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REVIEW

MiR-326: Promising Biomarker for Cancer

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Abstract: MicroRNAs (miRNAs) are small non-coding and highly conserved RNAs that act in biological processes including cell proliferation, invasion, apoptosis, metabolism, signal transduction, and tumorigenesis. The previously identified miRNA-326 (miR-326) has been reported to participate in cellular apoptosis, tumor growth, cell invasion, embryonic development, immunomodulation, chemotherapy resistance, and oncogenesis. This review presents a detailed overview of what is known about the effects of miR-326 on cell invasion, metastasis, drug resistance, proliferation, apoptosis, and its involvement in signaling pathways.

Keywords: MicroRNA, miR-326, tumor suppressor, cancer, metastasis, oncogene

Introduction

With short sequence length, non-coding and endogenous microRNAs (miRNAs) can regulate gene expression by binding to the 3′ untranslated region of target messenger RNAs (mRNAs), thus suppressing translation or degradation of the mRNA. MiRNAs participate in several significant biological processes, including cell differentiation, proliferation, apoptosis, and host response against viral infections.[1–3] All known or assumed protein-coding genes are reportedly expressed only in a small proportion of the entire genome, and miRNAs evidently constitute approximately 1–2% of all genes in worms, flies, and mammals.[2] A single miRNA can regulate expression of many genes.[4] Overall, gene expression that is regulated by miRNA may significantly contribute to overall regulation.

The goal of this review was to describe miRNA-326 (miR-326) activity in tumors, as miR-326 is downregulated in most tumors. Low expression of miR-326 is dramatically related with unfavorable prognosis, tumor development, metastasis, and progression. For example, downregulated miR-326 is positively correlated with the risk of metastasis in patients with gastric cancer, prostatic carcinoma, esophageal squamous cell carcinoma, and non-small lung cancer (NSCLC).[5–8] In this review, we summarize the promising effects of miR-326 on cell invasion, metastasis, drug resistance, proliferation, apoptosis, and its involvement in signaling pathways.

miR-326 in Invasion and Metastasis

Tumor invasion and metastasis processes are influenced by multiple factors. The progress of early tumor development into metastasis includes invading from the extracellular matrix to the stromal layers and then transferring to distant organs via infiltration from blood vessels to remote parenchymal tissues.[9,10] Studies have shown that miR-326 is involved in tumor cell invasion and metastasis in NSCLC, gastric cancer, breast cancer (BC), cervical cancer, osteosarcoma, glioma, colorectal cancer,

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endometrial cancer, prostatic carcinoma, and esophageal squamous cell carcinoma.[5,6,11–18]

In NSCLC, upregulated expression of miR-326 limits tumor metastasis by targeting a disintegrin and metalloprotease 17 (Adam17), nucleosome-binding protein 1 (NSBP1), and paired-like homeobox 2a (Phox2a).[11,19,20] As a member of the tumor necrosis factor converting enzyme family, Adam17 is an indispensable regulator of tumor metastasis.[21,22] Adam17 can adjust the expression of epidermal growth factor receptor by activating Notch1.[23] Studies found that miR-326 can inhibit NSCLC cell invasion, possibly by downregulating Adam17 expression level.[19,23] NSBP1 can regulate gene transcription by binding chromatin. It is distinctly expressed in various tissues and is a potential oncogene in diverse tumors.[24,25] Li et al reported that miR-326 hampered NSCLC cell invasion via inhibiting the protein level of NSBP1.[20] Phox2a essentiality was demonstrated in neurogenesis and in recent years its role in cancer has been described. Wang et al found that miR-326 slowed NSCLC metastasis by targeting Phox2a.[11] In gastric cancer, low expression of miR-326 was associated with clinical stage, tumor depth, lymph nodes, and distant metastasis. Survival analysis indicated that low expression of miR-326 was a prognostic factor for poor outcome for gastric cancer patients. Downregulation of miR-326 promoted metastasis by targeting Fascin 1 (FSCN1).[6] As an actin-binding protein, FSCN1 is often upregulated in different cancers, and overexpression of FSCN1 promotes tumor invasion and metastasis.[26–28] In BC, miR-326 was reported to repress cell metastasis by targeting B7-H3, an immunomodulin belonging to the B7 family. B7-H3 is highly expressed in various tumors and is correlated with adverse prognosis.[29,30] In cervical cancer, restoration of ETS domain-containing protein (ELK1) was reported to eliminate cell invasion due to miR-326 mimics, indicating that miR-326 suppressed cell invasion and metastasis by targeting ELK1.[31] As a member of the ETS oncogene family, ELK1 affects cytoskeleton transformation and tumor invasion.[32,33] As a conserved protein, nin one binding 1 (NOB1) affects RNA metabolism and protease function.[34,35] In osteosarcoma, glioma and colorectal cancer, miR-326 can target NOB1 to inhibit metastasis.[16,36,37] TWIST1, a transcription factor, affects tumorigenesis and tumor progression, especially in metastasis.[38] Analysis from data in *TargetScan* and *MicroRNA.org* identified TWIST1 as a potential target of miR-326, and luciferase reporter assays were consistent with the targeting of TWIST1 by miR-326. Forced expression of miR-326 significantly decreased the levels of TWIST1.[39,40] In endometrial cancer, in vitro assays revealed that knockdown of TWIST1 inhibited tumor cell invasion.[17] In prostatic carcinoma, miR-326 functioned as a tumor suppressor by negatively regulating Mucin1 (MUC1). Previous studies indicated that MUC1 is involved in the regulation processes of several specific miRNAs on tumor cell proliferation and invasion.[41] Furthermore, the miR-326/MUC1 axis inhibits prostatic carcinoma cell invasion partly via suppressing c-Jun amino terminal kinase (JNK) signaling activation.[5] In esophageal squamous cell carcinoma, Su et al reported that vascular endothelial growth factor-C reduced miR-326 expression and increased Src substrate cortactin (CTTN) protein level and invasive abilities, suggesting vascular endothelial growth factor-C upregulated CTTN expression through Src-mediated downregulation of miR-326.[18] Additionally, the expression of miR-326 was correlated with poor prognosis in esophageal squamous cell carcinoma patients.[8]

Thus, several studies have shown that miR-326 plays a role in inhibiting invasion and metastasis in a variety of tumor cells, but the specific mechanism has not been elucidated. More in-depth experiments should be performed to identify appropriate targets to tackle metastatic issue.

miR-326 in Cell Proliferation and Apoptosis

Tumor cells can exert complex effects on apoptosis and proliferation of malignant tumor cells.[42] Numerous genes may be involved in the regulation by miR-326 on cell apoptosis and proliferation, such as CyclinD1 (CCND1), fibroblast growth factor 1 (FGF1), son of sevenless homolog 1 (SOS1), and neuroblastoma RAS viral oncogene homolog (NRAS).[7,43,44]

As a pivotal cell cycle regulatory protein, CCND1 expression and cellular localization varies in human tumor cells. It is a gate-keeping protein that regulates conversion through the cell cycle restriction point between the G1 phase and the S phase. Consequently, changes in CCND1 gene amplification, posttranscriptional or posttranslational modifications, rearrangements, and variant polymorphisms can give rise to abnormal protein expression and increase risk of tumorigenesis.[45,46] In NSCLC, miR-326 may inhibit tumor proliferation by targeting CCND1. Sun et al found that miR-326 could suppress cyclin D1, thus facilitating the expression level of p57 and p21, which might explain the proliferation-inhibition property of miR-326.[7] Kaplan–Meier survival

analysis revealed that patients with low expression levels of miR-326 had shorter overall survival compared to patients with high expression levels of miR-326. These results demonstrated that down-regulation of miR-326 was associated with poor prognosis in NSCLC. As a member of the fibroblast family, FGF1 can facilitate the repair of damaged blood vessels and FGF family members can promote cancer cell growth and intensify chemotherapy resistance. FGF1 is overexpressed in a variety of tumors including NSCLC, ovarian, prostate, and breast cancers.[47,48] Studies have shown that miR-326 restrained FGF1 expression to modulate cell proliferation and apoptosis.[43] FGF family members target the fibroblast growth factor receptor (FGFR) to enhance tumor cell proliferation and invasion by controlling endothelial cells and pericytes in tumors.[49,50] The signaling pathways downstream of FGFR, i.e. mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), Ras, and JNK pathways, can promote cancer cell proliferation and distant dissemination, with participation in every step from tumorigenesis to oncogenesis.[51,52] In NSCLC, FGF1 increases the phosphorylation level of FGFR to activate PI3K and JNK, so decreased FGF1 regulated by miR-326 upregulation may inhibit the malignant behaviors of glioma cells by weakening the activities of PI3K/Protein kinase B (PI3K/AKT) and MAPK kinase (MEK) 1/2 pathways.[53,54] As a dual guanine nucleotide exchange factor for Ras and Rac1, SOS1 can convert inactive Ras-guanosine diphosphate to active status.[55] EGFR can regulate Ras to affect tumor cell apoptosis, metastasis, and tumorigenesis.[56,57] Additionally, nerve growth factor (NGF) can regulate cell proliferation through SOS1 stimulation and Ras signaling. NGF may promote endothelial cell migration and growth and influence cancerous angiogenesis.[58] NRAS, which belongs to the Ras family, is widely expressed in diverse cells, and NRAS activation may participate in SOS1 stimulation and be related to NGF and EGFR signaling. According to a previous study, SOS1 and NRAS are abundant in the NGF and EGFR signaling pathways.[59] Therefore, miR-326 might regulate tumor cell proliferation and angiogenesis through the NGF and EGFR signaling pathways. However, the detailed mechanisms underlying the regulation of these targeted genes by miR-326 have not been elaborated.

Involvement of miR-326 in Signaling Pathways

Hedgehog/Gli (Hh/Gli) Signaling Pathway

Changes in the Hh/Gli pathway can occur through both non-canonical and canonical mechanisms.[60] The activation of the canonical Hh/Gli pathway is controlled by the receptor Patched that inhibits the movement of Smoothened.[61] Sonic hedgehog (SHH) protein ligand can bind to Patched, thus moderating Smoothened inhibition, allowing activation of Hh/Gli that leads to Gli2 transcription factor activation and movement to the nucleus.[62] Gli2 can promote the transcription activity of Hh/Gli target genes, including Patched 1 and Gli1. Additionally, focal deletions, mutations, or gene amplifications that encode pathway components like Gli2, Smoothened, and Patched 1 have been shown to be involved in crucial oncogenic events in SHH-driven medulloblastoma (SHH-MB).[63–66] Other mechanisms of activation of non-canonical Hh/Gli pathways include p53/17p gene deletion, aberrant PI3K/Akt/S6 activation, histone methylation, and post-transcriptional modification of Gli1.[60,67–69] Additionally, miRNAs are crucial regulators of Hh/Gli signaling.[70] Suppressed expression of miR-326 and its host gene Arrestin B1 are characteristics of cancer-stem cells derived from SHH-MB. Overexpression of miR-326 and Arrestin B1 can inhibit the Hh/Gli pathway by targeting numerous activator components of this signaling pathway that Gli1, Gli2, and Smoothened require for cancer-stem cells activity.[71]

PI3K/Akt Signaling Pathway

As part of the Receptor Tyrosine Kinases signaling pathway, the PI3 kinase pathway has a significant function in stimulating tumor cell growth and proliferation. The members of this pathway alter the progress of cell malignant transformation through active participation in cellular biological activities such as cell differentiation, proliferation, cell migration, invasion, trafficking, and glucose homeostasis.[72,73] Preternatural signaling via the PI3 kinase pathway leads to a change in the expression level of transcription factors. Activation of the PI3 kinase pathway is characteristic of malignancies.[74] Dimerization and autophosphorylation occur due to complexing of growth factor ligands with membrane receptor tyrosine kinases, and the PI3 kinase is activated by catalytic conversion of phosphatidylinositol (3,4)-bisphosphate lipids to phosphatidylinositol (3,4,5)-trisphosphate.[75] PKB/Akt exhibits high affinity to phosphatidylinositol (3,4,5)-trisphosphate, allowing its infiltration of the plasma membrane. Phosphorylation of T308 by phosphoinositide-dependent kinase 1 initiates excitation, which is then completed by phosphorylation on the S473 residue, possibly due to the action of diverse proteins, such as mTOR.[76] Phosphorylated Akt then can induce downstream pathways that control cell proliferation and survival, including the phosphorylation and activation of MDM2 E3 ubiquitin

ligase transcription factor, NF-kB, and mTOR kinase, and the inactivation of pro-apoptotic protein BAD and FOXO1 transcription factor to promote tumorigenesis.[77,78] In addition to the protein coding genes, other mechanisms like aberrant activity of the PI3K pathway may affect miRNAs whose expression levels are influenced by the PI3 kinase signaling.[79] For example, miR-326 expression level is restrained by abnormal PI3 kinase signaling, resulting in downregulation in glioblastoma.[15,80]

RAS/ERK Signaling Pathway

In the RAS/extracellular signal-regulated kinase (RAS/ERK) pathway, Ras-family guanosine triphosphatases isoforms bind to RAF-family serine/threonine kinases, RAFs then interact with the dual-specificity MEK1 and MEK2, and then the MEKs together with MAPKs ERK1 and ERK2 participate in signaling for pivotal biological activities, including cell survival, proliferation, and differentiation leading to the development of carcinogenesis.[56] MEK can phosphorylate ERK1 and ERK2 (ERKs), which promotes the formation of homodimers, which are more stable than the labile heterodimers. The function of the dimerized ERKs has not yet been determined, and may affect diverse cell processes.[81] Dimerization can regulate ERK activity levels depending on the mono- or bi-phosphorylated state of the monomers.[82] Some studies have linked the phosphorylation state with the subcellular distribution of ERK. Dimerization was proposed as essential for ERK's nuclear translocation because mutations that alter dimerization of ERK2 reduced nuclear access.[83,84] However, the activities of ERKs to dimerize and then interact with nuclear pore proteins via the same structural motifs have not been clearly separated. Recent analysis showed that ERK dimers were detected predominantly in the cytoplasm, together with scaffold proteins that serve as ERK-dimerization platforms that allow ERKs to find their cytoplasmic substrates. ERK dimerization is also indispensable for cellular transformation and the transmission of tumorigenic signals by RAS/ERK pathway oncogenes.[85–87] Kang et al showed that miR-326 plays tumor-suppressive roles in melanoma by directly regulating KRAS and indirectly regulating the ERK signaling pathway.[88]

Effects of miR-326 on Drug Resistance

Chemotherapy is widely used to treat cancer, but various processes can prevent the effective killing of cancer cells by anticancer drugs, such as diversification in absorption, anomalous metabolism, and multidrug resistance (MDR). MDR presents a significant problem in chemotherapy, especially for patients who cannot sustain surgical resection or radiation therapy.[89,90] Studies have reported that miR-326 is involved in the MDR mechanism of hepatocellular carcinoma and BC, with two genes reported to be involved, ABCC1 and Bcl-xL.[12,91]

ABCC1 is a key efflux transporter and a member of the ABCC family, and can affect the absorption of drugs by cells. Suppression of ABCC1 expression has been linked to reduced tumor progression and chemotherapy resistance.[92,93] Adriamycin (ADM) is an effective and widely used drug for hepatocellular carcinoma and BC chemotherapy.[94,95] Ma et al found that miR-326 regulates the expression of the ABCC1 gene and ABCC1-mediated ADM-resistance in hepatocellular carcinoma. They transfected miR-326 mimic or negative control into HepG2 cells and then determined the cell survival rate by MTT assay. The result showed that transfection of miR-326 mimic significantly reduced cell viability compared with the negative control and mock transfection, and suggesting the underlying mechanism might be the blocking of MDR-related genes.[91] VP-16, a semi-synthetic derivative of podophyllotoxin, is one of the most effective antineoplastic agents used routinely in first-line combination chemotherapy treatment of small cell lung cancer, testicular cancer, and non-Hodgkin's lymphoma. Liang et al found that miR-326 could attenuate the expression of ABCC1 and sensitize BC cells to ADM and VP-16. They transfected miR-326 mimic into MCF-7/VP-16-resistant (MCF-7/VP) cells and determined the sensitivity of these mimic-transfected cells to VP-16 and ADM. MTT assay was performed with increasing concentrations of VP-16 and ADM with 48h treatment. The IC_{50} of MCF-7/VP cells resistant to VP-16 prior to transfection of miR-326 was 15.3 times higher than that of their parental cells, MCF-7. The IC_{50} of miR-326-transfected MCF-7/VP cells to VP-16 was 7.1 times lower than MCF-7/VP cells transfected with control ADM, and only 2.1 times higher than the MCF-7 parental cells. Transfection of MCF-7/VP with miR-326 also resulted in decreased resistance to ADM. The resistance of MCF-7/VP prior to transfection of miR-326 to ADM was 20 times higher than the MCF-7 parental cells. After MCF-7/VP cells were transfected with miR-326, their IC_{50} to ADM was 10 times lower than that of MCF-7/VP cells transfected with control oligonucleotide and only 1.9 times higher than the MCF-7 parental cells.[12] However, the details of the mechanism have not been determined. Another in vivo study also transfected miR-326 mimic or negative controls into HepG2 cells and similarly found significantly reduced cell viability compared with negative control cells and mock-transfected cells. They

found that miR-326 altered the protein expression of Bcl-xl by luciferase assay, suggesting that miR-326 might sensitize hepatocellular carcinoma cells to 5-Fluorouracil by targeting Bcl-xL, though the detailed mechanism remains unclear.[96] Bcl-xL belongs to the Bcl-2 family, which contains pro-apoptotic and anti-apoptotic (Bcl-2 and Bcl-xL) members.[97,98] An effective and widely used chemotherapeutic agent, 5-Fluorouracil is applied in treatment of colorectal cancer and other tumors including pancreatic cancer, esophageal cancer, gastric cancer, hepatic cancer, and BC. This drug disturbs DNA replication by replacing thymidine with fluorinated nucleotides that are incorporated into DNA, thus causing cell death.[99,100]

Conclusion

We discussed the potential roles of miR-326 in cell proliferation, apoptosis, migration, invasion, metastasis, and signaling pathways in diverse cancers. There is significant evidence that miR-326 can act as a tumor suppressor gene and is associated with tumor prognosis in many cancer types. We may be able to artificially inhibit tumor growth and metastasis using miR-326 mimics or synthetic agents, or to predict the prognosis of tumor patients by detecting the expression level of miR-326. Overall, we need to further explore the mechanisms by which miR-326 affects tumor suppression and study the molecules and pathways that interact with miR-326 to understand the roles of this RNA in cancer and to develop gene therapy strategies for clinical treatment.

Abbreviations

Adam17, A disintegrin and metalloprotease 17; ADM, Adriamycin; AKT, Protein kinase B; BC, Brease Cancer; CCND1, CyclinD1; CTTN, Src substrate cortactin; ELK1, ETS domain-containing protein; ERK, Extracellular signal-regulated kinase; FGF, Fibroblast Growth Factor; FGFR, FGF receptor; FSCN, Fascin 1; Hh/Gli, Hedgehog/Gli; JNK, c-Jun amino terminal kinase; MAPK, Mitogen-activated Protein Kinase; MDR, Multidrug Resistance; MEK, MAPK Kinase; MiRNA, MicroRNA; miR-326, miRNA-326; mRNA, Messenger RNA; MUC1, Mucin1; NGF, Nerve growth factor; NOB1, Nin one binding 1; NRAS, Neuroblastoma RAS Viral Oncogene Homolog; NSBP1, Nucleosome-binding protein 1; NSCLC, Non-small Lung Cancer; Phox2a, Paired-like homeobox 2a; PI3K, Phosphatidylinositol 3-kinase; SHH, Sonic Hedgehog; SHH-MB, SHH-driven Medulloblastoma; SOS1, Son of sevenless homolog 1.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Ambros V. The functions of animal MicroRNAs. *Nature*. 2004;431(7006):350–355. doi:10.1038/nature02871
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281–297. doi:10.1016/S0092-8674(04)00045-5
3. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new Era for the management of cancer and other diseases. *Nat Rev Drug Discovery*. 2017;16:203–222. doi:10.1038/nrd.2016.246
4. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of MiRNA regulation. *Genomics Proteomics Bioinformatics*. 2009;7:147–154. doi:10.1016/S1672-0229(08)60044-3
5. Liang X, Li Z, Men Q, Li Y, Li H, Chong T. MiR-326 functions as a tumor suppressor in human prostatic carcinoma by targeting Mucin1. *Biomed Pharmacother*. 2018;108:574–583. doi:10.1016/j.biopha.2018.09.053
6. Li Y, Gao Y, Xu Y, Ma H, Yang M. Down-regulation of MIR-3206 is associated with poor prognosis and promotes growth and metastasis by targeting FSCN1 in gastric cancer. *Growth Factors*. 2015;33:267–274. doi:10.3109/08977194.2015.1076406
7. Sun C, Huang C, Li S, et al. Hsa-MiR-326 targets CCND1 and inhibits non-small cell lung cancer development. *Oncotarget*. 2016;7(7):8341.
8. Hong CC, Chen PS, Chiou J, et al. MiR326 maturation is crucial for VEGF-C-driven cortactin expression and esophageal cancer progression. *Cancer Res*. 2014;74:6280–6290. doi:10.1158/0008-5472.CAN-14-0524
9. Cao H, Xu E, Liu H, Wan L, Lai M. Epithelial-mesenchymal transition in colorectal cancer metastasis: a system review. *Pathol Res Pract*. 2015;211:557–569. doi:10.1016/j.prp.2015.05.010
10. Zhang H, Jiang L, Sun D, Li J, Tang J. MiR-139-5p: promising biomarker for cancer. *Tumour Biol*. 2015;36(3):1355–1365. doi:10.1007/s13277-015-3199-3
11. Wang R, Chen X, Xu T, et al. MiR-326 regulates cell proliferation and migration in lung cancer by targeting Phox2a and is regulated by HOTAIR. *Am J Cancer Res*. 2016;6(2):173.
12. Liang Z, Wu H, Xia J, et al. Involvement of MiR-326 in chemotherapy resistance of breast cancer through modulating expression of multidrug resistance-associated protein 1. *Biochem Pharmacol*. 2010;79:817–824. doi:10.1016/j.bcp.2009.10.017
13. Cheng Y, Jiang S, Yuan J, Liu J, Simoncini T. Vascular endothelial growth factor C promotes cervical cancer cell invasiveness via regulation of MicroRNA-326/Cortactin expression. *Gynecol Endocrinol*. 2018;34(10):853–858. doi:10.1080/09513590.2018.1458304
14. Cao L, Wang J, Wang PQ. MiR-326 is a diagnostic biomarker and regulates cell survival and apoptosis by targeting Bcl-2 in osteosarcoma. *Biomed Pharmacother*. 2016;84:828–835. doi:10.1016/j.biopha.2016.10.008
15. Nawaz Z, Patil V, Paul Y, et al. PI3 kinase pathway regulated MiRNome in glioblastoma: identification of MiR-326 as a tumour suppressor MiRNA. *Mol Cancer*. 2016;15. doi:10.1186/s12943-016-0557-8

16. Wu L, Hui H, Wang LJ, Wang H, Liu QF, Han SX. MicroRNA-326 functions as a tumor suppressor in colorectal cancer by targeting the nin one binding protein. *Oncol Rep*. 2015;33:2309–2318. doi:10.3892/or.2015.3840
17. Liu W, Zhang B, Xu N, Wang MJ, Liu Q. MiR-326 regulates EMT and metastasis of endometrial cancer through targeting TWIST1. *Eur Rev Med Pharmacol Sci*. 201721(17):3787–3793.
18. Su CM, Su YH, Chiu CF, et al. Vascular endothelial growth factor-C upregulates cortactin and promotes metastasis of esophageal squamous cell carcinoma. *Ann Surg Oncol*. 2014;21:767–775. doi:10.1245/s10434-014-4009-7
19. Cai M, Wang Z, Zhang J, et al. Adam17, a target of Mir-326, promotes emt-induced cells invasion in lung adenocarcinoma. *Cell Physiol Biochem*. 2015;36:1175–1185. doi:10.1159/000430288
20. Li D, Du X, Liu A, Li P. Suppression of nucleosome-binding protein 1 by MiR-326 impedes cell proliferation and invasion in non-small cell lung cancer cells. *Oncol Rep*. 2016;35(2):1117–1124. doi:10.3892/or.2015.4403
21. Zunke F, Rose-John S. The shedding protease ADAM17: physiology and pathophysiology. *Biochim Biophys Acta Mol Cell Res*. 2017;1864:2059–2070. doi:10.1016/j.bbamcr.2017.07.001
22. Moss ML, Minond D. Recent advances in ADAM17 research: a promising target for cancer and inflammation. *Mediators Inflamm*. 2017;2017:1–21. doi:10.1155/2017/9673537
23. Baumgart A, Seidl S, Vlachou P, et al. ADAM17 regulates epidermal growth factor receptor expression through the activation of Notch1 in non-small cell lung cancer. *Cancer Res*. 2010;70:5368–5378. doi:10.1158/0008-5472.CAN-09-3763
24. Postnikov YV, Furusawa T, Haines DC, Factor VM, Bustin M. Loss of the nucleosome-binding protein HMGN1 affects the rate of N-Nitrosodiethylamine-induced hepatocarcinogenesis in mice. *Mol Cancer Res*. 2014;12:82–90. doi:10.1158/1541-7786.MCR-13-0392
25. Wei F, Yang F, Jiang X, Yu W, Ren X. High-mobility group nucleosome-binding protein 1 is a novel clinical biomarker in non-small cell lung cancer. *Tumour Biol*. 2015;36(12):9405–9410. doi:10.1007/s13277-015-3693-7
26. Zhang M, Zhao Z, Duan X, Chen P, Peng Z, Qiu H. FSCN1 predicts survival and is regulated by a PI3K-dependent mechanism in renal cell carcinoma. *J Cell Physiol*. 2018233(6):4748–4758. doi:10.1002/jcp.26264
27. Wang CQ, Tang CH, Wang Y, et al. FSCN1 gene polymorphisms: biomarkers for the development and progression of breast cancer. *Sci Rep*. 2017;7(1):15887.
28. Liu C, Gao H, Cao L, et al. The role of FSCN1 in migration and invasion of pituitary adenomas. *Mol Cell Endocrinol*. 2016;419:217–224.
29. Sun J, Guo YD, Li XN, et al. B7-H3 expression in breast cancer and upregulation of VEGF through gene silence. *Onco Targets Ther*. 2014;1979. doi:10.2147/OTT
30. Altan M, Pelekanou V, Schalper KA, et al. B7-H3 expression in NSCLC and its association with B7-H4, PD-L1 and tumor-infiltrating lymphocytes. *Clin Cancer Res*. 2017;23:5202–5209. doi:10.1158/1078-0432.CCR-16-3107
31. Zhang P, Kong F, Deng X, et al. MicroRNA-326 suppresses the proliferation, migration and invasion of cervical cancer cells by targeting ELK1. *Oncol Lett*. 2017;13(5):2949–2956.
32. Wang L, Peng Z, Wang K, et al. NDUFA4L2 is associated with clear cell renal cell carcinoma malignancy and is regulated by ELK1. *PeerJ*. 2017;5:e4065. doi:10.7717/peerj.4065
33. Kawahara T, Aljarah AK, Shareef HK, et al. Silodosin inhibits prostate cancer cell growth via ELK1 inactivation and enhances the cytotoxic activity of gemcitabine. *Prostate*. 2016;76:744–756. doi:10.1002/pros.v76.8
34. Luo L, Wang Y, Yin Y, Ge J, Lu X. Effects of NOB1 on the pathogenesis of osteosarcoma and its expression on the chemosensitivity to cisplatin. *Oncol Lett*. 2018;15(3):3548–3551.
35. Qi H, Wang Y. NOB1 gene as a potential biomarker in clinical outcomes and prognosis of patients with gastric cancer. *Clin Lab*. 2018;64(9):1469–1475. doi:10.7754/Clin.Lab.2018.180330
36. Wang J, Cao L, Wu J, Wang Q. Long non-coding RNA SNHG1 regulates NOB1 expression by sponging MiR-326 and promotes tumorigenesis in osteosarcoma. *Int J Oncol*. 2018;52(1):77–88. doi:10.3892/ijo.2017.4187
37. Zhou J, Xu T, Yan Y, et al. MicroRNA-326 functions as a tumor suppressor in glioma by targeting the Nin One Binding Protein (NOB1). *PLoS ONE*. 2013;8(7):e68469. doi:10.1371/journal.pone.0068469.
38. Zhao Z, Rahman MA, Chen ZG, Shin DM. Multiple biological functions of Twist1 in various cancers. *Oncotarget*. 2017;8(12):20380–20393. doi:10.18632/oncotarget.14608
39. Xu Y, Qin L, Sun T, et al. Twist1 promotes breast cancer invasion and metastasis by silencing Foxa1 expression. *Oncogene*. 2017;36:1157–1166. doi:10.1038/onc.2016.286
40. Lee KW, Yeo SY, Sung CO, Kim SH. Twist1 is a key regulator of cancer-associated fibroblasts. *Cancer Res*. 2015;75:73–85. doi:10.1158/0008-5472.CAN-14-0350
41. Xu X, Wells A, Padilla MT, et al. A signaling pathway consisting of MiR-551b, catalase and MUC1 contributes to acquired apoptosis resistance and chemoresistance. *Carcinogenesis*. 2014;2457–2466. doi:10.1093/carcin/bgu159
42. Nie F, Liu T, Zhong L, et al. MicroRNA-148b enhances proliferation and apoptosis in human renal cancer cells via directly targeting MAP3K9. *Mol Med Rep*. 2016;13:83–90. doi:10.3892/mmr.2015.4555
43. Ke J, Yao Y, Zheng J, et al. Knockdown of long non-coding RNA HOTAIR inhibits malignant biological behaviors of human glioma cells via modulation of MiR-326. *Oncotarget*. 2015;6. doi:10.18632/oncotarget.v6i26
44. Liu X, Song B, Li S, Wang N, Yang H. Identification and functional analysis of the risk MicroRNAs associated with cerebral low-grade glioma prognosis. *Mol Med Rep*. 2017;16(2):1173–1179. doi:10.3892/mmr.2017.6705.
45. Xu J, Lin DI. Oncogenic C-terminal Cyclin D1 (Ccnd1) mutations are enriched in endometrioid endometrial adenocarcinomas. *PLoS ONE*. 2018;13(7):e0199688. doi: 10.1371/journal
46. Dai J, Wei R-J, Li R, Feng J-B, Yu Y-L, Liu P-S. A study of CCND1 with epithelial ovarian cancer cell proliferation and apoptosis. *Eur Rev Med Pharmacol Sci*. 2016;20(20):4230–4235.
47. Liu P, Zhang R, Yu W, et al. FGF1 and IGF1-conditioned 3D culture system promoted the amplification and cancer stemness of lung cancer cells. *Biomaterials*. 2017;149:63–76. doi:10.1016/j.biomaterials.2017.09.030
48. Pirou C, Montazer-Torbati F, Jah N, et al. FGF1 protects neuroblastoma SH-SY5Y cells from P53-dependent apoptosis through an intracrine pathway regulated by FGF1 phosphorylation. *Cell Death Dis*. 2017;8:e3023–e3023. doi:10.1038/cddis.2017.404
49. Taeger J, Moser C, Hellerbrand C, et al. Targeting FGFR/PDGFR/VEGFR impairs tumor growth, angiogenesis, and metastasis by effects on tumor cells, endothelial cells, and pericytes in pancreatic cancer. *Mol Cancer Ther*. 2011;10:2157–2167. doi:10.1158/1535-7163.MCT-11-0312
50. Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *J Biochem*. 2011;149:121–130. doi:10.1093/jb/mvq121
51. Ornitz DM, Itoh N. The fibroblast growth factor signaling pathway. *Wiley Interdisciplinary Rev Dev Biol*. 4(3):215–266.
52. Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol Sci*. 2001;22:201–207. doi:10.1016/S0165-6147(00)01676-X

53. Zhao D, Lu Y, Yang C, Zhou X, Xu Z. Activation of FGF receptor signaling promotes invasion of non-small-cell lung cancer. *Tumour Biol*. 2015;36(5):3637–3642. doi:10.1007/s13277-014-3001-y
54. Li Q, Alsaidan OA, Ma Y, et al. Pharmacologically targeting the myristoylation of the scaffold protein FRS2α inhibits FGF/FGFR-mediated oncogenic signaling and tumor progression. *J Biol Chem*. 2018;293(17):6434–6448. doi:10.1074/jbc.RA117.000940
55. Baruzzi A, Remelli S, Lorenzetto E, Sega M, Chignola R, Berton G. Sos1 regulates macrophage podosome assembly and macrophage invasive capacity. *J Immunol*. 2015;195:4900–4912. doi:10.4049/jimmunol.1500579
56. Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. *Nat Rev Drug Discovery*. 2014;13:928–942. doi:10.1038/nrd4281
57. Liu F, Mischel PS. Targeting epidermal growth factor receptor co-dependent signaling pathways in glioblastoma. *Wiley Interdisciplinary Rev*. 2018;10(1):e1398.
58. Retamales-Ortega R, Oróstica L, Vera C, et al. Role of Nerve Growth Factor (NGF) and MiRNAs in epithelial ovarian cancer. *Int J Mol Sci*. 2017;18:507. doi:10.3390/ijms18030507
59. Nico B, Mangieri D, Benagiano V, Crivellato E, Ribatti D. Nerve growth factor as an angiogenic factor. *Microvasc Res*. 2008;75:135–141. doi:10.1016/j.mvr.2007.07.004
60. Yuan X, Cao J, He X, et al. Ciliary IFT80 balances canonical versus non-canonical hedgehog signalling for osteoblast differentiation. *Nat Commun*. 2016;7:11024.
61. Shao J, Xu L, Chen L, et al. Arl13b promotes gastric tumorigenesis by regulating smo trafficking and activation of the hedgehog signaling pathway. *Cancer Res*. 2017;77:4000–4013. doi:10.1158/0008-5472.CAN-16-2461
62. Sabol M, Car D, Musani V, et al. The hedgehog signaling pathway in ovarian teratoma is stimulated by sonic hedgehog which induces internalization of patched. *Int J Oncol*. 2012;41:1411–1418. doi:10.3892/ijo.2012.1554
63. Lee RTH, Zhao Z, Ingham PW. Hedgehog signalling. *Development*. 2016.
64. Faião-Flores F, Alves-Fernandes DK, Pennacchi PC, et al. Targeting the hedgehog transcription factors GLI1 and GLI2 restores sensitivity to vemurafenib-resistant human melanoma cells. *Oncogene*. 2017;36:1849–1861. doi:10.1038/onc.2016.348
65. Wang Y, Li Y, Hu G, et al. Nek2A phosphorylates and stabilizes SuFu: a new strategy of Gli2/Hedgehog signaling regulatory mechanism. *Cell Signal*. 2016;28(9):1304–1313. doi:10.1016/j.cellsig.2016.06.010
66. Grzelak CA, Sigglekow ND, McCaughan GW. GLI2 as a marker of hedgehog-responsive cells. *Hepatology*. 2015. 61(5):1770.
67. Northcott PA, Shih DJH, Peacock J, et al. Subgroup-specific structural variation across 1000 medulloblastoma genomes. *Nature*. 2012;488:49–56. doi:10.1038/nature11327
68. Pugh TJ, Weeraratne SD, Archer TC, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature*. 2012;488(7409):106.
69. D'Amico D, Antonucci L, Di Magno L, et al. Non-canonical Hedgehog/AMPK-mediated control of polyamine metabolism supports neuronal and medulloblastoma cell growth. *Dev Cell*. 2015;35(1):21–35. doi:10.1016/j.devcel.2015.09.008
70. Farooqi AA, Shu CW, Huang HW, et al. Trail, Wnt, Sonic Hedgehog, TGFβ, and MiRNA signalings are potential targets for oral cancer therapy. *Int J Mol Sci*. 2017;18:1523. doi:10.3390/ijms18071523
71. Miele E, Po A, Begalli F, et al. β-Arrestin1-mediated acetylation of Gli1 regulates Hedgehog/Gli signaling and modulates self-renewal of SHH medulloblastoma cancer stem cells. *BMC Cancer*. 2017;17(1):488. doi:10.1186/s12885-017-3477-0
72. Maryu G, Matsuda M, Aoki K. Multiplexed fluorescence imaging of ERK and Akt activities and cell-cycle progression. *Cell Struct Funct*. 2016;41:81–92. doi:10.1247/csf.16007
73. Faes S, Dormond O. PI3K and AKT: unfaithful partners in cancer. *Int J Mol Sci*. 2015;16:21138–21152. doi:10.3390/ijms160921138
74. Lien EC, Dibble CC, Toker A. PI3K signaling in cancer: beyond AKT. *Curr Opin Cell Biol*. 2017;45:62–71. doi:10.1016/j.ceb.2017.02.007
75. Insall RH, Weiner OD. PIP3, PIP2, and cell movement - Similar messages, different meanings? *Dev Cell*. 2001;1:743–747. doi:10.1016/S1534-5807(01)00086-7
76. Chen J, Zhao K-N, Li R, Shao R, Chen C. Activation of PI3K/Akt/MTOR pathway and dual inhibitors of PI3K and MTOR in endometrial cancer. *Curr Med Chem*. 2014;21(26):3070–3080. doi:10.2174/0929867321666140414095605
77. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/MTOR signaling in cancer. *Front Oncol*. 2014;4. doi:10.3389/fonc.2014.00064
78. Wu X-L, Wang L-K, Yang -D-D, et al. Effects of Glut1 gene silencing on proliferation, differentiation, and apoptosis of colorectal cancer cells by targeting the TGF-β/PI3K-AKT-MTOR signaling pathway. *J Cell Biochem*. 2018;119(2):2356–2367. doi:10.1002/jcb.v119.2
79. Slattery ML, Mullany LE, Sakoda LC, et al. The PI3K/AKT signaling pathway: associations of MiRNAs with dysregulated gene expression in colorectal cancer. *Mol Carcinog*. 2018;57(2):243–261. doi:10.1002/mc.v57.2
80. Wang R, Xu J, Xu J, et al. MiR-326/Sp1/KLF3: a novel regulatory axis in lung cancer progression. *Cell Prolif*. 2019;52:e12551. doi:10.1111/cpr.2019.52.issue-2
81. Deschênes-Simard X, Kottakis F, Meloche S, Ferbeyre G. ERKs in cancer: friends or foes? *Cancer Res*. 2014;74:412–419. doi:10.1158/0008-5472.CAN-13-2381
82. Yoo S-M, Cho SJ, Cho -Y-Y. Molecular targeting of ERKs/RSK2 signaling axis in cancer prevention. *J Cancer Prev*. 2015;20(3):165.
83. Tanimura S, Takeda K. ERK signalling as a regulator of cell motility. *J Biochem*. 2017;162:145–154. doi:10.1093/jb/mvx048
84. Jaiswal BS, Durinck S, Stawiski EW, et al. ERK mutations and amplification confer resistance to ERK-inhibitor therapy. *Clin Cancer Res*. 2018;24:4044–4055. doi:10.1158/1078-0432.CCR-17-3674
85. Dorard C, Vucak G, Baccarini M. Deciphering the RAS/ERK pathway in vivo. *Biochem Soc Trans*. 2017;45(1):27–36. doi:10.1042/BST20160135
86. Zahavi T, Maimon A, Kushnir T, et al. Ras-Erk signaling induces phosphorylation of human TLE1 and downregulates its repressor function. *Oncogene*. 2017;36:3729–3739. doi:10.1038/onc.2016.517
87. Kidger AM, Keyse SM. The regulation of oncogenic Ras/ERK signalling by dual-specificity mitogen activated protein kinase phosphatases (MKPs). *Semin Cell Dev Biol*. 2016;50:125–132. doi:10.1016/j.semcdb.2016.01.009
88. Kang K, Zhang J, Zhang X, Chen Z. MicroRNA-326 inhibits melanoma progression by targeting KRAS and suppressing the AKT and ERK signalling pathways. *Oncol Rep*. 2018;39(1):401–410.
89. Kartal-Yandim M, Adan-Gokbulut A, Baran Y. Molecular mechanisms of drug resistance and its reversal in cancer. *Crit Rev Biotechnol*. 2016;36(4):716–726.
90. Kibria G, Hatakeyama H, Harashima H. Cancer multidrug resistance: mechanisms involved and strategies for circumvention using a drug delivery system. *Arch Pharm Res*. 2014;37:4–15. doi:10.1007/s12272-013-0276-2
91. Ma J, Wang T, Guo R, et al. Involvement of MIR-133a and MIR-326 in ADM resistance of HepG2 through modulating expression of ABCC1. *J Drug Target*. 2015;23:519–524. doi:10.3109/1061186X.2015.1015536
92. Cole SPC. Targeting multidrug resistance Protein 1 (MRP1, ABCC1): past, present, and future. *Annu Rev Pharmacol Toxicol*. 2014;54:95–117. doi:10.1146/annurev-pharmtox-011613-135959

93. Cole SPC. Multidrug resistance Protein 1 (Mrp1, Abcc1), a "Multitasking" Atp-Binding Cassette (Abc,) transporter. *J Biol Chem*. 2014;289:30880–30888. doi:10.1074/jbc.R114.609248
94. Rivankar S. An overview of doxorubicin formulations in cancer therapy. *J Cancer Res Ther*. 2014;10(4):853–858. doi:10.4103/0973-1482.139267
95. Cagel M, Grotz E, Bernabeu E, Moretton MA, Chiappetta DA. Doxorubicin: nanotechnological overviews from bench to bedside. *Drug Discov Today*. 2017;22:270–281. doi:10.1016/j.drudis.2016.11.005
96. Ma J, Wang T, Guo R, et al. MicroRNA-133a and MicroRNA-326 co-contribute to hepatocellular carcinoma 5-fluorouracil and cisplatin sensitivity by directly targeting B-Cell lymphoma.Extra large. *Mol Med Rep*. 2015;12:6235–6240. doi:10.3892/mmr.2015.4134
97. Choi S, Chen Z, Tang LH, et al. Bcl-XL promotes metastasis independent of its anti-apoptotic activity. *Nat Commun*. 2016;7:10384.
98. de Jong Y, Monderer D, Brandinelli E, et al. Bcl-Xl as the most promising Bcl-2 family member in targeted treatment of chondrosarcoma. *Oncogenesis*. 2018;7. doi:10.1038/s41389-018-0084-0
99. Sara JD, Kaur J, Khodadadi R, et al. 5-fluorouracil and cardiotoxicity: a review. *Ther Adv Med Oncol*. 2018;10:1758835918780140. doi:10.1177/1758835918780140
100. Ji WB, Um JW, Ryu JS, et al. Clinical significance of 5-fluorouracil chemosensitivity testing in patients with colorectal cancer. *Anticancer Res*. 2017;37(5):2679–2682.