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MicroRNA-96: A therapeutic and diagnostic tumor marker

Hamid Reza Rahimi [1], Majid Mojarrad [1], Meysam Moghbeli [1]*

[1] Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Cancer has been always considered as one of the main human health challenges worldwide. One of the main causes of cancer-related mortality is late diagnosis in the advanced stages of the disease, which reduces the therapeutic efficiency. Therefore, novel non-invasive diagnostic methods are required for the early detection of tumors and improving the quality of life and survival in cancer patients. MicroRNAs (miRNAs) have pivotal roles in various cellular processes such as cell proliferation, motility, and neoplastic transformation. Since circulating miRNAs have high stability in body fluids, they can be suggested as efficient noninvasive tumor markers. MiR-96 belongs to the miR-183-96-182 cluster that regulates cell migration and tumor progression as an oncogene or tumor suppressor by targeting various genes in solid tumors. In the present review, we have summarized all of the studies that assessed the role of miR-96 during tumor progression. This review clarifies the molecular mechanisms and target genes recruited by miR-96 to regulate tumor progression and metastasis. It was observed that miR-96 mainly affects tumorigenesis by targeting the structural proteins and FOXO transcription factors.

Introduction

Cancer is still one of the main causes of death and remains an important health challenge globally. Although, improving the quality of life and increasing the life expectancy of cancer patients are the main goals of cancer therapy, the low efficacy of routine treatment modalities highlights the need to introduce novel specific therapeutic strategies (1). MicroRNAs (miRNAs) are a class of short non-coding RNAs (~22nt) involved in post-transcriptional regulation via mRNA degradation or translational suppression. They regulate cell proliferation, apoptosis, migration, and malignant transformation (2). Therefore, deregulation of miRNAs can be associated with tumor progression (3). They function as oncogene or tumor suppressors during neoplastic transformation. Regarding the tissue-specificity

of miRNA expressions, there are specific miRNA signatures in different tumors (4). Non-invasive or minimally invasive markers are required for early-stage tumor detection to improve the patient's survival. Circulating miRNAs have high stability in body fluids which can be suggested as efficient noninvasive tumor markers (5, 6). MiR-96 belongs to the miR-183-96-182 family that regulates cell motility and tumor progression as an oncogene or tumor suppressor by targeting various genes in solid tumors (7, 8). It can be regulated by ZEB1 (9), β-catenin (10), and epidermal growth factor receptor (EGFR) (11). In the present review, we discussed all of the miR-96 based tumor reports to clarify the molecular mechanisms of miR-96 during tumor progressions and metastasis (Figure 1) (Table 1).
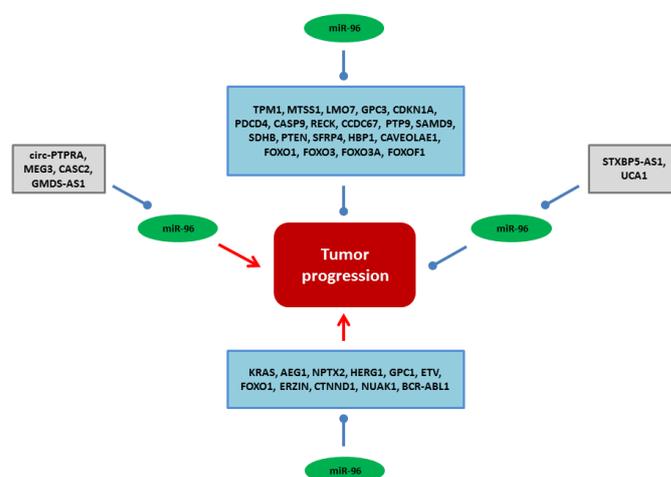

Figure 1. All of the molecular mechanisms and interactions of miR-96 during tumor initiation, progression, and metastasis

*Corresponding author: Meysam Moghbeli. Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Email: moghbelim@mums.ac.ir

Table 1. Molecular mechanisms of miR-96 during tumor progression

Study	Year	Type	Gene	Target	Mir-96 function	Samples
		Structural proteins				
GUO (7)	2012	Bladder	miR-96	FOXO1	Tumor suppressor	40 patients T24 cell line
TSAI (11)	2017	Prostate	miR-96	ETV	Tumor suppressor	69 patients PC3 and RasB1 cell lines
YAO (13)	2018	Osteosarcoma	miR-96	EZRIN	Tumor suppressor	4 patients MG-63 cell line
YU (14)	2015	Renal	miR-96	EZRIN	Tumor suppressor	63 patients CaKi-1 and 786-O cell lines
WU (17)	2017	Lung	miR-96	LMO7	Oncogene	BEAS-2B, A549, PC9, and H1299 cell lines
GAO (18)	2020	Breast	miR-96	CTNND1	Tumor suppressor	155 patients MDA-MB-231,MDA-Mb-246, T47D, and ZR-75-30 cell lines
YIN (22)	2020	Cholangiocarcinoma	miR-96	MTSS1	Oncogene	115 patients HuCCT1, HuH28, and RBE cell lines
XIE (23)	2018	Breast	miR-96	MTSS1	Oncogene	5 patients MCF7 and MDA-MB-231 cell lines
XU (24)	2016	Prostate	miR-96	MTSS1	Oncogene	PC3 and LNCaP cell lines
ZHANG (28)	2019	Glioma	MEG3	miR-96	Oncogene	30 patients GSC11, M059J, and D54 cell lines
LIU (33)	2018	Bladder	MEG3	miR-96	Oncogene	45 patients RT4, RT112, and T24 cell lines
GE (34)	2020	Colorectal	miR-96	TPM1	Oncogene	40 patients SW480 cell lines
LI (36)	2014	Pancreatic	miR-96	GPC1	Tumor suppressor	38 patients Panc-1, AsPC-1, and BxPC-3 cell lines
FEI (39)	2018	Lung	miR-96	GPC3	Oncogene	57 patients A549 and H460 cell lines
LIU (44)	2019	Ovarian	miR-96	CAVEOLAE 1	Oncogene	23 patients A2780, OVCAR3, SKOV3, and CAOV3 cell lines
FENG (51)	2014	Pancreatic	miR-96	HERG1	Tumor suppressor	20 patients PANC-1, SW1990, HPAC, CFPAC-1, and BxPC-3 cell lines
GAO (53)	2018	Breast	CASC2	miR-96	Oncogene	35 patients MCF7 and MDA-MB-231 cell lines
ZHAO (57)	2020	Lung	GMDS-AS1	miR-96	Oncogene	A549, SPCA-1, PC-9, H1975, and H838 cell lines
WEI (58)	2019	Lung	CIRC-PTPRA	miR-96	Oncogene	34 patients H23, H1755, and H522 cell lines
LIU (59)	2019	Thyroid	miR-96	CCDC67	Oncogene	78 patients TPC-1, K-1, BCPAP, and 8505c cell lines
		Transcription factors				
SONG (72)	2015	Thyroid	miR-96	FOXO1	Oncogene	60 patients TPC-1 and K-1 cell lines
YANG (73)	2019	Hepatocellular	miR-96	FOXO1	Oncogene	60 patients HepG2 cell line
YANG (75)	2020	Cervical	miR-96	FOXO1	Oncogene	83 patients C41, C33A, HeLa, CaSki, MS751, and HT-3 cell lines
YU (77)	2014	Prostate	miR-96	FOXO1	Oncogene	13 patients LNCaP and PC3 cell lines
LIN (79)	2010	Breast	miR-96	FOXO3A	Oncogene	23 patients MCF7, ZR-75-30, BT549, Bcap37, MDA-MB-453, and T47D cell lines
ZHOU (80)	2018	Pancreatic	UCA1	miR-96	Tumor suppressor	36 patients PANC-1, SW1990, and AS-PC1 cell lines
LI (81)	2015	Lung	miR-96	FOXO3	Oncogene	68 patients A549 and SPC-A-1 cell lines
YIN (82)	2020	Breast	miR-96	FOXO3	Oncogene	MCF7 and T47D cell lines
GAO (83)	2015	Colorectal	miR-96	FOXO1, FOXO3A	Oncogene	20 patients SW480 and SW620 cell lines
WANG (89)	2020	Oral	miR-96	FOXOF1	Oncogene	30 patients SCC1, SCC4, Tca8113, Cal-27, and HSC-2 cell lines

Continued Table 1

		Signaling pathways				
YAN (93)	2014	Glioma	miR-96	HBP1	Oncogene	U-87MG, U-251MG, U-373MG, and M059 cell lines
ZHANG (95)	2020	Cervical	miR-96	SFRP4	Oncogene	60 patients HeLa, SiHa, Me180, and MS751 cell lines
GUO (101)	2020	Pancreatic	UCA1	miR-96	Tumor suppressor	46 patients PANC-1, BxPC-3, Aspc-1, SW1990, and HUVECs cell lines
FENG (108)	2018	Glioblastoma	miR-96	AEG1	Tumor suppressor	U251 cell line
XIANG (111)	2020	Renal	miR-96	NPTX2	Tumor suppressor	8 patients RenCa, 786-O, and HEK293T cell lines
SHAO (112)	2019	Cervical	STXBP5-AS1	miR-96	Tumor suppressor	37 patients C33A, MS751, SiHa, HeLa, ME180, and CaSki cell lines
VAHABI (113)	2019	Head and neck	miR-96	PTEN	Oncogene	Cal27, FaDu, and H1299 cell lines
ZHAO (118)	2019	Thyroid	miR-96	SDHB	Oncogene	28 patients BCPAP, K-1, and TPC-1 cell lines
WU (120)	2016	Lung	miR-96	SAMD9	Oncogene	H358 and H23 cell lines
YU (126)	2010	Pancreatic	miR-96	KRAS	Tumor suppressor	PaCa-2, PANC-1, BxPC-3, and HeLa cell lines
		Phosphatases, kinases, and matrix metalloproteinases				
HONG (132)	2016	Breast	miR-96	PTPN9	Oncogene	10 patients MCF7 and MDA-MB-468 cell lines
MA (133)	2018	Cervical	miR-96	PTPN9	Oncogene	HeLa cell line
HUANG (137)	2014	Pancreatic	miR-96	NUAK1	Tumor suppressor	10 patients PaCa-2 and PANC-1 cell lines
HUANG (139)	2019	CML	miR-96	BCR-ABL1	Tumor suppressor	50 patients K562 and MEG01 cell lines
XIA (147)	2014	Esophageal	miR-96	RECK	Oncogene	145 patients TE-1, ECa-109, and EC-9706 cell lines
ZHANG (148)	2014	Breast	miR-96	RECK	Oncogene	38 patients MDA-MB-231, MCF7, MDA-MB-468, and T47D cell lines
GUO (149)	2014	Lung	miR-96	RECK	Oncogene	28 patients A549, SK-MES-1, and H1299 cell lines
		Apoptosis and cell cycle regulation				
IWAI (150)	2018	Hepatocellular	miR-96	CASP9	Oncogene	SNU387, SNU449, and HLF cell lines
GUO (152)	2018	Glioblastoma	miR-96	PDCD4	Oncogene	U-87MG, U-251MG, and A172 cell lines
LI (157)	2020	AML	UCA1	miR-96	Tumor suppressor	U937 and HL60 cell lines
WU (159)	2015	Bladder	miR-96	CDKN1A	Oncogene	60 patients T24 and EJ cell lines

Structural proteins

Structural proteins are involved in cell migration, adhesion, and ion homeostasis that can be regulated by miR-96 in tumor cells (Figure 2). EZRIN is a linker protein between the actin cytoskeleton and plasma membrane proteins participating in cell adhesion and migration. Its deregulation has been reported in metastatic tumors (12). It has been reported that miR-96 suppressed osteosarcoma (OS) cell invasion and proliferation while increasing apoptosis through EZRIN targeting. There was also miR-96 down-regulation in OS tissues in comparison with normal tissues (13). There was an inverse correlation between the
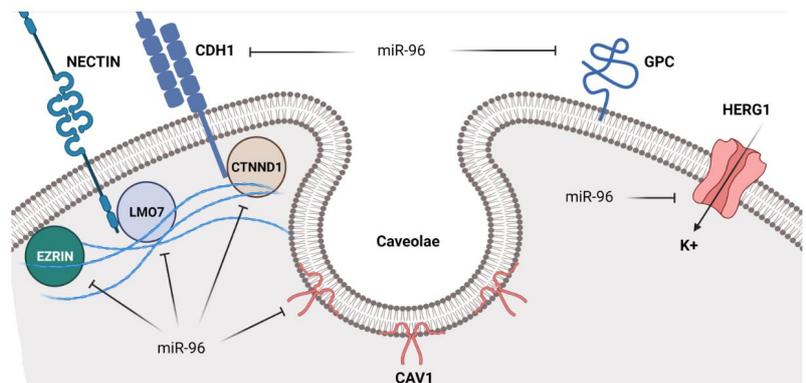

Figure 2. Role of miR-96 in regulation of structural proteins during tumor progressions

levels of miR-96 expressions and the invasive capability of renal cell carcinoma (RCC) cells. MiR-96 reduced the RCC cells invasion through EZRIN targeting (14). LMO7 belongs to the PDZ/LIM domain-containing family of proteins participating in protein-protein interactions by actin binding (15). It maintains the epithelial architecture by regulation of actin cytoskeleton in normal cells. However, various studies have reported LMO7 up-regulation during carcinogenesis in different tumor types (16). It has been shown that there was miR-96 up-regulation in lung cancer patients that was directly correlated with high-grade and metastatic lymph node tumors. Silencing of miR-96 reduced lung tumor cell migration and cisplatin resistance by LMO7 up-regulation (17). CTNND1 is a critical cell adhesion regulator through binding with the juxta-membrane region of the cadherin cytoplasmic tails. There was miR-96-5p down-regulation in BC tissues and cell lines. MiR-96-5p down-regulation was also associated with lower overall survival, TNM stage, and distant metastasis. MiRNA-95-5p reduced BC cell proliferation and metastasis via suppression of CTNND1 mediated WNT signaling (18).

MTSS1 regulates cytoskeletal dynamics and promotes membrane ruffle formation by interaction with actin and Rac proteins (19). It is an inhibitor of tumor metastasis by interaction with actin cytoskeleton associated with tumor progression in different organs (20, 21). There was significant miR-96 up-regulation in cholangiocarcinoma (CCA) tissues in comparison with normal margins that was significantly correlated with advanced TNM stage, shorter overall survival, lymph node metastasis, and poor differentiation. MiR-96 induced CCA cell proliferation and motility through MTSS1 targeting (22). There was a significantly higher serum miR96 level in breast cancer (BC) cells in comparison with controls that were reduced in patients with chemotherapy. MiR-96 down-regulated and up-regulated CDH1 and CDH2 in BC cells, respectively. It also inhibited BC cell migration through MTSS1 targeting (23). There was also a negative association between the serum levels of miR-96 and MTSS1 that was associated with survival in prostate cancer (PCa) patients (24). Long non-coding RNAs (lncRNAs) are involved in tumor progression and metastasis (25). Maternally expressed 3(MEG3) is a tumor suppressor lncRNA that is down-regulated in many cancers (26, 27). LncRNA MEG3 suppressed glioma cell proliferation and migration via miR96-5p sponging that resulted in MTSS1 up-regulation. Moreover, there was significant MEG3 down-regulation in glioma cells and tissues (28). MEG3 suppresses cell proliferation via p53 induction and TGF-β targeting (29, 30). Tropomyosin (TPM) is an actin-binding protein involved in inhibition of cellular transformation (31). TPM1 regulates the actin-myosin interaction (32). It has been reported that MEG3 inhibited bladder cancer (BCa) progression by regulating the miR-96/TPM1 axis. There was MEG3 down-regulation in BCa tissues that was correlated with high-grade and muscular invasion. MEG3 inhibited BCa proliferation while inducing apoptosis by miR-96 sponging and subsequent TPM1 up-regulation (33). There was miR-96 up-regulation in colorectal cancer (CRC) tissues. Suppression of miR96 increased oxaliplatin sensitivity in CRC cells by Bcl-2 down-regulation and the TPM1 and BAX up regulations (34).

Glypican-1 (GPC1) is a membrane-bound proteoglycan that is involved in organ development via regulation of extracellular growth factors, tumorigenesis, and angiogenesis (35). There was a significant miR-96-5p down-regulation in pancreatic cancer (PC) tissues that was associated with larger tumor sizes, poorer differentiation, and reduced survival. MiR-96 inhibited PC cell proliferation via GPC1 targeting (36). GPC3 belongs to the integral membrane proteoglycan family that is anchored with the membrane by a glycosylphosphatidylinositol. It is an important member of the extracellular matrix (ECM) involved in regulation of heparin-binding growth factor (37, 38). There was miR-96 up-regulation in non-small cell lung carcinoma (NSCLC) tissues in comparison with normal margins. MiR-96 increased NSCLC cell migration through GPC3 suppression (39). Caveolae1 (CAV1) is a structural component of caveolae involved in signal transduction and vesicular trafficking (40, 41). It has anti-tumor or oncogenic functions in different cancers (42, 43). There were significant miR-96-5p up-regulations in ovarian cancer (OC) tissues and cell lines in comparison with normal margins and cell lines. MiR-96-5p induced OC cell proliferation and migration via CAV1 targeting. It also up-regulated CCND1 by phosphorylation of AKT (44).

Ion channels are important structural proteins during tumor progression (45, 46). HERG1 belongs to the family of voltage-gated potassium channels that are up-regulated in various cancers (47, 48). It regulates cell proliferation and apoptosis (49, 50). HERG1 up-regulations in pancreatic tumor tissues were significantly associated with lymph node metastasis, TNM stage, and grade of differentiation. MiR-96 significantly inhibited pancreatic tumor cell proliferation and migration via HERG1 targeting (51).

SYVN1 is an E3 ubiquitin-protein ligase involved in elimination of unfolded proteins that are accumulated during ER stress. It promotes unfolded protein transportation to the cytoplasm where it uses the ubiquitin-proteasome process for protein degradation (52). There was significant CASC2 up-regulation in BC tissues compared with normal margins. CASC2 promoted apoptosis while inhibiting BC cell migration by miR-96-5p sponging that resulted in SYVN1 up-regulation (53).

As a deubiquitinase, CYLD has pivotal roles in microtubule stabilization by alpha-tubulin acetylation that is associated with cell proliferation, cytokinesis, migration, and angiogenesis (54-56). It has been reported that GMDS-AS1 inhibited lung tumor cell proliferation while inducing apoptosis. GMDS-AS1 up-regulated CYLD through miR-96-5p sponging (57).

CircPTPRA is a circular RNA transcribed from PTPRA. RASSF8 is a tumor suppressor with pivotal roles in maintenance of adherence junctions in epithelial cells and migration. There was circPTPRA down-regulation in NSCLC tumors compared with controls that was associated with invasive tumors and shorter survival rates. It sponged miR-96-5p to inhibit EMT in NSCLC cells through RASSF8 up-regulation (58). CCDC67 is a component of the deuterostome complex that is involved in centriole amplification in multi-ciliated cells. There were significant miR-96-5p up-regulations in papillary thyroid cancer (PTC) tissues and cell lines compared with normal margins and cells. MiR-96-5p induced PTC cell proliferation and migration by CCDC67 targeting (59).

Transcription factors

Forkhead transcription factors are critical factors during

embryogenesis (60). They are mainly considered tumor suppressors during tumor progressions (61). The Forkhead box O (FOXO) protein family has a critical role in regulation of PI3K/AKT signaling that mediates cell proliferation, differentiation, and tumorigenesis (62). FOXO proteins are involved in cell cycle regulation by up-regulations of p21Cip1 and p27Kip1 cell-cycle inhibitors, while CCND1 down-regulation results in G1/S arrest (63, 64). They also promote apoptosis by regulation of proapoptotic factors such as Bim and Fas ligand (65, 66). FOXO phosphorylation can result in DNA release and nucleus to cytoplasm translocation via 14-3-3 chaperones (67). FOXO1 is known as a tumor suppressor in the majority of cancers (68, 69), while it has an oncogenic function in female genital tract tumors (70). FOXO1 is mainly regulated by the PI3K/AKT pathway during thyroid tumorigenesis (71). There was miR-96 up-regulation in PTC tissues in comparison with normal samples. MiR-96 significantly induced PTC cell proliferation, while reducing 5FU mediated apoptosis by FOXO1 targeting that resulted in suppression of AKT/FOXO1/ Bim axis (72). There was miR-96 up-regulation in hepatocellular carcinoma (HCC) tissues and cell lines. MiR-96 induced AKT/GSK-3β/β-catenin axis via FOXO1 suppression in HCC cells. FOXO1 decreased tumor cell proliferation, invasion, and *in vivo* growth promoted by miR-96 in HCC. MiR-96 increased HCC progression via FOXO1 targeting. It also promoted β-catenin nuclear translocation (73). MiR-96 induced apoptosis via FOXO1 targeting in BCa cells (7), while suppressing camptothecin-induced apoptosis by FOXO1 targeting in PCa cells (74). p21 and p27 are critical factors involved in cell cycle regulation. MiR-96 was shown to induce cervical cancer (CC) cell proliferation through FOXO1 targeting. There were significant miR-96 up-regulations in CC tissues and cell lines that were associated with stage, grade, and lymph node invasion. MiR96 induced G1/S transition and cell proliferation in CC cells. Suppression of miR-96 increased apoptosis via p21 and p27 up-regulations (75). Another study showed that miR-96 was up-regulated by chemotherapeutic treatment that reduced the levels of FOXO1 expression and subsequent p21 down-regulation in GC cells (76). MiR-96 up-regulation was observed in PCa tissues in comparison with normal samples. MiR-96 induced PCa cell proliferation and clonogenicity through FOXO1 targeting (77).

FOXO3 belongs to the forkhead transcription factors involved in apoptosis, metabolism, and DNA repair (78). MiR-96 induced BC cell proliferation through FOXO3a targeting that reduced the levels of p27Kip1 and p21Cip1 expressions, while up-regulating CCND1. A significant miR-96 up-regulation was also reported in BC tissues compared with normal specimens (79). There was Urothelial Cancer Associated 1 (UCA1) up-regulation in PC tissues that was inversely associated with miR-96 expression. Silencing of UCA1 or FOXO3 inhibited pancreatic tumor cell invasion while promoting apoptosis. UCA1 increased pancreatic tumor cell invasion by miR-96 sponging that resulted in FOXO3 up-regulation (80). There was a significant miR-96 up-regulation in NSCLC tissues in comparison with normal specimens that was associated with TNM stage, grade of differentiation, and lymph node involvement. MiR-96 also induced NSCLC growth by FOXO3 targeting (81). The miRNA-96-5p up-regulation was also shown in BC tissues that induced cell proliferation through FOXO3 inhibition

(82). There was miR-96 up-regulation in CRC tissues compared with corresponding normal samples. MiR-96 also induced CRC cell proliferation through FOXO1 and FOXO3a targeting (83). FOXF2 has tumor suppressor function in a variety of tumors such as breast, gastric, and colorectal cancers (84-86).

Proteolytic enzymes with ECM degradation ability are also essential factors during tumor cell invasion (87). Matrix metalloproteinases (MMPs) are a family of proteases that degrade ECM during angiogenesis and invasion (88). There was miR-96-5p up-regulation in oral squamous cell carcinoma (OSCC) tissues in comparison with normal margins. MiR-96-5p significantly induced OSCC cell proliferation via CDK4 and CCND1 up-regulations while p27 down-regulation. MiR-96-5p also reduced the levels of MMP-2 and MMP-9 expressions, while up-regulating TIMP-1 in OSCC cells. It induced the EMT of OSCC cells through CDH1 down-regulation and CDH2 up-regulation. MiR-96-5p exerted all of the oncogenic functions in OSCC cells by FOXF2 targeting (89). There was also miR-96 up-regulation in PCa tissues in comparison with normal specimens which was associated with lymph node involvement, distant metastasis, and high PSA levels. MiR-96 increased PCa cell proliferation via FOXF2 targeting. Moreover, miR-96 down-regulation reduced PCa cell proliferation by suppressing the expressions of CCNA1, CDK2, and CDK4 (90).

Signaling pathways

WNT is a developmental signaling pathway that has critical roles in normal tissue homeostasis and neoplastic transformation (91, 92). MiR-96 is involved in regulation of the WNT signaling pathway during tumor initiation and metastasis (Figure 3). It has been shown that miR-96 induced the WNT pathway and glioma cell growth by suppression of HBP1 as an inhibitor of the WNT pathway (93). SFRP4 belongs to the secreted frizzled related proteins (SFRPs) associated with regulation of cell proliferation and motility through the WNT signaling pathway (94). There was miR-96-5p up-regulation in CC tissues that was significantly associated with clinical stages and lymph node invasion. MiR-96-5p induced cell migration, while inhibiting apoptosis in CC cells via SFRP4 targeting (95). CTNNB1 is the main downstream effector of the
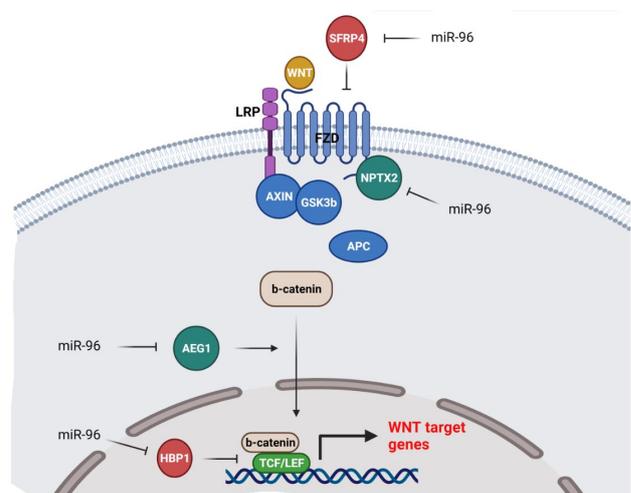

Figure 3. MiR-96 is involved in tumor progression and invasion through regulation of the WNT signaling pathway

canonical WNT pathway. It forms a complex with AXIN/APC/GSK3B in the absence of WNT ligands that results in CTNNB1 phosphorylation and subsequent degradation by the ubiquitin-proteasome system. However, WNT-FZD interaction prevents CTNNB1 degradation, and its nuclear accumulation causes the activation of TCF/LEF transcription factors and regulation of WNT target genes (96, 97).

Hypoxia is a pivotal feature of the tumor microenvironment during tumor progression (98). It promotes adaptive mechanisms by activation of hypoxia-inducible transcription factors that up-regulate various genes associated with angiogenesis, metastasis, and immune evasion (99, 100). AMOTL2 is an anti-angiogenic factor that regulates the actin cytoskeleton. It also suppresses the WNT signaling pathway via prevention of CTNNB1 nuclear translocation. It has been reported that there were higher levels of serum exosomal UCA1 in PC patients compared with healthy controls that were significantly associated with poor prognosis. Hypoxic pancreatic tumor cell-derived exosomal UCA1 promoted angiogenesis through regulation of miR-96-5p/AMOTL2/ERK1/2 axis (101).

During the EMT process, epithelial cells lose their polarity to convert to invasive mesenchymal cells (102). Astrocyte elevated gene-1 (AEG-1) is the activator of EMT-related signaling pathways such as SHH, TGF-b, and WNT (103-105). PI3K/AKT induction by AEG-1 can result in chemoresistance by MDR1 subsequent up-regulation (106). AEG-1 also increases the levels of LEF-1 expression as one of the critical WNT transcription factors. Moreover, AEG-1 promotes CTNNB1 nuclear translocation. Since, CTNNB1 phosphorylation by GSK3β results in its proteasomal degradation, AEG-1 can also induce CTNNB1 nuclear accumulation via GSK3β phosphorylation and inactivation (107). MiR-96 inhibited EMT and cell proliferation while inducing apoptosis via AEG-1 targeting in GBM cells (108). Neuronal pentraxin 2 (NPTX2) has a pivotal role in synapse formation and immune response (109). It also induces CRC cell proliferation and metastasis by a direct interaction with FZD6 that results in activation of the WNT signaling pathway (110). There was miR 96 down-regulation in RCC samples. MiR 96 reduced RCC cell invasion and proliferation via NPTX2 down-regulation (111).

PTEN is a protein phosphatase that suppresses the PI3K/AKT pathway by dephosphorylation of phosphoinositides. A significant STXBP5-AS1 up-regulation was observed in CC patients that was associated with poor prognosis. STXBP5-AS1 also inhibited CC cell proliferation by miR-96-5p sponging that resulted in PTEN up-regulation. Therefore, STXBP5-AS1 suppressed PI3K/AKT pathway via PTEN up-regulation in CC cells (112). There was miR-96-5p up-regulation in head and neck squamous cell carcinoma (HNSCC) specimens in comparison with normal margins. There were significantly higher levels of miR-96-5p expressions in HNSCC patients with TP53 mutations compared with wild types. MiR-96-5p up-regulation in mutant p53 carriers induced cell migration and chemo-resistance via PTEN targeting and activation of PI3K-AKT pathway in HNSCC (113). Succinate dehydrogenase (SDH) is a complex comprising SDHA-D components that is involved in the citric acid cycle of mitochondria by oxidation of succinate to fumarate (114, 115). It has also an important

role in electron transport (116). Deregulation of SDHB has been associated with reduced oxidative phosphorylation and tumor progression in which SDHB deficiency results in increased cell migration and invasion (117). SDHB is also involved in regulation of the AKT/mTOR signaling pathway by inhibition of AKT. There was miR-96-3p up-regulation in PTC tissues in comparison with benign tissues. The advanced stage PTC patients had higher levels of miR-96-3p in tumor tissues compared with normal margins. MiR96-3p induced PTC cell invasion and migration through regulation of the SDHB/AKT/mTOR axis (118).

Tumor necrosis factor-alpha (TNF-α) is a cytokine involved in apoptosis and inflammation. TNF-α activates the TNF receptors which subsequently triggers various signal transduction pathways including MAPK, ERK, and JNK (119). SAMD9 as an effector of TNF-α signaling is involved in inflammatory responses. It has been shown that miR-96 suppressed cisplatin-induced apoptosis through SAMD9 in NSCLC cells (120). KRAS is a GTPase belonging to the RAS oncogene family that is involved in cell growth and differentiation (121). It induces pancreatic tumorigenesis by activation of PI3K/AKT, ERK, and NF-κB signaling pathways (122-125). It has been reported that miR-96 targeted KRAS oncogene in pancreatic tumor cells. MiR-96 inhibited AKT signaling and cell migration while inducing apoptosis via KRAS down-regulation (126).

Phosphatases, kinases, and matrix metalloproteinases

Protein tyrosine phosphatases (PTPs) are considered pivotal regulators of different cellular processes and signal transductions. PTPN9 belongs to the classic PTPs involved in different cellular processes (127). It is involved in dephosphorylation and suppression of EGFR, ErbB2, and STAT3 (128, 129). Since, these factors have important functions during BC progression (130, 131); PTPN9 can be suggested as a tumor suppressor via inhibition of ErbB and STAT3 signaling pathways. A significant miR-96 up-regulation was shown in BC tissues. MiR-96 promoted breast tumor *in vivo* growth, proliferation, and motility by PTPN9 sponging (132). There was miR-96 up-regulation in CC tissues that was inversely related to the levels of PTPN9 expression. MiR-96 increased CC cellular proliferation via PTNP9 targeting (133).

NUAK1 belongs to the AMPK-related kinase (ARK) family that is activated by AKT. It regulates cell survival during glucose deprivation and also inhibits apoptosis mediated by nutrient starvation (134). It regulates death receptors via CASP8 and pro-CASP6 suppressions (135, 136). MiR-96 repressed PC cell proliferation and migration through NUAK1 targeting (137). Chronic myeloid leukemia (CML) is a neoplastic transformation associated with (9; 22) translocation that generates a constitutively active BCR-ABL1 tyrosine kinase (138). ABL1 is a proto-oncogene involved in cell proliferation, migration, and stress response. MiR-96 suppressed the BCR-ABL1 oncogene in CML (139). EGFR is another tyrosine kinase receptor that is activated by EGF that triggers several signal transduction pathways such as MAPK and AKT. Therefore, EGFR is a pivotal regulator of cell growth and migration. Aberrant EGFR signaling is commonly observed during tumor progression (140). ETV6 has a tumor suppressor function by TWIST1 down-regulation in metastatic PCa (141). It has been reported that there was a converse association between

the levels of ETV6 and miR-96 expressions in PCa tissues in which nuclear EGFR was correlated with ETV6 down-regulation. Nuclear EGFR increased the levels of miR-96 expressions that resulted in ETV6 targeting in PCa cells (11). EphrinA5 is a ligand for Eph receptors of tyrosine kinases that have important roles in regulation of angiogenesis and cell motility. It is an inhibitor of EGFR via inducing c-CBL binding and ubiquitylation (142). A significant miR-96 up-regulation was shown in HCC tissues in comparison with normal controls. MiR-96 increased HCC cell invasion and proliferation through ephrinA5 targeting (143).

MMPs are calcium-dependent proteases that have important roles in tissue remodeling, angiogenesis, and tumor cell metastasis. RECK is an MMP inhibitor involved in tumor metastasis by regulation of MMP-2 and MMP-9 (144, 145). Reduced levels of RECK expression are related to poor survival in different tumors (146). MiR-96 up-regulations were reported in esophageal cancer (EC) tissues and cell lines which was associated with clinical tumor stage and depth of invasion. MiR-96 reduced drug or irradiation responses in EC cells by RECK targeting (147). Significant miR-96 up-regulation was also shown in BC tissues in comparison with corresponding normal tissues. It also increased BC cell motility and proliferation by RECK inhibition (148). MiR-96 induced NSCLC cell growth and migration via RECK targeting. There was also miR-96 up-regulation in NSCLC tissues in comparison with normal tissues. Moreover, suppression of miR-96 reduced NSCLC cell proliferation (149).

Apoptosis and cell cycle regulation
Apoptosis and autophagy are the main cellular processes involved in regulation of the cell fate by protein and organelle turnovers. CASP9 is an initiator caspase required for the intrinsic apoptosis pathway. Due to the intracellular apoptotic stimuli, cytochrome c is released by mitochondria that form apoptosome by binding with Apaf-1. Then CASP9 will be activated by apoptosome that can subsequently activate executioner caspases to trigger apoptosis. A significant miR-96-5p up-regulation was observed in HCC tissues in comparison with normal samples. MiR-96-5p reduced the levels of FOXO1 expression in HCC cells. It increased doxorubicin resistance while inhibiting apoptosis through CASP9 targeting in HCC tumor cells (150). Programmed Cell Death 4 (PDCD4) is a tumor suppressor that activates BAX pro-apoptotic factors followed by cytochrome C mediated apoptosis (151). There was a significant miR-96 up-regulation in GBM cells. MiR-96 reduced GBM radiosensitivity through PDCD4 targeting (152). Autophagy is responsible for the maintenance of cellular homeostasis by elimination of dysfunctional cellular components using a lysosomal-mediated pathway. Therefore, this process can be associated with various biological processes and diseases (153, 154). Normally autophagy is an anti-apoptotic mechanism that maintains cell survival. Therefore, pro-apoptotic proteins should be inhibitors of autophagy. ATG7 belongs to the E1-like enzymes that suppress CASP9 translocation to the apoptosome resulting in apoptosis blocking (155, 156). It has been observed that UCA1 increased AML cell proliferation and autophagy by miR-96-5p sponging that resulted in ATG7 up-regulation (157). CDKN1A is a pivotal regulator of cell cycle progression that suppresses Cyclin/CDK2

complexes to mediate growth arrest due to DNA damages (158). There was miR-96 up-regulation in BCa tissues. MiR-96 reduced BCa cell proliferation while inducing apoptosis through CDKN1A down-regulation (159).

Conclusion
In the present review, we discussed all of the reports that have assessed the role of miR-96 in tumor initiation, progression, and invasion. This review clarifies the molecular mechanisms that are recruited by miR-96 to regulate tumor progression and metastasis. MiR-96 mainly exerts its role during tumorigenesis through targeting the structural proteins and FOXO transcription factors. Indeed, this review suggests miR-96 as an efficient diagnostic tumor marker in different cancers. Based on miR-96 as an oncogene or tumor suppressor, miR-96 or its inhibitors can be also suggested as novel therapeutic agents in cancer therapy. However, it is still required to perform animal studies in future research to find the efficiency of miR-96 targeted tumor therapy. More studies are also required to assess the miR-96 delivery method and long-term safety prior to medical practice as a novel therapeutic modality in cancer patients.

Authors' Contributions
MMoghbeli study conception and design; HRR and MMojarrad draft manuscript preparation; MMoghbeli critical revision of the paper; MMoghbeli supervision of the research; HRR, MMojarrad, and MMoghbeli Final approval of the version to be published.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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