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MicroRNAs in Invasion and Metastasis in Lung Cancer

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1. Introduction

Despite advances in diagnosis and treatment, the morbidity and mortality of lung cancer remains to mount up. The key factor of cancer associated morbidity and mortality is principally attributable to the development of metastases. Cancer cells depart their normal microenvironment from the primary tumor site through complicated and multistep processes disseminate and colonize distant organs [1]. However, the cellular and molecular machinery underlying metastasis is relatively poorly understood so far. In order to resist cancer dissemination, more effective therapeutic strategies are clearly required.

Cellular migration and invasion mechanism are commonly thought to be associated with Rho family GTPases [2-4], JAK-STAT [5-7], MAPK [8-10], Wnt [11-13], Notch pathway [14-16]. Recently, epithelial-mesenchymal transition (EMT) programs have become the focus of the mechanism of metastasis [1, 17-20]. EMT is an embryologically conserved genetic program by which epithelial cells down regulate intercellular tight junctions, loose polarity, express mesenchymal markers, and manifest a migratory phenotype [1]. In the EMT process, Rho family GTPases [21], JAK-STAT [22], MAPK [23], Wnt [24] and Notch [25] pathways may also play an important role. In recent years, emerging studies have highlighted the critical role of these pathways and their regulation by microRNAs (miRNAs) in cancer invasion and metastasis.

MiRNAs, short (18-24 nucleotides) non-coding RNAs, are derived from long transcripts pri-miRNAs and pre-miRNAs [26-30]. By targeting 3' untranslated regions (3'UTRs) of cognate mRNAs, miRNAs post-transcriptionally regulate gene expression and induce translational repression [29, 30]. Their specificity is determined by nucleotides 2-8 at the 5' end, termed the miRNA "seed sequence". To date, 1527 human miRNAs have been identified (Sanger miRBase 18 <http://www.miRbase.org/index.shtml>), forming less than 1% of all human genes, potentially regulating more than 10% of all protein coding genes [1]. Recently, miRNAs have

been discovered to play important roles in the invasion and metastasis of malignant tumors. [31-33]. Understanding specific characteristics of miRNAs would probably serve as predictive markers and as therapeutic strategies for patients with metastasis.

In light of these recent discoveries, the present article discusses how invasion and EMT pathways are regulated by miRNAs. We have classified invasion programs and key proteins involved in EMT according to the signaling pathway showed above and point out validated miRNAs regulating their expression and highlight critical knowledge gaps that remain to be addressed to enable improved understanding of the molecular mechanisms behind EMT and metastasis. A list of experimentally validated miRNAs regulating key proteins involved in invasion–metastasis programs or participating in some principal pathways can be found in Figure 1.

Figure 1. The experimentally validated miRNAs regulate key proteins involved in invasion–metastasis programs or participating in some principal pathways.

2. Rho family of GTPases

The Rho family of GTPases, a family of small (~21 kDa) signaling G protein, is a subfamily of the Ras superfamily [34]. In mammals, the Rho family is made of 20 members distributed

into eight subfamilies: Rho, Rac, Cdc42, Rnd, RhoU/V, RhoBTB, RhoH and RhoD/F. Almost all research involves the three most common members of the Rho family: Cdc42, Rac1 and RhoA [35]. Over expression of Rho GTPases is associated with reorganization of actin cytoskeleton, which plays an important role in cell migration, invasion and metastasis that are important aspects of cancer progression [36].

Emerging studies have indicated that miRNAs participate in the Rho GTPases signaling pathway. Among the tested miRNAs, the present articles demonstrated that miR-155, miR-185, miR-31 and miR-133a are associated with RhoA in cell migration and invasion. MiR-155 may play an important role in TGF- β -induced EMT and cell migration and invasion by targeting RhoA [37]. MiR-185 is a negative regulator of RhoA and Cdc42, and could inhibit proliferation and invasion of colorectal cancer cells [38]. The Effects of miR-31 on metastasis may be associated with concurrent suppression of integrin alpha 5, radixin, and RhoA phenocopies [39]. Chiba and his colleagues reported that RhoA expression is negatively regulated by miR-133a in bronchial smooth muscle cells [40].

Moreover, some studies discussed the regulation of cell migration and invasion by miRNA may be attribute to Rho-associated serine-threonine protein kinase (ROCK), one of the best characterized downstream effectors of Rho, that is activated when it selectively binds to the active GTP-bound form of Rho [41, 42]. As with Rho, ROCK has been implicated in altering cell migration and invasion during tumor cell metastasis [43, 44]. Yu and his colleagues indicate that downregulation of miR-205 resulted in an increase in Rho-ROCKI activity, phosphorylation of the actin severing protein cofilin, and a corresponding diminution of filamentous actin [45].

A number of articles reported that some miRNAs regulate cell migration and invasion by targeting Rac and Cdc42. Recently, microRNA-142-3p, a new regulator of Rac1, suppresses the migration and invasion of hepatocellular carcinoma cells [46]. The regulation of cancer cell migration by MiR-10b may be attribute to activate Rac by targets Tiam1 [47]. MiR-151 exerts this function by directly targeting RhoGDI, a putative metastasis suppressor in hepatocellular carcinoma (HCC), thus leading to the activation of Rac1, Cdc42 and Rho GTPases [48]. Liu and his colleagues have found that miR-137 may have a tumor suppressor function by directly targeting Cdc42 to inhibit the proliferation and invasion activities of colorectal cancer cells [49, 50]. MiR-206 may suppress invasion and migration of MDA-MB-231 cells in vitro partly via regulating actin cytoskeleton remodelling by downregulating Cdc42 [51]. MiR-29 activates p53 by targeting p85 alpha and Cdc42 [52].

In addition, MiR-21 targets the tumor suppressor Rho B and regulates proliferation, invasion positively in colorectal cancer cells [53, 54]. Jiang and his colleagues have indicated that miR-138 plays an important role in tongue squamous cell carcinoma cell migration and invasion by concurrently targeting Rho C and ROCK2 [36]. Studies on the association of Rho with miRNAs highlight the importance of miRNAs in invasion and metastasis of malignant tumors.

3. JAK-STAT

The JAK-STAT signaling pathway transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell. JAK, short for Janus Kinase, is a family of intracellular, nonreceptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway. As a key component of the JAK/STAT pathway, Signal Transducer and Activator of Transcription, an important transcription factors, is activated by JAK [55, 56]. In JAK and STAT family, emerging studies have indicated that JAK2/STAT3 pathway is well-established regulators of cell migration, and has been implicated in the process of tumor cell invasion and metastasis [57].

Some studies have indicated that miRNAs participate in the JAK-STAT signaling pathway. MiR-375 may function as a tumor suppressor to regulate gastric cancer cell proliferation potentially by targeting the JAK2 oncogene [58]. MiR-125b suppresses the proliferation and migration of osteosarcoma cells through downregulation of STAT3 [59]. Transfection of precursor miR-199a-3p into osteosarcoma cell lines significantly decreased cell growth and migration. Duan and his colleagues observed decreased mTOR and STAT3 expression in miR-199a-3p transfected cells [60]. Yan and his colleagues indicated that miR-20a regulates STAT3 at the post-transcriptional level, resulting in inhibition of cell proliferation and invasion of pancreatic carcinoma [61].

4. MAPK pathway

The Mitogen Activated Protein Kinase (MAPK) pathway is a frequent event in tumorigenesis. MAPKs have been implicated in cell migration, proteinase induction, apoptosis, and angiogenesis, events that are essential for successful completion of metastasis [8]. The presence of at least six MAPK in yeast suggests that there are more in mammals: extracellular signal-regulated kinases (ERK1, ERK2), c-Jun N-terminal kinases (JNKs), p38 isoforms, ERK5, ERK3/4, ERK7/8. In vivo and in vitro studies have confirmed that three major subgroups of MAPK including ERK1/2, JNK, and p38, are specifically involved in invasion and metastasis [9, 10, 62].

Mounting studies have indicated that miRNAs participate in the MAPK signaling pathway. MiR-143 plays an important role in prostate cancer proliferation, migration and chemosensitivity by suppressing KRAS and subsequent inactivation of MAPK pathway [63]. MiR-17-5p significantly activates the p38 kinase pathway [64]. Raf kinase inhibitory protein suppresses a cascade of metastasis signalling involving LIN28 and let-7 [65]. Zhu and his colleagues found that miR-101 targets MAPK phosphatase 1 to regulate the activation of MAPKs in macrophages [66]. MiR-146a suppresses tumor growth and progression by targeting EGFR pathway and in a p-ERK-dependent manner in castration-resistant prostate cancer [67]. Liu and his colleagues indicated that miR-21 induces tumor angiogenesis through targeting PTEN, leading to activate AKT and ERK1/2 signaling pathways [68,69]. EGFR promotes lung tumorigenesis by activating miR-7 through a Ras/ERK/Myc pathway that targets the ETS2 transcriptional repressor ERF [70].

5. Wnt signaling pathway

Wnt signaling pathway controls tissue polarity and cell movement through the activation of RhoA, JNK, and nemo-like kinase (NLK) signaling cascades. The Wnt gene family is a group of developmental genes that encode cysteine-rich glycosylated proteins [71]. Aberrant activation of Wnt signaling pathway in human cancer leads to more malignant phenotypes, such as abnormal tissue polarity, invasion, and metastasis [72].

A number of studies have indicated that miRNAs participate in the Wnt signaling pathway. MiR-200a is a new tumor suppressor that can regulate the activity of the Wnt/ β -catenin signaling pathway [73]. MiR-371-373 expression is induced by lithium chloride and is positively correlated with Wnt/ β -catenin-signaling activity in several human cancer cell lines [74]. MiR-27 directly targeted and inhibited adenomatous polyposis coli (APC) gene expression, and activated Wnt signaling through accumulation of β -catenin [75]. Kapinas and his colleagues reported that miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop [76]. MiR-17-5p plays an important role in breast cancer cell invasion and migration by suppressing HBP1 and subsequent activation of Wnt/ β -catenin [77]. Kennell and his colleagues demonstrated that miR-8 family members play an evolutionarily conserved role in regulating the Wnt signaling pathway [78].

6. Notch signaling pathway

The Notch signaling pathway is a conserved ligand–receptor signaling pathway. Notch genes encode single-pass transmembrane proteins that can be activated by interacting with a family of its ligands. To date, four Notch receptors have been identified in mammals, including human, such as Notch-1-4. It has been well known that Notch signaling plays important roles in maintaining the balance involved in cell proliferation, survival, apoptosis, and differentiation which affects the development and function of many organs [79]. Therefore, dysfunction of Notch prevents differentiation, ultimately guiding undifferentiated cells toward malignant transformation. Indeed, many observations suggest that alterations in Notch signaling are associated with invasion and metastasis in many human cancers [14-16].

Mounting studies have indicated that miRNAs participate in the Notch signaling pathway. MicroRNA-23b is capable of inducing tolerogenic properties of dendritic cells in vitro through the inhibition of the Notch1 and NF- κ B signalling pathways [80]. MicroRNA-181 promotes natural killer (NK) cell development by regulating Notch signaling [81]. MiR-124a mediates stroke-induced neurogenesis by targeting the JAG-Notch signaling pathway [82]. Pang and his colleagues demonstrated that miR-34a affected cell invasion by regulating expression of urokinase plasminogen activator through Notch [83]. MiR-206 targets Notch 3, activates apoptosis, and inhibits tumor cell migration and focus formation [84]. MiR-1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling [85]. Some studies indicated that the ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells [86, 87].

7. EMT

Several oncogenic pathways (Rho GTPases, JAK-STAT, MAPK, Wnt and Notch) may induce EMT [21-25]. In particular, the association of those pathways with EMT has been shown to activate EMT-inducing transcriptional regulators such as the members of the Snail family, the zinc finger transcription factors (ZEB), Transforming growth factor beta (TGF- β), Twist and Slug.

Members of the Snail family of transcriptional regulators, namely Snail 1 and Snail 2, have emerged as a key regulatory factor of EMT. The zinc finger transcription factors ZEB1 and ZEB2 also make a pivotal contribution to this regulation. TGF- β , a major inducer of EMT, exists in at least three isoforms called TGF- β 1, TGF- β 2 and TGF- β 3. It cooperates with stem cell pathways like Wnt, Ras and Notch to induce EMT [88, 89]. Twist, a basic helix-loop-helix transcription factor, exists in at least two isoforms called Twist 1 and Twist 2. Twist proteins promote EMT by turning-down the expression of epithelial specific proteins, such as the E-cadherin and by up-regulating the expression of mesenchymal markers such as the N-cadherin, the vimentin and the smooth-muscle actin [90]. Slug, a zinc finger transcription factor, whose product belongs to the Snail family of developmental regulatory proteins, is transcriptional repressors of E-cadherin and induces EMT [1].

Emerging studies have indicated that miRNAs participate in the EMT. The miR-106b-25 cluster targets Smad7, activates TGF- β signaling, and induces EMT in human breast cancer [91]. MiR-27 promoted EMT by activating the Wnt pathway [92]. MiR-221/222 targeting of trichorhinophalangeal 1 (TRPS1) promotes EMT in breast cancer [93]. MiR-194 inhibits EMT of endometrial cancer cells by targeting oncogene BMI-1 [94]. Let-7d negatively modulates EMT expression and also plays a role in regulating chemo-resistant ability in oral cancer [95]. MiR-200b and miR-15b regulate chemotherapy-induced EMT in human tongue cancer cells by targeting BMI-1 [96]. Kumarswamy and his colleagues found that miR-30a targets Snail1, inhibits invasion and metastasis, and is downregulated in non-small cell lung cancer (NSCLC) [97]. Vetter and his colleagues indicated that miR-661 expression in Snail 1-induced EMT contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers [98]. Some studies indicated that the miR-200 family and miR-205 regulate EMT by targeting ZEB1 and SIP1 [99, 100].

8. MicroRNAs in invasion and metastasis in lung cancer

Lung cancer is the leading cause of death among the malignant tumors worldwide, and the incidence of lung cancer is increasing. Tumor invasion and metastasis are the critical steps in determining the aggressive phenotype of human cancers. Mortality of tumor patients results mainly from cancer cells spreading to distant organs. In order to resist cancer dissemination, more effective therapeutic strategies are clearly required. However, the cellular and molecular machinery, underlying invasion and metastasis by miRNA in lung cancer, is relatively poorly understood. In light of these recent discoveries, we have classified the experimentally validated miRNAs regulating the invasion and metastasis of lung cancer and showed in Figure 2.

Figure 2. The experimentally validated miRNAs regulate the invasion and metastasis in lung cancer.

In light of these recent discoveries, the present article indicated that miRNAs participate in invasion and metastasis in lung cancer. Zhu and his colleagues indicated that MTA1 functions in regulating the invasive phenotype of lung cancer cells and this regulation may be through altered miRNA expression, such as miR-125b, miR-210, miR-103, miR-194 and miR-500 [101]. Hu and his colleagues reported that MiR-193b modulated proliferation, migration, and invasion of NSCLC [102]. A p53/miR-34 axis has been found that it regulates Snail1-dependent cancer cell EMT [103]. MiR-378 is associated with NSCLC brain metastasis by promoting cell migration, invasion and tumor angiogenesis [104]. MiR-30a targets Snail1, inhibits invasion and metastasis, and is downregulated in NSCLC [105]. Expression level of miR-206 was inversely correlated with metastatic potential of lung cancer [106]. Roybal and his colleagues demonstrated that miR-200 Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1 [107]. Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in NSCLC [108]. In our previous studies, we found that hsa-miR-125a-3p and hsa-miR-125a-5p are downregulated in NSCLC and have inverse effects on invasion and migration of lung cancer cells [109]. Zhang and his colleagues reported that miR-21 post-transcriptionally downregulates the expression of tumor suppressor PTEN and stimulates growth and invasion in NSCLC [110]. Crawford and his colleagues indicated that MiR-126 alters lung cancer cell phenotype by inhibiting adhesion,

migration, and invasion and the effects on invasion may be partially mediated through Crk regulation [111]. The deep mechanisms of miRNAs in invasion and metastasis which contribute to lung cancer are worthy of further investigation.

9. Conclusion and future perspective

Despite recent advances in diagnosis and treatment, lung cancer remains a leading cause of death among the malignant tumors worldwide, and the incidence of lung cancer is increasing. Even so, no improvement in prognosis has been observed if the patient presents with metastases at diagnosis. A better understanding of the mechanism of tumor cell invasion is critical for the development of more effective treatments for metastatic cancer. In recent years, emerging studies have attested to the association between miRNAs and the mechanism in critical processes during cancer dissemination, and we have summarized many of these in the present manuscript. Here, we have condensed much of this early work, and highlight key deregulated miRNAs targeting molecules involved in Rho family GTPases, JAK-STAT, MAPK, Wnt, Notch pathway and transcriptional control of EMT. In the future, a more complete dissection of the pathways controlled by miRNAs may offer new insights on metastasis, and highlight promising areas for the development of novel anti-cancer therapies.

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10. References

- [1] Sreekumar R, Sayan BS, Mirnezami AH, Sayan AE. MicroRNA Control of Invasion and Metastasis Pathways. *Front Genet* 2011; 2: 58.
- [2] Baranwal S, Alahari SK. Rho GTPase effector functions in tumor cell invasion and metastasis. *Curr Drug Targets* 2011; 12 (8):1194-201.
- [3] Struckhoff AP, Rana MK, Worthylake RA. RhoA can lead the way in tumor cell invasion and metastasis. *Front Biosci* 2011; 16: 1915-26.

- [4] Narumiya S, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev* 2009; 28 (1-2): 65-76.
- [5] Lai SY, Childs EE, Xi S, Coppelli FM, Gooding WE, Wells A, Ferris RL, Grandis JR. Erythropoietin-mediated activation of JAK-STAT signaling contributes to cellular invasion in head and neck squamous cell carcinoma. *Oncogene* 2005; 24 (27): 4442-9.
- [6] Zhao Y, Zhang J, Xia H, Zhang B, Jiang T, Wang J, Chen X, Wang Y. Stat3 is involved in the motility, metastasis and prognosis in lingual squamous cell carcinoma. *Cell Biochem Funct.* 2012; 30 (4): 340-6.
- [7] Devarajan E, Huang S. STAT3 as a central regulator of tumor metastases. *Curr Mol Med* 2009; 9 (5): 626-33.
- [8] Reddy KB, Nabha SM, Atanaskova N. 9. Gomes LR, Terra LF, Wailemann RA, Labriola L, Sogayar MC. Role of MAP kinase in tumor progression and invasion. *Cancer Metastasis Rev* 2003; 22 (4): 395-403.
- [9] Del Barco Barrantes I, Nebreda AR. Roles of p38 MAPKs in invasion and metastasis. *Biochem Soc Trans* 2012; 40 (1): 79-84.
- [10] Zhang S, Guo W, Ren TT, Lu XC, Tang GQ, Zhao FL. Arsenic trioxide inhibits Ewing's sarcoma cell invasiveness by targeting p38 (MAPK) and c-Jun N-terminal kinase. *Anticancer Drugs* 2012; 23 (1): 108-18.
- [11] Sarkar FH, Li Y, Wang Z, Kong D. The role of nutraceuticals in the regulation of Wnt and Hedgehog signaling in cancer. *Cancer Metastasis Rev* 2010; 29 (3): 383-94.
- [12] Huang D, Du X. Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination. *World J Gastroenterol* 2008; 14 (12): 1823-7.
- [13] Neth P, Ries C, Karow M, Egea V, Ilmer M, Jochum M. The Wnt signal transduction pathway in stem cells and cancer cells: influence on cellular invasion. *Stem Cell Rev* 2007; 3 (1):18-29.
- [14] Asnaghi L, Ebrahimi KB, Schreck KC, Bar EE, Coonfield ML, Bell WR, Handa J, Merbs SL, Harbour JW, Eberhart CG. Notch signaling promotes growth and invasion in uveal melanoma. *Clin Cancer Res* 2012; 18 (3): 654-65.
- [15] Bailey JM, Singh PK, Hollingsworth MA. Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenic proteins. *J Cell Biochem* 2007; 102 (4): 829-39.
- [16] Wang XQ, Zhang W, Lui EL, Zhu Y, Lu P, Yu X, Sun J, Yang S, Poon RT, Fan ST. Notch1-Snail1-E-cadherin pathway in metastatic hepatocellular carcinoma. *Int J Cancer* 2012 131 (3):E163-72.
- [17] Shih JY, Yang PC. The EMT regulator slug and lung carcinogenesis. *Carcinogenesis* 2011; 32 (9): 1299-304.
- [18] Wells A, Chao YL, Grahovac J, Wu Q, Lauffenburger DA. Epithelial and mesenchymal phenotypic switchings modulate cell motility in metastasis. *Front Biosci* 2011; 16: 815-37.
- [19] Savagner P. The epithelial-mesenchymal transition (EMT) phenomenon. *Ann Oncol* 2010; 21 Suppl 7:vii89-92.
- [20] Cano CE, Motoo Y, Iovanna JL. Epithelial-to-mesenchymal transition in pancreatic adenocarcinoma. *ScientificWorldJournal* 2010; 10: 1947-57.

- [21] Savagner P. Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. *Bioessays* 2001; 23 (10): 912-23.
- [22] Yadav A, Kumar B, Datta J, Teknos TN, Kumar P. IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. *Mol Cancer Res* 2011; 9 (12): 1658-67.
- [23] Wu WS. The signaling mechanism of ROS in tumor progression. *Cancer Metastasis Rev* 2006; 25 (4): 695-705.
- [24] Neth P, Ries C, Karow M, Egea V, Ilmer M, Jochum M. The Wnt signal transduction pathway in stem cells and cancer cells: influence on cellular invasion. *Stem Cell Rev* 2007; 3 (1): 18-29.
- [25] Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. *Curr Drug Targets* 2010; 11 (6): 745-51.
- [26] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993; 75 (5), 843-54.
- [27] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003; 425 (6956), 415-9
- [28] Kong W, Zhao JJ, He L & Cheng JQ. Strategies for profiling microRNA expression. *J Cell Physiol* 2009; 218 (1), 22-5
- [29] Hutvagner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the *let-7* small temporal RNA. *Science* 2001; 293 (5531), 834-8.
- [30] Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* 2001; 106 (1), 23-34.
- [31] Crawford M, Brawner E, Batte K, Yu L, Hunter MG, Otterson GA, Nuovo G, Marsh CB, Nana-Sinkam SP. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. *Biochem Biophys Res Commun* 2008; 373 (4), 607-12.
- [32] Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Res* 2008; 18 (3), 350-9.
- [33] Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008; 451 (7175), 147-52.
- [34] Bustelo XR, Sauzeau V, Berenjeno IM. GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo. *Bioessays* 2007; 29 (4): 356–370.
- [35] Boureux A, Vignal E, Faure S, Fort P. Evolution of the Rho family of ras-like GTPases in eukaryotes. *Mol Biol Evol* 2007; 24 (1): 203–16.
- [36] Rathinam R, Berrier A, Alahari SK. Role of Rho GTPases and their regulators in cancer progression. *Front Biosci* 2011; 16: 2561-71.

- [37] Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, Cheng JQ. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. *Mol Cell Biol* 2008; 28 (22):6773-84.
- [38] Jiang L, Liu X, Kolokythas A, Yu J, Wang A, Heidbreder CE, Shi F, Zhou X. Downregulation of the Rho GTPase signaling pathway is involved in the microRNA-138-mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma. *Int J Cancer* 2010; 127 (3): 505-12.
- [39] Valastyan S, Chang A, Benaich N, Reinhardt F, Weinberg RA. Concurrent suppression of integrin alpha5, radixin, and RhoA phenocopies the effects of miR-31 on metastasis. *Cancer Res* 2010; 70 (12): 5147-54.
- [40] Chiba Y, Tanabe M, Goto K, Sakai H, Misawa M. Down-regulation of miR-133a contributes to up-regulation of RhoA in bronchial smooth muscle cells. *Am J Respir Crit Care Med* 2009; 180 (8): 713-9.
- [41] Kamai T, Tsujii T, Arai K, Takagi K, Asami H, Ito Y, Oshima H. Significant association of Rho/ROCK pathway with invasion and metastasis of bladder cancer. *Clin Cancer Res* 2003; 9 (7): 2632-41.
- [42] Bishop AL, Hall A. Rho GTPases and their effector proteins. *Biochem J* 2000; 348 Pt 2:241-55.
- [43] Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol* 2003; 4 (6), 446-56.
- [44] Salhia B, Rutten F, Nakada M, Beaudry C, Berens M, Kwan A, Rutka JT. Inhibition of Rho-kinase affects astrocytoma morphology, motility, and invasion through activation of Rac1. *Cancer Res* 2005; 65 (19), 8792-800.
- [45] Yu J, Peng H, Ruan Q, Fatima A, Getsios S, Lavker RM. MicroRNA-205 promotes keratinocyte migration via the lipid phosphatase SHIP2. *FASEB J* 2010; 24 (10): 3950-9
- [46] Wu L, Cai C, Wang X, Liu M, Li X, Tang H. MicroRNA-142-3p, a new regulator of RAC1, suppresses the migration and invasion of hepatocellular carcinoma cells. *FEBS Lett* 2011; 585 (9): 1322-30.
- [47] Moriarty CH, Pursell B, Mercurio AM. miR-10b targets Tiam1: implications for Rac activation and carcinoma migration. *J Biol Chem* 2010; 285 (27): 20541-6.
- [48] Luedde T. MicroRNA-151 and its hosting gene FAK (focal adhesion kinase) regulate tumor cell migration and spreading of hepatocellular carcinoma. *Hepatology* 2010; 52 (3): 1164-6.
- [49] Liu M, Lang N, Qiu M, Xu F, Li Q, Tang Q, Chen J, Chen X, Zhang S, Liu Z, Zhou J, Zhu Y, Deng Y, Zheng Y, Bi F. miR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. *Int J Cancer* 2011; 128 (6): 1269-79.
- [50] Chen Q, Chen X, Zhang M, Fan Q, Luo S, Cao X. miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. *Dig Dis Sci*. 2011; 56 (7): 2009-16.
- [51] Liu H, Cao YD, Ye WX, Sun YY. Effect of microRNA-206 on cytoskeleton remodelling by downregulating Cdc42 in MDA-MB-231 cells. *Tumori* 2010; 96 (5): 751-5.
- [52] Park SY, Lee JH, Ha M, Nam JW, Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. *Nat Struct Mol Biol* 2009; 16 (1): 23-9.

- [53] Liu M, Tang Q, Qiu M, Lang N, Li M, Zheng Y, Bi F. miR-21 targets the tumor suppressor RhoB and regulates proliferation, invasion and apoptosis in colorectal cancer cells. *FEBS Lett* 2011; 585 (19): 2998-3005.
- [54] Connolly EC, Van Doorslaer K, Rogler LE, Rogler CE. Overexpression of miR-21 promotes an in vitro metastatic phenotype by targeting the tumor suppressor RHOB. *Mol Cancer Res* 2010; 8 (5): 691-700.
- [55] Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. *Science* 2002; 296 (5573): 1653–5.
- [56] Looyenga BD, Hutchings D, Cherni I, Kingsley C, Weiss GJ, Mackeigan JP. STAT3 Is Activated by JAK2 Independent of Key Oncogenic Driver Mutations in Non-Small Cell Lung Carcinoma. *PLoS One* 2012; 7 (2): e30820.
- [57] Devarajan E, Huang S. STAT3 as a central regulator of tumor metastases. *Curr Mol Med.* 2009; 9 (5): 626-33.
- [58] Ding L, Xu Y, Zhang W, Deng Y, Si M, Du Y, Yao H, Liu X, Ke Y, Si J, Zhou T. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. *Cell Res* 2010; 20 (7): 784-93.
- [59] Liu LH, Li H, Li JP, Zhong H, Zhang HC, Chen J, Xiao T. miR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3. *Biochem Biophys Res Commun* 2011; 416 (1-2): 31-8.
- [60] Duan Z, Choy E, Harmon D, Liu X, Susa M, Mankin H, Hornicek F. MicroRNA-199a-3p is downregulated in human osteosarcoma and regulates cell proliferation and migration. *Mol Cancer Ther* 2011; 10 (8): 1337-45.
- [61] Yan H, Wu J, Liu W, Zuo Y, Chen S, Zhang S, Zeng M, Huang W. MicroRNA-20a overexpression inhibited proliferation and metastasis of pancreatic carcinoma cells. *Hum Gene Ther* 2010; 21 (12): 1723-34.
- [62] Gomes LR, Terra LF, Wailemann RA, Labriola L, Sogayar MC. TGF- β 1 modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. *BMC Cancer* 2012; 12: 26.
- [63] Xu B, Niu X, Zhang X, Tao J, Wu D, Wang Z, Li P, Zhang W, Wu H, Feng N, Wang Z, Hua L, Wang X. miR-143 decreases prostate cancer cells proliferation and migration and enhances their sensitivity to docetaxel through suppression of KRAS. *Mol Cell Biochem* 2011; 350 (1-2): 207-13.
- [64] Yang F, Yin Y, Wang F, Wang Y, Zhang L, Tang Y, Sun S. miR-17-5p Promotes migration of human hepatocellular carcinoma cells through the p38 mitogen-activated protein kinase-heat shock protein 27 pathway. *Hepatology* 2010; 51 (5): 1614-23.
- [65] Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM, Minn AJ, Rosner MR. Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. *EMBO J* 2009; 28 (4): 347-58.
- [66] Zhu QY, Liu Q, Chen JX, Lan K, Ge BX. MicroRNA-101 targets MAPK phosphatase-1 to regulate the activation of MAPKs in macrophages. *J Immunol* 2010; 185 (12): 7435-42.
- [67] Xu B, Wang N, Wang X, Tong N, Shao N, Tao J, Li P, Niu X, Feng N, Zhang L, Hua L, Wang Z, Chen M. MiR-146a suppresses tumor growth and progression by targeting

- EGFR pathway and in a p-ERK-dependent manner in castration-resistant prostate cancer. *Prostate*. 2012; 72 (11): 1171-8.
- [68] Liu LZ, Li C, Chen Q, Jing Y, Carpenter R, Jiang Y, Kung HF, Lai L, Jiang BH. MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1 α expression. *PLoS One* 2011; 6 (4): e19139.
- [69] Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, Olson EN. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. *Cancer Cell* 2010; 18 (3): 282-93.
- [70] Chou YT, Lin HH, Lien YC, Wang YH, Hong CF, Kao YR, Lin SC, Chang YC, Lin SY, Chen SJ, Chen HC, Yeh SD, Wu CW. EGFR promotes lung tumorigenesis by activating miR-7 through a Ras/ERK/Myc pathway that targets the Ets2 transcriptional repressor ERF. *Cancer Res* 2010; 70 (21): 8822-31.
- [71] Dale TC. Signal transduction by the Wnt family of ligands. *Biochem J* 1998; 329 (Pt 2): 209-23.
- [72] Katoh M. WNT/PCP signaling pathway and human cancer. *Oncol Rep* 2005; 14 (6): 1583-8.
- [73] Su J, Zhang A, Shi Z, Ma F, Pu P, Wang T, Zhang J, Kang C, Zhang Q. MicroRNA-200a suppresses the Wnt/ β -catenin signaling pathway by interacting with β -catenin. *Int J Oncol* 2012; 40 (4): 1162-70.
- [74] Zhou AD, Diao LT, Xu H, Xiao ZD, Li JH, Zhou H, Qu LH. β -Catenin/LEF1 transactivates the microRNA-371-373 cluster that modulates the Wnt/ β -catenin-signaling pathway. *Oncogene*. 2012; 31 (24): 2968-78.
- [75] Wang T, Xu Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. *Biochem Biophys Res Commun* 2010; 402 (2): 186-9.
- [76] Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *J Biol Chem* 2010; 285 (33): 25221-31.
- [77] Li H, Bian C, Liao L, Li J, Zhao RC. miR-17-5p promotes human breast cancer cell migration and invasion through suppression of HBP1. *Breast Cancer Res Treat* 2011; 126 (3): 565-75.
- [78] Kennell JA, Gerin I, MacDougald OA, Cadigan KM. The microRNA miR-8 is a conserved negative regulator of Wnt signaling. *Proc Natl Acad Sci U S A* 2008; 105 (40): 15417-22.
- [79] Wang Z, Li Y, Ahmad A, Azmi AS, Banerjee S, Kong D, Sarkar FH. Targeting Notch signaling pathway to overcome drug resistance for cancer therapy. *Biochim Biophys Acta* 2010; 1806 (2): 258-67.
- [80] Zheng J, Jiang HY, Li J, Tang HC, Zhang XM, Wang XR, Du JT, Li HB, Xu G. MicroRNA-23b promotes tolerogenic properties of dendritic cells in vitro through inhibiting Notch1/NF- κ B signalling pathways. *Allergy* 2012; 67 (3): 362-70.
- [81] Cichocki F, Felices M, McCullar V, Presnell SR, Al-Attar A, Lutz CT, Miller JS. Cutting edge: microRNA-181 promotes human NK cell development by regulating Notch signaling. *J Immunol* 2011; 187 (12): 6171-5.

- [82] Liu XS, Chopp M, Zhang RL, Tao T, Wang XL, Kassiss H, Hozeska-Solgot A, Zhang L, Chen C, Zhang ZG. MicroRNA profiling in subventricular zone after stroke: MiR-124a regulates proliferation of neural progenitor cells through Notch signaling pathway. *PLoS One* 2011; 6 (8):e23461.
- [83] Pang RT, Leung CO, Ye TM, Liu W, Chiu PC, Lam KK, Lee KF, Yeung WS. MicroRNA-34a suppresses invasion through downregulation of Notch1 and Jagged1 in cervical carcinoma and choriocarcinoma cells. *Carcinogenesis* 2010; 31 (6): 1037-44.
- [84] Song G, Zhang Y, Wang L. MicroRNA-206 targets notch3, activates apoptosis, and inhibits tumor cell migration and focus formation. *J Biol Chem* 2009; 284 (46): 31921-7.
- [85] Kwon C, Han Z, Olson EN, Srivastava D. MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling. *Proc Natl Acad Sci U S A* 2005; 102 (52): 18986-91.
- [86] Brabletz S, Bajdak K, Meidhof S, Burk U, Niedermann G, Firat E, Wellner U, Dimmler A, Faller G, Schubert J, Brabletz T. The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. *EMBO J* 2011; 30 (4): 770-82.
- [87] Vallejo DM, Caparros E, Dominguez M. Targeting Notch signalling by the conserved miR-8/200 microRNA family in development and cancer cells. *EMBO J* 2011; 30 (4):756-69.
- [88] Fuxe J, Vincent T, Garcia de Herreros A. Transcriptional crosstalk between TGF- β and stem cell pathways in tumor cell invasion: role of EMT promoting Smad complexes. *Cell Cycle* 2010; 9 (12): 2363-74.
- [89] Wendt MK, Allington TM, Schiemann WP. Mechanisms of the epithelial-mesenchymal transition by TGF-beta. *Future Oncol* 2009; 5 (8): 1145-68.
- [90] Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res* 2008; 68 (10): 3645-54.
- [91] Smith AL, Iwanaga R, Drasin DJ, Micalizzi DS, Vartuli RL, Tan AC, Ford HL. The miR-106b-25 cluster targets Smad7, activates TGF- β signaling, and induces EMT and tumor initiating cell characteristics downstream of Six1 in human breast cancer. *Oncogene* 2012; 31(50): 5162-71.
- [92] Zhang Z, Liu S, Shi R, Zhao G. miR-27 promotes human gastric cancer cell metastasis by inducing epithelial-to-mesenchymal transition. *Cancer Genet* 2011; 204 (9): 486-91.
- [93] Stinson S, Lackner MR, Adai AT, Yu N, Kim HJ, O'Brien C, Spoerke J, Jhunjhunwala S, Boyd Z, Januario T, Newman RJ, Yue P, Bourgon R, Modrusan Z, Stern HM, Warming S, de Sauvage FJ, Amler L, Yeh RF, Dornan D. TRPS1 targeting by miR-221/222 promotes the epithelial-to-mesenchymal transition in breast cancer. *Sci Signal* 2011; 4 (177): ra41.
- [94] Dong P, Kaneuchi M, Watari H, Hamada J, Sudo S, Ju J, Sakuragi N. MicroRNA-194 inhibits epithelial to mesenchymal transition of endometrial cancer cells by targeting oncogene BMI-1. *Mol Cancer* 2011; 10: 99.
- [95] Chang CJ, Hsu CC, Chang CH, Tsai LL, Chang YC, Lu SW, Yu CH, Huang HS, Wang JJ, Tsai CH, Chou MY, Yu CC, Hu FW. Let-7d functions as novel regulator of epithelial-

- mesenchymal transition and chemoresistant property in oral cancer. *Oncol Rep* 2011; 26 (4): 1003-10.
- [96] Sun L, Yao Y, Liu B, Lin Z, Lin L, Yang M, Zhang W, Chen W, Pan C, Liu Q, Song E, Li J. MiR-200b and miR-15b regulate chemotherapy-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting BMI1. *Oncogene* 2012; 31 (4): 432-45.
- [97] Kumarswamy R, Mudduluru G, Ceppi P, Muppala S, Kozlowski M, Niklinski J, Papotti M, Allgayer H. MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snai1 and is downregulated in non-small cell lung cancer. *Int J Cancer*. 2012; 130 (9): 2044-53.
- [98] Vetter G, Saumet A, Moes M, Vallar L, Le Béhec A, Laurini C, Sabbah M, Arar K, Theillet C, Lecellier CH, Friederich E. miR-661 expression in SNAI1-induced epithelial to mesenchymal transition contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers. *Oncogene* 2010; 29 (31): 4436-48.
- [99] Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; 10 (5): 593-601.
- [100] Xiong M, Jiang L, Zhou Y, Qiu W, Fang L, Tan R, Wen P, Yang J. The miR-200 family regulates TGF- β 1-induced renal tubular epithelial to mesenchymal transition through Smad pathway by targeting ZEB1 and ZEB2 expression. *Am J Physiol Renal Physiol* 2012; 302 (3): F369-79.
- [101] Zhu X, Zhang X, Wang H, Song Q, Zhang G, Yang L, Geng J, Li X, Yuan Y, Chen L. MTA1 gene silencing inhibits invasion and alters the microRNA expression profile of human lung cancer cells. *Oncol Rep*. 2012; 28 (1): 218-24.
- [102] Hu H, Li S, Liu J, Ni B. MicroRNA-193b modulates proliferation, migration, and invasion of non-small cell lung cancer cells. *Acta Biochim Biophys Sin*. 2012; 44 (5): 424-30.
- [103] Kim NH, Kim HS, Li XY, Lee I, Choi HS, Kang SE, Cha SY, Ryu JK, Yoon D, Fearon ER, Rowe RG, Lee S, Maher CA, Weiss SJ, Yook JI. A p53/miRNA-34 axis regulates Snail1-dependent cancer cell epithelial-mesenchymal transition. *J Cell Biol*. 2011; 195 (3): 417-33.
- [104] Chen LT, Xu SD, Xu H, Zhang JF, Ning JF, Wang SF. MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis. *Med Oncol* 2012; 29 (3):1673-80
- [105] Kumarswamy R, Mudduluru G, Ceppi P, Muppala S, Kozlowski M, Niklinski J, Papotti M, Allgayer H. MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snai1 and is downregulated in non-small cell lung cancer. *Int J Cancer*. 2012; 130 (9): 2044-53.
- [106] Wang X, Ling C, Bai Y, Zhao J. MicroRNA-206 is associated with invasion and metastasis of lung cancer. *Anat Rec* 2011; 294 (1): 88-92.
- [107] Roybal JD, Zang Y, Ahn YH, Yang Y, Gibbons DL, Baird BN, Alvarez C, Thilaganathan N, Liu DD, Saintigny P, Heymach JV, Creighton CJ, Kurie JM. miR-200

- Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1/VEGFR1. *Mol Cancer Res* 2011; 9 (1): 25-35.
- [108] Ceppi P, Mudduluru G, Kumarswamy R, Rapa I, Scagliotti GV, Papotti M, Allgayer H. Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in non-small cell lung cancer. *Mol Cancer Res* 2010; 8 (9): 1207-16.
- [109] Jiang L, Huang Q, Zhang S, Zhang Q, Chang J, Qiu X, Wang E. Hsa-miR-125a-3p and hsa-miR-125a-5p are downregulated in non-small cell lung cancer and have inverse effects on invasion and migration of lung cancer cells. *BMC Cancer* 2010; 10: 318.
- [110] Zhang JG, Wang JJ, Zhao F, Liu Q, Jiang K, Yang GH. MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). *Clin Chim Acta* 2010; 411 (11-12): 846-52.
- [111] Crawford M, Brawner E, Batte K, Yu L, Hunter MG, Otterson GA, Nuovo G, Marsh CB, Nana-Sinkam SP. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. *Biochem Biophys Res Commun*. 2008; 373 (4): 607-12.