1** Dysregulated miR-4295 in various cancers.

System	Type	Level	Expression	Reference
Respiratory system	NSCLC	10 tumor tissues of smokers, 10 tumor tissues of former smokers and 9 tumor tissues of non-smokers from GEO (GSE53519)	Downregulation	14,20
Urinary system	BC	Paired tumor and normal tissues; Hcv29, UM-UC-3, Blu87, T24, Hbc, UROtsa, and ISO-treated T24T cell lines	Upregulation	13,17,41
Digestive system	PC	PC3 and DU145 cell lines	Upregulation	16
	PDAC	AsPC-1, Panc-1, BxPC-3, and SW1990 cell lines	Upregulation	42
	GC	MKN-28, NCI-N87, SGC-7901, MKN-45, and BGC-823 cell lines	Upregulation	15
Circulatory system	IH	24 tumor tissues and matched adjacent tissues; HUVEC cell line	Upregulation	22
Nervous system	Glomas	20 tumor tissues and matched normal tissues; U87 and U251 cell lines	Upregulation	37
Others	HNSCC	92 tumor tissues and adjacent normal tissues; FaDu, Hep2, SCC22B, and SCC154 cell lines	Upregulation	43
	Osteosarcoma	25 paired tumor and adjacent normal tissues	Upregulation	24

NSCLC: non-small cell lung cancer; BC: bladder cancer; ISO: isorhapontigenin; PC: prostate cancer; PDAC: pancreatic ductal adenocarcinoma; GC: gastric cancer; IH: infantile hemangioma; HNSCC: head and neck squamous cell carcinoma; HUVEC: human umbilical vein endothelial cells.

### Cell cycle regulation of miR-4295 related genes

In the human glioma cell lines (U87 and U251), knockout of miR-4295 will significantly inhibit the cell cycle.<sup>37</sup> Similarly, in the human bladder cancer cell lines (T24 and Hbc), inhibition of miR-4295 expression reduces the percentage of cells in the G1/G0 phase observably.<sup>17</sup> These results indicate that miR-4295 can regulate the G0/G1 transition and accelerate the cell cycle.

### Cell migration regulation of miR-4295 related genes

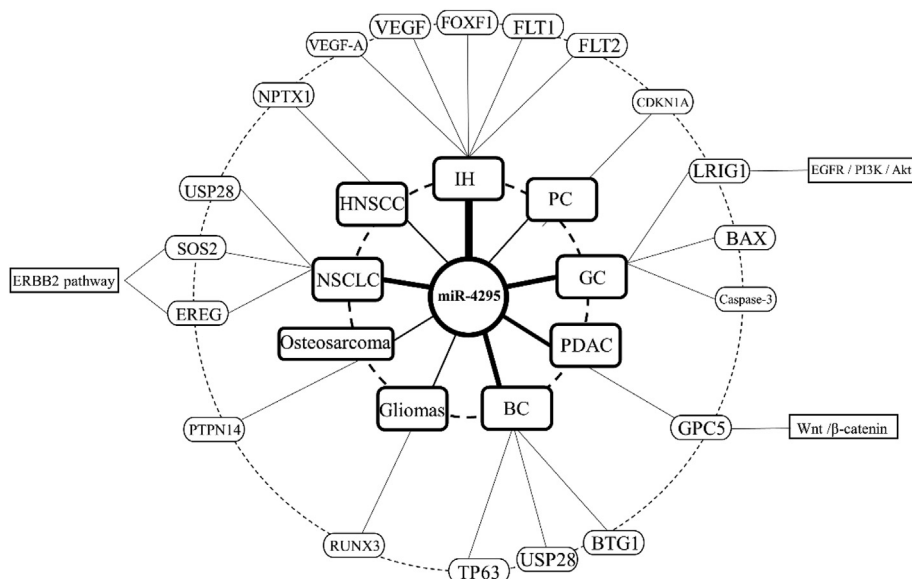
In human bladder cancer cells (T24 and Hbc), miR-4295 not only accelerates the cell cycle by down-regulating BTG1 but also promotes the migration of cancer cells.<sup>17</sup> At the same time, targeted inhibition of NPTX1 by miR-4295 can also promote the migration of HNSCC cells (FaDu and Hep2).<sup>43</sup> In addition, propranolol can down-regulates the expression of miR-4295 and this kind of inhibition finally reduces the cell migration of HUVEC.<sup>22</sup> These indicate that miR-4295 plays an important role in promoting cancer cell migration.

### Malignant EMT regulation of miR-4295 related genes

As previously described, in urothelial tumor UROtsa cells, miR-4295 binds the 3' UTR of TP63 mRNA to inhibit its expression,<sup>13</sup> and the down-regulation of TP63 is critical for malignant EMT.<sup>46</sup> In the HNSCC cell lines (FaDu and Hep2), after treated with the miR-4295 inhibitor, the expression of mesenchymal markers (N-cadherin and vimentin) was decreased, while the expression of epithelial marker (E-cadherin) was up-regulated.<sup>43</sup> These results indicate that miR-4295 can promote the EMT process.

### Cell invasion regulation of miR-4295 related genes

In PDAC cells, miR-4295 down-regulates the expression of GPC5 and then promotes the Wnt/ $\beta$ -catenin signal transduction pathway and increases cancer cell invasion, at the same time, down-regulation of miR-4295 expression also inhibits the cell invasion of PDAC oncocytes.<sup>42</sup> In osteosarcoma Saos-2 and MG-63 cell lines, overexpressed miR-4295 and targeted inhibition of PTPN14 significantly promoted cancer cell migration. We already know that PTPN14 is involved in cytoplasmic localization, which inhibits metastasis by changing protein transport. Inhibition of PTPN14 increases the secretion of growth factors and cytokines, and increases the expression of EGFR and FLT4 on the cell surface.<sup>47</sup> Interestingly, miR-4295 also promotes the production of EGFR through other pathways such as increasing the expression of EREG, indicating that miR-4295 is a complex network for tumor regulation.<sup>15</sup> Besides, PTPN14 can promote cancer cell invasion by inhibiting YAP activity in the Hippo signaling pathway.<sup>26</sup>

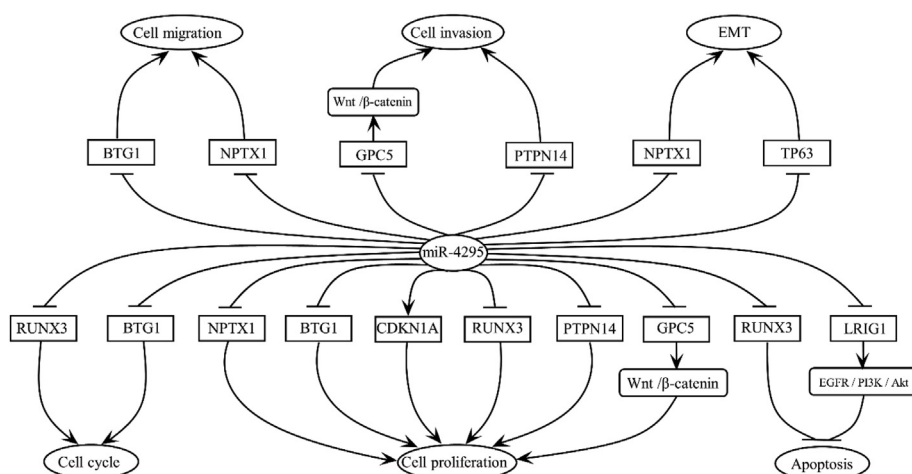


**Figure 2** miR-4295 plays a role in multiple cancers through different pathways. miR-4295 inhibits the expression of TP63 and BTG1 in bladder cancer, and inhibits the expression of USP28 to exert a tumor suppressing effect. miR-4295 also promotes the expression of CDKN1A in prostate cancer. In gastric cancer, miR-4295 down-regulates the expression of BAX and Caspase-3 and inhibits EREG/PI3K/Akt signaling pathway by targeted down-regulation of LRIG1. miR-4295 inhibits GPC5 expression and promotes Wnt/ $\beta$ -catenin signaling pathway in pancreatic ductal adenocarcinoma. In IH, miR-4295 up-regulates the expression of VEGF-A, VEGF, FLT1, FOXF1 and FLT2. In gliomas, miR-4295 reduces the expression of RUNX3. In HNSCC, miR-4295 reduced the expression of NPTX1. In osteosarcoma, up-regulation of miR-4295 reduce the expression of PTPN14 to promote oncogenesis. In non-small cell lung cancer, low expression of miR-4295 reduces the expression of SOS2 and EREG, whereas miR-4295 inhibits the expression of USP28 in non-small cell lung cancer, and acting as a tumor suppressor.

### Apoptosis regulation of miR-4295 related genes

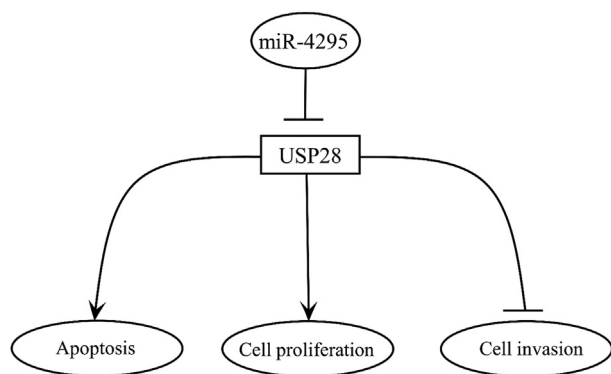
In the human glioma cell lines (U87 and U251), up-regulated miR-4295 expression can not only promotes proliferation and cell cycle, but also inhibits apoptosis causing glioma

ultimately.<sup>37</sup> In pediatric hemangiomas, miR-4295 can also inhibits apoptosis in HUVEC cells.<sup>22</sup> At the same time, in the treatment of gastric cancer, cisplatin can reduce the expression of miR-4295 and promote the expression of LRIG1. Inhibiting of miR-4295 expression impedes the



**Figure 3** Regulation of miR-4295 on biological behavior of cancer cells through a variety of pathway. miR-4295 exerts multiple effects on cancer cells through different target genes. miR-4295 promotes cell migration by inhibiting the expression of BTG1 and NPTX1. miR-4295 promotes cell invasion and EMT by inhibiting the expression of GPC5, NPTX1, TP63, and PTPN14, respectively. Furthermore, miR-4295 promotes cell cycle by inhibiting the expression of RUNX3 and BTG1, and promotes cell proliferation by inhibiting the expression of 5 genes (*NPTX1*, *BTG1*, *RUNX3*, *GPC5*, and *PTPN14*) and promoting the expression of CNKN1A. Moreover, miR-4295 can also inhibit apoptosis by inhibiting the expression of RUNX3 and LRIG1.





**Figure 4** miR-4295 inhibits cancer by targeting USP28. miR-4295 can directly inhibit the expression of USP28, preventing non-small cell lung cancer and bladder cancer by promoting apoptosis and cell proliferation, and inhibiting cell invasion.

EGFR/PI3K/Akt signaling pathway, which finally induce apoptosis in gastric cancer cells (MKN-28 and MKN-45).<sup>15</sup> In other words, miR-4295 play a tumor-promoting role in apoptosis regulation.

### Cancer inhibition function of miR-4295 related genes

At present, it has been reported that miR-4295 also has a tumor-suppressing effect, which is related to USP28 (Fig. 4). As a carcinogenic factor, USP28 is involved in the development of cancers such as gastric cancer<sup>34</sup> and bladder cancer.<sup>48</sup> In non-small cell lung cancer, Zhang L et al first discovered that miR-4295 targets USP28 and promotes cell proliferation and induces apoptosis,<sup>14</sup> while Luo Y et al found that isosaponin (ISO) can increase the expression of Dicer protein, causing the over-expression of miR-4295. Subsequently, over-expressed miR-4295 targets USP28 to inhibit invasion of bladder cancer cells, thereby demonstrating a tumor suppressor effect.<sup>41</sup> Interestingly, miR-4295 exhibits dual function in bladder cancer (BC) cells. Specifically, it inhibits stem cell-like properties of cancer cells at an early stage, thereby exerting a tumor suppressor effect<sup>41</sup>; and then promotes cancer progression in advanced bladder cancer.<sup>17</sup> At present, whether miR-4295 will produce anti-cancer effects through other genes is an important direction for future research.

### Antioxidative damage of miR-4295 related genes

There is a research shows that, in human trabecular meshwork (HTM) cells, lycium barbarum polysaccharides (LBP) treatment can up-regulate the expression of miR-4295, thereby activating PI3K/AKT and ERK signaling pathways and preventing oxidative damage caused by high concentrations of H<sub>2</sub>O<sub>2</sub>.<sup>49</sup> miR-4295 plays an important role in the treatment of glaucoma with LBP. Oxidative damage caused by H<sub>2</sub>O<sub>2</sub> is not only related to glaucoma but also has a complex effect in the development of cancer. On the one hand, low concentrations of H<sub>2</sub>O<sub>2</sub> promote cancer cell

proliferation<sup>50</sup> and invasion<sup>51</sup>; on the other hand, excessive H<sub>2</sub>O<sub>2</sub>-mediated oxidative damage causes nuclear and mitochondrial DNA damage, and up-regulates Caspase-3 and pro-apoptotic protein BAX, leading to apoptosis in cancer cells.<sup>52</sup> Interestingly, the expression of Caspase-3 and BAX was also up-regulated in gastric cancer cell lines (MKN-28 and MKN-45) with suppressed expression of miR-4295.<sup>15</sup> However, there are no study on miR-4295 and oxidative damage in cancer currently. Oxidative damage is the driving factor for cancer cell apoptosis.<sup>53</sup> By activating the PI3K/AKT and ERK signaling pathways, miR-4295 can antagonize the oxidative damage<sup>49</sup> of HTM cells in glaucoma caused by high concentrations of H<sub>2</sub>O<sub>2</sub>. In gastric cancer cell lines MKN-28 and MKN-45, miR-4295 can activate EGFR/PI3K/AKT signaling pathway to inhibit cell apoptosis.<sup>15</sup> It can be seen that the PI3K/AKT pathway is a common pathway for miR-4295 to antagonize H<sub>2</sub>O<sub>2</sub> oxidative damage and inhibit cell apoptosis. The downstream effector of the PI3K/AKT pathway is mTOR, which can inhibit ROS and protect cancer cells from oxidative stress.<sup>54</sup> In summary, miR-4295 inhibits cell apoptosis through the PI3K/AKT pathway may be related to the inhibition of oxidative damage of cancer cells, which also implies that miR-4295 has oncogenic properties that inhibit cancer cell apoptosis. However, whether miR-4295 induces apoptosis through oxidative stress-related pathways remains to be verified.

### miR-4295 has important functions in diagnosis, prognosis, and treatment

The expression of miR-4295 in bladder cancer,<sup>17</sup> infantile hemangioma,<sup>22</sup> glioma<sup>37</sup> and head and neck squamous cell carcinoma<sup>43</sup> was significantly higher than that in adjacent tissues. These results suggest that miR-4295 may be a diagnostic indicator for a variety of cancers. However, it is worth noting that miR-4295 is down-regulated in lung cancer,<sup>20</sup> and the effects of expression in different stages of bladder cancer are also inconsistent.<sup>17,41</sup> A new clinical study<sup>55</sup> shows that miR-4295 can be an effective prognostic indicator for propranolol and pingyangmycin combined with pulsed dye laser in the treatment of childhood hemangiomas. Interestingly, the expression of miR-4295 varies in different types of hemangiomas, and the expression of miR-4295 in cavernous hemangioma is significantly higher than that of capillary hemangioma.<sup>55</sup> These results suggest that miR-4295 may be a promising indicator for future diagnosis and treatment of cancer.

miR-4295 plays a dual role in cancer and it also exhibits different prognostic effects (Table 2). First, promoting the expression of miR-4295 can shorten the survival of patients with NSCLC and therefore has a poor prognosis.<sup>20</sup> In addition, miR-4295 expression was significantly associated with tumor size, TNM stage, and lymph node metastasis of HNSCC, and high expression of miR-4295 was a prognostic risk factor for HNSCC patients.<sup>43</sup> In glioma, miR-4295 overexpression was positively correlated with clinical stage, and miR-4295 expression in stage IV tumors was higher than stage I tumors and normal tissues.<sup>37</sup> In HNSCC, miR-4295 is significantly elevated in clinical stages III and IV, while high miR-4295 expression means lower overall

**Table 2** Prognostic role of miR-4295 in different cancers.

Cancer type	Sample	Factors	Relationship	Reference
NSCLC	Survival of NSCLC patients from Kaplan–Meier Plotter database	OS	Negative correlation	20
Gliomas	20 matched patients	Clinical stage	Positive correlation	37
HNSCC	92 matched patients	Clinical stage, Tumor size, TNM stage, lymphatic metastasis	Positive correlation Negative correlation	43 43
BC	564 high miR-4295 expression patients and 186 lower expression of miR-4295 patients from Kaplan–Meier Plotter database	OS	Positive correlation	41
NSCLC	53 high USP28 expression patients and 17 lower expression of USP28	OS	Positive correlation	14

NSCLC: non-small cell lung cancer; BC: bladder cancer; HNSCC: head and neck squamous cell carcinoma; OS: overall survival.

survival (OS).<sup>43</sup> On the other hand, Luo Y et al compared the OS of bladder cancer patients with different miR-4295 expression and found that the high expression of miR-4295 was positively correlated with the survival time of patients with BC.<sup>41</sup> And up-regulation of miR-4295 may improve OS in patients with NSCLC.<sup>14</sup> All these results indicate that miR-4295 is involved in cancer development and cancer prognosis.

miRNA transcription is regulated by RNA Pol II-related transcription factors and epigenetic regulatory factors.<sup>56</sup> Several studies have reported that the expression of miR-4295 is regulated and can be used for disease treatment. For example, GRh2 alters the expression of miR-4295 at the transcriptional level and inhibits the growth of prostate cancer cells.<sup>16</sup> Propranolol was found to reduce the expression of miR-4295 in HUVEC, thereby inhibiting the development of IH.<sup>22</sup> Decreasing the expression of miR-4295 can promote the sensitivity of GC patients to the chemotherapy drug cisplatin. In addition, the proto-oncogene N-myc promotes glioma development by binding to the miR-4295 promoter.<sup>37</sup> ISO also promotes the development of bladder cancer by promoting the expression of Dicer protein to promote the maturation of miR-4295.<sup>41</sup> LBP treatment can up-regulate the expression of miR-4295 to prevent oxidative damage caused by high concentrations of H<sub>2</sub>O<sub>2</sub>.<sup>49</sup> These results suggest that miR-4295 may be a novel therapeutic target for disease.

### Interaction of miR-4295 related genes and various drugs

We also screened for the drugs related to the downstream genes of miR-4295. Hypermethylation of CpG island in RUNX3 may cause colorectal cancer patients to be resistant to two chemotherapeutic drugs, irinotecan and leucovorin.<sup>57</sup> While the effect of miR-4295 on RUNX3 is consistent with the methylation of RUNX3 CpG island, all of which down-regulate the expression of RUNX3,<sup>37</sup> suggesting that miR-4295 may be one of the causes of chemotherapy drug resistance in patients

with colorectal cancer. It is worth noting that the down-regulation of miR-4295 can enhance the sensitivity of gastric cancer patients to the chemotherapeutic drug cisplatin.<sup>15</sup>

The high transcription level of EREG is a predictor of good prognosis in the treatment of metastatic colorectal cancer with cetuximab.<sup>58</sup> The protein expression level of EREG is a predictive marker of the therapeutic effect of panitumumab in advanced colorectal cancer patients with RAS wild-type.<sup>59</sup> The expression of EREG is also elevated during the down-regulation of miR-4295 by the chemotherapy drug cisplatin to promote apoptosis in gastric cancer cells.<sup>15</sup> This suggests that miR-4295 may affect the efficacy of the drug by affecting the expression of EREG.

Pentosan polysulfate sodium is an anti-VEGF drug<sup>60</sup> that treats prostate cancer by reducing the expression of VEGF in prostate cancer. Ranibizumab, bevacizumab, aflibercept, and other drugs are VEGF inhibitors, and their mechanism for treating IH is similar to that of propranolol, which reduces the expression of VEGF.<sup>22</sup> miR-4295 up-regulates VEGF expression in IH, so miR-4295 may affect the effects of these anti-VEGF drugs. While phenytoin, cilastazol, and gentamicin can increase VEGF expression, miR-4295 may have a synergistic effect on them. FLT-1 and FLT-2 are the VEGF receptor and fibroblast growth factor receptor, respectively. They have been found to be useful in a variety of cancer therapies<sup>61–63</sup> such as lung cancer,<sup>64</sup> thyroid cancer,<sup>65</sup> prostate cancer, etc.<sup>66</sup> The up-regulation of miR-4295 on these two genes may affect the effects of these antagonists. Therefore, miR-4295 may play a role in the treatment of diseases as a new target.

### Conclusions and future perspectives

There is increasing evidence that miRNAs are involved in a variety of biological processes.<sup>5</sup> Over-expressed miR-4295 promotes tumor development by directly targeting TP63, RUNX3, GPC5, CNKN1A, BTG1, NPTX1, LRIG1 and PTPN14, and functions as an oncogene. However, miR-4295 also targets USP28 to inhibit the progression of non-small cell lung cancer. miR-4295 is over-expressed in most of the

cancer tissues, and the prognosis of patients is significantly correlated with the level of miR-4295. Propranolol, lycium barbarum polysaccharides, ginsenoside, isorhapontigenin, cisplatin, and other drugs can affect the expression of miR-4295, and the biological effects of downstream genes suggest that miR-4295 may be a potential therapeutic target.

We have identified three signaling pathways related to miR-4295, including N-myc/miR-4295/RUNX3, miR-4295/GPC5/Wnt/ $\beta$ -catenin, and miR-4295/EGFR/PI3K/Akt, but whether the existence of other signaling pathways related to miR-4295 remains to be explored. In the current study, miR-4295 is down-regulated in tumors such as NSCLC and up-regulated in other cancers, such as BC and PC, which indicate the paradoxical roles of miR-4295 in human cancer. In addition, the role of non-coding RNA (ncRNA) on the abnormal expression of miR-4295 in cancer cannot be ignored. Although there is no research report on ncRNA targeting miR-4295, the study of the ncRNA/miRNA/mRNA signaling pathway is of great significance for understanding the role of miRNA-4295 in cancer. However, more research is needed on a large number of different cancer samples to determine this observation. In addition, miR-4295 may interact with various drugs through related genes, which will make miR-4295 a new target for disease treatment. Due to concerns about the safety of miRNAs, attempts to use miRNAs as a cancer gene therapy tool are still few.<sup>67</sup> In this review, we present the potential diagnostic, prognostic, and therapeutic value of miR-4295 in cancer, which may provide insight into future research.

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## Conflict of interests

The authors declare that they have no conflict of interest.

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