The role of microRNA in metastatic processes of non-small cell lung carcinoma

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Background. MicroRNAs are small non-coding one-stranded RNA molecules that play an important role in the post-transcriptional regulation of genes. Bioinformatic predictions indicate that each miRNA can regulate hundreds of target genes. MicroRNA expression can be associated with various cellular processes leading to the metastasis of malignant tumours including non-small cell lung carcinoma. This review summarizes current knowledge on the role of microRNAs in NSCLC metastasis to the brain and lymph nodes.

Methods. A search of the NCBI/PubMed database for publications on expression levels and the mechanisms of microRNA action in NSCLC metastasis.

Results and Conclusion. Dysregulation of microRNAs in NSCLC can be associated with brain and lymph node metastasis. There are differences in microRNA expression profiling between NSCLC with and without metastases but it is currently not possible to reliably predict the site of metastasis in NSCLC. Based on data from RNA microarrays, bioinformatics analysis is able to predict the target genes of highlighted microRNAs, providing us with complex information about cancer cell features such as enhanced proliferation, migration and invasion. Such microRNAs may then be knocked-down using siRNAs or substituted with miRNA mimics. RNA microarray profiling may thus be a useful tool to select up- or down-regulated microRNAs. A number of authors suggest that microRNAs could serve as biomarkers and therapeutic targets in the treatment of NSCLC metastasis.

Key words: microRNA, non-small cell lung carcinoma, brain metastasis, lymph node metastasis

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INTRODUCTION

Lung cancer is one of the most frequently diagnosed malignant tumours in developing and developed countries and the leading cause of cancer-related mortality worldwide¹. Non-small cell lung cancers (NSCLC) account for approximately 80% of all cases of lung cancers and have a 5-year survival rate of only 16% (ref.²) and almost 2/3 of patients with NSCLC are diagnosed at an advanced stage of disease³. 25% of non-small cell lung cancers metastasize to brain⁴. For this reason, predicting the metastatic potential of primary tumours is of paramount importance in providing potential prophylactic therapy.

In spite of ongoing research, there are currently no reliable biomarkers for predicting metastatic spread to different sites. One of the best candidates for this purpose are microRNAs since they are differentially expressed in different tissues.

MicroRNAs are small noncoding molecules of 19-24 nucleotides in length⁵. About 1/3 of human genes are mediated by miRNAs which negatively regulate gene expression by interfering with mRNA translation^{6,7}. Increasing evidence indicates that miRNAs may be a key player in the regulation of tumour cell invasion and metastasis in a variety of human cancers including NSCLC (ref.⁸). Nearly 50% of microRNAs are located in fragile regions of the human genome⁹. Each miRNA has many target genes

within pathways, while several miRNAs can regulate the same gene^{10,11}. Some microRNAs can regulate oncogenes/tumour suppressors as well^{12,13}. Some are differentially expressed in histological subtypes of cancers suggesting a dual function¹⁴. MiRNAs are ubiquitously deregulated in all kinds of cancers. By modulating either multiple targets or entire pathways, and by having unique expression profiles and greater stability in biological samples, miRNAs have quickly gained diagnostic and therapeutic value¹⁵.

The current review summarizes the potential role of selected miRNAs in the process of tumour spread to different sites and highlights future perspectives on verifying the role of different miRNAs in the process of tumour progression.

The role of miRNAs in brain metastasis

Few miRNAs have been studied in association with brain metastasis so far.

Western blot and RT-qPCR analyses (Hwang et al.¹⁶) showed that brain metastatic PC14PE6/LvBr4 cells had mesenchymal characteristics and higher invasiveness than PC14PE6 cells. These authors also found that miR-95-3p was down-regulated, cyclin D1 was up-regulated and actin binding LIM protein family member 2 (ABLIM2) was reduced in PC14PE6/LvBr4 cells. ABLIM2 is closely associated with cytoskeletal organization and biogenesis

and is the host gene of miR-95. MiR-95-3p suppressed cyclin D1 expression through direct binding to 3' UTR its mRNA and suppressed the invasiveness and proliferation of PC14PE6/LvBr4 cells. PE14PE6/LvBr4 cells showed decreased E-cadherin expression and N-cadherin upregulation, indicating that PE14PE6/ LvBr4 cells underwent EMT. Consistent with *in vitro* metastatic cells, the levels of miR-95-3p and ABLIM2 mRNA were decreased in brain metastatic tissues compared to lung cancer tissues, and higher cyclin D1 expression was connected to poor prognosis.

Surgical specimens and clinical records were obtained from 25 lung adenocarcinoma patients who had undergone surgical removal of primary tumours (N = 11) or brain metastases (N = 14) (ref.¹⁶). Since actin binding proteins are of importance in actin polymerization and are dynamically controlled during metastatic processes, Hwang et al. suggest that downregulation of ABLIM2 is closely related to the cytoskeletal organization of cancer cells¹⁶.

Cyclin D1 is an oncogene frequently over-expressed in various cancers including lung cancer¹⁷⁻²⁰. Cyclin D1 plays a key role in processes leading to acquisition of metastatic potential such as migratory and invasive properties¹⁶. Over-expression of cyclin D1 in glioma cells increases matrix metalloproteinase (MMP) activity and cellular motility²¹. Cyclin D1 is also down-regulated by miR-545 in lung cancer tissues compared to adjacent normal tissues and inhibits proliferation *in vivo* and *in vitro*²². Cyclin D1 is a key regulator of the cell cycle progression from the G1 phase to the S phase and is suspected to have an important role in the process of carcinogenesis and cancer progression²³. Macrophages, fibroblasts, and epithelial cells have, after depletion of cyclin D1, enhanced adhesion and reduced migration²⁴⁻²⁶, a process essential for metastasis. Cyclin D1 protein levels are induced by β -catenin overexpression and reduced in cells overexpressing the cadherin cytoplasmic domain. Increased β -catenin levels may thus promote neoplastic conversion by triggering cyclin D1 gene expression and consequently, uncontrolled progression into the cell cycle²⁷.

Chen et al. compared NSCLC tissues with brain metastasis (BM) to NSCLC tissues without BM and provided evidence that miR-378 overexpression is associated with NSCLC brain metastasis by promoting cell migration, invasion and tumour angiogenesis. Detailed investigation of the human NSCLC cell line A549 revealed that miR-378 promotes these properties by up-regulation of VEGF, MMP-2 and MMP-9, the key metalloproteinases involved in angiogenesis and blood vessel invasion through the extracellular matrix²⁸. Hua, et al. demonstrated that miR-378 binds to VEGF 3' UTR competing with miR-125a for the same seed region and promotes VEGF expression in a model of human nasopharyngeal carcinoma cells²⁹.

Arora et al. demonstrated that miR-330-3p expression was increased in NSCLC patient tissues and was involved in NSCLC brain metastasis³⁰. Liu et al. reported that miR-330-3p is up-regulated in NSCLC patient tissues compared to normal adjacent tissues. These authors carried out *in vitro* studies proving that over-expression of

miR-330-3p significantly enhanced cell proliferation in A549 and H23 cells transfected with miR-330-3p mimics compared to control miRNA mimic transfected cells. MiR-330-3p over-expression resulted in fewer cells in the G0/G1 phase and more cells in the G2/M and S phase. Their data also showed that the early growth response protein 2 (EGR2) expression level was decreased in miR-330-3p overexpressing cells compared to control cells. Moreover, EGR2 knockdown significantly increased cell proliferation. Taken together, the results of these authors show that EGR2 is a target of miR-330-3p (ref.³¹).

Unoki and Nakamura suggest that EGR2 is able to suppress growth in a variety of cancer cells significantly by mediating the PTEN signalling pathway³².

The results of the experiments of Remon et al. revealed three microRNAs involved in NSCLC brain metastasis. These are miR-197, miR-184 and miR-423-5p over-expressed in NSCLC BM+ patients compared to a control group of NSCLC BM- patients³³. MiRNA-197 overexpression is a potential biomarker for early detection of lung cancers and also correlates with metastasis³⁴. Elevated miR-197 expression is correlated with reduced Fus1 expression in NSCLC tumour, confirming that miR-197 targets the 3' UTR of the Fus1 transcript³⁵ and with reduced p53 tumour suppressor gene³⁶. FUS1 induces apoptosis through the activation of the intrinsic mitochondrial-dependent and Apaf-1-associated pathways and inhibits the function of protein tyrosine kinases including EGFR and AKT among others, in lung cancer³⁷. Inhibition of miR-197 promotes upregulation of p53-dependent apoptosis in lung cancers³⁶. MiR-184 suppresses cell proliferation by modulating cell cycle transition signals such as C-Myc, blocking cell growth mediated by PI3K/AKT/C-Jun pathway. MiR-184 is positively modulated by CCDC19 in NSCLC (ref.³⁸). These results are with accordance with an experiment on hepatocellular carcinoma where miR-184 overexpression increased cell proliferation, tumourigenicity and cell cycle progression. SOX7 was identified as a direct target of miR-184. The interaction led to downregulation of the SOX7 protein³⁹. SOX7 was found to be down-regulated in lung cancer⁴⁰. This protein together with SOX17 and SOX18 mediates various developmental processes and tumourigenesis⁴¹. SOX7 is a transcription factor that binds to β -catenin and negatively regulates its activity⁴². MiR-423-5p has been found to be related to cell proliferation and invasion in gastric cancer cells⁴³. There are no data describing miR-423-5p in lung cancers.

Microarray analysis of 5 primary and 3 brain metastatic lung adenocarcinoma samples performed by Zhao et al. showed five upregulated microRNAs: miR-9*, miR-1471, miR-718, miR-3656, miR-720 and 3 down-regulated microRNAs: miR-214, miR-145, miR-23a. The most significantly deregulated microRNAs: miR-145, miR-214 and miR-1471 were subsequently validated in an additional 35 primary and 8 BM lung adenocarcinoma samples using TaqMan quantitative PCR. Taken together these authors used 51 samples. 40 primary lung adenocarcinoma samples (20 samples with lymph node metastasis, 20 samples without) and 11 BM from lung adenocarcinoma. Using

functional assay Zhao et al. found that the expression of miR-145 can regulate the proliferation ability of A549 and SPC-A1 cells in vitro and is related to lymph node metastasis, migration and invasion. These results suggest that miR-145 may have a cell type specific function and play an important role in the process of BM in lung adenocarcinoma⁴⁴. Previous studies indicate that miR-145 acts as a metastasis suppressor, is down-regulated in NSCLC cells and has more direct targets. MiR-145 acts as tumour suppressor, inhibiting migration and invasion via inhibition of fascin 1 protein expression in NSCLC cells⁴⁵. The motility and invasiveness of human cancers can be associated with complex rearrangements of the actin cytoskeleton that are governed by several actin binding proteins. One actin-binding protein that has been linked to the invasive behaviour of carcinomas is fascin which serves to aggregate F actin into bundles⁴⁶. FSCN1 thus contributes to cell interactions, migration, intracellular movements, motility and cell invasion⁴⁷. Ye et al. showed that low miR-145 silenced by DNA methylation promotes NSCLC cell proliferation, migration and invasion by targeting mucin 1 (ref.⁴⁸). Mucin 1 is a heterodimeric protein that regulates critical pathways in oncogenesis including cell proliferation, self-renewal, tissue invasion, and apoptosis⁴⁹. Hsa-miR-145 targets EGFR and nucleoside diphosphate linked moiety X-type motif 1 (NUDT1) in lung adenocarcinoma cells. The expression of EGFR and NUDT1 was significantly down-regulated after miR-145 transfection in human lung adenocarcinoma cells⁵⁰. The role of NUDT1, also known as MTH1, is to hydrolyze 8-oxo-dGTP to the monophosphate form⁵¹. 8-oxo-dGTP is highly mutagenic, altered by oxidative stress and one of the main endogenous sources for spontaneous mutagenesis⁵². The induction of NUDT1 expression has been linked to oxidative stress and cellular proliferation and persistent oxidative stress in cancer cells may stimulate cellular proliferation⁵³. NUDT1 has been shown to be over-expressed in various human tumours including lung cancer⁵³⁻⁵⁶. Huber et al. deduced that since NUDT1 is thought to prevent incorporation of oxidized nucleotides into DNA, inhibitors of NUDT1 should increase the content of genomic 8-oxo-dGTP and thus induce DNA damage in cancer cells⁵⁷. MiR-145 may be thus of importance in cancer treatment.

Arora and colleges performed microarray profiling on samples of clinically matched NSCLC from seven patients with brain metastasis and six without brain metastasis. Statistical analysis and qRT-PCR validation confirmed eight microRNAs to be significantly differentially expressed. Of these, miR-328 and miR-330-3p were able to correctly classify BM⁺ versus BM⁻ patients. In vitro studies carried out on A549 cell line showed that A549 that over-expressed miR-328, had several signalling pathways affected such as interleukin-1 (IL-1) signalling, VEGF signalling, and PDGF signalling. VEGF/IL1 signalling pathway, and several of the miR-328 target genes play a key role in controlling this signalling pathway leading to loss of cell adhesion and increased migration. These target genes include PRKCA, VEGFD, NOTCH1, IL1-alpha, IL1-beta, and PLC-gamma among others. PRKCA (protein kinase C, alpha), which was one of the genes

up-regulated in A549-328 cells, was also over-expressed at the protein level in A549-328 cells compared to A549-empty cells. Moreover, A549-328 cells had significantly increased cell migration compared to A549 cells which were significantly reduced after PRKCA knockdown³⁰. IL1-beta via PRKCA induces the expression of uPA (urokinase plasminogen activator), which leads to migration of A549 NSCLC cells⁵⁸. uPA is a plasminogen activator, one of the serine proteases originally described as matrix- and fibrin-degrading enzymes. The expression of such proteases is strictly regulated and involved in physiological and pathological processes such as cancer invasion and metastasis⁵⁹. Notch signalling, comprised of four receptor isoforms (Notch1, Notch2, Notch3, Notch4) and five ligands members (Delta-like 1, Delta-like 3, Delta-like 4, Jagged-1 and Jagged-2), is crucial for cell proliferation, differentiation and apoptosis⁶⁰. Deregulated Notch signalling leads to various diseases, such as T cell leukemia and lung cancer^{61,62}. VEGF expression in lung cancer cells could be attributed to increased activity of the JAK/STAT3 signalling pathway which is associated with decreased survival rate⁶³. Overexpression of VEGF in NSCLC may predict poor prognosis⁶⁴. PLC-gamma has been found to be connected to tumour cell motility required for invasiveness and metastasis⁶⁵. Activation of this protein lies upstream of cytoskeletal reorganization. PLCγ is at the convergence point of various signalling pathways leading to spreading and motility⁶⁶.

MiRNAs involved in lymph node metastasis

An example of a microRNA involved in lymph node metastasis is cited by Meng et al. who claim that miR-31 is upregulated in lung adenocarcinoma tissues from patients with lymph node metastases compared with those without lymph node metastases. In vitro assay on human lung ADC cell lines H23, H1573, and H2228 showed that miR-31 increases cell migration, invasion and proliferation in an ERK1/2 signalling-dependent manner. Meng et al. observed reduction in ERK1/2 signalling in miR-31 knockdown cell line, suggesting that ERK1/2 pathway activation is a main mediator of the miR-31-induced invasive phenotype. After miR-31 overexpression in H23 cells, the expression of several EMT-activating markers such as Vimentin, TWIST1 and SNAI1 were increased⁶⁷.

Vimentin is a type III intermediate filament, a typical marker for the mesenchymal cell⁶⁸. It maintains the cell shape in a quiescent cell, is involved in the highly dynamic remodelling of the cytoskeleton in motile cells⁶⁹ and is upregulated during EMT in epithelial cells⁷⁰, resulting in more mesenchymal phenotype and motile behaviour⁷⁰⁻⁷². Gain of vimentin expression and loss of E-cadherin expression are considered to be the most important molecular markers of EMT (ref.⁷³). Twist is a helix-loop-helix transcription factor which promotes EMT by down-regulating E-cadherin directly or interacting with other transcription factors⁷⁴. Phosphorylation and nuclear localization of SNAI1, governed by PI3K and Wnt signalling pathways, are critical in SNAI1 regulation. SNAI1 has a key role in the regulation of EMT. SNAI1-induced EMT involves the loss of E-cadherin and claudins with accompanying

upregulation of vimentin and fibronectin, among other biomarkers⁷⁵. However, it remains unclear whether there is a connection between ERK signalling activation and EMT (ref.⁶⁷).

Lymphovascular invasion is positively correlated with the increased expression of miR-183 in lung adenocarcinoma tissues compared to lung adenocarcinoma without lymphovascular invasion. Together with miR-183, miR-210 was also shown to be upregulated and miR-34 was down-regulated in LAD compared to normal control lung tissue by microarray method. Pak et al. also showed that miR-183 expression was significantly higher in adenocarcinoma with EGFR exon 19 mutations than those without mutation. Pak et al. assume that miR-183 could play an oncogenic role in conjunction with EGFR amplification, PTEN loss, and AKT activation⁷⁶. miR-183 knockdown of tumour cell lines caused deregulation of a miRNA network composed of miR-183-EGFR-PTEN in synovial sarcoma, RMS, and colon cancer cell lines and the zinc finger transcription factor early growth response-1 (EGR-1) (ref.⁷⁷), known as a gene essential for growth, proliferation, or differentiation, is a direct regulator of PTEN (ref.⁷⁸). MiR-183 has a potential oncogenic role through the regulation of 2 tumour suppressor genes, EGR1 and PTEN and the deregulation of this fundamental miRNA regulatory network may be central to many tumour types⁷⁷.

MiR-181b expression in 126 pairs of surgically removed NSCLC tissues and their corresponding normal lung tissues was measured by real-time quantitative RT-PCR. The results showed that miR-181b expression was significantly down-regulated in NSCLC tissues compared to their normal counterparts. The low miR-181b expression was found to be closely correlated with larger tumour size, higher p-TNM stage and positive lymph node metastasis of NSCLC patients⁷⁹. Neuropilin-1 (NPR-1) was identified as a target of miR-181b and down-regulated by miR-181b. NPR-1 is a mediator of lung branching and angiogenesis in embryonic development and angiogenesis in cancer^{80,83}. NPR-1 overexpression was found in NSCLC tissues compared to normal adjacent tissues and this demonstrated that NPR-1 might be closely correlated with invasion and metastasis of NSCLC (ref.⁸⁴).

Sun et al. compared normal lung tissues, NSCLC-lymph node positive and -lymph node negative. The results demonstrated that the relative levels of miR-124 were decreased in NSCLC-lymph node positive tissues. MiR-124 was also suppressed in highly aggressive cell lines H522 and H1975. MYO10 was subsequently identified as a novel functional downstream target of miR-124, and was up-regulated in node-positive NSCLC tissues. Knockdown of MYO10 inhibited cell migration, whereas forced MYO10 expression markedly rescued miR-124-mediated suppression of cell metastasis. Further experiments showed that miR-124 is down-regulated by NF- κ B (ref.⁸⁵). Myo10 is a motor protein best known for its role in filopodia formation⁸⁶. To escape the primary tumour, cancer cells also build structures called invadopodia, actin-rich protrusions with proteolytic activity are capable of digest-

ing surrounding extracellular matrix. Myo10 is the key component of invadopodia⁸⁷.

Sun et al. continued with the research and found increased levels of IL-6 and IL-8 through NF- κ B, in highly invasive cells. They demonstrated that the IL-6-mediated activation of the NF- κ B pathway could suppress the expression of miR-124, promoting MYO10 expression and subsequent cell aggressiveness⁸⁵. It was also demonstrated that IL-6-mediated STAT3 activation could suppress the expression of miR-124 by downregulation of HNF4 α , which could increase miR-124 levels by binding to the promoter⁸⁸. Another study, Xie et al. revealed that miR-124 is under-expressed in NSCLC samples compared to adjacent normal tissues. Their experiments also indicated that miR-124 directly targeted and decreased SOX8 in NSCLC cell lines. Moreover, increased SOX8 expression was detected in tumour samples which correlated to lymph node metastasis⁸⁹. There are few reports on SOX8 in cancer. Zhang et al. reported that SOX8 promoted cancer cell proliferation in vitro and that its expression correlated with elevated β -catenin levels in hepatocellular carcinoma⁹⁰.

There is another microRNA whose biological function in NSCLC remains controversial. Zhu et al. showed that miR-224 expression levels were significantly down-regulated in NSCLC compared to corresponding noncancerous lung tissues and in human NSCLC cell lines A549, H460, 95D, and H358 compared to normal human bronchial epithelial cell line 16HBE. In addition, decreased miR-224 expression was significantly lower in samples with lymph node metastasis and advanced TNM stage. Low miR-224 levels were found in 60% of patients with lymph node metastasis, and in 28.6% of patient samples without lymph node metastasis. Furthermore, transfection of miR-224 mimics in NSCLC A549 cells was able to reduce cell proliferation, invasion, and migration, and promote cell apoptosis⁹¹. On the other hand, Cui et al. compared primary lung adenocarcinomas with lymph node metastasis and primary lung adenocarcinomas without lymph node metastasis. Of 12 up-regulated miRs, only miR-31 positively associated with lymph node metastasis and negatively correlated with survival in patients with LAD. Further analysis using NSCLC cases (LAD+SCC) and normal adjacent tissues showed that miR-224 was significantly up-regulated in both LAD and SCC compared to normal tissues. In addition, Cui et al. compared ADC patients with available staging and miRNA expression data, patients with and patients without LNM, and found that miR-224 was markedly up-regulated in patients with LNM compared to those without LNM. They further evaluated miR-224 expression in metastasized lung cancer tissues and primary lung cancer tissues and found stronger miR-224 expression in metastasized lung cancers compared with primary lung cancers. Further analysis showed that miR-224 expression promotes cell migration, invasion, and proliferation by directly targeting and significantly decreasing tumour suppressors TNF α -induced protein 1 (TNFAIP1) and SMAD4 in both LAD and SCC. Moreover, they found promoter hypermethylation and activated ERK signalling to be involved in the regulation of miR-224 expression in

NSCLC. Up-regulated miR-224 facilitates tumour progression by shifting the equilibrium of the partially antagonist functions of TNFAIP1 and SMAD4 to enhanced invasion and growth in NSCLC (ref.⁹²). It has been reported that TNFAIP1 stimulates DNA polymerase delta activity in vitro, suggesting that TNFAIP1 might be involved in DNA synthesis and apoptosis^{92,93}. SMAD4 mediates TGF-beta signal transduction⁹⁴. Aberrant TGF-beta signal transduction is a common feature of lung cancers. Inactivation of SMAD4 is thought to play a role in the inactivation of TGF-beta signalling⁹⁵. Cui et al. reported that caspase3 (CASP3) and caspase7 (CASP7) are targets of miR-224 in NSCLC, and that miR-224 promotes lung cancer cell proliferation and migration in part by directly targeting CASP7 and downregulating its expression. In addition, miR-224 attenuated TNF- α induced apoptosis by direct targeting of CASP3 resulting in reduction of cleaved PARP1 expression in lung cancer cells⁹⁶. Both CASP3 and CASP7 are effector caspases that perform the downstream execution steps of apoptosis by cleaving important cellular substrates⁹⁷. CASP7 is highly related to CASP3. These two caspases are activated in both death receptor- and mitochondria- induced apoptosis^{98,99}.

Ge et al. used the RT-PCT method to assess the expression levels of miR-148b. The results showed that the expression of miR-148b was decreased in NSCLC tissues compared to corresponding adjacent normal lung tissues. A low miR-148b expression was measured in 77.5% of NSCLC samples LNM+ and in only 38.2% of NSCLC samples LNM-, suggesting that miR-148b might be associated with lymph node metastasis¹⁰⁰. Serum mir-148b together with miR-148a, miR-152 and miR-21 were dysregulated in NSCLC patients compared to healthy controls. The first three microRNAs may associate with advanced stage, large tumour size, malignant cell differentiation, and lymph node metastasis. The microRNA levels were as follows: low mir-148b expression was observed in 55.1% of NSCLC LNM+ patients and in 44% of NSCLC LNM- patients; low miR-148a levels were found in 57.5% of NSCLC LNM+ patients and in 32% of NSCLC LNM- patients; the miR.152 was down-regulated in 54.3% of NSCLC LNM+ and in 48% of NSCLC LNM- patients. High expression of miR-21 correlated only with large size tumour and advanced cancer stage¹⁰¹.

Functional assays demonstrated that miR-148a decreased Rho-associated protein kinase 1 (ROCK1) protein expression in NSCLC cells and inhibited EMT (ref.¹⁰²). MiR-148b functions as a suppressor of cell proliferation and migration in NSCLC. The suppression is caused by the means of direct targeting a pro-oncogene, a carcinoembryonic antigen (CEA) (ref.¹⁰³), widely upregulated in NSCLC specimens. This pro-oncogene is involved in cell-cell recognition and modulates cellular processes that range from the shaping of tissue architecture to neovascularization¹⁰⁴. Su et al. described the ADAM metallopeptidase domain 17 (ADAM17) as a direct target of miR-152, down-regulated in NSCLC. ADAM17 inversely correlates with miR-152 in NSCLC tissues. ADAM17 silencing by miR-152, induced suppression of cell proliferation, colony formation, migration and invasion¹⁰⁵. Another direct tar-

get of miR-152 was fibroblast growth factor 2 (FGF2). FGF2 knockdown suppressed cell proliferation, colony formation, migration and invasion in the A549 NSCLC cell line. FGF2 also plays essential roles in tumour angiogenesis¹⁰⁶.

Wan et al. showed significant downregulation of miR-409-3p in primary lung adenocarcinoma, compared to corresponding noncancerous tissues, and this correlated with aggressive phenotype, poor prognosis and higher incidence of LNM. 60.5% of NSCLC LNM+ patients had low miR-409-3p expression and only 39.5% of NSCLC LNM- patients had low miR-409-3p expression. Further investigations indicated that miR-409-3p directly targeted the hepatocyte growth factor receptor (c-Met). C-Met inversely correlated with miR-409-3p expression in human lung adenocarcinoma tissues¹⁰⁷. C-Met is a receptor tyrosine kinase that after binding with hepatocyte growth factor, activates the Akt signalling pathway among others¹⁰⁸. C-Met plays critical roles in growth, motility, migration and invasion^{109,110} and has been found to be aberrantly activated in various human cancers via mutation, amplification and/or protein expression¹¹¹.

Yu et al. observed down-regulation of miR-193-3p and miR-193-5p in NSCLC; and in highly metastatic cells A549, H1299 and SPC-A-1sci compared to NSCLC weakly metastatic cells LC-21, SPC-A-1 and H358 in vitro. Decreased levels of these miRNAs significantly associated with tumour node metastasis and lymph node metastasis. Median expression levels of miR-193-3p in NSCLC samples with metastasis were 0.768 ± 0.714 and in NSCLC samples without metastasis 1.340 ± 0.978 . A similar situation was observed in measurement of miR-193-5p where the median expression 1.769 ± 1.443 was lower in NSCLC samples with metastasis and higher expression 2.882 ± 2.822 was found in NSCLC samples without metastasis. ERBB4 and S6K2 were identified as direct targets of miR-193-3p and PIK3R3 and mTOR were the direct targets of miR-193-5p. All four targets are potential metastasis promoters. MiR-193a-3p/5p might function as a tumour suppressor and inactivate PIK3R3 /Akt/mTOR signalling pathway and ERBB signalling pathway¹¹². The ErbB signalling network instructs the execution of key cellular processes, such as cell survival, proliferation and motility, through the signals of defined strength and duration. In contrast, unabated ErbB signalling disrupts tissue homeostasis that leads to cell transformation¹¹³. The PI3K/Akt/mTOR signalling pathways are crucial to many physiological and pathological conditions, such as cell proliferation, survival, differentiation, adhesion, motility and invasion¹¹⁴. Dysregulation of the PI3K/AKT/mTOR pathway is involved in lung tumourigenesis and it has been associated with high grade tumours (G3-G4) and advanced disease (stage III) (ref.¹¹⁵). Accordingly, inhibitors of PI3K signalling have been suggested as potential therapeutic agents in NSCLC. Dereglulation of this pathway occurs through a variety of mechanisms including activation of tyrosine kinase receptors upstream of PI3K, PIK3CA amplification as well as mutations in KRAS, PI3K or AKT, or loss of negative regulation by the tumour suppressor gene PTEN (ref.¹¹⁴).

He et al. showed that the expression levels of miR-675-5p in NSCLC tissues were significantly reduced compared to those in adjacent non-cancerous tissues. 89% of NSCLC metastasis positive patients and 61% of NSCLC metastasis negative patients had low expression levels of miR-675-5p suggesting that there is a negative correlation with lymph node metastasis. Further assay showed that G protein-coupled receptor 55 (GPR55) was a direct target gene of miR-675-5p. GPR55 up regulation contributes to aggressive behaviour in various cancer types. Overexpression of miR-675-5p can lead to the down regulation of GPR55 and its signalling pathway. The study of He et al. also examined the expression of GPR55 signalling downstream target genes and found that the expression of p-ERK, cyclin D1, MMP9 and MMP2 were decreased in NSCLC A549 and HTB-182 cells that stably over-expressed miR-675-5p (ref.¹¹⁶). P-ERK is an important component in cell survival. When p-ERK was inhibited, cell viability decreased and the expression of p-c-Jun and cyclin D1 was reduced¹¹⁷. Cyclins act together with their CDK partners to drive cells from one phase of the cell cycle to the next¹¹⁸. Expression of cyclin D1 is induced by numerous mitogens. For example, stimulation of the PI3K/Akt pathway leads to activation of NF- κ B, a transcription factor that induces production of cyclin D1 (ref.¹¹⁹). Current data indicate that cyclin D1 overexpression is a pivotal element in the process of malignant transformation in the lung and other tissues¹¹⁸. Matrix metalloproteinases are the key mediators of the extracellular matrix that regulates multiple cellular functions required for tumour invasion¹²⁰⁻¹²³. MMP9 is an important matrix proteinase that degrades collagen type IV, the major component of the basement membrane. Overexpression of MMP9 often facilitates cancer metastasis. MMP-2 has also been reported to maintain extracellular matrix rigidity and promote motility and proliferation^{124,125}.

Interesting results were also presented by Wang et al. who measured the expression levels of miR-29b. Quantitative RT-PCR analysis of fresh normal lung tissue versus lung tumour tissue showed that the relative expression of miR-29b in normal tissues was -0.463. The expression level of miR-29b in NSCLC patients with lymphatic metastasis was -2.389 ± 1.302 and in NSCLC patients without lymphatic metastasis was higher: -1.505 ± 0.799 . Moreover, in vitro studies revealed that miR-29b suppresses cell proliferation, migration and invasion in A549 cells indicating that upregulation of this microRNA might be involved in the metastasis of NSCLC. MiR-29b targeted matrix metalloproteinase 2 (MMP2) and phosphatase and tensin homolog (PTEN). Following experiments showed that miR-29b regulated MMP2 negatively but there were no obvious changes in PTEN mRNA or protein expression levels in miR-29b transfected or miR-29b inhibitor transfected cells¹²⁶. PTEN regulates tumour cell growth, cell cycle, apoptosis and metastasis by regulating multiple signalling pathways negatively^{127,128}.

Tang et al. aimed to identify the relationship between miR-203 expression and its clinicopathological significance in NSCLC patients. The relative level of miR-203 was 6.12 ± 6.25 in NSCLC tissues, remarkably more down-

regulated than that of paired normal tissues: 7.88 ± 5.56 . MiR-203 expression negatively correlated with lymphatic metastasis. The relative level was 7.89 ± 5.67 in NSCLC patients without lymphatic metastasis and 4.69 ± 6.37 in NSCLC lymphatic metastasis. In vitro experiments confirmed the suppressive role of miR-203 mimic on the cell growth of H460, A549, H1299, PC9 and H292 NSCLC cells¹²⁹. Other authors have described miR-203 target genes in NSCLC. Wang et al. demonstrated that miR-203 played a role in proliferation, apoptosis and migration by targeting PKC α (ref.¹³⁰). PKC α has been shown to bind and phosphorylate the scaffold protein discs large homolog 1 (DLG1) and promote cell migration in NSCLC cells¹³¹. Jin et al. identified survivin as a direct gene inhibited by miR-203 (ref.¹³²). Survivin is a suppressor of apoptosis in the inhibitor of apoptosis protein gene family¹³³. Saini et al. suggest that survivin regulated by miR-203 promotes metastasis of prostate cancer¹³⁴. Wang et al. reported that miR-203 might restrain cell proliferation and migration and promote cell apoptosis of lung cancer cells by targeting SRC, a non-receptor tyrosine kinase which plays an essential role in cancer development since it can promote survival, angiogenesis, proliferation and invasion pathways¹³⁵.

According to Ren et al., miR-92a belongs to the group of microRNAs that are significantly upregulated in NSCLC compared to adjacent normal tissues. Its expression is connected with lymph node metastasis. 3.33 ± 0.42 was the miR-92a expression in NSCLC lymph node positive patients. In contrast, 2.11 ± 0.29 only was the expression level of miR-92a in NSCLC lymph node negative patients. Upregulation of miR-92a by miR-92a mimic in A549 cells significantly enhanced migration and invasion, while downregulation of miR-92a by anti-miR-92 in A549 cells significantly inhibited migration and invasion as expected. TargetScan6.2 and miRanda, western blotting and qRT-PCR showed that PTEN, a tumour suppressor gene, is a direct target of and down-regulated by miR-92a (ref.¹³⁶). It is established that PTEN functions as a negative regulator of the PI3K/Akt pathway via dephosphorylation of PI (3,4,5)P3 (ref.¹³⁷).

Chen et al. evaluated the expression of miR-30b in NSCLC (ref.¹³⁸). The median $\pm$ SD expression levels of miR-30b were associated with lymph node metastasis as follows: NSCLC patients LNM+ had 0.2147 ± 0.0670 levels; NSCLC patients LNM- had 0.3129 ± 0.1234 levels. Lower levels of miR-30b were in NSCLC compared to adjacent normal tissue. Furthermore, these authors evaluated the expression of collagen triple helix repeat containing 1 (Cthrc1). The median $\pm$ SD expression levels of Cthrc1 inversely correlated with miR-30b expression: NSCLC patients LNM+ had 2.4555 ± 0.3352 expression levels and NSCLC patients LNM- had 2.1952 ± 0.3290 levels. In vitro examinations revealed that miR-30b inhibits cell invasion and migration through targeting and downregulating Cthrc1 of A549 and Calu-3 cells. Not only was the expression of Cthrc1 decreased, MMP-2 and MMP-9 expression was also reduced while TIMP-1 expression was increased in miR-30b mimic transfected A549 and Calu-3 cells¹³⁸. TIMP1 regulates extracellular

matrix catabolism but the major effect in tumour tissue is promotion of cell growth and anti-apoptotic activity¹³⁹.

Ectopic up-regulation of *Cthrc1* in cancer cells might result in elevated invasive and proliferative abilities. The biological effect of *Cthrc1* on metastasis and proliferation is mediated by the activation of the Wnt/ β -catenin pathway¹⁴⁰.

Interesting research was reported by Yan et al. who evaluated expression of miR-761 not only in NSCLC patients' tissues and cell lines but also in NSCLC patients' serum. MiR-761 was significantly upregulated in both NSCLC serum and tissues compared to normal participants and paired noncancerous tissues. High expression levels of miR-30b were measured in 66.6% of NSCLC LNM+ patients while there were no NSCLC LNM- patients with high expression levels of miR-761 at all. Ectopic expression of miR-761 promoted cell proliferation and metastasis in human lung cancer H460 cells, while miR-761 inhibitor reduced proliferation rates and metastasis in human lung cancer H23 cell line. Furthermore, luciferase reporter assay and functional analyses indicated that miR-761 directly targeted *ING4* and *TIMP2*. Expression of miR-761 is negatively correlated with *ING4* and *TIMP2* in NSCLC (ref.¹⁴¹). *TIMP2* promotes upregulation of E-cadherin/ β -catenin complex, inhibits MMP-2 blood vessel formation, inactivating receptor tyrosine kinases including VEGFR-2 and FGFR-2 through de-phosphorylation mediated by SHP-1 of A549 cells¹⁴². *ING4* was found to down-regulate interleukin IL-6, IL-8, MMP-2 and MMP-9 expression in the human lung adenocarcinoma cell line A549 (ref.¹⁴³). *ING4* has been shown to interact physically with the p65 subunit of nuclear factor (NF)- κ B and regulate angiogenesis through transcriptional repression of NF- κ B-responsive genes in brain tumours¹⁴⁴.

Noticeable down-regulated expression of miR-375 was detected in 89% of NSCLC samples compared to matched healthy tissue. The means $\pm$ SD Δ CT value of miR-375 in NSCLC tissue was 12.13 ± 1.62 compared with 8.47 ± 0.86 in adjacent healthy tissue. Decreased level of miR-375 was significantly correlated with advanced disease stage and lymphatic metastasis. The mean $\pm$ SD Δ CT value of miR-375 in NSCLC tissues with positive lymphatic metastasis was 14.39 ± 1.52 and in NSCLC tissues with negative lymphatic metastasis was 10.22 ± 1.05 (ref.¹⁴⁵). Moreover, the plasma level of miR-375 was examined. The levels of plasma miR-375 were significantly lower in 61.5% of NSCLC patients with metastasis than those in 42.4% of patients without metastases¹⁴⁶. The downregulation of miR-375 is mainly caused by aberrant promoter hypermethylation¹⁴⁷. MiR-375 could bind directly to the 3'-untranslated region of *AEG-1*, an important oncogene involved in multiple aspects of cancer development and progression¹⁴⁸. *AEG-1* induces epithelial-mesenchymal transition in lung cancer through activating Wnt/ β -catenin signalling¹⁴⁹.

Lan et al. described the validated clinical significance of miR-133a expression and clinicopathological significance in NSCLC. The evaluated mean $\pm$ SD $2^{-\Delta\Delta Cq}$ miR-133a

expression by qRT-PCR in NSCLC tissues was 2.0108 ± 1.3334 , significantly lower than that of the adjacent non-cancerous tissue 3.6430 ± 2.2625 . MiR-133a expression was 1.8035 ± 1.3079 in NSCLC patients with lymph node metastasis than in those without 2.0787 ± 1.3777 . The expression was also negatively correlated to tumour size, TNM stages and EGFR protein expression¹⁵⁰. An example of the one microRNA targeting more genes provides the experiments revealing the biological functions of miR-33a. This microRNA, down-regulated in malignant lung cancer cells, directly suppresses multiple oncogenic membrane receptors including IGF-1R, TGFBR1 and EGFR thus inhibits cell invasive capacity in NSCLC (ref.¹⁵¹). Wang et al. found that the expression levels of miR-133a were higher in NSCLC cells with low invasiveness (EKVX and H23) than in those with high invasive ability (A549, H441 and H1299) suggesting that miR-133a is dysregulated in lung cancer and that the expression of miR-133a negatively correlates with cell invasiveness. The expressions of EGFR and IGF-1R were decreased by the ectopic expression of miR-133a in CL1-5 and A549 cells. Downregulation of TGFBR1 expression was observed in A549 cells but not in CL1-5 cells. MiR-133a regulates AKT activity through suppressing its downstream targets of IGF-1R and TGFBR1. MiR-133a regulates IGF-1 and TGF- β -mediated downstream signalling through down-regulating their receptors. In vivo studies showed that miR-133a suppresses cancer metastasis in mice model¹⁵⁴. IGF-1R signalling pathway has important roles in tumorigenesis, metastasis, and resistance to existing forms of anti-cancer therapy. Targeting the IGF signalling pathway might be one evolving anticancer therapy with potential efficacy¹⁵². TGFBR1 pathway is critical for cell growth and differentiation. In the pathway, TGF- β binds directly to TGFBR2, and is then recognized by TGFBR1, which is phosphorylated and activated by TGFBR2 (ref.¹⁵³). TGF- β signalling alterations have been identified in development and progression of several epithelial-type human malignancies, including NSCLC (ref.¹⁵⁴⁻¹⁵⁶). Constitutively decreased type I TGF- β receptor (TGFBR1) expression is emerging as a novel tumour-predisposing phenotype¹⁵⁷. EGFR signalling plays a role in cell growth, differentiation, survival and proliferation¹⁵⁸. The strength and duration of the signal are strictly controlled and dysregulation of this signalling pathway can lead to carcinogenesis^{159,160}.

MiRNA involved in bone metastasis

Guo et al. showed that the expression of miR-21 in NSCLC patients with bone metastasis was significantly higher than that in patients with bone tuberculosis. This study demonstrated that miRNA-21 promoted H2170 NSCLC cell proliferation by inhibiting cell apoptosis via the increase of COX-19 expression¹⁶¹. COX-19 is a protein that affects COX subunit assembly, which subsequently influences COX activity¹⁶². Alteration of COX activity induces cell apoptosis¹⁶³.

The microRNAs involved in NSCLC metastasis formation are summarized in Fig 1.

Fig. 1. Altered microRNA expression in NSCLC leading to metastasis spread to brain, lymph nodes and bones.

Current in vitro studies selecting potential miRNAs involved in the process of migration and metastasis of NSCLC cell lines.

Based on the information that the expressions of miR-193b in cancer tissues are significantly lower than those in normal lung tissues, Hu et al. further investigated the effects of miR-193b on A549 cell proliferation, migration, invasion and potential target genes. The results showed that miR-193b inhibits cell proliferation and triggers G1 phase arrest, suppresses the migration and invasion and represses the expression of CCND1 and urokinase-type plasminogen activator (uPA) directly in A549 cells with miR-193b transfection. The data showed that the expression levels of CCND1 and uPA in NSCLC tissues were higher than those in adjacent normal tissues¹⁶⁴. Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle¹⁶⁵. CCND1 forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, and therefore controls cell cycle G1/S transition. Mutations and overexpression of this gene altering the cell cycle progression, are observed in a variety of tumours and may contribute to tumourigenesis^{166,167}. The uPA is a serine protease which catalyzes the conversion of the inactive zymogen plasminogen into the active broad-spectrum plasmin. Plasmin degrades matrix proteins and activates other proteases, including matrix metalloproteinases¹⁶⁸. Through degrading the extracellular matrix proteins, uPAs regulate cancer progression processes including cancer cell motility, extracellular matrix invasion, and metastasis¹⁶⁹.

Savita and Karunakaran generated a stable clone of miR-106b-25 cluster and the empty vector in NSCLC

H1299 cells. Expression of β -TRCP2 mRNA was significantly lower in H1299 with miR-106b-25 cluster than in empty vector cells. SNAIL protein expression was higher in H1299 with miR-106b-25 cluster than in empty vector cells. MiR-106-25 cluster induced migration, invasiveness, and colony formation of H1299 cells. These data indicate that MiR-106-25 cluster may play an important role in the metastasis of human NSCLC (ref.¹⁷⁰). β -TRCP is a member of the ubiquitin ligase complex that ubiquitinates many proteins such as IjBa, WEE1, b-catenin and SNAIL (ref.¹⁷¹).

MiR-33a, an intronic miRNA located within the sterol regulatory element-binding protein 2 (SREBP-2) gene, is expressed at low levels in the metastatic NCI-H1299 NSCLC cell line and is inversely correlated with the expression of its target gene, Twist1. Twist1 knockdown blocks EMT-related metastasis and forced expression of miR-33a inhibits lung cancer metastasis in a xenograft animal model suggesting that miR-33a targets Twist1 and inhibits invasion and metastasis in NSCLC clinically. MiR-33a is found to be at low levels in NSCLC patients and down-regulation of miR-33a predicts poor prognosis¹⁷². Twist1 promotes EMT in part, by directly repressing E-cadherin expression by recruiting the nucleosome remodelling and deacetylase complex for gene repression and by upregulating Bmi1, AKT2, YB-1, etc. (ref.¹⁷³).

The metastasis-associated gene 1 (MTA1) is one of the metastasis-related genes which is over-expressed in numerous carcinomas including NSCLC (ref.¹⁷⁴). For this reason, Zhang et al have tried to identify microRNAs that suppress malignancy in HEK293T, SPC-A-1 and 95D NSCLC cells by targeting MAT1. Among candidate mi-

Table 1. Contribution of dysregulated microRNAs and their downstream target genes to metastatic processes of NSCLC tissues and cells.

MicroRNA	Affected gene	Biological function of the gene
miR-95-3p ↓	cyclin D1 ↑	cell cycle progression
	ABLIM2 ↓	cytoskeletal organization
miR-378 ↑	VEGF ↑	angiogenesis
	MMP-2 ↑	angiogenesis
	MMP-9 ↑	angiogenesis
miR-330-3p ↑	EGR2 ↓	proliferation
miR-197 ↑	FUS1 ↓	apoptosis inducer
miR-184 ↑	SOX7 ↓	tumorigenesis
miR-145 ↓	fascin1 ↑	invasion
	NUDT1 ↑	oxidative stress
	EGFR ↑	growth
miR-328 ↑	PRKCA ↑	adhesion
	VEGFD ↑	adhesion
	NOTCH1 ↑	adhesion
miR-31 ↑	vimentin ↑	EMT
	TWIST1 ↑	EMT
	SNAI1 ↑	EMT
miR-183 ↑	EGFR ↑	growth and proliferation
	PTEN ↓	growth and proliferation
	AKT ↑	growth and proliferation
miR-181b ↓	NPR-1 ↑	angiogenesis
miR-124 ↓	MYO10 ↑	migration
	SOX8 ↑	proliferation
miR-224 ↑	TNFAIP1 ↓	invasion proliferation and migration
	SMAD4 ↓	invasion, proliferation and migration
	CASP7 ↓	invasion, proliferation and migration
miR-148b ↓	CEA ↑	proliferation and migration
miR-148a ↓	ROCK1 ↑	EMT
miR-152 ↓	ADAM17 ↑	invasion, proliferation and migration
	FGF2 ↑	invasion, proliferation and migration
miR-409-3p ↓	c-Met ↑	growth, motility, invasion, migration
miR-193-3p ↓	ERBB4 ↑	survival, motility, proliferation
	S6K2 ↑	survival, motility, proliferation
miR-193-5p ↓	PIK3R3 ↑	survival, motility, proliferation
	mTOR ↑	survival, motility, proliferation
miR-675-5p ↓	GPR55 ↑	cell survival
miR-29b ↓	MMP2 ↑	angiogenesis
miR-203 ↓	PKCα ↑	proliferation, apoptosis, migration
	survivin ↑	apoptosis
miR-92a ↑	PTEN ↓	growth and proliferation
miR-30b ↓	CTHRC1 ↑	invasiveness and proliferation
	MMP-2 ↑	angiogenesis
	MMP-9 ↑	angiogenesis
	TIMP-1 ↓	growth and antiapoptosis
miR-375 ↓	AEG-1 ↑	cancer development and progression
miR-21 ↑	COX-19 ↑	proliferation and apoptosis
miR-193b ↓	CCND1 ↑	cell cycle progression
	uPA ↑	cell motility, extracellular matrix invasion
miR-106b-25 ↑	β-TRCP2 ↓	inhibition of migration
	SNAI1 ↑	migration
miR-33a ↓	Twist1 ↑	invasion
miR-125-3p ↓	MAT1 ↑	proliferation, migration and invasion
miR-144 ↓	AP4 ↑	invasion
miR-410 ↑	BRD7 ↓	proliferation, migration and invasion

croRNAs, miR-125-3p was shown to post-transcriptionally regulate MTA1 in NSCLC cells and thus inhibit the proliferation, migration and invasion of NSCLC cells through MTA1 targeting and downregulation. Expression levels of miR-125-3p and MTA1 protein were examined in NSCLC tissues and matched normal tissues. There was a significant negative correlation between the expression of miR-125-3p and MTA1 in NSCLC tissues¹⁷⁵. MTA1 expression is associated with an invasive and metastatic phenotype based on microarray data¹⁷⁶. It is a key regulator of the DNA damage response, and is involved in EMT (ref.¹⁷⁷).

Recent studies have reported that activating protein-4 (AP4) is upregulated in NSCLC and predicts poor prognosis in such patients¹⁷⁸⁻¹⁸⁰. AP4 has been shown to regulate cancer metastasis in NSCLC via regulating mTor/p21 and transforming the growth factor β (TGF β) receptor signalling pathway to increase EMT to augment cell invasiveness. Bioinformatics analyses and further luciferase-reporter assay revealed that miR-144 targeted AP4 mRNA. MiR-144 overexpression inhibited AP4-mediated cell invasiveness, while miR-144 depletion increased AP4-mediated cell invasiveness in the human A549 NSCLC line. The levels of miR-144 and AP4 inversely correlated in patient specimens¹⁸⁰.

Tang et al. detected miR-25 expression in A549 cells, NSCLC and non-cancerous tissues. The expression of miR-25 in A549 cells and NSCLC tissues was significantly higher than that in normal MRC5 lung fibroblast cells and non-cancerous lung tissue. The aim of their study was to investigate the functional role of miR-25 in vitro and in vivo. Downregulation of miR-25 markedly inhibited A549 cell proliferation, induced G1 cell cycle arrest, increased cisplatin sensitivity, and suppressed the growth of tumours in a xenograft model, possibly through downregulation of its direct target cell division cycle 42 (CDC42). MiR-25 significantly down-regulated CDC42 in A549 cells¹⁸¹. CDC42 activation results in the stimulation of a variety of signalling cascades altering cellular processes such as cytoskeletal remodelling, establishment of cell polarity, regulation of cell migration and cell proliferation, as well as modification of transcriptional programs¹⁸². Controversially, Zhang et al. showed that CDC42 was over-expressed in NSCLC tissues¹⁸³.

MiR-410 can function as a tumour suppressor or oncogene in some malignancies. To determine its role in NSCLC, Li et al. compared the expression levels NSCLC tissues with adjacent normal tissues and four NSCLC cell lines (A549, SPC-A1, H1299 and H1650) with the normal bronchial epithelial 16HBE cell line. Expression of miR-410 was up-regulated in both human NSCLC tissues and cell lines. Overexpression of miR-410 promoted cell proliferation, migration, and invasion of A549. In addition, bromodomain-containing protein 7 (BRD7) was a direct target of miR-410. MiR-410-mediated downregulation of BRD7 led to increased Akt phosphorylation. Akt activation partially blocked miR-410-induced cell proliferation¹⁸⁴. BRD7 is down-regulated in various malignancies¹⁸⁵⁻¹⁸⁷. However Chen and Yu found that BRD7 expression was significantly higher in NSCLC tissues

compared to normal lung tissue and also in an NSCLC lymph node metastasis group than that in the non-lymph node metastasis group¹⁸⁸. BRD7 plays critical roles in various cellular processes such as development, differentiation, growth, and metabolism^{189,190}. BRD7 might serve as a tumour suppressor by promoting p53 function and by reducing the survival signalling mediated by PI3K/Akt activation¹⁹¹.

The biological functions of selected microRNAs described in this review are summarized in Table 1.

CONCLUSION

At this time, it is not possible to reliably predict the site of metastasis in NSCLC. Microarray technology allows us to analyze the expression levels of hundreds of RNAs in different tissues. There are differences in microRNA expression profiling between NSCLC with and without metastases. The RNA microarray profiling may thus be a useful tool to select up- or down-regulated microRNAs and consider them as unique biological characteristics and potential markers.

Based on the data from RNA microarrays, bioinformatics analysis is able to predict the target genes of highlighted microRNAs which provide us with complex information about cancer cell features such as enhanced proliferation, migration and invasion. Such microRNAs may then be knocked-down using siRNAs or substituted with miRNA mimics.

ABBREVIATIONS

NSCLC, non-small cell lung carcinoma; ABLIM2, actin binding LIM protein family member 2; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; BM, brain metastasis; EGR2, early growth response protein 2; FUS1, FUS RNA binding protein; SOX7, sex determining region Y; FSCN1, FUS RNA binding protein; NUDT1, nucleoside diphosphate linked moiety X-type motif 1; EGFR, epithelial growth factor receptor; IL-1, interleukin complex 1; PRKCA, protein kinase C, alpha; JAK, janus kinase; uPA, urokinase-type plasminogen activator; STAT3, signal transducer and activator of transcription 3; TWIST1, twist family bHLH transcription factor 1; SNAI1, snail family zinc finger 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; RMS, Rhabdomyosarcoma; NPR-1, neuropilin-1; MYO10, myosin X; HNF4 α , hepatocyte nuclear factor 4, alpha; SOX8, SRY (sex determining region Y)-box 8; TNM, tumour node metastasis; LNM, lymph node metastasis; TNFAIP1, TNF α -induced protein 1; CASP, caspase; CEA, carcinoembryonic antigen; ADAM17, ADAM metalloproteinase domain 17; FGF2, fibroblast growth factor 2; c-Met, hepatocyte growth factor receptor; PIK3R3, phosphoinositide-3-kinase, regulatory subunit 3; KRAS, Kirsten rat sarcoma viral oncogene homolog;

GPR55, G protein-coupled receptor 55; NF- κ B, nuclear factor of kappa light polypeptide gene enhancer in B-cells; PTEN, phosphatase and tensin homolog; DLG1, discs large homolog 1; SRC, SRC proto-oncogene, non-receptor tyrosine kinase; Cthrc1, collagen triple helix repeat containing 1; TIMP-1, TIMP metalloproteinase inhibitor 1; ING4, inhibitor of growth family, member 4; TGFBR1, type I TGF- β receptor; TGF- β , transforming growth factor, beta 1; COX-19, COX19 cytochrome c oxidase assembly factor; CDKs, cyclin-dependent kinases; WEE1, WEE1 G2 checkpoint kinase; SREBP-2, sterol regulatory element-binding protein 2; MTA1, Metastasis-associated gene 1; AP4, activating protein-4; CDC42, cell division cycle 42; BRD7, bromodomain-containing protein 7.

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