Review Article

Molecular determinants of lung cancer metastasis to the central nervous system

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Abstract: Lung cancer remains the leading cause of cancer-related mortality worldwide. The propensity for metastasis to the central nervous system (CNS) is a major clinical hurdle contributing to the low five-year survival rate of advanced disease. CNS metastases significantly outnumber primary brain tumors and carry a dismal prognosis in part due to the inability of therapeutic agents to cross the blood brain barrier. Standard treatment using radiation has been largely ineffective in improving mortality, suggesting the need for new agents targeting the critical metastatic drivers. The genetic and molecular events governing CNS metastasis from the lung are poorly understood at this time. This review highlights genetic events associated with CNS dissemination from the lung and molecular mechanisms associated with CNS metastasis. *In vivo* model systems that faithfully recapitulate escape from the lung and colonization of the CNS are described as tools for understanding the metastatic phenotype and for testing new therapeutic agents. A deeper understanding of the mechanisms of lung cancer metastasis to the CNS is needed to elucidate novel therapeutic avenues towards the improvement of the mortality associated with advanced stage lung cancer.

Key Words: Lung cancer; CNS metastases; molecular determinants

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Introduction to lung cancer and CNS metastasis

In 2013, lung cancer is expected to affect 246,000 people and result in 164,000 deaths in the USA (1). Worldwide, lung cancer kills close to 1.5 million people per year (2). The five-year survival rate for advanced stage lung cancer is less than 10% (3). Advanced stage lung tumors are the most likely tumor type to disseminate to the central nervous system (CNS). There have been estimates that 50% of the patients diagnosed with either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC), the two major histological types of lung cancer, will develop metastatic brain lesions. Interestingly, the differing histological subtypes of lung cancer disseminate to the CNS at different rates (*Table 1*). For SCLC, more than 10% of patients present clinically with CNS involvement (10). The overall survival for patients diagnosed with metastatic CNS involvement is dismal, usually ranging from 3-6 months. Thus, improved clinical management of advanced lung cancer patients necessitates an understanding and therapeutic interventions towards metastatic disease, particularly in the brain.

Metastatic brain lesions outnumber primary brain tumors more than 10:1, with 50% of all CNS metastases arising from lung cancer (11,12). CNS metastases carry a clinical burden of morbidity and mortality, but also acute neurological deficits, cognitive impairment and seizures (12,13). Strikingly, the incidence of CNS metastases

Table 1 Reported incidence of CNS metastasis from primary lung cancers by histologic subtype

Histologic subtype	Incidence of CNS metastasis	References
Small cell lung cancer	13.5-59%	(4-7)
Adenocarcinoma	6.6-43%	(8,9)
Squamous cell carcinoma	5.2-13%	(8,9)
Large cell carcinoma	8.3%	(8)
Undifferentiated	41.0%	(9)
NSCLC-NOS	7.4%	(8)

appears to be on the rise, due to factors ranging from an aging population, increased CNS screening after cognitive warning signs, and improvements in treatment of systemic disease (12). The brain presents a unique challenge to therapeutic interventions given that the blood brain barrier (BBB) restricts access to many therapeutic compounds, especially bulky antibodies. Therapeutic interventions derived from understanding the molecular processes of CNS metastases will have to overcome hurdles not faced in many of the other disseminated tumor sites.

Clinical management of primary lung cancer with CNS metastasis

Clinical management of metastatic lung cancer continues to be a significant challenge. Specifically, the majority of lung cancer patients (~50%) are diagnosed with local and/or distant metastasis, which has a median survival of 7-11 months (14-16). The brain is a common site for metastasis in NSCLC patients, present in 25-30% of patients at diagnosis and the majority (40-50%) of patients will develop brain metastases during the course of their disease (14-16). The presence of brain metastases comes with a dismal patient outcome; overall survival for these patients is 2 months with palliative steroid treatment (14-16). While platinum-based therapies have positive benefits for metastatic NSCLC at other sites, application of these therapies for metastatic NSCLC brain lesions is limited, due to the inefficient transport of therapeutics across the BBB (16). Instead, radiation is the treatment of choice for metastatic NSCLC brain lesions, provided radiation therapy is compatible with the chosen systemic therapy. However, even with radiation, survival remains poor, with median survival at 7.6 months (16). Alternatively, a small subset of metastatic NSCLC patients (7%) is found to have a solitary brain lesion either at initial diagnosis or at recurrence (14,15,17). The course of treatment differs for these patients, with primary treatment focused on the brain metastases. Evidence indicates that localized therapy for the brain lesion, in the form of surgical resection of the brain metastasis with whole brain radiation, followed by standard treatment of the primary NSCLC lesion (surgery, surgery with adjuvant chemo/radiation), can improve survival for these patients, increasing median survival time from 2 to 7-27 months (14,15,17). More recently, targeted therapy for adenocarcinoma patients with epidermal growth factor receptor (EGFR) mutations has been shown to control metastatic NSCLC within the brain (16,18), suggesting that targeted therapies may have value in combating CNS metastasis. With such a severe mortality rate, there is an urgent clinical need to understand the mechanisms that govern lung cancer metastasis to the brain so we can identify therapeutic vulnerabilities.

Genetic mechanisms associated with CNS metastasis from the lung

Hanahan and Weinberg characterized genomic instability and mutations as one of the enabling characteristics of cells that facilitates the acquisition of hallmarks of cancer (19). In the course of carcinogenesis, cells acquire several genetic alterations, such as mutations, gene deletions, copy-number aberrations or chromosomal rearrangements, that are associated with the transition from a pre-neoplastic lesion to an invasive tumor and finally progression to the metastatic state (20,21). Even though lung cancer is the most frequent primary site that metastasizes to brain (22), very little is known about the genetic aberrations associated with CNS metastasis from the lung. The next section summarizes the available data on genetic aberrations of matched primary and CNS metastatic lung cancer specimens, and these findings are summarized in *Table 2*.

Somatic gene mutations and CNS metastasis

In the majority of the lung cancer studies, somatic mutations (including EGFR, KRAS, TP53, and many others) were identified on primary lung tumors to understand the genetic basis of the disease (35-41). The genetic landscapes of lung tumor subtypes are now being surveyed by next-generation sequencing towards understanding driver mutations (42-45). However, very few studies have interrogated matched primary and metastatic tumor specimens to correlate the metastatic potential of tumors with somatic mutations.

Table 2 Genetic and chromosomal aberrations associated with CNS metastasis

Locus	Alteration	References
EGFR	Mutation	(23-25)
EGFR	Copy number gain	(26)
KRAS	Mutation	(25,27-29)
Ch2q	Loss	(30,31)
Ch4q12-q32	Loss	(32)
Ch5q35	Amplification	(33)
Ch10q23	Amplification	(33)
Ch11p15	Imbalance	(34)
Ch17q23-24	Amplification	(33)
Ch18q	Loss	(30,31)
Ch22q	Loss	(30,31)

Additionally, investigations of matched primary and metastatic tumor specimens have focused primarily on the mutational status of only EGFR or KRAS. In regard to the EGFR studies, a recent review by Burel-Vandenbos *et al.* thoroughly summarized EGFR mutation status in lung cancer brain metastasis (23). In East Asian cohorts, known to have a higher prevalence of EGFR mutations, activating mutations were found in 44-63% of brain metastases. In Caucasian cohorts, with a low overall prevalence of EGFR mutations, activating mutations were found 0-2% of brain metastases. Eichler and colleagues demonstrated that patients with brain metastasis were more likely to have primary tumors with EGFR mutations (24). Few patient-matched primary and brain metastatic tumor sets have been explored for EGFR mutation status. Four studies with small sample sizes have suggested a discordant EGFR mutation rate between primary and brain metastatic tumors between 0 and 32% (23). There are reported instances of EGFR mutations in CNS metastatic tumors not seen in the patient-matched primary tumor (25), however the impact of technical detection limits of the mutations remains a question.

There is very little data available on the mutation status of KRAS in primary lung cancer with corresponding brain metastasis. Cortot *et al.* reported that 2 out of 13 patients with brain metastasis demonstrated KRAS mutation at codon 12 (G12C) (27). Of the two patients with KRAS mutation, one patient demonstrated KRAS mutation in both primary and corresponding brain metastasis while the other patient demonstrated gain of KRAS mutation in metastatic lesions. However, they were not able to verify the gain of mutation determined using direct sequencing in one patient, using mutant-enriched PCR. In a similar study, Kalikaki *et al.* showed gain of KRAS mutation at codon 12 (G12S) in one metastatic brain tumor sample as compared to corresponding primary lung tumor sample out of two analyzed matched specimens (25). Matsumoto *et al.* found a KRAS mutation in 2 out of 19 metastatic tumors at codon 12 (G12C) (28). However, they didn't have matched primary lung tumor available for KRAS mutation analysis. Finally, a recent study by Munfus-McCray and co-workers found that 23.5% of analyzed metastatic lung tumors with KRAS mutation metastasize to brain (29).

In summary, available data do not establish any clear correlation between EGFR and KRAS mutation status of primary lung tumors and their propensity to metastasize to the CNS. Additional studies are needed to further investigate the link between gene mutations in primary tumors and their potential for CNS dissemination.

Chromosomal imbalances associated with CNS metastasis

Despite the advent of next-generation sequencing and array-based comparative genomic hybridization (aCGH), few studies have been conducted examining genomic aberrations associated with brain metastasis from the lung. In one of the first studies of its kind Shiseki *et al.* investigated 22 brain metastases and 23 early-stage, primary lung tumors from 43 patients (10 matched primary and brain metastasis samples) for allelic losses at 40 loci in 10 chromosomes using restriction fragment length polymorphism (RFLP) (30,31). They demonstrated that in brain metastasis, a significant ($P<0.05$) incidence of allelic losses (>60%) was observed at loci on chromosomes 2q, 18q, and 22q. Takahashi *et al.* investigated 8 primary lung tumors, their 14 corresponding metastases and 8 corresponding normal lung tissues using SNP array analysis (34). In 5 primary lung tumors and their 7 corresponding brain metastasis, a majority (≥81%) of allelic imbalances were similar between primary and matched metastasis. Allelic imbalance at 11p15 was most frequently observed when the genetic imbalance only occurred in the metastatic lesion. In a recent study, Lee and co-workers investigated 18 primary NSCLC and their corresponding brain metastasis for copy number alterations using molecular inversion probe (MIP) technology (33). Using comparative MIP analysis they found that amplification of chromosomal regions 5q35, 10q23, and 17q23-24 in primary lung adenocarcinomas was significantly associated with development of early brain

metastasis.

Sun *et al.* investigated EGFR copy number variations in NSCLC primary tumors and corresponding brain metastasis using fluorescence *in situ* hybridization (FISH) analysis and demonstrated high frequency of gain in EGFR copy number was present in NSCLC primary (62%) and brain metastases (64%) (26). Further, a relatively high level of concordance (84%) for EGFR copy number status was observed between primary tumor and corresponding brain metastasis. Conversely, 9 cases (16% of total) demonstrated discordance between EGFR copy number status between primary tumor and corresponding brain metastasis; in 6 of these, brain metastasis sites had a gain in copy number.

Wrage *et al.* discovered that lung cancer patients in a bone marrow positive group (patients tested positive for disseminated tumor cells in bone marrow) show loss in 4q12-q32 as compared to lung cancer patients in a bone marrow negative group using aCGH, signifying a role of 4q deletion in metastasis (32). Additionally, they performed FISH analysis for 4q21 on tissue microarray with 36 brain metastases and demonstrated that 39% of samples show one allele loss of 4q, whereas gains were only found in 6% of tumors (32). Their comprehensive FISH analysis on 43 primary lung tumor and 35 brain metastasis tumors showed significant loss of 4q21 in brain metastases as compared to primary lung tumors. This data would suggest that a metastasis suppressor gene(s) for lung cancer metastasis to brain could be present on chromosome 4q.

In summary, while multiple genomic aberrations have been reported for lung cancer metastasis to the brain, there is little concordance and few data sets. Next-generation sequencing projects employing larger sample sizes of patient-matched primary and brain metastasis may identify new genomic aberrations driving brain metastasis that can be exploited both for patient prognosis and to guide treatment options.

Molecular mechanisms associated with CNS metastasis

Despite the frequency of CNS metastasis from primary lung tumors, the molecular mechanisms governing this complex process are not well understood. Here we will discuss genes and/or signatures that have been associated with CNS metastases from studies using human clinical samples or *mouse* models of tumor growth and metastasis.

Identification of genes associated with CNS metastasis using clinical specimens

Kargi *et al.* examined 30 NSCLC cases, including 15 excised brain metastases and determined that CD44 protein was significantly inversely related to metastatic potential (46). CD44 isoforms play a role in cell adhesion and are dysregulated in a number of tumor types (47). Kikuchi and colleagues performed expression profiles on 16 metastatic brain foci compared to 37 primary NSCLC tumors and 244 genes showed significantly differential expression between brain metastasis and primary lung tumors (48). Several cytoskeletal protein genes and genes associated with cell movement were differentially up-regulation in the metastases including metallothionein 2A (MT2A), fascin homolog 3 (FSCN3), microtubule-associated protein 7 (MAP7) and CXCL13. Grinberg-Rashi *et al.* analyzed 142 NSCLC tumors and found that N-cadherin, kinesin family member C1 (KIFC1) and bromodomain PHD finger transcription factor (BPTF/FALZ) expression was predictive of brain metastasis (49). N-Cadherin was over-expressed in brain metastasis. This protein has been associated with cells undergoing epithelial-to-mesenchymal transition, and cell invasiveness (50). In addition, a recent report showed that loss of E-cadherin expression was significantly associated with brain metastasis (51). NSCLC patients who developed brain metastasis during follow-up compared to NSCLC patients with no evidence of brain metastasis displayed low E-cadherin expression. Finally, KIF1C, a kinesin family member known to be associated with cell movement (52), was also over-expressed in brain metastasis, while FALZ, a transcription factor with chromatin remodeling properties (53), was under-expressed in brain metastasis.

Another family of genes with strong associations to tumor cell migration and invasion are the chemokine receptors. Chemokine receptors and their cognate ligands are up-regulated in a number of cancers and have been demonstrated to play vital, non-redundant roles in cancer metastasis from multiple primary tumors [reviewed in (54)]. Thus, chemokine receptors have been examined for a role in lung metastasis to the CNS. In 32 patients with solitary brain metastasis from NSCLC, 90% of primary tumors and 100% of brain metastases expressed CXCR4, significantly higher than NSCLC without distant metastases or primary brain tumors (55). Another chemokine receptor associated with lung cancer and metastatic spread is CX3CR1. Protein expression of CX3CR1 was elevated in NSCLC compared

to SCLC (56). While CX3CR1 positivity was significantly associated with number of metastatic sites, paradoxically CX3CR1-negative lung adenocarcinomas were more likely to have disseminated to the brain. The studies summarized above indicate that metastatic colonization of the CNS from lung is a complex process that employs dysregulation of a number of genes known to play a role in cell migration and invasion.

The role of constitutive receptor tyrosine kinase activation in cancer biology is well established. A significant amount of research in lung cancer has focused on the ERBB family members, especially EGFR. The dysregulation of this receptor through mutation or amplification is a known driver of some lung cancers, and serves as a frontline therapeutic target. The role of the ERBB receptors in brain metastasis is less appreciated. It is known the ERBB2 expression in breast cancer is associated with worse prognosis and brain metastasis (57-59). A report by Sun and colleagues examined the protein expression patterns of EGFR, ERBB2, ERBB3, and their ligands in 50 NSCLC primary tumors and corresponding brain metastases (26). The metastases displayed significantly higher protein expression of EGF and amphiregulin in the nucleus. The phosphorylation of EGFR and ERBB3 was elevated in the membrane of the brain metastases compared to primary tumors. In contrast, transforming growth factor-alpha and neuregulin demonstrated significantly higher expression in primary tumors compared to brain metastases. Thus, ERBB family members and ligands are differentially expressed in primary tumors versus brain metastases. In another study, the phosphorylation status of 128 signaling proteins was examined in 42 brain metastases from breast and NSCLC patients by reverse phase protein microarray. The NSCLC metastases exhibited elevated relative levels of the EGFR/ERK network (60). The breast cancer metastases showed higher activation of the ERBB2/IGFR-Akt network compared to lung cancer metastases. Thus, there appears to be a role for EGFR in the brain metastatic phenotype of lung tumors, and this pathway is under investigation as a therapeutic target.

Another receptor tyrosine kinase associated with tumor cell invasion and metastasis is the hepatocyte growth factor receptor (c-MET). c-MET and its ligand, hepatocyte growth factor (HGF), have been associated with tumor progression and metastasis in many solid tumor types (61). The protein expression of c-MET was observed in ~30% of adenocarcinomas and c-MET gene amplification is seen in 10% of adenocarcinomas (62). Increased activity of c-MET can occur via oncogenic activation of KRAS (63), while gene amplification of c-MET is often related to resistance to EGFR-tyrosine kinase inhibitors (TKIs). The expression of c-MET and/or HGF has been associated with therapeutic resistance against EGFR-TKIs (64-66), cisplatin (67), and radiation (68). Expression of c-MET was more common in poorly differentiated adenocarcinomas compared to well-differentiated tumors (62). Benedettini *et al.* demonstrated that c-MET expression and phosphorylation were associated with the development of brain metastasis, and enriched in brain metastases compared to patient-matched primary tumors (69). Thus, the HGF/c-MET pathway may offer unique therapeutic vulnerabilities against brain metastases.

In SCLC, the most highly aggressive lung cancer subtype with strong predilection for metastasis to the brain, placental growth factor (PLGF) and vascular endothelial growth factor receptor 1 (VEGFR1) expression levels were recently associated with brain metastasis (70). Elevated serum levels of PLGF were detected in SCLC patients with brain metastasis compared to SCLC patients without brain metastasis. This elevated expression of PLGF was also seen in the brain metastasis tissue. PLGF triggered VEGFR1 signaling and promoted SCLC cell trans-endothelial migration *in vitro*. Depletion of PLGF via shRNA technology inhibited brain metastasis in an *in vivo* model system. Thus, the VEGF member PLGF may play a role in the invasive nature of SCLC.

Identification of genes associated with CNS metastasis using mouse models

Transgenic mouse technology has become a powerful tool for investigating the contribution of a gene or genes in development and disease pathology, particularly cancer. Manipulating expression of genes involved in human NSCLC with conditional alleles or transgenes has led to a variety of genetically-engineered mouse models (GEMM) (71). Several GEMMs have been developed carrying reported mutated oncogenes of NSCLC (e.g., $EGFR^{L858R}$,T790M, $ERBB2^{YVMA}$, EML4-ALK chimera, $PIK3CA^{H1074R}$, $KRAS^{G12V}$, c-MET), in the presence or absence of deletion of tumor suppressors such as TP53, P16 or LKB1 (71). Several of these models have shown the capacity for metastatic spread into lymph nodes, the surrounding chest cavity and even into distal organs such as bone (72). Despite these aggressive model systems, metastatic colonization of the CNS has remained elusive.

The lack of CNS metastasis may be accounted for by the shortened survival times of these aggressive models, or the lack of SCLC models, the lung histologic subtype most likely to present with CNS metastasis. It will be interesting to see whether expression of SCC- or SCLC-specific oncogene mutations will result in GEMMs of lung cancer cell dissemination to the brain.

While the development of GEMMs with reproducible brain metastasis has proven elusive to date as outlined above, a number of cell line models (both syngeneic and xenograft) have been generated with resultant CNS metastatic features. These models have used multiple injection routes including inter-cardiac injection and lung orthotopic injection. Two groups have reported the ability of A549 lung cancer cells to colonize the brain when injected in the bloodstream or orthotopically implanted into the lungs of immune-deficient mice (73,74). Another cell line with reported brain metastatic phenotype is NCI-H250, a SCLC model (70). H250 cells injected into the internal carotid artery presented with brain metastasis in 5 of 18 mice, with a suppression of PLGF activity completely abrogating brain metastasis. One drawback of using human cell lines in the mouse model is the lack of complete immune response. To overcome this challenge, syngeneic models have been generated using Lewis lung carcinoma (LLC) cells. These cells have the ability to produce metastatic tumors from orthotopic injection to multiple organs, including a low incidence of brain metastasis (75). Injection of the LLC cells into the internal carotid artery could also produce brain metastatic lesions (76).

Multiple labs have generated a number of site-specific metastatic models across multiple cancer types. *In vivo* selection of lung tumor cells with brain colonizing potential, followed by extraction/expansion in culture, and re-introduction *in vivo* has the ability to produce cell subclones with enhanced 'brain seeking' potential. In 2004, Yoshimasu *et al.* described an *in vivo* model of CNS metastasis using the SCC cell line EBC-1 (77). Ventricular injection of parental EBC-1 cells produced low incidence of both brain and bone metastasis. Extraction of these metastatic cells and repeated *in vivo* selection produced EBC-1 subclones with enhanced potential to colonize the brain or bone. The highly brain metastatic subclone of EBC-1 cells expressed significantly higher expression of integrin alpha-3 compared to EBC-1 parental cells or EBC-1 cells metastatic to the bone (77). Suppression of integrin alpha-3/beta-1 significantly diminished brain metastasis using the *in vivo* model. ADAM9, a member of the "a disintegrin and metalloprotease" family has also been associated with brain metastasis from NSCLC. The ADAM family members regulate cell-cell and cell-matrix interactions (78,79). ADAM9 mRNA was highly expressed in EBC-1 brain metastatic lines compared to EBC-1 parental and bone metastatic lines (73). Over-expression of ADAM9 in A549 cells enhanced micro-metastatic foci in the brain. Another SCC cell line capable of brain metastasis *in vivo* is HARA (80). Again, *in vivo* selection after cardiac injection resulting in brain lesions produced a subclone with enhanced brain metastatic potential. This *in vivo* model has been used to begin to understand the recruitment and interplay between astrocytes and metastatic cells in metastatic growth (81).

The Massague lab has generated a number of site-specific metastatic models across multiple cancer types. In H2030 and PC9 cells (adenocarcinoma cell lines driven by KRAS and EGFR mutations, respectively), inter-cardiac injection of these cells, extraction and expansion in culture of brain lesions, and multiple rounds of *in vivo* selection produced cells that seeded the brain 100% of the time (82). One feature of this model is the ability to orthotopically implant cells into the lungs with resultant brain metastasis. The brain seeker lines compared to the parental cell lines displayed enriched activity of the WNT/TCF pathway. Genes in this pathway associated with metastasis were lymphoid enhancer-binding factor 1 (LEF1), homeobox B9 (HOXB9), and bone morphogenetic protein 4 (BMP4). The knockdown of LEF1 or HOXB9 significantly decreased the ability of the brain seeker lines to form metastases. Huang and colleagues used the PC9 brain seeker line to test a toxin directed at EGFR and urokinase receptor (uPAR) (83). They found that immunotoxin administration prolonged mouse survival.

Role of microRNAs in brain metastasis

miRNA biomarkers associated with CNS metastasis

MicroRNAs (miRNAs) are noncoding endogenous RNA species that regulate gene expression at the post-transcriptional level [reviewed in (84)]. Dysregulation of miRNAs has been linked to the development and progression of multiple cancer types, but the role of miRNAs in CNS metastasis remains an emerging area of research. The stability of miRNAs in tissues and fluids makes them attractive candidates for use in predictive and prognostic markers (85,86). Multiple groups have explored

Table 3 Selected molecular targets with potential therapeutic agents

Gene	Drugs	References
EGFR	Cetuximab, erlotinib, gefitinib, afatinib, dacomitinib	(93)
ERBB2	Afatinib, dacomitinib, lapatinib	(93)
N-cadherin	ADH-1	(94)
VEGFR1	Vandetanib, sorafenib, sunitinib, axitinib, cabozantinib, pazopanib	(95)
CX3CR1	F1 hCX3CL1 analog	(96)
MET	Onartuzumab, tivantinib	(93)
CXCR4	Plerixafor	(95)

miRNA expression as biomarkers to stratify patients or identify CNS metastasis. Lu *et al.* (87) profiled miRNAs extracted from surgically resected Stage I lung tumors in search of signatures predictive of recurrence and relapse-free survival. Ten miRNAs were found to be differentially expressed in samples from patients who subsequently developed brain metastases: miR-1, -29c, -30d, -145*, -148a*, -187, -218, -375, -450b-3p, and -708*. Teplyuk *et al.* explored miRNA signatures in cerebrospinal fluid (CSF) to detect and distinguish CNS malignancies (88). Members of the miR-200 family (including miR-141 and miR-200a/b/c) were highly expressed in the CSF of patients with metastases from breast or lung but not in other pathologic conditions. Recently, Arora *et al.* demonstrated that miR-328 and miR-330-3p expression significantly distinguished seven patients with brain metastasis from six patients without brain metastasis (89). Thus, miRNA profiles may help to identify lung cancers more likely to metastasize to the brain.

CNS metastasis mechanisms associated with miRNA function

There are now efforts towards understanding the mechanistic role(s) of miRNAs in the brain metastatic phenotype. For instance, miR-378 has been reported to promote survival, invasion and migration in A549 cells through MMP2, MMP9 and VEGF (90). Arora *et al.* showed that overexpression of miR-328 increases NSCLC migration via regulation of PRKCA, a member of the VEGF-IL1 family (89). Cheng *et al.* have elucidated a PRKCA-dependent mechanism through which IL1-beta induces uPA expression and cellular migration in A549 NSCLC cells (91). Others report that miR-146a exerts a mechanistically similar metastasis-suppressing function (92). Overexpression of miR-146a inhibits the degradation of β-catenin and acts to suppress hnRNPC, which in turn reduces expression of uPA, uPAR, MT1-MMP and MMP1. Both increased expression of β-catenin and suppression of hnRNPC served to inhibit invasion and migration. The role of miRNAs in lung cancer metastasis to the CNS is just beginning to be understood, and may offer novel therapeutic targets against advanced lung cancer.

Therapeutic opportunities against CNS metastasis

There is an urgent need to improve the clinical outcomes for patients who present with or develop CNS metastasis from primary lung tumors. With improved systemic treatment of primary lung lesions and enhanced imaging modalities, the incidence or detection of CNS metastasis will continue to increase. Current clinical regimens of surgery plus radiation (for solitary brain lesion) or radiation for multi-focal lesion have demonstrated infrequent clinical success. Selected targets that have been associated with CNS metastasis with known pharmacologic inhibitors are listed in *Table 3*. Demonstrated clinical benefits using EGFR-targeted therapies towards mutant EGFR expressing CNS lesions (97) gives credence to improved therapeutics with enhanced understanding of the genetics and molecular mechanisms of the CNS phenotype. The targeting of other ERBB family members may also prove to be a viable strategy for combating CNS metastases from lung or breast primary tumors (26). CNS lesions arising from mutant KRAS-expressing primary lesions may be susceptible to combined targeting of MAPK and PI3K signaling or SRC inhibitors. The demonstration that c-MET expression is associated with CNS metastatic events opens the possibility of exploiting c-MET inhibitors (98,99) in this setting. It is therefore crucial to further understand the driver events of CNS metastasis through the collection of patient-

matched primary and metastatic lesions for investigation as well as continued development of *in vivo* models capable of faithfully recapitulating the CNS metastatic phenotype from primary lung tumors. It will also be critical to test the ability of these agents to cross the BBB to affect the desired target.

Conclusions

Lung cancer continues to be a leading cause of cancer-related mortality throughout the world. The five-year survival rate for advanced, especially metastatic, disease is dismal. The colonization of the brain as a metastatic site contributes to the mortality of this disease, resulting in a dramatically reduced survival expectation. A thorough understanding of the genetics and molecular mechanisms that govern CNS metastasis from the lung are far from complete at this time. The emergence of next-generation sequencing along with the collection of patient-matched primary and CNS metastatic lesions offers a path forward towards a more complete understanding of the metastatic process and novel therapeutic avenues. Targeted approaches as seen with EGFR-targeted therapeutics positively affecting patient outcome with CNS metastasis offers hope that a full understanding of the CNS metastatic process will lead to better therapeutics and improved patient survival.

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